AGROFOOD

20 - 21 June 2019, Istanbul

INTERNATIONAL CONFERENCE ON AGRONOMY AND FOOD SCIENCE & TECHNOLOGY

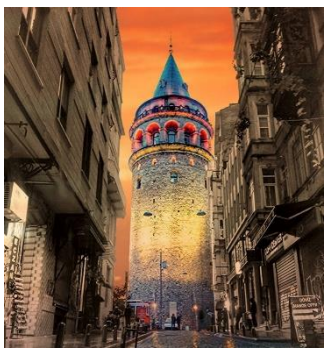
20 – 21 June 2019

Double Tree by Hilton Hotel, Avcılar, Istanbul, Turkey

www.agrofoodconference.org

AGROFOOD CONFERENCE E-BOOK (POSTER PRESENTATIONS)

galaksi



Welcome to Istanbul

We are honored to invite you to the **International Conference on Agronomy and Food Science and Technology (AgroFood),** which will be held on 20-21 June 2019 in Istanbul. The aims of this multi-faceted event are;

To present and discuss the latest innovations and trends,

To provide an appropriate environment to share the results of the researches on agriculture and food and the experiences gained in these fields,

Bringing together leading scientists, researchers and academics.

Food and agricultural industries have an important place in the economic life of all countries, regardless of their level of development. There is no substitute for these industries producing foodstuffs and raw materials for human nutrition. The global climate change, rapid decline of agricultural lands, serious declining the soil fertility and the threatening rise of food prices hint scenarios of a global food crisis in the near future. For this reason, the areas of Food and Agriculture Science, Processing and Technology continue to improve each passing day. In order to improve new technologies and innovations in the field of food and agriculture, it is necessary to establish a strong interdisciplinary cooperation by employing fundamental sciences such as biology, chemistry, physics and engineering. Therefore, the AgroFood Conference offers you a platform to present your ideas and researches on all related disciplines such as Food Science and Technology, Aquaculture Science, Nutrition Science, Animal Science, Horticulture, Agriculture and Food Biotechnology.

The AgroFood – Istanbul'2019 conference that is going to be launched on 20 June 2019 in Avcılar, Istanbul, Turkey, provides a fruitful, international and unique forum for exchange of views among participants currently working in the areas of agriculture and food science and technology.

We hope you enjoy the AgroFood – Istanbul'2019 conference and your stay in Istanbul, Turkey.

Chairman and Editor

Dr. Okan ÖZKAYA

Co-Chairman and Editor

Dr. Kemal ŞEN



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Dr. Stéphanie ROUX	Paris Institute of Technology For Life	FRANCE
Dr. Yekta GÖKSUNGUR	Ege University	TURKEY

The ***International Conference on Agronomy and Food Science and Technology (AgroFood)*** is a peer reviewed international conference.

Keynote Speakers

Dr. Arun K. BHUNIA

Purdue University, Indiana, USA

Dr. Mar VILANOVA de la TORRE

Spanish National Research Council, Pontevedra, SPAIN

Dr. Brijesh TIWARI

Teagasc Food Research Centre, Dublin, IRELAND

Dr. Stéphanie ROUX

Paris Institute of Technology For Life, Paris, FRANCE

Conference Topics

We invite abstracts in all areas related to agriculture and food engineering, including, but not limited to:

Food Science and Technology

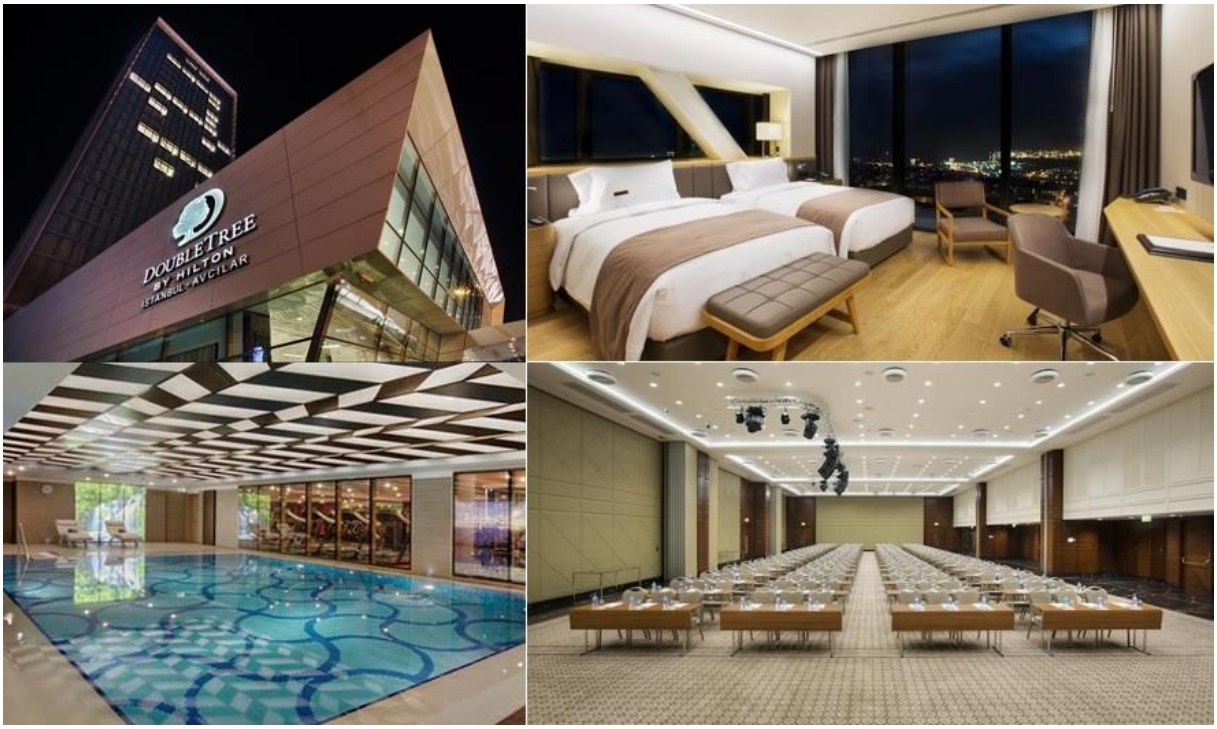
- Food Assurance and Safety
- Food Production Technologies
- Food Microbiology
- Food Chemistry
- Food Biotechnology
- Food Rheology, Texture and Shelf Life
- Food Additives, Bioactive Ingredients and Antioxidants
- Food Engineering Basic Operations
- Applications of Enzymes and Foods
- Nanotechnology and Food Applications
- Functional Foods
- Traditional Food
- Process Simulation and Optimization
- Food Packaging

Agricultural Science and Technology

- Agricultural Science and Technology
- Plant Nutrients and Soil Fertility
- Plant Breeding and Breeding
- Plant Ecology and Physiology
- Industrial Plants and Bio-Diesel Production
- Alternative Plant Production Systems
- Plant protection
- Field Crops
- Horticultural Science and Technology
- Animal Science and Technology
- Agricultural Machinery and Innovation
- Seafood
- Environmental Problems and Solutions in Agriculture
- Climate Change and Its Effects on Agricultural Production
- Agricultural Biotechnology
- Post-harvest applications and technologies in agricultural products

Conference Venue

The 5 star Double Tree By Hilton Hotel, Avcılar, Istanbul.



The Social Programme

Welcome Lunch and Opening Ceremony

The participants (delegates, students and listeners) warmly invited to join us for the Opening Ceremony that takes place in the main hall of the Double Tree By Hilton Hotel, after the conference registration. After the Opening Ceremony, the organization committee will give an award to the Keynote speakers. After the award ceremony, the participants will go to the lunch meeting.

Gala Dinner, Bosphorus Cruise Tour & Night Show

The gala night offers a luxurious dinner cruise night out on the Bosphorus in Istanbul. You will enjoy delicious gourmet foods, while you dance with the panaromic view of the Bosphorus that separates the two continents Europe and Asia.

Enjoy as you cruise The Bosphorus and see historical places such as Dolmabahce Palace, Bosphorus Bridge, Ortakoy, Rumeli Fortress, Fatih Bridge, Beylerbeyi Palace, Maiden's Tower and also Istanbul's very famous night clubs as you dine the night away. No other Istanbul experience can match this unique combination of romantic ambiance. We combine fresh food with unparalleled views of Istanbul and live entertainment to create Istanbul‘s leading Bosphorus Dinner Cruise. After the dinner, you can enjoy the entertaining music and traditional show programs or simply relax with a drink and enjoy the view.

International Conference on Agronomy and Food Science and Technology (AgroFood)

20 - 21 June 2019, DoubleTree by Hilton Hotel - Avcılar, Istanbul, Turkey

CONFERENCE PROGRAMME

Time	Thursday, 20 June 2019
08:00-11:00	***Conference Registration***
11:00-12:00	***Openning Ceremony***
12:00-13:00	***Welcome Lunch***
13:00-13:20	**Keynote Speaker: Dr. Arun K. BHUNIA, Purdue University, USA** Global challenges in microbial food safety and solutions
13:20-13:40	**Keynote Speaker: Dr. Brijesh TIWARI, Teagasc Food Research Centre, IRELAND** Cold plasma technology for improving shelf life of foods
13:40-14:00	**Keynote Speaker: Dr. Mar VILANOVA, Spanish National Research Council, SPAIN** New applications to improve the grape aroma
14:00-14:20	**Keynote Speaker: Dr. Stéphanie ROUX, Paris Institute of Technology For Life, FRANCE** Modeling as a tool to understand and predict the extent of reactions in food products
14:20-14:45	***Coffee Break***

Time	Thursday, 20 June 2019			
	Hall A & Session 1 Chair: Dr. Arun K. Bhunia	***Hall B & Session 1 Chair: Dr. Brijesh K Tiwari***	***Hall C & Session 1 Chair: Dr. Stéphanie Roux***	***Hall D & Session 1 Chair: Dr. Silvius Stanciu***
14:45-15:00	Impact of Environmental and Host-Induced StressonPathogenesis,Disease, and Microbial Detection A. K. Bhunia	LC-DAD-ESI-MS/MS characterization of elderberry flower (Sambucus nigra) phenolics extracted with various solvents T. Uzlasir, P. Kadiroğlu, S. Selli, H. Kelebek	Improvement of microwave-vacuum drying system and mathematical modeling of pear A. Polat, O. Taşkın, N. İzli	Researches on the incidence of pesticide residues in food products on the Romanian market S. Stanciu
15:00-15:15	Investigation of the microbial profile of argan, bergamot, nigella sativa seed, pomegranate seed and ginger oil I. Guldal, S. Karabiyikli	Antioxidant andbiological activitiesof blackthorn extracts B.M. Popović, B. Blagojević, R.Ž. Pavlović, N. Mićić, S. Bijelić, B. Bogdanović, A.T. Serra	Combined effects of novel technologies on vacuum impregnation treatments of foods A. Görgüç, E. Gençdağ, F.M. Yılmaz	The Levels of 5-hydroxymethylfurfural in different kinds of pekmez N. İçli
15:15-15:30	Modelling effect of freeze-thaw stress on the growth of wine yeasts isolated from Turkish region H.A.Karaoglan,N. DeveliIsikli, N. Coksoyler, F. Ozcelik	Impact of honey color from Jordanian flora on total phenolics and flavonoids contents and antioxidant activity M. Al-Dabbas, H. Otoom	Effect of micronutrients on the bioavailability of flavonoids S. Kamiloglu	The Levels of organochlorine pesticides in milk; The situation in Turkey N. İçli
15:30-15:45	Detection of beta-lactamase genes blaA and blaB and ampicillin susceptibility of Yersinia enterocolitica from food S. Arslan, H.G. Kabakcı	Determination of some sugar contained products and amount of advanced glycation ends products via high-performance liquid chromatography – spectrophotometric B. Yusufoğlu, M. Yaman, E. Karakuş	The Effect of impregnation pre-treatments on drying kinetics of saffron milk cap (lactarius deliciosus) E. Gençdağ, A. Görgüç, F.M. Yılmaz	Investigation of the availability of image processing as an alternative method to spectrophotometry for prediction of hmf content in honey for different temperatures T. Türkoğlu, S. Sabancı, A. Baltacı, S.N. Dirim
15:45-16:00	Monitoring of microbial changes during incubation in the yoghurts prepared with different culture concentrations by DNA melting curves B. Çetin, H. Aktaş, H. Meral	Thermal inactivation of listeria monocytogenes in Inegöl and kasap köfte (traditional Turkish meatballs) A. Kemah, A. Dikici, S.B. Bozatlı	The Effect Synthetic Antioxidants on the Oxidative Stability of Sunflower and Corn Oils T. Keceli Mutlu	How Safe Are Romanian Foods to GMOs Contamination? S. Stanciu, C. I. Bichescu
16:30-17:30	***Bus Transfer From the Conference Hotel to the Cruise Tour***			
17:30-19:00	***Free Time in Eminonu District***			
19:00-23:00	***Bhosphorus Tour & Gala Dinner & Turkish Night Show***			

Time	Thursday, 20 June 2019			
	Hall E & Session 1 Chair: Dr. Cem Baltacıoğlu	***Hall F & Session 1 Chair: Dr. Hande Baltacıoğlu***	***Hall G & Session 1 Chair: Dr. Haşim Kelebek***	***Hall H & Session 1 Chair: Dr. Yusuf Z. Menceloğlu***
14:45-15:00	Drying characteristics of white cheese dried by different methods Ö. Köprüalan, M. Koç, N. Koca, F. Kaymak-Ertekin	Image processing techniques in the food applications H. Yüksel, S.N. Dirim	Effectof picklingon some biochemical properties of brassica vegetables: cabbage, cauliflower and broccoli R. Arslan, A. Güleç, H. Tokbaş, E.C. Eroglu, S. Aksay	Magnetic iron oxide nanoparticle-based nanobiotechnology applications in agri-food production K. Gokduman
15:00-15:15	Effects of explosive puff drying and hot air drying on quality characteristics of nutritive pumpkin chips Ö. Altay, Ö. Köprüalan, A. Bodruk, F. Kaymak-Ertekin	Effect of mild temperature-long time (MTLT) treatment on bioactive compounds in peach juice H. Baltacioglu	A new dehydration technique: Refractance window drying Ö. Süfer	Effects of carbon dots extracted from sugar beet molasses on the growth of different plants S. Dinç, M.Z. Karapçin, M. Kara, E. Tuncay
15:15-15:30	Influence of edible coatings on some physicochemical properties of dried banana (Musa cavendish) A. Aygün, M. K. Uslu, M. Torun, T. Aygün	Selective factors among the green technologies for extraction of bioactive compounds C. Değerli, S.N. El	Effect of dryingmethods onquality characteristics of black myrtle (myrtus communis l.) H. Tokbaş, R. Arslan, A. Güleç, S. Aksay	Comparison of antioxidant activities of carbon dots synthesized from different carbon sources M. Kara, S. Dinç, S. Kolaylı, C. Birinci
15:30-15:45	Determination of thin layer drying kinetics and mathematical modeling of bay (laurus nobilis l.) leaves H. Tokbas, H.İ. Ekiz	Optimization of oil body isolationfrom pomegranate seeds I. Tontul, D. Sert	Sourdough bread with pumpkin pomace M. Şimşek Aslanoğlu, Ö. Süfer, H. Demir, B. Eke, R. Koşay	Nanoengineering in Agriculture and Food Science Y.Z. Menceloglu
15:45-16:00		Optimization of mucilage extraction from Chia seeds S.A. Tontul		
16:30-17:30	***Bus Transfer From the Conference Hotel to the Cruise Tour***			
17:30-19:00	***Free Time in Eminonu District***			
19:00-23:00	***Bhosphorus Tour & Gala Dinner & Turkish Night Show***			

Time	Friday, 21 June 2019			
	Hall A & Session 2 Chair: Dr. Mar Vilanova	***Hall B & Session 2 Chair: Dr. Bülent Çetin***	***Hall C & Session 2 Chair: Dr. Vesna Levkov***	***Hall D & Session 2 Chair: Dr. Dragana Stamenov***
08:30-08:45	Assessment of Representativeness of Aromatic Extracts for Aroma Characterization: A study of Caper G. Güçlü, A. S. Sönmezdağ, S. Selli, H. Kelebek	GMOs in theRepublic ofMoldova. Legislative Framework and Public Perception of Genetically Modified Food M. Munteanu Pila, S. Stanciu	STEC Survival and synthetic gastric fluid resistance during storage of experimentally produced frankfurters S.B. Bozatli, A. Dikici, B. Ergönül	Extracellular protease genes in motile aeromonas species isolated from oncorhynchus mykiss S. Arslan, F. Özdemir
08:45-09:00	Effect of Geographic Conditions on Pine honey Carbon Isotope Value, Texture and Color Properties O. Sevindik, G. Guclu, M. Yetişen, H. Kelebek, S. Selli	Food Safety and Assurance Through Halal Certification: The Malaysian Experience S. Md Shaarani	Retention of vitamin B6 during different cooking methods in fish and chicken meats J. Çatak	Microbial inoculants in cabbage productioneffect on cabbage yield and soil microbiological activity T. Hajnal Jafari, S. Đurić, D. Stamenov
09:00-09:15	Comparison of Different Techniques Used in Determination of Aroma Active Compounds in Foods by GC-O E. Ereli, K. Şen	Investigation of microbial changes of yoghurts prepared with different culture concentrations by molecular and classical methods during storage H. Aktaş, B. Çetin	The effect of grape vinegar on the some physicochemical and textural properties of chicken breast meat K. Ünal, A. Cabi, E. Alagöz, C. Sariçoban	Detection of genes involved in biofilm formation in staphylococcus aureus isolates from meat and quantification of their biofilm formation S. Arslan, C.B. Eken
09:15-09:30	GC-MS-Olfactometric Characterization of Key Odorants in Pine Honey from Two Different Regions of Turkey G. Guclu, O. Sevindik, H. Kelebek, S. Selli	Molecular and phenotypic characterization of siderophore production in yersinia enterocolitica isolates from meat and milk products S. Arslan, F. Özdemir	Incorporation of selected herbal plant extracts to suppress glyceamic impact of cane sugar C.H.Samarasinghe, M.A. Jayasinghe, S.P.A.S. Senadheera, I. Wijesekara,	Chemical composition, biological activity and microbial diversity of kombucha İ.Y. Sengun, A. Kirmizigul
09:30-09:45	Effects of matrix composition on perception of strawberry flavour in model dairy gels P. G. Owusu-Darko, A. Paterson, J. Piggott	Extraction,Processing and Functional Properties of Pistachio Oil T. Mutlu Keceli	Chemical investigation and biological activities of the lamiaceae plant: Thymus fallax E. Eroğlu-Özkan, M. Boğa, Y. Yeşil	Production of probiotic beverage with oat milk containing grape juice using lactobacillus rhamnosus G. Tiryaki Gündüz, H. Didar Sözbir
09:45-10:00		Processing, Usage and Functional Properties of Peanut Oil T. Mutlu Keceli	Nutritive Value And Health Benefits Of The Modified Eggs With Bioactive Components N. Gjorgovska, K. Filev, S. Grigorova, D. Abadjieva, V. Levkov	Total phenolic contents, antioxidant and antimicrobial activities of koruk (unripe grape, vitis vinefera l.) products I.Y. Sengun, B. Ozturk
10:00-10:30	***Coffee Break***			

Time	Friday, 21 June 2019			
	Hall E & Session 2 Chair: Dr. Chinyere B. B. Okebalama	***Hall F & Session 2 Chair: Dr. Yunus Emre Tuncil***	***Hall G & Session 2 Chair: Dr. Ayşe Özyılmaz***	***Hall H & Session 2 Chair: Dr. Bekir Gökçen Mazı***
08:30-08:45	Investigation of socio-economic characteristics of wheat producers on certified seed use: The case of Ankara province C. Cevher, B. Altunkaynak, S. Coşkun Cevher	Structures features of dietary fibers of Turkish hazelnut (corylus avellana l.) and hazelnut skin Y.E. Tuncil	A preliminary study of micro element accumulation of four fish species from North Eastern Mediterranean A. Özyılmaz	Determination of some chemical and physical properties of structured lipids produced by enzymatic interesterification of beef tallow with corn oil A.B. Aktaş, B. Ozen
08:45-09:00	Mapping distribution of soil quality parameters and indexes using geostatistical methods in the harran plain A.V. Bilgili, Y. Kaplan	Wheat starch-lauric acid complexation in the presence of bovine serum albumin S.S.Severcan, B. OskaybasEmlek, A. Özbey, K. Kahraman	Hepatosomatic Index (HSI) and crude protein contents of four cartilaginous fish species from the Northeastern Mediterranean A. Özyılmaz	Expression of full-length and mature αamylase from bacillus sp. in p. pastoris and characterization of the recombinant enzyme F. Erden-Karaoğlan, M. Karaoğlan
09:00-09:15	Fungicides usage status and current problems of growers in Sanliurfa province A. Bilgili, A. Aksu Altun, C. Yetkin, S. Ipekcioglu	Effects of Salt Substitue on Mineral Contents of Circassian Cheese During Storage Y. Parlak, N. Güzeler	Leptin receptor (LEPR) gene polymorphism in some of the donkey populations of Turkey R. Işık, F. Özdil	D-tagatose 3-epimerase enzyme production for converting D-fructose into D-allulose E. Parıldı, B. Devrim Özcan, E. Dikkaya, O. Kola, M.R. Akkaya, N. Cengiz
09:15-09:30	Site Specific PoultryManure Recommendation for Cucumber (Cucumis Sativus L.) and It's Effect On The Fertility of an Ultisol after Three Years Continuous Application C.B. Okebalama, K.C. Ogunezi, I.C. Agbo, K.C. Asogwa, and B. Marschner	Method of determination of water-soluble protein in Bulgarian semi-hard yellow cheese T.Kolev, G.Dicheva, T. Angelova, S. Laleva, N. Naydenova	The Nutritional Quality of Freshwater Fish of Republic of Moldova M. Munteanu Pila, S. Stanciu	Effects of Cinnamon on Type-2 Diabetes Mellitus E. F. Topdas, A. Odunkıran, M. Sengul
09:30-09:45	Cutting Force and Energy Requirement of Boğazkere Grape (Vitis vinifera l.) Cane A. Sessiz, A. K. Eliçin, Y. Bayhan	Comparison of polyphenolic characteristics and cytotoxic activities of fermented morus alba cultivars from Turkey M.F. Seyhan, A. Diren		
09:45-10:00	Shear Force and Energy Requirement of Rice Stem R. Esgici, F. G. Pekitkan, A. Sessiz			
10:00-10:30	***Coffee Break***			

	Hall A & Session 3 Chair: Dr. Serkan Selli	***Hall B & Session 3 Chair: Dr.Yusuf Z. Menceloğlu***	***Hall C & Session 3 Chair: Dr. Bige İncedayı***	***Hall D & Session 3 Chair: Dr. Timea Hajnal Jafari***
10:30-10:45	Contraverses in the determination of antioxidant activity in food and biological samples-from individual determinations to PCA analysis B.M. Popović, R.Ž. Pavlović, B.Blagojević, N. Mićić, T. Jurić	Investigation of anti-fungal activity of encapsulated Saccharomyces boulardii and clove oil emulsion in strawberries at postharvest stage A. Hacioglu, F. Poyraz, A. Orcen, B. Berber Orcen	Drying kinetics and quality characteristics of hot air dried potato cv. jelly under different process parameters N. Türkmen Erol, B. İncedayi, F. Sari, Ö.U. Çopur	The effects of radicle pruning on plant growth and some physiological parameters of bean under drought stress E. Yıldırım, M. Ekinci, S. Örs, R. Kul
10:45-11:00	Effect of Different Harvest Period on Phenolic Compounds and Some Important QualityParameters of KırmızıPistachio Variety T. Uzlasir, A. S. Sönmezdağ, G. Güçlü, P. Kadiroğlu, S. Dıblan, N. Cengiz, S. Selli, H. Kelebek	Effect of different harvesting time on quality and phytochemicals in prickly pear (opuntia ficus indica) E.C. Eroglu, R. Arslan, A. Güleç, S. Aksay	Nutritional potential characterization and bioactive properties of caper products B. Seyhan, B. İncedayi	Combine effects of drought and cadmium metal stress on lettuce seedling M. Ekinci, E. Yıldırım, S. Örs, R. Kul
11:00-11:15	The Phenolic Composition and Antioxidant Activities of Red Beetroots H. K. Şaşmaz, P. Kadiroglu, S. Selli, H. Kelebek	Effect of Photoselective Nets on Harvest Quality and Physicochemical Characteristics of apples cv.'Fuji' O.Ozkaya, N. Dölek, B. Imrak, A. Küden, Ö. Dündar, H. Demircioğlu	The Effect of the Addition of Different Concentrations of Four Hydrocolloids on Sensory Properties of Strawberry Jam E. S. Erten, T. Erten	Developmentofschemes for provided services of forest shelterbelts in dry climate A. Chikalanov, S. Peteva, Y. Petkov, M. Lyubenova, I. Todorova
11:15-11:30	Volatile Composition of Althaea officinalis L. Flowers G. Guclu, O. Sevindik, H. Kelebek, S. Selli	LongLastingSanitation of Postharvest Storage Areas by using Sol Gel Chemistry N. Ö. Şanlı and Y. Z. Menceloglu	Effect of cultivar type and maturation level on sorption isotherms of apricots B. Öztürk, F. Seyhan	The increase of productive capacity of acidic soil by the application of microorganism and liming D. Stamenov, S. Đurić, T. Hajnal Jafari
11:30-11:45	Impact of Ultrasonic Frequencies on Phlorotannin Content of Seaweeds V. Ummat, C. P. O'Donnell, G. Rajauria B. K. Tiwari	Active Modified Atmosphere Pallet Cover Treatment Effects on Storage and Shelf Life Quality of Strawberry E. Korkut, E. Kurt, N. Yavuz, O. Ozkaya		
11:45-12:00		Cold Storage of O'gosho Persimmon A. E. Özdemir, D. Kılıç, C. Toplu, A. Çelik, S. Ş. Arslan, H. Yıldırım		
12:00-13:00	***Lunch Break***			

Time	Friday, 21 June 2019			
	Hall E & Session 3 Chair: Dr. Salih Aksay	***Hall F & Session 3 Chair: Dr. Michael Wisniewski***	***Hall G & Session 3 Chair: Dr. Sema Başbağ***	***Hall H & Session 3 Chair: Dr.Türkan Keçeli Mutlu***
10:30-10:45	The effect of different extraction techniques on color values of peach pomace C. Baltacıoğlu, İ. Okur, B. Harmankaya, D. Bayrakçı, F. Turan, H. Koşok	Theeffect ofhumidification on seed germination and seedling emergence of sweet corn seeds E. Ozden, A. Unlu, I. Demir	Determination of Agronomic and Technological Properties Varieties of Flax in Sowing Different Times (Linum usitatissimum L) N. Tayınmak, S. Basbag, R. Ekinci	The effect of continuous system ultraviolet light application on the quality of yoghurt with surface cream M.Ildız, G. Tiryaki Gündüz, M. Urgu Öztürk, D. Kışla, S. Ünlütürk, N. Koca
10:45-11:00	Extraction ofanthocyanins fromgrape pomace by using supercritical carbon dioxide E. Koçak, F. Turan, G. Ova, F. Pazir	Effects of different diets on parasitization performance oftrichogramma spp. (hymenoptera: trichogrammatidae) N. Özder, E. Tayat	Association between skin pigmentation, physiological parameters and adaptation of dairy goats to environmental stress D.J. Agossou, S. Göncü, N. Koluman	Physicochemical properties of Gemlik type black olives obtained from the market C. Demir, O. Gürbüz
11:00-11:15	Determination of bioactive properties of protein and pigments obtained from spirulina platensis R. Arslan, E.C. Eroglu, S. Aksay	Establishment of in vitro clonal cutting production protocol of laurel (laurus nobilis l.) S.D.Royandazagh, E.C.Pehlivan, H. Parmak	Potentials and challenges for future development of milk production and industrial processing in Republic of Benin D.J. Agossou, O. Zannou, C.G. Tchemadon, M.D. Aboudoulaye, A.Fatondji, S. Agossou, Y. Toukourou, N. Koluman	Detecting of 3-MCPD in edible oil by near infrared spectroscopy (NIR) H. Basdogan, S. Karasu, R.M. Yildirim
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11:30-11:45		Biological Control of Fusarium graminearum and Sclerotinia homoeocarpa F.T. Benn. on Turfgrass V. Askin, A. Coskuntuna		The Changes in Quality Criteria of Some Edible Oils During Frying T. Mutlu Keceli
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14:45-15:00	Production Method and Some Properties of Kolot Cheese Y. Parlak, N. Güzeler		A Traditional Fermented Food: Kumiss B. Zorlugenç, E. Ereli	
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3	Bioactive compounds in water extracts prepared from rosehip containing herbal blends N. Petkova, M. Kirchev, M. Ognyanov, M. Stancheva, P. Denev
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AGROFOOD
20 - 21 June 2019, Istanbul

Microbiological control of local and imported foodstuffs- Algiers

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ABSTRACT

Listeria are ubiquitous bacteria found almost everywhere; in the soil, in epiphyte on plants, water, etc. Highly resistant, they can survive cleaning-disinfection treatments and thus persist in production workshops of the food industry. Our work was carried out at the level of the bacteriology of water and food-Enterobacteria lab at the Pasteur Institute of Algeria during the months of May and June 2014.
We worked on different types of foods including:
Bovine carcasses, cuts of meat, milk powder, soft drinks, fruit juices, ready meals and preserves.
Objectives: Research and enumeration of harmful microorganisms.
Research and identification of pathogenic germs (*salmonella, listeria*)
Material and results: on a 40th of analyzed samples it was noted that:
Total sprouts were present at normal levels except for a single sample of beef (imported); coliforms (total and resistant) and SRA were in the standards; *Staphylococci* exceeded the norms in some samples but without presence of *S. aureus* species (catalase negative); the grows raised especially in soft drinks; S*almonella* was absent in all samples (suspected presence of *Salmonella arizonae* in one sample) and total absence of *Listeria* in all samples.
Every link in the food chain is affected by microbiological laboratory tests. Before production, the laboratory analysis of the food makes it possible to validate that the product corresponds to the requirements and complies with the regulations in force. At the end of production, the microbiological analysis of food makes it possible to unseal any possible contamination and thus protect the consumer from food poisoning or even more serious infections - often fatal – especially in pregnant women.

Keywords: Agribusiness, Intoxication, Listeria, Listeriosis, Microbiology.

AGROFOOD
20 - 21 June 2019, Istanbul

Whitening Effect of the Extracts from *Locusta migratoria*

Hyun Jin Kim*, Ga Hyun Lim, Seong Yeon Kim, Jeong Eun Kim, Yu Beom Lee and Do Ik Kim

Insect & Sericultural Research Institute, Jeollanamdo Agricultural Research & Extension, KOREA

ABSTRACT

This study was conducted to investigate the inhibitory effect of tyrosinase and melanin for the development of whitening cosmetics. The *Locusta migratoria* used for the test were directly raised in the laboratory. They were collected after 20 ~ 30 days of the final molting and then freeze drying. The lysates of B16F0 melanoma cells containing tyrosinase were incubated with DOPA for 1 hour. Tyrosinase activity was measured as described in the material and methods. The cells were cultured in the presence of the extracts at concentration of 1000 ug/mL for 72 hours. The concentration of arbutin was 500 ug/mL. The determination of melanin content was measured as described in the materials and methods. The extracts were separated into male and female *Locusta migratoria.* They were removed each fat and extracted with water and 70% ethanol and concentrated. Tyrosinase of the *Locusta migratoria* extracts was inhibited by 19.6% at concentrations of 1000 ug/mL in female extract. The melanin biosynthesis was inhibited in a dose dependent manner at a concentration of 100 ~ 1000 ug/mL. As a result, the whitening effect of ethanol extracts of female *Locusta migratoria* was proved to be possible to make functional whitening cosmetic materials

Keywords: *Locusta migratoria*, Melanin biosynthesis, Tyrosinase, Whitening effect

Acknowledgment: Collate acknowledgements in this separate section at the end of the abstract. List here those individuals who provided help during the research (e.g., funding the project, providing language help, writing assistance or proof reading the article, etc.).

AGROFOOD
20 - 21 June 2019, Istanbul

Production method and quality characteristics of pet food with *Locusta migratoria* powder

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ABSTRACT

The Korean animal market is booming with an annual population of 7 million, and a population of 10 million. The companion animal feed market was 889 billion won, of which 642 billion won for the dogs, 236.6 billion won for the dogs, and 10.4 billion won for the other companion animals. Over 70% of the feed market is dominated by imported brands, and high-priced organic and premium products dominate the import market. Some insects known as pet food are high protein foods, especially locusts and insects with a high protein content of over 70%. This study was carried out to develop a new pet food by using various functional ingredients of edible insects. The grasshopper used in this study contains a high protein content of more than 75% and 10% fat. This is a significantly higher amino acid content that has the effect of improving the quality and increasing the immunity. Therefore, wet type feed was prepared by adding 1 ~ 5% of locust powder and the quality characteristics such as sensory evaluation, nutrients and functional ingredients were investigated. And microbial changes were measured to establish storage conditions. Protein content was 5% higher than non - added, 0.2% higher in fat, more than 56% in amino acid, and glutamic acid and proline were detected. Amino acids, which have the effect of improving hair quality, were more than twice as high as no additives.

Keywords: Insect product, *Locusta migratoria*, Pet food, Quality characteristics

Acknowledgment: Collate acknowledgements in this separate section at the end of the abstract. List here those individuals who provided help during the research (e.g., funding the project, providing language help, writing assistance or proof reading the article, etc.).

AGROFOOD
20 - 21 June 2019, Istanbul

A Traditional Drink: Nogay Tea

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ABSTRACT

Noghays are Kipchak Turks living in the North Caucasus. They, who migrated to various regions for different reasons throughout history, currently reside in Turkey, Crimea, Romania, Stavropol and Dagestan with Karachay-Cherkessia Republic affiliated to Russian Federation. In Tukey, they live in Ankara, Konya, Istanbul, Osmaniye, Adana, Çorum, Afyon, Eskişehir, Bursa, Kütahya, Gaziantep and Isparta. Noghays have their own significant culture accumulations. Nogay tea is one of the most important traditional food cultures of Noghays from past to present. It is also known as "Ayakşay" or "Tatar Tea" among the people. To prepare Nogay tea, black tea is brewed. Milk is added to the brewed tea and the obtained mixture is boiled. When boiling begins, black pepper and salt are added to the tea. It is thoroughly boiled and then filtered. Tea can be served in a glass or bowl. A little butter or cream can be placed on it while serving. This tea has been consumed by Noghays for many years to treat diseases such as colds and influenza by improving the immune system. It can be said that these positive health effects are caused by the ingredients such as milk, black tea and butter found in Nogay tea. According to the information in literature, this tea contains proteins, antioxidant compounds like polyphenols, minerals such as calcium and phosphorus, vitamin A, vitamin B (especially B2 and B12) and essential fatty acids found in butter. It is seen that there is little data on the chemical composition and health effects of Nogay tea in literature and more detailed studies are needed.

Keywords: Ayakşay Tea, Nogay Tea, Tatar Tea, Traditional Drink.

AGROFOOD
20 - 21 June 2019, Istanbul

An Alternative Functional Food: Donkey Milk

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ABSTRACT

The interest in the use of donkey milk for human nutrition is increasing day by day because of its unique composition and distinctive functional properties. Today, in some countries, especially in European countries, donkey milk is located in the market and is mostly used in the feeding of elderly and convalescent people. Because the chemical composition of donkey milk is similar to human milk, it can also be used as an alternative for feeding babies who do not tolerate to bovine milk. In comparison with other dairy animals' milk, donkey milk has low in total solid (8-10%) and protein (1.5-1.8%) contents and high in lactose level (6-7%). Its fat percentage changes from 0.28% to 1.82% and its lipid fraction is characterized by high levels of both linoleic and linolenic acids. Donkey milk also contains 5.8-7.4% lactose, and 0.3-0.5% ash. Donkey milk contains high concentrations of peptide-bound amino acids, especially essential amino acids. The levels of valine and lysine in donkey milk are higher than that of Friesian cows, buffaloes, goats, ewes, camels, and mares. The whey protein fraction of donkey milk contains less β-lactoglobulins and more α-lactalbumin and immunoglobulins than cow milk. Average concentrations of Ca, P, K, Na and Mg in donkey milk are 807.09 mg/L, 638.42 mg/L, 746.61 mg/L, 140.94 mg/L and 81.69 mg/L, respectively. The concentration of macro elements in donkey milk is lower than that of conventional dairy types and higher than human milk. Donkey milk consumption is useful in the treatment of human immune diseases, as it induces the release of inflammatory and anti-inflammatory cytokines from normal human peripheral blood lymphomononuclear cells. Nitric oxide induction by the consumption of donkey milk can be also useful in the prevention of atherosclerosis.

Keywords: Atherosclerosis, Chemical Composition, Donkey Milk.

AGROFOOD
20 - 21 June 2019, Istanbul

Antioxidant activity of some edible flowers from Bulgaria

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[2] Institute of Organic Chemistry with Centre of Phytochemistry-BAS, Laboratory of Biologicaly Active Substances, BULGARIA

ABSTRACT

Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace the synthetic ones. The edible flowers represent a valuable alternative source of bioactive compounds in this regard and have been used as food and herbs with increasing interest. The antioxidant properties of 7 edible flowers of the South Bulgaria, including *Viola tricolor* L., *Cucurbita pepo* L., *Sambucus nigra* L., *Calendula officinalis* L., *Hibiscus rosa-sinensis* L., *Rosa damascene* Mill., and *Allium ursinum* L. were evaluated by six different methods. The contents of flower chemicals, such as total phenolics and total flavonoids content, were determined as well. The results showed that among all investigated extracts the highest antioxidant activity was found in the *Rosa damascene* and *Viola tricolor* ones. By comparing decoction and infusion as methods of extraction, the decoction ones revealed to be the most appropriate in respect of the evaluated compounds. The established total polyphenol content in the decocts of *Rosa damascene* and *Viola tricolor* was 56.66 ± 0.48 and 135.82 ± 1.50 mg GAE/g dw and the total flavonoids were 28.60 ± 0.43 and 15.87± 0.52 mg QE/g dw, respectively. The present research extends the traditional knowledge and revealed an opportunity to obtain biological active substances of the nature and edible flowers in particular.

Keywords: antioxidant activity, edible flowers, water extracts.

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Nonhormonal additives for animal nutrition – an alternative in animal reproductivity (A mini review)

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ABSTRACT

Nowadays, a modern trend of the animal husbandry is to increase the reproductive potential of livestock and poultry. There is a large interest worldwide in discovering new and safe nonhormonal biologically active substances obtained from natural sources, which will substitute the synthetic hormones. The positive influence on reproduction in livestock of the biologically active substances of natural origin such as isoflavones, carotenoides, minerals, phytohormones, PUFAs, etc. represent a potential alternative. These substances are derived from various plant sources and micro and macro algae as well and could provoke positive changes in animal reproduction practices, corresponding to the modern EU requirements for quality and safe for the human health nutrients.

Keywords: livestock, nonchormonal biologically active substances, reproduction.

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Formulation of a Selective Medium for the Isolation and Enumeration of *Lactobacillus bulgaricus*

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ABSTRACT

Lactobacillus delbrueckii ssp. *bulgaricus*, an important specie of lactic acid bacteria is widely used in the dairy industry, particularly in yogurt production. Commonly used in mixed cultures; especially with Streptococcus thermophilus, *L. bulgaricus* is mandatory for traditional yogurts and has several health benefits which meet the current prerequisites of probiotic bacteria. The current standard medium, de Man, Rogosa and Sharpe (MRS) is insufficiently selective for *L. bulgarcius* species and is prone to underestimating true bacteria count. Consequently, there is a need for a better, and a more reliable medium for the differential enumeration of L. bulgaricus. The objective of this study was to formulate an agar medium effective for the differential isolation and enumeration of *L. bulgaricus*. Modified reinforced clostridial medium (mRCM) was formulated by adding 0.025% CaCl2, 0.01% uracil, 0.2% Tween 80, 0.5% fructose, 0.5% dextrose, 1% maltose and 0.25% pyruvate to RCM. The inclusion of 0.04% aniline blue dye in the mix influenced the improved morphology and differentiation of L. bulgaricus colonies observed in mixed yogurt culture. Cell recovery and bacterial count of L. bulgaricus in tested yogurt brands using mRCM-BLUE were higher than in the standard medium MRS as mRCM-BLUE largely inhibited the growth of other bacterial species (*Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidus* and *Lactobacillus reuteri*) present in the yogurt. Our results suggested mRCM-BLUE could be recommended as a selective agar medium for a more accurate differentiation and enumeration of *L. bulgaricus* in dairy products.

Keywords: Isolation, *L. bulgaricus*, Mixed culture, Selective media

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Pectin and Whey Protein Concentrate Minimizes the Generation of Acid Whey in Greek Style Yogurt

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ABSTRACT

The production of Greek yogurt generates large amounts of an environmentally harmful waste product known as acid whey. Therefore, the Greek yogurt industry is searching for a solution to decrease the production of acid whey. The purpose of this study was thus to investigate the effects of pectin and whey protein concentrate (WPC) on the production of acid whey during GSY production. Acid whey production was measured by calculating the water holding capacity (WHC). First, pectin (0.05%) and WPC (1%) were added to skim milk for the production of GSY. The yogurt mixes were then heated at 90°C for 10 min, inoculated with 3.0% of starter culture, incubated at 40°C for 4 h (pH ~ 4.6), and then refrigerated overnight at 5°C. A control yogurt sample was prepared without the addition of these ingredients. The yogurt made with pectin and WPC had a significantly higher WHC ($P < 0.05$) and lower syneresis than the control. The WHC of yogurt with both pectin and WPC was ~ 56%, which was 23% higher than the control (33%). Similarly, yogurt supplemented with both pectin and WPC exhibited 15% less susceptibility to syneresis compared to the control. Native PAGE analysis revealed an interaction between pectin and the WPC. Pectin hinders the formation of large oligomeric aggregates of whey protein which correlates with an increase in WHC and a decrease in syneresis. Our results demonstrated that pectin and WPC could be used as additives to reduce the generation of acid whey in the production of Greek style yogurt.

Keywords: Acid whey, Greek yogurt, Pectin, Whey protein

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Bactericidal Activity of Copper-Ascorbic Acid Mixture Against *Staphylococcus aureus* spp.

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ABSTRACT

The purpose of this study was to investigate the bactericidal activity of copper (2.5, 5, 10 ppm, v/v) and ascorbic acid (0.2, 0.3%, v/v) alone and a mixture of copper-acid against *S. aureus* ATCC 25923 (methicillin-susceptible) and ATCC 700698 (methicillin-resistant). Approximately 4.0 log CFU/mL of each strain was individually inoculated into brain heart infusion (BHI) broth containing copper, acid, and a copper-acid mixture. Samples were incubated for 24 h at 37ºC and turbidities were estimated visually. A 0.1 ml of sample was surface plated onto BHI agar from tubes lacking turbidity, and plates were incubated for 24 h. Inner membrane permeability of *S. aureus* strains was determined by measuring β-Galactosidase activity during 24 h incubation period by using ONPG as substrate. Results showed that a mixture of 0.3% acid with 10 ppm copper was bactericidal for *S. aureus* strains. Based on these results, we further conducted a time-kill assay by inoculating ~ 5.0 log CFU/ mL of each strain into copper, acid, and a copper-acid mixture. The time-kill study revealed a > 3.0 log CFU/mL reduction in both strains after 24 h, indicating that the copper-acid mixture was bactericidal. The β-Galactosidase activity in sample treated with Cu-AA mixture was maximum whereas the activity was found to be negligible in individual treatment of Cu and AA. These results indicate that bacterial cell membrane permeability is damaged when *S. aureus* cells are exposed to Cu-AA mixture. A copper-acid mixture can be an effective sanitizer to minimize the risk associated with *S. aureus.*

Keywords: Ascorbic acid, Bactericidal activity, Copper, MRSA, *Staphylococcus aureus*

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Preparation and Storage Temperature Effect on Polyphenolic Content and Antioxidant Activity of Cornelian Cherry Fruit Jam

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ABSTRACT

Compounds of polyphenolic nature, found in fruits and fruit products, possess high antioxidant capacity against free radicals and reactive oxygen/nitrogen species. Therefore, cornelian cherry (Cornus mas L.) fruit, known as a rich source of polyphenols, was used for the preparation of traditional fruit jams. The goal of this study was to determine the influence of preparation and storage temperatures of fruit jams on their polyphenolic content and antioxidant potential. Therefore, fruit jams were prepared by cooking and non-cooking method. Prepared jams were stored at different temperatures (+20°C, +4°C, -20°C, -72°C) over six months period. Influence of storage temperature on chemical composition and antioxidant potential of jams was assessed using spectrophotometric methods. Total phenols and flavonoids in water extracts were determined using Folin-Ciocalteu and aluminium chloride assays, respectively. Antioxidant potential of the extracts was evaluated by using DPPH, ferric reducing antioxidant power (FRAP) and superoxide dismutase activity (SOD) assays. Significantly higher content of total phenols/flavonoids and antioxidant activity was determined in fruit jams prepared by the cooking method, with an emphasis on jam stored at -72°C. No significant differences were observed among jams prepared by the same method, stored at different temperatures. In conclusion, higher temperature in the process of preparation has induced an increase in polyphenolic content which can be explained by temperature-induced degradation of polymeric polyphenols into adequate monomeric molecules. Lowering the temperature of storage has prevented further degradation of monomeric polyphenols which resulted in preserved antioxidant potential during the time.

Keywords: Antioxidant, Cornelian cherry, Polyphenols, Storage

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Cornelian cherry leaf as an underestimated source of phytochemicals

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ABSTRACT

Cornelian cherry (*Cornus mas* L.) is a flowering shrub belonging to the family Cornaceae and native to the southern parts of Europe and Asia. All parts of cornelian cherry, including fruits, branches, and leaves are considered to be of nutritional and pharmacological importance, especially in the treatment of diabetes and gastrointestinal disorders. However, literature data covering the detailed phytochemical profile of *Cornus mas* leaves are scarce. Therefore, the focus of this study was on the investigation of antioxidant activity and bioactive principles in the water (CMW) and 70% ethanolic (CME) extracts of cornelian cherry leaves. DPPH, ABTS, and FRAP assays were employed for screening the antioxidant activity of extracts, while different classes of phenolic compounds (hydroxycinnamic acids, flavonoids, tannins, anthocyanins, and total phenols) were identified by HPLC-PDA analysis and spectrophotometric determination. Both extracts were rich in phenolics, but a significantly higher amount of phenolic components was obtained in CME (243.34 mg GAE/g dry extract) than in CMW (176.68 mg GAE/g d.e.), probably due to the diverse polarity of constituents of the leaves. HPLC-PDA analysis revealed that derivatives of caffeic acid and quercetin were the major components in both extracts. Considering antioxidant capacity, the investigated extracts exhibited a strong reducing power on Fe^{2+} ion as well as radical scavenging potential, with IC_{50} values comparable with reference compounds, BHT and ascorbic acid. These findings suggest that leaves of cornelian cherry could be regarded as a novel source of phytochemicals with remarkable biopotential.

Keywords: *Cornus mas* L., antioxidant activity, phenolic profile, HPLC-PDA analysis.

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Antioxidative Effect of Dry Distilled Rose Petals Extract in Bulgarian Type Dry Fermented Sausages with Reduces Nitrate Content

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ABSTRACT

The aim of this study was to explore the dry distilled rose petals extract (DDRPE) addition in Bulgarian lukanka type dry fermented sausages with a half reduced nitrate content. The antioxidative effect of addition of DDRPE to lukanka filling mass on colour characteristics (L*, a*, b*), pH, acid value (AV), peroxide value (POV), thiobarbituric acid reactive substances (TBARS) and sensory quality of the sausages was studied. The experiment was made with four samples: control sample C100 with 0.500 g/kg nitrate without addition of DDRPE, control sample C50 with 0.250 g/kg nitrate without addition of DDRPE (control sample with a half reduced nitrate content), experimental sample R1 with 0.250 g/kg nitrate and addition of 1.140 g/kg DDRPE and experimental sample R2 with 0.250 g/kg nitrate and addition of 2.280 g/kg DDRPE. The examinations were made dynamically on 1, 10 and 18 day of the sausage processing. At the end of the experiment (18^{th} day) was found that the colour brightness (L*) was the highest in samples C100 and R1, while the redness (a*) and yellowness (b*) values were higher in samples R1, only. The pH was the lowest in samples R2 and R1. An increase in AV during the studied period (18 days) was evidence for outgoing lipolysis in all samples (C100, C50, R1 and R2). In comparison to use of 2.280 g/kg DDRPE (samples R2), the addition of 1.140 g/kg DDRPE (samples R1) demonstrates better inhibition of oxidative changes in lipid fraction (POV, TBARS) of dry fermented Bulgarian sausages. Samples C100 had the best sensory properties, followed by sensory cores of samples R2 and R1 respectively.

Keywords: Dry fermented sausages, Nitrate reduction, *Rosa damascena* Mill, Oxidative stability, Sensory properties.

1. INTRODUCTION

In the last years much attention has been paid to develop new meat products which may prevent the risk of diseases (Zhang *et al.*, 2010) as so call healthy food. Different innovations in the meat industry were targeted at reducing the content of unhealthy substances (Toldrá, 2011) i.e. to replace or reduce the nitrite content in meat products (Balev *et al*., 2014; Bulambaeva *et al.*, 2014; Vlahova-Vangelova *et al.*, 2014). Nitrates are used as an auxiliary material in the colouring of meat products, i.e. to give their characteristic pinkish red colour (Zhang *et al*., 2007). Nitrite also has preservative properties, inhibiting the growth of some microorganisms, and *Clostridium botulinum*, causing some of the worst food poisonings (Davidson & Harrison, 2002). Despite the benefits of nitrite incorporation, residuals have a negative effect on the quality of meat products by their ability to form nitrosamines - substances with a strong carcinogenic effect (Sebranek & Bacus, 2007).
Our research has shown that extracts of dry distilled rose petals (DDRPE) can be used for the production of cooked sausages with 50% reduced nitrate content (Vlahova-Vangelova *et al.*, 2014). The DDRPE up to 0.05% does not change the sensory properties and stabilizes the colour of the cross-cutting surface of sausages with 50% reduced nitrate content (Balev *et al.*, 2014).
An interesting by-product, which is a waste in the production of Bulgarian rose (*Rosa damascena* Mill) oil and rose water, is the distillate rose petals. It is a typical raw material that has been shown to contain a wide range of phenolic antioxidant components - flavonoids with synergistic effect (Shikov *et al.*, 2008) with proven antioxidant and antibacterial properties (Özkan *et al.*, 2004). The DDRPE are good source of polyphenols (Kammerer *et al.*, 2005) too.
The high antioxidant activity of the phenolic compounds is due to the activity of free radicals and the chelate activity of the transition metals (Mukai *et al*., 2005; Andjelković *et al.*, 2006). The antioxidant effect of phenolic compounds in complex biological systems such as meat is difficult to predict because it depends on their ability to remove free radicals from the system by interacting with transition metal ions (Dragoev, 2009). All the above examples of the use of dry distillate rose petals when it is incorporated into meat products, show the significant potential of this by-product for incorporation in functional meat products with potential health effects.
Last but not least, the recovery of this by-product would help solve major environmental problems related to environmental pollution. By utilizing the by-products from the rose oil production in the perfumery industry, it will contribute for development the waste-free technologies and thus to deliver significant economic, environmental and social and health benefits. Therefore the aim of this study was to explore the DDRPE addition in Bulgarian lukanka type dry fermented sausages with a half reduced nitrate content.

2. MATERIAL AND METHODS

2.1.Meat raw materials

The chilled to 0 - 4ºC beef topside (pH 6.60) and pork chest (pH 6.55) were used in this experiment. The beef and pork meat were supplied by the company Kartevi brother's Ltd, village Benkovski, district Plovdiv, Bulgaria.

2.2.Ingredients and additives

The sodium chloride (salt), sugar, spices and sodium nitrate (E250) were bought from the local market. The dry distilled rose (*Rosa damascena* Mill) petals extract was produced in the Department of Food Preservation and Refrigeration Technology, Technological Faculty, University of Food Technology, Plovdiv, Bulgaria.

2.3.Sample preparation

Samples of the Bulgarian lukanka type dry fermented sausages were produced according to the following recipe: beef round with fat up to 10% - 600 g/kg, pork shoulder with fat up to 5% - 200 g/kg, pork belly with fat up to 50% - 200 g/kg, cooking salt - 23 g/kg, sugar - 3 g/kg, black pepper (*Piper nigrum*) - 3 g/kg, cumin (*Cuminum cyminum*) - 3 g/kg. The four different samples were prepared by addition of: control samples C100 - 0.500 g/kg sodium nitrate without addition of DDRPE, control samples C50 - 0.250 g/kg sodium nitrate without DDRPE, experimental samples R1 - 0.250 g/kg sodium nitrate with addition of 1.140 g/kg DDRPE, experimental samples R2 - 0.250 g/kg sodium nitrate with addition of 2.280 g/kg DDRPE. The examinations were made dynamically on 1, 10 and 18 day of the sausage processing.

2.4.Colour characteristics

Colourimeter Konica Minolta model CR-410 (Konica Minolta Holding, Sending, Inc., Tokyo, Japan), was used to evaluate the CIE L*, a*, b* colour properties of sausages (Hunt *et al.* 2012) on 1st, 10th and 18th day of processing at 8 - 15ºC.

2.5. pH value

The pH value of the sausage samples was determined by pH-meter MS 2004, equipped by pH combination recorder S 450 CD (Sensorex pH Electrode Station, USA) (Young *et al.*, 2004).

2.6.Lipid extraction

Total lipids were extracted from the sausage filling mass by Bligh & Dyer (1959) method and after evaporation of the eluent immediately were used for next lipid analyses.

2.7.Acid value

As a standard of the rate of lipolysis, the acid value (AV) of the extracted lipids was measured following EN ISO 660:2001 procedure (Kardash & Tur'yan, 2005).

2.8. Peroxide value

The presence of primary lipid oxidation products was expressed by the peroxide value (POV). It was measured using procedure described by Shantha & Decker (1994). The double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd, Cambridge, UK) was used.

2.9.2-Thiobarbituric acid reactive substances

TBARS were determined by the method described by Botsoglou *et al.* (1994). The double beam UV-VIS spectrophotometer Camspec model M550 (Camspec Ltd, Cambridge, UK) was used.

2.10. Sensory analysis

The sensory characteristics of the samples were determined after opening the packages. A panel consisting of five members with proven tasting abilities (Meilgaard *et al.*, 1999) was used. The samples were scored using 1 to 5 scales.

2.11. Statistical analysis

The data of different samples were analyzed independently by SAS software (SAS Institute, Inc. 1990). The Student-Newman-Keuls multiple range test was used to compare differences among

means. Mean values and standard errors of the mean were reported. Significance of differences was defined at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1.Colour characteristics

At the end of the experiment (18th day) was found that the colour brightness (L*) was the highest ($P < 0.05$) in samples C100 and R1 (Table 1), while the redness (a*) and yellowness (b*) values were higher ($P < 0.05$) in samples R1, only (Tables 2 and 3).

At the beginning of the experiment (1d) the highest colour brightness (L*) was found in filling mass of dry fermented sausages with 100% nitrate content (samples C100). In comparison, the colour brightness (L*) in experimental samples R1 and R2 were 5.7% and 5.4% lower ($P \geq 0.05$) (Table 1). Different trend was found at 10-th day of sausage production. Compared to samples C50 the addition of 1.14 g/kg DDRPE in dry fermented sausages with half-reduced nitrate content (R1) increases the colour brightness (L*) with 8.9% ($P < 0.05$). In experimental samples R2 with addition of 2.28 g/kg DDRPE the established colour brightness (L*) was closest to those of samples C100. At the end of the experiment (18 d) the colour brightness (L*) in two experimental samples (R1 and R2) decreased with 11.64 % and 6.07 % respectively in comparison with 10-th day (Table 1). At 18-th day the colour brightness (L*) was the lowest in samples C50 and R2, followed by samples R1 which was closer to sausages with 100% nitrate content (control samples C100). In conclusion, for the entire period (18 days) the colour brightness (L*) was most stable in dry fermented sausages with half-reduced nitrate content with addition of 1.14 g/kg DDRPE (R1) with total decrease of 8.2% (Table 1).

Table 1. Changes in the colour brightness (L*) of dry fermented sausages during 18 days of their processing (expressed as means ± SEM).

Samples	**Colour brightness (L*)**		
	The phase of drying and fermentation, d		
	1d	**10 d**	**18d**
1	2	3	4
C 100	45.06±0.10 **c,z**	39.51±0.10 **a,w**	40.38±0.29 **b,z**
C 50	43.59±0.16 **c,y**	40.92±0.31 **b,y**	37.58±0.22 **a,x**
R 1	42.65±0.20 **b,x**	44.58±0.58 **c,z**	39.39±0.28 **a,y**
R 2	42.76±0.23 **c,x**	40.36±0.15 **b,x**	37.91±0.26 **a,x**

a, b, c Means in the same row with different superscript letters differ significantly ($P < 0.05$).
x, y, z Means in the same column with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

At first day of the experiment little deviations of the colour redness (a*) was found. No significant difference ($P \geq 0.05$) in colour redness (a*) of the samples with half-reduced nitrate content (samples C50, R1 and R2) was found. The only exception were samples C100 which had with 7.4% higher colour redness (a*) compared to samples C50 ($P < 0.05$). After 10-th day the colour redness (a*) decreased in all studied samples. At the 10-th day the lowest colour redness (a*) was established in dry fermented sausages with half-reduced nitrate content with addition of 1.14 g/kg DDRPE (samples R1), followed by samples C50. In comparison, the colour redness (a*) in control samples C100 was 24% higher than those in samples R1 ($P < 0.05$) (Table 2). Different trend was determined at the end of the experiment (18 day). In all studied samples colour redness (a*) increased except in samples

C100. The addition of 1.14 g/kg DDRPE (R1) again showed stabilizing effect with slightest change in colour redness (a*) for the entire experimental period (Table 2).

Table 2. Changes in the colour redness (a*) of dry fermented sausages during 18 days of their processing (expressed as means ± SEM).

Samples	**Colour redness (a*)**		
	The phase of drying and fermentation, d		
	1d	**10 d**	**18d**
1	2	3	4
C 100	19.57±0.25 **b,z**	16.50±0.02 **z,x**	16.45±0.10 **a,w**
C 50	18.22±0.03 **c,w**	15.70±0.29 **x,x**	17.27±0.08 **b,y**
R 1	18.33±0.03 **c,x**	13.30±0.16 **w,x**	17.69±0.08 **b,z**
R 2	18.52±0.17 **c,y**	16.17±0.13 **y,x**	16.57±0.01 **b,x**

a, b, c Means in the same row with different superscript letters differ significantly ($P < 0.05$).
x, y, z Means in the same column with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

On the 1-st day of experiment (in the filling mass of sausages) were not established significant ($P \geq 0.05$) differences in the colour yellowness (b*) of samples with half-reduced nitrate content (C50, R1 and R2). The only exception was made in control samples C100 which had with 8.2% higher colour yellowness (b*) compared to samples C50 ($P < 0.05$) (Tables 3). After 10 days significant ($P < 0.05$) decrease of colour yellowness (b*) was found in all studied samples. At the 10-th day the lowest was colour yellowness (b*) in dry fermented sausages with 100% nitrate content (control samples C100). The highest colour yellowness (b*) for the same studied period was established in sausages with half-reduced nitrate content (samples C50) and in sausages with addition of 2.28 g/kg DDRPE (samples R2). At the end of the experiment (18 day) the different trend was established. In two of studied samples C100 and R1 colour yellowness (b*) increased ($P < 0.05$), while in samples C50 and R2 significantly ($P < 0.05$) decreased compared to 10-th day (Table 3). During the studied period (18 days) the most stable colour yellowness (b*) was found of the dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R1) (Table 3). The observed changes in the colour characteristics of dry fermented sausages with reduced nitrate content and addition of DDRPE can be explained by antioxidant and antibacterial properties of the used flavonoids (Özkan *et al.*, 2004). Playing as antioxidants flavonoids can stabilize the cross-section surface color of the sausages. They can improve the colour stability by fortification of sausages with polyphenol copigments naturally occurring in rose petals (Mollov et al., 2007).
On the other hand their antibacterial activity suppresses growth of putrefactive microflora preventing the myoglobin oxidation process and transformation of red pigments to gray brown colour metmyoglobin.

Table 3. Changes in the colour yellowness (b*) of dry fermented sausages during 18 days of their processing (expressed as means ± SEM).

Samples	Colour yellowness (b*)		
	The phase of drying and fermentation, d		
	1d	10 d	18d
1	2	3	4
C 100	9.79±0.15 ^{c,y}	4.47±0.03 ^{a,x}	5.53±0.27 ^{b,z}
C 50	9.05±0.05 ^{c,x}	4.94±0.04 ^{b,y}	4.46±0.09 ^{a,x}
R1	9.05±0.05 ^{c,x}	4.77±0.22 ^{a,y}	5.55±0.03 ^{b,z}
R2	9.07±0.10 ^{c,x}	5.04±0.03 ^{b,z}	4.76±0.06 ^{a,y}

$^{a, b, c}$ Means in the same row with different superscript letters differ significantly ($P < 0.05$).
$^{x, y, z}$ Means in the same column with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

3.2.pH value

At the start of the experiment (1d), the pH of the four studied samples was not significantly ($P \geq 0.05$) different (Table 4). After 10 days, pH in all studied samples (C100, C50, P1 and P2) decreased. Compared to the first day, the significant ($P < 0.05$) decrease with 3% of pH was found in dry fermented sausages with 100% nitrate (control samples C100) and with 50% reduced nitrate content (samples C50). Only the pH of dry fermented sausages with half-reduced nitrate content and addition of 1.14 g/kg DDRPE (R1) did not significantly ($P \geq 0.05$) different between first and 10-th day of the experiment (Table 4). At the 18-th day the pH values of control samples C100 and half-reduced nitrate content (samples C50) were increased. In opposite, a decrease of pH values in dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R1) and 2.28 g/kg DDRPE (samples R2) was established (Table 4). Compared to control samples C100, the addition of 1.14 g/kg DDRPE stabilize the pH of dry fermented sausages. In the finish product the lowest pH values were determined in samples R2 and R1. The extracts of dry distilled rose petals exhibit a slightly acidic reaction. Responsible for it are contained in the extract 22 kaempferol and quercetin glycosides (Schieber *et al.*, 2005).

Table 4. Changes in pH of dry fermented sausages during 18 days of their processing (expressed as means ± SEM).

Samples	pH		
	The phase of drying and fermentation, d		
	1d	10 d	18d
C 100	6.24±0.05^{b,x}	6.05±0.04^{a,x}	6.30±0.05^{b,y}
C 50	6.26±0.04^{c,x}	6.08±0.03^{b,x,y}	6.26±0.07^{c,y}
R 1	6.19±0.07^{a,x}	6.16±0.04^{a,z}	6.12±0.05^{a,x}
R 2	6.23±0.07^{c,x}	6.11±0.03^{b,y,z}	6.06±0.04^{b,x}

$^{a, b, c}$ Means in the same row with different superscript letters differ significantly ($P < 0.05$).
$^{x, y, z}$ Means in the same column with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

3.3.Acid value

The increase of AV during the studied period (18 days) was evidence for outgoing lipolysis in all studied samples (C100, C50, R1 and R2). The highest free fatty acid content was found at 10 day in control samples C100, followed by dry fermented sausages with addition of 2.28 g/kg DDRPE (samples R2) and 1.14 g/kg DDRPE (samples R1). At the end of the experiment the total increase in AV was 1.30 times for control samples C100, 1.46 times for samples C50, 1.54 times for samples R1 and 1.72 times for samples R2. The comparison between AV of samples C50 (with a half-reduced nitrate content only) and dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R1) show that the differences were not significant ($P \geq 0.05$) (Table 5). The addition of 2.28 g/kg DDRPE (samples R2) influence negatively on lipolytic changes in dry fermented sausages and on 18-th day of the experiment the AV was with 1.28 times higher than those of control samples C100 (Table 5). The conclusion was made that an increase of AV during the studied period (18 days) is an evidence for outgoing lipolysis in all samples (C100, C50, R1 and R2) and ½ reduction of the nitrate content support the fatty acid liberation process. In this case the results show the addition of 1.14 or 2.28 g/kg DDRPE stimulate the lipolytic changes during 18 days of processing of Bulgarian lukanka type dry fermented sausages.

Table 5. Lipolytic changes of represented by the acid value (AV) of dry fermented sausages during 18 days production (expressed as means ± SEM).

Samples	**Acid value (AV), mg KOH/g**		
	The phase of drying and fermentation, d		
	1d	**10 d**	**18d**
1	2	3	4
C 100	0.72±0.01 **a,x**	0.98±0.02 **b,z**	0.94±0.03 **b,x**
C 50	0.70±0.03 **a,x**	0.76±0.02 **b,x**	1.02±0.05 **c,y**
R1	0.72±0.01 **a,x**	0.91±0.01 **b,y**	1.11±0.05 **c,y**
R2	0.71±0.01 **a,x**	0.92±0.01 **b,y**	1.21±0.01 **c,z**

a, b, c Means in the same row with different superscript letters differ significantly ($P < 0.05$).
x, y, z Means in the same column with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

3.4.Peroxide value

The results for changes of peroxide value (POV) of the all samples show the typical induction period with pick on 10-th of day. At the end of the experiment (18 days) a decreasing of primary lipid oxidation products was found. The 1.44, 2.47, 2.80 and 2.18 times reductions of POV in samples C100, C50, R1 and R2 respectively were found. At the 18-th day of the experiment significantly ($P < 0.05$) lower POV was established in Bulgarian lukanka type dry fermented sausages with addition of 1.14 g/kg DDRPE (samples R1). The POV of control samples C100 and samples C50 and R2 (sausages with addition of 2.28 g/kg DDRPE) did not significantly ($P \geq 0.05$) different at the end of the experiment (18 day) (Table 6). The result allow us to conclude the addition of low concentration (1.14 g/kg) DDRPE (samples R1) demonstrates ability to inhibit hydroperoxides formation and show antioxidative action against accumulation of primary lipid oxidation products in Bulgarian lukanka type dry fermented sausages at the conditions of this experiment. Thus established phenomenon can be explained by synergistic effect and antioxidant activity of the phenolic compounds of the DDRPE (Shikov *et al.*, 2008) in terms of evacuating of free radicals from the system in the presence of transition metals as chelating agents (Mukai et al., 2005; Andjelković et al., 2006).

Table 6. Changes in the primary products of lipid peroxidation represented by the peroxide value (POV) of dry fermented sausages during 18 days production (expressed as means ± SEM).

Samples	Peroxide value (POV), meqv O_2/kg		
	The phase of drying and fermentation, d		
	1d	10 d	18d
1	2	3	4
C 100	2.00±0.22^{b,y}	3.48±0.07^{c,z}	1.38±0.28^{a,y}
C 50	3.27±0.03^{b,x}	3.02±0.36^{b,x}	1.32±0.22^{a,y}
R1	1.87±0.18^{b,y}	2.95±0.44^{c,x}	0.67±0.11^{a,x}
R2	2.78±0.21^{b,z}	3.27±0.15^{c,y}	1.27±0.06^{a,y}

$^{a, b, c}$ Means in the same row with different superscript letters differ significantly ($P < 0.05$).
$^{x, y, z}$ Means in the same column with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

3.5.TBARS

In all examined samples secondary product of lipid oxidation (expressed by TBARS) increased during first ten days of the processing of Bulgarian lukanka type dry fermented sausages. Up to 10-th day the malonaldehyde formation was highest in samples C50 with half-reduced nitrate content only and in samples R2 (dry fermented sausages with addition of 2.28 g/kg DDRPE) and the significantly ($P < 0.05$) lower in control samples C100. At the end of the experiment the TBARS of control samples C100 and samples C50 stabilize around 0.78 - 0.88 mg MDA/kg (Table 7). In comparison with those levels the TBARS of the samples R1 were a little bit higher ($P < 0.05$) (Table 7) and the highest (1.41 mg MDA/kg) in samples R2. The results show that the addition of 1.14 and especially 2.28 g/kg DDRPE had not inhibitory effect on secondary products of lipid oxidation in examined dry fermented sausages even stimulate the oxidation process. In previous researches Balev *et al.* (2014) and Vlahova-Vangelova *et al.* (2014) established the positive effect of DDRPE addition in cooked sausages, but in Bulgarian lukanka type dry fermented sausages this effect cannot be confirmed.
The conclusion was made that in comparison to 2.280 g/kg DDRPE (samples R2) supplementation, the addition of 1.140 g/kg DDRPE (samples R1) demonstrates better inhibition effect on the lipid oxidative changes (POV and TBARS) of the Bulgarian lukanka type dry fermented sausages. In conclusion the addition of 2.280 g/kg DDRPE (samples R2), nor of 1.140 g/kg DDRPE (samples R1) have an inhibition effect on the accumulation of secondary lipid oxidation products (expressed by TBARS) during 18 days of processing of Bulgarian lukanka type dry fermented sausages.

Table 7. Changes in the lipid peroxidation secondary products represented by the 2-thiobarbituric acid reactive substances (TBARS) of dry fermented sausages during 18 days of their processing (expressed as means ± SEM).

Samples	TBARS, mg MDA/kg		
	The phase of drying and fermentation, d		
	1d	10 d	18d
1	2	3	4
C 100	0.60±0.03 ^{a,y}	0.75±0.02^{b,x}	0.78±0.05 ^{b,x}
C 50	0.54±0.04 ^{a,y}	1.10±0.08 ^{c,z}	0.88±0.05 ^{b,x}
R1	0.55±0.02 ^{a,x}	0.97±0.02 ^{b,y}	0.95±0.05 ^{b,y}
R2	0.52±0.02 ^{a,x}	1.10±0.03 ^{b,z}	1.41±0.02^{c,z}

$^{a, b, c}$ Means in the same row with different superscript letters differ significantly ($P < 0.05$).
$^{x, y, z}$ Means in the same column with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

3.6.Sensory quality

The results from sensory analysis indicated that the control samples C100 were evaluated with the highest sensory scores, followed by sensory cores of samples R2 and R1 respectively (Table 8). The differences between means of sensory scores of samples R2 and R1 from one hand and control samples C100 from the other one were significant ($P < 0.05$) but not so great and deviated around 0.25 units except at the texture scores where the differences were not found (Table 8). Those results allows us to conclude that the half-reduction of nitrate content in dry fermented sausages is the main factor affected on decreasing of the sensory quality of Bulgarian lukanka. The addition of 1.14 or 2.28 g/kg DDRPE to the sausage filling mass to some extent helps preserve the sensory characteristics. This is not enough to compensate for the 50% reduction of nitrate in the Bulgarian lukanka formulation probably because that DDRPE flavonols, despite structural similarity, have different antioxidant effects (Wang *et al.*, 2006).

Table 8. Sensory scores of the dry fermented sausages - final product on day 18 of the experiment (expressed as means ± SEM).

Samples	Surface cross-sectional view	Colour	Texture	Odour	Flavor	Overall experience
1	2	3	4	5	6	7
C 100	4.90 ± 0.10 ^{z}	4.95 ± 0.05 ^{z}	4.90 ± 0.10 ^{x}	4.90 ± 0.05 ^{z}	4.90 ± 0.10 ^{z}	4.91± 0.06 ^{z}
C 50	4.30 ± 0.15 ^{x}	4.00 ± 0.10 ^{x}	4.95 ± 0.12 ^{x}	4.40 ±0.20 ^{x}	4.50 ± 0.07 ^{x}	4.43± 0.13 ^{x}
R 1	4.65 ± 0.10 ^{y,z}	4.70 ± 0.08 ^{y}	4.90 ± 0.10 ^{x}	4.70 ± 0.10 ^{y}	4.70 ± 0.10 ^{y}	4.74 ± 0.10 ^{y}
R 2	4.80 ± 0.05^{z}	4.80 ±0.07 ^{y,z}	4.90 ± 0.10 ^{x}	4.80 ± 0.10 ^{y,z}	4.75 ± 0.08 ^{y}	4.81 ± 0.08 ^{y,z}

^{x,y} - index showing data with statistical different value in columns ($p < 0.05$)
SEM- standard error of the mean.

4. CONCLUSION

The addition of 1.140 g/kg DDRPE to the filling mass of Bulgarian lukanka type dry fermented sausages with a half-reduction of nitrate content contributes to improve the colour characteristics and to reduce the pH and peroxide value.
The addition of 1.140 g/kg DDRPE to the filling mass of Bulgarian lukanka type dry fermented sausages with a half-reduction of nitrate content cannot prevent the outgoing lipolysis and inhibit the TBARS accumulation.
The addition of 1.140 and 2.280 g/kg DDRPE to the filling mass of Bulgarian lukanka type dry fermented sausages with a half-reduction of nitrate content is not so effective to protect the same sensory quality such as addition of 100 % sodium nitrate.

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Changes of Fatty Acid Profiles and Sterols, Tocopherols and Carotenoids Content of Pork by Antioxidant Type Phytonutrients

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ABSTRACT

In recent years, there has been interest in applying natural antioxidant phytonutrients to improve pork nutritional value. Therefore, the purpose of this study was to determine the effect of 3.5 and 7.5 mg dihydroquercetin (DHQ) from *Larix sibirica Ledeb* (samples D1 and D2. respectively), or 0.255 and 0.545 g dry distilled rose (*Rosa damascena* Mill.) petals (DDRP) (samples R1 and R2. respectively)/kg live weight/day added as supplements to pigs commercial diet until the last 45 d prior to harvest on fatty acid composition, content of sterols, tocopherols and carotenoids in *Longissimus lumborum* and *Semimembranosus* muscles, backfat and leaf fat stored 24 h at 2 ± 1°C. An increasing of unsaturated fatty acids was found in m. *Longissimus lumborum* and leaf fat with 11.0% at samples D1 and R2 respectively, in backfat with 4.7% at samples D1 and in m. *Semimembranosus* with 3.9% at samples R1. The sterols in m. *Longissimus lumborum* were increased with 2.3 times independently of R1. R2. D1 or D2 supplementation, but in leaf fat were not determined significant ($p \geq 0.05$) differences and a decreasing were found in backfat. The significant ($p < 0.05$) increasing of tocopherols was estimated in two muscles at samples R2 and D2 but decreasing of their content in backfat and leaf fat. Four studied phytonutrients supplementations decrease the carotenoids in muscles and backfat including leaf fat samples and m. *Semimembranosus* D2 sample were indicated 1.32 times increasing of carotenoids.

Keywords: dihydroquercetin, lipids, pork, *Rosa damascena*, fatty acids.

1. INTRODUCTION

In the food pyramid the meat is categorized as food, from which the human body supplies essential amino acids for the synthesis of endogenous proteins. (Chan, 2004). Unfortunately, the media is speculating on the risks to human health of certain dangers in meat products. For most users, red meat has a negative image because it is widely believed that meat contains many fats and is a major source of saturated fatty acid in diet (Valsta *et al.,* 2005). It is discussed as responsible for modern life-style diseases in developed countries. The ratio of ω-6 / ω-3 polyunsaturated fatty acids (PUFA) is another risk factor for cancer as well as coronary heart disease (Williams, 2000). It is therefore appropriate to look for mechanisms to lower levels of saturated fatty acids and cholesterol to make the meat a functional food, and hence more attractive and healthier.

One of the main approaches to healthy eating is the reduction of certain ingredients from food, perceived as components with a negative effect. Numerous studies have demonstrated that it is possible to change the negative image of meat by eliminating or severely lowering the fat content and some supplements (Fernandez-Ginës *et al.*, 2005; Demeyer & Astiasaran, 2007). In this connection, Arihata (2006), Perez-Alvarez (2008) and Zhang *et al.* (2010) offer a number of strategies on the possibilities of creating new functional meat products. They consist in reducing the content of unhealthy substances such as, sodium chloride, residual nitrite (Honikel, 2008) and fat and an increase in the content of substances with a health effect, such as ω-3 polyunsaturated fatty acids, natural antioxidants, probiotics and biologically active peptides (Toldra & Reig, 2011; de Ciriano *et al.*, 2013). In the literature are discussed several approaches to creating new functional meat products. The first is by modifying the total chemical composition of the carcass obtained. The second is by manipulating the meat raw materials. The third approach is by re-formulating the composition of meat products by reducing the sodium, nitrite, fat, cholesterol, energy value of the product, and incorporating certain functional ingredients. The second and third approaches to obtaining functional foods by using ingredients that improve the technological properties and quality of the finished product during its production are widely used in the meat industry. On the other hand, the composition of the carcass obtained depends on factors such as sex, degree of protection, age, etc. By selecting breeds and creating new genetic lines it is possible to reduce the fat content of the fatty tissue of the slaughtered animals. Some strategies alter the composition of carcass meat associated with a change in protein, vitamin content, and fatty acid content that has been insufficiently studied. In recent years, approaches have been proposed for the production of carcass meat with a modified chemical composition (Nieto *et al.,* 2010, Nasir & Grashorn, 2010, Yan *et al.,* 2010, Hashemi & Davoodi, 2010, Özturk *et al.,* 2012). The approach to the application of various natural antioxidants to the development of lipid-protein oxidation deterioration in meat and meat products (Falowo *et al.*, 2014) is also discussed.

Dihydroquercetin, also known as taxifolin, is a powerful natural bioflavonoid with antioxidant action and a capillary protector exhibiting P-vitamin activity. As a substance with a high degree of biological activity, it has a wide range of positive effects on the metabolic reactions and dynamics of various pathological processes that have been identified in a number of studies. Dihydroquercetin has proven radioprotective (Bischoff, 2008), antioxidant (Kurth & Chan, 1951), capillary protector (Rice-Evans *et al.,* 2000), hepatoprotective, gastroprotective (Sugihara *et al.,* 2000), radioprotective (Teselkin *et al.*, 1998), anticoagulant (Plotnikov *et al.,* 2003) and antiinflammatory (Yamamoto *et al.*, 1984) properties and inhibits the oxidation of LDL-cholesterol in blood serum (Kostyuk *et al.,* 2003). cardioprotective, membranoprotective, angioprotective, lipid lowering, antiallergic, detoxication,

neuroprotective, immunomodulatory, retinoprotective and endocrinological (Artem'eva *et al.,* 2015) properties.
Another interesting by-product, which is a waste in the production of Bulgarian rose oil and rose water, is the extract of distilled rose petals. It is a typical Bulgarian raw material for which there is evidence that it contains a wide range of antioxidant components with synergistic effect (Shikov *et al.,* 2008). The *Rosa Damascena* Mill extract has strong antioxidant properties (Shikov *et al.,* 2012) because it is rich in flavonoids (Shikov *et al.,* 2008). It has been used to stabilize the color of fruit juices (Mollov *et al.,* 2007).
According to preliminary data, after obtaining of 1 kg of rose oil, as a by-product is obtained about 50 kg of rose petals waste. With an average annual yield of 1500 kg of rose oil in Bulgaria, approximately 75000 kg of rose petals can be expected. It is now discarded without being used. On the one hand this can lead to environment pollution. Another possibility is to load the business with additional costs for its use. If suitable application of *Rosa Damascena* Mill extract in livestock and the food industry is found, this can lead to significant economic, social, hygienic and ecological effects.
An experiment with broiler chickens aiming at detecting changes in the composition of the minced meat after enrichment of the poultry feed with dry pressed distilled rose petals and d ihydroquercetin was made (Balev *et al.,* 2015).
In recent years, there has been interest to apply natural antioxidant phytonutrients as a means of improving the nutritional value of pork. Therefore, the purpose of this study was to determine the effect of 3.5 and 7.5 mg dihydroquercetin (DHQ) from *Larix sibirica* Ledeb (samples D1 and D2 respectively), or 0.255 and 0.545 g dry distilled rose (*Rosa damascena* Mill.) petals (DDRP) (samples R1 and R2. respectively)/kg live weight/day added as supplements to pigs commercial diet until the last 45 d prior to harvest on fatty acid composition, content of sterols, tocopherols and carotenoids in *Longissimus lumborum* and *Semimembranosus* muscles, backfat and leaf fat stored 24 h at 2 ± 1°C.

2. MATERIAL AND METHODS

2.1. Ethics statement

This experiment was conducted in accordance with Art. 14 of Part V. Breeding and Livestock Units form European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, regulations of the European Union (Council Directive 2008/120/EC; Council Directive 2010/63/EC; Commission Recommendation 2007/526/EC; Commission Implementing Decision 2012/707/EU; Commission Implementing Decision 2014/11/EU; Council Regulation (EC) No 1/2005; Council Regulation (EC) No 1099/2009; Council Regulation (EC) No 1255/97) and the national legislation of the Republic of Bulgaria (Veterinary Activity Act, 2006; Ordinance No 15 of 3 February 2006; Ordinance No 16 of 3 February 2006; Ordinance No 20 of 1 November 2012; Ordinance No 21 of 14 December 2005). The experiment was approved by the Bulgarian Scientific Ethics Committee and requirements of the Council Directive 2010/63/EC were met.

2.2. Animal housing and diets treatments

The experiment was conducted at the State Enterprise Experimental Farm at Agricultural Institute, Shumen, Bulgaria. Danube White pigs were created by crossing Bulgarian White, Large White, Landras and Pietren, then crossing with Hampshire sires. Resulting offspring were randomly allocated to five treatments (one control and four experimental), each containing of 24 animals. Control (C) pigs received a typical commercial diet. The other four experimental samples were fed the commercial diets containing either 3.5 mg/kg live weight per day dihydroquercetin (D1); 7.5

mg/kg live weight per day dihydroquercetin (D2); 0.255 g/kg live weight per day dry distilled rose petals (*Rosa damascene*) (R1); or 0.545 g/kg live weight per day dry distilled rose petals (R2).

2.3. Animals

Animals care began at weaning, when pigs were 35 days of age. Pigs were equalized by origin, age, weight and sex and allocated to one of the treatments, 8 pigs accommodated together, all in one stable. The feeding and health status of the pigs were regularly monitored throughout the period after weaning. When reaching an average live weight of 33 kg on June 6, 2017, at 97 day after birth, all pigs were weighed again and moved to another stable equipped with individual boxes with feeders and drinkers. The pigs were equalized again by sex, age and live weight. During the fattening period, the animals were reared in accordance with the requirements of Ordinance No 21 of 14.12.2005. The experiment was divided in two sub periods, with growing period starting at about 33 kg live weight and ended at about 72 kg live weight. No treatment was applied at this period. The fattening period starting at 72 kg live weight at 155 day after birth and ended at about 110 kg live weight. The enrichment of the diets with both concentrations of dihydroquercetin and dry compressed distilled rose petals started at this time and lasted 45 days, till 200 day after birth of pigs, when they were slaughtered.

2.4. Diets

Forty-five days prior to slaughter, or at approximately 72 kg, pig diets were weighed daily before being placed in individual feeders, twice a day. Residual feed was monitored daily and was weighed and subtracted from of the daily amount of feed consumed. Daily dose of the supplements were calculated according to previous day weight gain, weighed and mixed with feed mixture and given to the animals with the morning feeding. Water was provided at libitum and was assured by nipple drinkers.

2.5. Supplements

Dried and powdered dihydroquercetin was provided by the company Flavitlife Bio JSCo (Sofia, Bulgaria). Distilled rose petals were supplied by Damascena rose oil distillery, village of Skobelevo, municipality of Pavel Banya, Stara Zagora district, part of Bulattars Production Company Ltd (Sofia, Bulgaria). After compression, petals were dried and ground to particle size < 0.4 mm.

2.6. Animal performance

Animals were weighed every two weeks. Average weight gain and the feed consumption was calculated. Temperature, relative humidity and velocity of the air, and harmful gases, such as carbon dioxide, ammonia, hydrogen sulphide and methane were monitored daily.

2.7. Harvesting

At the end of the feeding period, pigs were identified and transported to a processing plant (Unanimp Ltd., Voyvodinovo village, Maritza municipality, Plovdiv district). After 18 h of lariage, pigs were showered and harvested in accordance with normal industry processing procedures and in accordance with requirements of Article 9(3) of the Ordinance No 15 of May 8, 2009. Carcasses were split and chilled. After a 24 hr chilled to 4°C carcasses were moved to a cold storage where they remained until fabrication. Muscle and fat tissues were collected 24 hr after harvesting.

2.8. Lipid extraction

Total lipids were extracted from the chopped and minced muscle and fat tissues by Bligh & Dyer (1959) method and after evaporation of the eluent immediately were used for next lipid analyses.

2.9. Determination of fatty acid profils and sterols

The experimentals for the fatty acid composition of total triacylglycerols (BDS EN ISO 5508:2000) and sterols (BDS EN ISO 12228:2000) were performed by GC gas chromatographic system Thermo Scientific, Trace GC ultra model with FID detector.

2.10. Determination of tocopherols and carotenoids

The tocopherols and carotenoids were determined by high performance Liquid Liquid Chromatography (ISO 9936:1997) of HPLC Gas Chromatography System Hitachi, Elite la Chrome with Fluorescence Detection.

2.11. Statistical analysis

The results of the experiments were processed using the variation statistics methods with different software packages Microsoft Excel 5.0, JMP, v.7 and MINITAB 16 (SAS Institute, Inc. 1990). The comparison of the values of the various indicators was done by the Student and ANOVA t-experimental of all experimental samples with the control one on the one hand and inside the roups by factor levels (low and high concentration of the corresponding antioxidant additives) for statistically significant differences at the level of confidence $\alpha = 0.05$ ($p < 0.05$), respectively. $\alpha = 0.01$ ($p < 0.01$).

3. RESULTS AND DISCUSSION

3.1. Effect of the suplementation with dihydroquercetin or dry compressed distilled rose petals on the fatty acid composition of the muscle and fatty tissues

The addition of dihydroquercetin and dry compressed distilled rose petals has little effect on the fatty acid composition of m. *Semimembranosus*, 1d post-mortem (0 - 4°C). Compared to the control samples C, in the experimental the samples D1 (pigs fed with feed supplemented with 0.242 g dry compressed distilled rose petals/kg body weight) the share of unsaturated fatty acids was increased by 3.9%. In the other four (C, D1, D2 and R2) samples, the difference in the amounts of unsaturated fatty acids ranges around 1% (Table 1).

Table 1. Fatty acid composition of *m. Semimembranosus* 1d *post mortem* (0-4°C)

Fatty acids, %	*m. Semimembranosus,* 1d *post mortem*				
	Control sample (C)	**Experimental sample (D1)**	**Experimental sample (D2)**	**Experimental sample (R1)**	**Experimental sample (R2)**
Lauric acid C12:0	0.1±0.02	0.1±0.01	0.1±0.01	0.1±0.02	0.1±0.02
Myristic acid C 14:0	1.6±0.08	1.8±0.07	1.8±0.05	1.8±0.05	1.7±0.04
Pentadecanoic acid C 15:0	-	0.1±0.01	-	-	0.1±0.01
Palmitic acid C 16:0	29.4±1.10	29.0±1.0	29.1±1.10	28.1±0.8	28.9±0.8
Palmitoleic acid C 16:1	2.6±0.01	2.4±0.05	3.2±0.04	3.3±0.05	3.4±0.04
Margaric acid C 17:0	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01
Margarine oleic acid C17:1	0.3±0.01	0.3±0.015	0.2±0.01	0.3±0.01	0.3±0.01
Stearic acid C 18:0	12.1±0.40	12.6±0.25	13.2±0.15	11.4±0.30	12.0±0.20
Oleic acid C 18:1	51.9±1.80	51.9±1.60	51.2±1.40	53±1.10	51.7±1.10
Linoleic acid C 18:2	0.7±0.01	0.5±0.05	0.1±0.05	0.6±0.05	0.8±0.02
Linolenic acid C 18:3	0.7±0.04	0.7±0.04	0.5±0.05	0.8±0.04	0.6±0.05
Arachic acid C 20:0	0.3±0.01	0.3±0.02	0.2±0.01	0.2±0.04	0.1±0.02
Beheenoic acid C 20:2	0.1±0.01	0.1±0.01	0.2±0.01	0.2±0.01	0.1±0.01
Saturated fatty acids (SFA)	**43.7**	**44.1**	**44.6**	**41.8**	**43.1**
Unsaturated fatty acids (UFA)	**56.3**	**55.9**	**55.4**	**58.2**	**56.9**
Monounsaturated fatty acids (MUFA)	***54.8***	***54.6***	***54.6***	***56.6***	***55.4***
Polyunsaturated fatty acids (PUFA)	***1.5***	***1.3***	***0.8***	***1.6***	***1.5***

* The statistical analysis was conducted in rows using one-way ANOVA. The presence of statistically significant differences between the mean values ($p < 0.05$) are denoted by the same letters, $^{a, b, c, d, e}$ – $p < 0.05$

Feeding of pigs with feed supplemented by dihydroquercetin or dry compressed distilled rose petals changes the fatty acid composition of the total lipids extracted from m. *Longissimus lumborum* (Table 2). On the first post mortem day, the content of unsaturated fatty acids in the different samples varies between 1 and 4%. Compared to the control samples C, the share of monounsaturated fatty acids increased by 11%, 5.5% and 4.4%, respectively, for samples D1 with 3.5 mg of dihydroquercetin/kg body weight, D2 – with 7.5 mg of dihydroquercetin/kg body weight and R2 - with 0.545 g dry compressed distilled rose petals/kg body weight.

Table 2. Fatty acid composition of *m. Longissimus lumborum* 1d *post mortem* (0 - 4°C)

Fatty acids, %	***m. Longissimus dorsi,* 1d *post mortem***				
	Control sample (C)	**Experimental sample (D1)**	**Experimental sample (D2)**	**Experimental sample (R1)**	**Experimental sample (R2)**
Lauric acid C12:0	-	0.1±0.01	0.1±0.01	-	0.1±0.01
Myristic acid C 14:0	1.7±0.04	1.7±0.04	1.8±0.03	1.8±0.01	1.8±0.02
Pentadecanoic acid C 15:0	0.1±0.01	-	-	-	0.1±0.01
Palmitic acid C 16:0	30.7±1.20	29.7±1.0	31.3±1.0	31.8±0.8	31.5±0.9
Palmitoleic acid C 16:1	2.0±0.10	2.9±0.05	2.8±0.10	2.7±0.05	3.0±0.10
Margaric acid C 17:0	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01
Margarine oleic acid C17:1	0.3±0.05	0.2±0.05	0.2±0.05	0.2±0.05	0.2±0.05
Stearic acid C 18:0	16.2±0.70	13.0±0.80	14.6±0.80	14.6±0.50	14±0.40
Oleic acid C 18:1	45.1±1.60	49.5±1.40	47.0±1.00	45.3±1.0	46.3±1.20
Linoleic acid C 18:2	2.7±0.05	1.5±0.15	1.1±0.15	2.1±0.20	1.7±0.20
Linolenic acid C 18:3	0.6±0.05	0.9±0.05	0.6±0.05	0.8±0.10	0.7±0.10
Arachic acid C 20:0	0.3±0.01	0.2±0.01	0.2±0.02	0.3±0.01	0.3±0.01
Beheenoic acid C 20:2	0.1±0.01	0.1±0.01	0.1±0.01	0.2±0.01	0.1±0.01
Saturated fatty acids (SFA)	**49.2**	**44.9**	**48.2**	**48.7**	**48.0**
Unsaturated fatty acids (UFA)	**50.8**	**55.1**	**51.8**	**51.3**	**52.0**
Monounsaturated fatty acids (MUFA)	***47.4***	***52.6***	***50.0***	***48.2***	***49.5***
Polyunsaturated fatty acids (PUFA)	***3.4***	***2.5***	***1.8***	***3.1***	***2.5***

* The statistical analysis was conducted in rows using one-way ANOVA. The presence of statistically significant differences between the mean values ($p < 0.05$) are denoted by the same letters, $^{a, b, c, d, e}$ – $p < 0.05$

The use of feed supplemented with dihydroquercetin or dry compressed distilled rose petals alters the fatty acid composition of the backfat (Table 3). The content of unsaturated fatty acids in backfat obtained from pigs received the feed supplemented with 3.5 mg and 7.5 mg of dihydroquercetin/kg body weight (samples D1 and D2) increased by 4.7% and 2.2%, respectively. In contrast, the share of saturated fatty acids in backfat from pigs fed with feed supplemented with 0.545 g of dry compressed distilled rose petals/kg body weight (samples R2) decreased by 4.64%. The lower concentration of the dry compressed distilled rose petals (samples R1) does not change significantly ($p \geq 0.05$) the fatty acid composition of the backfat.

Table 3. Fatty acid composition of *backfat* 1d *post mortem* (0 - 4°C)

Fatty acids, %	***Backfat*, 1d *post mortem***				
	Control sample (C)	**Experimental sample (D1)**	**Experimental sample (D2)**	**Experimental sample (R1)**	**Experimental sample (R2)**
Lauric acid C12:0	0.1±0.01	0.1±0.01	0.1±0.01	-	0.1
Myristic acid C 14:0	1.8±0.20	1.7±0.15	2.3±0.20	2.1±0.20	2.0±0.15
Pentadecanoic acid C 15:0	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01
Palmitic acid C 16:0	32.5±1.0	30.3±0.7	38.9±1.0	32.9±1.0	34.8±1.0
Palmitoleic acid C 16:1	1.2±0.20	1.0±0.10	1.9±0.20	1.2±0.15	1.2±0.15
Margaric acid C 17:0	0.4±0.05	0.5±0.07	0.5±0.08	0.5±0.07	0.6±0.07
Margarine oleic acid C17:1	0.4±0.02	0.5±0.02	0.4±0.02	0.3±0.03	0.3±0.02
Stearic acid C 18:0	18.7±0.70	18.6±1.50	10.6±1.10	17.9±1.50	18.5±1.40
Oleic acid C 18:1	38.8±1.00	42±1.60	39.5±1.0	40.7±120	38.7±1.0
Linoleic acid C 18:2	4.6±0.10	3.9±0.20	4.6±0.20	3.2±0.20	2.9±0.30
Linolenic acid C 18:3	0.9±0.05	0.7±0.05	0.6±0.07	0.5±0.10	0.3±0.04
Arachic acid C 20:0	0.2±0.01	0.3±0.05	0.3±0.02	0.2±0.01	0.2±0.01
Beheenoic acid C 20:2	0.3±0.01	0.3±0.01	0.2±0.01	0.4±0.05	0.3±0.01
Saturated fatty acids (SFA)	**53.8**	**51.6**	**52.8**	**53.7**	**56.3**
Unsaturated fatty acids (UFA)	**46.2**	**48.4**	**47.2**	**46.3**	**43.7**
Monounsaturated fatty acids (MUFA)	***40.4***	***43.5***	***41.8***	***42.2***	***40.2***
Polyunsaturated fatty acids (PUFA)	***5.8***	***4.9***	***5.4***	***4.1***	***3.5***

* The statistical analysis was conducted in rows using one-way ANOVA. The presence of statistically significant differences between the mean values ($p < 0.05$) are denoted by the same letters, $^{a, b, c, d, e}$ – $p < 0.05$

The addition of dihydroquercetin or dry compressed distilled rose petals to the forage changes the fatty acid composition of leaf fat (Table 4). Compared to the control samples C, the content of unsaturated fatty acid increased by 11% in the pig fed with forage supplemented with 0.545 g of dry compressed distilled rose petals/kg of body weight (samples R2). In opposite, the content of unsaturated fatty acids in the samples of leaf fat obtained from pigs fed with feed supplemented with 7.5 mg of dihydroquercetin/kg body weight (samples D2) and 0.252 g of dry compressed distilled rose petals/kg of body weight (samples R1) decterase with 9 %. Nearest to the fatty acid composition of the leaf fat to control samples C were samples D1 - pigs fed with feed enriched with the addition of 3.5 mg dihydroquercetin/kg body weight.

Table 4. Fatty acid composition of *leaf fat* 1d *post mortem* (0 - 4°C)

Fatty acids, %	*Leaf fat,* **1d** *post mortem*				
	Control sample (C)	**Experimental sample (D1)**	**Experimental sample (D2)**	**Experimental sample (R1)**	**Experimental sample (R2)**
Lauric acid C12:0	0.1±0.01	0.1±0.01	0.1±0.01	0.2±0.01	0.1±0.01
Myristic acid C 14:0	2.2±0.20	2.3±0.10	2.2±0.20	2.3±0.20	2.1±0.20
Pentadecanoic acid C 15:0	0.1±0.01	0.2±0.01	0.1±0.01	0.1±0.01	0.1±0.01
Palmitic acid C 16:0	33.7±1.0	33.5±0.70	34.0±0.80	34.8±0.60	32.1±1.00
Palmitoleic acid C 16:1	1.3±0.30	1.4±0.40	1.9±0.40	1.3±0.40	1.6±0.40
Margaric acid C 17:0	0.4±0.03	0.6±0.05	0.4±0.05	0.5±0.05	0.4±0.04
Margarine oleic acid C17:1	0.2±0.01	0.5±0.03	0.4±0.08	0.2±0.03	0.3±0.04
Stearic acid C 18:0	17.2±0.60	18.7±0.50	20.3±0.60	19.4±0.60	13.8±0.60
Oleic acid C 18:1	42.1±1.20	37.9±1.20	38.1±1.10	38.7±1.20	46.5±1.20
Linoleic acid C 18:2	1.9±0.20	4.1±0.50	1.5±0.10	1.8±0.20	2.2±0.10
Linolenic acid C 18:3	0.2±0.01	0.5±0.03	0.5±0.03	0.3±0.03	0.3±0.02
Arachic acid C 20:0	0.2±0.01	0.1±0.01	0.2±0.01	0.2±0.01	0.2±0.01
Beheenoic acid C 20:2	0.4±0.02	0.1±0.01	0.3±0.02	0.2±0.01	0.3±0.01
Saturated fatty acids (SFA)	**53.9**	**55.5**	**57.3**	**57.5**	**48.8**
Unsaturated fatty acids (UFA)	**46.1**	**44.5**	**42.7**	**42.5**	**51.3**
Monounsaturated fatty acids (MUFA)	***43.6***	***39.8***	***40.4***	***40.2***	***48.5***
Polyunsaturated fatty acids (PUFA)	***2.5***	***4.7***	***2.3***	***2.3***	***2.8***

* The statistical analysis was conducted in rows using one-way ANOVA. The presence of statistically significant differences between the mean values ($p < 0.05$) are denoted by the same letters, $^{a, b, c, d, e}$ – $p < 0.05$

3.2. Effect of the suplementation with dihydroquercetin or dry compressed distilled rose petals on the content of sterols, tocopherols and carotenoids of the muscle and fatty tissues

The monitoring of the changes in sterols content in m. *Semimembranosus* (Table 5), show no significant differences between control samples C and experimental samples D1 (pigs fed with feed supplemented with 3.5 mg dihydroquercetin/kg body weight) and experimental samples D2 (pigs fed with fodder enriched with the addition of 7.5 mg dihydroquercetin/kg body weight). It was found that in the experimental samples R1 (pigs fed with feed supplemented with 0.242 g dry compressed distilled rose petals/kg body weight) and samples R2 (pigs fed with feed supplemented with 0.545 g dry compressed distilled rose petals/kg body weight), content of sterols of m. *Semimembranosus* decreases 1.88 times. Unlike those result the sterols content of m. *Longissimus lumborum* was the lowest in the control samples C (Table 5). All other experimental samples (R1, R2, D1 and D2, $p \geq 0.05$) showed more than 2.3 times higher values than the controls.

The content of sterols in the backfat was highest in control samples C. Compered to C sterols in D1 and D2 were 1.6 times lower and in samples R1 and R2, 4 times lower respectively. There were no significant differences ($p \geq 0.05$) between the content of sterols of the experimental samples D1 and D2 as well as the experimental samples R1 and R2.

The content of sterols in all experimentaled samples leaf fat did not have significant differences ($p \geq 0.05$) (Table 5).

The feeding of pigs with feed supplements of dihydroquercetin or dry compressed distilled rose petals has a different effect on the tocopherol content (Table 5) in the oxidative and glycolytic types of muscles. In the oxidative m. *Semimembranosus*, the least content of tocopherols were recorded in the experimental sample R2 (pigs fed with 0.242 g dry compressed distilled rose petals/kg body weight), 4.00 mg/kg. Tocopherols in the control samples C were 3 times higher than those in the experimental samples R2, and 5 times higher in the experimental samples R1 (20 mg/kg). Comparing to the

experimental samples R2, 6.25 times and 13.5 times highest tocopherols content was found in the experimental samples D1and D2, respectively (Table 5). The lowest content of tocopherols in the m. *Longissimus lumborum* (glycolic muscle type) were found in the control samples C 5.00 mg/kg (Table 5). Three times higher was the content of tocopherols in the experimental samples D1, D2 and R1, with no significant differences ($p \geq 0.05$) between them. The highest content of tocopherols was determined in an experimental samples R2, more than 5.4 times compared with the control samples C.

The content of tocopherols of the backfat of experimental samples D1 was 13.00 mg/kg no significant differences ($p \geq 0.05$) was found between samples C, D2 and R1, with approximately 1.8 times higher tocopherols compared to D1. The highest content of tocopherols was found in the backfat of a experimental samples R2 (pigs fed with feed supplemented with 0.545 g dry compressed distilled rose petals/kg body weight) over 2.9 times compared with the experimental samples D1 (pigs fed with feed with addition of the 3.5 mg dihydroquercetin/kg body weight).

The similar changes of the content of tocopherols in leaf fat from samples of the experimental pigs were determined. At least the tocopherols were contained in the experimental samples D1 - 4.00 mg/kg. In the samples R2, the content of tocopherols was 3.5 times higher than those of samples D1. In addition the content of tocopherols of the control samples C and the experimental samples D1 were about 5 times higher comparing with samples D1.

The highest content of tocopherols in the leaf fat was found in samples R1 (pigs fed with feed supplemented with 0.242 g dry compressed distilled rose petals/kg body weight) - over 11.2 times in comparison with the experimental samples D1 (pigs fed with feed supplemented with 3.5 mg dihydroquercetin/kg body weight).

When track changes in the content of carotenoids in oxidative muscle type m. *Semimembranosus*, the following trend was noticed: the lowest content of carotenoids in the experimental samples D1 and R1 were 6.90 and 8.10 mg/kg, respectively. In the control samples C and in the experimental samples R2 the content of carotenoids was over 3.5 times higher, and in the experimental samples D2 - more than 4.5 times higher compared to the samples D1 and R1(Table 5).

In the glycosylic type m. *Longissimus lumborum*, content of carotenoids was least in experimental samples D2 and R1, with no significant ($p \geq 0.05$) differences between them. On the next place according to the content of carotenoids were arranged experimental samples D1 and R2 with slightly above 1.3 times higher levels. The highest content of carotenoids was determined in the control samples C - over 1.6 times.

The lower content of carotenoids was found in the backfat of the experimental samples R2 - 3.70 mg/kg. Content of carotenoids of the experimental samples D1 was 2.8 times higher than those in the experimental samples R2, and in the experimental samples R1 and D2 content of carotenoids was 5.4 times higher. The highest content of carotenoids was found in the control samples C, about 8 times, compared to the experimental samples R2 (Table 5).

In leaf fat the lowest content of carotenoids was found in control samples C and experimental samples D1, with no significant ($p \geq 0.05$) differences between them. In experimental samples R1, R2 and D2, content of carotenoids was 1.3 times higher.

Table 5. Content of sterols, tocopherols and carotenoids in m. Semimembranosus, m. Longissimus dorsi, backfat and leaf fat (1d post mortem, 0-4 ° C)

Sampes	Sterols, g/100 g	Tocopherols, mg/kg	Carotenoids, mg/kg
m. Semimembranosus, 1d post mortem			
Control sample (C)	0.32±0.07	12.00±1.50	24.50±2.50
Experimental sample (D1)	0.32±0.07	25.00±2.20	6.90±1.20
Experimental sample (D2)	0.30±0.05	54.00±3.50	32.40±2.10
Experimental sample (R1)	0.15±0.05	20.00±2.50	8.10±1.60
Experimental sample (R2)	0.17±0.05	4.00±0.80	23.80±2.00
m. Longissimus lumborum, 1d post mortem			
Control sample (C)	0.12±0.03	5.00±0.80	36.20±2.80
Experimental sample (D1)	0.28±0.03	15.00±1.50	30.20±2.00
Experimental sample (D2)	0.32±0.03	16.00±1.30	22.60±1.80
Experimental sample (R1)	0.25±0.03	15.00±1.70	18.90±1.40
Experimental sample (R2)	0.23±0.03	27.00±2.20	29.90±1.30
Backfat, 1d post mortem			
Control sample (C)	0.16±0.03	21.00±1.90	29.70±1.30
Experimental sample (D1)	0.10±0.01	13.00±2.00	10.60±1.00
Experimental sample (D2)	0.08±0.03	24.00±2.20	13.60±1.00
Experimental sample (R1)	0.04±0.02	23.00±2.50	15.70±1.10
Experimental sample (R2)	0.05±0.02	38.00±3.00	3.70±0.80
Leaf fat, 1d post mortem			
Control sample (C)	0.06±0.01	19.00±1.70	7.90±0.80
Experimental sample (D1)	0.07±0.02	4.00±0.50	8.70±0.60
Experimental sample (D2)	0.04±0.01	23.00±2.50	10.60±0.80
Experimental sample (R1)	0.04±0.01	45.00±3.00	11.10±0.70
Experimental sample (R2)	0.08±0.03	14.00±1.50	11.50±0.50

* The statistical analysis was conducted in rows using one-way ANOVA. The presence of statistically significant differences between the mean values ($p < 0.05$) are denoted by the same letters, $^{a, b, c, d, e}$ – $p < 0.05$

4. CONCLUSION

The addition of low and high concentrations of dihydroquercetin or dry compressed distilled rose petals has a different effect on the fatty acid profile of the muscle and fatty tissues. The share of unsaturated fatty acids increases with 3.9% in m. *Semimembranosus* samples R1; with 11.0% in m. *Longissimus lumborum* samples D1; with 11.0% in leaf fatsamples R2 and with 4.7% in backfat samples D1.

The content of sterols in m. *Semimembranosus* decreases 1.88 times at samples R1 and R2 only, and at samples D1 and D2 is equal with control samples C. The content of sterols in the backfat decreases 1.6 times at samples D1 and D2 and 4 times at samples R1 and R2, respectively. Conversely, the content of sterols in m. *Longissimus lumborum* increases 2.3 times independently of R1, R2, D1 or D2 supplementation, and in leaf fat don't found significant differences of sterols content.

The significant increasing of tocopherol content was estimated in m. *Semimembranosus* samples D2 and in m. *Longissimus lumborum* and backfat samples R2, as well as in R1 samples from leaf fat.
The fourth examined phytonutrient supplementations decrease the content of carotenoids in two muscles and backfat excluding in m. *Semimembranosus* samples D2. The opposite, the fourth phytonutrient treatments provoke a significant increasing of the content of carotenoids in leaf fat.

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Enrichment of Antioxidant Compounds of Fluid Bed and Freeze Dried Apples by Spirulina platensis

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ABSTRACT

Apple is one of the most consumed fruit in the world. *Annurca* apple is a typical fruit of Campania (Italy), that is extensively studied for its high antioxidants level. The aim of this study was to determine the effects of fluid bed drying and freeze drying on antioxidant activity. Moreover apples samples of different shapes, dipped in 0.5-1-5% *Spirulina* solutions, were dried and their antioxidant activity was monitored. Antioxidant activity was maximum at 1% *Spirulina* concentration (phenolic content: 1023,42±52,33 mgGAE/100g Dry weight, ABTS value: 11,75±0,66 mmolTE/100g Dry weight) and it decreased with drying (fresh apple phenolic content:2300.89 ± 886.33 mgGAE/100g Dry weight, highest phenolic content with in dried products:1023,42±52,33 mgGAE/100g Dry weight, for ABTS values in fresh apple: 19,25±3,06 mmol TE/100g Dry weight and the highest ABTS value in dried products was 11,75±0,66 mmolTE/100g Dry weight). Freeze dried samples had higher antioxidant activity than fluid bed dried samples (for phenols: highest value of Freeze dried sample was 1023,42±52,33 mgGAE/100g Dry weight and Fluid Bed dried samples was 984±57,37 mgGAE/100g Dry weight, for ABTS values highest Freeze dried sample value was found as 11,75±0,66 mmolTE/100g Dry weight and Fluid Bed dried samples' highest value was 6,91±1,41 mmolTE/100g Dry weight). Shape differences did not affect the antioxidant activity significantly for both fresh and fluid bed samples. This study shows *Spirulina* can increase antioxidant activity of dried apples.

Keywords: Apple, Freeze Drying, Fluid Bed Drying, *Spirulina*, Antioxidant

1.INTRODUCTION

Apple is one of the most commonly consumed fruit in the world; in particular, Italy is the 6th world biggest apple producer country (De Ros, 2012).'Annurca' has been known since ancient Roman times and it is one of the most important cultivars in Southern Italy. Today it is the most commonly grown cultivar in the Campania region. It accounts for 95% of the southern Italy and 3–4% of the national apple production (Lo Scalzo et al. 2001).The Annurca apple is a variety with a "Protected Geographical Indication". These apples are extremely rich in catechin, epicatechin, and chlorogenic acid and display a stronger antioxidant activity compared with other varieties (Fini et al. 2007).
Spirulina platensis is one of the most studied micro algae species. It has been used for many years as industrial product and in scientific material. *Spirulina*is used in industry for single cell protein, as a feed product for fishery and as a food source. Several *Spirulina* sp. based foods and supplementshave been found in the markets, subsidized by theFood and Drugs Administration (FDA), andclassified as GRAS (generally recognized as safe) (Lemes et al. 2012). *Spirulina platensis*~~includes~~is characterized by high content of protein, ~~main~~ unsaturated fatty acids, vitamins, minerals, antioxidant compounds etc.*Spirulina* containsmore calcium than milk, more B_{12} than cow liver, vitamin A,B_2, B_6, H, K and all essential minerals and enzymes thanother food stuffs (Fox 1986). The powerful antioxidantproperties of *Spirulina* are due to its phycocyanin content (Özyurt et al. 2015). It is also reported that reportedthat *Spirulina* polysaccharides have a stimulating effect on DNA repair mechanism, which might explain the radiologicalprotection effect mentioned several times in relation to *Spirulina*. A double-blind study on human subjects showed antioxidant and lipidlowering ability of *Spirulina platensis* treatment in chronic obstructive pulmonary disease (Ismail et al. 2015).
~~H~~as not been usedat the current state of knowledge, *Spirulina platensis*has not been usedfor increasing mainly antioxidant compounds in another food yet.
Dried apples provide much more antioxidant compounds for each weight cause of removing water cause of this we studied different drying methods for more efficiency to get highest content of antioxidant activity. It was selected that fluid bed dryer and freeze drier to see the hot air and vacuum drying effect on the material and for comparing antioxidant results. For enriching of antioxidant compounds *Spirulina platensis* was used and it compared with fresh and dried apples antioxidant activities. *Spirulina* include high antioxidant content. In this study it is aim that to see different drying methods how affect the antioxidant compound and with *Spirulina* will antioxidant content increase as expected or not and how the drying method affect antioxidant content of apples with *Spirulina* solution.

2.MATERIAL AND METHOD

Annurca apples used in this study were purchased from local markets and stored at 4°C until analyses. *Spirulina platensis* (dried powder) handled from Universita Degli Studi di Napoli Federico II, Food Science and Technology laboratory. It stored dark area and at room temperature until using.

2.1 Sample preparation

Before drying, apples were washed, pealed, cutted as parallelopipped (1x1x3cm), cubic (1x1x1cm) and sliced as circle (Diameter= 3cm, width= 0.5cm) and were dipped in different concentrated *Spirulina* solutions. However, cubic and circle shape apples were dipped in 1% *Spirulina* solutions. Because, firstly parallelopidded samples dipped and dried to see the maximum effective *Spirulina* solution for antioxidant compounds. For paralellopipped solutions was prepared %0.5-1 and %5 concentration. Every shape of apple dipped *Spirulina* solution in 100ml beherglasses of distilled water at room temperature and samples were dipped 1 hour at 300 rpm. Sample and solution ratio was kept 1:2 for all studies. Then, excess solution removed carefully with paper and were applied two different drying technologies: freeze drying and fluid bed drying.

2.2 Method

2.2.1 Drying technologies

2.2.1.1 Freeze Drying

Apples were freezed at -18^{o} C overnight, then, were dried using a freeze dryer Alpha 1-2 LD plus at -50^{o}C, for 16 h. It was possible to obtain a final moisture content (wet dry basis) of about 7.5±1.7%

2.2.1.2 Fluid Bed Drying

To define the better drying parameters, preliminary apples were dried in a Fluid Bed Drying (Sherwood Scientific, Cambridge Scientific Park) at different air temperature: 60; 65; 70; 75^{o}C. Then, according to drying time and quality indices, 72^{o}C and 7th level of air flow speed were selected as parameters to adopt for the preparation of dried apples.

2.2.2 Moisture

The apples moisture content was determined by gravimetric method. 1g of fresh or treated sample was put in oven for 24 hours at 105^{o}C (Inter continentalequipments) and was accurately weighted at regular time interval until constant weight was reached (Cuccurullo et al. 2018). Three samples from each apple were measured. The moisture content was calculated as:

$$\%moisture = \frac{W_I - W_F}{W_I} \times 100$$

Were W_I=initial weight[g]; W_F=final weight[g].

2.2.3 Antioxidant Compounds

2.2.3.1 Extraction

In order to extract antioxidants from apples, 1g of sample and 10 ml or 20 ml of methanol solution (70% v/v) for fresh and dried samples respectively, were added in a falcon. Successively, Ultra-Turrax (IKA T18-Basic) was applied for 1 min at level 4 of speed and then the obtained mixture was put in a sonic bath (Argo Lab DU 45) for 30 minutes at power 6 and centrifugated by ALC Multispeed Refrigerated Centrifuge PK 131Rat 10000 rpm for 10 min. The obtained supernatant was filtered and conveniently diluted in methanol 70% (v/v) (1:2 for fresh samples and 1:8 for dried samples) for successive analyses.

2.2.3.2 ABTS

ABTS method is based on the reduction of the $ABTS^{\bullet+}$ activity by the antioxidants contained in the sample.A solution of 7.4 mM $ABTS^{\bullet+}$ (5 mL) mixed with 140 mM $K_2S_2O_8$ (88µL) was prepared, stabilized for 12h at 4°C and then mixed with ethanol (1:88,v/v). Subsequently, 100 µL of supernatant obtained from the above extraction and further diluted 1:10, were added to 1 mL of diluted $ABTS^{\bullet+}$

,incubated for 2.5 min and the absorbance was read at 734 nm. The standard curve was linear between 0 and 20 µM Trolox. Results were expressed as µmol of TroloxEquivalent/100 g dried weight (DW) (Miller and Rice-Evans, 1997).

2.2.3.3 Phenol Test

Total polyphenolic amount was evaluated by using Folin−Ciocalteu's assay as reported by Singleton et al (1999). with some modifications. In a falcon (15 ml), 100 µl of diluted methanolic extract, 100 µl of Folin−Ciocalteu's phenol reagent, 1000 µl of Na_2CO_3 and 400 µl of distilledwater were added and shaken. After 6 min, 625 µL of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted with 500 µL of distilled H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm. All biological replicates of samples were analyzed in triplicate. Total phenolic content of apples was expressed as milligrams of gallic acid equivalents (GAE) per 100 g DW.

All the colorimetric determinations were carried out by Jasco V-550 UV/VIS Spectrophometer.

2.2.3.1 Statistical Analysis

Samples of fresh, freeze dried and fluid dried apples results were expressed as mean value ±standard deviation. Differences among evaluated parameters in samples were determined by using SPSS (Statistical Package for Social Sciences) Package 6, version 20.0. Significance was determined by comparing samples and fluid bed drying through a factorial analysis of variance (one-way ANOVA) with Duncan post hoc test at a significance level of 0.05. For determination of stability test, significance of values for samples treated by fluid bed drying were evaluated for each week of analysis by Duncan post hoc test at a significance level of 0.05.

3.RESULT AND DISCUSSION

During all processes it was aimed that to see how *Spirulina* content affect antioxidant activity. 0.5-1 and 5% *Spirulina* solution applied for general studies and after these studies 1% *Spirulina* solutions has found more effective and to see effect of different shape of apples cubic and circle shaped apples dried in both fluidbed and freeze dryer with 1% *Spirulina* solution.

Antioxidant results were given and results were compared with fluid bed dried apple, freeze dried apple and fresh apple.

3.1 Phenol Results:

Table.1: Phenol results of all samples (FA: Fresh Apple, FBDA: FluidBed Dried Apple, FDA: Freeze Dried Apple, C: Circle Shape, Cubic: Cubic Shape)

Sample	**mg GAE/100g Dry Weight**
FA 1% spirulina	2300.89 ± 886.33
FA	1263.83± 57.09
FBDA	777.72 ± 116.00
FDA	924,54±11,73
FBDA 0.5% spirulina	778.27 ± 46.93
FBDA 1% spirulina	788.01 ± 46.00
FBDA 5% spirulina	984±57,37
FDA 0,5% spirulina	953,65±61,33
FDA 1% spirulina	1008,09±74,85

FBDA 1% spirulina C	803,19±62,46
FDA 1% spirulina C	965,54±32,70
FBDA 1% spirulina Cubic	817,52±27,85
FDA 1% spirulina Cubic	1023,42±52,33

According to table, fresh apple with 1% *Spirulina* found most effective phenol result. When apples dried, freeze dried samples showed more protection for phenol values. 1% freeze dried samples was found highest values. Freeze dried and cubic shaped samples' phenol values was the highest score within all dried samples as mean value 1023,42 mg GAE/100g dry weight. Circle shaped and freeze dried samples' value (965,54 mgGAE/100g dry weight) found less than paralellopipped one (1008,09 mgGAE/100g dry weight) but for fluid bed dried samples, including 5% *Spirulina* solution sample showed higher score as expected (984 mg GAE/100g dry weight) and for different shape studies cubic shape fluid bed dried samples (871,52 mgGAE/100g dry weight) has slightly more phenol content than circle (803,19 mgGAE dry weight) and paralellopipped shaped samples (FBDA 1% *Spirulina*: 788,01 mgGAE/100g dry weight) were showed lest phenol contents for different shape studies.

3.1.1.2 ABTS

Sample	**mmolTE/100g DryWeight**
FA	11,82±0,84
FA 1% spirulina	19,25±3,06
FBDA	4,95±1,09
FDA	10,30±0,82
FBDA 0.5% spirulina	5,13±0,56
FBDA 1% spirulina	5,54±1,33
FBDA 5% spirulina	6,91±1,41
FDA 0,5% spirulina	10,71±0,87
FDA 1% spirulina	11,20±0,73
FBDA 1% spirulina C	5,38±0,92
FDA 1% spirulina C	11,22±0,55
FBDA 1% spirulina Cubic	5,50±0,74
FDA 1% spirulina Cubic	11,75±0,66

Fresh apple with 1% *Spirulina* showed highest ABTS value (19,25 mmolTE/100g dry weight). Among to dried samples freeze dried and cubic shaped samples had the most ABTS value as 11,75 mmolTE/100g dry weight. For different shaped freeze dried samples circle shaped sample had slightly higher value (11,22 mmolTE/100g dry weight) than paralellopipped shaped (11,20 mmolTE/100g dry weight) samples. Fluid bed dried samples protected ABTS values (6,91mmolTE/100g dry weight) less than freeze dried samples. In fluidbed drying heat transfer applied with 72^{o}C and as it is known heat affect lots of valuable products. Phenolic and antioxidant compounds has been lost cause of this heat applying. Eventhough increasing of *Spirulina* content in apples provided much more antioxidant activity with 5% *Spirulina* in fluid bed dried samples less than freeze dried samples cause of this heat damage problem. ABTS content was affected by *Spirulina* concentration according to fluidbed and freeze drying. In fluid bed drying studies 5% *Spirulina*

samples' had highest ABTS value (6,91 mmolTE/100g dry weight) and paralellopipped shaped samples found highest ABTS (5,54 mmolTE/100g dry weight) unlikely freeze drying. However, the difference between cubic shaped (5,50 mmolTE/100g dry weight) and paralellopipped samples (5,54 mmolTE/100g dry weight) have not been found significantly. For both antioxidant compounds freeze drying showed more protection and cubic shaped samples had higher results but it is not absolute and values were not quite much different than each other.

In freeze drying of apples enriched with 5% of *Spirulina*, was experienced foam problem during the study and after during the drying process water did not remove effectively and moisture content found as 3 or 4 times higher than other ones. Therefore, antioxidant studies did not carry out for this concentrate. Fig.2 reported images of apples which are enriched with %5 *Spirulina*.

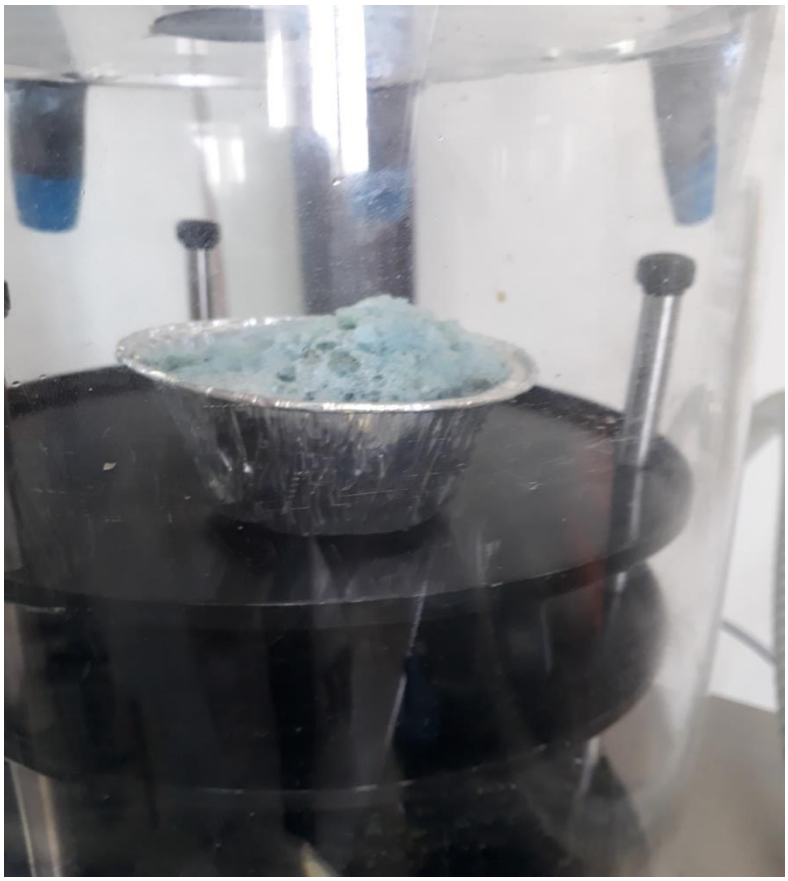

Fig.2: Enriched with %5 *Spirulina* solution sample's foam problem in freeze dryer

However, when sample was enriched with 5% *Spirulina*, the green color was too marked and not acceptable. It is estimated that *Spirulina* got more accumulated on surface of material and effecting drying and diffusion for these products and results between enriched with %1 and %5 *Spirulina* showed similarities.

There is no study mentioned about enriching with *Spirulina* on apple and drying *Annurca* apple. In addition, fluid bed drying have not mentioned significantly for apple in liturature. Altisent et al. 2014, studied with six different kind of fresh apple. The results showed fresh apple total phenol content similarly with *Annurca* apple. Lavelli et al. 2010, studied on dried apple (which type was not

mentioned in the study) and enriching with green tea extract for antioxidant activity improving. Hot air drying done in the study and total phenol result found as 8100mg/kg dried weight. Fluid bed drying samples in our study without enriching with *Spirulina* found similarly to that value and all enriched samples showed much more antioxidant results. For all freeze dried samples showed higher values than the study as well. In antioxidant activity determination study with Golden delicious apple has been done by Tarko et al. 2009. In study microwave applied as pretreatment and hot air drying was the main drying. ABTS values determined in study for fresh and dried apples. When comparing with *Annurca* apples all results found has lower ABTS values. Dalmau et al. 2017, studied on *in vitro* digestion of Golden apple and was determined also antioxidant activities. Convective drying and freeze drying applied on apples which are 1cm dimensioned cubic apples. Freeze dried samples showed more antioxidant compounds than convective dried samples. When comparing with our samples, all type of apples was found higher antioxidant contents especially freeze dried and *Spirulina* solution enriched samples.

Consequently, a few studies done can be reach for drying apple antioxidant activity and none of them are applied on *Annurca* apples. It is determined in this study that antioxidant activity can be improve by *Spirulina platensis*. Drying studies showed antioxidant activity decreased in all treatments. However, freeze dried samples had highest antioxidant content and changing of apple shapes did not find mainly different than each other. Sustainable antioxidant sources will more take place in future studies and algae represent one of those sources.

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Physicochemical properties of high oleic sunflower oil with herbs

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ABSTRACT

Physico-chemical and optical properties of cold pressed sunflower highly oleic oil with admixtures from basilicum (Ocimum basilicum), rosemary (Rosmarinus officinalis), oregano (Origanum vulgare), truffle and walnut oil have been investigated. The aim of the present study is to make a comparative analysis of the composition, optical and chemical characteristics of cold-pressed sunflower samples with and without herbal supplements to assess the antioxidant and oxidative properties. Different methods were used such as liquid chromatography, express optic methods such as fluorescence, colorimetry and all.. The peroxidative, iodine and saponification values are determined for each of the 8 investigated samples. By using the spectroscopic data the content of pigments as β- carotene and chlorophyll are calculated. The smallest oxidation stability determined by Rensamat shows the sample with walnut oil equivalent to 16.1 h. The oxidative stability values of the tested oils determine the need of stabilizers for some of them to be used when they are included in dressings, sauces and others. Perhaps the highest increase in the peroxide value with the addition of olive oil 12.26 meq O2/kg. Adding herbs to the sunflower oil increases the content of polyunsaturated fatty acids, β-carotene and chlorophyll. The sunflower oil with basilicum (Ocimum basilicum) shows a fluorescence peak at about 555 nm, which is due to the high β-carotene content. All samples show a peak between 741 nm and 746 nm range, which is due to pigments different from chlorophyll, and those containing herbs and a chlorophyll peak at about 683 nm.

Keywords: cold-press sunflower oil, fluorescence, liquid chromatography, colorimetry

1. INTRODUCTION

The cold pressed oils contain calcium, copper, iron, magnesium, manganese, phosphorus, selenium, zinc, vitamins from B-group, and vitamin E, and have antioxidant activity that reduces oxidative stress leading to cell damage. They are characterized by a lower content of saturated fatty acids and a higher content of polyunsaturated fatty acids. A number of studies have shown that higher intake of saturated fatty acids leads to an increase in plasma cholesterol (*Akoh & Min, 2002, Akoh, 2006, Bockisch, 1998),* which is a major factor in the formation of cholesterol plaques on the blood vessels, for disruption of blood flow and increased risk of cardiovascular disease *(Cautreels et al., 2006).* On the other hand, it is suggested that some polyunsaturated fatty acids are indispensable for the human body. Their main functions are related to the fact that they are structural components of biomembranes. For that purpose, research is being carried out to enrich the vegetable oils traditionally used for healthy nutrition with biologically active substances by adding herbs to them. On the one hand, herbs can increase the antioxidant properties of oils and on the other - enhance their oxidative stability, as they are rich in alkaloids, glycosides, polysaccharides, tannins, flavonoids, essential oils and vitamins. Tocopherols content also vary in the vegetable oils, ranging between 2.5 and 12 mg / kg of total daily energy intake, as these quantities meet the body's essential fatty acid needs. According to *Garrett & Grisham, 2013* recommendations, the intake of essential fatty acids is appropriate to be combined with the intake of complete proteins whose role as natural antioxidants in living organisms is to inactivate free radicals and reduce the oxidation processes of unsaturated fatty acids (*Gunstone, 2002; Lawrence, 2010).*

The purpose of the present investigation is to make a comparative analysis of the composition, optical and chemical characteristics of cold-pressed sunflower samples with and without herbal supplements to assess the antioxidant and oxidative properties by using applied photonics.

2. MATERIAL AND METHODS

High-oleic sunflower oil as well as pressed sunflower oil "Sunfluro" with basilicum (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), truffle and walnuts supplied by ‘Pliska Oil’ were studied. The concentration of the herbs in the samples is a trade secret of the company and therefore is not commented in the exhibition. Oils are in use on the date of the research. They are stored under refrigeration conditions, after each analysis the air chamber between the oil and the cap is flushed with nitrogen and the cap is sealed with a vaporized film to reduce the risk of oxidation.

Determination of color parameters and pigments
The sample were poured into a 10-mm thick dish. The color parameters in two colorimetric systems XYZ and CIE Lab have been measured by Lovibond PFX 880 (UK).

2.1. Fluorescence spectra

The source used to measure the fluorescence spectra is 390 nm light emitting diode (LED). A fiber optic spectrometer Brolight with sensitivity in the (200-1100) nm range and a resolution of about 8

nm was used to measure the fluorescence spectra. The oil samples were placed in a cuvette 10 mm x 10 mm and irradiated by LEDs.

2.2 Determination of the chemical parameters

The following physico-chemical parameters are defined for characterization of the oils

• Peroxide number (PN) - ISO EN 3960:2008;
• Acid number (AN) and total acidity, according to ISO EN 660:2009;
• Saponification value - ISO EN 3657:2002;
• • The relative density according to BS ISO 6883:2003;
• Oxidative stability or OSI (Oil Stability Index), according to ISO 6886:2008

2.3 Oxidative stability. Oxidative stability of oils was determined by measuring of IP, using conductometric detection of volatile acids. Rancimat apparatus Methrom 679 (Methrom, Herisau, Switzerland) was used at 100^{o}C and an air flow rate 20 l/h.

2*.4. Determination of refractive index* The refractive indexes of oils were measured using an Abbe refractometer (Carl Zeiss, Germany) at 20°C.

2.5. Analysis of fatty acids. The fatty acid composition of oils was determined by gas chromatography (GC) after transmethylation of the respective sample with 20 g.kg-1 H_2SO_4 in absolute CH_3OH at 50˚C. FAME were purified by TLC on 20x20 cm plates covered with 0.2 mm silica gel 60 G (Merck, Darmstadt, Germany) layer with mobile phase n-hexane: diethyl ether (97:3, v/v). GC was performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 75 m x 0.18mm x 25 μm a capillary column Supelco FP - 2560 and a flame ionization detector. The column temperature was programmed from 140 °C (5 min), at 4 °C/min to 240°C, at 3.0 °C/min to 250°C (9 min), at 40°C/min to 230°C (1 min); injector and detector temperatures were kept at 270 °C and 280 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL/min; split was 1:50. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions.

2,6, Determination of color parameters and pigments The sample were poured into a 10-mm thick dish. The color parameters in two colorimetric systems XYZ and SIE Lab have been measured by Lovibond PFX 880 (UK).

3. RESULTS AND DISCUSSION

The fatty acid composition of the oil extracts tested is presented in Table 1. It is one of the key features characterizing the nutritional value of oils and their oxidative stability during storage and heat treatment, which is essential for a healthy diet. The addition of basilicum, oregano or rosemary (Rosmarinus officinalis) to sunflower oil reduces the percentage of saturated and monounsaturated fatty acids and increases the content of polyunsaturated fatty acids. Unlike herbal supplements, the addition of walnut oil increases saturated fatty acids and polyunsaturated acids, and reduces the content of monounsaturated ones.

Table 1 Fatty acid composition of cold pressed sunflower oil with addition of herbs or walnuts oil

Type	Fatty acids composition, %		
	saturated	monounsaturated	polyunsaturated
Sunflower oil+ basilicum	5,06	73,96	20,58
Sunflower oil + walnut oil	6.78	65.89	27.33
Sunflower oil + rosemary	6.02	74.2	19.78
Sunflower oil+ oregano	5.75	70.52	23.73
Sunflower oil+ truffle	6.07	74.65	19.28

Table 2 Physico-chemical parameters of cold pressed sunflower oil with addition of herbs or walnuts oil

Property	Sunflower oil+ basilicum	SO+ oregano	SO+ truffle	SO+ walnut oil	SO+ Rosemary	SO
SN, mg KOH/g	164,13	166.45	168.11	161,72	167,5	157,02
PN, meq O_2/kg	7,95	4.42	4.13	4,17	3,68	5,05
IN, gl/100g	103,61	106.4	101.8	108.8	102 ,3	101,1
d, 20 °C	0,9142	0.9095	0.9104	0,9101	0,91	0.9134
Oxidative stability, h	20	20	20	16,1	17,9	22
β, ppm	1210, 69	0	98.39	110.09	136.55	90,67
Chlorophil, ppm	3944.4	3969.3	0.208	0.741	10.83	0,235

The analysis of the data takes into account the differences in the indicators of the sunflower oil tested with herbal supplements or walnut oil. The addition of rosemary (Rosmarinus officinalis), basil (Ocimum basilicum) and oregano significantly increases the chlorophyll content. β-carotene content is higest for oil with basil and rosemary, as opposed to walnut oil and truffle. The samples with additives did not differ significantly by relative density and iodine number. The peroxide and oxidation stability results suggest that there are real prerequisites for more active oxidation processes in sunflower oil with and walnut oil. The addition of herbs leads to reducing the value of peroxide number. In some technological processes such as pasteurisation, roasting, evaporation, etc. this deviation of the indicator is within the permissible limits for determining the quality and safety of the oils. *(Popov & Ilinov, 1986, Hadzhiyski, 1987; Jebe et al., 1993).*

The adsorption and fluorescence spectra were obtained for λex = 390 nm and are represented respectively in Figures 1 and 2.

The addition of walnut oil or truffle in the cold-pressed sunflower oil slightly alter its absorption rate but preserves the position and shape of the peak in the range of 400 nm to 500 nm. Models of basil or rosemary have a clear absorption peak of about 670 nm. It is highly intense in the sample with basil and much weaker in the sample with rosemary. Oil with basil also has two other absorption bands with a center around 534 nm and 614 nm, probably due to the high amount of β-carotene in the sample and the presence of pigments other than chlorophyll *(Duppy et al., 2005).*

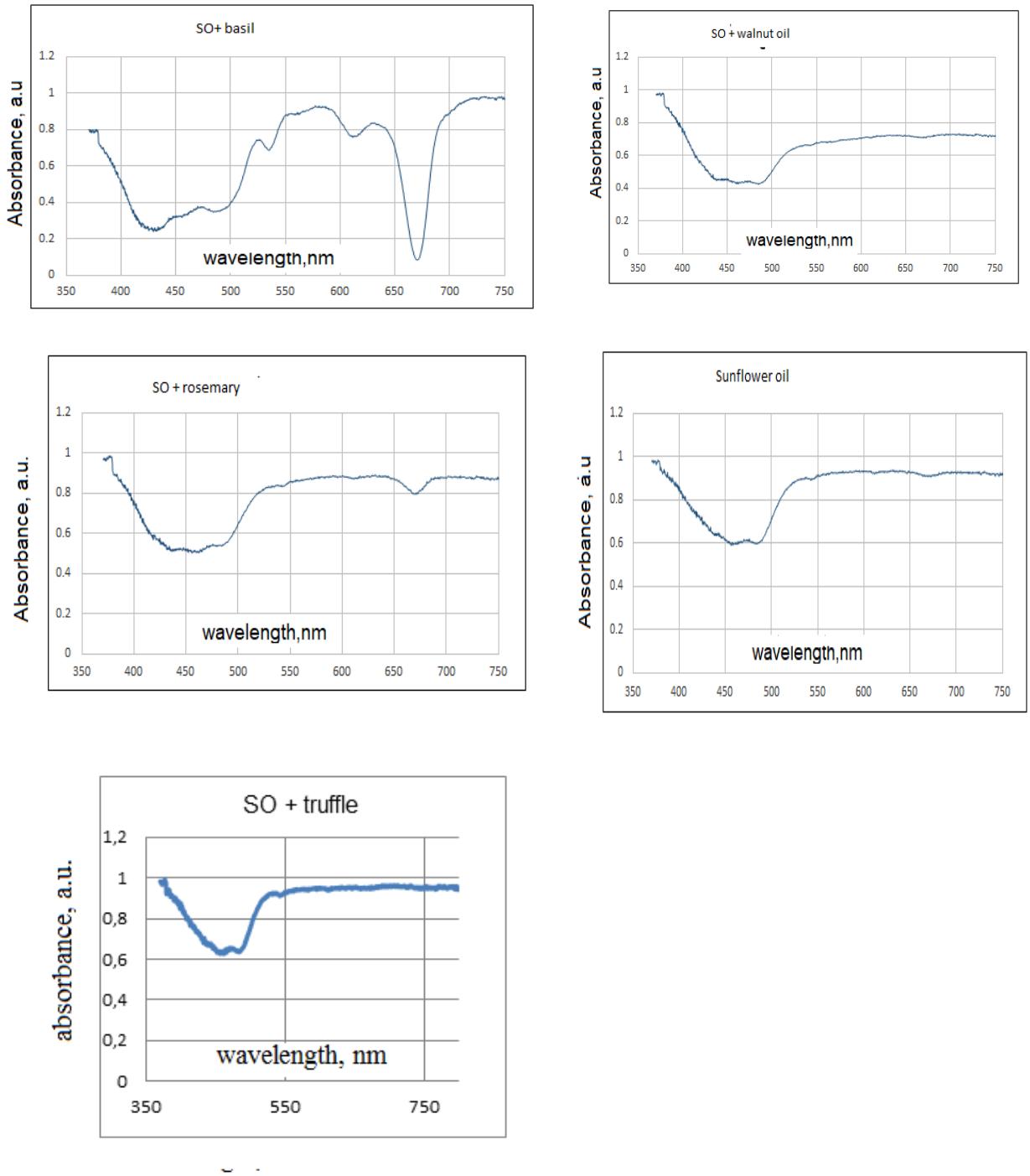

Figure 1. Adsorption spectra of cold pressed sunflower oil with addition of herbs or walnuts oil

The results of the fluorescence spectra can be analyzed and conclusions can be drawn about the nature and changes in the composition of the oils during the processing and storage processes. Vegetable oils include three major groups of natural fluorophores - tocopherols, chlorophylls and phenols *(Sikorska et al., 2005).*

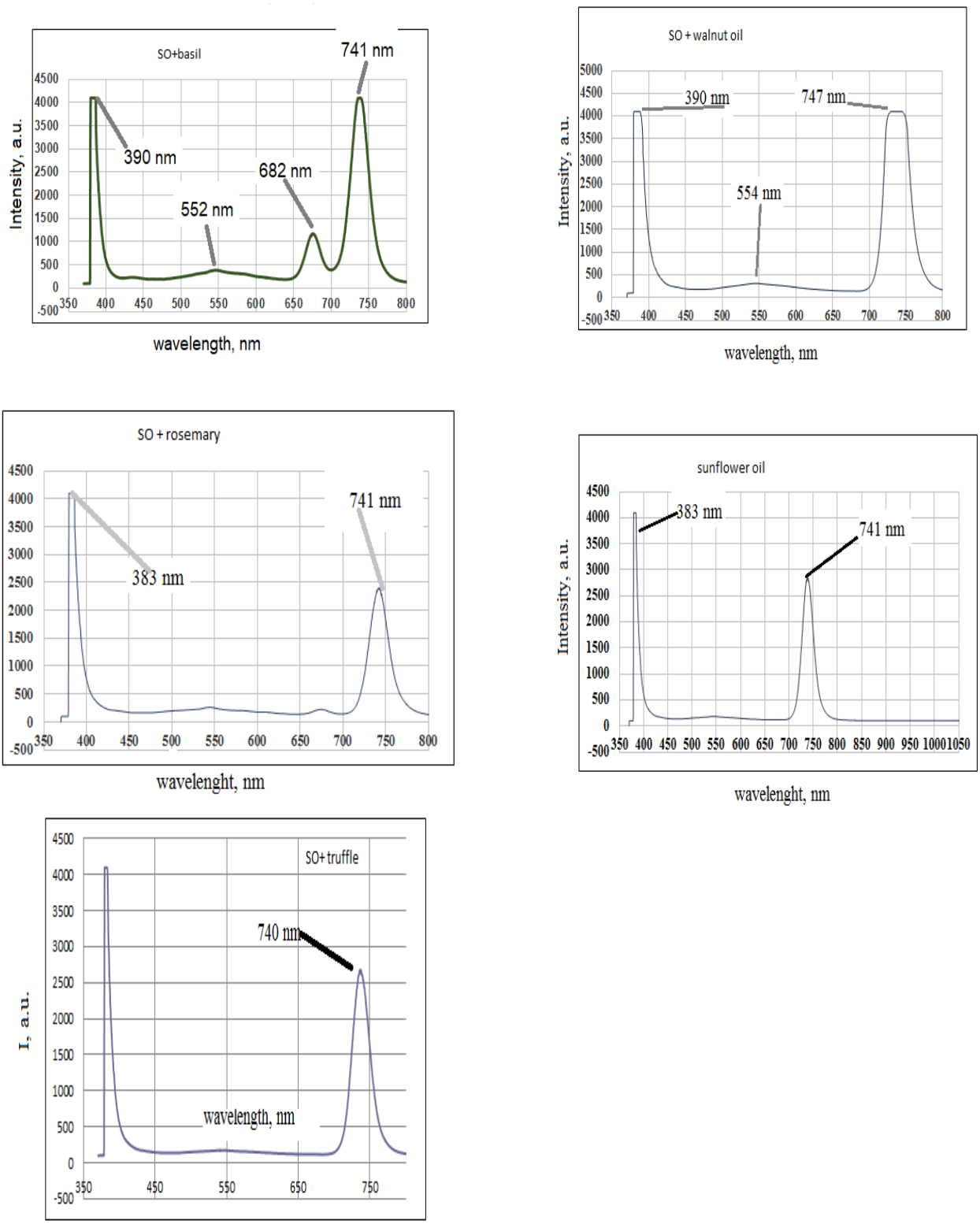

Figure 2. Fluorescence spectra of cold pressed sunflower oil with addition of some herbs or walnuts oil

The addition of rosemary *(Rosmarinus officinalis)* or basil (*Ocimum basilicum*) leads to an increase in the chlorophyll content and to a fluorescence peak at about 683 nm. The peak is more intense for the sample with basil and is much weaker for the oil with rosemary (*Rosmarinus officinalis*). All samples show a fluorescence peak of about 741 nm and 746 nm due to pigments other than chlorophyll. Oils with high contnt of β-carotene exhibit a fluorescence peak at about 555 nm (*Bingning Li et al., 2015)* due to the increase in primary and secondary oxidation products.

4. CONCLUSION

The applied photonics can be used for qualitative determination of the fatty acid composition, pigments and oxidation processes during the storage and processing of the oils. The addition of herbs leads to an increase in pigments and β-carotene content in investigated high oleic sunflower oil. Walnut oil contributes for substantially reduced oxidative stability of the sample and makes it suitable only for sauces and salads.

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Fluorescence spectra and antioxidant activity of juices from dogwood (cornus mas)

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ABSTRACT

The aim of the present work was to measure the content of some biologically active substances, the antioxidant stability and to determine dependencies between them and intensity of fluorescence emission for two excitation wavelengths in UV region - 265 nm and 295 nm, respectively. The juices were prepared from frozen fruits of dogwood (Cornus mas) and were filtered. Inulin with mass 1g, 2g, 3g and lactulose with concentration 0.5 ml, 1 ml and 1,5 ml were added to the juices. The addition of inulin in the juice does not change the color parameters in CIELab colorimetric system. The brightness decreases when the concentration of lactulose increases. The total content of polyphenols and anthocyanins does not depend on the presence of inulin or lactulose. The fluorescence spectra of juice samples with added inulin was measured using steady-state regime of spectrofluorometer FloroLog3 (HORIBA JY, France) and the specific features were observed in the following spectral regions at cited excitation wavelengths:

1. for excitation wavelength λ=295 nm - the peak is related to anthocyanins content.
2. for excitation wavelength λ=265 nm - the peak is related to galic acid content
3. for excitation wavelength λ=295 nm-the peak is related to tannins content.
4. for excitation wavelength λ=295 nm-the peak is related to presence of different types of fruit pigments.

The regression models with positive and negative correlation between some biological active substances and the fluorescence maxima were observed, color parameters and antioxidant properties are developed.

1. INTRODUCTION

Traditionally in Bulgaria fruits are often used in form of juices, syrups, jams and compotes. The natural juices from wild forest berry are attractive drinks. To our knowledge there are no studies which give the relation between the parameters of applied photonics and antioxidant properties. There are though a few data about phenolic and antioxidant levels of traditional Bulgarian juices from the supermarket (*M. Todorova et all, 2018*).

The fruits of dogwood have a tart flavor, and the ripened fruits are sour-sweet. The dogwoods are rich in sugars, from which fructose and glucose predominate, while sucrose is less pronounced. From the vitamin composition the highest is the content of vitamin C, which increases with the ripening of the fruits. The dogwood blooms in February and March and its fruits ripen from July to October. The fruits are dark red in color and have a slightly sour taste *(Denev P et all, 2011).*

In the present work will be investigating the physicochemical properties of fresh juice with inulin or lactulose from the traditional Bulgarian forest-fruit – dogwood (Cornus mas). Numerous scientific studies have shown that daily use of juices with inulin leads to a reduction in the amount of pathogenic bacterias such as Salmonella, Staphylococcus and others. It also improves the exchange of lipids - cholesterol, triglycerides and phospholipids in the blood. This reduces the risk of developing cardiovascular disease, strengthens the immune system and is considered very good at reducing atherosclerosis. By activating the circulatory system, inulin also contributes to the removal of salts of heavy metals from the body *(Dinkova, P et all, 2014).*

The aim of the presented study was to analyze the relationships between the optic parameters and antioxidant activity of total phenolic content as well as to compare the antioxidant potential of investigated samples with juices from the Bulgarian supermarket.

2. MATERIAL AND METHODS

2.1 *Materials*

The dogwood (Cornus mas) purchased from the area of Velingrad were used for this study. Fruits (600 g) were thawed and milled using a MR 300 Minipimer compact blender (Braun, Kronberg, Germany). The pulp was pressed to obtain a fruit fluid. The sample of 600 g of pulp was then squeezed. The obtained pressed juice was pasteurized by heating at high temperature in order to perish pathogenic organisms. Formulations of pasteurized raspberry juice with inulin (1 g, 2 g, and 3 g) and lactulose (0.5 ml, 1 ml and 1.5 ml) have been developed.

2.2. *Methods*

1. ***Content of polyphenols and anthocyanins:*** 0.5 - 1 ml fresh plant material was triturated with quartz sand and 2 - 5 ml 70% methanol in a mortar, quantitatively transferred to a flask with a reflux refrigerator. It is extracted three times for 20 min at 700C. The content of anthocyanin pigment concentration expressed as cianidin-3-glucose equivalent, is calculated. The contents of biologically active substances such as anthocyanin and phenolic components in the juice samples were measured spectrophotometrically.

2. ***Antioxidant activity:*** Ferric reducing antioxidant power (FRAP) assay was used for determining the antioxidant activity. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated, juice. The DPPH method - The method is performed according to *(Goupy et al, 2003).* The working solution of the DPPH radical is prepared with ethanol, the extinction of the control at 517 nm being 0.900 ± 0.010. The experiment was performed by adding the test samples to 2 ml of the solution. Their output at 517 nm was measured after one-hour incubation in a dark place at room temperature. Reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm.

3. **Fluorescence spectra and color measurements:** The fluorescence spectra are measured at excitation wavelengths 265 nm and 295 nm with FloroLog3 (HORIBA JY, France). The color characteristics based on the CIELab colorimetric system were determined by spectrophotometer Konica Minolta CR-400 / 410. They are measured by using reflectance spectrum with a cuvette of an 8 mm length. The color parameters a, b and brightness L of the tested samples were measured. All measurements have been carried out at room temperature and the average value has been taken from 3 measurements.

4. **Sensor analysis:** The organoleptic evaluation on the samples was performed using a grading system. Samples are provided for organoleptic evaluation by tasters, each completing a tasting card. Raw materials are evaluated by the following: appearance, aftertaste, smell, consistency, red color, tart taste, side taste, sweet taste, sour taste, characteristic taste, fruity taste, pronounced taste, sweet taste and expected taste

3. RESULTS AND DISCUSSION

Total polyphenolic content, anthocyanin concentration and antioxidant activity of the tested juices are presented in figures 1, 2 and 3.

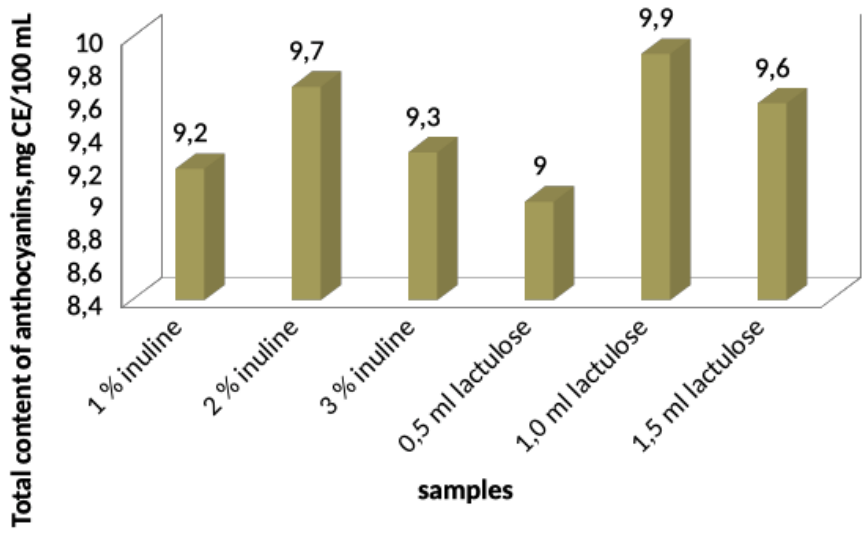

Figure 1. Total anthocyanin content of drinks from dogwood (Cornus mas).

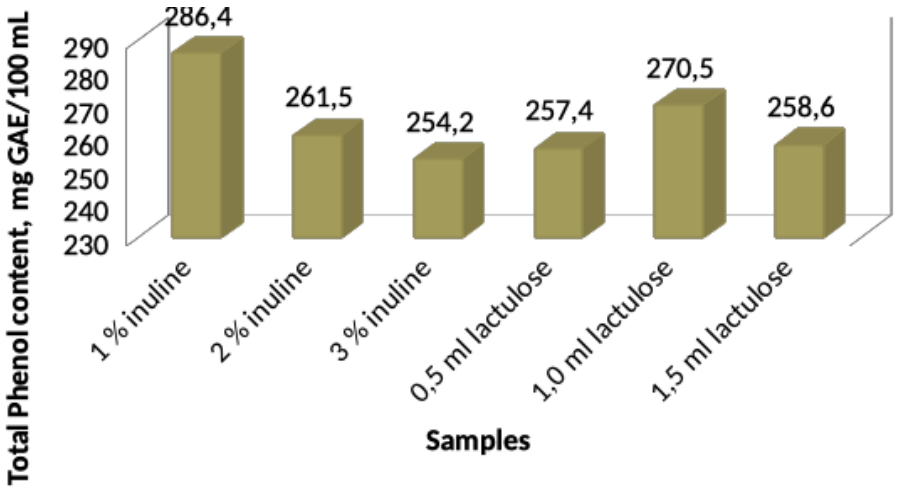

Figure 2. Total phenolic content of dogwood (*Cornus mas)* juices.

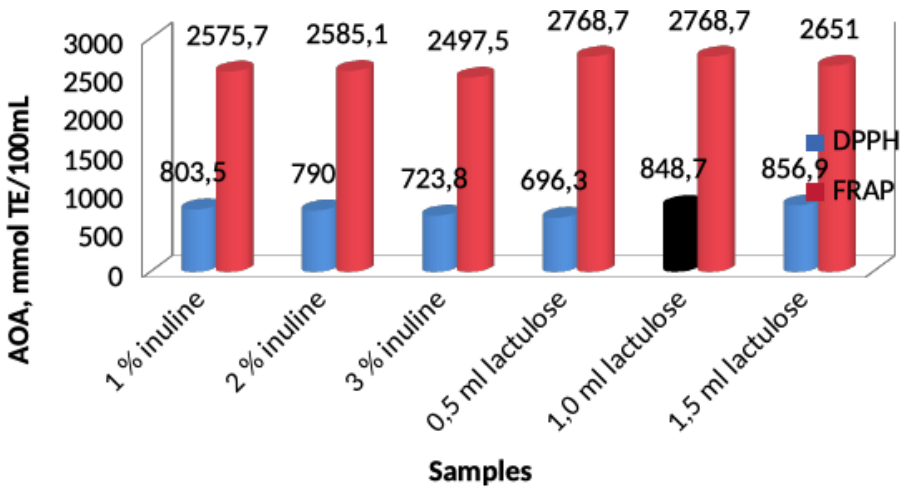

Figure 3. Antioxidant activity of investigated juices by using DPPH and FRAP methods.

The highest total phenolic content was established for juice from dogwood *(Cornus mas)* with 1% inulin (286.4 mg GAE/100ml), followed by juice with 1 ml lactulose. For the other samples with inulin or lactulose the total phenolic content was in the interval [(254 -262) mg GAE/100 mL].
The total phenol content of juices from dogwood (Cornus mas) is greater than blank currant juices (281.3 mg GAE/100 ml) *(Milan N et all, 2011).* Comparing these results with literature, similar values were reported for cherry juices produced in Germany (*Q. Lui et all, 2014*), (270.4–499.8) mg GAE/100 mL and for cherry and blackcurrant juices produced in Croatia (*D. Bonerz et all, 2007*) - (205.443 and 277.094) mg GAE/100 mL.

The content of anthocyanins in the juices from dogwood (Cornus mas) with addition with inulin or lactulose is in narrow limits (9 – 9.9) mg CE/100mL. It is approximately two times greater than this of the compotes (4,74 mg CE/100 mL) and natural fruits juices (3,61 mg CE/100 mL) (*M. Todorova et all, 2018*), but it is smaller to this of the juices from black currant (75.736–92.092) mg CE/100 mL *(L. Jakobek et all, 2007, I.L. Nielsen et all, 2003*). From figure 3 is evident that by increasing of the concentration of lactulose in investigated samples, the antioxidant activity by DPPH method increases while the antioxidant activity by the FRAP method has approximately the constant value. With increasing the concentration of inulin the antioxidant activity by DPPH method decreases.

The optical properties of juices from dogwood were investigated by using the applied photonic methods and searching the relations between the optical properties, antioxidant activity, total phenolic content and color parameters. The fluorescence spectra were obtained for excitation wavelengths 265 nm and 295 nm. From Fig. 4 it is evident that there are three main fluorescence peaks: the peaks in the intervals (355-365) nm and (365- 380) nm are connected with phenolic content *(K. Wlodarska et all, 2016)*. The longer wavelength band with a very low intensity at about (680– 700) nm corresponds to different types of fruit pigments *(K. Wlodarska et all, 2016).* The dependences between total phenol content or anthocyanins and the ratio between the intensity of emission and intensity of excitation for exciting wavelengths 265 nm and 295 nm have been found. Similar linear dependencies are measured between lightness and total content of anthocyanins and between content of β-carotene and color parameter a. The relationships are presented in Table 1.

Table 1. Dependencies between chemical parameters and optical characteristics	
Analytical relations	R^2
$I_{368-380/265} = -1204.4 . Total\ phenolic\ content + 356325$	0.768
$I_{350-365/295} = 3.10^5 . Anthocyanins + 6.72$	0.895
$L = -0.8071\ Anthocyanins + 31.257$	0.972
$TPC = -0.006 . I_{268-380/265} + 287.83$	0.7675
DPPH → 5.5697.TPC+1097	0.676

The good correlation between the total anthocyanin content and the antioxidant activity, determined by DPPH method (R2 = 0.6) of the tested juice samples was confirmed (DPPH= 48.12.Anthocyanins-613.22). In (*M. Todorova et all, 2018*) authors found the significant correlation coefficient between the anthocyanins content and antioxidant capacity for rose wine. The varying power of dependencies can be explained by the different technology of wine and juice production.

The color characteristics of the investigated juices have been measured in SIELab color system. The data for color parameters a and b and lightness L were presented on figure 5. The inulin does not lead to significant reduction of lightness L and color characteristics a and b. The addition of lactulose leads to decreasing of lightness L and increasing of color characteristics a and b.

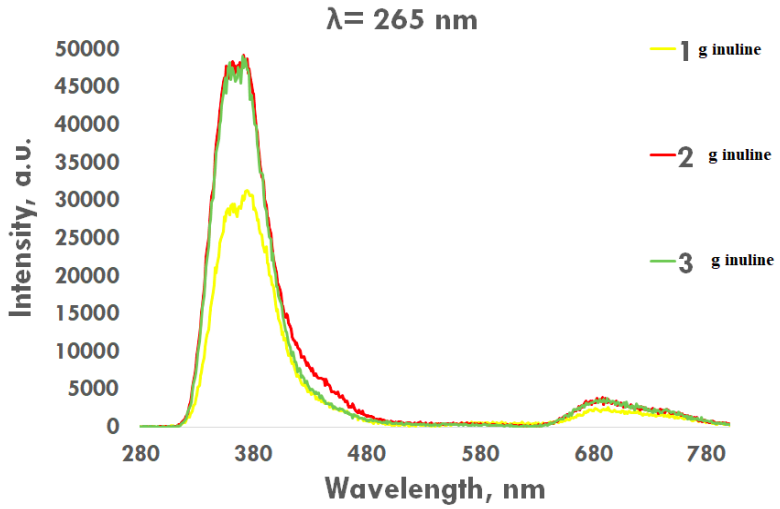

Figure 4 . Fluorescence spectra of dogwood juice with inulin

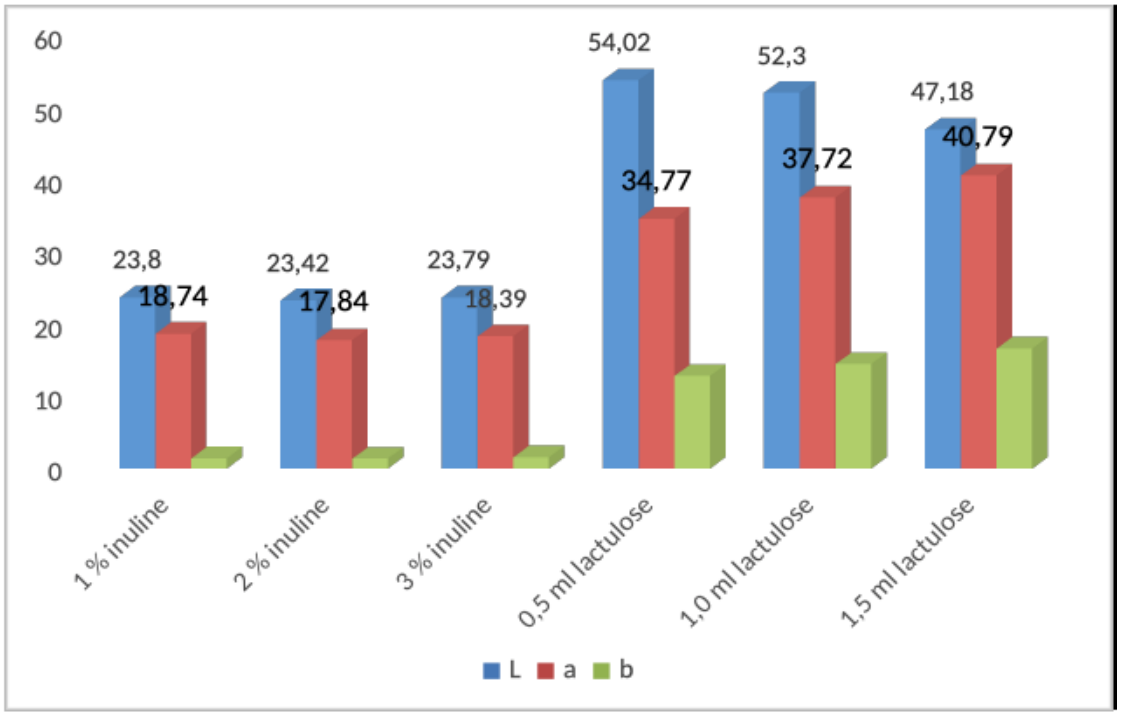

Figure 5 Color characteristics in SIELab color system for dogwood juice

Based on the sensory analysis it can be summarize that increasing the concentration of inulin leads to an increase in indicators sweet taste, smell of the dogwood juice and influences on the its consistency. We can recommend that a juice from dogwood should be prepared with 3% inulin. A dogwood juice concentration of 1.5 ml of lactulose leads to elevation of sweetener indicator, improves odor and consistency and decreases aftertaste. The results are presented on the figures 6 and 7.

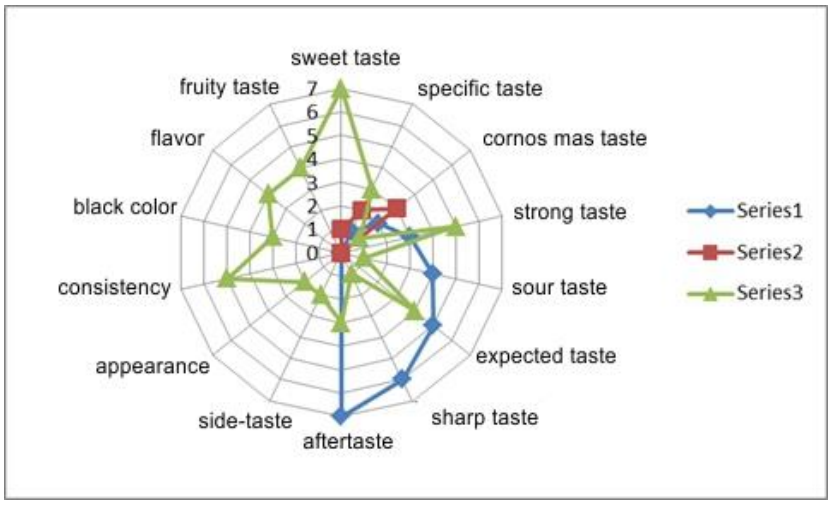

Figure 6. Sensory profile of Cornus mas juice with added inulin (1g, 2g and 3g)

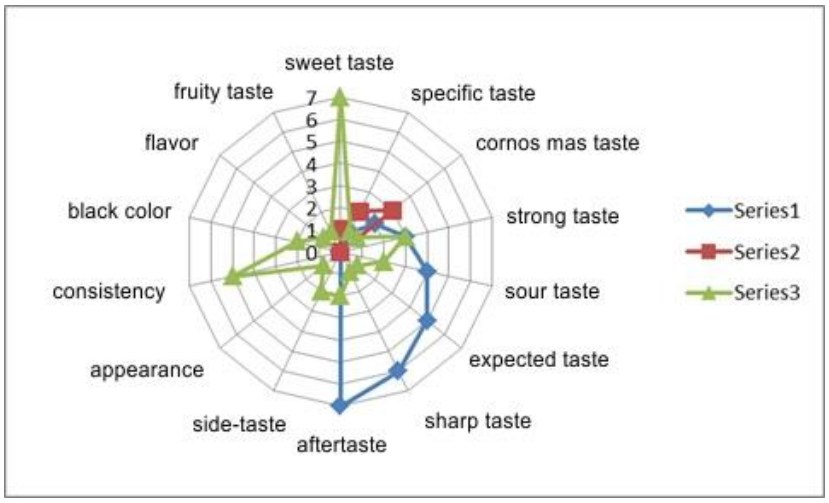

Figure 7 Sensory profile of Cornus mas juice with added lactulose (0.5 ml, 1 ml and 1.5 ml)

4. CONCLUSIONS:

These results suggest that the investigated juices contain a high content of a different group of polyphenols, which have a potent antioxidant capacity. The obtained data indicate that the juices having additional ingredients (natural polysaccharides with different concentrations) have slight variations in the extent of the observed antioxidant capacity. The linear dependencies between intensity of the first or the second fluorescence peak and antioxidant activity determined by DPPH are obtained.

ACKNOWLEDGEMENTS

№ 18001 "Explicit method for the study of the relation between optical properties and antioxidant effect of extracts of medicinal plants and beverages from traditional fruit for Bulgaria" (Competitive Session 2018 of the Scientific Research Fund at the Medical University - Varna).

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The effects of drying methods on the quality parameters of potatoes and red beetroot purée

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ABSTRACT

The aim of this study is to investigate the influence of conventional and foam mat drying methods on the quality parameters of a non-dairy solid probiotic product based on potatoes (*Solanum tuberosum* L.) and red beetroot (*Beta vulgaris* L.). The vegetable purée was used as fermentation substrate for the potentially probiotic *Lactobacillus delbrueckii* Lb 12 strain. All the purée samples were evaluated by the rheological, microstructural, microbiological and phytochemical properties point of view. The samples had a pseudoplastic behaviour fitted on two models: Herschel – Bulkley and Power law. Rheological low amplitude oscillatory measurements revealed a low resistance to the applied strain for fresh puree sample, while the reconstituted one showed to be stiffer (higher elastic and viscous moduli) and more strain resistant. In the case of fresh sample the strain value at G'/G" intersection registered 0.6% while the reconstituted one – 7.05%. The instrumental texture of the samples revealed that the samples obtained by foam mat drying were the most similar with the fresh purée. Confocal laser microscopy revealed vegetal tissues fragments with a rich content of bioactive compounds and lactic acid bacteria aggregated in biofilms. Colour parameters have not changed significantly. After 28 days of storage at 4°C, the total number of Lb 12 was 8.9 log CFU/g in the fresh purée and 8.04 log CFU/g in dried purée powder. The fresh potatoes with beetroot puree sample registered among of 14.83 mg β-xantine/g dw, 447.409 mg β-catotene/g dw and 299.593 g lycopene/g dw.

Keywords: *Lactobacillus delbrueckii*, potatoes, probiotic, purée, red beetroot

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Yeasts and moulds species evolving during production of traditional Beaten cheese

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ABSTRACT

The yeasts and moulds present in traditional ewe's milk beaten cheese produced in two different farmhouses were the object of survey in this study. The changes in their number during manufacturing and ripening were followed and the isolation and determination of the isolates were also made. After serial dilution with sterile physiological solution samples of milk and cheese were cultivated on YGC agar by incubation at 25˚C for 5 days. The isolated colonies were determinate based on their morphological and physiological characteristics. The yeasts were present during the whole process of cheese production and ripening. Their number in the milk used for cheese production in first and second farmhouse were 3.71-4.38 log cfu/ml and 4.13-4.47 log cfu/ml respectively. The yeasts were most present in beaten cheese in the samples collected during the dry salting (5.04-5.47 log cfu/g) and 10 days brining (5.09-5.31 log cfu/g) in the first farmhouse and during the dry ripening (4.77-5.04 log cfu/g) in the cheese samples collected from the second farmhouse. *Trichosporon pulullans, Debariomyces hansenii, Pichia polymorpha and Kluyveromyces lactis* were prevailing species in traditional beaten cheese. The moulds were not continually present in examined traditional cheese indicating of its additional contamination. The highest numbers of moulds were count in cheese samples collected from curd (4.69 log cfu/g), dry ripening (3.77 log cfu/g), dry salting (4 log cfu/g) and 10 days brining (4.17 log cfu/g). The prevaling species were determinate as *Aspergilus niger, Fusarium oxysporum, Alternaria tenuis, Curvularia lunata, Penicillium spinulosum.*

Key words: traditional beaten cheese, yeasts species, moulds species, dynamic of microorganisms

1. INTRODUCTION

Beaten cheese is a product with relatively good nutritional value used in the daily diet of the population in Macedonia. It can be produced industrially mostly from cow's milk by adding starter cultures, yet on the market traditional beaten cheese is also found produced from raw cow's as well as sheep's milk. Traditional beaten sheep cheese is produced almost on the entire territory of Macedonia, in small quantities in family farms or farmhouses, and it is mostly sold on the green markets or directly from these farms. It is semi-hard cheese that ripens and is kept in brine. It is yellowish and holes of different sizes and patterns can be noticed when cut. What is specific about it is its high concentration of salt. The production technology itself is also specific in the process of curd breaking in small pieces by means of a special device, this process being called "beating", thus providing the name of the cheese itself - Beaten cheese (Sulejmani *et al*., 2014a). The application of traditional tools and production technology, as well as the use of raw sheep's milk without the addition of starter cultures, combined with the characteristics of the geographical region and animal nutrition influence the production of cheese variations that differ in taste and aroma (Micari *et al*., 2007). The analyses of traditional Beaten cheese so far have been focused on physicochemical characteristics, proteolysis and volatile cheese components (Sulejmani *et al*., 2014a, 2014b), while analyses of Levkov *et al*., (2014, 2017) have been more focused on the microbiological characteristics of cheese, in particular, characteristics of lactic acid bacteria. More detailed data related to the presence of yeasts and moulds in traditional Beaten sheep cheese is not available. Traditional cheeses contain original (unique) microorganisms (Beresford, 2001) that develop and change during its ripening as a result of the change that occurs in terms of nutrients and environmental conditions (Wiliams *et al*., 2002). These microorganisms participate in a complex phenomenon called "ripening" composed of a wide range of biochemical reactions (Gardini *et al*., 2006). According to Beresford *et al.*, (2001) microorganisms in cheese are mainly divided into two main groups: starter lactic acid bacteria and secondary microorganisms. Yeasts play an important part in the group of secondary microorganisms. They develop well in spite of the unfavorable physical and chemical conditions generated during cheese ripening such as decrease in pH, reduction of moisture content, high salt concentration, low storage temperature (Lopandic *et al*., 2006). They can adapt well to the conditions in the cheese due to their ability to ferment and assimilate the lactose, as well as (assimilate) organic acids produced by the lactic acid bacteria. Additionally, they also have certain proteolytic and lipolytic abilities (Ferreira and Viljoen, 2003). Yeast proteases and lipases participate in the formation of some aromatic components in cheese, but yeasts if present in large quantity can cause spoilage of cheese because they participate in the formation of excess gas, unpleasant smell, as well as softening and changing cheese color (Gardini *et al.*, 2006). Moulds can be used as a starter culture in the production of certain types of cheese such as Roquefort, Blue, Stilton, Gorgonzola, Camembert, Brie, etc. Their role is to participate in the creation of precursors of aroma components in cheeses which contribute to the improvement and emphasis on their taste and smell as well as modification of their structure (Wouters *et al*., 2002). The presence of moulds in the cheese is often undesirable because they reduce its nutritional value and can be toxic due to the ability to produce mycotoxins which present a risk to human health (Hymery *et al*., 2014). During the production process of traditional cheese, the additional input of moulds is caused by cheese production tools, air, shelves and walls in the facilities where cheese is made, ripened and preserved (Basílico *et al*., 2002).

Since there is not much information about the dynamics and presence of yeasts and moulds in traditional Beaten sheep cheese, the aim of the research has been to determine the changes in their number during cheese production and ripening, as well as to isolate the yeasts and moulds species from different cheese production stages, and to identify them based on their morphological and physiological characteristics.

2. MATERIALS AND METHODS

2.1. Cheese sampling

The analyses were made on traditional Beaten cheese produced from raw ewe's milk in two different farmhouses (farmhouse 1 and farmhouse 2) situated in the village in the south region of N. Macedonia. The samples were taken within three successive cheese production processes (repetitions) in each farm (in total, 6 cheeses were analyzed). The cheese production technology was similar in both farmhouses. Samples of milk, curd, curd preheating and after draining, during dry ripening and dry salting and after 10, 30 and 45 days brining they were taken aseptically and kept under refrigerated condition till the analytical process. Yeasts and moulds analyses were performed within 24 hours after sampling.

2.2 Yeasts and moulds content and isolation

Aliquot of 10 ml of milk or 10 g of curd and cheese samples were taken. Serials of decimal dilutions of milk were made with a sterile physiological solution. Cheese samples were homogenized with 90 ml of sterile 2% sodium-citrate solution and then decimal dilutions with sterile physiological solution were made. Yeast extract Glukose Chloramphenicol agar (YGC agar) was inoculated with prepared samples and incubated under aerobic conditions at 25˚C for 5 days. Isolates of yeasts and moulds were obtained by random picking from YGC agar plates and were purified by consecutive subculturing on YGC agar. Purified isolates were kept on Malt Extract Agar and subcultured in the interval of one month. Determination of yeasts was made according to their morphological characteristics (appearance and color of colonies, appearance of cells under microscope and their size), the way of reproduction, and capability of spore formation as well as their physiological abilities such as fermentation and assimilation of glucose, galactose, lactose maltose and sucrose ability to form pellicle, ring or sediment. Identification was made according to the Lodder ând Kreger-van Rij, (1952) and Kreger-van Rij (1984).

Morphological characteristics of moulds (appearance and color of colonies) and morphology of the vegetative mycelium were analyzed by slide observation under the microscope Nikon E 80. Determination of moulds was performed according to the (Gilman, 1957) and (Funder**,** 1953)

3. RESULTS AND DISCUSSION

The analyses of Beaten sheep cheese have shown the presence of yeasts throughout the entire production process in both variants. Their presence is noted earlier in the phase, in the milk from which the cheeses are made (Fig.1 and Fig.2), being present in smaller number in the farmhouse 1 (3.7-4.4 log cfu/ml) as compared with the milk from the farmhouse 2 (4.1-4.4 log cfu /ml). From the graphs it can be noted that the number of yeasts in the stages of cheese production is not dramatically changed. An increase in the number of yeasts is noticed in the cheese samples taken after dry salting from the farmhouse 1, as for the number of yeasts in the farmhouse 2, it is the biggest in the phase of dry ripening. After the dry salting phase, the number decreases for one logarithmic unit compared to the cheese produced in the farmhouse 2.

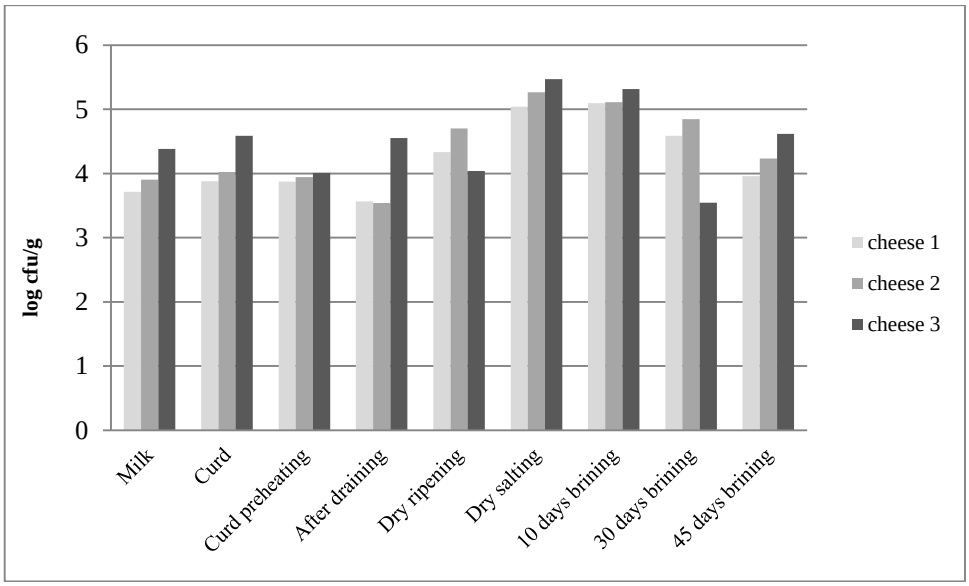

Figure 1. Growth of yeasts during Beaten cheese production and ripening in the farmhouse 1

During brining, the number of yeasts gradually decreases and after 45 days brining of the cheese from farmhouse 1, it ranges within the limits of 3.9-4.6 log cfu/g, while in the cheese produced in the farmhouse 2 the number ranges from 2.4-3.5 log cfu/g. The obtained results are in accordance with the studies of Kołakowski *et al*., (2012), Padilla *et al.*, (2014), but the number of yeasts in the milk for the production of beaten cheese is higher than that compared to the milk used to produce Pecorino Crotonese cheese (1.07 log cfu/g) (Gardini *et al*., 2006). Similarities in the yeasts dynamics of Beaten cheese are observed in relation to the results of Özdemir *et al*., (2010).

The presence of yeasts and moulds at the beginning of ripening of Karin Kaymagi cheese was 3.60-5.54 log cfu/g while in the final product was 4.81-6.53 log cfu/g. Peščić-Mikulec and Jovanović (2005) detected high proportion of yeasts in Serbian white traditional cheese as well. In the Italian sheep cheese Fiore Sardo, Di Cagno *et al.*, (2003) found the presence of the yeasts amounting to 10^4cfu/g. The increase in the number of yeasts during the cheese ripening (Fig. 1 and Fig. 2) can occur as a result of higher temperature (25°C) in the facilities where cheese is produced and ripened. They can be further added to the milk or cheese because they are widely spread in the premises where cheese is ripened, on the production surfaces, in the ripening equipment, even in the air (Cardoso *et al*., 2015). But it can also be additionally added through the brine where cheese is kept and ripened (Kołakowski *et al*., 2012). The higher number of yeasts especially in the initial stage of cheese maturing occurs probably as a result of the interaction with lactic acid bacteria, which reaches a maximum level in this production period (Levkov *et al*., 2014). Yeasts have a positive effect on the ripening process by stimulating the growth of the lactic acid bacteria through the ability to synthesize vitamins and amino acids (Fereirra and Viljoen, 2003). According to the authors, lactic acid bacteria with their metabolism enable the creation of organic acids used by the yeasts.

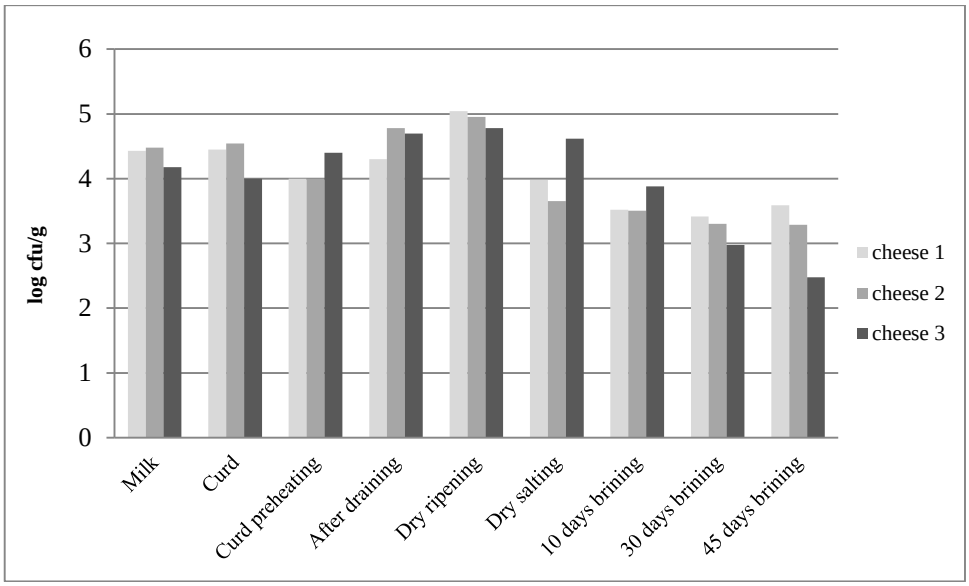

Figure 2. Growth of yeasts during Beaten cheese production and ripening in the farmhouse 2

Although yeasts are able to survive in restrictive conditions existing in Beaten cheese, after 45 days of the ripening process high salt concentration (3.8±0.1%-7.1±0.2%) (Levkov *et al*., 2014) affects the decrease of yeasts. This process is especially noticeable in the cheese of the farmhouse 2 (Fig. 2). Gardini *et al*., (2006) indicates that high concentrations of NaCl can influence the metabolytic activities of yeasts as well as species succession.

The types of yeast isolated from the Beaten cheese produced in both farmhouses are very similar, but the cheeses produced in farmhouse contains slightly more different yeast species. Out of all production phases of beaten cheese made in farmhouse 1 there were a total of 98 yeast isolates, while from cheese samples collected from farmhouse 2, 76 isolates were allocated. Isolates of cheese samples from farmhouse 1are determined as: *Torulopsis famata* (2%), *Trichosporon pulullans* (40.8%), *Debariomyces hansenii* (9.2%), *Torulopsis candida* (7.1%), *Kluyveromyces lactis* (4.1%), *Rhodotorula minutes* (3.1%), *Pichia polymorpha* (3.1%), *Trichosporon* sp. (23.5%), *Oosporidium sp*. (7.1%). Isolates of the yeasts of beaten cheese from the three production batches of farmhouse 2 according to the morphological and physiological characteristics are determined as: *Trichosporon pulullans* (26.3%), *Pichia polymorpha* (11.8%), *Rhodotorula glutinis* (5.3%), *Rhodotorula minute* (2.6%), *Rhodotorula rubra* (2.6%), *Kluyveromyces lactis* (3.9%), *Kluyveromyces marxianus* (1.3%), *Debariomyces hansenii*, *Trichosporon cutaneum* (3.9%), *Torulopsis candida* (3.9%), *Candida intermedia* (3.9%), *Trichosporon* sp (28.9%). It is noticeable that the genus *Trichosporon* is dominant, especially with the type *Trichosporon pullulans*. These species are present in all stages, starting from the process of milk production to the phase of delivering the final product. Representatives of the genera *Torulopsis, Debariomyces, Oosporidum* were found in milk, coagulum, in the ripening of the cheese dough and during salting. During the brining process, the representatives of following genera were isolated: *Trichosporon, Torulopsis, Debariomyces, Candida, Kluyveromyces* and *Pichia*. The species *Trichosporon pullulans, P. polimorpha, Rhodotorula minuta, Rh. glutinis, Rh. rubra* were isolated from the brine.

Some of defined types of yeasts are also isolated from other types of traditional cheeses according to the researches of Freitas and Malcata (2000), Ouadghri *et al*., (2014), Cardoso *et al*., (2015). Most probably the yeast isolated from the traditional Beaten cheese has a significant role in the formation of its taste and smell, which indicates their constant presence in milk and cheese in the whole

processing and ripening stages. The species such as *K. lactis*, *D. hansenii*, *S. cerevisiae* are capable of fermenting lactose and galactose, and can also utilize more carbon and nitrogen sources and produce alcoholic, acidic and cheesy flavor (Gardini *et al*., 2006). The influence of yeasts on the ripening of Beaten cheese should be additionally determined by examining the proteolytic and lipolytic abilities of the isolated species. Studies by Wojtatowicz *et al*., (2001), Gardini *et al*., (2006), Ouadghri *et al*., (2014) show that yeasts have different abilities to participate in cheese ripening due to differences in their ability to break down cheese proteins and fats which depends on the activity of the intra and the extracellular enzymes. It is thought that species with extracellular proteolytic enzymes contribute to proteolysis of cheese (Gardini *et al*., 2006). In the Beaten cheese made in both farmhouses, the presence of fungi is not continuous during the preparation and ripening of cheese. The results of the research show that in the samples taken from the initial stage of production such as milk, curd and after curd heating, only in the second and the third repetition moulds were noticed in the quantity from 2.3 to 2.6 log cfu/ml and 2.0-2.3 log cfu/g respectively (Fig. 3). In the samples taken from the farmhouse 2, the highest number of molds was observed in the curd from the first repetition of 4.7 log cfu/g (Fig. 4).

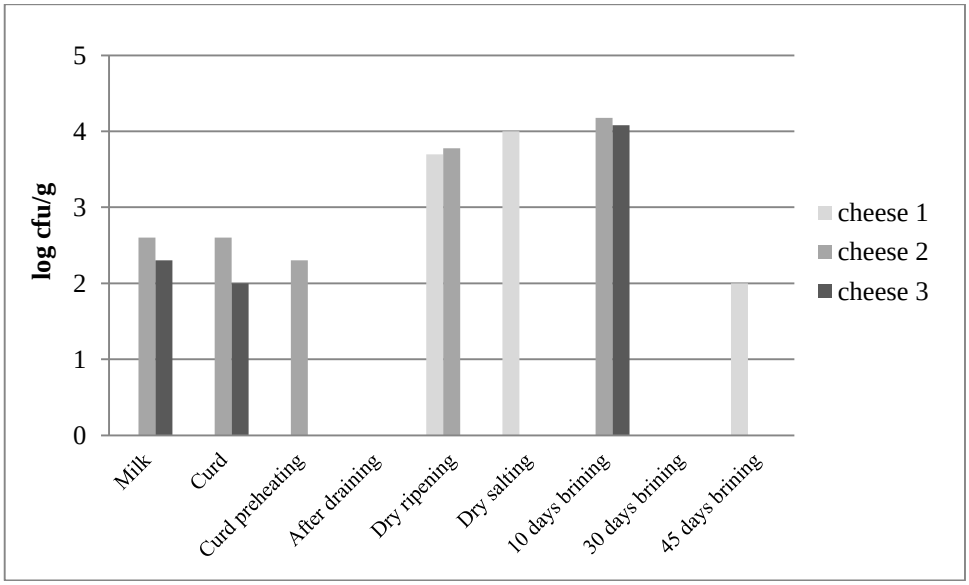

Figure 3. Growth of moulds during Beaten cheese production and ripening in the farmhouse 1

More specifically, moulds' spores can penetrate into the milk, the coagulum and the cheese dough through the air from the place where the milking process takes place, and also where cheese is produced and ripened. Also, the milk tanks, the wooden tools for breaking milk coagulum as well as the wooden inventory in the premises for production and ripening of cheese, the walls of the premises, all afore mentioned are natural habitats of fungi and their spores (Banjara *et al*., 2015)

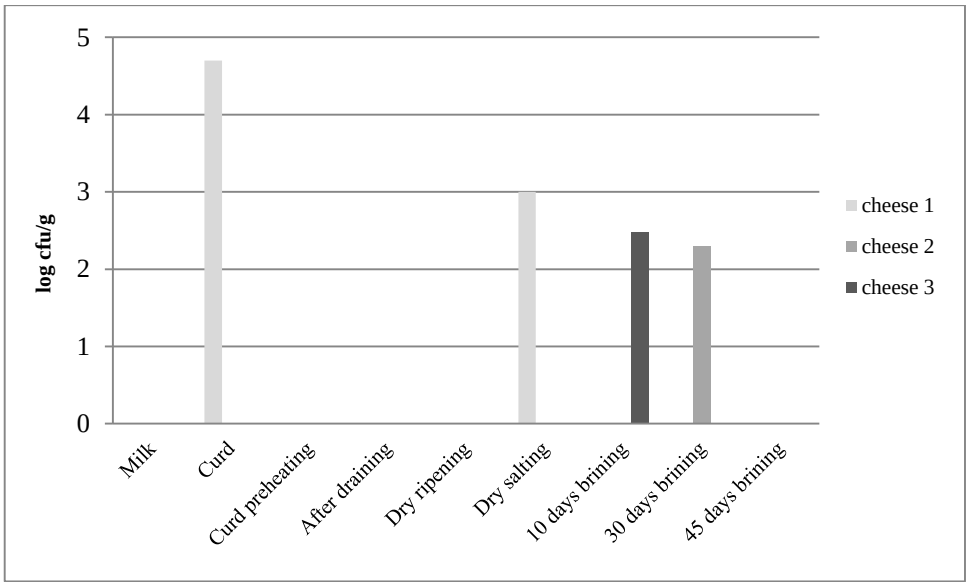

Figure 4. Growth of moulds during Beaten cheese production and ripening in the farmhouse 2

From the cheese of the two farmhouses, a total of 11 isolates of fungi were defined: *Aspergilus niger* (18.2%), *Fusarium oxysporum* (36.4%); *Alternaria tenuis* (9.1%); *Curvularia lunata* (9.1%); *Penicillium griseofulvum* (9.1%)*; Penicillium spinulosum* (9.1%).

The presence of moulds during ripening and salting indicates their adaptability to low pH, high concentration of salt and high concentration of fat (Fox *et al*., 2000, Basílico *et al*., 2001). The number of fungi in both variants of beaten cheese was similar to the number of fungi in the Karin Kaymagi cheese (Özdemir *et al*., 2010). In the traditional Montasio cheese, the moulds were present during the whole cheese production process (Marino *et al*., 2003). The authors believe that they inhabit the walls and shelves where cheese is kept. Their proper and regular cleaning can cause mould removal and growth control. Freitas and Malcata (2000) in the Idiazábal cheese found the presence of mould of the genus of: *Penicillium, Cephalosporium, Aspergillus, Geotrichum, Pullularia, Mucor, Candida, Acremonium*. In the Roncal cheese, existing moulds belonged to the following nine genera: *Penicillium, Cephalosporium, Aspergillus, Geotrichum, Pullularia, Mucor, Paecilomyces, Candida, Acremonium*. Kure and Skaar (2000) isolated and identified visible moulds of two types of Norwegian semi-hard cheese Jarlsberg and Norvegia. In both cheeses, the most commonly isolated species was *P. roqueforti ssp. roqueforti*. In addition, 69.8% of the isolates belonged to the genus *Penicillium*, as follows: *P. solitum, P. commune, P. palitans*. Of the two types of cheese, other types of fungi belonging to the following genus were also isolated: *Alternaria, Aureobasidium, Cladosporium, Epicoccum, Mucor, Geotrichum, Phoma* and *Ulocladium*.

During the research, the moulds were not noticeable on the surface of the cheese samples. However, isolated species of *Aspergillus, Penicillium, Fusarium* are known producers of mycotoxins that can be dangerous for human health. Cheese contamination with mycotoxins is most commonly due to the use of contaminated milk for cheese preparation or less frequently as a result of the growth of fungi that produce mycotoxins Hymery *et al*., (2014), Banjara *et al*., (2015). Although mycotoxogenic moulds are isolated from traditional beaten cheese, they do not pose a threat to the health of people who consume cheese, as the synthesis of mycotoxins in moulds differs, that is, not every species is capable to produce those (Hymery *et al*. 2014). However, it is necessary to take greater care in reference to hygiene in the locations where milking takes place, as well as in places where milk is processed and produced into cheese. Regular cleaning and sanitation is also required with appropriate devices for such purposes.

4. CONCLUSION

Yeasts isolated from beaten cheese and their continuous presence in all stages of production indicate their significant role in the process of cheese ripening and formation of its taste and smell, especially because of the dominance of *Trichosporon pullulans, Debariomyces hansenii, Kluyveromyces lactis* that might create precursors of aromatic compounds. The difference in the dynamics and species composition between the cheeses produced in the two farmhouses was expected, since for the most part yeasts originate from the equipment and the environment in which cheese is produced. However, further researches need to be conducted in order to determine the proteolytic and lipolytic properties of the isolated species. Moulds on the other hand, are result of cheese contamination from the environment where it is produced and ripened. In order to maintain good quality of cheese, regular hygiene and sanitation of the equipment, devices and premises where cheese is prepared is one of the top priorities.

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New Sugar-Free Product on the Basis of Almond Flour and Pumpkin Pulp

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ABSTRACT

The effect of almond flour on rheological, textural properties and bioactive compounds of a new roulade with pumpkin pulp were evaluated. The rheological behavior of tested dough's showed a rather good structural regeneration during forced flow for almond based dough similar to the control. Also the low amplitude oscillatory tests revealed a stiffer structure for the almond based dough and a high resistance to the applied strain for the control. When simulating temperature treatment with quasi-static temperature ramp, it was observed that although almond based dough presented a stiffer structure al low temperatures, after macromolecules denaturation and gel formation the G` values for the almond based sample was significantly lower in comparison to control sample. Also during the same test it was observed a higher dough extension (gap increase) in the gelatinization temperature range for the almond based dough. Firmness, adhesiveness, resilience, gumminess and chewiness were determined from the stress-displacement curve. Textural analysis revealed firmer texture for dough's with added almond flour, before and after baking as well. Total carotenoids and β-carotene content were 44.296 mg/g dw and 40.9 mg/g dw, respectively.

Keywords: carotenoids, pumpkin pulp, rheological and textural properties, sugar free product

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Safety of phytomedicines for pregnant women marketed in the region of Tlemcen (northwestern of Algeria)

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Herbal medicine is an increasingly popular therapy for the treatment of various disorders of the pregnant woman. It is perceived by the general public as a medicine without any risk even though it is not. This work aims to listing phyto-medicines provided for pregnant women in the region of Tlemcen in Algeria and verifying their safety of use.

A survey was conducted in 8 pharmacies and non-pharmaceutical chemist's in the region of Tlemcen. Then, a bibliographical research was made to collect informations about the listed phymedicines and their safety for the pregnant woman [1] [2].

This survey allowed us to inventory 65 phyto-medicine of which the comparison of the data of the note with the bibliographical data showed that 12 % of them can be used without risks by the pregnant women, 25% are not recommended and contraindicated and 15 % are lacking information about their gestational use.

The obtained results helped us supply a support to every pregnant woman, wishing to heal herself by phytomedicines. These results were alarming what requires establishing rigorous regulations to confer to pregnant women a safety use of natural products protecting them from the slightest danger.

Key words: Phytomedicine, phytotherapy, pregnancy, Tlemcen,.

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Phytochemical composition of black cumin (*Nigella sativa* L.) seeds from Turkey as an unconventional source for the food industry

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ABSTRACT

Phytochemical composition of black cumin seeds (*Nigella sativa L.*) of Turkish origin was studied to evaluate their potential as a source of biologically active components in foods thus establishing a scientific basis for the development of innovative foods, nutraceuticals, and pharmaceuticals. The seeds of *Nigella sativa L.* were characterized (on a dry-weight basis) by the content of crude fat (37.00%), essential oil (0.59%), crude protein (22.10%), fiber (4.20%), and total carbohydrates (31.30%). The main constituents of the essential oil were *p*-cymene (26.01%), thymoquinone (21.09%), *α*-thujene (9.92%), thymol (8.42%), and *γ*-terpinene (5.16%). The unsaturated fatty acids of *Nigella sativa L.* seeds were linoleic acid (43.43%) andoleic acid (21.56%). Palmitic acid (9.62%) represented the major part of the saturated fatty acids. Arginine (20.12 g/100 g protein), glutamic acid (16.25 g/100 g protein) and leucine (10.01 g/100 g protein) were the major amino acids. Cystine was the minor amino acid (0.42 g/100 g protein). Black cumin seeds contained vitamins (3358 µg/100g), including niacin (2323 µg/100 g). The predominant minerals were Ca (3540.00 µg/g), K (3265.00 µg/g), and P (2234.01 µg/g). The information is a valuable source for diversification of foods and adding a functional aspect to them.

Keywords: Turkish *Nigella sativa* L, phytochemical composition.

1. INTRODUCTION

Nigella sativa L., known as black cumin, is an annual herbaceous plant belonging to Ranunculaceae family. The plant originates from the Mediterranean region but also grows in many countries of Europe and Asia (Saleh et al., 2018). *N. sativa* has many biological effects as anti-inflammatory, antihyperlipidemic, and displays antimicrobial, anticancer, antioxidant, antidiabetic, antihypertensive effects as well as benefits on the reproductive, digestive, immune and central nervous system such as anticonvulsive and analgesic activity (Ermumcu and Şanlıer, 2017; Toma et al., 2010; Khan et al., 2011; Kooti et al., 2016; Sharma et al., 2009).

According to Takruri and Dameh (1998), proteins, fats, dietary fiber, iron, zinc, phosphorus, calcium, thiamin, cyanine, folic acid, isoquinoline alkaloids, nigelin, and nigelinin are involved in the composition of *N. sativa* seeds. Other constituents of the seeds are saponins, flavonoids, alkaloids, and others (Sharma et al., 2009; Randhawa and Alghamdi, 2011; Mendi, 2018).

The seeds of *N. sativa* contain essential oil (from 0.5 to 1.5%), with the main constituents thymoquinone, *p*-cymene, *α*-thujene, *α*-pinene and others, depending on the origin of the raw material (Akram, 1999; Damianova et al., 2003; Mozaffari et al., 2000; Stoyanova et al., 2003; Kaskoos, 2011). The essential oil obtained from these seeds has proven antimicrobial activity (Bourrel et al., 1995a, b, Damianova et al., 2003, Babayand et al., 2006). For example, the total extracts and essential oil of *Nigella Sativa* L. seeds display an inhibitory effect, up to 100% depending on the used concentration, on pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Hosseinzadeh et al., 2007). The crude extract of *Nigella Sativa* L. seeds on multiple antibiotic-resistant bacteria has been studied by Morsi (2000) and found to be effective. Also antifungal and an inhibitory effect on the production of aflatoxins produced by different species of *Aspergillus* have been noted (El-Nagerabia et al., 2012).

The amount of vegetable fat in the seeds is up to 35%, the major fatty acids are linoleic, oleic, and palmitic acids (Abdel-Aal and Attia, 1993; Atta, 2003; Perifanova-Nemska et al., 2002; Ustun et al. 1990). According to Khan et al. (2011), the seeds of *N. sativa* L. are a source of phenolic compounds that are at high levels, defining their pharmaceutical, functional (antioxidative and antibacterial), and technological potential.

The seeds have a protein content of up to 27%, about 20% cellulose and other substances (Abdel-Aal and Attia, 1993; Al-Gaby et al., 1998). The characteristic aroma of these seeds and the slightly spicy flavor of the spice determines the wide use in various culinary and confectionery products (Takruri and Dameh, 1998). A good understanding of the biologically active profile of a number of the natural raw product, as well as their aromatic and taste properties, determine the significance of their use.

Due to their potential application in a variety of functional foods, the objective of the study was to determine the phytochemical composition of *N. sativa* L. seeds from the plants growing under the environmental conditions of Turkey (i.e. soil composition, specific regional weather conditions, etc.).

2. MATERIAL AND METHODS

2.1. Plant Material

N. sativa seeds were purchased from local markets in Mersin, Turkey, and then stored in a cool, dark cabinet (at 5 - 7 ºC). The total moisture content of the seeds (5.20% ± 0.15) was determined by the gravimetric method using a drying oven at 105 ± 2 °C. The biologically active substances in the samples were analyzed and the values were represented on the basis of absolute dry weight.

The absolute weight of 1000 randomly selected seeds was measured using an electronic precision

balance (Denver Instrument). The seeds were finely ground using a laboratory mill (Retsch SK 100 Grinder) into particles of 0.5-1.0 mm size.

2.2. Obtaining of the essential oil

15g of black cumin seeds was ground (0.5 – 1.0 mm) in a laboratory mill (IKA 11 basic, Retsch SK 100). The essential oil was isolated by hydrodistillation for 3 h in a Clevenger apparatus. The extracted oil was further dried using anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis. The essential oil yields are expressed on an absolute dry weight basis.

2.3. Fatty acid composition

Fatty acid methyl esters were obtained by hydrolysis using a 2 M methanolic potassium hydroxide solution, and extraction with n-heptane, in accordance with ISO 5509 method (ISO 5509:2000) and following a procedure described in previous work (Barreira et al., 2009). The fatty acids profile was evaluated using a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, a flame ionization detector (FID), and a Chrompack CP-9050 auto-sampler. The separation was performed using a 50 m x 0.25 mm i.d. fused silica capillary column coated with a 0.19 μm film of CP-Sil 88 (Chrompack). Helium was used as carrier gas at an internal pressure of 120 kPa. The results are expressed as the relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area, and assuming that the detector response was the same for all compounds.

2.4. Gas chromatography-Mass spectrometry (GC-MS) analysis of the essential oil

GC-MS analyses were performed using an Agilent Technologies 6890 N Network GC System equipped with a DB-Wax capillary column (60 m x 0.25 mm x 0.25 μm) and DB-23 capillary column (60 m x 0.25 mm x 0.25 μm) and interfaced with an Agilent 5973 Network Mass Selective Detector. The oven temperature was programmed at 165 °C at a rate of 5 °C/min and held at 165 °C for 10 min, then programmed to 190 °C at a rate of 5 °C/min and held at 190 °C for 55 min. The split ratio was 30:1; transfer line temperature was 280 °C; ion source temperature was 210 °C; carrier gas helium at a linear velocity of 1.5 mL/min; ionization energy 70eV; scan range 15-550 am. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

2.5. Proximate analysis of the seeds

Nigella sativa seeds were analyzed for their crude fat, crude protein, dietary fiber, and total carbohydrates. The total nitrogen was analyzed using the Kjeldahl method, and crude protein content was calculated using a nitrogen conversion factor of 5.30 (AOAC, 2000).
Total crude fat was determined after extraction with diethyl ether for 16 h in a Soxhlet apparatus (AOAC, 2000). The dinitrophenol method was utilized in the analysis of total carbohydrates (Ross, 1959) using a spectrophotometer. The total dietary fibers were determined (AOAC, 2000).

2.6. Amino acids composition

The protein was hydrolyzed to free amino acids as 300 mg of dried seeds were placed in a glass ampule with a 5 mL 6N hydrochloric acid solution. The ampule was thoroughly sealed and left in a drying chamber at 105 °C for 24 h. The ampule content was then transferred to a crystallizer and dried in a vacuum chamber at 40-50 °C. After evaporation of the water, the residue was fully diluted

in 10 mL 20 mM hydrochloric acid. The solution was filtered through a paper filter and 20 µL of the collected filtrate was derivatized with an AccQ-Fluor kit (WATO52880, Waters Corporation, USA). Initially, 60 µL of AccQ-Fluor borate buffer was added to the filtrate and homogenized. Then, 20 µL of AccQ-Fluor reagent was added and the sample was homogenized again for 30 s. Before injection, the solution was heated in a water bath at 55 °C. The resulting AccQ-Fluor amino acid derivatives were separated by an ELITE LaChrome high-performance liquid chromatography (HPLC) (Hitachi) equipped with a diode array detector (DAD) and a reverse phase column C18 AccQ-Tag (3.9 mm x 150 mm) operating at 37 °C. The volume of the injected sample was 20 µL and the elution was made with a gradient system of two mobile phases: A – buffer (WATO52890, Waters) and B – 60% acetonitrile. Amino acids were detected at 254 nm. Subsequently, the chemical score was calculated. Subsequently, the chemical score was counted, based on the FAO (1985) pattern (threonine = 3.4; valine = 3.5; leucine = 6.6; isoleucine = 2.8; tyrosine + phenylalanine = 6.3; lysine = 5.8) (FAO/WHO, 1991).

2.7. Determination of mineral composition

Macro and micro minerals were determined according to a validated laboratory method using microwave mineralization of 1 g plant material with 3 mL of 0.2% HNO_3 and 2 to 3 mL H_2O_2. The mineralized sample was filtered and analyzed by ICP-OES SPECTROFLAME (Spectro Analytical Instruments) with a monochromator, linear range from 165 to 440nm, Nebulizer type Minhard, coolant gas- 42 bar, auxiliary gas - 26 bar. The measured intensities were compared with the intensity of a series of standard solutions.

2.8. Determination of vitamins

The water-soluble vitamins from the B group were extracted according to a previously described method (AOAC, 1990). The chromatographic system was equipped with a Shimadzu HPLC and photodiode array detector. Supelcosil LC 18 DB column (250 mm × 4.6 mm, 5 µm; Sigma, USA) were used for separation of vitamins).

2.9.Statistical analysis

The measurements were performed in triplicate and the results were presented as the mean value of the individual measurements with the corresponding standard deviation (SD), using Microsoft Excel.

3. RESULTS AND DISCUSSION

The results for the *N. sativa* seed composition of the essential oil (0.59% ± 0.01), crude fat (37.00% ± 0.04), crude protein (22.10% ± 0.41), crude fiber (4.20% ± 0.15), and carbohydrate (31.30% ± 0.02), are similar with the results reported earlier (Guler et al., 2006; Matthaus and Özcan, 2011; Stoyanova et al., 2003).

Table 1 shows the fatty acids profile of *N. sativa* seed oil. Eleven fatty acids are identified, which accounts for 100% of the seed oil. The ratio of unsaturated and saturated fatty acids is 74.59: 25.51 and the ratio of monounsaturated fatty acids to polyunsaturated fatty acids is 24.76: 52.83.

Table 1. Fatty acid composition of *Nigella sativa* L. seeds oil

	Fatty acids	Content, %
C $_{12:0}$	Lauric acid	6.53 ± 0.01
C $_{14:0}$	Myristic acid	1.18 ± 0.00
C $_{16:0}$	Palmitic acid	9.62 ± 0.02
C $_{18:0}$	Stearic acid	8.18 ± 0.03
C $_{18:1}$	Oleic acid	21.56 ± 0.03
C $_{18:2}$	Linoleic acid	43.43 ± 0.04
C $_{18:3}$	Linolenic acid	5.38 ± 0.01
C $_{20:2}$	Eicosadienoic acid	3.52 ± 0.03
C $_{16:1}$	Palmitoleic acid	0.20 ± 0.00
C $_{20:4}$	Arachidonic acid	0.30 ± 0.00
C $_{20:2}$	Eicosadienoic acid	0.10 ± 0.00

The results show that the major portion of the fatty acids is represented by linoleic (43.43%), oleic (21.56%), palmitic (9.62%), and lauric (6.53%), therefore the seed oil is considered as linoleic-oleic type. The obtained data is comparable to that obtained by other researchers (Nergiz and Otles, 1992; Perifanova-Nemska et al., 2002; Khan et al., 2011; Kaskoos, 2011) but it is in contrast with the data presented by Ustun et al. (1990) for oleic-linoleic type seed oil. The results showed that the content of linoleic acid in *N. sativa* from Turkey (43.43%) was lower than that of Morocco (58.5%) for oleic acid (23.8%) and palmitic acid (13.1%) as reported by Gharby et al. (2015).

Comparing our data with the data existing in the literature, it can be noted that there are differences in the quantities of the specified substances; these differences can be explained by the origin of the raw material, the conditions for its cultivation and the method of processing, as well as the methodologies for conducting the analyzes.

The chemical composition of *N. sativa* essential oil constituents is presented in Table 2. Fourteen essential oil constituents, representing 83.81% of the total essential oil, were identified: 9 were in concentrations over 1% and the other 5 constituents represented less than 1% of total essential oil (Table 2). The main constituent was *p*-cymene (26.01%), whose values are higher than previously reported (Khan et al., 2011). The content of thymoquinone (21.09%) was significantly higher than that reported by other researchers (8.7%) (Khan et al., 2011; Salem, 2001). The values of α-thujene (9.92%), thymol (8.42%), and γ-terpinene (5.16%) are comparable to those reported by Khan et al. (2011).

Table 2. Chemical composition of *Nigella sativa* L. seeds essential oil

Compound	RI	Content,%
α-Thujene	915	9.92 ± 0.09
α-Pinene	923	2.28 ± 0.01
Sabinene	947	2.18 ± 0.01
β-Pinene	958	4.32 ± 0.03
β-Myrcene	974	0.61 ± 0.00
α-Phellandrene	983	0.76 ± 0.00
Limonene	991	0.71 ± 0.00
p-Cymene	1005	26.01 ± 0.25
γ-Terpinene	1009	5.16 ± 0.04
Terpinolene	1014	0.23 ± 0.00
Carvone	1078	0.32 ± 0.00
Thymoquinone	1088	21.09 ± 0.19
Thymol	1185	8.42 ± 0.08
Carvacrol	1232	1.80 ± 0.01

Monoterpene hydrocarbons, phenylpropanoid hydrocarbons, and oxygenated monoterpenes were the dominant group in the essential oil (Figure 1), followed by oxygenated phenylpropanoids.

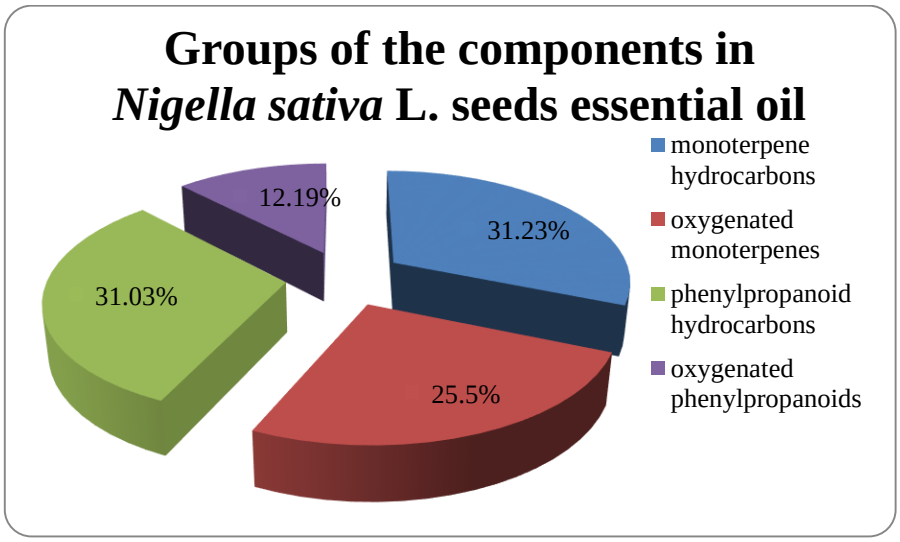

Figure 1. Groups of the components in *Nigella sativa* L. essential oil

The noted differences between the quantities of the identified constituents, as well as in the groups of compounds identified in our study, and data reported in the published literature could be explained by the origin of the raw material and Turkey's particularities in the climatic, soil and growing conditions.

The amino acid composition of the protein fraction and the calculated chemical score (Table 3) indicate that the seeds of *N. sativa* from Turkey stand out with significantly higher amino acid levels of glutamine (16.27 to 7.78%), proline (6.31% to 1.58%), alanine (2.05% to 1.47%), leucine (10.01% to 2.04%), and arginine (20.12% to 2.86%) compared to those reported by Michel et al. (2010).

Table 3. Amino acid composition of *Nigella sativa* L. seeds protein fraction

Amino acids	Content (g/100 g protein)	Chemical score
Asp	3.29 ± 0.02	
Ser	1.72 ± 0.21	
Glu	16.25 ± 0.12	
Gly	4.18 ± 0.05	
His	0.52 ± 0.02	
Arg	20.12 ± 0.07	
Thr	0.60 ± 0.14	5.66
Ala	2.05 ± 0.11	
Pro	6.31 ± 0.12	
Cys	0.42 ± 0.04	
Tyr	5.92 ± 0.02	
Val	3.14 ± 0.05	1.11
Met	5.55 ± 0.04	
Lys	7.12 ± 0.02	0.81
Ile	7.09 ± 0.01	0.39
Leu	10.01 ± 0.02	0.66
Phe	6.51 ± 0.01	0.51

* tyrosine + phenylalanine

Only the reported quantities of asparagine (3.07%), serine (1.31%), cysteine (0.75%) by Michel et al. (2010) was comparable to those obtained in our study. *N. sativa* seeds originating from Turkey are a source of niacin (2323 µg/100 g), and the macroelements Ca (3540.00 µg/g), K (3265.00 µg/g) and P (2234.01 µg/g) were the predominant minerals (Table 4).

Table 4. *Nigella sativa* seeds vitamin and mineral content

Vitamins, µg/100g	
B1 (Thiamin)	735.00 ± 0.01
B2 (Riboflavin)	42.00 ± 0.00
B6 (Pyridoxine)	253.00 ± 0.00
PP (Niacin)	2323.00 ± 0.01
Folate	5.00 ± 0.00
Minerals, µg/g	
Na	578.71 ± 0.01
Mg	1057.12 ± 0.01
Al	35.12 ± 0.01
P	2234.01 ± 0.01
K	3265.00 ± 0.01
Ca	3540.00 ± 0.01
Mn	19.96 ± 0.01
Fe	95.42 ± 0.01
Zn	86.87 ± 0.01
Cu	8.61 ± 0.01
Se	1.21 ± 0.01

Our data indicate that the predominant elements are Ca (3540.00 µg/g), followed by K (3265.00 µg/g),

P (2234.01 μg/g) and Mg (1057.12 μg/ g) whereas the reported results by Sultan et al. (2009) are lower for K (808.00 μg/g), Ca (570.00 μg/g) P (543.00 μg/g) and Mg (265.00 μg/g).

4. CONCLUSION

The results obtained in our study show that the main constituent in the black cumin seeds from the Mersin area of Turkey was *p*-cymene, followed by thymoquinone that was significantly higher than other black cumin samples originating from different countries. Both *p*-cymene and thymoquinone display, apart of antioxidant and antimicrobial properties, anti-cancer properties a fact which make the black cumin seeds from Turkey (Mersin area) a valuable natural resource for the pharmaceutical industry. Moreover, the minerals Ca, K, P, and Mg are the predominant in these seeds, thus giving them the best potential to be used in foods with functional properties.
Based on the identified specific composition of the *N. sativa* seeds from Turkey, this study provides information on the composition particularities of these seeds and the possibility for their application as a source of biologically active substances to support the human health and diet. The total content in biologically active compounds or the separated compounds found in these seeds indicates that *N. sativa* seeds from Turkey could have a broad spectrum of applications in various foods, also as technological aids, and in the pharmaceutical and dietary products.

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Rheological and sensory properties of glazes prepared with carob and cocoa powders

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ABSTRACT

Different types of glazes are used in the food industry for improving the quality of the bakery and confectionery products due to their textural properties. The aim of this study was to evaluate some rheological and descriptive sensory properties of cocoa and carob glazes during storage. Four glaze formulations with different quantity of cocoa and carob powder were prepared. Resulting flow curves were fitted to Ostvald-de-Waele rheological models. It was determined that carob glazes have similar rheological properties like the cocoa glazes, which were typical for non-Newtonian fluids. The results show that the shear stress and the viscosity of the samples were shear rate dependent and in all cases, the behavior was shear-thinning (or pseudoplastic). The hysteresis loop area determined that the most narrow loops were found in the systems with cocoa powder, which are stronger and more shear-resistant. The sensory evaluation of appearance, colour, flavour, taste, consistency, sweetness, aftertaste, and overall perception was also performed. As a result, the carob powder could be used as a potential substituent of cocoa powder due to its high nutritional value and pleasant sensory properties.

Keywords: Carob, cocoa, glaze, rheology, sensory evaluation.

1. INTRODUCTION

Due to the globalization and increasing interdisciplinary focus on food production, the distribution of various foods around the world undergoes significant changes. The understanding of different tastes, textures, and possibilities for various food applications determine the necessity for a good knowledge of their properties (Ojeda et al., 2018). A very important segment of food production and distribution is the overall consumer perception (Hong et al., 2014; Chambers et al., 1981; Lawless et al., 2013).
The use of non-traditional raw materials and substitutes of already known food products may be a way of creating new products through the use of raw materials with functional potential (Ayaz et al., 2009). Today, increasing interest is given to the use of raw materials with proven health effects (Youssef et al., 2013b), which can successfully combine biologically active (Ayaz et al., 2009) and textural indicators (Grosso, 2011), ensuring manufacturing a product with high quality. The nutritional value of raw materials extends the scope of the development of confectionery products with functional potential (Loullis & Pinakoulaki, 2017).
Cocoa is a major ingredient in the production of chocolate and chocolate derivatives, defining the specific flavor properties of the products (Aprotosoaie et al., 2016). The carob becomes more pronounced in the food industry because of its nutritional value. The high content of phytosterols and bioactive compounds in the carob and aromatic flavours make it a potential ingredient for food systems as a substitute for cocoa (Youssef et al., 2013b). Cocoa is mainly used in a wide range of confectionery products as a cocoa glaze (Youssef et al., 2013a; Buckman & Viney, 2002). The glazes perform various technological functions (Loullis & Pinakoulaki, 2017), preventing the loss of moisture from the interior of pastry products (Jahromi et al., 2012), the air penetration and the induction of oxidation processes (Buckman & Viney, 2002), giving them a good commercial appearance (Ojeda et al., 2018). The use of glazes helps to preserve the aroma contained in the product (Jahromi et al., 2012) as well as in the formation of the nutritional and biological value of the products. The use of glazes has a positive effect on the shelf life of confectionery products, on the development of undesirable microflora on the surface of the product (Jahromi et al., 2012; Buckman & Viney, 2002).
For the production of glazes, ingredients such as milk, eggs, sugar, vegetable oils, flavorings, and others are used (Grosso, 2011). Each of the ingredients involved in glazing has an influence on its rheological properties (Steffe, 1996; Aprotosoaie et al., 2016).
Knowledge of the rheological properties in the creation of new products is of major importance for the food industry (Youssef et al., 2013b). In this field, it is important to know the processes of the stratification and film-forming properties of glazes. An important condition for obtaining a confectionery glaze with good quality is the possibility of obtaining a surface-laid glazing film of a certain thickness (Grosso, 2011), which depends on the viscosity and the glaze yielding border area. For the optimal glazing properties of the fluid, the glazing method and the type of the product for which it is intended is of major importance (Grosso, 2011). Carob is considered as a potential substitute of cocoa, because of its nutritional composition, the growing demand for cocoa and its rising costs. It is difficult to find scientific information on the rheological properties of confectionary glazes obtained by replacing the cocoa powder with carob powder. This information can be important for the food industry, both from an economic, technological and health point of view.
The aim of this study is to perform a rheological and sensory evaluation of glazes prepared with carob and cocoa powders.

2. MATERIALS AND METHODS

2.1. Materials

Standard raw materials such as cocoa powder (Gaio Plovdiv), carob powder (Bio Class, Bulgaria), cocoa butter (Dragon Superfoods), glucose (Biznes Kashti OOD), sugar (Zaharni zavodi AD) are authorized by the Ministry of Health as manufactured in Bulgaria, and drinking water - complying with the Ordinance for Amendment and Supplement to Ordinance No. 9 of 2001 on the quality of water intended for drinking and household purposes (promulgated, SG, issue 30 of 2001, amended and supplemented, 87 in 2007, issue 1 of 2011, issue 15 of 2012 and No. 102 of 2014).

2.2. Methods

2.2.1. Preparation of glazes

The formulation composition of the glazes is presented in Table 1. The determined amounts of water and crystalline sugar are heated to a boiling point, then the cocoa or carob powder is added at a temperature of 96-98 °C for 6-8 minutes. Glucose and cocoa butter are added to the resulting mass. After addition of the glucose and the cocoa butter, the mixture is removed from the heating. The resulting mass is homogenized well to obtain a smooth consistency, characteristic luster, and viscosity.

Table 1. Glaze formulations

Ingredients	Amount based on:			
	Cocoa glaze, [%]		Carob glaze, [%]	
	A	B	A	B
Water	21.28	22.98	21.28	22.98
Glucose	42.54	45.97	42.54	45.97
Cocoa butter	10.64	11.50	10.64	11.50
Sugar	10.64	11.50	10.64	11.50
Cocoa powder	14.90	8.05	-	-
Carob powder	-	-	14.90	8.05

*The amounts of the ingredients involved are presented on a weight basis of the glaze

2.2.2. Rheological properties of the glazes

A comparative characterization of the main parameters and properties between different glazes with cocoa and carob powder was made in order to determine the uses of carob powder. These glazes are produced with two percentages of cocoa (A and B) and carob powder (A and B).
The viscosity of the samples was defined with „Rheotest 2“, Germany, with a share rate in a range from 0.17 to 72.9 s^{-1} at 30 °C. All of the samples were stored at 27 °C and analyzed during the first, third and fifth day.
The dynamic viscosity (η) was calculated using the formula (Steffe, 1996, Rao, 1999):

$$\eta = \frac{\tau}{D} \qquad (1)$$

where: τ is the shear stress, Pa;
D – is the shear rate, s^{-1}.

The thixotropic areas were also calculated.

2.2.3. Sensorial evaluation of the glazes

The descriptive test for quantitative sensory profiling was used to establish the sensory characteristics (appearance, colour, flavour, taste, consistency, sweetness, aftertaste, overall perception) of the glazes. The glaze samples were ready 8 h before the evaluation. Samples of different glazes were kept in coded plates covered with aluminium foil. Twelve trained panellists were selected to guarantee evaluation accuracy. The intensity of each sensory characteristic was recorded on a ten-point linear scale after 1 h orientation sessions of the panellists, where they specified terminology and anchor points on the scale. The coded samples were shown simultaneously and evaluated in a random order among the panellists.

2.2.4. Statistical analysis

Depending on the type of the studied characteristic from 3 to 12 repetitions of each measurement were done. Data were presented as mean values ± standard deviation. The chosen level of significance for the data analysis was $\alpha = 0.05$.
According to the aim of the study, it was necessary to do comparisons between several glazes. In order to choose the appropriate method for statistical analysis, the Shapiro-Wilk test for normal distribution was performed. It showed that the samples were not from normally distributed populations. Hence the non-parametrical Wilcoxon Signed Rank Test was used in order to conduct the necessary comparisons.

3. RESULTS AND DISCUSSION

3.1. Rheological properties of the glazes

Flow curves of the glazes with cocoa and carob powder were shown for the first day (Figure 1), for the third day (Figure 2.), and for the fifth day (Figure 3).

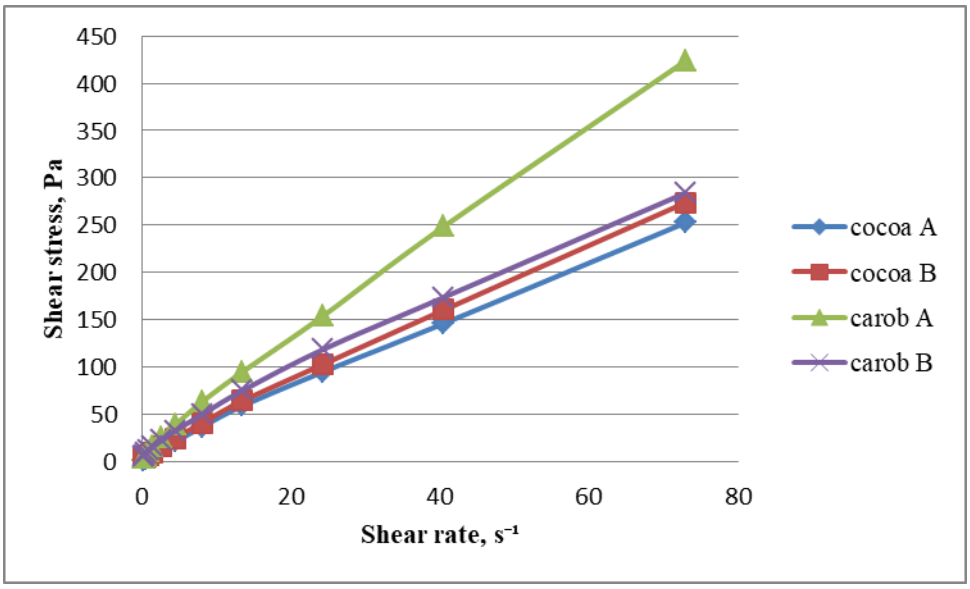

Figure 1. Flow curves of glazes depending on the shear rate at 30 °C, on the first day

The graphical correlation showed that the samples rheologically represent a non-Newtonian fluid. The results show that the shear stress and the viscosity of the samples were shear rate dependent and in all cases, the behavior was shear-thinning (or pseudoplastic).
Each ingredient, added in the glaze formulation reverses the viscosity of the final product. Higher shear stress values on the first day of the carob B sample is higher than the rest of the samples. The higher shearing stress is due to the wider contact surface of the carob powder fine particles and the functional structuring properties of the sugar in the matrix structure of the fatty acids involved in the formulation (Mongia & Ziegler, 2000).

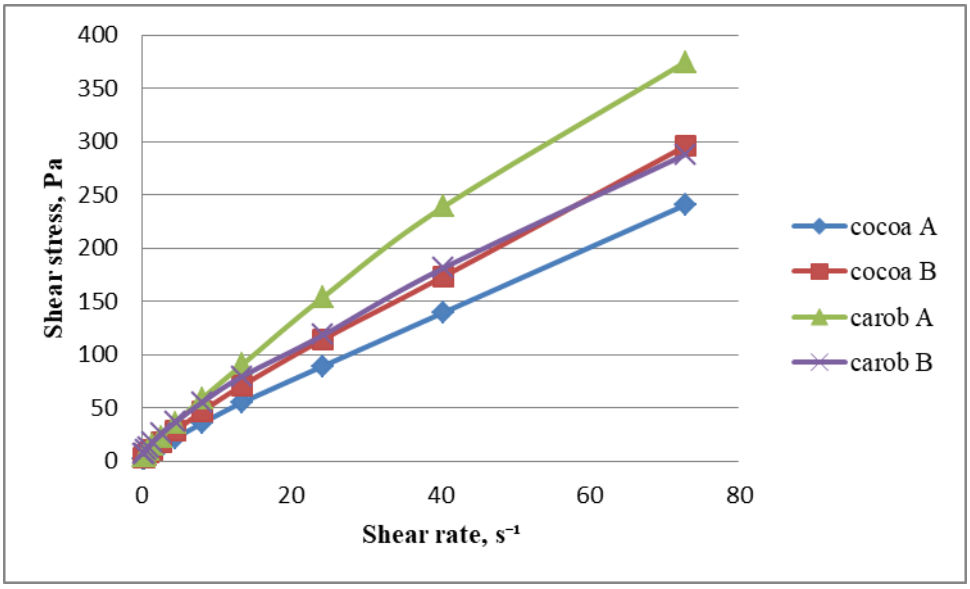

Figure 2. Flow curves of glazes depending on the shear rate at 30 °C, on the third day

The results show that the distribution and size of the glaze particles from cocoa A, B, and carob B

have less pronounced polydispersity, confirming the high homogeneity of the system (Toro-Vazquez et al., 2004). In the carob glaze A, high shear stress values were recorded. This behavior of the studied systems (cocoa A, cocoa B, and carob B) is similar to the chocolate glazes studied earlier by Schantz & Rohm (2005), which indicated that the use of emulsifiers can improve the monodispersity of the system. The effect of the added emulsifier was achieved with the addition of cocoa butter having pronounced surfactant properties. Taking into account the cutting force on the second day of the test, it is noted that the cocoa B and carob B samples have comparable values. The glaze with lower percentage participation of cocoa was characterized by the lowest shear values, which confirms the strong bonding forces between the carob and the liquid phase in the system during storage.

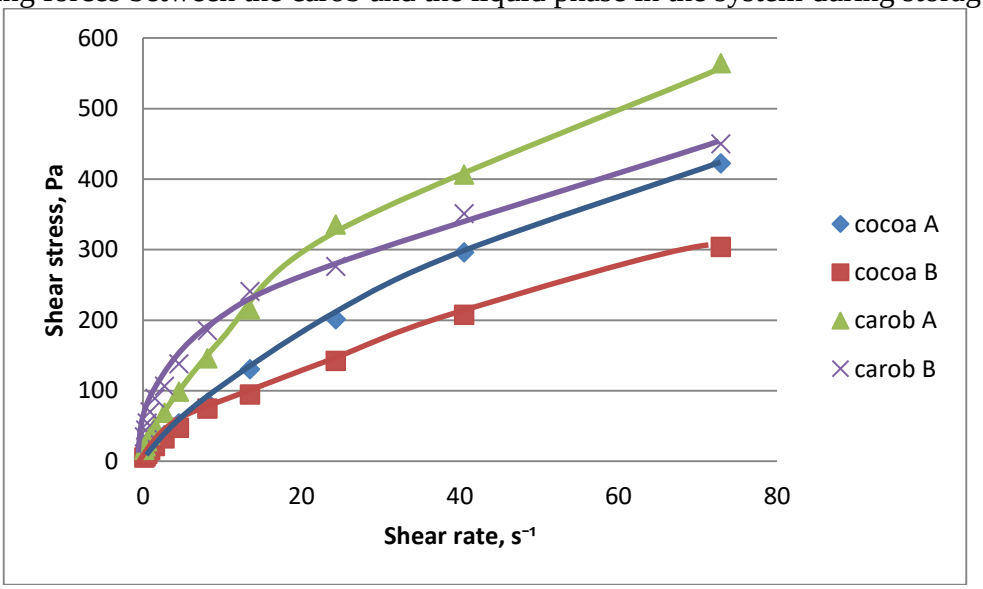

Figure 3. Flow curves of glazes depending on the shear rate at 30 °C, on the fifth day

Viscosity is also defined as a basic rheological property of glazes. The values of the viscosity of the samples according to the rate gradient (D) in the temperature range examined are reflected in the logarithmic coordinates of Figures 4, 5 and 6.

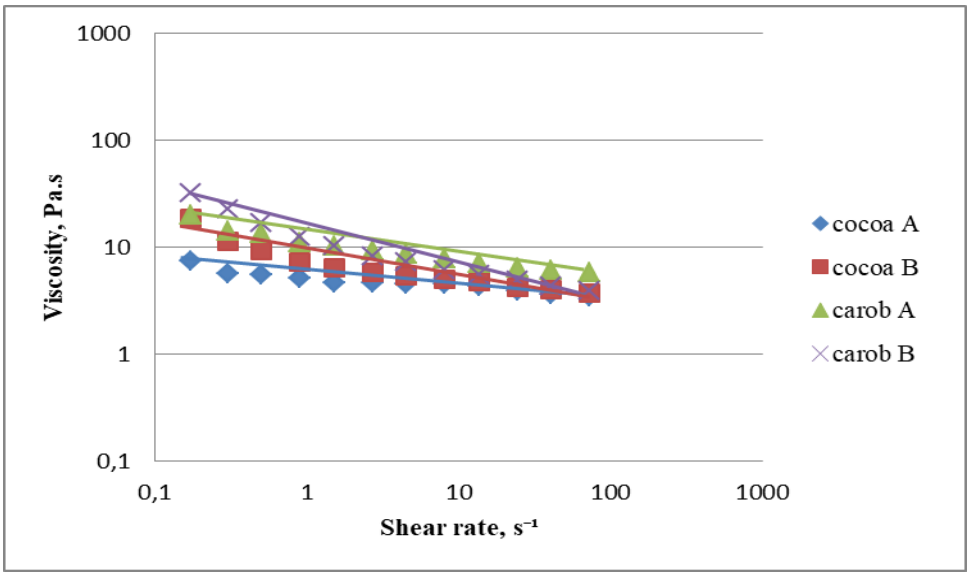

Figure 4. Viscosity of glazes depending on the shear rate at 30 °C, on the first day

The reported close viscosity values on the first day of the study showed good dispersion strength of the solid fractions in the system (Sikora et al., 2003).
The transition from solid to liquid for the test samples occurs smoothly. The most rapid process is observed in the cocoa sample A, and the smoothest the process is recorded in the carob sample A. This change in consistency is due to the increasing level of sugar and to the decreasing amounts of solid particles in the matrix. The process can be explained by the inclusion of lower amounts of suspended solids that change the density of the system (Fernandesa et al., 2013).

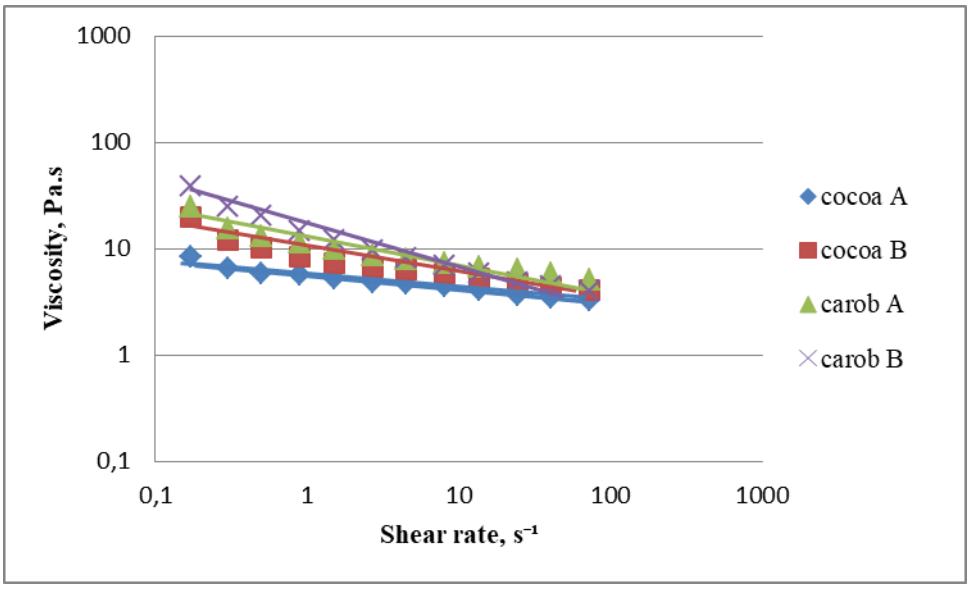

Figure 5. Viscosity of glazes depending on the shear rate at 30 °C, on the third day

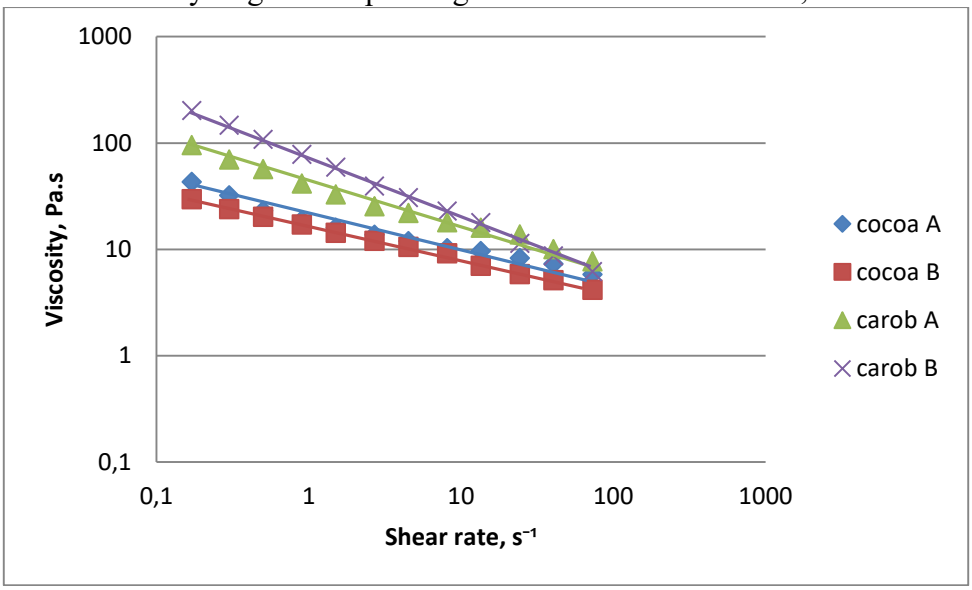

Figure 6. Viscosity of glazes depending on the shear rate at 30°C, on the fifth day

The graphically expressed dependence confirms that analyzed glazes have non-Newtonian viscosity and viscosity depends on the shear rate (D).

$$\eta = K.D^{n-1}$$

Where

η is the viscosity of the sample, Pa.s;

D-share rate, s^{-1};

K - consistency coefficient;

n- flow behavior index.

In this case with the increase of share rate, the viscosity decreases.

It is evident from the above figures that in logarithmic coordinates the dependence is linear and is expressed by Ostwald-de Waele's model of the viscosity (Figura and Teixeira, 2007).

The values of the coefficients in equation and hysteresis loop areas depending on the type of samples are shown in Table 2.

Table 2. Values of coefficients K, n, R^2 and hysteresis loop area at 30°C

Storage	Samples №	K (Pa.s^n)	n (-)	R^2	Hysteresis loop area (Pa.s^{-1})
1-st day	Cocoa A	5.316	0.8995	0.9987	93.1
	Cocoa B	8.355	0.7764	0.9892	89.8
	Carob A	11.717	0.8135	0.9976	451.4
	Carob B	13.570	0.6654	0.9889	367.0
3-th day	Cocoa A	5.719	0.8641	0.9990	249.5
	Cocoa B	9.347	0.7761	0.9921	524.3
	Carob A	12.052	0.7805	0.9931	721.7
	Carob B	15.514	0.6403	0.9715	689.9
5-th day	Cocoa A	20.329	0.6985	0.9941	2695.1
	Cocoa B	16.548	0.6817	0.9995	1364.7
	Carob A	42.242	0.6069	0.9957	5126.1
	Carob B	73.496	0.4259	0.9985	5591.1

The parameters of the Ostwald-de-Waele model for analyzed glazes fitted to the determined flow curves and measured areas of the hysteresis loops are presented in Table 2. The multiple correlation coefficients R^2 informed about the very good fitting of the Ostwald-de-Waele model, which is widely used in the analysis of various food systems. The coefficients of determination (R^2) obtained were

high and varied from 0,9715 to 0,9995. The consistency coefficient (K) and flow behaviour index (n), obtained by fitting of the power law and Ostwald-de-Waele model to the experimental shear stress-shear rate data are given in Table 2 (Gibiński et al., 2006). Values for flow behaviour indices, n, were below 1, which was indicative of the pseudoplastic nature. The flow behaviour index (n) is between 0,4259 and 0,8995. The smaller n values determined the greater departure from the Newtonian behaviour, increasing proportionally to the storage time (Chhinnan et al., 1985). Consistency coefficient K can also be used as a criterion of viscosity. On the first day, the viscosity increases from sample 1 to sample 4, and this dependence is preserved after storage, except sample 2 on the fifth day, which has the lowest value. Increase of the viscosity was also observed during sample storage analyzing.

The results obtained show that glazes with a lower percentage of carob exhibited more pronounced molecular mobility at 30 °C throughout the storage period. The analysis demonstrated that the transition from solid to liquid occurs at slightly lower stress values in carob A sample.

Higher stress values were recorded in samples with a higher percentage of dry matter due to increased viscosity in the glaze matrix.

There was a change in thixotropic behaviour in all systems. The thixotropic effect is described as a viscosity decrease with time at a constant shear rate (Gouveia et al., 2008).

There is no strict dependence between the samples hysteresis loop areas at the same storage time but the results showed that the most narrow loops were found for the systems with cocoa powder (cocoa A and cocoa B). Thus, the structure of such glazes was the strongest and shear-resistant. It was obvious that by increasing the storage time of the samples shear-resistant decreases. Samples 3 and 4 on the fifth day had the highest value of the hysteresis loop area which means the weakest and the shear-resistant value.

3.2. Sensorial evaluation of the glazes

Sensory analysis evaluation was performed in order to determine the optimum sensory characteristics of the glazes in terms of the panellists' preferences. The results of the sensory evaluation are given in Tables 3, 4, 5 and 6. Each table represents a comparison between the mean scores of a pair of glazes for all 8 attributes. As mentioned above the non-parametrical Wilcoxon Signed Rank Test was used in order to conduct the comparisons between every two glazes. The last column of each of the four tables represents the two-tailed p-values of the test. Those of them followed by S are less than the significance level, which means that there is a significant difference between the scores on the corresponding row.

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Table 3. Mean scores of the attributes ratings of carob glazes

Attributes	Carob A	Carob B	p-values for Wilcoxon test
Appearance	7.82 ±1.43*	7.93 ±1.31	0.3681
Colour	7.83±0.99	7.79±1.05	0.6384
Flavour	7.19±1.30	7.03±1.99	0.6455
Taste	7.02±1.59	6.88±1.71	0.3222
Consistency	7.43±1.28	7.42±1.64	0.9203
Sweetness	7.14±1.49	6.28±1.87	0.0001 S**
Aftertaste	6.70±1.95	6.81±1.43	0.9200
Overall perception	7.20±1.56	6.67±2.00	0.0032 S

Note: *Values are mean score ± standard deviation; **p-values followed by S are less than $\alpha = 0.05$, hence the mean scores on the corresponding row are significantly different according to the Wilcoxon test ($\alpha = 0.05$).

The sensory evaluation of glazes highlighted the tasters' assessment of organoleptic criteria, which takes preference to carob A sample. The taste scores of carob A sample showed higher results (7.02 ± 1.59) in comparison to the carob B sample (6.88 ± 1.71). The presence of a higher concentration of dry matter in the food system may exhibit a suppressing effect on the product's taste (Buckman & Viney, 2002), which is taken into account in the presence of carob. Reverse dependence is seen in the cocoa glaze, where the cocoa A sample is reported to have lower tastes (7.51 ± 1.57) than cocoa B sample (7.85 ± 1.23). The presence of higher amounts of soluble substances in the cocoa composition implies an increase in the sensation of a more pronounced taste in increasing the concentration of dry matter (Gouveia et al., 2008).
Similar results for the sweetness, aftertaste, and overall perception values were reported for the carob A sample, probably explained by the higher amounts of insoluble carbohydrates in the carob composition (Youssef et al., 2013a).

Table 4. Mean scores of the attributes ratings of cocoa glazes

Attributes	Cocoa A	Cocoa B	p-values for Wilcoxon test
Appearance	7.97±1.10*	7.83±0.95	0.1527
Colour	7.83±1.52	7.78±1.01	0.4295
Flavour	7.25±1.40	7.77±1.10	0.0065 S**
Taste	7.51±1.57	7.85±1.23	0.0610 S
Consistency	7.49±1.45	7.46±1.21	0.9283
Sweetness	7.30±1.42	7.30±1.17	0.8415
Aftertaste	7.29±1.58	7.08±1.58	0.4839
Overall perception	7.65±1.34	7.73±1.34	0.7263

Note: *Values are mean score ± standard deviation; **p-values followed by S are less than $\alpha = 0.05$, hence the mean scores on the corresponding row are significantly different according to the Wilcoxon test ($\alpha = 0.05$).

According to the results, the cocoa A and cocoa B glazes differ in flavour and taste and cocoa B glaze has better sensorial ratings.

Table 5. Mean scores of the attributes ratings of carob A and cocoa A glazes

Attributes	Carob A	Cocoa A	p-values for Wilcoxon test
Appearance	7.82 ±1.43*	7.97±1.10	0.2420
Colour	7.83±0.99	7.83±1.52	0.2301
Flavour	7.19±1.30	7.25±1.40	0.3900
Taste	7.02±1.59	7.51±1.57	0.0036 S**
Consistency	7.43±1.28	7.49±1.45	0.2500
Sweetness	7.14±1.49	7.30±1.42	0.0740

Aftertaste	6.70±1.95	7.29±1.58	0.0091 S
Overall perception	7.20±1.56	7.65±1.34	0.0041 S

Note: *Values are mean score ± standard deviation; **p-values followed by S are less than $\alpha = 0.05$, hence the mean scores on the corresponding row are significantly different according to the Wilcoxon test ($\alpha = 0.05$).

The carob A and cocoa A glazes show statistically significant different scores for their taste, aftertaste and overall perception. The average values of the individual cocoa A and carob bean glazes are comparable, and the reported differences in values can be explained by traditional taster eating habits (Yanes et al., 2002).

Table 6. Mean scores of the attributes ratings of carob B and cocoa B glazes

Attributes	Carob B	Cocoa B	p-values for Wilcoxon test
Appearance	7.93 ±1.31*	7.83±0.95	0.3222
Colour	7.79±1.05	7.78±1.01	0.7718
Flavour	7.03±1.99	7.77±1.10	0.0001 S**
Taste	6.88±1.71	7.85±1.23	4.34E-05 S
Consistency	7.42±1.64	7.46±1.21	0.9283
Sweetness	6.28±1.87	7.30±1.17	4.42E-05 S
Aftertaste	6.81±1.43	7.08±1.58	0.2460
Overall perception	6.67±2.00	7.73±1.34	3.08E-05 S

Note: *Values are mean score ± standard deviation; **p-values followed by S are less than $\alpha = 0.05$, hence the mean scores on the corresponding row are significantly different according to the Wilcoxon test ($\alpha = 0.05$).

Differences in taste in the various samples may be as a result of the surface strength observed in the rheological characteristics of the systems where the suspended particles enhance the sweet taste of the glaze (Fernandesa et al., 2013).

4. CONCLUSIONS

The production of glazes with good rheological properties and acceptable sensory evaluation gives ground for the application of the glazes with the participation of carob powder in the composition of pastry products. The rheological behaviour of the studied glazes with carob demonstrated that in spite of the large amounts of suspended particles in the matrix, they re-established their rearrangement for a short time, achieving equilibrium distribution. This process of recovering the system is important to ensure equilibrium stability. Future research is forthcoming to create model technological solutions related to the specific application of glazes, as well as to analyze their functional properties.

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The Effect of Different Extraction Methods on Color Values of Sour Cherry Pomace

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ABSTRACT

Sour cherry fruit (*Prunus cerasus* L.) is consumed by the consumer with taste and is a fruit that is produced in our country. Sour cherry is rich in anthocyanins, which are the best known natural food dyes. One of the positive properties of anthocyanins in the food industry is that they reduce the use of artificial colorants in foods. In this study, microwave-assisted extraction (MAE) (900W for 30, 60 and 90 sec.), and ultrasonic-assisted extraction (UAE) (for 5,10 and 15 min with a power of 100%) was used as novel processes as well as conventional solvent extraction (CSE) was performed as a traditional method. Color values (L^*, a^*, b^*) and browning index (BI) the extracts obtained from sour cherry were performed. The effect of extraction method on color values was evaluated. The highest L^* value in color analysis was observed as 20.35±1.25 in classical extraction. a^* value that denotes redness was obtained as 24.29±2.14. Lower values were obtained from UAE and MAE than conventional solvent extraction. BI value decreased as application time increased in MAE. The lowest value of 48.95±3.14 was reached in 10 minutes in UAE.

Keywords: Color, extraction, sour cherry, total phenolic content

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Antimicrobial activities of *Salvia sclarea* L. and *Salvia aethiopis* L. essential oils

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ABSTRACT

In this study, antimicrobial activities of the essential oils of *Salvia sclarea* L. and *Salvia aethiopis* L., grown in Eskisehir were investigated using two different methods (disc diffusion and broth dilution) against seven different microorganisms (*Bacillus subtilis* ATCC 6037, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 1103, *Escherichia coli* O157:H7 ATCC 43895, *Listeria monocytogenes* Scott A, *Salmonella* Typhimurium NRRLB 4420, *Staphylococcus aureus* 6538P). According to disc diffusion method, the highest inhibition zones were observed against *S. aureus* as 18.5±4.95 mm and 23.5±9.19 mm, for essential oils of *Salvia sclarea* and *Salvia aethiopis*, respectively. The MIC values of the essential oils were determined using 96-well microplates, containing the final concentrations of essential oils in the range of 0.02%-10% (v/v). The results showed that the MIC values of the samples were ranged between 5% and >10% depending on the test cultures used. *E. faecalis* and *E. coli* were the most sensitive microorganisms against *Salvia sclarea* essential oil (MIC of 5%). *Salvia sclarea* essential oil had bactericidal activity at concentrations of 10% (v/v) against *S. aureus* while no bactericidal activity was observed on other test cultures for the concentrations under 10%. Besides, no bactericidal activity at concentrations under 10% were observed for *Salvia aethiopis* essential oil. As a result, the essential oil of *Salvia sclarea* was found more effective than the essential oil of *Salvia aethiopis*, in terms of antimicrobial activities.

Keywords: *Salvia sclarea* L., *Salvia aethiopis* L., antimicrobial, essential oil

1. INTRODUCTION

The plants contain natural compounds such as phytochemicals, phenols, polyphenols, essential oils and micronutrients (Tajkarimi *et al*., 2010; Gyawali & Ibrahim, 2014). Essential oils are produced for defense against microorganisms and they have been researched worldwide as potential sources of novel antimicrobial compounds, alternatives to agents promoting food preservation (Belletti *et al*., 2004; Alviano & Alviano, 2009; Astani *et al*., 2010; Safaei-Ghomi & Ahd, 2010; Solórzano-Santos & Miranda-Novales, 2012). These antimicrobial and antioxidant agents are aromatic oils obtained by the distillation from different parts of the plants such as flowers, buds, leaves, fruits, bark, seeds (Jayasena & Jo, 2013; Calo *et al*., 2015; Cui *et al*., 2015). Essential oils that have characteristics flavor and odor, are used as preservatives in the food industry to prevent spoilage and ensure food safety (Solórzano-Santos & Miranda-Novales, 2012; Firuzi *et al*., 2013). They consist of low molecular weight organic compounds such as terpenes, terpenoids, phenylpropanoids (Bakkali *et al*., 2008; Hyldgaard *et al*., 2012).

Salvia species are one of the most important aromatic plants of the Lamiaceae family, with approximately 900 species in the world (Fu *et al*., 2013; Cui *et al*., 2015; Tohma *et al*., 2016). They include various secondary metabolites such as sterols, flavonoids, sesquiterpenoids and diterpenoids (Askun *et al*., 2010). *Salvia* essential oils contain various important components such as 1,8-cineole, camphor, borneol, α-thujene, α-pinene, β-pinene and camphene (Fu *et al*., 2013). They are commonly used in the food industry as antimicrobial agent and a preservative against food spoilage (Cui *et al*., 2015). Besides, *Salvia* species are also used in cosmetics, perfumery and pharmaceutical industries (Tohma *et al*., 2016). In several studies, it has been reported that essential oils of *Salvia* species have antimicrobial effects against various microorganisms. The chemical composition and structure of essential oils play an important role in the determination of their antimicrobial activity (Taârit *et al*., 2012). *Salvia* species have positive impact on human health with antibacterial, antifungal, antioxidant, anti-inflammatory, antidiabetic, antitumoral, anxiolytic, antiplasmodial, hypoglycaemic and anticarcinogenic effects (Kamatou *et al.*, 2008; Esmaeili & Sonboli, 2010; Gürsoy *et al*., 2012; Jeshvaghani *et al*., 2015; Cui *et al*., 2015; Tohma *et al*., 2016). Hence, *Salvia* species traditionally used as a therapeutic agent against many diseases such as stomachache, asthma, analgesic, sore throat, cold, bronchitis (Poyraz *et al*., 2017).

In the previous studies, the antimicrobial properties of *Salvia* species collected from different geographical regions have been investigated. However, to the best of the knowledge of authors, there is no study on the antimicrobial activities of *S. sclarea* L. and *S. aethiopis* L. essential oils grown in Eskisehir in open literature. Hence, the objective of this study was to investigate the antibacterial activities of essential oils of *S. sclarea* L. and *S. aethiopis* L. against seven different bacteria (*Bacillus subtilis* ATCC 6037, *Escherichia coli* ATCC 1103, *Escherichia coli* O157:H7 ATCC 43895, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* Scott A, *Staphylococcus aureus* 6538P, *Salmonella* Typhimurium NRRLB4420) using disc diffusion and broth dilution methods.

2. MATERIAL AND METHODS

2.1 Collection of the plant materials

S. sclarea L. and *S. aethiopis* L. were collected around Mihalliccik/Eskisehir in 2018. Plant samples were identified by Prof. Dr. Ersin YUCEL. In the first step, the samples were washed under tap and

distilled water, then left to dry on paper filter at room temperature. The materials were grained after drying through a hammer mill and stored in tight plastic containers for further use.

2.2 Extraction of the plant materials

The essential oils of *S. sclarea* L. and *S. aethiopis* L. were obtained using hydrodistillation method. 100 g samples were placed in a Clevenger-type apparatus with 2 L of double distilled water and then hydrodistilled for 3 hours. The samples were stored at 4℃ until use (Baydar *et al*., 2004).

2.3 Antimicrobial activity

2.3.1 Bacterial strains

The antimicrobial activity of *S. sclarea* L. and *S. aethiopis* L. essential oils were investigated against seven different microorganisms (*B. subtilis* ATCC 6037, *E. coli* ATCC 1103, *E. coli* O157:H7 ATCC 43895, *E. faecalis* ATCC 29212, *L. monocytogenes* Scott A, *S. aureus* 6538P, *S.* Typhimurium NRRLB 4420). These cultures were obtained from Ege University, Food Engineering Department, Food Microbiology Research Laboratory. Stock cultures were transferred to Tryptic Soy Broth (TSB, pH 7.3±0.2, Oxoid) for the activation and incubated at 37°C for 24 hours. The optimized bacterial cultures (DEN-1 McFarland Densitometer, Grant-bio), equivalent to 0.5 McFarland turbidity standard, were used in the analyses.

2.3.2 Disc diffusion method

The disc diffusion method was used for the determination of the preliminary antibacterial activity of essential oils (Deng *et al*., 2014). Firstly, suspension of test bacteria was spreaded on Mueller Hinton Agar (MHA, pH 7.3±0.2, Oxoid) plates. Paper discs (6 mm in diameter) impregnated with the essential oils of *S. sclarea* (40 mg/mL) and *S. aethiopis* (40 mg/mL) were placed on the surface of the bacteria inoculated plates. Then the prepared plates were incubated at 37℃ for 24 hours and the diameter of the inhibition zones (DIZ) was measured after incubation. Ampicillin and gentamycin (10 µg/mL) were used as positive controls and sterile water as negative control. The experiment was done in triplicate.

2.3.3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The antibacterial activities of essential oils of *S. sclarea* and *S. aethiopis* were also tested by micro-dilution method (Deng *et al*., 2014). MIC values of the essential oils were determined using 96-well "U" type sterile microplates. 100 µL Mueller Hinton Broth (MHB, pH 7.3±0.2, Oxoid) was added to each well. After that, 100 µL of essential oil dissolved in DMSO (5%) was transferred into the wells of the first row. Serial two-fold dilutions of the samples prepared with MHB (a total volume of 200 µl) were dispensed into wells of the microplate. The final concentrations of the samples in the wells were; 10%, 5%, 2.5%, 1.25%, 0.625%, 0.313%, 0.156%, 0.078%, 0.039%, 0.020% (v/v), respectively. After dilution period, 100 µL of inoculum were added to each well. The plates were incubated at 37℃ for 18 hours. Then, 20 µL of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC, Merck) was added to each well and the plates were incubated at 37℃ for 30 minutes. The lowest concentration of the sample necessary for inhibit visible growth of the culture (no color formation) was determined as MIC value.

The minimum bactericidal concentration (MBC) of the samples were taken from the first wells, where no growth was observed and streaked on Mueller-Hilton Agar (MHA, pH 7.3±0.2, Oxoid). Each experiment was performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Diameter inhibition zone (DIZ) values of the samples

The preliminary antibacterial activity of *S. sclarea* and *S. aethiopis* essential oils were investigated against seven bacteria (Table 1). Essential oil of *S. sclarea* showed inhibition zones ranging between 7±0 mm-18.5±4.95 mm on test bacteria, while *S. aethiopis* essential oil had inhibitive effect against test bacteria except *S.* Typhimurium with DIZ values in the range of 8.5±2.5 mm-23.5±9.19 mm. Both of the essential oil samples showed the highest inhibition effects against *S. aureus*. the results also showed that *S. sclarea* essential oil showed high inhibition zone against *B. subtilis* (18±0 mm) while *S. aethiopis* essential oil showed low inhibition effect on the same test microorganism (8.5±2.5 mm).

In a study performed by Ogutcu *et al*. (2008), inhibitive effect of essential oil of *S. sclarea*, grown in Erzurum, was investigated against fifty-five microorganisms. However, no inhibitive effect was observed for test microorganisms. In another study, antimicrobial effects of chloroform and acetone extracts of *S. sclarea* collected from Kahramanmaras, were determined against fifteen different microorganisms. DIZ values of chloroform and acetone extracts of *S. sclarea* were ranged between 7-9 mm and 7-22 mm, respectively, while no inhibitive effect was determined against *L. monocytogenes* and *E. coli* for both extracts (Gulcin *et al*., 2004). In another study, the antimicrobial activity of *S. aethiopis* (collected from Bingol) that impregnated with different concentrations of disc (30, 60 and 90 µL) was examined against various microorganisms (*B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *B. megaterium* DSM 32, *Enterobacter aerugenes* ATCC 13048, *E. coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 13883, *Candida albicans* ATCC 10231, *Yarrowia lipolytica* and *Saccharomyces cerevisiae*). The most sensitive microorganism was found as *S. aureus* for concentrations of 30 and 60 µL (DIZ values of 9 and 11 mm, respectively) and *Y. lipolytica* for concentration of 90 µL (DIZ value of 13 mm). However, it was determined that *S. aethiopis* did not have an inhibitory effect against *B. subtilis* ATCC 6633, *B. megaterium* DSM 32, *K. pneumoniae* ATCC 13883, *C. albicans* ATCC 10231 and *S. cerevisiae* in all three concentrations (Tohma *et al*., 2016). These studies showed that DIZ values of *Salvia* species show differences depending on the plant type, oil composition and concentration, oil extraction method and test microorganisms.

Table 1. DIZ values of *S. sclarea* and *S. aethiopis* essential oils against test microorganisms (mm)

Test Microorganisms	***Salvia sclarea***	***Salvia aethiopis***	**P.C.**		**N.C.**
			Amp.	**Gen.**	**S.W.**
B. subtilis ATCC 6037	18 ± 0	8.5 ± 2.5	29 ± 1.0	29.5 ± 0.5	6 ± 0
E. coli ATCC 1103	8 ± 0	9 ± 3.0	20.5 ± 0.5	21.5 ± 1.5	6 ± 0
E. coli O157:H7 ATCC 43895	7 ± 0	9 ± 3.0	11.5 ± 0.5	18 ± 0	6 ± 0
E. faecalis ATCC 29212	9.5 ± 0.5	10.5 ± 1.5	21 ± 1.0	14.5 ± 0.5	6 ± 0
L. monocytogenes Scott A	11.5 ± 1.5	10.5 ± 4.5	28 ± 2.0	28.5 ± 1.5	6 ± 0
S. aureus 6538P	18.5 ± 4.95	23.5 ± 9.19	35.5 ± 0.5	22.5 ± 0.5	6 ± 0
S. Typhimurium NRRLB 4420	7.5 ± 0.5	6 ± 0	18 ± 2.0	20 ± 0	6 ± 0

*P.C.: positive control; N.C.: negative control; Amp.: Ampicillin; Gen.: Gentamycin; S.W.: sterile water

3.2 MIC and MBC values the samples

The MIC values of essential oils of *S. sclarea* and *S. aethiopis* were examined against seven different microorganisms using 96-well microplates (Table 2). *E. coli* and *E. faecalis* were the most sensitive microorganisms against essential oil of *S. sclarea* (MIC of 5%). Besides, essential oil of *S. sclarea* showed an inhibitive effect at 10% concentration against *E. coli* O157:H7, *S. aureus* and *S.* Typhimurium. However, essential oil of *S. aethiopis* did not show inhibitive effect at concentrations under 10% against test microorganisms except *E. faecalis* and *L. monocytogenes*. Although MIC value of *S. sclarea* essential oil was observed as 10% against *S. aureus*, no bactericidal activity was determined at concentrations under 10% against other microorganisms (Table 2). However, essential oil of *S. aethiopis* showed no bactericidal effect against test microorganisms at concentrations under 10%.

In a study conducted by Cui *et al*. (2015), the antimicrobial activity of *S. sclarea* L. essential oil was examined against seven different microorganisms (*S. aureus*, *B. subtilis*, *E. coli*, *S.* Typhimurium, *K. pneumonia*, *P. aeruginosa* and *B. pumilus*). MIC and MBC values of essential oil was determined against all test microorganisms as 0.05% and 0.1%, respectively. In another study, the antifungal activity of essential oil of *S. sclarea* L., collected from Russia, was investigated against seven different *Candida* species. MIC values of *S. sclarea* essential oil was ranged between 6 and 15 µg/mL. Besides, minimum fungicidal concentration (MFC) of essential oil against *Candida* species was found as ranging between 12 and 28 µg/mL (Jirovetz *et al*., 2013). In the study performed by Firuzi *et al*. (2013), the antibacterial effects of extracts of different *Salvia* species, collected from Iran, were investigated against *E. coli*, *K. pneumoniae*, *S. typhi*, *B. subtilis*, *S. epidermidis* and *S. aureus*. MIC values of *S. sclarea* extract was ranged between 0.31 and 5 mg/mL, while extract of *S. aethiopis* did not show antibacterial effect against test microorganisms except *K. pneumoniae* (MIC of 10%) and *S. typhi* (MIC of 5%). The results of this study showed that extract of *S. sclarea* is more effective than *S. aethiopis* extract against test microorganisms, as similar to our results. In another study, antimicrobial activity of ethanol extract obtained from different parts (flower, leaf and stem) of *S. aethiopis* L., grown in Serbia, was determined against nine different microorganisms. MIC values of extracts of flower, leaf and stem found as 80-200 µg/mL, 60-200 µg/mL and 120-200 µg/mL, respectively (Velickovic *et al*., 2002).

Table 2. MIC and MBC values of *S. sclarea* and *S. aethiopis* essential oils against test microorganisms (%, v/v)

Test Microorganisms	*Salvia sclarea*		*Salvia aethiopis*	
	MIC	**MBC**	**MIC**	**MBC**
B. subtilis ATCC 6037	>10	>10	>10	>10
E. coli ATCC 1103	5	>10	>10	>10
E. coli O157:H7 ATCC 43895	10	>10	>10	>10
E. faecalis ATCC 29212	5	>10	10	>10
L. monocytogenes Scott A	>10	>10	10	>10
S. aureus 6538P	10	10	>10	>10
S. Typhimurium NRRLB 4420	10	>10	>10	>10

4. CONCLUSION

In this study, antimicrobial activities of essential oil of *S. sclarea* and *S. aethiopis*, collected form Eskisehir, were determined against seven different microorganisms. Essential oil of *S. sclarea* showed bactericidal activity against *S. aureus* 6538P. Besides, essential oil of *S. sclarea* had higher antimicrobial activity than essential oil of *S. aethiopis*. The results of this study showed that the antimicrobial activity may change depending on plants properties and test microorganisms used. Further studies on the effect of essential oils of *S. sclarea* and *S. aethiopis* in food applications are needed.

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Effect of Somatic Cells Count of Raw Milk on the Carbonyl Compounds and Low Molecular Weight Fatty Acids Formation in Ayran

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ABSTRACT

The present study aimed to provide scientific information for the effect of somatic cells count (SCC) of raw cow milk on the carbonyl compounds and low molecular weight fatty acids formation in ayran. Test ayran samples were produced from three different batches of cow milk with low (up to 400,000 cells.mL^{-1}), medium (between 500,000 and 600,000 cells.mL^{-1}) and high (above 1000,000 cells.mL^{-1}) SCC, respectively. The results obtained showed, that the SCC of raw milk did not affect significantly the content of the main components, the active acidity and the lactic acid concentration in the ayran produced. Volatile organic compounds in ayran samples were determined by gas chromatography-mass spectrometry (GC–MS) analysis. The aldehydes hexanal and heptanal, the ketones acetone, diacetyl, acetoin, 2-heptanone and 2-nonanone, and butanoic and hexanoic fatty acids were identified and quantified in all studied samples. In the ayran samples from batches with higher SCC (between 500,000 and 1000,000 cells.mL^{-1}), a lower content of diacetyl, acetoin, hexanal, and heptanal, and a higher content of acetone, 2,3-pentanedione and butanoic acid were established. The results obtained showed, that the increase of SCC of raw cow milk had a negative effect on the profile of the carbonyl compounds in ayran, which may lead to deterioration in its sensory quality and, respectively, to shelf life reduction.

Keywords: Ayran, Carbonyl compounds, Low molecular weight fatty acids, Sensory quality, Somatic cells.

1. INTRODUCTION

Raw milk quality is not only important in terms of food safety but also a factor affecting the quality of the processed product. Many studies have been carried out to determine the effect of SCC on the yield and quality of milk and dairy products (Cooney *et al.*, 2000; Le Maréchal *et al.,* 2011). It is well known that the higher SCC in raw milk causes adverse effects on raw milk and dairy products quality (Andreatta *et al.,* 2007). Mastitis disease causes economic loss due to low yield in dairy farming. There are various problems in terms of yield losses and shelf life as a result of using the milk of animals with mastitis within the industry. Juozaıtıene *et al.* (2006) conducted a study to investigate the effects of the SCC on milk yield in Holstein cows grown in the Lithuanian region. As a result of the study, when SCC was above 100,000 to 800,000 cells.mL^{-1}, they reported a loss of 14.4% in milk yield, 14.7% in milk fat and 9.1% in milk protein.

Subclinical mastitis alters the composition of the milk in addition to suppressing milk yield (Bramley *et al.*, 1992; Harmon, 1994). Unlike milk production loss, there is a direct relationship between SCC and milk quality. According to Harmon (1994), mastitis or elevated SCC is associated with a decrease in lactose, casein and fat in milk because of reduced synthetic activity in the mammary tissue. The largest negative consequences of the presence of SCC are related to shorter shelf life and less sensory content or undesirable organoleptic characteristics of the final product, due to enzymatic activities of somatic cells (Töpel, 2004).

The use of milk containing high SCC in yogurt production leads to negative effects on physical and chemical properties of the yogurt. The high presence of SCC in milk affects the activity of yogurt fermentation (Tamime and Robinson, 1999), and can even stop this process. Fernandes et al. (2007) studied the effect of SCC in raw milk on the chemical and physical properties of yogurt. The authors found that SCC in milk did not increase the extent of proteolysis of the resulting yoghurt. Over the 30 d of storage there was no change in viscosity of yoghurt manufactured from milk with low and intermediate SCC. In contrast, viscosity of yoghurt with high SCC increased with storage time. The investigations showed that increased SCC in milk led to an increase in free fatty acids in the resulting yoghurt during storage for 30 days that may result in a decrease in its shelf life. Based on these results the authors suggested that raw milk used to produce yoghurt should not contain more than 400,000 cells.mL^{-1}.

Studies on the impact of SCC on the quality of ayran are however scarce. Moreover, the impact of the high SCC in milk on the profile of carbonyl compounds and low molecular weight fatty acids has not yet been studied. Therefore, the aim of the present work was to determine the effects of SCC levels in raw cow milk on the carbonyl compounds and low molecular weight fatty acids formation in ayran.

2. MATERIAL AND METHODS

2.1. Milk samples

Raw bulk milk samples were collected from small-scale dairy farms affiliated with Diary Producer Associations in Biga district of Çanakkale province, (Turkey). More than 100 samples were brought to the laboratory of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) at 4 ℃ every week. SCC, TVC and composition of milk samples were measured. All analyses of raw milk were carried out in triplicate. For experimental ayran samples preparation were selected three different batches of raw milk with low (<400,000 cells.mL^{-1}), medium (between 500,000 and 600,000 cells.mL^{-1}) and high (1000,000 cells.mL^{-1}) SCC, respectively.

2.2. Ayran samples

Ayran samples were produced according to traditional method for Turkey from cow milks with different somatic cell counts according to the fallowing procedure: the raw milks from three batches (L, M and H) with three different SCC were accepted into the pilot dairy processing plant of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) and the platform tests (dry matter, fat, acidity and antibiotics) were carried out, clarification and fat ratio standardization procedures were performed at 55-60 ℃. Homogenization was carried out at 55 °C under 180 kg/cm^2 pressures. Standardization procedure (6%) of dry matter is realized by adding water and pasteurization process is performed at 95 °C for 5 minutes. Milk was cooled down to a fermentation temperature of 42-43 °C. Starter culture (1%) consisting of *Str. thermophilus* (30%) and *Lb. bulgaricus* (70%) bacteria is added in a process tank. After mixing inoculated milk is incubated at 41-43 °C until pH value reaches 4.50. After incubation, the curd was broken by stirring and rapid cooling to 4 °C in the processing tank. Simultaneously a 0.6% salt was added. After cooling ayran was poured in plastic caps and stored at refrigerated temperatures for 15 days.

2.3. Determination of SCC of raw milk

Bactocount IBCm (Bentley Instrument, USA) device was used for SCC determination. The milk fat, protein, lactose and total solids content of studied milk samples were measured by using Infrared Milk Analyzer 150 (Bentley Instrument, USA). The instrument was calibrated with certified reference milk samples from Italy Acredite Dairy Laboratories A.I.

2.4. Physicochemical analysis of ayran

The milk fat, protein, lactose and dry matter content of studied ayran samples were measured by using Infrared Milk Analyzer 150 (Bentley Instrument, USA). pH values were measured by pH meter. Lactic acid content of milk and ayran samples was calculated on the basis of the results for titratable acidity determined by titration method according to BNS 1111-80.

2.5. Analysis of volatile organic compounds in ayran samples

Volatile organic compounds in microalgae pastes were extracted by solid-phase microextraction (SPME) for gas chromatography-mass spectrometry (GC–MS) analysis. 0.2 g of the wet microalgal biomass was added to a 40 mL amber colored screw top vial with hole cap PTFE/silicon septa (Supelco, Bellafonte, USA), and 0.5 g of NaCl was added to the vial to increase the detection sensitivity of the SPME extraction technique, due to “salting-out” effect (Guichard, 2002; Zhang *et al.*, 2009). In order to optimize the technique, the effects of various parameters, such as the extraction time (20, 40 and 60 min) and the extraction temperature (40, 60 and 80 °C) in a water-bath were studied carefully for extraction efficiency. Based on the detection number of peaks with maintained consistency, the highest peak area and the best extraction conditions were selected as follows: 20 min of extraction time, 40 °C extraction temperature in water-bath. The vials were kept at 40 °C in a water-bath for 20 min to equilibrate the volatiles in headspace. Then, a SPME (2 cm to 50/30 μm DVB/Carboxen/PDMS, Supelco, Bellafonte) needle was inserted into the vial and exposed to the headspace for 20 min at 40 °C in a water-bath. The fiber type 50/30 μm DVB/Carboxen/PDMS was chosen for the extraction of volatile organic compounds due to its high sensitivity and extraction efficiency as tested by many researchers (Santos et al., 2016; Van Durme et al., 2013). The sample

was then immediately injected into the GC-MS. VOCs were separated by GC-MS using a non-polar column (DB5 30-m length x 0.32-mm i.d. x 0.25-µm film thickness; J&W Scientific, Folsom, CA). The GC–MS system consisted of an HP 6890 GC and 7895C mass selective detector (Agilent Technologies, Wilmington, DE, USA). The GC oven temperature was programmed to gradually increase from 40 °C to 230 °C at a rate of 10 °C min-1 with initial and final hold times of 3 and 15 min, respectively. Helium was used as a carrier gas at a flow rate of 1.5 mL/min.
Mass selective detector (MSD) conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range 35–350 amu; scan rate, 4.45 scans/s.
An internal standard mixture consisting of 2-methyl-3-heptanone (for neutral/basic compounds) and methyl nonanoate (for ester compounds) was added to all samples for quantification. A stock solution of the internal standard (IS) mixture was prepared at 0.82 µg/mL and 0.875 µg/mL in high-performance liquid chromatography (HPLC) grade methanol, respectively and samples were spiked with 10 µL of IS mixture (or 8.2 ng for 2-methyl-3-heptanone and 8.75 ng for methyl nonanoate).
Mean relative abundance (µg/kg) = concentration of IS x peak area of compound/peak area of IS.

2.6. Statistical analysis

Computer processing of the results was performed by using the program Microsoft Excel 2010. All determinations were carried out in triplicate and data were subjected to analysis of variance (ANOVA). ANOVA was carried out with the General Linear Models (GLM) with a significant level of $P<0.05$ (Draper and Smith, 1998). The Fischer's test with a significant difference set at $P<0.05$ was used to compare sample values (Kenward, 1987).

3. RESULTS AND DISCUSSION

Physicochemical analysis of ayran samples produced from milk with different SCC

The three raw milk batches used for ayran production, marked as L, M, and H, were characterized by a low (210 000 cells.mL^{-1}), medium (495 000 cells.mL^{-1}) and high (970 000 cells.mL^{-1}) somatic cell counts, respectively (Table 1). It can be seen that the three ayran batches were characterized by similar dry matter, fat, protein and lactose content. No statistically significant ($P<0.05$) differences were established in the pH values and the lactic acid content between samples L, M, and H. The results showed that the SCC values in the raw milk did not affect significantly ($P<0.05$) the content of the main components, the pH and the lactic acid concentration in the ayran produced. Our results were in agreement with the findings of Fernandes *et al.* (2006) and Oliveira *et al.* (2002) who reported that, the level of SCC does not seem to affect pH and titrable acidity, fat and protein content or microbiological characteristics of cows yogurts.

Table 1. SCC of raw milk and physicochemical characteristics of ayran samples.

Characteristics / Samples	SCC of raw milk, cells.mL^{-1}	Dry matter, %	Milk fat, %	Milk proteins, %	Residual lactose, %	pH	Lactic acid, %
Batch L	210 000	13.20±0,38	3.8±0,24	3.28±0,20	3.77±0,25	4.40±0,06	0.680±0,024
Batch M	495 000	13.26±0,40	3.7±0,19	3.27±0,18	3.87±0,28	4.35±0,05	0.682±0,032
Batch H	970 000	13.25±0,41	3.8±0,17	3.35±0,22	3.75±0,21	4.38±0,07	0.684±0,029

Analysis of carbonyl compounds and low molecular weight fatty acids in ayran samples produced from raw milk with different SCC

The analysis of the carbonyl compounds in the ayran test samples led to the identification and quantitative evaluation of the aldehydes hexanal and heptanal, and the ketones acetone, diacetyl, acetoin, 2-heptanone, and 2-nonanone (Figure 1, Figure 2 and Figure 3). Data were also obtained on the butanoic and hexanoic fatty acid content. The results on the content of the carbonyl compounds and low molecular weight fatty acids analysed in the ayran test samples have been presented in Figure 4. The quantitative analysis of the carbonyl compounds contained in the test samples of ayran showed the highest concentrations of acetone, diacetyl, acetoin, butanoic and hexanoic fatty acids. The concentrations of 2,3-pentanedione, hexanal and 2-heptanone were comparatively lower, and the contents of heptanal and 2-nonanone were the lowest. Practically, heptanal was only found in the ayran samples made from milks with lower SCCs (batch L).

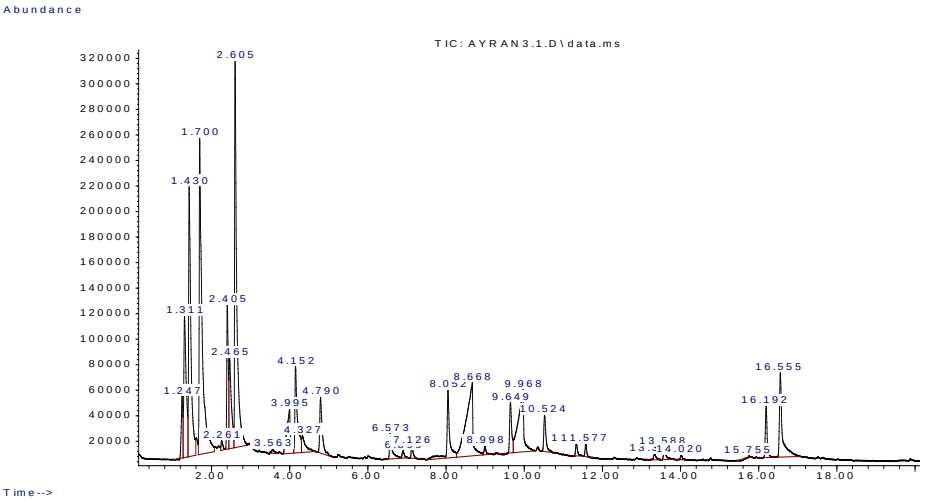

Figure 1. Chromatogram of carbonyl compounds and low molecular weight fatty acids in ayran samples produced from raw caw milk with low SCC (Batch L).

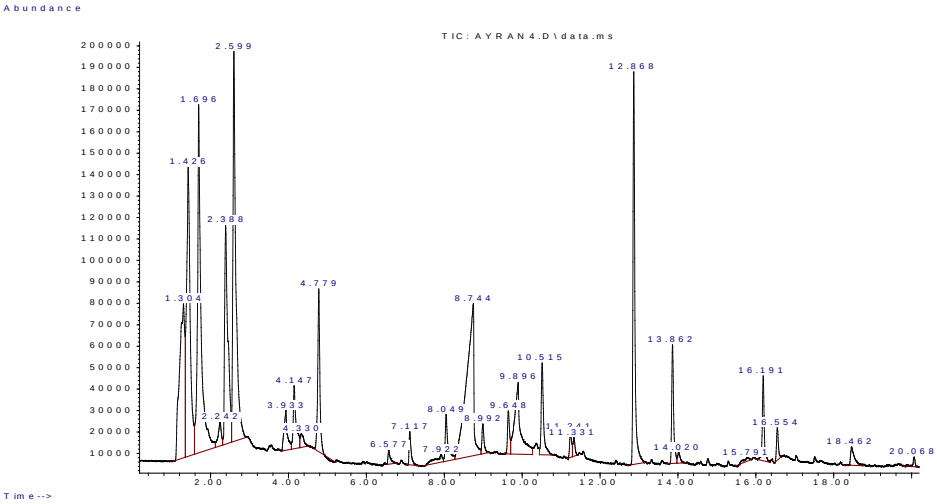

Figure 2. Chromatogram of carbonyl compounds and low molecular weight fatty acids in ayran samples produced from raw caw milk with medium SCC (Batch M).

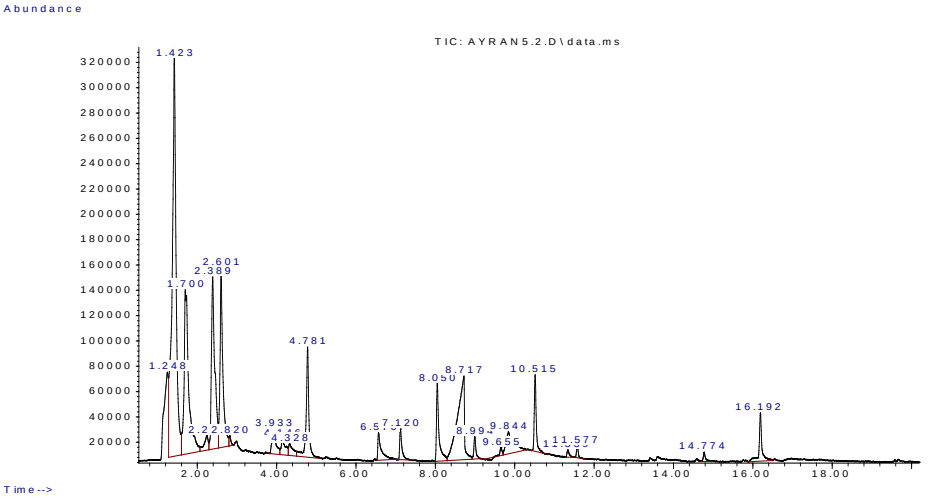

Figure 3. Chromatogram of carbonyl compounds and low molecular weight fatty acids in ayran samples produced from raw caw milk with high SCC (Batch H).

In the present study no statistically significant differences ($P<0.05$) were established in the concentrations of acetone, 2-heptanone, 2-nonanone, butanoic and hexanoic acid in the ayran samples made from milks with low or medium SCCs (batches L and M). The ayran samples from batches L and M demonstrated statistically significant differences ($P<0.05$) in the concentrations of diacetyl, 2,3-pentanedione, acetoin, hexanal and heptanal. In the ayran samples from batch M, a lower content of diacetyl, acetoin, hexanal, and heptanal, and a higher content of 2,3-pentanedione compared to batches L were established. The same trend was observed in the ayran samples with a higher SCC (batch H). They showed increased concentrations of acetone and butanoic acid compared to the ayran samples with low and medium SCCs (batches L and M). This study did not establish any statistically significant differences ($P<0.05$) in the hexanoic acid and 2-nonanone content in all test samples of ayran.

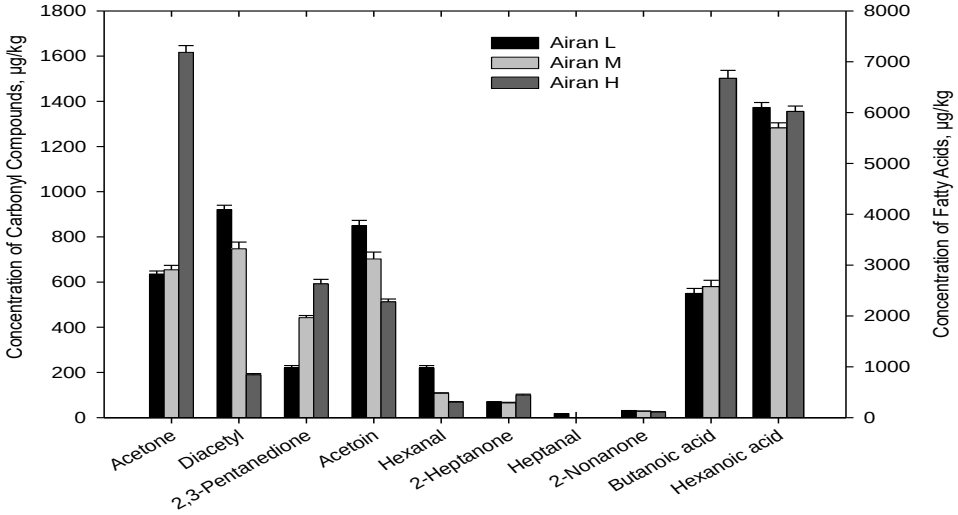

Figure 4. Carbonyl compounds and low molecular weight fatty acids contents of test ayran samples (batches L, M and H).

These results (Figure 4) showed that even within the limits up to 500,000 cells.mL^{-1}, the somatic cell count in the milk affected the carbonyl compound profile in the ayran. The SCC increase in the milk was related to a reduction in the content of the compounds which had a positive effect upon the ayran aroma, such as diacetyl and acetoin, at the expense of the increased share of compounds such as acetone, 2,3-pentanedione and butanoic acid. The trends observed showed that the somatic cell count in the milk had considerable influence on the progress of the biochemical processes leading to the formation of the substances which form the ayran aroma.

The higher butanoic acid content in the ayran samples made from milks with higher SCCs could be explained by the occurrence of lipolytic processes, which are more intensive in mastitis milk. The most important consequence of lipolysis in dairy products is the development of off-flavours associated with rancidity, which is caused mainly by short chain FFA (Deeth, 1993; Muir, 1996). Vivar-Quintana et al. (2006) also reported that the SCC exceeding 3,000,000 cells.mL^{-1} in ewes yogurt can induce off-flavor development.

4. CONCLUSION

The results obtained in the present study showed that the SCC of the raw milk did not have a significant effect on the composition of ayran. With the increase of SCC in milk a negative changes in the profile of the carbonyl compounds in ayran were induced. Lower concentrations of diacetyl, acetoin, hexanal, and heptanal, and a higher concentrations of acetone, 2,3-pentanedione and butanoic acid in ayran samples produced from raw milks with higher SCC were established. Such changes in the profile of the carbonyl compound of ayran could lead to the appearance of sensory defects and, respectively, to shelf life reduction. The carbonyl compound profile in the ayran was influenced by the SCC values of raw milk, even within the limits up to 500,000 cells.mL^{-1}.

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Effect of Somatic Cells Count of Raw Milk on the Proteolysis in Farmers Cheese

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ABSTRACT

The aim of the present study was to investigate the effect of somatic cells count (SCC) of raw cow milk on the proteolysis in Farmers cheese. Test cheese samples were produced from three different batches of cow milk with low (up to 200,000 cells/cm^3, batch L), medium (between 500,000 and 600,000 cells/cm^3, batch M) and high (above 1500,000 cells/cm^3, batch H) SCC, respectively. Test cheese samples were aged and cold stored at 4±1 °C for 3 and 7 months, respectively. The proteolysis in Farmers cheese was evaluated on the basis of the nitrogen fractions ratios as water-soluble to total nitrogen (WSN/TN), non-casein (soluble at pH=4.6) to total nitrogen (NCN/TN), non-protein (soluble in tricloroacetic acid) to total nitrogen (NPN/TN), as well as the changes in the free amino groups content. The results obtained indicated for more intensive proteolysis during the ageing and cold storage of cheeses made from milk with a SCC exceeding 500,000 cells/cm^3. At the end of the experiment (10th month), the WSN/TN values in the cheese samples from batches L, M, and H reached 18.6±0.8 %, 20.2±0.9 % and 28.4±0.8 %, respectively. For the same period, the free amino groups content in the cheeses from batches L, M and H reached 42.4±1.4 mg/100 g; 59.1±1.3 mg/100 g and 83.6±1.5 mg/100 g, respectively. The increased proteolysis in Farmers cheese made from milks with higher SCC could induce the appearance of sensory quality defects and thus to reduce the cheese shelf life.

Keywords: Farmers cheese, Free amino groups, Nitrogen fractions, Proteolysis, Somatic cells.

1. INTRODUCTION

The abundance of locally produced foods in Turkey is accepted as a part of the cultural richness. The local products especially need to be protected and industrialized. At the same time, it is also an important issue for the region. Providing and increasing raw milk quality is very important both in terms of product quality and human health. By obtaining raw milk quality many benefits will be achieved. At first, microbial degradation will be prevented, and human health will be protected. In addition, keeping animal diseases such as Mastitis under control will prevent losses in milk yields and animals.

Raw milk quality is not only important in terms of food safety but also a factor affecting the quality of the processed product. Many studies have been carried out to determine the effect of SCC on the yield and quality of milk and dairy products, especially cheeses (Cooney *et al.*, 2000; Le Maréchal *et al.*, 2011). It is well known that the higher SCC in raw milk causes adverse effects on raw milk and dairy products quality. The impact of SCC of raw milk on cheese quality is related mainly with the impairing milk coagulation properties, increasing moisture content in most cheeses and inducing cheese off-flavor development (Andreatta *et al.*, 2007; Mazal *et al.*, 2007, Vianna *et al.*, 2008). The largest negative consequences of the higher SCC are related to shorter shelf life and less sensory content or undesirable organoleptic characteristics of the final product, due to enzymatic activities of somatic cells (Töpel, 2004). Mikulec *et al.* (2012) have reported yield loss and quality deficiencies in cheeses produced from mastitis milks with high SCC obtained from Simmental breed cattle. Cheese production from milk with high SCC has been reported to be lower than from low SCC milk (Everson, 1980). The proteolytic effects of somatic cells on main caseins in cheese are different if cheeses are made with raw or pasteurized milk. The effects of somatic cells on cheese ripening also depend on the pretreatment of milk. Mikulec *et al.* (2012) have researched the effects of SCC of milk from the Simmental breed used for cheese production in Serbia on the cheese making process. The authors stated that cheese samples made from milk with low SCC had positive effects on the yield and on cheese structure. Sılva *et al.*, (2012) found the significant increase in the total protein ratio of whey from high SCC milk, and as a result, the loss of yield in cheese was observed.

The aim of the present study was to determine the effects of SCC levels in raw cow milk on the proteolysis in Farmers cheese.

2. MATERIAL AND METHODS

2.1. Milk samples

Raw bulk milk samples were collected from small-scale dairy farms affiliated with Diary Producer Associations in Biga district of Çanakkale province, (Turkey). More than 100 samples were brought to the laboratory of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) at 4 ℃ every week. SCC, TVC and composition of milk samples were measured. All analyses of raw milk were carried out in triplicate. For experimental ayran samples preparation were selected three different batches of raw milk with low (<400,000 cells/cm^3), medium (between 500,000 and 600,000 cells/cm^3) and high (1000,000 cells/cm^3) SCC, respectively.

2.2. Farmers cheese

Farmers cheese samples were produced according to traditional methods from cow milks with different somatic cell counts according to the fallowing procedure: the raw milks from three batches (L, M and H) with three different SCC were accepted into the pilot dairy processing plant of Çanakkale Onsekiz Mart University-Biga Highschool (Turkey) and the platform tests (dry matter, fat, acidity and antibiotics) were carried out, pasteurization procedures were realized at 68 °C for 15 minutes. Milk was cooled down to coagulation temperature of 35-36 °C. Starter culture consisting of 70% Streptococcus thermophilus and 30% Lactobacillus bulgaricus and cheese rennet were added. After 90 minutes of coagulation, the curd was sliced into nut sized curd grains and a portion of the whey is removed. After 5 minutes of curd ventilation process, curd and the remaining whey were heated at 41-42 °C for 15 minutes. At the end of the heating process, raw cheese is pressed for 2-3 hours without applying pressure. Yong cheeses are then removed from the molds and salted in 16% NaCl solution at 15-18 ℃ for 24 hours. After salting young cheese was taken out, dried and packed in polyamide/polyethylene foil under vacuum at 90-99.8 Pa. Ripening took place in these packages at t=4±1 ℃ and relative humidity of 75-80% for 3 months. After that the cheese samples were subjected to cold storage at t=4±1 ℃ for another 7 months (up to the 10th month after production).

2.3. Determination of SCC and Total viable count of raw milk

Bactocount IBCm (Bentley Instrument, USA) device was used for SCC determination. The milk fat, protein, lactose and total solids content of studied milk samples were measured by using Infrared Milk Analyzer 150 (Bentley Instrument, USA). The instrument was calibrated with certified reference milk samples from Italy Acredite Dairy Laboratories A.I.
Total viable count (TVC) was determined by using Plate Count Agar medium according to ISO 4833-2:2013. Inoculated petri dishes were subjected to incubation at 30 ℃ for 48 to 72 hours and colony forming units (CFU) were counted on petri dishes.

2.4. Physicochemical analysis of cheese

Fat content of cheese samples was determined according to Gerber-Van Gulik method (BNS 1671-89). Dry matter and water content was determined by heating samples at 105 ℃ to constant weight. Sodium chloride content of cheese was measured according to BNS 8274-82. pH values of cheeses were determined by using of pH meter MS 2011 (Microsyst, Plovdiv, Bulgaria), equipped with a pH electrode Sensoret (Garden Grove, CA, USA). Lactic acid content of cheese samples was measured by titration method according to BNS 1111-80.

2.5. Water soluble nitrogen (WSN), Noncasein nitrogen (NCN), nonprotein nitrogen (NPN) and total nitrogen (TN) content

Water soluble nitrogen, noncasein nitrogen, nonprotein nitrogen and total nitrogen content were determined by the Vakaleris and Price method (1959) modified to suit the specific conditions of the analysis. For water soluble nitrogen (WSN) and noncasein nitrogen (NCN) determination, approximately 5 g of cheese was extracted in 100 mL deionized water or sodium acetate buffer (pH 4.6), respectively. The homogenate was agitated at ambient temperature for 2 h and filtered. The nitrogen fraction soluble in 12% trichloracetic acid (TCA) was considered the nonprotein nitrogen (NPN). To determine the NPN content, approximately 5 g of cheese were homogenized in 40 mL sodium acetate buffer (pH=4.6), the homogenate was agitated at ambient temperature for 2 h, then 10

mL of 60% TCA was added and the homogenate was filtered through Watman No 42 paper. Nitrogen determination was performed in duplicate by the Kjeldahl method using a Kjeltec Auto 1030 Analyzer (Tecator Sweden) combined with the Digestion System 20. Total protein (TP) was calculated as total nitrogen multiplied by coefficient of 6.38.

2.6. Free amino groups in cheese samples

Water extracts of cheese samples were prepared according to the procedure described by Mayer *et al.* (1998). Free amino groups of amino acids were determined by the reaction with ninhydrin with cadmium in cheese water extract according to the procedure described by Folkertsma and Fox (1992).

2.7. Statistical analysis

Computer processing of the results was performed by using the program Microsoft Excel 2010. All determinations were carried out in triplicate and data were subjected to analysis of variance (ANOVA). ANOVA was carried out with the General Linear Models (GLM) with a significant level of $P<0.05$ (Draper and Smith, 1998). The Fischer's test with a significant difference set at $P<0.05$ was used to compare sample values (Kenward, 1987).

3. RESULTS AND DISCUSSION

Physicochemical and microbiological analysis of raw milk and cheese samples with different SCC

The results from the physicochemical and microbiological analysis of the raw milk used for obtaining the cheese test samples have been presented in Table 1. The three raw milk batches used were marked as L, M and H, and were characterized by a low, medium and high SCC, respectively. No statistically significant ($P<0.05$) differences were found in the total viable count values of the three test batches of raw milk. Probably, this was related to the similar hygiene conditions during the obtaining and storage of the raw milk used in the present study. In this case, the higher SCC in batches L and M was most probably due to the increased share of mastitis milks added to the bulk milk.

Table 1. Microbiological and physicochemical characteristics of raw milk used for production of cheese samples.

Parameter / Samples	SCC, Cells/cm^3	TVC, CFU/cm^3	Dry matter, %	Fat, %	Proteins, %	Lactose, %	pH
Batch L	106 000	$9.4\pm0.5.10^5$	12.45±0.12	3.67±0.09	3.21±0.07	4.62±0.04	6.57±0.07
Batch M	556 000	$9.7\pm0.7.10^5$	12.42±0.16	3.66±0.08	3.28±0.06	4.58±0.05	6.59±0.05
Batch H	1 533 000	$1.2\pm0.3.10^6$	12.41±0.13	3.73±0.08	3.30±0.08	4.47±0.03	6.61±0.05

It can be seen (Table 1) that regardless of the differences in SCC, the content of milk fat, proteins and dry matter in the three batches was similar. Slightly lower lactose values were observed in the milks with high SCCs (batch H) compared to the other milk batches. No statistically significant ($P<0.05$) differences were established in the pH values between the three batches of raw milk used in the current study for obtaining the test samples of cheese.
The results of the physochemical analysis of the cheese samples have been presented in Table 2.

Table 2. Physicochemical characteristics of cheese samples.

Parameters / Samples	Moisture content, %	Dry matter, %	Fat, %	NaCl, %	Proteins, %	pH	Lactic acid, %
Batch L	47.48±0.42	52.52±0.46	32.12±0.32	2.15±0.12	18.2±0.27	5.84±0.05	0.846±0.044
Batch M	47.52±0.45	52.48±0.38	31.67±0.28	2.11±0.09	18.6±0.22	5.81±0.05	0.855±0.057
Batch H	48.19±0.58	51.81±0.41	32.13±0.45	2.08±0.11	17.9±0.31	5.85±0.04	0.837±0.038

It is evident that the cheeses in all batches were characterised by similar values of the dry matter and moisture content respectively, milk fat content, proteins, and NaCl. Mazal *et al.* (2007) also reported that the SCC did not affect the protein and fat contents of the Prato cheese or the fat loss to the whey. No statistically significant ($P<0.05$) differences were established in the pH values and lactic acid content between the cheeses in batches L, M and H. This indicated a similar acidification rate diring production of the cheese test samples.
The results obtained showed that the SCC in the raw milk had no significant influence on the content of the main components, active acidity and lactic acid concentration in the cheese produced.

Effect of SCC in raw milk on the proteolysis during ageing and cold storage of Farmers cheese

The proteolytic processes occurring in the cheese matrix during ageing and cold storage are accompanied by partial hydrolysis of the milk proteins to lower molecular weight compounds: polypeptides, dipeptides, free amino acids, biogenic amines, ammonia and other low molecular weight compounds. This process plays an important role in the formation of the characteristic taste, aroma and texture of the ripened cheese. In the test cheese samples these processes were catalyzed by the rennet enzyme (chymosin) and the native proteases of milk, such as plasmin, on the one hand, and by the proteolytic enzymes (proteinases, polypeptidases, etc.) of the starter and non-starter microflora on the other hand. From this point of view, the proteolysis in the cheese was largely affected by the growth and proteolytic activity of the lactic acid microflora.
For evaluation of the proteolysis in the test cheese samples in the present study, the ratios of the different nitrogen fractions, i.e. water-soluble to total nitrogen (WSN/TN), non-casein (soluble at pH=4.6) to total nitrogen (NCN/TN), non-protein (soluble in tricloroacetic acid) to total nitrogen (NPN/TN), as well as the changes in the free amino groups were established. The WSN/TN and NCN/TN provided information on the share of the milk proteins that had undergone hydrolytic changes in the process of cheese ageing. They are commonly used for evaluation of the cheese ripening degree since they provide a general characteristic of the proteolysis.
The results on the changes in WSN/TN and NCN/TN in the cheese samples studied have been presented in Figure 1 and Figure 2, respectively. It can be seen that the values of the two indicators increased during the ageing and cold storage of all test samples. This tendency was the most pronounced in the cheese samples made from milks with SCC exceeding 500,000 cells/cm^3 (batch H).

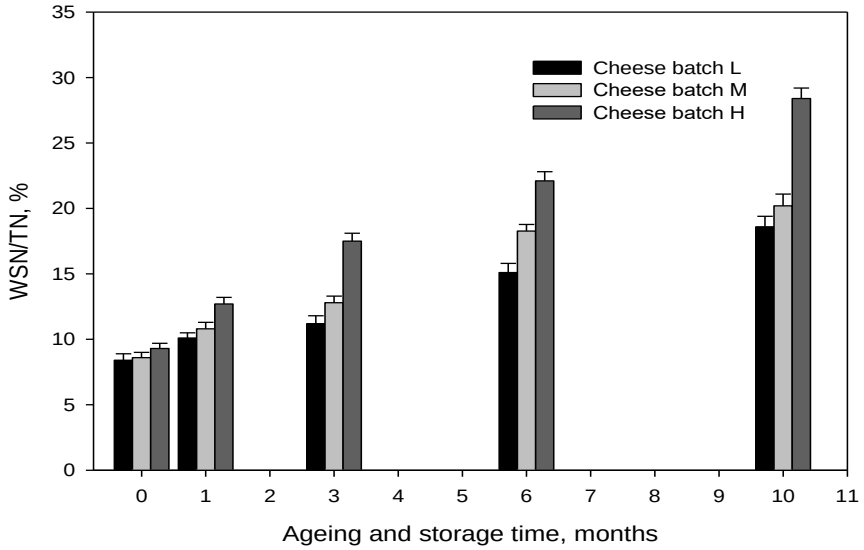

Figure 1. Changes in the WSN/TN ratio during ageing and storage of Farmers cheese produced from raw milk with low (Batch L), medium (Batch M) and high SCC (Batch H).

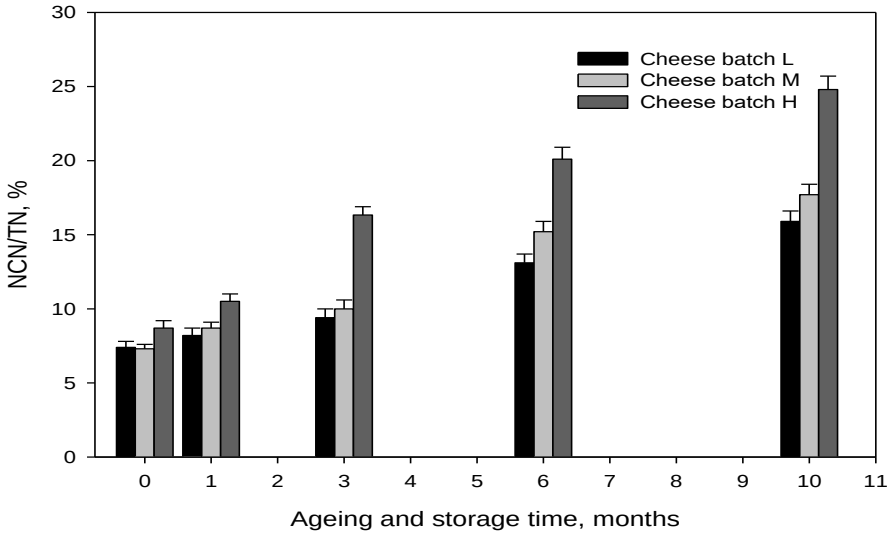

Figure 2. Changes in the NCN/TN ratio during ageing and storage of Farmers cheese produced from raw milk with low (Batch L), medium (Batch M) and high SCC (Batch H).

Within the 3-month ageing period, the WSN/TN values of the cheeses from batches L and M increased from 8.4±0.5% to 11.2±0.6% and from 8.6±0.4% to 12.3±0.5% respectively. For the same period, the NCN/TN values for the test samples from batches L and M increased from 7.4±0.4% to 9.4±0.6% and from 7.3±0.3% to 10.0±0.6% respectively. At the end of the ageing process (third month), no statistically significant ($P<0.05$) differences were established in the WSN/TN and NCN/TN values between the batch L and batch M cheeses. In contrast, the batch H cheeses had significantly ($P<0.05$) higher values of these two indicators at the end of ageing: 17.5±0.6% and 16.3±0.5%, respectively, for WSN/TN and NCN/TN. These results indicated more intensive proteolysis during the ageing of cheeses made from milk with a SCC exceeding 500,000 cells/cm^3.

Other authors (Andreatta *et al.*, 2007; Wedholm *et al.*, 2008; Marino *et al.*, 2005) also reported for higher levels of proteolysis in cheeses made with high SCC regardless the cheese type. According to Le Maréchal *et al.* (2011) mastitis milk influences primary proteolysis of caseins. The authors reported for increased proteolysis of β-caseins during the early stages of cheese ageing which induces an accelerated breakdown of α_{S1}-casein. Mazal *et al.* (2007) found that the pH 4.6 SN (NCN) was significantly higher in Prato cheeses produced from high-SCC milk, indicating a more intensive proteolysis in fresh cheese from high-SCC milk (5 d after manufacture). According to other authors, plasmin and its activators were probably incorporated into the curd (Fox *et al.*, 2000) and could thus have participated in the breakdown of the cheese protein (Klei *et al.*, 1998; Kalit *et al.*, 2002).
An increase in the WSN/TN and NCN/TN values was also observed during the cold storage of the cheese test samples (from the 3rd to the 10th month). At the end of the cold storage (10th month), the WSN/TN values in the cheese samples from batches L, M, and H reached 18.6±0.8%, 20.2±0.9% and 28.4±0.8%, respectively. For the same period, the NCN/TN values in the cheeses from batches L, M and H reached 15.9±0.7%, 17.7±0.7% and 24.8±0.9%, respectively. These data indicated that the trend towards more intensive proteolysis in the cheeses made from milk with high SCCs observed in the ageing process continued during their cold storage, too.
The results obtained on the changes in the NPN/TN ratio and the content of free amino groups in the cheese samples studied have been presented in Figure 3 and Figure 4, respectively. The changes in the values of these two indicators reflected the deep hydrolysis of the proteins in the cheese matrix to low molecular weight compounds such as dipeptides, free amino acids, biogenic amines, ammonia, etc. (the depth of cheese ageing, respectively).

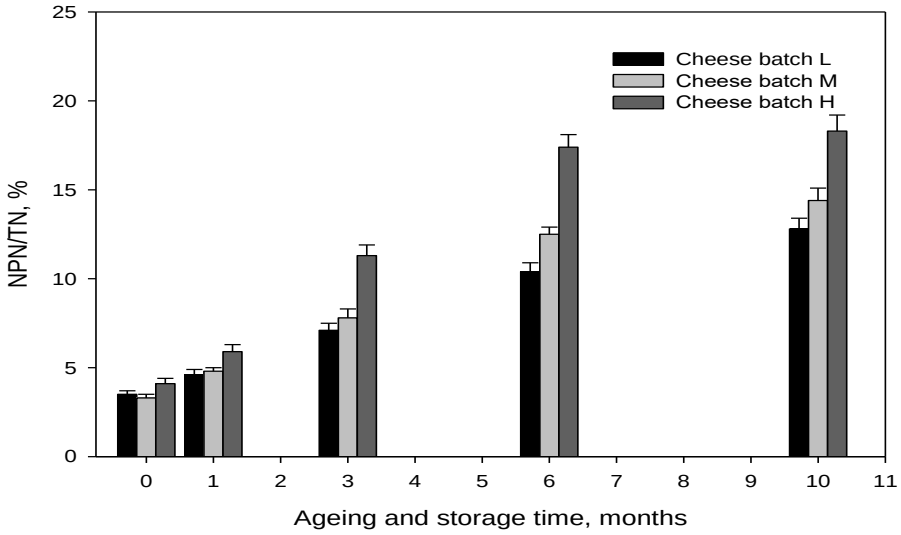

Figure 3. Changes in the NPN/TN ratio during ageing and storage of Farmers cheese produced from raw milk with low (Batch L), medium (Batch M) and high SCC (Batch H).

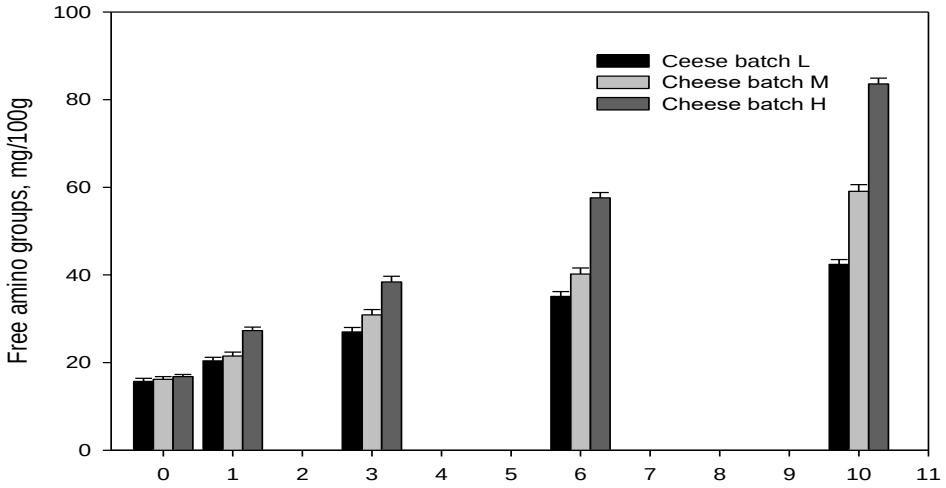

Figure 4. Changes in the free aminogrups content during ageing and storage of Farmers cheese produced from raw milk with low (Batch L), medium (Batch M) and high SCC (Batch H).

The data obtained (Figure 3 and Figure 4) showed that the values of the two indicators increased during the ageing and cold storage process in all test samples. Again similarly to the two preceding indicators (WSN/TN and NCN/TN), this trend was the most pronounced in the cheese samples made from milks with SCCs exceeding 500,000 cells/cm^3 (batch H).

Within the 3-month ageing period, the NPN/TN values of the cheeses from batches L and M increased from 3,5±0,2% to 7,1±0,4% and from 3,3±0,2% to 7,8±0,5% respectively. For the same period, the free amino groups content of the test samples from batches L and M increased from 15.7±0.7 mg/100 g to 27.1±0.8 mg/100 g and from 16.2±0.6 mg/100 g to 30.9±0.9 mg/100 g respectively. At the end of the ageing process (third month), no statistically significant ($P<0.05$) differences were established in the NPN/TN and free amino groups content between the batch L and batch M cheeses. In contrast, the batch H cheeses exhibited considerably higher values ($P<0.05$) of these two indicators at the end of ageing: 11.3±0.6% and 38.4±1.2 mg/100 g, respectively, for NPN/TN and free amino groups content.

These results showed that the highest degree of proteolysis of caseins to low molecular weight compounds was observed during the ageing of milks with SCCs exceeding 500,000 cells/cm^3. These results were in accordance with the findings of Mazal *et al.* (2007) who established that NPN values were significantly higher in high SCC whey than in low SCC whey. The authors found that the cheese from high SCC milk showed, on average, more intense proteolysis, as measured by the significantly higher NCN content. According to Fox *et al.* (2000) proteolysis is characterized by primary and secondary reactions that are followed by increases in NCN and NPN, respectively. As a result of degradation of the small peptides a simple amine components were liberated, favoring a significant increase in NPN values. Kalit *et al.* (2002) also observed a more intense proteolysis during ageing in the cheese from milk with a high SCC. Other studies have demonstrated higher proteolysis in Swiss cheese (Cooney *et al.*, 2000) and in Podravec cheese (Kalit *et al.*, 2002) manufactured from milk with high levels of SCC.

An increase in the NPN/TN ratio and free amino groups content was also observed during the cold storage of the cheese test samples (from the 3rd to the 10th month). At the end of the cold storage (10th month), the NPN/TN values in the cheese samples from batches L, M, and H reached 12.8±0.6%; 14.4±0.7% and 18.3±0.9%, respectively. For the same period, the free amino groups content in the cheeses from batches L, M and H reached 42.4±1.4 mg/100 g; 59.1±1.3 mg/100 g and

83.6±1.5 mg/100 g, respectively. These data indicated that the trend towards more intensive hydrolysis of the proteins to low molecular weight compounds in the cheeses made from milk with high SCCs observed in the ageing process continued during their cold storage, too.
The more active proteolytic process established in this study during the ageing and cold storage of cheeses made from milks with SCCs exceeding 500,000 cells/cm^3 was a prerequisite for the appearance of taste defects, which was also confirmed by the organoleptic analysis of the samples investigated (data not shown). The greater hydrolysis of the α_{s1}-CN in cheese made with high-SCC milk could be responsible for the defects in Cheddar cheese texture described by other authors (Grandisson and Ford, 1986; Mitchell et al., 1986; Rogers and Mitchell, 1994).

4. CONCLUSION

The results obtained in the present study showed that the SCC of the raw milk has a significant effect on the proteolysis during ripening of Farmers cheese. Higher levels of proteolysis were established in cheeses made from raw milk with SCC exceeding 500,000 cells/cm^3. After 10 months of ripening and cold storage the WSN/TN, NCN/TN and NPN/TN values of these samples reached 28.4±0.8 %, 24.8±0.9% and 18.3±0.9%, respectively. For the same period, the free amino groups content of cheeses from high SCC milk reached 83.6±1.5 mg/100 g. The more intensive proteolytic process during the ageing and cold storage of Farmers cheese made from milks with higher SCC is associated with the appearance of sensory quality defects and cheese shelf life reduction. Therefore, the appropriate control of SCC in raw cow milk is important both for the cheese quality and storability.

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Growth and Activity of Probiotic Bacteria in Fermented Milks Fortified with Polyphenol Extract from Strawberry By-product

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ABSTRACT

The objective of the present study was to investigate the effect of polyphenol-enriched extracts from strawberry press residues (SPE) on lactic acid production and the viability of *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus* and *Streptococcus thermophilus* in fermented milks during 2 weeks of refrigerated storage. Ethanol-water strawberry extracts (0.39 mg GAE/mL) were added into milk-starter culture mixture (S1 and S2) and incubated at 44±2 ºC until the pH reached 4.5. Fermented milks, without the addition of phenolic compounds, were used as controls. All fermented milks were stored at 4±2 ºC for 15 days and samples were analyzed for pH, lactic acid content, and residual lactose content and viable counts of yogurt bacteria. A decrease in pH values (from 4.40±0.06 to 4.05±0.11) and an increase in the lactic acid concentration (from 8.33±0.10 g/L to 11.12±0.41 g/L), was observed in all studied samples. Addition of polyphenol-enriched extracts from strawberry by-product did not affect significantly ($p>0.05$) the viability of *Lactobacillus spp.* and *S. thermophilus* during the refrigerated storage. It was found that the fermented milks fortified with polyphenol extract from strawberry by-product preserved a high *S. thermophilus* and *Lactobacillus spp.* counts (above Log 8) through refrigerated storage and this can ensure health benefits to be delivered to consumers on daily consumption of these products.

Keywords: Fermented Milks, Functional Foods, Post-acidification, Probiotic Bacteria, Strawberry Polyphenols.

1. INTRODUCTION

Fermented milks have high consumer acceptance and positive health promoting ingredients such as ingested live microorganisms (probiotics) included in starter culture (Allgeyer *et al.*, 2010). The viable and active yogurt bacteria are considered to be beneficial for human health (Shah, 2000), and according to National Yoghurt Association (NYA) regulations for refrigerated yogurts, the cultures must be viable at the end of the stated shelf life (Chandan and O'Rell, 2006). Probiotic microorganisms have been primarily incorporated into dairy products (Alexandraki *et al.*, 2013) and it has been demonstrated that the survival of probiotic cultures in milk products can be increased by ingredients that are rich in phenolic compounds (Ma *et al.*, 2015). Polyphenols have a large interest since they are mainly associated with the prevention of cancer and coronary heart diseases (Servili *et al.*, 2009). Antioxidant and anti-inflammatory properties are also reported for dietary polyphenols (Covas *et al.*, 2006). Phenolic compounds are known as antimicrobial agents, inhibiting the growth of pathogenic bacteria and fungi (O'Connella and Fox, 2001) as well as lactic acid bacteria (Rodríguez *et al.*, 2009). The biological benefits of polyphenols depend on their bioavailability. For example, a significant portion of dietary polyphenols is not absorbed in the small intestine and can thus interact with colonic microbiota (Manach *et al.*, 2004). These compounds reach the colon, where they are deglycosylated and metabolized by microbial enzymes (Velderrain-Rodríguez *et al.*, 2014). Their metabolites are also described as modulators of human gut microbiota (Cardona *et al.*, 2013). Polyphenols may act as promoting factors of growth, proliferation or survival for beneficial gut bacteria – mainly *Lactobacillus* strains - and thus exerts prebiotic actions that inhibit the proliferation of some pathogenic bacteria such as *Salmonella* and *Helicobacter pylori* species (Hervert-Hernández and Goñi, 2011). Probiotic bacteria and polyphenols have already demonstrated health-promoting properties (Maukonen and Saarela, 2014).

It has been found that in the production of probiotic functional products polyphenols may influence the metabolic activity of microorganisms as well as the *in vitro* and *in vivo* viability of probiotic starter cultures (Duda-Chodak *et al.*, 2008). From a technological point of view, the incorporation of polyphenol-enriched extracts can be an effective way to increase the health benefits of functional foods such as fermented milk (Servili *et al.*, 2011).

Fruits are well known for their functional potential and more recently, fruit by-products have gained attention due to their higher nutritional contents in comparison to their respective edible portion (Can-Cauich *et al.*, 2017). Sójka *et al.* (2013) reported that industrial strawberry press residues that remain after juice processing amounts to about 4% of the initial weight of raw material. By-products, remaining after processing fruits and vegetables in the food processing industry, still contain a significantly huge amount of phenolic compounds. Some studies have already been done on by-products, which could be potential sources of antioxidants (Volf and Popa, 2004).

The objective of the present study was to evaluate the growth and activity of *Lactobacillus bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13 in fermented milks fortified with polyphenol-enriched extracts from strawberry press residues (SPE) during 15 days of refrigerated storage.

2. MATERIAL AND METHODS

2.1. Materials

Plant materials: The strawberry by-products were supplied by Santulita Ltd. (Sofia, Bulgaria). The press residues were stored frozen at -18 ℃ until used to produce the polyphenol extracts.

Bacterial strains: The starter cultures of lactic acid bacteria used in the present study consisted of *Lactobacillus delbrueckii* subsp. *bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13. They belong to the laboratory collection of the Department of Microbiology at the UFT, Plovdiv.

Polyphenol extract from strawberry press residues: Polyphenol-enriched extracts from strawberries were obtained by adsorption technology. To remove the sugars, salts and amino acids, the extracts were purified using a column (465×30 mm) filled with Amberlite XAD 16 HP. The purified extracts were lyophilized for 48 h.

Yogurt samples: The control and experimental batches of fermented milks were prepared in laboratory conditions (Department of Milk and Dairy Products Technology at the UFT, Plovdiv, Bulgaria) from a single vat of milk according to the following procedure: cow's milk (M = 3.7%) was heated to 95 ℃ for 15 min, cooled to t=45±1 ℃ and divided into two lots: one experimental lot (Batches S1 and S2) fortified with polyphenols to 0.39 mg GAE/mL using SPE, and an unfortified control lot (Batches K1 and K2). The experimental and control batches of milk were inoculated with 2% Bulgarian yogurt starter culture consisted of *Lb. bulgaricus* : *Str. thermophilus* in ratio of 1:5 (samples K1 and S1) and starter culture consisted of *Lb. bulgaricus* : *Lb. rhamnosus* : *Str. thermophilus* in ratio of 0.5:0.5:5 (samples K2 and S2). All samples were packaged in containers and incubated at t=44±1 ℃ until the pH reached 4.6. At the end of the incubation, the fermented milks were cooled down to approximately 4 ℃ and then stored at the same temperature for 15 days.

2.2 Methods

Microbiological analysis: Viable cell counts of *Str. thermophilus* S13, *Lb. delbrueckii* subsp. *bulgaricus* S19 and *Lb. rhamnosus* YW were determined every 3rd day during the storage period by cultivations on synthetic culture media M17 and MRS (Merck, Darmstadt, Germany). The methodology described in Standard EN ISO 7889 was followed. The samples were prepared according to ISO 8261:2001. *Lb. bulgaricus* and *Lb. rhamnosus* were counted on MRS (Merck, Darmstadt, Germany) spread plates in which the pH had been adjusted to pH 5.4 and incubated at 41 ℃ for 48 h. *Str. thermophilus* was counted on M17-lactose (Merck, Darmstadt, Germany) spread plates after incubation at 37 ℃ for 48 h.

Physicochemical analyses: The pH values of the samples were determined with an MS 2011 pH meter (Microsyst, Plovdiv, Bulgaria) equipped with a Sensoret pH electrode (Garden Grove, CA, USA). The lactic acid content was determined by the titration method (ISO 11869:1997). The residual lactose content was calculated on the basis of the results for initial lactose content in milk and lactic acid formation during the storage period.

Statistical analysis: The results reported in the present study are expressed as the mean values of at least three analytical determinations. The coefficient of variation expressed as percentage ratios

between the standard deviations and the mean values was found to be <5% in all cases. The means were compared using one-way ANOVA performed with Microsoft Excel and Tukey's test at a 95% confidence level.

3. RESULTS AND DISCUSSION

The results for the changes in the starter lactic acid bacteria count, the pH values, lactic acid and residual lactose concentrations during refrigerated storage of controls and polyphenol-enriched fermented milks are presented in Figure 1 and Figure 2. During the storage period of fermented milk samples, from the 1st to the 15th day, a constant tendency to decrease in the pH values from 4.40±0.06 to 4.05±0.11 and an increase in the lactic acid concentration from 8.33±0.10 g/L to 11.12±0.41 g/L, respectively, were observed. All fermented milk samples showed a more intense decrease in pH and lactic acid concentration from day 1 to day 9 and a slower change between the 9th and 15th day (Figure 1 and Figure 2). The residual lactose content of the control and test samples at the end of the refrigerated storage was in the range of 31.44±0.39 g/L. It could be seen that (Figure 1 and Figure 2) during the refrigerated storage period the pH values and lactic acid concentration in the controls and polyphenol-enriched fermented milks did not differ significantly ($P < 0.05$). This indicates that the addition of polyphenol extracts from strawberry presses residues in fermented milk did not affect the post-acidification process. Our results were in agreement with the findings of Pelaes Vital *et al.* (2015), who reported that during the storage period of polyphenol-enriched fermented milks the pH values were similar to those of the respective control samples.

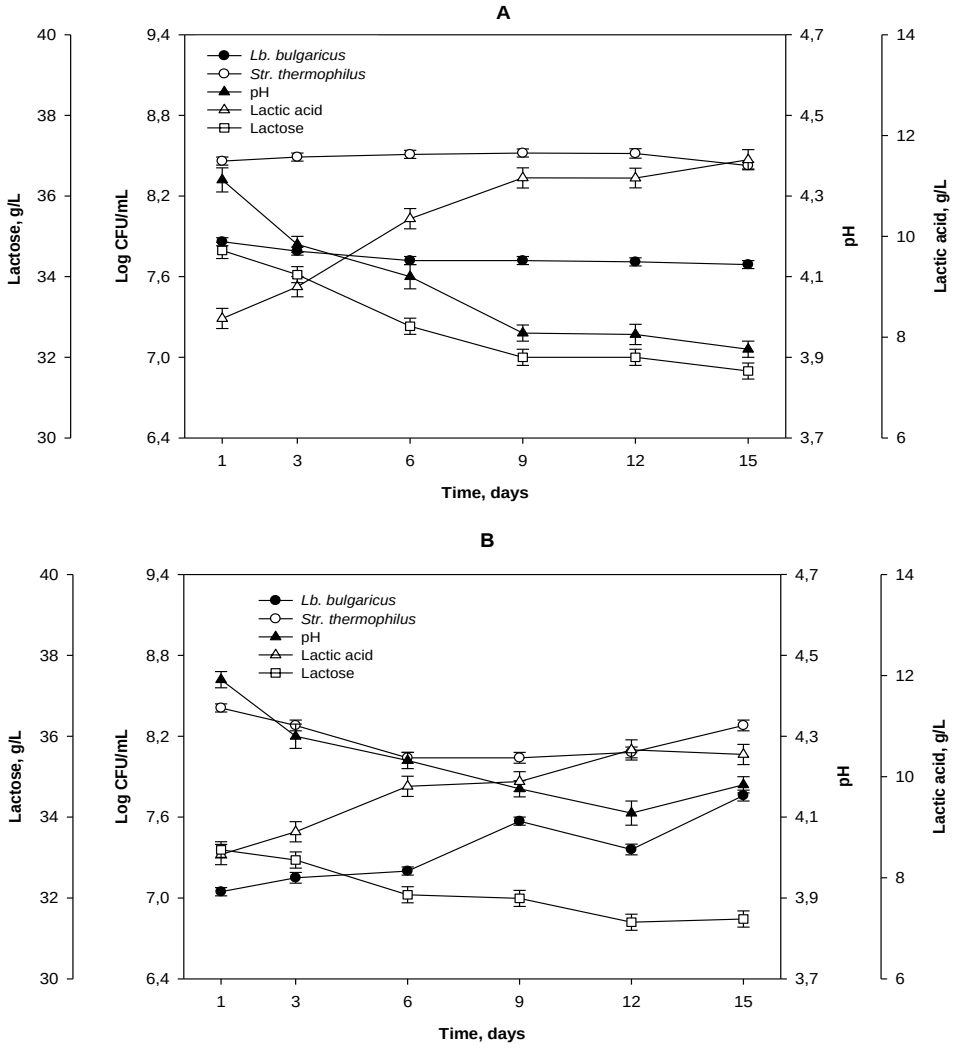

Figure 1. Growth and acidification activity of the starter culture during the storage period of the control sample – K1 (A) and polyphenol-enriched sample – S1 (B).

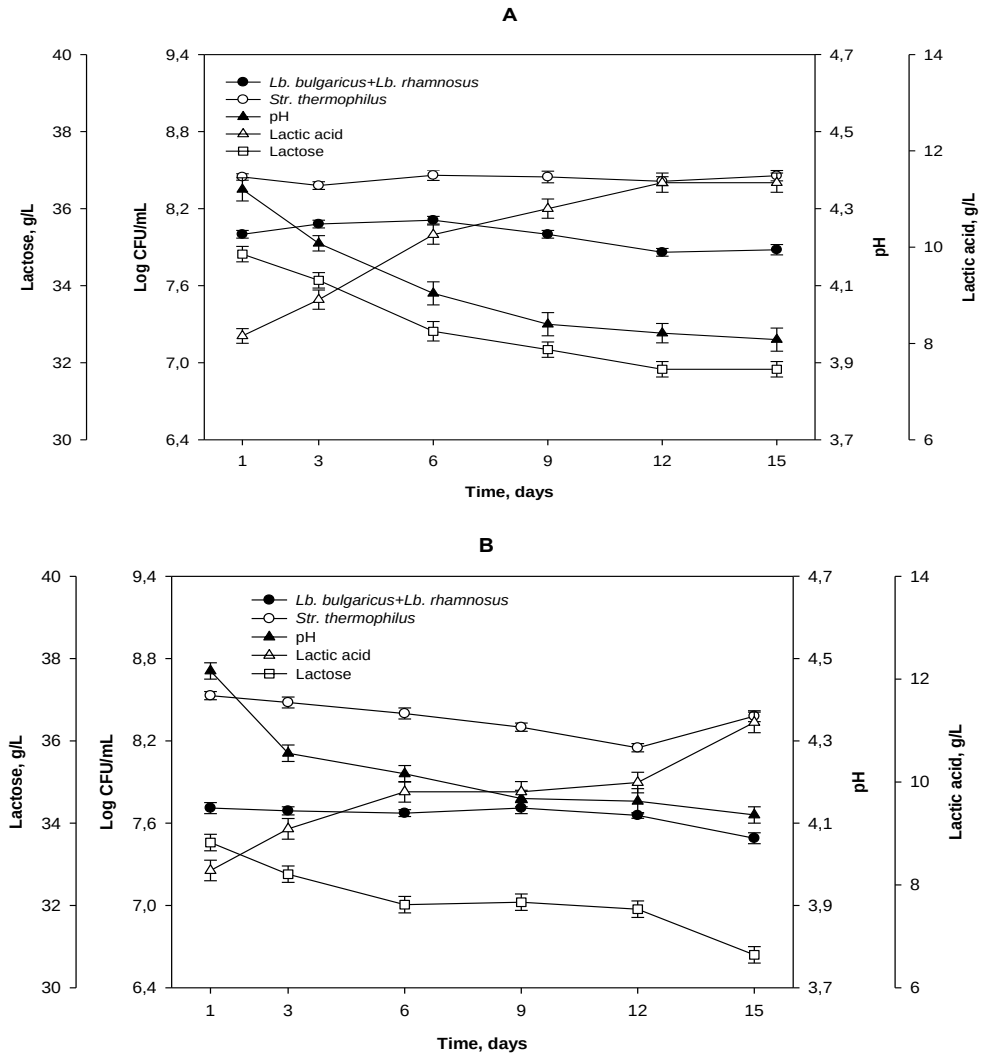

Figure 2. Growth and acidification activity of the starter culture during the storage period of the control sample – K2 (A) and polyphenol-enriched sample – S2 (B).

According to Caleja *et al.* (2016), the analysis of this parameter in fermented milk is very important in terms of product safety. The results obtained in the present study (Figure 2) indicated that the presence of *Lactobacillus rhamnosus* YW in the starter culture did not influence significantly ($P<0.05$) the lactic acid production during the refrigerated storage, as evidenced by the similar pH values and lactic acid concentration of samples K1 and K2 as well as S1 and S2.

The enrichment of fermented milks with plant by-products is a good approach to improve the viability of lactic acid bacteria (Frumento *et al.*, 2013). Muniandy *et al.* (2017) investigated the effect of adding green, white and black tea extracts on the viability of *Lb. bulgaricus* and *Str. thermophilus* in fermented milk during 15 days refrigerated storage. The authors found that the addition of tea extracts did not significantly affect the viability of lactic acid bacteria. In the present study, good survival of probiotic strains of lactic acid bacteria during the storage period was observed. No significant differences ($P<0.05$) in the *Streptococcus thermophilus* S13 counts in the control and polyphenol-enriched fermented milks was observed. Similar results were reported by other authors (Servili *et al.*, 2011). A slight decrease in the count of lactobacilli was found, which for the entire storage period was within about Log 0.5. It was found that the presence of *Lactobacillus rhamnosus* YW in the

starter culture was associated with a higher count of lactobacilli at the end of the refrigerated storage (samples K2 and S2). This could be due to its better survival during refrigerated storage compared to *Lactobacillus delbrueckii* subsp. *bulgaricus* S19.
The results for total count of lactic acid bacteria in polyphenol-enriched fermented milks during the storage period are shown in Table 1.

Table 1. Total count of lactic acid bacteria in polyphenol-enriched fermented milks during the storage period.

Parameters	Total count of lactic acid bacteria, CFU/mL					
Time, day / Samples	**1**	**3**	**6**	**9**	**12**	**15**
K1	$3.6.10^8$	$3.7.10^8$	$3.7.10^8$	$3.8.10^8$	$3.8.10^8$	$3.2.10^8$
S1	$2.7.10^8$	$2.0.10^8$	$1.3.10^8$	$1.5.10^8$	$1.4.10^8$	$2.5.10^8$
K2	$3.8.10^8$	$3.6.10^8$	$4.2.10^8$	$3.8.10^8$	$3.4.10^8$	$3.6.10^8$
S2	$3.9.10^8$	$3.5.10^8$	$3.0.10^8$	$2.5.10^8$	$1.9.10^8$	$2.7.10^8$

It could be seen that on the 15th day of refrigerated storage a high level of *S. thermophilus* and *Lactobacilllus spp*. counts – in the range of $3.0\pm0.4.10^8$ CFU/mL was observed, which is an important prerequisite for the functional characteristics of the fermented milk. During refrigerated storage, no significant difference ($P<0.05$) in the total count of lactic acid bacteria between the control and experimental samples was observed (Table 1). These results indicated that, the addition of polyphenol-enriched extracts from strawberry press residues (SPE) did not affect the survival of the probiotic lactic acid bacteria during 15 days of cold storage. Our results were in agreement with other studies (Santos *et al.*, 2017; Yadav *et al.*, 2017) that found that the addition of grape seed extract did not affect the viability of *Streptococcus* and *Lactobacillus spp.* counts during 14 days of cold storage.

4. CONCLUSION

The results reported in the present study showed that the fortification of milk with polyphenols extracted from strawberry press residues had no significant effect on the post-acidification during 15 days of refrigerated storage. The two types of polyphenol-fortified yogurt samples (S1 and S2) preserved high *S. thermophiluls* and *Lactobacilllus spp.* counts – in the range of $3.0\pm0.4.10^8$ CFU/mL throughout the refrigerated storage. Such high count of viable probiotic bacteria ensures the potential health benefits to consumers upon regular consumption. In addition, the utilization of waste from fruit industries can help to reduce the environmental impacts caused by its disposal.

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Antioxidant Capacity of Yogurt Fortified with Polyphenol Extract from Strawberry Pomace

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ABSTRACT

The aim of this work was to evaluate the antioxidant capacity of yogurt fortified with polyphenol extract from strawberry pomace. Functional fermented milk (S1 and S2) fortified with phenolic compounds (0.39 mg/mL) extracted from strawberry pomace, and fermented with two combinations of lactic acid bacteria – *Lactobacillus bulgaricus* S19 and *Streptococcus thermophilus* S13 (S1) and *Lactobacillus bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13 (S2) were manufactured. Yogurts without the addition of phenolic compounds were used as controls (K1 and K2). All yogurts were then refrigerated (4±2 ℃) for 15 days and samples were analyzed for changes in anthocyanin compounds, total extractable polyphenols content and antioxidant activity. The LC-MS analysis showed the presence of 6 individual anthocyanin compounds in the yogurts fortified with polyphenol extracts. The concentrations of most abundant anthocyanins pelargonidin 3-glucoside and cyanidin 3-glucoside were 59.68±1.79 mg/100 g and 8.46±0.25 mg/100 g, respectively. During the cold storage, high levels of polyphenol compounds and high antioxidant capacity of yogurts fortified with polyphenol extracts were established, which is an important prerequisite for their functional characteristics.

Keywords: Anthocyanins, Antioxidant activity, Polyphenol Extracts, Strawberry Pomace, Yogurt.

1. INTRODUCTION

Polyphenols have been well studied and confer many important benefits such as protection against oxidative stress and degenerative diseases. Experimental data have shown that most of these health effects are attributed to the antioxidant properties of polyphenol compounds (Han *et al.*, 2007). Berry fruits such as strawberry, blackberry, cranberry, and raspberry are the well-known sources of polyphenolic components with high antioxidant activity (Coisson *et al.*, 2005).

Strawberry (*Fragaria* × *ananassa*, Duch.) is among the most widely consumed fruits in the world (Martínez-Ferri *et al.*, 2014). The natural antioxidants present in strawberry make it an important natural source of bioactive compounds such as vitamins and phenolic compounds (Forbes-Hernández *et al.*, 2016). The phenolic compounds in strawberry are mainly anthocyanins, flavonols, flavanols, and hydroxycinnamic and ellagic acid derivatives (Aaby *et al.*, 2007).

Manufacturing of products derived from the strawberry generates high volumes of agro-waste which represents an environmental problem in the production areas that requires different technical waste management solutions (Šaponjac *et al.*, 2015). Some studies have already been done on the utilization of vegetable by-products as potential sources of polyphenols with antioxidant activity (Volf and Popa, 2004). The natural antioxidants available in these sources protect the human body against free radicals and oxidative stress (Tohma and Gulcin, 2010).

The combination of phenolic compounds, especially when extracted from natural sources (like strawberry pomace) and probiotic lactic acid bacteria may represent an innovative approach for the development of functional fermented milks.

Yogurt is a source of bioactive peptides which are formed during fermentation, but generally has a limited antioxidant activity. Therefore, yogurt with added antioxidants from natural sources appears to be a convenient food format to satisfy consumer interest in original yogurt nutrients, beneficial effects of starter cultures, and health benefits of added antioxidants. For this reason, several attempts to produce yogurts fortified with natural antioxidant-rich extracts have been undertaken; including supplementation with polyphenol-rich strawberry extract (Karaaslan *et al.*, 2011; Singh *et al.*, 2013; Sun-Waterhouse *et al.*, 2013).

However, it is important to assess whether the claimed health benefits are maintained within food mixtures during shelf life. Fruit and milk is one such mixture in which the antioxidant capacity of fruit constituents can be delivered in combination with the health benefits of milk (Wegrzyn *et al.*, 2008). Therefore, the aim of this study was to investigate the changes in total polyphenols and total antioxidant capacity of polyphenol-enriched yogurts fermented with two combinations of lactic acid bacteria during the storage period. The LC-ESI-MS characterization of changes in anthocyanins in yogurts was also investigated.

2. MATERIAL AND METHODS

2.1. Materials

Plant materials: The strawberry pomaces were supplied by Santulita Ltd. (Sofia, Bulgaria). The press residues were stored frozen at -18 ℃ until used to produce the polyphenol extracts.

Polyphenol extract from strawberry press residues: Polyphenol-enriched extract from strawberry pomace (SPE) was obtained by adsorption technology. To remove the sugars, salts and amino acids, the extract was purified using a column (465×30 mm) filled with Amberlite XAD 16 HP. The purified extract was lyophilized for 48 h.

Bacterial strains: The starter cultures of lactic acid bacteria used in the present study consisted of *Lactobacillus delbrueckii* subsp. *bulgaricu*s S19 (*Lb. bulgaricus*), *Lactobacillus. rhamnosus* YW (*Lb. rhamnosus*) and *Streptococcus thermophilus* S13 (*Str. thermophilus*). They belong to the laboratory collection of the Department of Microbiology at the UFT, Plovdiv.

Yogurt samples: The control and experimental batches of yogurts were prepared in laboratory conditions (Department of Milk and Dairy Products Technology at the UFT, Plovdiv, Bulgaria) from a single vat of milk according to the following procedure: cow's milk (M = 3.7%) was heated to 95 ℃ for 15 min, cooled to t=45±1 ℃ and divided into two lots: one experimental lot (Batches S1 and S2) fortified with polyphenols to 0.39 mgGAE/mL using SPE, and an unfortified control lot (Batches K1 and K2). The experimental and control batches of milk were inoculated with 2% Bulgarian yogurt starter culture consisted of *Lb. bulgaricus* : *Str. thermophilus* in ratio of 1:5 (samples K1 and S1) and starter culture consisted of *Lb. bulgaricus* : *Lb. rhamnosus* : *Str. thermophilus* in ratio of 0.5:0.5:5 (samples K2 and S2). All samples were packaged in containers and incubated at t=44±1 ℃ until the pH reached 4.6. At the end of the incubation, the yogurts were cooled down to approximately 4 ℃ and then stored at the same temperature for 15 days.

2.3 Methods

Total polyphenols, total monomeric anthocyanins, and antioxidant capacity tests: All measurements were performed with a Helios Omega UV–vis spectrophotometer equipped with VISION*lite* software (all from Thermo Fisher Scientific, Madison, WI, USA). Preparation of the yogurt samples for analysis: In a 50 mL flask were added 20 mL of the yogurt sample and filled up to the mark with methanol (99%). After overnight extraction at 10 ℃, the mixture was filtered through a paper filter. The extraction was performed in triplicate.
The total polyphenol (TPP) content was determined by the method of Singleton and Rossi (1965) modified according to the specific conditions of the analysis. The results were expressed as mg gallic acid equivalents (GAE) per 100 mL of yogurt. The total antioxidant capacities were determined by the DPPH (2.2-diphenyl-1-picryl hydrazyl hydrate) and FRAP (ferric reducing antioxidant power) assays modified according to the specific conditions of the analysis. The results of both tests were expressed as μmol Trolox equivalents (TE) per 100 mL of yogurt.

UHPLC-DAD and LC-MS analysis: The extraction of polyphenol compounds from yogurt samples were performed according to Redeuil *et al.,* (2009) with some modifications. Yogurt fortified with SPE (1 g) was homogenized with 30 mL of methanol acidified with formic acid (9:1 v/v) and centrifuged at 13,000 rpm for 5 min. The samples were kept at -20 ℃ during 2 h to allow protein precipitation. The slurry was then centrifuged at 4000 ×g at 4 ℃ for 20 min. The supernatant was filtered through a paper filter and diluted with deionized water in ratio 1:1. From the so prepared solution, 10 mL were taken and filtered using a 5 kDa cutoff membrane (5000 MWCO) and centrifugated at 6000 ×g for 30 min to remove soluble proteins. The extracts were evaporated with nitrogen at 35 °C to a volume of 2 mL. Samples were stored at 4-6 ℃ until analyzes.
The separation of strawberry anthocyanins was performed using a Nexera UPLC system (Shimadzu, Kyoto, Japan) equipped with two model LC-30AP high pressure pumps, a model DGU-20A5R degasser, a model SIL-30AC autosampling unit (cooled at 10 ºC), a model CTO-20AC column oven (set at 40 ℃) and a model SPD-M20A diode array detector. The column used was an Acquity HSS T3 (Waters, Ireland) (150×2.1 mm; 1.8 μm particle size) equipped with a security guard column. The mobile phase consisted of 5% (v/v) formic acid in water (eluent A) and 5% (v/v) formic acid in

acetonitrile (eluent B). The monitoring was performed at 520 nm at a flow rate of 0.4 mL/min. The injection volume was 5 μL and the samples were membrane-filtered (0.45 μm) prior to the analyses. The identification was confirmed by mass spectrometric analysis. Therefore, the same UPLC method as described above was applied using an Acquity UPLC system (Waters Milford, MA, USA), consisting of a binary pump (BSM), an autosampler (SM) cooled at 20 ℃, a column oven (CM) set at 40 ℃, a diode array detector (PDA) and Acquity TQD triple-quadrupole mass spectrometer with an electrospray interface (ESI), operating in positive mode. An Acquity UPLC HSS T3 column with 1.8 μm particle sizes (150×2.1 mm) and guard column (5×2.1 mm) was used for separation. The mobile phase consisted of 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B). The injection volume was 5 μL. The monitoring was performed at 520 nm at a flow rate of 0.4 mL/min. The mass spectrometer was tuned using a delfinidin-3-O-glucoside solution.

Statistical analysis: The results reported in the present study are expressed as the mean values of at least three analytical determinations. The coefficient of variation expressed as percentage ratios between the standard deviations and the mean values was found to be <5% in all cases. The means were compared using one-way ANOVA performed with Microsoft Excel and Tukey's test at a 95% confidence level.

3. RESULTS AND DISCUSSION

The LC-ESI-MS characterization of the anthocyanins in yogurts fortified with SPE (S1 and S2) on 1st and 15th days of refrigerated storage are presented on Figure 1, while the identification of the peaks is shown in Table 1 and Table 2. Identification of anthocyanins was carried out using LC-MS data (retention times, molecular mass and MS^2 ion fragments). The main anthocyanin compound in both polyphenol-enriched yogurts was pelargonidin-3-glucoside. The content of this anthocyanin accounted for more than 70% of total anthocyanins in both yogurts. Giampieri *et al.* (2012) also reported that pelargonidin-3-glucoside is the major anthocyanin in strawberries, independent from genetic and environmental factors and that glucose seems to be the most common substituting sugar in strawberry anthocyanins.

From the results in the tables, it can be seen that the concentration of all anthocyanin compounds in yogurt samples were about 50% lower than the theoretically calculated value (TCV). Probably, this is due to the formation of associations between polyphenols and milk proteins. These data were in agreement with the studies of other authors (Oliveira *et al.*, 2015; Sun-Waterhouse *et al.*, 2013). Anthocyanins are highly unstable and easily susceptible to degradation (Oliveira *et al.*, 2015). In yogurts, the stability of anthocyanins may be influenced by storage temperature, pH, other polyphenol compounds, milk fat content as well as by the presence of proteins (Gris *et al.*, 2007; Wallace and Giusti, 2008). Viljanen *et al.* (2005) found that in the aqueous phase anthocyanins can be associated with proteins.

In the present study, during the refrigerated storage of the samples, a significant ($P<0.05$) decrease in cyanidin 3-glucoside, pelargonidin 3-glucoside, and pelargonidin 3-(malonyl) glucoside was observed. Probably some of these anthocyanins were metabolized by lactic acid bacteria to lower molecular compounds.

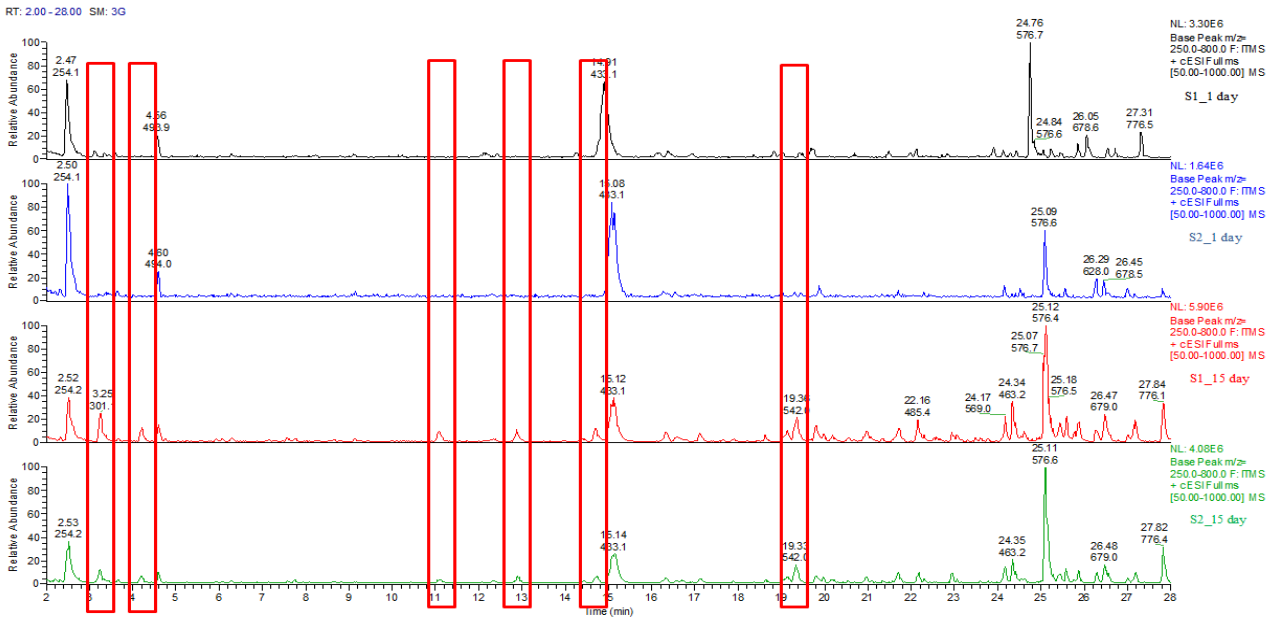

Figure 1. LC-ESI-MS characterization of changes in anthocyanins in yogurt fortified with SPE (S1 and S2) on 1st and 15th days of refrigerated storage.

Table 1. Identification and quantification of anthocyanin compounds in yogurts fortified with SPE and fermented with a starter culture of *Lactobacillus bulgaricus* S19 and *Streptococcus thermophilus* S13 (sample S1).

№	t_R [min]	Compound	Content mg/100 g**		
			TCV*	1 day	15 day
1	3.25	Unknown	-	-	trace
2	4.22	Dimer of coumaroylquinic acid	-	-	trace
3	11.11	Unknown Unknown	-	-	trace
4	12.31	Cyanidin 3-glucoside	8.46±0.25	4.68±0.14	4.20±0.13
5	12.89	Feruloyl hexoside	-	-	trace
6	14.71	Unknown	-	-	trace
7	15.05	Pelargonidin 3-glucoside	59.68±1.79	35.53±1.07	28.30±0.85
8	16.54	Pelargonidin 3-rutinoside	4.80±0.14	2.83±0.08	2.61±0.08
9	19.36	Unknown	-	-	1.60±0.05
10	19.86	Pelargonidin 3-(malonyl)glucoside	6.38±0.19	3.77±0.11	3.17±0.10
11	24.76	Unknown	-	1.20±0.04	1.13±0.03
		Total:	**79.31±2.38**	**48.01±1.44**	**41.02±1.23**

*Theoretically calculated value
** mean ± SD (n = 3)

Table 2. Identification and quantification of anthocyanin compounds in yogurts fortified with SPE and fermented with a starter culture of *Lactobacillus bulgaricus* S19, *Lactobacillus rhamnosus* YW and *Streptococcus thermophilus* S13 (sample S2).

№	t_R [min]	Compound	Content mg/100 g**		
			TCV *	1 day	15 day
1	3.25	Unknown	-	-	trace
2	4.22	Dimer of coumaroylquinic acid	-	-	trace
3	11.11	Unknown Unknown	-	-	trace
4	12.31	Cyanidin 3-glucoside	8.46±0.25	5.01±0.15	4.28±0.13
5	12.89	Feruloyl hexoside	-	-	1.23±0.04
6	14.71	Unknown	-	-	trace
7	15.05	Pelargonidin 3-glucoside	59.68±1.79	35.67±1.07	29.36±0.88
8	16.54	Pelargonidin 3-rutinoside	4.80±0.14	2.84±0.09	2.66±0.08
9	19.36	Unknown	-	-	1.36±0.04
10	19.86	Pelargonidin 3-(malonyl)glucoside	6.38±0.19	4.06±0.12	3.33±0.10
11	24.76	Unknown	-	1.51±0.05	1.18±0.04
		Total:	**79.31±2.38**	**49.09±1.47**	**43.40±1.30**

*Theoretically calculated value
** mean ± SD (n = 3)

It was found that the stability of anthocyanins in yogurts can be influenced by lactic acid bacteria used as a starter culture. During the fermentation process, microorganisms can produce enzymes causing hydrolysis of anthocyanins to less stable aglycones (Buchert *et al.*, 2005). Furthermore, the degradation of anthocyanins is facilitated by hydrogen peroxide produced by lactic acid culture (Yüksekda *et al.*, 2004). In the present study, during refrigerated storage, no significant ($P<0.05$) change in concentration of pelargonidin 3-rutinoside was observed.

On the first day of the storage period, a new unknown compound with a retention time of 24.76 min was established. From the fact that this compound was not found in the added strawberry extract (data not shown) it can be concluded that it is a product of the metabolic activity of lactic acid bacteria durig fermentation of milk.

In the present study, no significant ($P<0.05$) effect of the starter culture composition (presence or absence of *Lb. rhamnosus*) on the changes in the anthocyanin profile in the yogurt samples fortified with SPE was observed.

The changes in the total extractable polyphenol content of yogurts fortified with SPE (S1 and S2) during refrigerated storage at 4±2 ℃ were studied. Data are shown on Figure 2. The results are presented as mean values obtained from three parallel measurements.

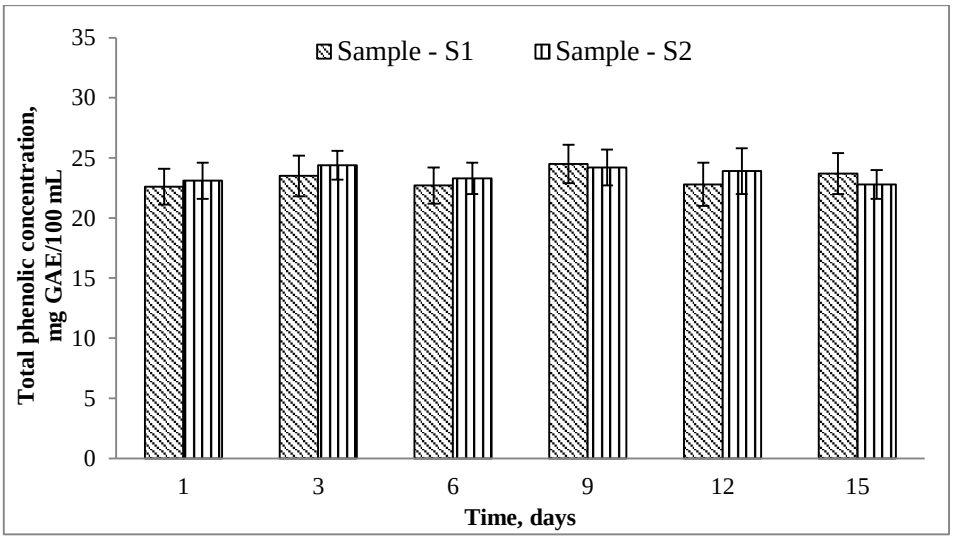

Figure 2. Total polyphenol content in polyphenol-enriched yogurts during refrigerated storage.

It could be seen that the total polyphenol content of the test samples did not change significantly ($P<0.05$) during the storage period. These results were in agreement with the findings of Karaaslan *et al.* (2011) who reported the good stability of the grape bioactive compound in the enriched yogurts during refrigerated storage. In the same way, the analysis in the present study evidenced the stability of total phenolic concentration during the storage period.

The content of total extractable polyphenols in yogurts fortified with SPE was found to be 23.6±1.9 mg GAE/100 mL. In the present study, based on the *in vitro* inhibitory and prebiotic effect of the polyphenol extract from strawberry pomace against the test strains of probiotic lactic acid bacteria (data not shown), the optimal concentration of polyphenols in milk was determined to be 0.390 mg/mL (Dimitrova *et al.*, 2019). Therefore, the expected content of polyphenols in the experimental yogurts has to be 39.0 mg/100 mL. Many of researchers (Olivera *et al.*, 2015; Xiao *et al.*, 2011), have reported that, when added to milk, some of the polyphenols form complexes with milk proteins, especially whey proteins. As a result, the total extractable polyphenols in yogurt is 30% - 40% lower than the theoretically calculated. Singh *et al.*, (2012) investigated the total polyphenol content in fermented milk "Dahi" enriched with strawberry polyphenol extract. The authors found that total extractable polyphenols comprises only 34% of the theoretical value of added strawberry polyphenol in aqueous dahi extract (whey portion), whereas the rest (66%) was retained in the gel matrix. This explains the lower values of the total extractable polyphenols found in the present study in milk supplemented with polyphenol strawberry extract. In addition, Trigueros et *al.* (2014) investigated the interactions of polyphenols with milk proteins in pomegranate fermented milks. The authors found that the degree of interaction of polyphenols with milk proteins depends on the type of phenolic compounds. The results obtained by the authors showed that 84.73% of the total anthocyanins remained associated with the proteins on the first day and 90.06% after 28 days of refrigerated storage, indicating the high affinity of anthocyanins towards milk proteins. Therefore, we can conclude that the polyphenol compounds in strawberry extract (mainly anthocyanins) interact with milk proteins. Thus, the lower values of total extractable polyphenols in yogurts fortified with SPE can be explained. In the present study, the similar values of total polyphenol content in samples S1 and S2, indicated that the presence of *Lb. rhamnosus* YW in starter culture did not affect significantly ($P<0.05$) the changes in polyphenol content of yogurts.

The DPPH• scavenging activities and Fe^{3+} reducing power (FRAP) of control and polyphenol-enriched samples during refrigerated storage are shown on Figure 3 and Figure 4, respectively.

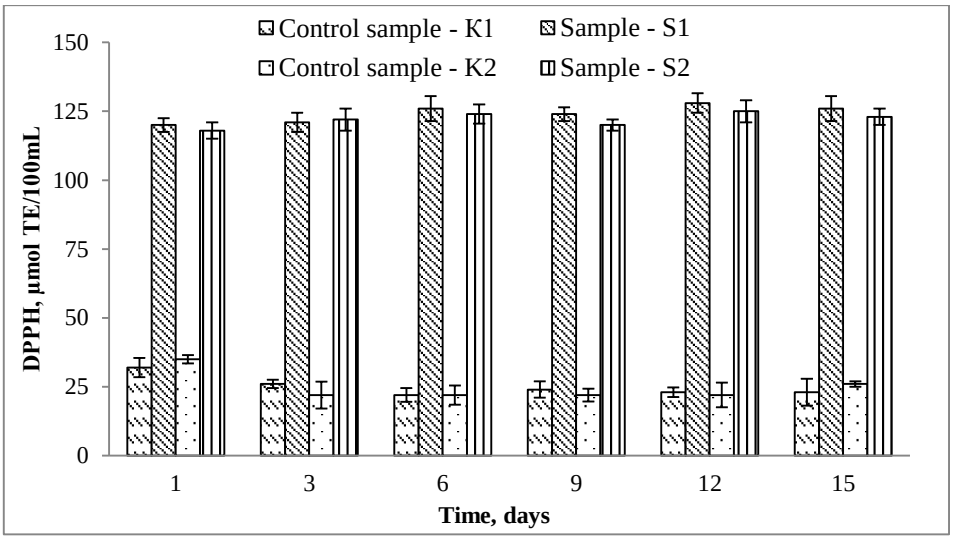

Figure 3. DPPH radical scavenging value of yogurts fortified with SPE (S1 and S2) and the respective controls (K1 and K2) during refrigerated storage.

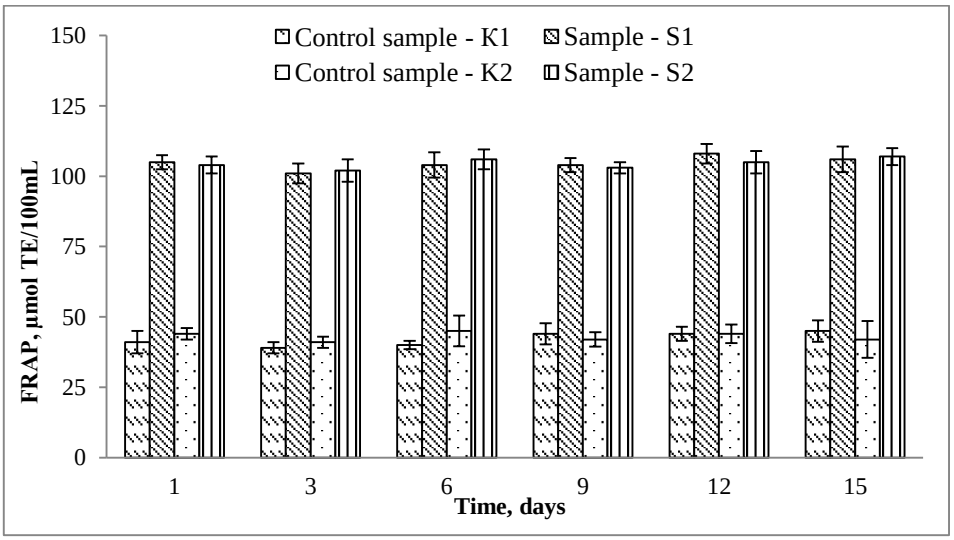

Figure 4. Ferric reducing power (FRAP) of yogurts fortified with SPE (S1 and S2) and the respective controls (K1 and K2) during refrigerated storage.

The results obtained showed that the antioxidant capacities determined by the DPPH and FRAP assays of the control samples (K1 and K2) at the beginning of storage was 33.0±2.7 µmol TE/100 mL and 43.1±3.0 µmol TE/100 mL, respectively. This is probably due to the presence of casein, whey proteins, urate, vitamin C together with lactic acid bacteria (Chen *et al.*, 2003). There were no statistically significant differences ($P < 0.05$) in DPPH and FRAP values of milk fermented with the two starter cultures - *Lb. delbrueckii* subsp. *bulgaricus* S19 + *Str. thermophilus* S13 and *Lb. delbrueckii* subsp. *bulgaricus* S19 + *Lb. rhamnosus* YW + *Str. thermophilus* S13. Therefore, the differences in the composition of the starter microflora did not significantly affect the antioxidant potential of yogurts. During the storage period, a decrease of DPPH values in the control samples (K1 and K2) was observed and at the 15th day they reached 24.7±3.3 µmol TE/100 L. In contrast, the values from the FRAP test remained constant throughout the refrigerated storage and at 15th day were about 42.8±4.5 µmol TE/100 mL. A possible explanation of this fact is the different mechanisms of the antioxidant activity of these two tests (Qureshi *et al.*, 2017).

Antioxidant activity of yogurts fortified with SPE (S1 and S2) was observed to be seven to nine folds higher as compared to control samples (K1 and K2). The DPPH and FRAP values in the yogurts fortified with SPE were 119.0±6.7 μmol TE/100 mL and 104.5±5.2 μmol TE/100 mL, respectively.
Several authors have reported a positive correlation between the total polyphenol content and antioxidant activity of fruit polyphenol extracts (Caillet et al., 2006; Skrede et al., 2004). In the present study, the higher polyphenol levels in the polyphenol-enriched yogurts resulted in a higher antioxidant capacity as quantified by DPPH and FRAP assays (Figure 3 and Figure 4). At the end of the refrigerated storage of the polyphenol-fortified yogurts, DPPH and FRAP values were 124.6±7.0 μmol TE/100 mL and 106.4±4.1 μmol TE/100 mL, respectively. These data indicate for the high antioxidant capacity of yogurts fortified with polyphenol extract from strawberry pomace, which is an important prerequisite for their functional properties.

4. CONCLUSION

The results reported in the present study showed that the polyphenol-fortified yogurts had significantly higher antioxidant activity than the controls. There were no statistically significant changes ($P<0.05$) in antioxidant capacity and total polyphenol content during the 15 days storage at refrigeration temperature (4±2 °C). Thus, the yogurts fortified with polyphenol extract from strawberry pomace can serve as a potential functional food.
During the refrigerated storage of the samples, a significant ($P<0.05$) decrease in cyanidin 3-glucoside, pelargonidin 3-glucoside, and pelargonidin 3-(malonyl) glucoside was observed in contrast to pelargonidin 3-rutinoside which remains stable.
Furthermore, the starter culture composition (presence or absence of *Lb. rhamnosus*) did not affect the polyphenol content, the antioxidant activity and the changes in the anthocyanin profile in the yogurt samples fortified with SPE.

ACKNOWLEDGMENT

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Quince Seed Mucilage as Edible Coating for Mandarin Fruit; Determination of the Quality Characteristics during Storage

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ABSTRACT

Nowadays consumers mostly prefer minimally processed fruits and vegetables due to their beneficial and nutritious effect on human health. Edible coatings can be used to prevent deterioration in minimum processed fruits and vegetables and to increase the quality and shelf life of these products. Increased consumer demands for higher quality food in combination with the environmental need to reduce disposable packaging waste have led to increased interest in research into edible films and coatings. In this study, the use of quince (*Cydonia oblonga*) seed mucilage was investigated as an edible coating to extend the shelf-life of mandarin fruit presented to the consumers in ready form for consumption. For this purpose, the seeds of quince were obtained manually and extracted without waiting. The mucilage obtained from the quince seeds extract were used for covering the mandarin slices after the fruits were peeled and separated into slices. Physical, chemical and sensory characteristics of fruits coated with edible film and uncoated fruit as control were investigated during storage at 4°C for 10 days. Coating significantly delayed softening, reduced weight loss and maintained color values of fruits. Sensory characteristics of coated mandarin such as color, taste and texture were much better preserved. At the end of storage time, the values of antioxidant activity and total phenolic content of coated samples were significantly higher than control. The results of this study indicated that usage of edible coatings will be effective for mandarin fruit preserving and quality characteristics of mandarin fruits maintained longer by edible coating.

Keywords: Edible Coating, Mucilage, Quince Seed

1. INTRODUCTION

Fruits and vegetables are rich sources of vitamins, minerals and dietary fiber that are involved in many metabolic activities in the body. They play a major role in human health and nutrition because of having many useful compounds with antioxidant properties related to the prevention of various types of cancer and some degenerative diseases (Ncama et al., 2018). However, fruits and vegetables continue respiration after harvesting. Fruits and vegetables start to use their own substances such as carbohydrates, proteins, fats and organic acids since they are separated from the plant. This situation causes undesirable changes in the sensory properties such as color, aroma, taste and texture and increase loss of weight and nutritional value. Therefore, biological process occurring in post-harvest fruits and vegetables should be prevented as much as possible (Yousuf et al., 2017).

Minimal processing of fruits and vegetables includes peeling, cutting and slicing. Thus, minimally processed fruit and vegetable products can be presented to consumers without the need for preparation for consumption. Minimally processed fruit-vegetable technology is required to provide the closest form to fresh product and to maintain this structure for a long time. Therefore, it is aimed to retard the enzymatic reactions and inhibit the microbiological activity (Lin and Zhao, 2007). Many different methods such as coating, modified or controlled atmosphere packaging, chemical applications, UV irradiation and low temperature storage have been developed in order to prevent loss of nutritional value during the process, meet customer demands and increase shelf life (Yousuf et al., 2017).

Edible films and coating is one of the innovative methods used to protect product weight, sensory qualities and nutritional value with minimum loss and control microbial spoilage during storage in processed fruits and vegetables (Hassan et al. 2018). Edible film and coatings are produced from hydrocolloids such as proteins, polysaccharides, lipids and/or composites. Coating materials are dissolved in a solvent such as water, alcohol or organic solvent. Also plasticizers, antibrowning agents, flavors, antioxidants, antimicrobials or coloring agents are used to improve the functional properties of the film coating (Suput et al., 2015).

Quince is a fruit that has high nutritive value and beneficial influence on human health. It is stated that the fruit itself and its peel have free radical binding and antioxidant properties. Fruits give positive results in the treatment of inflammatory bowel diseases and stomach ulcer. Also, leafs are used as sedative and seeds are used as emulsifying agent in cosmetic industry (Ritzoulis et al., 2014). There are about 10 seeds in a fruit. There are hydrocolloids that will cause the formation of mucilage when the seeds are kept in water. Cellulose, water-soluble carbohydrates, amino acids are the components of the mucilage structure (Kirtil and Oztop, 2016).

In this study, it is aimed to use the mucilage obtained from the quince fruit to cover mandarin samples with the films formed. In addition, the effectiveness of quince seed mucilage (QSM) edible film in extending the shelf life of minimally processed mandarin fruits was determined. For this purpose sensory quality, weight loss, color, firmness, antioxidant activity and total phenolic content and of fruits coated with edible film and uncoated fruit as control were investigated during storage at 4°C for 10 days. In the literature review, there is no study about the application of mucilage edible film obtained from quince seed to fruits and vegetables. QSM has been only applied to marine products as an edible film coating (Jouki et al., 2013). Therefore, this study is expected to be important and original among limited studies.

2. MATERIAL AND METHODS

2.1. Materials

Quince and mandarin fruits were purchased from local market in Izmir. The seeds of the quince were obtained manually. 10 grams of seed were mixed with 3 times the weight of seeds with ethanol (96% w/w) for 5 minutes with constant mixing speed. Liquid quince seed mucilage was extracted with distilled water (Seed/Water, 1/30). Mucilage was obtained by mixing in the blender for 15 minutes at 45 °C and at 1100 rpm. In the final step, the solution was filtered with a cheese cloth. Mucilage obtained was stored in the refrigerator (at 4 °C) prior to film forming (Jouki et al., 2013).

2.2. Methods

Preparation of QSM Film: 1% mucilage and 35% glycerol (w/w) based on QSM weight was dissolved under constant stirring (750 rpm, 15 minutes) at 45 °C. Solution was homogenized using Ultra Turrax (12000 rpm, 5 minutes) to obtain an emulsion and emulsion was centrifuged (6000 rpm, 3 minutes) to decrease air bubbles (Jouki et al., 2013).

Coating Application: Mandarins were peeled and separated into slices. Mandarin slices were dipped in film solution for 2 min and dried in room temperature (23 °C). Control samples were dipped in distilled water (Hashemi et al., 2017). Coated and uncoated samples were put in plastic boxes and stored in refrigerator (4 °C) for 10 days. At days 0, 2, 4, 7 and 10, samples were evaluated for physical, chemical and sensory properties.

Sensory Evaluation: Coated and uncoated samples were analyzed by using scoring test (Altuğ and Elmacı, 2015). Panelists were trained for sensory panel and samples were presented in coded plates. The panelists were given a 5-point scoring scale for appearance, texture and taste. Samples with scores equal or higher to 3 were considered acceptable.

Weight Loss: Weight losses of coated and uncoated samples were measured gravimetrically and compared during storage. Cumulative weight loss was expressed as percentage loss of the original fresh weight (Guerreiro et al., 2017).

Firmness: Firmness of the samples was evaluated using the texture analyzer (Stable Microsystems, Surrey, UK). Cylindrical aluminum probe (2mm diameter) was used for texture analysis. Values were expressed as newton (N) (Sogvar et al., 2016).

Color: Color of fruits was measured by a Minolta Chromameter CR-300 (EC Minolta, Japan) using the CIELab scale (L*, a* and b*).

Antioxidant Capacity: Antioxidant activity was determined by the 2,2-diphenyl-1- picryl-hidrazil (DPPH) radical-scavenging method (Robles-Sánchez et al., 2013). Changes in the absorbance of the samples and standards were measured at 515 nm by spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage.

Total Phenolic Content: Total phenolic content were determined by the Folin-Ciocalteu method described by Oms-oliu et al. (2008). The absorbance values of the samples were measured against

80% methanol-water mixture at 765 nm by spectrophotometer. The total phenolic content of the samples were calculated using the gallic acid standard.

3. RESULTS AND DISCUSSION

Sensory characteristics such as appearance, texture and taste significantly and directly affect the decision of the product to be purchased by consumers (Altuğ and Elmacı, 2015). As shown in Fig. 1, results of the samples showed that appearance, texture and taste characteristics decreased during storage time as expected. However, sensory evaluation of coated and uncoated samples revealed significant ($p \leq 0.05$) differences in appearance, texture and taste at the end of the storage time. Appearance and taste scores of uncoated samples were under average after 7th day but coated samples' scores were equal or above average during storage period. It was observed that texture scores of coated samples at 10th day and uncoated samples after 7th day were less than limit points. Sensory panel results indicated that film coating had a positive effect on sensory quality of the mandarins. Similarly, sensory scores of cactus mucilage coated strawberries had higher values compared to uncoated samples at the end of storage time (Del-valle et al., 2006).

In post-harvest period, respiration causes loss of weight in fruits and vegetables due to loss of water (Sogvar et al., 2016). As shown in Fig. 2, weight loss increased during storage time in all treatments. However, uncoated samples showed higher weight loss compared to the coated samples during storage. Results obtained indicated that coating acted as a barrier against water loss and reduced weight loss significantly ($p \leq 0.05$) throughout storage period. In a study conducted by Trevino-Garzaa et al. (2017) the efficiency of edible coating on pineapple fruits was investigated and weight loss of uncoated samples was found to be significantly higher compared to the coated samples.

Firmness of the control fruits decreased significantly ($p \leq 0.05$) with storage time (Fig. 3). Coating had significant effect ($p \leq 0.05$) on retarding fruits firmness loss compared to uncoated samples after 7th and 10th days of storage. The comparison of the results in Fig. 3 showed that usage of edible film coating were effective for mandarin firmness. Azaraksh et al., (2014) studied the effect of edible coating on firmness of pineapple fruits and similarly found that firmness of coated samples was higher than uncoated samples.

Significant increase ($P \leq 0.05$) of L* parameter was observed in uncoated samples at the end of storage (Table 1). However, coated samples showed lower L* values. In general, a* values decreased and b* values increased in all samples. However, coated samples had more stable values of a* and b* during storage. Similarly, it was observed that L* values were maintained in coated pine apple samples (Trevino-Garzaa et al., 2017), a* and b* values of coated strawberry and apple samples found to be more stable compared to uncoated samples during the storage period (Del-valle et al., 2006; Rojas-Graü et al., 2007).

Changes in the percentage of DPPH radical inhibited by antioxidants and total phenolic content in all samples are shown in Table 2. Coated samples had highest antioxidant capacity value at 4th day of storage. Uncoated samples had lowest antioxidant capacity value at 10th day of storage. In general, the unstable results for all samples were obtained during storage period but the coated samples had higher values at the end of the analysis. Similarly, Serrano et al., (2006) determined that aloe vera gel coated grape samples found to have higher antioxidant capacity than uncoated samples. Total phenolic contents of coated pears found to have significantly higher values than uncoated samples during storage period (Oms-oliu et al., 2008).

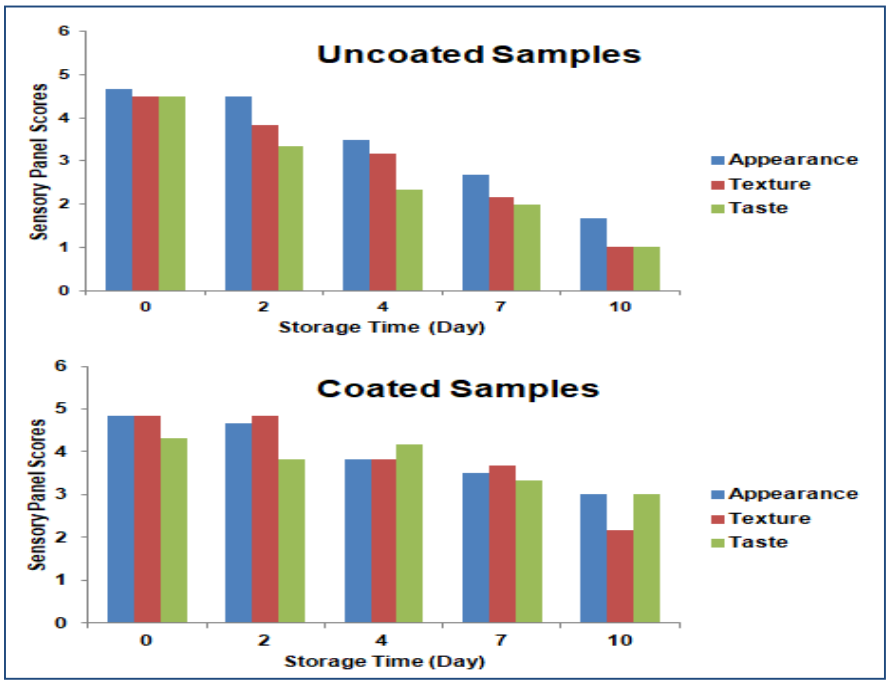

Figure 1. Sensory analysis scores of coated and uncoated samples

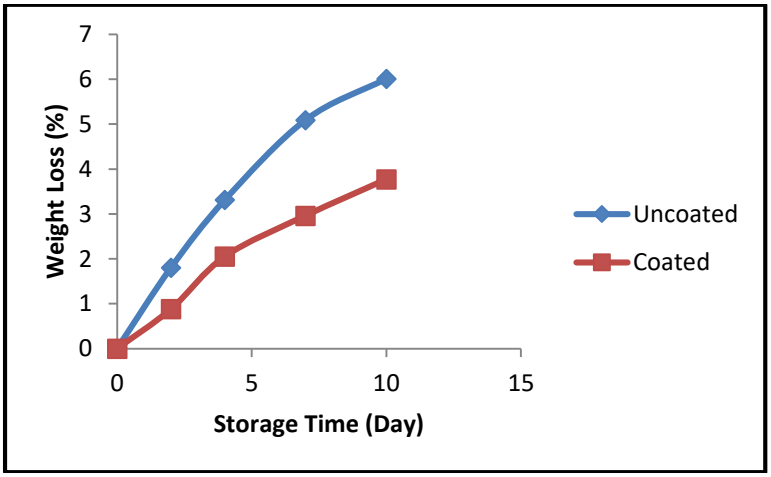

Figure 2. Weight loss of coated and uncoated samples

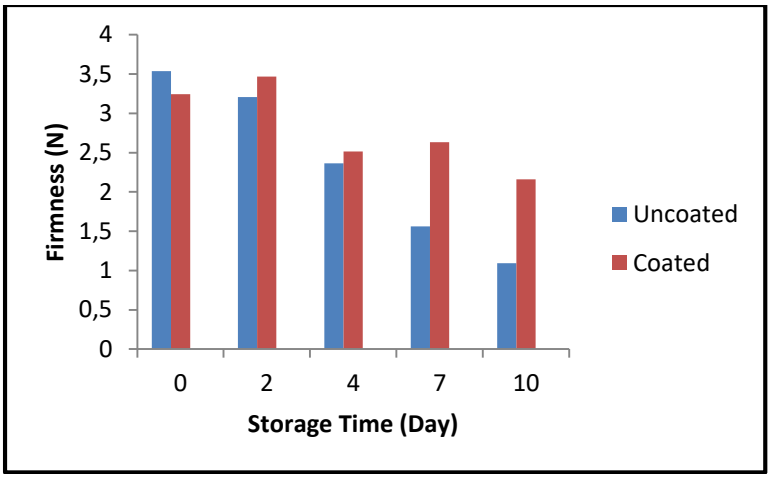

Figure 3. Firmness values of coated and uncoated samples

Table 1. Color values of coated and uncoated samples

	L*		a*		b*	
Day	Uncoated	Coated	Uncoated	Coated	Uncoated	Coated
0	46,14	45,78	5,01	4,92	23,54	23,61
2	49,65	46,24	5,11	3,89	25,13	24,32
4	50,15	45,27	4,81	4,08	28,07	24,78
7	51,81	46,17	4,14	4,22	25,47	23,84
10	52,09	46,44	4,24	4,37	27,94	24,71

Table 2. Antioxidant capacity and total phenolic content of coated and uncoated samples

	Antioxidant capacity (%)		Total phenolic content (mg/ml)	
Day	Uncoated	Coated	Uncoated	Coated
0	59,16	57,25	0,9491	1,0172
2	50,69	52,39	0,8041	0,8886
4	63,26	63,38	0,9297	0,7544
7	47,89	50,94	0,6428	0,6816
10	39,26	45,26	0,4434	0,5431

4. CONCLUSION

This study showed that QSM edible film coating helped to protect the quality and extend the shelf-life of minimally processed mandarin by preventing the weight loss, softening, and by delaying alteration of color. In addition, sensory panel evaluations indicated that coating helped to preserve characteristics such as appearance, texture and taste. Coating process improved the quality and prolonged the shelf-life of mandarins for 10 days compared with uncoated samples. Thus, it can be concluded that QSM edible coating could be used as a potential application in the food industry to maintain overall quality and extend the shelf-life of the minimally processed fruits.

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The Role of Global Climate Change on Pasture and Rural Economy

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ABSTRACT

The scientific evidence point to climate change is very important impact on life on the World. Global warming and global climate change have become more pronounced since the 1980s. This problem have some important socioeconomic effects, such as less water resources, drought, desertification, and decrease in crop yield. Global climate change and environmental changes will have very important consequences for vegetation. This affects crop yield, arises in development and international trade. In contemporary countries, pastures are perceived as very important natural resources for both animal husbandry and forage protection. Pastures have crucial entities rich biodiversity on ecological sustainability and rural development. Pastures also are very important sources for environmental protection and for the conservation of plant genetic resources. Therefore, protection of pastures is important in terms of agricultural economy and rural development. However, pastures are lost day by day as a result of climate change and global warming, improper practices and allocations to other uses. Pastures management is primal importance rural development; maladministration of the grazing not only damages the pasture, but it increases erosion. This situation can cause serious damage to agricultural land. Therefore, pasture management is important in terms of economy. Especially, in this study it is aimed to investigate the impact of climate change on pastures in Turkey for the period 1980- 2018. In order to investigate the possible effects of climate changes on pastures the relationship between agricultural productivity, temperature, rainfall and size of pasture areas will estimated by using formal statistics.

Keywords: Global climate change, Pastures, Pasture Management Rural development, Economic Impact

1. INTRODUCTION

"To learn about climate change, you first must know what climate is."

NASA

Global climate change is very important in all country. The main source of global climate change is human-induced influences in atmospheric composition. Climatic factors influence biological, and ecological structure, in addition to human, agriculture, natural determinants. Therefore, understanding the linkages between climatological and ecological change will help determined strategies.

In addition to the average characteristics of all weather conditions that have been experienced or observed over many years anywhere in the Earth, the climate, the temporal distributions of their occurrence frequency, the observed extreme values, violent events and all is defined in the form of a combination of variability types (Türkeş, 2008). The climate of a place is mainly determined by its latitude, which is the height of the sea level and the distance to the ocean. Although the standard average duration of the climate is 30 years, other periods can be used depending on the purpose. Whether people are aware or not (daily, monthly or yearly) the climate has a significant impact on their lives (Yıldırım et al., 2016). Climate change is the change that occurs in the climate as a result of human activities that alter the composition of the atmosphere directly or indirectly in addition to the natural variability of the climate observed in a comparable time period (an average of 30 years according to the World Meteorological Organization) (Başoğlu, 2014).

In the world's 4.5 billion-year history, the changes occurring between astronomical or geological time zones, which occur very slowly at human level, are also expressed as natural climate variability. Although these two concepts can be separated through definitions, the observations being recorded are very short, but the recent changes occur around the long-term real changes (climate change) or a fixed average The subject of the differences (climate variability) is not yet clear (Hatık, 2015).

Global warming is a phenomenon of climate change characterized by a general increase in average temperatures of the Earth, which modifies the weather balances and ecosystems for a long time. It is directly linked to the increase of greenhouse gases in our atmosphere, worsening the greenhouse effect (https://solarimpulse.com/global-warming-solutions). With the emergence of the problem of global warming, serious disasters have begun to happen on the earth. As a matter of fact, climate change, which is the biggest global environmental problem today, is seen as the most important factor that threatens live life on earth (Karakaya et al., in preparation).

Global mean temperature has increased by 0.6°C (in Europe by about 1.2°C) over the past 100 years, and the 1990s was the warmest decade for 150 years. Sea level rose by 0.1–0.2 meters, globally and

inEurope, during the last century. It is projected to rise by an additional 0.1–0.9 meters by 2100. Other signs include a retreat of mountain glaciers and a decrease of snow cover (EEA, 2003:91). All these indicators show that our world is at a great risk.

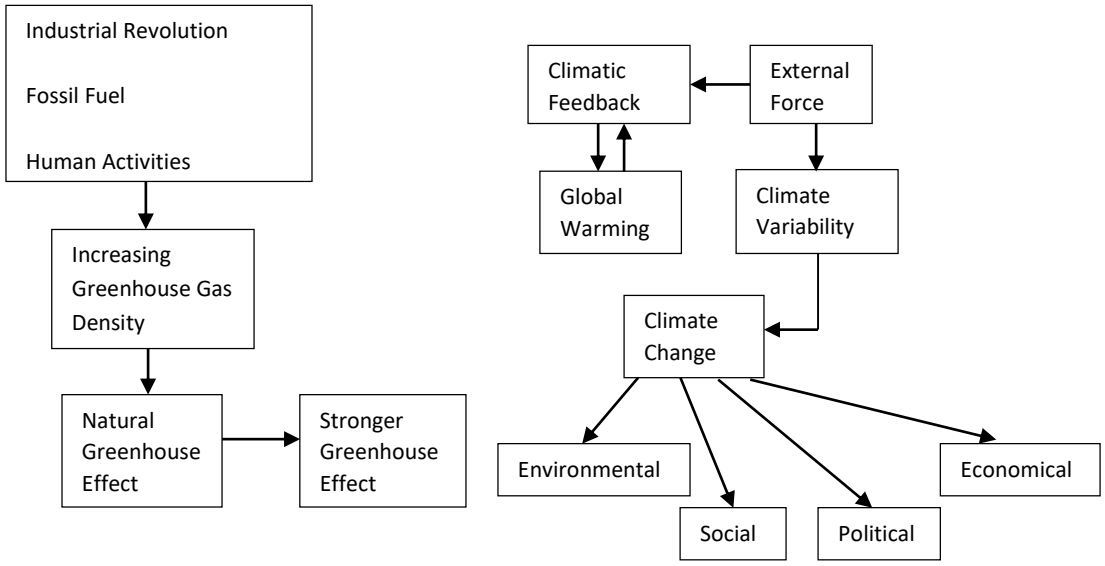

Figure 1. Climate Change Process (Başoğlu, 2014)

Since the 1970s, the climate change has become one of the main agenda topics of the countries due to the noticeable improvement of general ecological problems, developments in science and technology and the work of ecological economists. In 1979, the I. World Climate conference and in 1980, Madden, Ramanthan and Hansen's "human activities are causing climate change," the explanations brought together academics and policymakers and the international Meetings have begun. These meetings have been conducted to determine the causes and effects of climate change and the determination of national, regional and global measures to be taken to prevent this (Bayramoğlu et al., 2017).

It is effective on many areas such as water resources, forest and vegetation, biodiversity, agriculture and human health. On the other hand, the increasing adverse effects of climate change in the recent years such as changing the rainfall regime, temperature increases, drought, desertification and natural disasters; It is an important threat to the economy by affecting productivity and growth rates in agriculture (Hayaloğlu,2018).

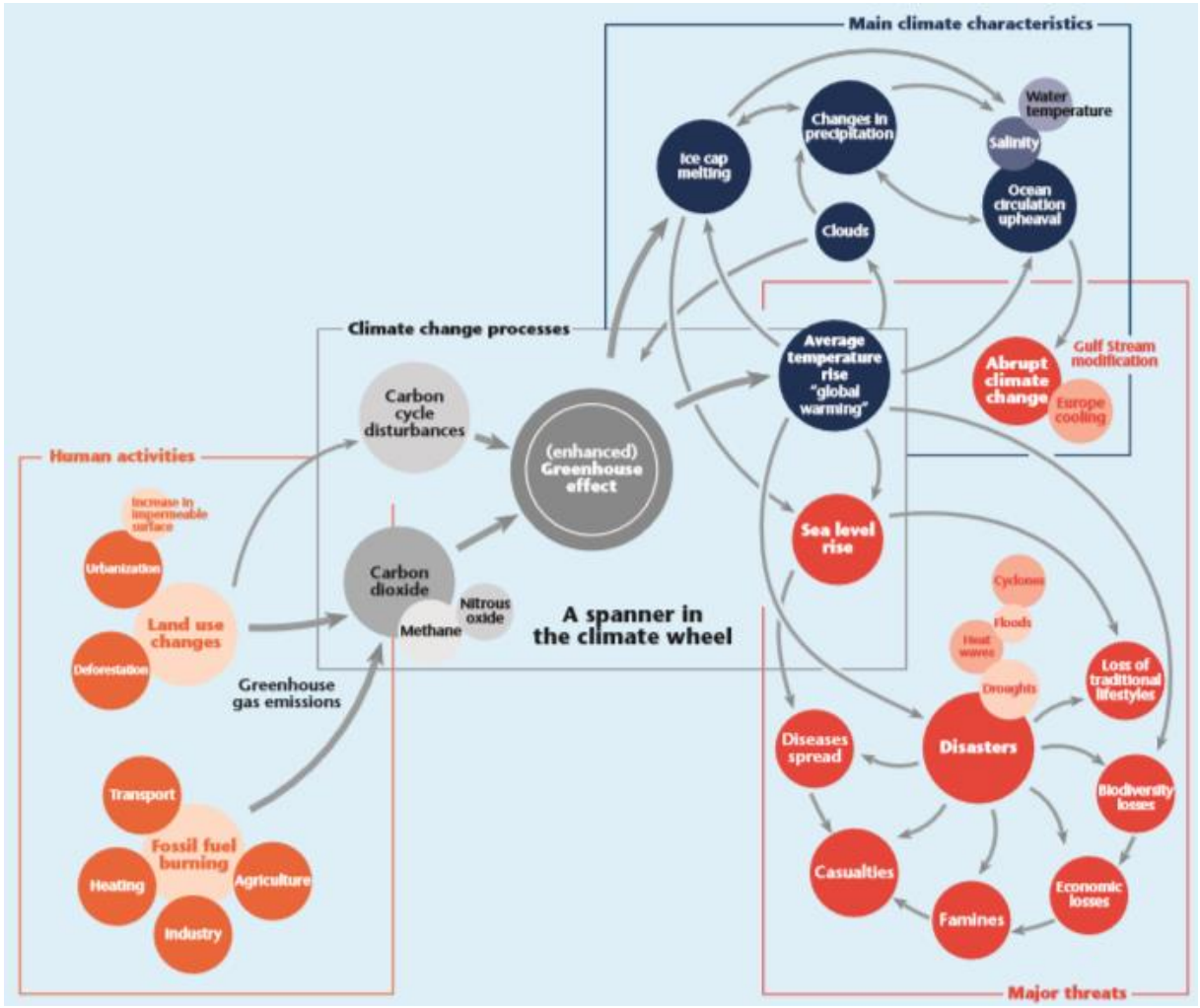

Figure 2. Climate Change: Processes, Characteristics and Threats (World Water Development Report 3, 2009:69)

While there are many factors that lead to climate change, human-induced greenhouse emissions within these factors have an important effect. The biggest share of greenhouse emissions is carbon dioxide gas. Factors such as economic growth, population, energy consumption, carbon emissions resulting from the use of fossil fuels and deforestation are factors that determine carbon emissions (Karakaya et al., in preparation)

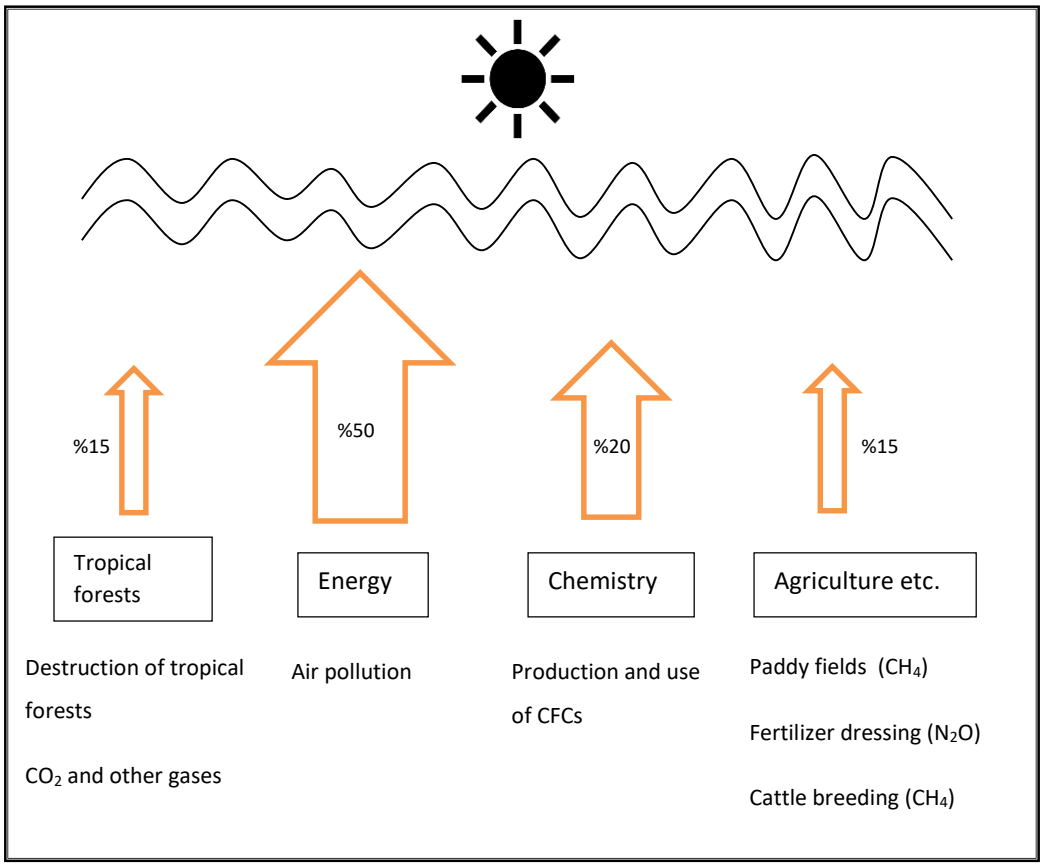

Figure 3. Causes of Human Sources Greenhouse Gases (Öztürk, 2002)

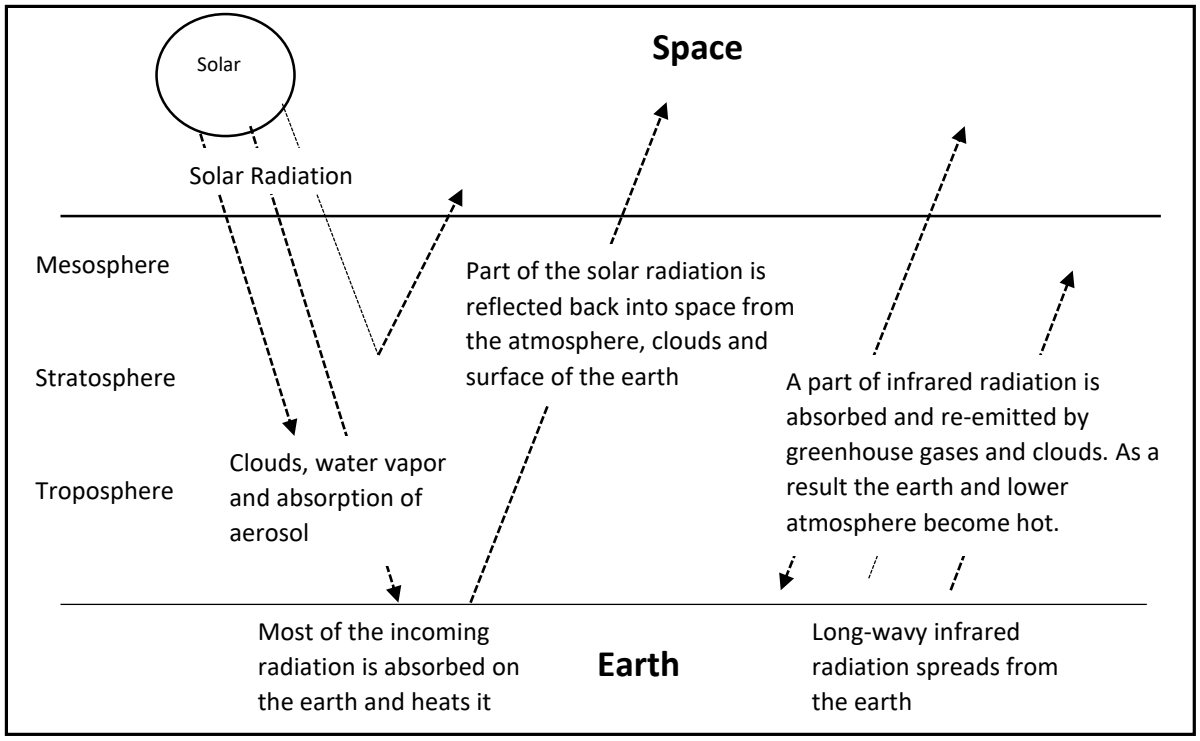

Figure 4. Schematic Representation of Greenhouse Effect (Çetiner et al., 2000)

2. MATERIAL AND METHODS

Studies on the effects of theoretical and applied greenhouse gases are extremely important. Global climate change and its impact on ecosystems and biodiversity are detailed in this study.

Demir (2009), has examined global climate change and its impact on the ecosystem and biodiversity of Turkey in this situation how it affected. Accordingly, terrestrial and marine ecosystems and biological diversity are under very serious risk. Especially, tropical and middle latitude forests, plant and animal species of a significant will be damaged. This will affect to the adaptation process negatively and in the meantime the new conditions of new species that the will be possible.

Hayaloğlu (2018), to analyzed the impact of climate change on agriculture sector and economic growth for the 10 countries most affected by climate change according to the Global Climate Change Risk Index. Panel data analysis technique was used in the study for the period 1990-2016. The results obtained showed that climate change has a negative effect on economic growth and agricultural value added in certain countries.

Kırış and Toprak (2015), examined the role of its role in preventing climate change and sustainable development of forests in Turkey in order to reflect its importance in the solution of environmental problems and climate change on forest ecosystems. In this study, they emphasized that they have significant effects on climate change by decreasing the radiation effect of forests and balancing the temperature change, balancing the formation of precipitation and humidity, cleaning the air and reducing the pollution and being the source of oxygen.

Aydın and Sarptaş (2017), the geographic-climatic suitability of maize, safflower, canola (rape), cotton, wheat and switchgrass was projected for 2070, and the current and future conditions were compared. Accordingly, the methodology may aid for planners and land managers to understand changes in climatic suitability of plants from present to future.

Yıldırım et.al (2016), examined to determine effects of climate change in future on Turkey's poppy production that is influential country on poppy production and opium alkaloids trade in the world. To this end, temperature and precipitation data (1961-2013) of climate parameters are used. One of climate change models; HadGEM-2ES RCP8.5 scenario were used to determine changes that may be occurred in the future. Using temperature and precipitation projections for 2015-2040, 2040-2070 and 2070-2099 periods, possible changes are shown in poppy cultivation areas for the future.

Gürkan et.al. (2017), the effects of climate changes tried to determine on sunflower and cotton production which have an important role in agricultural production in Turkey. This study, covers the period between 2006 and 2016. According to the obtained results, it has been determined that the changing climate conditions cause decrease in the sunflower (for oil) yield up to 20% and in the cotton (seed cotton) yield up to 14% in Turkey.

In the study of Kanber et.al. (2010), Effects of Global Climate Change on Water Resources and Agricultural Irrigation, the effects of decreasing water resources, formation of salinity-alkalinity problem in soils and decreasing agricultural areas and yield were evaluated.

Climate change is causes to cause stress on water and other resources, badlands, polution land, desertification, proliferation of pests, diseases in agricultural crops, and the destruction of coastal ecosystems by sea levels rising. Climate change affects whole humanity and economic activities of human directly or indirectly. Among the sectors that most severely affected by climate change is the agriculture. There is risk and uncertainty in agriculture. Climate agrees as an independent variable in the agricultural product function and has a limited forecast situation. The climatic conditions required to be suitable for the expected agricultural activity.

Climate change will be higher in the coming years. Pasture areas will be narrowed, herd farming will be removed and the barn-farm will cause significant changes in the main plant tissue of the region.

Therefore, the forest boundary will be turned into cultivation.

Change in Pastures

It is estimated that there are 3.4 billion hectares of pasture in the world. 12 percent of these pasture areas are in China and 11 percent are in Australia. At a smaller rate, 7 percent of those in the past are in the US and 6 percent are in Brazil. Turkey has 14.6 million hectares of pastureland (https://tr.boell.org/tr). The pasture areas are very well protected in countries where animal husbandry develops. Because these pasture areas are very important for sustainable agricultural production. However, in recent years, particularly in Turkey, pastures began to decline. Rangelands are owned by the state in Turkey. Therefore, it is used as investment areas.

The biggest change occurred in grassland areas in basic land assets in Turkey and this continuous change took place to the detriment of these areas. In the past 70 years there has been a 61.5% decrease in natural pasture and pasture areas (Gökkuş, 2018: 6). The decrease in these pastures is accelerated by increasing global warming and climate change.

Table 1. Changes of Pasture Areas by years

CHANGE OF PASTURE AREAS									
REGİONS	**1970 Village Services**		**1991 Agricultural Census**		**2001 TUIK Counter**		**1998 – 2014 2016**		**Dry Herb Yield** - (Kg/ha)
	Area (ha)	**%**	**Area (ha)**	**%**	**Area (ha)**	**%**	**Area (ha)**	**%**	
Aegean	1.027.900	1.32	615.900	0.79	802.879	1.33	388.846	0.46	600
Marmara	463.600	0.59	564.100	0.72	552.662	0.71	280.619	0.35	600
Mediterranean	1.002.400	1.29	434.300	0.56	659.334	0.85	501.765	0.66	500
Central Anatolia	5.884.200	7.54	3.890.300	4.99	4.570.182	5.86	3.726.055	4.32	450
Black Sea	1.99.3.100	2.56	1.556.000	1.99	1.533.605	1.97	1.073.371	1.36	1.000
Eastern Anatolia	2.165.100	11.75	4.573.400	5.86	5.485.449	7.03	3.824.257	4.32	900
Southeastern Anatolia	9.162.100	2.78	743.600	0.95	1.012.576	1.30	553.256	0.68	450
Total	**21.698.400**		**12.377.600**		**14.616.687**		**10.348.169**		

Note: Turkey faces in the calculation was taken as a measurement of 78 million hectares. (2001 General Agricultural Census for meadow and pasture land, Ministry of Agriculture and Forest for others).

Sources: T.C. Tarım ve Orman Bakanlığı, https://www.tarimorman.gov.tr/., https://www.tarimorman.gov.tr/Konular/Bitkisel-Uretim/Cayir-Mera-ve-Yem-Bitkileri, Mera Alanlarının Yıllar İtibariyle Değişimi, Erişim 27.05.2019.

Turkey's meadows and pastures assets was 44.2 million hectares in 1940. This amount decreased to 28.7 million hectares in 1960 (https://tr.boell.org/tr). According to the table, pasture areas were severely reduced. In particular, tourist facilities, industrial facilities or shopping malls, mass housing projects are carried out in these areas. Therefore, livestock breeding was negatively affected. Detection of Pasture work still continues in Turkey. because pasture losses are continuous due to rent. According to the pasture area of distribution is very striking in Turkey. Particularly pasture areas have decreased rapidly in the Aegean and Marmara region. This decrease is caused by urban transformation and touristic construction. The land prices are high in the Aegean and Marmara region. In addition, industrial investments are concentrated in these regions. These regions also have a high rate of urbanization. Therefore, agricultural production and livestock have declined considerably. For this reason, projects for conservation and development of pastures are important.

Table 2. Between 2000-2014 annual Turkey General Pasture Improvement and Management Project Studies

Years	Number of Projects (units)	The project area
1998-2002	46	83.527
2002-2014	1.037	4.982.074
Total	1.083	5.065.601

Sources: T.C. Tarım ve Orman Bakanlığı, https://www.tarimorman.gov.tr/., https://www.tarimorman.gov.tr/Konular/Bitkisel-Uretim/Cayir-Mera-ve-Yem-Bitkileri, Mera Alanlarının Yıllar İtibariyle Değişimi, Erişim 27.05.2019.

The degraded pasture should improve and restorated for liverstock. Over the years, importance has been attached to project design, but studies are still not sufficient. The pastures must converted to high quality pastures for a way of making economic use of pastures.

The Reduction of precipitation has a negative impact for pastures. Especially, The increase in temperature and reduction of precipitation are shown in whole seasons and the increase is very dangers.

Topuz (2018), Turkey average values for the period 1955-2013, for winter precipitation showed a significant reduction trend was started in 1971 autumn rains began in 1981 it shows an increasing trend.

After 1988, a general decreasing trend in precipitation was considerable severe. A statistical significant increase trend in the series of annual temperatures and significant decrease in the series of the annual precipitation 29 meteorological stations were determined(Altın, 2012: 21).

3. RESULTS AND DISCUSSION

Agricultural productivity and pastures assets are important to be sensitive to global climate change. Climate changes will have dangers consequences for global vegetation. Especially, To the extent that agricultural productivity and pasture area are affected, there can be important economic consequences. There is significant determinations about the effects of global change on the vegetation and animal productivity of pasture and rangeland ecosystems. Pasture areas have decreased rapidly in regions where tourism sector is prominent due to regional distribution of pastures and high land prices. Therefore, agriculture and animal husbandry sector is affected negatively. Pasture areas, which are important for agriculture and livestock sector, are the basis of regional economic development.

As a result, the rainfall values are important to measure the severity of a drought in Turkey. Drought has significant economic, social and environmental impacts. In order to investigate the possible effects of climate changes on pastures the relationship between agricultural productivity, temperature, rainfall and size of pasture areas are very important.

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Factors Effecting Of Reproductive Biology and Fruit Settings In Olive

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ABSTRACT

Temperature is the most important environmental factor affecting the flowering in olives. Hartmann (1953) showed that winter coldening is mandatory for flower development. In the researches, it was observed that the yield and quality increased when the olive was watered twice during the end of flowering and core hardening (Akça, A. 2012). Olive cultivation cannot be done in very high places. Since the olive trees bloom late and enter very early, the amount and quality of fruit is not good. To minimize periodicity; It is recommended to give importance to the pruning of the crops in the year (Lavee, 1999; Anonymous, 2008; Özkaya, 2008). In olive cultivation, it is possible to obtain a higher success with machine harvest on the most suitable dates. The selection of varieties in the garden and their genetic characteristics are also very important. Researchers reported that foreign dusting would benefit even in self-conforming varieties in order to obtain sufficient and high quality products (Mete, N., 2009). As a result; Turkey has great importance for the olive and olive oil. Considering this study; The most important job that should be done is to start the standard production by determining the above properties in the olive growing gardens. If this situation is realized, the olive and olive oil production of our country will increase further and the foreign trade will increase and the olive will bring more added value to the country's economy.

Keywords: Biology, fertilization, harvest, olive, pollination.

1. INTRODUCTION

The olive (Oleea europaea L.), which has been cultivated since ancient times and has an important place in the Mediterranean cuisine, and show also an important place in the source of many legends in History, Ancient Writings and holy books have been included. As a matter of fact, after the flood of Noah, The Olive is considered as a symbol of peace because of the return of a white pigeon with an olive branch in its mouth as a sign of the end of the flood. The olive, which has been increasing day by day in human health nutrition, is the oldest known product imported from prehistoric times (Özkaya et al., 2004) (Pouyafard, 2013) that has began in the eastern Mediterranean six thousand years ago (Zohary and Hopf, 2000; Therios, 2009) (Figure 1). Olive is a typical Mediterranean climate plants. Although the rich plant of poor soils has some ecological demands restricting production (Pansiot and Rebour, 1964).

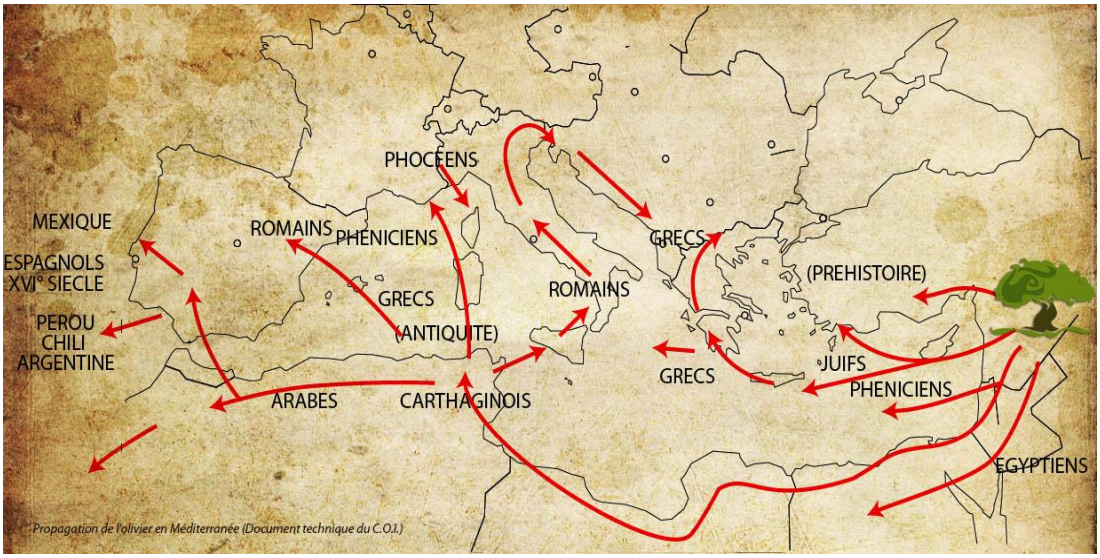

Figure 1. Countries where olive is grown (Anonymous 2016a)

The biological cycle in olive plant is completed in two years and the crop in the trees is formed on shoots growing in the previous year. Vegetative development takes place in the first year and generative development follows in the second year (Varol 2006) (Figure 2). According to the scientific calendar of the olive tree, it is the period in which the flower buds of March continue to differ and the crown leaves and male organs of the flower are formed (Kaya 2006).

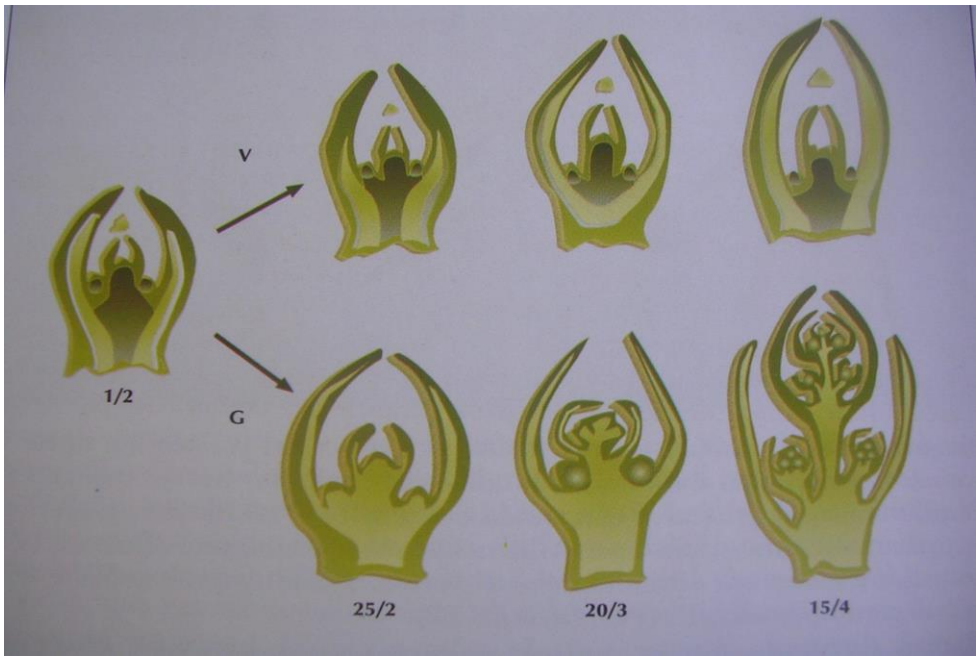

Figure 2. Development of vegetative shoots in olive (Kıvrak, 2015)

The time interval from the first flower bunch variation on the flower bunch to the last one has been a week or two (Lavee, 1999). The rest period ends in April. In this month, the buds that complete their morphological formations start to open and flower clusters from within these buds (Pansiot and Rebour 1964). Blooming period of olives is usually the beginning of May-mid June for mediterranean climate. In June, the olive flower opening is completed and fruit binding takes place. In July, the olive plant grows rapidly with the increase in temperature. In August, fruit growth continued and seed hardening was observed (Varol 2004). September is when the olive fruit begins to collect fat. In October, the rate of photosynthesis decreases as a result of decreasing temperatures. During this period olive fruit continues to be lubricated and remarkable color change is observed in the fruit. In November, as the photosynthetic activity slows down, the olive fruit continues to turn black and maturation occurs in this month (Kaya, 2006).

2. MATERIAL AND METHODS

Morphology of olive flower (structure) the entire O. Europaea is unificated and consists of 4 compound sepals forming a dish at the base of the flower. They have a combined 4 white petals on their floor and fall together at the end of flowering. The flower has 2 stamens (male organ), each with a large, yellow two-lobed Antine. The ovary (ovum) is located in the centre of the sepal dish, each of which contains 2 ovules (seed outline). ” Style " (stylus); fairly large stigma (female Hill) and flat, short and thick. Stigma's form varies between different varieties and is slightly fractured by many types of extremes. The length of the style is also dependent on the variety. Pollen is in the form of a barrel, a three-piece view of the length. Anther and pollen of all olive varieties are bright yellow (Lavee, 1999) (Figure 3).

Figure 3. Branch of olive, flowering and flower structure (Cansu Gencer, unpublished thesis work)

Olive flowers often pollute with the wind. The climatic conditions during flowering are critical for pollination and fruit handling. It has been proven that pollen tube development was prevented in the ovarium when the temperature increased above 30 °C during flowering (Griggs et al. 1975, Fernandez-Escobar et al. 1983). Both the hot and dry climate during flowering and the cool and humid climate can lead to a marked increase in the number of Parthenocarpic fruits (Lavee, 1999).
In this study, the factors affecting the fruit attitude in olive were investigated. These factors are the genetic characteristics of Olives (being self infertile/productive), periodicity, temperature, rainfall and irrigation, altitude and elevation, nutrition, pruning and dilution, harvest.

3. RESULTS AND DISCUSSION

According to various researchers studies, it is determined that internal and external conditions have effects on the stimulation of flowering in olives (de Almedia, 1940). In the year following the year of yield in olive flower, bud formation is very little, also during the development period due to the female organ (ovary) undergoes drop (disappear) flower consists of only male bodies (Urio, 1959).

a. Temperature

The temperature is the most crucial environmental factor affecting flowering olives. Hartmann (1953) showed that the winter chill was mandatory for flower growth. In the heated greenhouses where the winter chill was eliminated, there was no blooming in the growing olives. Studies have shown that the temperature of 2-4 ^{0}C lower than and 15-19 ^{0}C higher than the temperature of the flowering periods when it comes down to the most (Salih Ülger, 1997).
In flower bud, there is little need for cold in order to differentiate. If the cold is sufficiently high, there is also a difference in the high temperatures that are not alarming. The sensitivity of olive to stimulating conditions depends on the product of the previous year. The trees that make up more crops need a longer and more precise cooling. There is an increase in male flower ratio in the

insufficiency of stimulant conditions (Morettin, 1951) (Salih Ülger, 1997).
Take Owusu et al. (2004) examined the climatological conditions required to grow the olive tree in Ghana. In the western regions of Ghana, olives grow better at temperatures between 32.3°C and 40.1°C and can be grown because of the favorable effects of this temperature on germination, vegetative development, flowering and fruit maturation (Çolakoğlu, 2009).
Aguilera and Valenzuela (2012) conducted a 3-year study on microclimical fluctuations in the flower and pollen production rates of olive trees, Iberian Peninsula, pollen grains, flowers, flower clusters and fruit branches, and the possible effects of microclimmatic conditions have been analyzed. Olive trees can give half a million flowers per tree. Pollen production per anther picual variety is more than 60000. Low temperature and high rainfall conditions are the most suitable animals for breeding in olive trees. As altitude increases and fertilizer applications in olive trees pollen formation and reproduction increases.

b. Rainfall and irrigation

Research has shown that yield and quality increased when the Olive was irrigated twice during flowering and core hardening. Furthermore, it is stated that olives remove the most potassium from the soil, remove the most nitrogen with pruning and take into account the amount of the product of the tree in fertilization (Akça, A. 2012).
In the case of fruit trees, water stress decreases in fruit holding period and increases in periodicity (Beede and Goldhamer, 1994); fruit decline occurs in fruit development period, if it occurs at the beginning of June and July, the seed-size decrease occurs (Lavee et al., 1990).

c. Elevation-Altitude

Olive cultivation can not be done in very high places. It is due to the fact that, in places like this, the weather is usually cold and the snowfall is severe. In addition, the amount and quality of the fruit is not good, as the Olive opens late flowers and gets to rest very early. Olive grows at a height of up to 1000 m in the countries in the Mediterranean basin. Olive is grown in South America in Mexico, Argentina and Uruguay at 1200-1600 m, even in micro-climates of 2000 m (Özeker, 2006). In our country, the highest place where olives grow (1157 m) is Cudi Mountain (individual interview, Mücahit Taha Özkaya).

d. Plant Feeding (Fertilizer)

The researchers examined relationship between the level of olive leaf with periodicity. In early studies, it was found that starch content in fruitless trees was higher in winter than in fruitless trees (Anagnostopoulous and Balonos, 1933, Harley et al., 1942).
Sarmiento et al. (1976) found that carbs in olive trees with more products dropped during the growth period.
Özbaykal (1995) investigated the effects of urea, ammonium sulphate and ammonium nitrate on yield, quality and Plant Nutrient Intake in Adana cultivars, domates and Gemlik cultivars applied in two different doses (0.5 kg/tree and 1.0 kg/ tree) from soil for 4 years. As a result of his analysis of olive fruits, the researcher stated that all fertilizers have a positive effect in terms of fruit length, fruit width, meat weight and core weight.

e. Pruning and periodicity

In Turkey, it is difficult and costly to take care for the olive groves on the mountain, on the hill and on the slope, because olive groves are inadequate in terms of maintenance and mineral nutrients and therefore the effect of periodicity has not been reduced (Tunalıoğlu et al., 2003).
In order to minimize periodicity; it is recommended to give importance to crop pruning during the

year or this reason, it is stated that more shoots on the tree should be diluted or application of tip extraction is suggested (Lavee, 1999; anonymous, 2008; Özkaya, 2008).
Ahmad et al. (2009) investigated the effects of "Islamabad" in Pakistan between 1 February-1 March 15-day intervals on the excellent flowering percentages (PF), fruit yield (FS), fruit harvest (FH), fruit size (FSI) and fruit weight (FW) between 2004-2005 in the studies on the effects of "Islamabad" on the fruit culture in Uslu olive cultivars. In both years, the percentage of all data has increased. The highest number of PF was recorded on February 15 and followed by a bracelet on March 1 in 2005.

f. Fruit Dilution

In the study conducted to improve fruit quality with chemical fruit dilution in 'Gemlik' olive cultivars, it was determined that the years of application and K - NAA at different levels have significant effects on the investigated properties. There has been a significant increase in the application dose of 180 ppm compared to the average yield control. Especially in the first year of the trials (no year), depending on the application dose, a stable increase in yield was observed, especially in the second year, according to the control of the level of 180 ppm was very high yield increase. In this respect, it was concluded that 150-180 ppm and even higher doses of NAA applications may be useful in 'Gemlik' olive cultivars (Çiğdem, 2014), in order to control periodicity to a certain extent and especially to reduce the loss of yield in the year, even if it is not fully inhibited (Çiğdem, 2014).
The application of 200-250 ppm NAA for fruit dilution 12-18 days after full flowering yields positive results (Sibbett and Martin 1981). The 80-240 ppm doses of NAA and NAD in Israel were applied 4, 8, and 12 days after full blooming of Manzanillo, Arida-5 and Kalamata cultivars. The NAA application has increased the fruit size and quality of the NAD (Lavee and Spiegel-Roy 1967) while setting the amount of the product (Salih Ülger, 1997).

g. Harvesting

Sol and Floraensa (1997) harvested Arbequina variety olives, which were grown in Spain between 1990-1993, mechanically between 19 November and 13 February, and on 3 different dates. Yield values were 8.38 kg/tree, 13.80 kg/tree and 14.42 kg/tree respectively from early harvest period to late harvest period. They stated that a high yield, a high harvest percentage and the best economic yield were achieved in late harvest. As a result of the researches carried in Turkey, the most suitable dates were determined for machine harvests by the olive harvest and harvest efficiency (Figure 4).
Çiçek and et al. (2012) harvest with stripping in research (1. method), beating the harvest with the rod (2. method), harvest with combed Machine (3. method) and harvest with branches shaking (4. method) four different harvesting methods were used. The yield and business success values were investigated by applying 4 different harvesting methods in 12 different trees with the same characteristics for Ayvalik olive cultivars grown as fat in the field studies. As a result of research 1. and 2. it has been determined that methods should not be preferred in terms of business success. In terms of the most appropriate method for business success is the 3rd method. It was evaluated in terms of yield, which has proved that methods had no effect on yield ($p>0.05$).

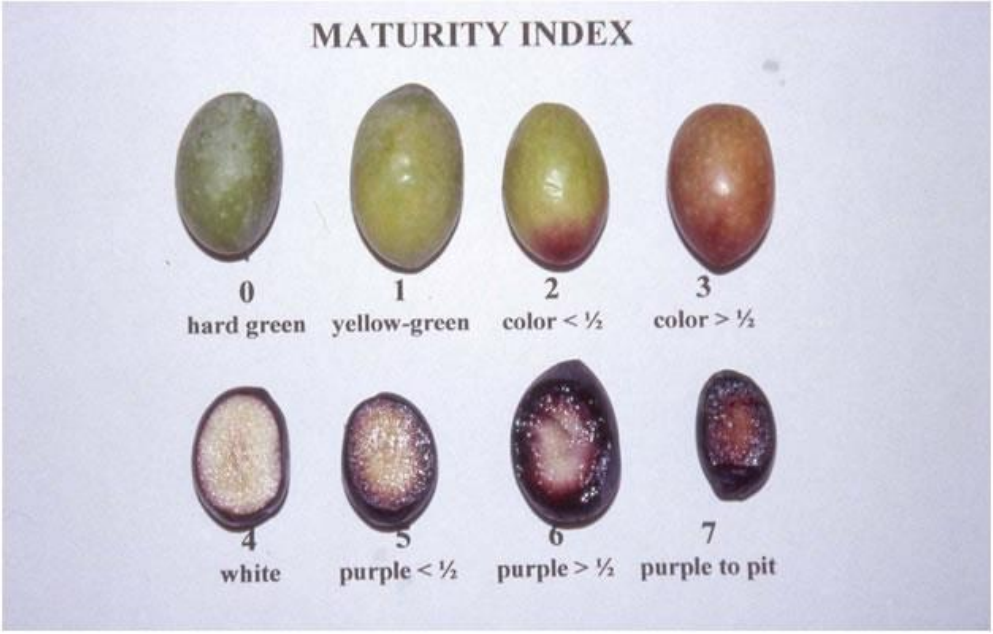

Figure 4. Fruit maturity status (Anonymous 2016b)

h. Genetic and effective Pollination Period

Mete, (2009), in his study between 2007 and 2008, fertilization biology of Domat, Edincik Su, Eşek zeytini (Ödemiş), Kilis yağlık, Samanlı, Uslu and Arbequina cultivars were investigated. For this purpose, free pollination, mutual pollination and automatic applications, self-efficiency of varieties were investigated and appropriate pollinators were determined. As a result of the research, Arbequina, Edincik Su and Samanlı varieties were self-fertile, Domat and Uslu varieties were partially self-fertile, Eşek zeytini (Ödemiş) and Kilis Yağlık varieties were self-inefficient and evaluated in three groups. In addition, researchers have reported that foreign pollination will be beneficial even for a variety of self-polluting products (Mete, N., 2009).

Mozo-Garcia et al. (2007) they conducted their research in the cities of Castilla - La Mancha, Real and Toledo, which are Spain's second largest olive producer. Two different time climate data were obtained: flowering intensity, flower powder index in the atmosphere and flowering and fruit attitudes. In statistical analyses, the annual flower powder index was determined to be the most important variable in both cities affecting the final production. On the other hand, they found that the maximum temperature in March and the amount of rain in October were the climate data that most affected annual production. It is stated that a significant step has been taken in predicting future olive production with the model using climate and flower powder data in Castilla-La Mancha region.

In southern Europe (Cordoba, Spain) the trends and changes of olive's flowering season in 30 years were examined as “annual pollen index” (PI) , “pollen season start” (PSS), “peak date” (PD), “pollen season end” (PSE) and “pollen season end” (PSE). In traditional linear regression analysis, a seasonal trend research procedure based on STL and ARIMA model was applied. Linear regression results probably show a correct trend with the effect of temperature rise. Findings have shown that the olive reproductive cycle has changed significantly over the last 30 years due to climate change.

In some varieties, parthenocarpic fruit (beaded fruit) formation is observed in varying proportions according to years. Lavee (1998) attributed this to the factors such as non-functional flower dust pollination, inhibition of the growth of the flower dust grass tubing in the tracheal tube, and abnormal development of the ovary (Figure 5).

Figure 5. Beaded fruit development (Anonymous 2016c)

4. CONCLUSION

According to the results of the research, increasing the flowering and fruit attitude depends on internal and external factors. These are the genetic characteristics of Olives (being self infertile/productive), periodicity, temperature, rainfall and irrigation, altitude and elevation, nutrition, pruning and dilution, harvest. In order to increase flowering and yield, lighting should be increased, cooling needs should be met, orchards should not be installed at very high altitudes, there should be no lack of water during pollination and fertilization. In order to prevent or reduce the periodicity of flower bud formation in the present, pruning and harvesting should be done in a way that does not harm the eyes of wood and flowers. The fertilizers that will be given in a regular way in the trees in the product Age make them efficient and balanced. Genetically, the garden should have some kind of pollinator, the main type of pollinator between the time of flowering should coincide with varieties.

As a result, Turkey is of great importance in terms of olive and olive oil. Therefore, productivity and quality needs to be increased further. This situation can be especially with the increase of fertilization and fruit attitudes. The most important task to be done is to determine these characteristics in the gardens where olive cultivation is carried out and to switch to standard production. If this is realized, the production of olive and olive oil will increase further and the possibility of foreign sales will improve and the Olive will provide added value to the economy of the country.

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AGROFOOD
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Research On Increasing The Value Added In Oliviculture

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ABSTRACT

Turkey, among the world's major regions, although olive olive cultivation, agricultural techniques and technology due to the lack of progress in our country, has not developed in such a way that it deserves (Anonim, 2019a). Olives must be completely devoid of bitter taste. In processing, olive husk, thin and elastic, hardness and durability of fruit meat should be permanent (Marsilio, 2002). Barut (2000), In this study, it was found that low acid content in black table olives and high protein, fat and sugar content were among the most important quality criteria. Many factors such as flavor, taste and odor in olive oil composition, diversity, maturation, harvesting, post-harvest storage are determined (Papadimitriou ve ark., 2006). As a result, the processes for the production of high quality olives and olive oil should be followed in accordance with the factors such as waiting, handling and processing, starting from the supply of seedlings, taking into account climate and growing conditions, and after harvesting and harvesting determined on time. While one of the most important stages in olive production is to relieve the acidity, the most important factor in olive oil is known as high polyphenol and low acid. The aim of this article is to shed light on other studies in order to raise the added value especially considering that our country can be grown in five regions.

Keywords: Fruit, health, olive, production, quality.

1. INTRODUCTION

Turkey, among the world's major regions, although olive olive cultivation, agricultural techniques and technology due to the lack of progress in our country, has not developed in such a way that it deserves. Olive trees, the root systems after you access a certain size formed tubers is lost to renovate themselves through property, it contains polyphenols, antioxidants, such as adaptation to stress conditions of flavanoid and five in our country can make it in different parts of the tree that bears its own unique features (Anonim, 2019a).
Aegean region: Balıkesir in the Gulf "Ayvalik", İzmir, Aydin and Muğla “Memecik” olive variety is leading. Gemlik, Çakır, Çilli, Dilmit, Erkence, Eşek zeytini, İzmir sofralık, Kiraz, Memeli, Tavşan yüreği, other varieties of the region. Marmara region: olive grove dates back to ancient times. Olives Gemlik assortment including; Edincik su, Beyaz yağlık, Çelebi (İznik), Çizmelik, Erdek yağlık, Eşek zeytini, Samanlı, Şam, Karamürsel su, Siyah Salamura. Mediterranean region: Olives in this region, in competition with other crops. The most important varieties; Topak Ulak, Çelebi (Silifke), Halhalı (Hatay), Karamani, Sarı Habeşi, Sarı Ulak, Saurani ve Sayfi. Southeast Anatolia region: important varieties; Kilis Yağlık, Nizip Yağlık, Halhalı, Eğriburun (Nizip), Kan Çelebi. Other varieties; Belluti, Halhalı, Hamza Çelebi, Hırhalı Çelebi, Yağ Çelebi ve Yün Çelebi Zoncuk (Anonim, 2019a) (Figure 1).

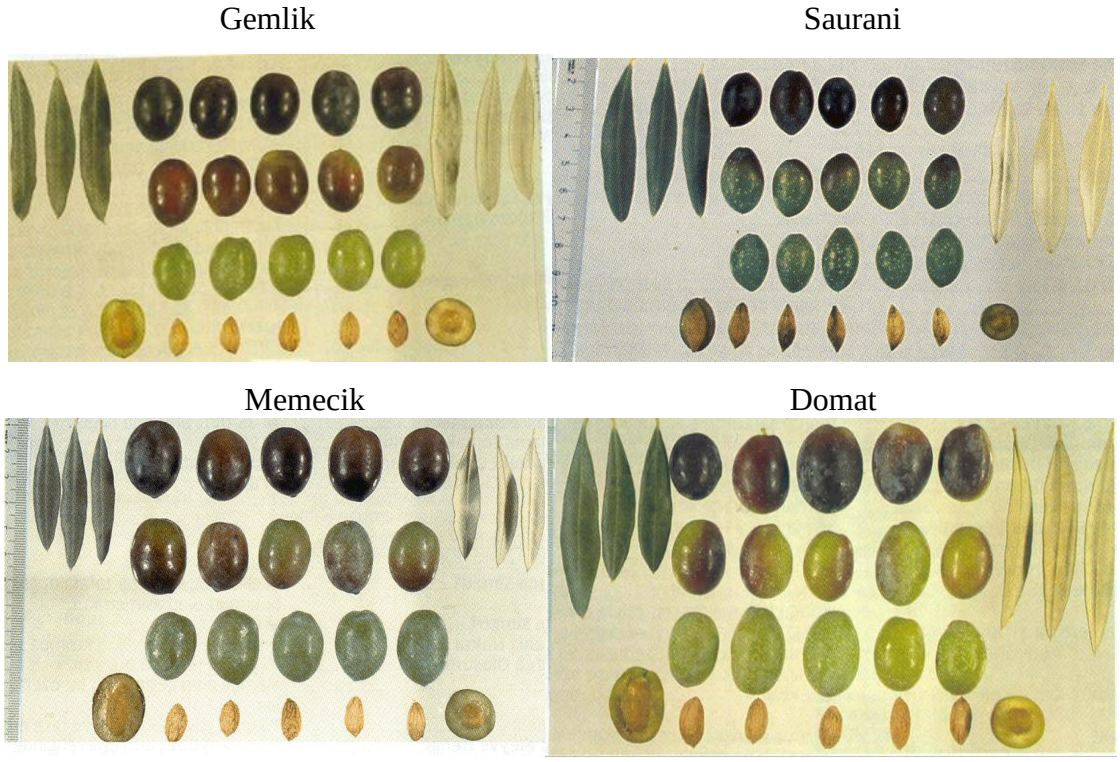

Figure 1. Some important varieties (Anonim, 2019a)

2. MATERIAL AND METHODS

Olive seedlings production stages must haves: some varieties of selection, the rootstock and the creation of the parcel must be known to the replication method pen, reproductions and formation of the practice ground, pre-sales certification processes (Özkaya, 2003).
Olive oil composition changes, such as flavor, taste and smell, kind of like the ripening, harvesting,

post-harvest storage is determined by many factors. The most important quality problems that may appear unsaturated fatty acids oxidation occurring due to oxidative rancidity and consequently causing unwanted taste and taste components in the oil. Olive oil oxidation resistance and oxidative decomposition degree (Mateos et al., 2005) and fatty acid composition and phenolic components, tocopherols, carotenoids and chlorophyll depends on the concentration of natural antioxidants (Papadimitriou et al., 2006). The quality of food products; a collection of properties that are acceptable by consumers (lens; the product's energy, vitamins, minerals, toxin content and freshness and color, shape, taste and subjective; the smell of the product etc.) (Dölekoğlu, 2003) (Özdoğan, 2018). In addition to the physiological necessity of food consumption quality people, secure and reliable. Companies must certify their production and brands in accordance with national regulations (Zırapli, 2008) (Özdoğan, D. ve Tunalıoğlu R., 2017) (Table 1).

Table 1. The properties examined in sensory analysis of table olives (IOC, 2010) (Özdoğan, 2018).

Character	Sensory perception
Negative features	Abnormal fermentation, fattening, bitterness, cooking effect, soap, metallic, earthenware, vinegar (wine) taste
Descriptive taste character	Salty, bitter, acidic taste
Tissue properties	Hardness, fiber, crispness

Abnormal superficial spots in olives, wrinkled, pest damage and abnormal color should not be. Color, variety, maturity and processing according to the desired tone and uniform must be. Olives must be completely devoid of bitter taste. In processing, olive husk, thin and elastic, hardness and durability of fruit meat should be permanent (Marsilio, 2002). Barut (2000), In this study, it was found that low acid content in black table olives and high protein, fat and sugar content were among the most important quality criteria.

Geographical indications are protected in order to ensure that agricultural and food products used for human consumption in general in the world and in EU regulations have a certain quality and quality. The quality and characteristics of the registered product and its products are not the persons or institutions that make the registration, but the quality and characteristics of the registered product (Yıldız, 2008) (Özkaya ve ark., 2010).

The amount of α-tocopherol (vitamin E) in olive oil may vary depending on variety, maturity, storage conditions and storage time. The amount of tocopherol in the first period of harvest is high and the amount of late harvest oil is less (Gimeno et al., 2002) (Sevim, 2011). The pain is caused by oleureopein content and therefore is not eaten without treatment. This process also causes softness in vision. Olives collected during certain periods are kept in the brine. It is processed in aerobic or anaerobic conditions after PH adjustment with lactic or acetic acid. Color, appearance, etc. in terms of structure is fixed (Mafra et al., 2007) (Table 2).

Table 2. Olive fruit, leaves, olive oil, black water and pirina polyphenol amounts (ppm) (Farag, 2003) (Sevim, 2011).

Products	Amount of polyphenol (ppm)
Olive Fruit	495
Olive leaf	250
Olive oil	195
Glaucoma	170
Pirina	73

Processes for the production of olives and olive oil start from the supply of seedlings, taking into account the climate and growing conditions, timely identified harvest and post harvest, factors such as transportation and processing. In this study, the above factors have been examined one by one and the ones that need to be done in order to produce quality products have been investigated.

3. RESULTS AND DISCUSSION

Olive and olive oil production is a labor-intensive agricultural activity as in other agricultural activities. Regular maintenance of olive trees, pest and disease control, etc. breeding requirements are very important. The correct determination of the harvest time, the olives should be collected individually and carefully from the branches in order to prevent damage, and the healthy ones should be carried to operation in the safe boxes at the latest 24 hours by extracting the olives from the damaged and rotten grain. Dane processing with modern technology, hygiene rules and the use of special storage conditions increase the quality (Tunalıoğlu and Özkaya, 2014).

Olive plant needs favorable environmental conditions for high yield and climate factor is very important in terms of aquaculture (Çolakoğlu, 1992). Characteristics such as fruit size, ripening cycle, resistance against casting, which vary in all varieties, affect table olive quality (Seferoğlu, 1997). In order to establish a physiological balance in vegetative and generative development of the olive tree and to extend its economic life, it needs pruning, taking into account the present or absent years (Fontanaza, 1988). Irrigation and fertilization application is also important in olives. Biological pests such as olive fly, olive stew, environmental conditions and deficiencies in agronomic methods cause significant product losses and quality degradation in olives (Harp and Keçeli, 2008) (Özdoğan, 2018). In addition to the formation of injuries and spots in the olives harvested from the pole, the shoots of the olive tree are also damaged. Harvested olives should never be kept in the sun. Even in the shade, olive oil has been waiting for a long time. Harvested olives should be moved to operate in plastic containers not deep, and should not be moved in stacks or bags. Olive oil, which is waited in stacks for a long time, has deteriorated due to heat (Söylemez et al., 2010). The phenolic contents of oils obtained from early harvested olives are higher. It was observed that the total phenol and O-diphenol contents of the oil were decreased as the maturation level increased (Skevin et al., 2003) (Harp ve Keçeli, 2008).

After the harvest, the process of longitudinal and extraction, the processes applied in olive processing and packaging also affect the quality of the final product. Inappropriate hygienic conditions in small enterprises, variety of variations, uncontrolled additives use are some of the factors that affect quality (Korukluoğlu, 2006). It is very important that acidification, pH, salt concentration, yeast mildew growth and circulation processes of olives are performed at regular intervals during processing and fermentation process applied in table olive technology is carried out effectively (Tetik, 2006; Harp ve Keçeli, 2008). In Table Olives, in addition to cans with lacquer, vacuum and plastic containers are

also used as packing material. In poorly laced cans, the shelf life of the product is reduced, and in plastic packaging made of poor quality material, it causes the taste of Olives to change during the storage period. Packaged olives should be stored in moisture-free environment (Trigger, 2006; Harp and Felt, 2008). Strong light has a negative effect on vitamins, such as chlorophyll and ß-carotene, which are color substances. Oxygen in the air oxidizes the oils over time. For this reason, oils should not be left exposed for a long time. Olive oil should be stored in a dark and cold environment (10-15 °C) to slow degradation (Kiritsakis, 1998; Kayahan and Tekin, 2006) (Keçeli, 2008).

Şimsek and Yalçın, 2008. In order to determine the effects of different modes of transportation and waiting periods on olives, they used the Memecik variety which was grown widely in Aydın region. The harvested olives were filled in boxes and nylon bags and divided into two groups. Some of the samples carried, without any waiting, while the other 7, 14 and 21 days waiting, moisture, shape index, friction coefficients and % oleic acid values were investigated. As a result of the study, it was observed that the loading time and the loading time were statistically effective on the parameters studied.

Kutlu and Şen, 2011, in order to investigate the effect of different harvest times on the quality of fruit and olive oil, Manisa also harvested fruit samples at different times in a study conducted on Gemlik olive varieties. Meat/core ratio reached high values in the third and fourth harvest time. As harvest time progressed, increase in oil amount, decrease in moisture amount was observed. The free fatty acid has shown a slight increase as maturity progresses, giving values below 1%. The most important fatty acid in olives, oleic acid has not changed with maturation. Palmitic and linolenic acid decreased while palmitoleic and linoleic acid increased as harvest time progressed. It has been concluded that it would be appropriate to harvest in December for the late November and late December of the Year for the edible Gemlik olive varieties in the region.

Oleureopein is abundant in unripe olive fruits and leaves, and the pain is greater in these products. In processed olives and olive oil, the acidity decreases as hydroxytozol, which is the product of oleuropeinin decomposition, is more abundant (El ve Karakaya, 2009). For this reason, chemical (alkali - diluted NaOH) and enzymatic hydrolysis are applied for the immediate consumption of Olives after harvest (Marsillo and Lanza, 1998). Water wash takes place several times to get away from excess alkalinity. Then 10-13% (W/V) NaCl solution is added to the fruit and waited for months for fermentation (Brenes de Castro, 1977) (Yorulmaz ve Tekin, 2008).

To make olives edible, it is necessary to relieve their suffering. For this reason, after treating olives with alkaline waters and washing them with ordinary clear water, it is canned in salted water (brine). Olives to be removed from the pain are put in concrete pools, tap or glazed containers. The olive oil is poured over the olives and waited for 4-6 hours. When two-thirds of the fleshy part of the olive changes the color, it is necessary to empty the water. In olives, it is left in water for three days by changing its water three times a day. The last water should be clear and sparkling. The most recent olives (1-8) are prepared and cooled in hot brine, preserved for a long time without spoiling (Üstüner, 1982) (Figure 2).

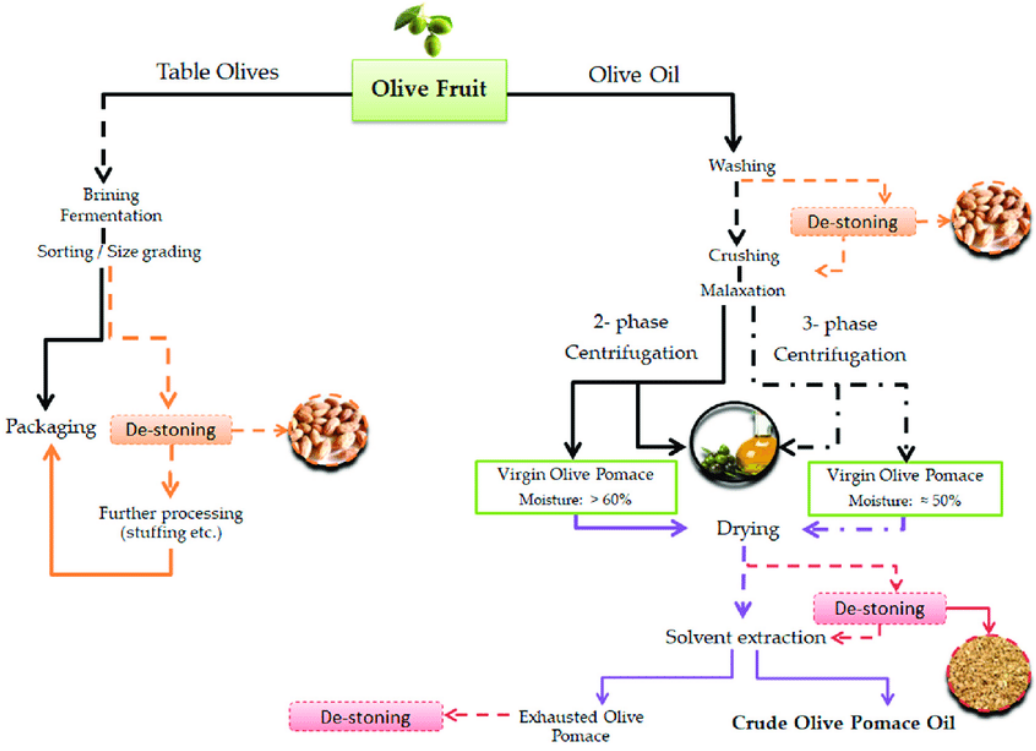

Figure 2. General processing lines for table olive and olive oil (Anonim, 2019c)

It has been reported that the contents of oleuropein are reduced by drying up to 15-20% of the contents of the olives after pre-treatment such as waiting in brine, holding in water, folding in dry salt, drilling and/or drawing. In Italy, a method called Ferrandina has been reported to produce black olives with table tables for many years (Marsilio et al., 2000, Gambella et al., 2000). In this method, the black olives which were harvested as mature over time are kept in boiling water at 90 ^{0}C for 5-10 minutes and then kept in 10% dry salt for 3 days. The olives were then dried at 50 ^{0}C for about 17 hours in the dryer (Cardoso et al., 2009). However, the researchers reported that drying and table olive production were not widespread in the industry (Özdemir et al., 2015). It has been reported that the olives have the highest polyphenol content. The results of sensory analysis of the olives that were applied to the drilling process were the highest (Piga et al., 2005) (Özdemir ve ark., 2015).

In the industry, it is reported that olives are treated with alcali 3 times in order to relieve the pain. Alcaline ratio varies according to the type of Olives, although 1. (%2), 2. (1.0-1.5%) and 3. (0.5-1.0%) in the form of alcali applied. 3. in the costume, olives should be washed immediately and removed from alcaline when the costume is processed up to 2/3 of the grain (Aktan and Kalkan, 1999) (Ekici ve Velioğlu, 2004).

Barak and Yaşar, 2008. In their study, the total polyphenol content of 4 different commercial olive oil samples was compared, but no significant differences were observed. However, it is known that oils derived from early harvested olives contain more phenolic content. Polyphenols are effective materials for durability of samples. The time and storage conditions after degreasing cause changes in the amount of polyphenols. Because the phenolic compounds, heat, light, moisture, trace elements such as the effect of the reaction of autooxidation that occurs, they are consumed over time. Due to the large solubility in water, it is known that most of the polyphenols in olives were transferred to Olive land water during the oil removal process (Figure 3).

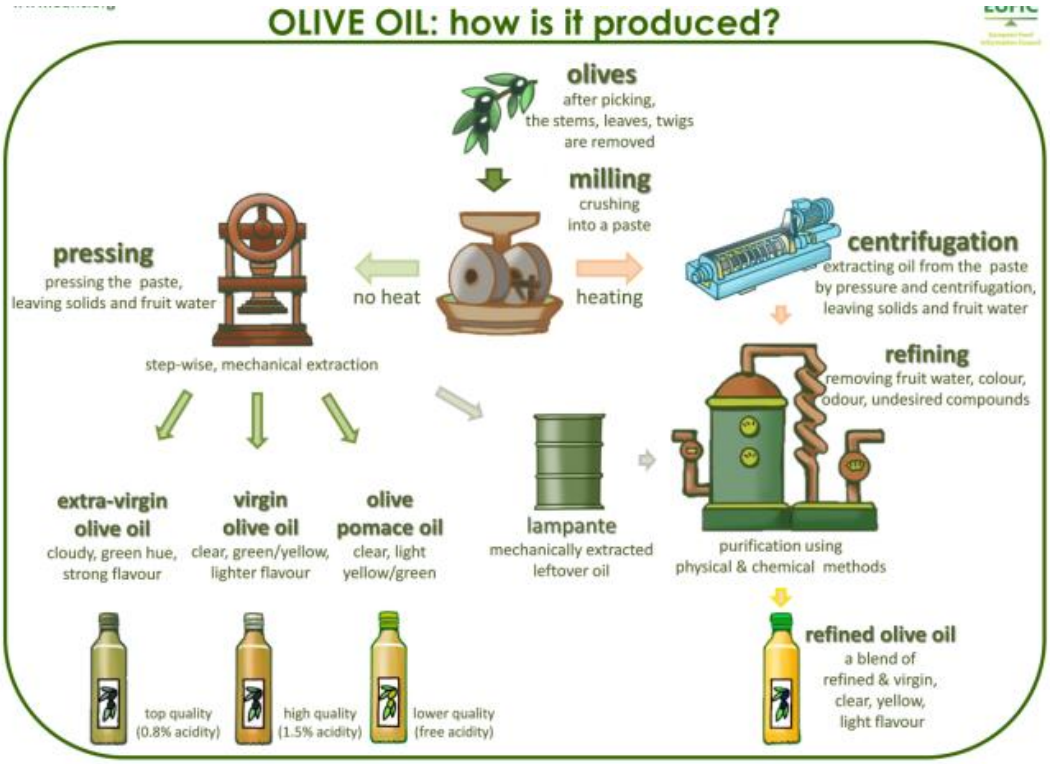

Figure 3. Olive oil production stages (Anonim, 2019b)

High amounts of monounsaturated fatty acids in olive oil structure and has longer shelf life than other vegetable oils. However, if olives are not processed on the same day, fermentation and oxidation are inevitable to begin. The excess water added to the olive paste causes the water soluble polyphenols to move away from the oil and deteriorate the quality (Fadıloğlu and Göğüş, 2009). Polyphenols increase the oxidative stability of oil, improves sensory properties. Researchers; the relationship between daily intake of phenolic agents in olive oil, especially hydroxytizol and its derivatives, and cardiovascular diseases and cancer (Boskou, 2006; Yorulmaz ve Tekin, 2008). (Table 3).

Table 3. Major and minor components in olive (Granados-Principal ve ark., 2010.)

<table>
<tr><td rowspan="9">Major components (saponifiable fraction);
Oleic acid,
palmitic acid,
linoleic acid,
stearic acid,
palmitoleic acid,
linolenic acid,
myristic acid</td><td>Minor components (non-saponified fraction);</td></tr>
<tr><td>1. Non-glyceride esters and waxes</td></tr>
<tr><td>2. Aliphatic alcohols</td></tr>
<tr><td>3. Triterpen alcohols: eritrodiol and uvaol</td></tr>
<tr><td>4. Sterols: β-sitosterol, kampesterol, stigmasterol</td></tr>
<tr><td>5. Hydrocarbons: squalene, volatile hydrocarbons (phenanthrene, pyrene, fluoranthrene), carotenoids (β-carotene and lycopene)</td></tr>
<tr><td>6. Pigments: chlorophylls and feofitins (a and b)</td></tr>
<tr><td>7. Volatile compounds</td></tr>
<tr><td>8. Phenolic compounds:
a) Lipophilic: Tocopherols and tokotriends (α, β, γ)
b) Hydrophilic: Phenolic acids; benzoic such as benzoic, gallic, vanylic acid; cinnamic, caffeic, coumaric acid etc. sinnamic species</td></tr>
<tr><td colspan="2">Phenolic alcohols; hydroxytyrosol, tyrosol and glycosides</td></tr>
<tr><td colspan="2">Secoiridoids on; oleuropein and aglycone, ligstroside aglycone, hydroxytyrosol and tyrosol</td></tr>
<tr><td colspan="2">Flavonoids: apigenin, luteolin</td></tr>
</table>

4. CONCLUSION

As a result, the processes for the production of high quality olives and olive oil should be followed in accordance with the factors such as waiting, handling and processing, starting from the supply of seedlings, taking into account climate and growing conditions, and after harvesting and harvesting determined on time. In addition to the quality and reliability of its products, taste, aroma and appearance are important for consumers. While one of the most important stages in olive production is to relieve the acidity, the most important factor in olive oil is known as high polyphenol and low acid.

This article focuses on how to plan better quality and appropriate production in olive and olive oil, what can be done to better pay for the consumer's expectations and the labor that is spent. The aim of this article is to shed light on other studies in order to raise the added value especially considering that our country can be grown in five regions.

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Evaluation of Antioxidant Activity and Total Phenols Content in *Acerola* (Barbados cherry)

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ABSTRACT

Barbados cherry, having a red, pink or yellow color, is a tropical plant species. The plant has the antioxidant content; vitamin C is high nutrients too. Having high vitamin C content, in addition to the use of ascorbic acid and antioxidant in food and feed constantly increase the demand for Barbados cherry. Several studies reported that in addition to vitamin C, fruit is a good source phytochemicals such as anthocyanins, phenolic compounds, and dietary carotenoids. This study intended to assess antioxidant capacity of this plant. For this purpose different solvents with different polarities (water, 50% methanol, 70% ethanol). Water bath and ultrasound assisted extraction were used as extraction method and total phenolic contents of the extracts of *barbados cherry* were identified by Folin-Ciocalteu modified. Also the medicinal plant were evaluated for antioxidant potential using DPPH free radical scavenging assay and reducing power activities of plant extracts were observed by means of FRAP assay. Results shown that the antioxidative activity depend on the extraction methods of applied fruit extracts (as ripe, less ripe and immature) and decreases in the order methanol >ethanol> water.

Keywords: Barbados cherry, Folin-Ciocalteu, DPPH, FRAP

1. INTRODUCTION

Free radicals that occur as a result of various reactions in the body can cause various damages to the body. These damages can be to tissues, organs and systems. Many researchers reported that anthocyanin contents in the medicinal plant decrease the free radical effects (Doa QD and et al, 2014; Güçlü K and et al, 2006). Plant material consists of multi-component mixtures, and that‘s why their separation and determination still creates problems. The medicinal efficacy of medicinal plants is highly depended on the method of extraction or extracts preparation. Among the extraction methods, in this study ultrasound assisted and water bath extraction were used; methanol, ethanol and water were chosen as a solvent. Barbados cherry grows in Central and South America including Brazil and ıt is principally known for its high vitamin C content (Ergun M and et al, 2014). The fruit is grown in some areas of the Turkey but there is no study on the antioxidant properties of Barbados cherry grown in Turkey. In the light of this information the plant specimen were obtained from a farmer in Dalaman, Mugla; and determined the antioxidant capabilities. Fruit is divided into 3 groups as ripe, immature, and less ripe. The objective of this work was to investigate the effects of solvents on the extraction of phenolic contents from Barbados cherry and investigate the antioxidant activity of the extracts by *in vitro* methods. For this purpose, total amount of phenolic contents in acerola was determined as gallic acid equivalent by Folin-Ciocalteau method. Antioxidant activity of acerola was assessed by various in vitro assays such as DPPH (2,2-diphenyl-1- picrylhydrazyl) and FRAP (ferric reducing antioxidant power). Results were determined as Trolox equivalent.

2. EXPERIMENTAL

Barbados cherry fruits which were obtained from a farmer in Dalaman, Mugla, Turkey. Folin-Ciocalteu’s reagent, iron (III) chloride hexahydrate, methanol, sodium carbonate were purchased from Merck (Darmstadt, Germany). DPPH, (Trolox), gallic acid (GA), (TPTZ) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). The rest of chemicals, including sodium carbonate, sodium hydroxide, ferric chloride, methanol, ethanol, acetonitrile and petroleum ether were of analytical grade and obtained from Merck. Deionized and pure water (Millipore-Q System) was used for the study.

2.1. Preparation of plant extracts

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization (Doa QD and et al, 2014). Also solvent effects and extraction conditions on phenolic contents of medicinal plant were studied in our previous studied (Alkaya DB and et al, 2019). The fruit samples (as ripe (R), immature (I), and less ripe (LR)) were grinding to obtain a homogenous material with using a blender. The extraction of hydrophilic compounds uses polar solvents such as methanol (MET), ethanol (ET) and water (W). 500 mg fruit material powders were mixed with the 5 mL of solvents (i.e. 50% methanol, 70% ethanol and water). The extraction temperature and time were 60 ^{o}C, 60 min for ultrasound assisted extraction [U] and water bath extraction [S]. The extraction procedure was repeated three times and these three extracts were combined, the volume was completed to 15 mL. The extracts were filtered using Whatman blue band filter paper and stored at -20^{o}C until analysis. The extraction procedure was as described by Güçlü K and et al (Güçlü K and et al, 2006).

2.2. Total phenolic contents

Total phenolic content of *barbados cherry* was determined with Folin-Ciocalteu reagent according a modified method using GA as phenolic compound standard (Karadağ E and et al, 2009). In brief, 100 µL extract solution was added to 4.0 mL of distilled water afterwards 100 µL Folin-Ciocalteu reagents was added into the mixture. Each mixture was incubated for 5 min at 30ºC and then 800 µL Na_2CO_3 (6%) solution was added. Subsequently the mixture was incubated for 30 min at 30 ºC and then the absorbance was measured in 685-760 nm by using a spectrophotometer (UV 1601, Shimadzu Co., Ltd., Kyoto, Japan).The results were expressed as milligram GA equivalents per mL sample dry weight (Fig. 1). The calibration equation for GA is $A = 0.0019x – 0.0114$ ($R^2 = 0.999$). Each test was repeated three times.

2.3. Ferric reducing/antioxidant power (FRAP) assay

In this study the method was described by Benzie and Strain (Benzie and Strain,1999) with some modifications. The working solutions, used on the day of preparation were: FRAP reagent:acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in HCl, 40 mM) and $FeCl_3$ (20 mM). The required sample was added to the FRAP reagent (10:1:1, v:v:v) incubated at 37 ºC for 4 min. The absorbance of the mixture was read at 580-600 nm domains. The antioxidant capacity based on the ability to reduce ferric ions of the sample was expressed as milimolar trolox equivalents per sample by using a calibration graph (Fig. 2). The calibration equation for trolox is $A = 0.0016x + 0.0416$ ($R^2 = 0.9915$). Each test was repeated three times.

2.4. Determination of radical scavenging ability by using DPPH Method

The antioxidant activity of the extract was measured with the DPPH method with slight modifications (Yilmaz and Seyhan, 2017). The extract 1.5 mL and and 0.1 mM DPPH solution (1.5 mL) was mixed together in a test tube. The reaction tubes were incubated at 30˚C for 30 min. (200 rpm). The decrease in absorbance was measured at 515-528 nm domains by using spectrophotometer. The results were expressed as milimolar of trolox equivalent antioxidant capacity (TEAC) per mL sample dry weight (Figure 3). The calibration equation for trolox is $A = -0.0127x + 0.5628$ ($R^2 = 0.9989$). Each test was repeated three times.

3. RESULTS AND DISCUSSIONS

This study intended to assess the antioxidant potential of *barbados cherry* extracts, obtained by two extraction methods and also in different extraction solvents, was evaluated and the results were compared.

3.1. Total phenolic content

The values of total phenolic content for analyzed samples are presented in Fig 1. The methanol extract of *barbados cherry* showed the highest phenolic concentration contrarily water (for ultrasound assisted extraction) and ethanol (for water bath extraction) extract of this plant showed lowest antioxidant activity. As indicated in Fig. 1, the total polyphenols values varied from 0.64-1.47 mg GAE /mL.

3.2. Antioxidant capacities

Modern pharmacology demonstrated that antioxidant activity of *barbados cherry* species and has potential activity to protect the body from some disease. The antioxidant capacity of were widely studied *in vitro by* follows: DPPH and FRAP method [Güçlü K and et al, 2006].

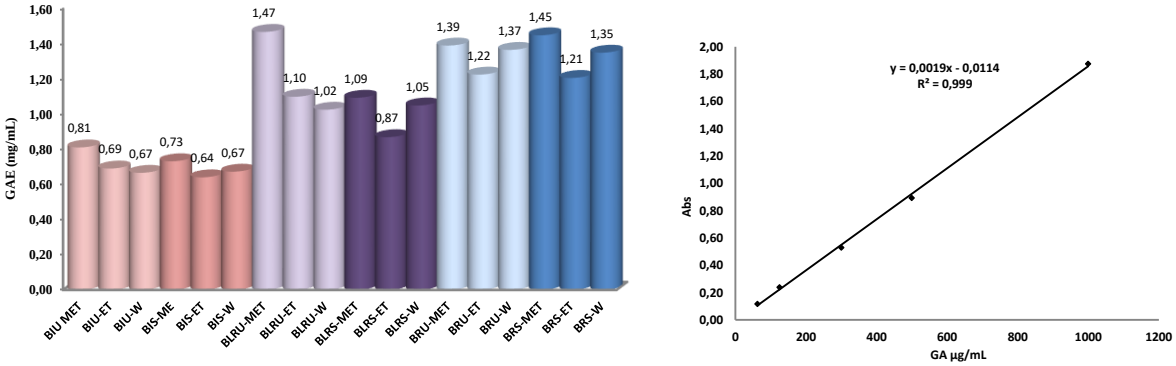

Figure 1.Total phenolic contents in the *Barbados cherry* extracts expressed in terms of gallic acid equivalent (mg of GaA/g of extract)

The FRAP values of studied samples are shown in Fig 2. The best antioxidant capacity was acquired using methanol. Contrarily, water (for ultrasound assisted extraction) and ethanol (for water bath extraction) extract contains considerably smaller concentration of phenols. As indicated in Fig 2, the FRAP values varied from 5.49-29.53 mM Trolox/mL

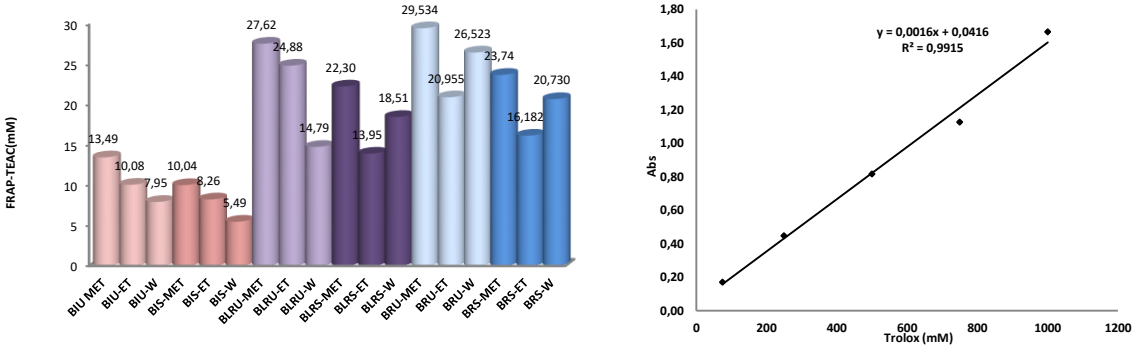

Figure 2. FRAP of *Barbados cherry* extracts.

The DPPH values of sample are shown in Fig. 3. The highest value was determined for methanolic extract. By contrasts, the lowest antioxidant capacity was obtained by using water water (for ultrasound assisted extraction) and ethanol (for water bath extraction) extract as solvent. As indicated in Table 3, the DPPH values varied from 0.17-6.12 mM TEAC/mL.

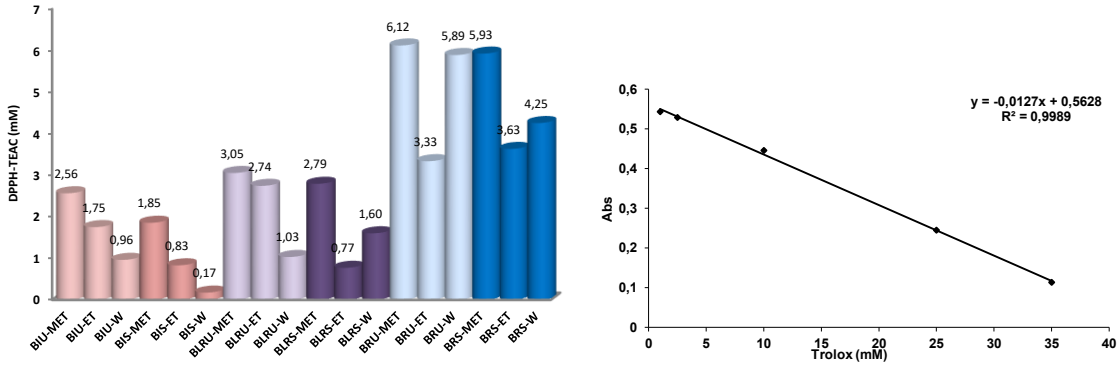

Figure 3. DPPH scavenging activity of investigated *Barbados cherry* extracts

4. CONCLUSIONS

The results presented in this study provide information on *Barbados cherry* had the antioxidant capacity of extracts. The best antioxidant potential of extract was evaluated using different antioxidant tests. In this study, it was also observed that the antioxidant activity of methanol extracts is higher than those of water, and ethanol solvents. In addition, the results of the ultrasound assisted extraction method showed higher antioxidant capacity than the water bath extraction method. Comparatively, the data obtained clearly indicate that the ripe fruit extract have contents than immature and less ripe extract. Therefore, this aqueous fraction will have highest amount of antioxidant compounds which are thought to be phenol in nature. Among the tested extraction methods, the antioxidant capacity of the plant extracts the highest value of antioxidant capacity of *barbados cherry* in water extract. In vitro antioxidants of the polyphenol extracted with the optimum conditions showed high antioxidative activities. In the light of this information the result of this study showed that 1)The maturity of fruit; (2) different solvents used in extraction resulted in differences in compositions and antioxidant activities of the extracts 3) the method and conditions of extraction affected antioxidan activity. For extracting flavonoids from ripe fruit methanol performed better than etanol and water.

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Using of Hydrocolloids in Meat and Meat Products

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ABSTRACT

Recently, consumer demand towards less fat and more functional foods is increasing. For this purpose, Hydrocolloids in meat products, used to compensate undesirable effects of fat reduction, salt reduction, improve functional properties and freeze/thaw process. Hydrocolloids are used in batter systems as functional components to improve adhesive batter performance and reduce oil uptake. Hydrocolloids can be obtained synthetically or isolated from animals, vegetables and microbial sources. Polysaccharides form a major group depending on the source for hydrocolloid applications. Hydrocolloids of animal origin such as gelatin gelatin also find various applications in food industry. Hydrocolloids are proteins or polysaccharides in some cases with functional properties such as gelling, stabilizing, thickening, film forming, dispersing and texture modifying that are successfully used in food products. Hydrocolloids are used by the meat industry as water-binding and texture-modifying agents in many different types of meat products but especially in low-fat processed meats. In this review, the effect of hydrocolloids on meat and meat products will be examined.

Keywords: Hydrocolloids, Meat, Polysaccharides.

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1. INTRODUCTION

Hydrocolloids are high molecular weight polymer compounds (polysaccharides/ proteins) that used by the food industry for improve the rheological and textural characteristics of food systems (Regand and Goff, 2002). Nowadays, Hydrocolloids widely used to perform a number of functions such as gelling aqueous solutions, thickening, stabilising foams, inhibiting ice, sugar crystal formation, emulsions and dispersions and the controlled release of flavours, etc. (Williams and Phillips, 2000). Thickening property of hydrocolloids occurs by disperse in water. The degree of thickening varies with the nature, type and concentration of hydrocolloids (Maity *et al.*, 2018). Hydrocolloids such as are starch, guar gum, xanthan, locust bean gum, gum Arabic, gum tragacanth, gum karaya and cellulose derivatives are used as thickeners (Saha and Bhattacharya, 2010). Gelling involves association or cross-linking of the polymer chains to form a three dimensional network that traps or immobilizes the water within it to form a rigid structure that is resistant to flow (Maity *et al*., 2018). A hydrocolloid ingredient may act as an emulsifying agent, as a stabilizing agent, or in both of these roles. Hydrocolloids cannot act as primary emulsifying agents because polysaccharides are predominantly hydrophilic in molecular character, and most hydrocolloids are not surface-active. However, there is really only one hydrocolloid± namely, gum arabic ± which is commonly employed as an emulsifying agent (Dickinson, 2009). The most widely used polysaccharide emulsifiers in food applications are, modified starches, modified celluloses, some kinds of pectin, some galactomannans and gum arabic (Acacia senegal) (Garti and Reichman, 1993; Dickinson, 2003)

Hydrocolloids in meat products are used to improve cooking efficiency, reduce the cost of the formulation, increase moisture retention, modify the texture of the product, and improve the freeze-thaw stability. The development of low-fat meat products increased with the use of these ingredients. The purpose of the hydrocollaids in emulsion type meat products is to adjust viscosity, to form gel, to stabilize emulsions and to inhibit contraction. Recently the consumption of food products with reduced fat and food containing functional ingredients is more and more increased. Excessive consumption the traditional meat products.

May cause increase risk of obesity and some types of cancer because of have approximately 20– 30% fat content. Saturated fat rate in meat products is closely related to high blood cholesterol and coronary heart diseases. Fat in meat products has a major effect on some properties of the products such as flavor, texture and juiciness. It also improves consumer overall acceptability (Tobin et al., 2013). Meat proteins are good emulsifying agent in meat systems. Production a stable meat emulsion is based on that meat proteins surround the fat globules. Myosin that one of the meat protein fraction is the major structural protein for fat emulsification and WHC.

2. USİNG OF HYRDOCOLLOİDS İN MEAT PRODUCT

2.1. Classification of Hydrocolloids

Hydrocolloids can be classified in four broad groups: gums, starch and derivates, celluloses and derivates and others. Starch is a member of the `polysaccharide' group of polymers. The most important sources of starch are potato, maize, Tapioca, wheat and rice. Starch consists of two polysaccharide components: amylose and amylopectin. As soon as a suspension of starch granules is heated in excess water, the amylopectin double helical structures are disappear and the granule swells (Taggart, 2009). Cellulose occur of two repeating anhydroglucose units combined with 1,4 glycosidic linkages. There are some modified types of cellulose approved as a food additive. For exampe,

Carboxymethyl cellulose soluble in both neutral and cold water, creates a a clear, colorless solution with neutral taste (the use of hydrocolloids in processed meat systems). Gums are used in the food industry to regulate viscosity and form gels. There are many gum group: There are many gum; tragacanth gum, carrageenan, pectin, alginate,galactomanan and xanthan gum (guar gum and locust bean gum). Many studies have been carried out on gums. Guar gum is a galactomannan obtained from the endosperm of the Cyamopsis tetragonolobus seed; the specific polysaccharide component of guar gum is guaran, a galactomannan where about one-half of the b-d-mannopyranosyl main-chain units, joined by (1-4) bonds, contain an a-d-galactopyranosyl side chain attached at O-6. Guar molecules produce high viscosity solutions alone or mixed with xanthan solutions (BeMiller and Whistler, 1996). Gum tragacanth is an exudate from the stem of the bush like plant Astragalus species, and it is one of the few natural plant sources of L-fucose-substituted polysaccharides (Abbasi *et al*.,2019). Also there are different classification forms of hydrocolloids (Figure 1).

1. According to the source:
   - Marine origin/algal (seaweed) gums: agar, carrageenans, alginic acid, laminarin.
   - Plant origin: shrubs/tree exudates (gum ghatti, gum arabica,, gum tragacanth, gum karaya,albizia and khaya gums), seed gums (starch, cellulose, guar gum, locust bean gum, guar gum, amylose), extracts (pectin, larch gum), tuber and roots (potato starch).
   - Animal origin:chondroitin sulfate, chitin and chitosan, hyaluronic acid.
   - Microbial origin (bacterial and fungal): emulsan, xanthan, dextran, pullulan, zanflo, curdian, Baker's yeast glycan,krestin, schizophyllan, lentinan, scleroglucan.
2. According to the charge:
   - Non-ionic seed gums: xanthan, cellulose, guar, amylose, tamarind,, arabinans, galactomannans, locust bean.
   - Anionic gums: agar, tragacant, arabic, carrageenans, karaya, gellan, algin,pectic acid

3. Semi-synthetic:
   - Starch derivatives: hetastarch, starch phosphates, starch acetate.
   - Cellulose derivatives: hydroxy ethylcellulose, methylcellulose (MC), carboxy methyl cellulose (CMC), hydroxypropyl methylcellulose (HPMC), microcrystalline cellulose (MCC).
4. According to shape Linear:
   - Algins, amylose, cellulose, pectins. Branched: short branches (xanthan, xylan, galactomanan); branch-on-branch (amylopectin, gum arabic, tragacanth).
5. According to manomeric units in chemical structure
   - Homoglycans—amylose, arabinanas, cellulose; diheteroglycans—algins, carragennans, galactomannans; tri-heteroglycans—arabinoxylans, gellan, xanthan; tetra-heteroglycans—gum arabic, psyllium seed gum; penta-heteroglycans—ghatti gum, tragacanth.

Carrageenan is obtained from seaweed and red algae (Trius et al., 1996). It is a hydrocolloid consisting mainly of the sodium, potassium, calcium, magnesium and ammonium sulfate esters of galactose and 3,6-anhydrogalactose copolymers (Glicksman, 1983). The three major carrageenans are kappa, iota, and lambda carrageenans. lambda carrageenan is not a gelling agent (Lockwood, 1985). All the carrageenans are soluble in hot water and temperatures above 70 °C. Only X-carrageenan and sodium salts of κ and i-carrageenans are soluble in cold water (Glicksman, 1983). Kappacarrageenans forms gel structure in the presence of gel-inducing cation (Moirano, 1977).

2.2 Use of hydrocolloids in Sausage and Fermented sausage

Hydrocolloids are generally used as encapsulation of starter bacteria (Muthukumarasamy and Holley, 2006), an fat replacement, casing material (Liu *et al*., 2007) in sausage and fermented sausage products.

Maximum fat levels of traditional frankfurters and similar cooked sausages are 30%. (USDA-FSIS, 1990). However, reduced fat sausages contain according to traditional sausages 50% less fat (14–15%). Low-fat products must contain no more that 10% fat, while extra-lean products must be under 5% fat (Andrès *et al*., 2006). The dietary recommendations of several different organizations and scientific committees for nutrition are important about healthy cured raw sausage (Aranceta and Serra-Majem, 2001; Beriain *et al.*, 2011). Beriain *et al*. (2011) researced that variations in the quality of low-salt, inulin enriched Pamplona-style chorizo, in which some of the pork back fat was replaced with olive oil. Four different sausage formulations were prepared (50% of the pork back fat was replaced with olive oil emulsified with alginate and 58% of the sodium chloride was replaced with 20% potassium chloride and 38% calcium chloride). Méndez-Zamora *et al.* (2015) investigated influence of different concentrations of inulin and pectin as fat substitutes. Shear force, fracturability, hardness, gumminess, and chewiness values of sausage contain low fat with 30% inulin and low fat with 15% inulin + 15% pectin were slightly lower than those of the control. Gum tragacanth has been reported to have both emulsifying and stabilizing properties. Also it helped to facilitate emulsification (Garti and Reichman,1993).

Various studies have been carried out on protein-based hydrocolloids such as soy protein, milk powder, casein on sausage and fermented sausage (Ensor *et al*., 1987; Lyons *et al.*,1999). Lyons *et al*. (1999) observed that TGase and casein could increase hardness of chicken sausage significantly ($p<0.05$), while mixed phosphate and κ-carrageenan had no increasing effect ($p<0.05$). In a study by Hughes *et al*. (1998), addition of whey protein or tapioca starch reduced cook loss and increased emulsion stability. In a similar study, the effects of different fat level (5%, 10% and 20%) and soy protein (SP), wheat gluten (WG) or whey powder (WP) on textural and sensory characteristics, binding properties, color of cooked beef sausages were evaluated. Addition of SP, WP and WG increased WHC and emulsion stability (ES). The most effective additive on WHC and ES was SP (Serdaroglu and Ozsumer, 2003).

Several studies were performed to develop edible casings with Hydrocolloid for sausage (kaynak) Liu *et al.*, (2007) casings from pectin and incorporated with 2.5% corn oil and 5% olive oil were compared with gelatin/sodium alginate blends with same concentration of corn oil and olive oil. Sensory analysis of sausages showed that pectin casings were more preferred than gelatin/sodium alginate blend casing for sausage manufacture.

2.3. Use of Hydrocolloids in Fish Product

Protein-carbohydrate interactions determine functional properties in foods with high protein content such as meat and fish products. Gel-forming capability is required to obtain fish products, such as surimi-based products (water-washed pastes) or restructured products (from whole-minced pastes). Carbohydrates, such as starches and gums, provide to the formation of the continuous matrix by interacting with proteins and water in the fish paste. Adding carbohydrates into a formulation could modify the capacity of salt to solubilisemyofibrillar proteins, which would affect the mechanical and functional properties of gels.Several hydrocolloids such as carrageenan, konjac and starch are typically used to improve the mechanical properties of surimi gels (Gomez-Guillén *et al.*, 1997; Park, 2000).

Starch

Starch is used to evaluate the wetness, stickiness, serving temperatures and/or thermal stability during

storage in the formulation of surimi products. Starches is used as partially fish proteins replacement and maintaining desired gel characteristics at a lower cost (Hunt *et al*., 2009).Chai and Park (2007) reported that Potato starch slightly improved the strength of surimi seafood gels treated with ohmic heating, but it had a detrimental effect on whiteness, as compared to wheat starch.

Adding acetylated rice starch to surimi pastes (starch 40-80 g/kg product) increased the freeze-thaw stability of surimi pastes, but reduced the gel strength and expressible moisture content of surimi gels (Jung *et al*., 2007).

Modified tapioca starch (hydroxypropylated distarch phosphate), added at 10 g/kg to bigeye snapper (*Priacanthus tayenus*) mince paste. reduced the negative changes caused by five freeze-thaw cycles, improving the mechanical, functional, and overall properties of restructured products, as compared to products with no starch. Restructured products containing 10 g/kg modified starch showed a finer matrix with smaller strands at all freeze-thawcycles used, observed by scanning electron microscopy (SEM), as compared with the control minced gel (Tuankriangkrai and Benjakul, 2010).

Gums

Carrageenan and konjac are highly compatible with fish muscle meals, but others gums have a negative effect on texture and some especial considerations must be taken into account. On this regard, locust bean and xanthan gum at a 0.25/0.75 ratio has been proposed to improve the mechanical properties of surimi gels from silver carp (Ramírez *et al*., 2002).

Alginates weaken surimi gels when incorporated (Lee *et al*., 1992). However, alginates are commonly used for obtaining raw restructured fish products by the cold binding technique under chilling or freezing conditions. The effectiveness of sodium alginate as cold binder can be improved by adding a low concentration (1 g/kg) of $CaCl_2$, whereas a higher concentration (10 g/kg) reduces the binding capability of the alginate (Moreno *et al*., 2009, 2010).

Konjac glucomannan (KGM) has been proposed to be an efficient cryoprotective agent for fish myofibrillar proteins. This gum was able to decrease the muscle protein denaturation/aggregation from grass carp (*Ctenopharyngodon idella*) during storage at -18 C, improving the amount of salt-extractable protein. Adding KGM at 10 g/kg showed the same cryoprotective effect as a conventional cryoprotectant (100 g/kg sucroseesorbitol, 1:1). Adding 15-20 g/kg KGM allowed an increase inwater-holding capacity, breaking force, and deformation of surimi gels, although the whiteness decreased and the colour became darker (Xiong *et al*., 2009).

Pectins

Pectins are classified as high methoxyl (HM) or low methoxyl (LM) pectins. Both types of pectins have been reported to not improve the mechanical and functional properties of surimi. Moreover, both pectins induced a disruptive effect during gelling. However, amidated LM (ALM) pectin is compatible with surimi gels and improves gelling properties. Results suggest that electrostatic proteinepectin interactions induced a disruptive effect when HM and LM pectinwere added. Amidation could improve the polarity of LM pectin. Thus, hydrogen bonds between amidated LM pectin and protein could form a more compatible proteinecarbohydrate system (Barrera *et al.*, 2002). Gels are normally formed at concentrations of 1 g/kg. Two types of LM pectins are produced commercially: (a) ordinary LM pectins, prepared by acid treatment in ethanol or isopropanol, and (b) amidated pectins, prepared with ammonia in alcoholic suspensions of pectin (Morris, 1998).

HM pectins improved the gelling capacity and thermal stability of whey protein concentrate in the pH range of 4.6-8.5 when the protein concentratewas above 80 g/kg. Protein-pectin interactions improved solubility, emulsification, gelation, and foaming behaviour of whey protein concentrates (Mishra *et al*., 2001). Pectins have been used as cryoprotectants in surimi (Sych *et al.*, 1990b; Ueng and Chu, 1996).

LM pectin improved the hardness of surimi gels and decreased the shear strain and cohesiveness, but had no significant effect on shear stress, springiness, or water-holding capacity. The mechanical

properties of surimi gels containing LM pectin were not affected by adding calcium.
ALM pectin showed a beneficial effect on the mechanical properties of surimi and restructured fish products, as compared to LM pectins (Barrera *et al*., 2002; Uresti *et al.*, 2003). ALM pectins are reported to work better under acidic conditions (pH 3.2-3.6), while non-amidated LM pectin works in the pH range of 2.8-6.5.
Hydrogen bonds between ALM pectin and myofibrillar proteins could result in an improvement in the mechanical properties, specifically hardness and breaking force, of fish gels when ALM pectins are added at 10 g/kg.
Fibre
Dietary fibre obtained from plants is considered a functional ingredient because it provides several health benefits beyond bowel regularity.
Adding 20-40 g/kg GDF (red grape dietary fibre concentrate) to minced fish muscle from horse mackerel (*Trachurus trachurus*) improved water and oil retention, lipid stabilisation, and cooking yield during frozen storage at -20 C for six months. Although GDF showed a good dispersion in the protein matrix according to scanning electronic microscopy (SEM), the matrix was more discontinuous compared to control samples and was associated with an increase in aggregation of myofibrillar proteins during frozen storage. Samples containing 20 g/kg GDF scored highest in overall acceptance compared to control samples (Sanchez-Alonso *et al*., 2007; Sanchez-Alonso and Borderias, 2008). Adding fibre into minced hake (*Merluccius merluccius*) and horse mackerel (*T. trachurus*) muscle increased the WHC when water was not added to maintain the moisture constant, but even in these conditions, rigidity and cohesiveness were lower for products containing fibre than control samples (Sanchez-Alonso *et al*., 2007). Chicory root inulin, a soluble fibre, has been used as an additive in restructured fish products with a detriment to mechanical properties, specifically hardness. This negative effect can be avoided by adding 20 g/kg carrageenan, but it was not compatible with MTG (Cardoso *et al*., 2007a, b, 2008; Cardoso, Mendes, Pedro, & Nunes, 2008; Cardoso *et al*., 2009). Pea fibre can be added to minced fish and surimi up to 40 g/kg to obtain restructured products without modifying the textural and functional properties. It was compatible with up to 20 g/kg carrageenan, allowing increased hardness of restructured hake products. Pea fibre was compatible with MTG at 1 g/kg or above, and it improved the textural properties of surimi gels from Atlantic mackerel (*Scomber scombrus*) and chub mackerel (*Scomber japonicus*) (Cardoso *et al*., 2007a, b, 2008; Cardoso *et al*., 2009).
Others
Adding 10 g/kg chitosan to kamaboko gels from grass carp (*Ctenopharyngodon idella*) allowed an increase in hardness, springiness, cohesiveness, chewiness, adhesiveness, whiteness, WHC, and TBA while decreasing peroxide value and bacterial Growth (Mao and Wu, 2007; Wu & Mao, 2009).

2.4. Use of Hydrocolloids as Cryoprotectants

A variety of carbohydrate compounds, including most of the mono- and disaccharides evaluated, and several lowmolecular- weight polyols, many amino acids and carbosylic acids were found to be cryoprotective. Nucleotides (ATP, ADP, and IMP) have been shown to exert a protective effect on fish actomyosin stored at -20°C (Jhiang et al., 1987). Watanabe *et al*. (1988) demonstrated the cryoprotective ability of certain surfactants, particularly certain polyoxyethylene sorbitan esters and sucrose esters, in preventing loss of gel-forming ability in surimi.
Sucrose and/or sorbitol, typically alone or mixed 1:1 and added at 8% w/w to leached fish muscle, serves as the primary cryoprotectant commercially used in manufacture of surimi from Alaskan pollack (Park *et al*., 1988). In a subsequent study (Park *et al*., 1990), when the salt was omitted from

the cryoprotected sample, the gel forming abilities of both the pre- and post-rigor muscles were maintained at near unfrozen levels by polydextrose addition.

Freezing and frozen storage are important techniques for long term preservation of animal products such as surimi, Processed meat and fish muscle preparations. However, freezing generally result in a loss of protein functionality by damaging muscle protein and inducing protein denaturation (Maity *et al*., 2018). Polyols have been extensively used as a cryoprotectant in frozen animal food preparations (Sych *et al*., 1990a; Park *et al*., 1996; Jasra *et al*., 2006). Sultanbawa and Chan (1998) suggested effectiveness of sugar and polyol such as sorbitol, lactitol blends as cryoprotectants in ling cod surimi during frozen storage. Chen *et al*. (1991) found stability in chicken surimi during cold storage (-18C) added with 1:1 (w/ w) sucrose/sorbitol mixture for 2 months. The protein denaturation induced by freezing was found to be reduced when sorbitol, glucose syrup, sucrose and sucrose/sorbitol (1:1, w/w) mixture was added at 8% (w/w) in cod-surimi (Sych *et al.*, 1990a). Goeller *et al*. (2004) optimized level of sorbitol into intact fish muscle and monitored the cryoprotection during freezing and thawing as change in myosin Ca2C ATPase activity. Authors reported that excellent cryoprotection was found when 60% sorbitol was incorporated into intact fish muscle during freezing.

Cryoprotectants were also found to be very effective in preventing surimi quality deterioration by lowering water activity (aw), decreasing protein freeze denaturation, and reducing ice crystal mechanical damages during super chilling storage (Liu *et al*., 2014). A quantity as low as 1% modified tapioca starch could also reduce the breaking force, deformation and expressible moisture content of bigeye snapper mince gels subjected to different freeze-thaw cycles (Tuankriangkrai and Benjakul, 2010). Malto-dextrins used at 8% in Alaska Pollock Surimi were found to protect the surimi against freeze-thaw stress (Carvajal and Lanier, 1996).

The oligosaccharides were also found to retain mechanical properties of surimi stored at -18C for 3 months (Auh et al., 1999). Gum Arabic and k-carrageenan were found to be the best cryo-protectants for storage of beef at -18C (Akkose and Aktas, 2008).Kovacevic et al. (2011) used thermal analytical techniques to prove the cryoprotective effects of polydextrose on chicken surimi. They reported that highest level of polydextrose (10%) exhibited greatest cryoscopic depression of initial freezing point (Ti) and increase in thermal transition temperature (Tp).

3. CONCLUSION

Hydrocolloids such as agar, carrageenans, alginic acid, starch, cellulose, guar gum, locust bean gum, chitosan, soy protein, milk powder can be effectively applied in meat products.

Hydrocolloids are generally used for encapsulation of starter bacteria, an fat replacement, casing material in sausage and fermented sausage products, for cryoprotectant in frozen meat products, for gel-forming in fish products such as surimi-based products.

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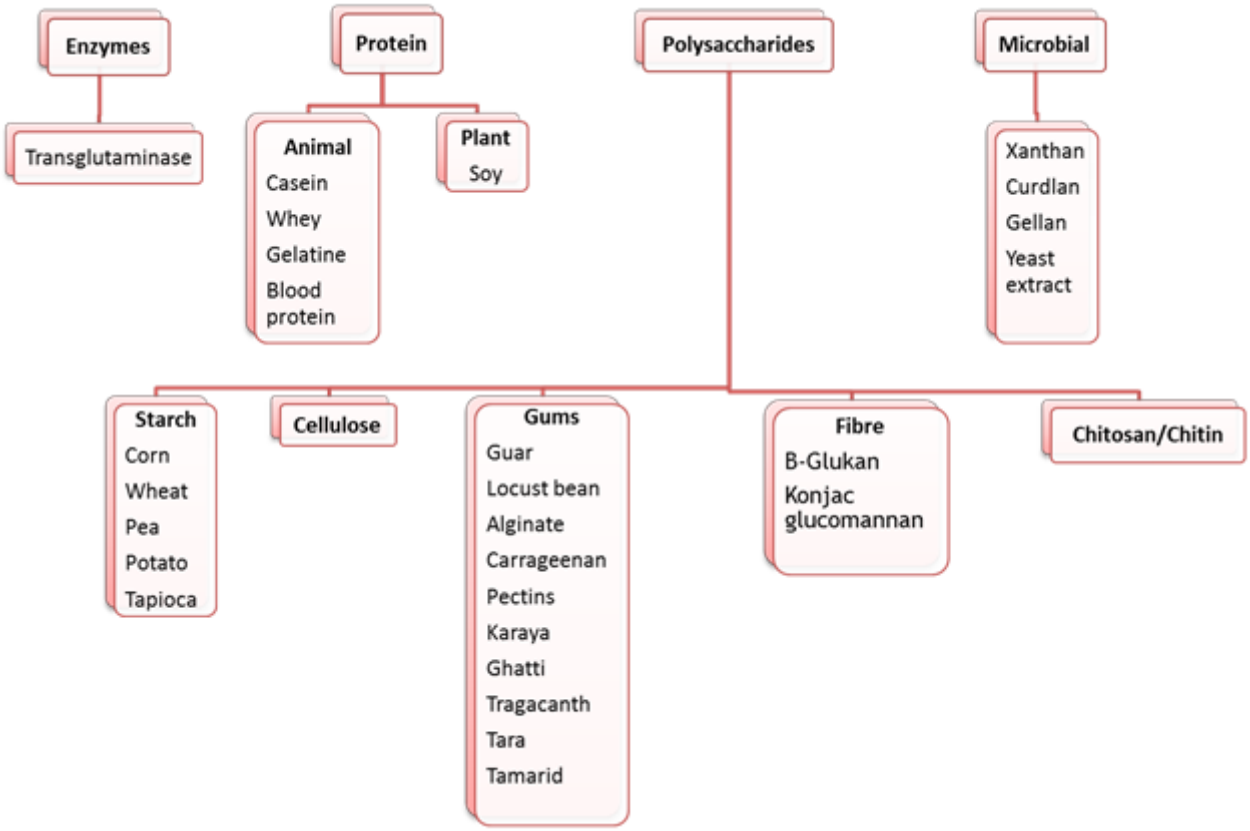

Figure 1. Resource of different types of hydrocolloids used in the food industry

AGROFOOD
20 - 21 June 2019, Istanbul

Unravelling Maillard reaction and caramelization extents in a cake model by the quantitative analysis of reaction substrates, intermediates and products for baking at different levels of temperature and convection

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ABSTRACT

Baking cereal products triggers a large number of reactions, mainly across the Maillard reaction and caramelization ways, and leads to a multitude of quality-related or health-related compounds. The formulation and structure complexity of this kind of products makes very difficult to discriminate the different reactions pathways, whereas studying too simple systems gives results quite far from reality. Therefore, this study uses a cake model structurally identical to a real one but completely non-reactive and in which reaction precursors (glucose alone or with leucine) are thus specifically added. After baking in controlled conditions of temperature (3 levels) and convection (2 levels), quantitative kinetics for 20 reaction markers are acquired either by on-line-TD-GC-MS for volatile compounds (quantified in the oven air) or by UHPLC-CAD/DAD/MS for compounds extracted from the matrix. Results regarding the substrates disappearing and products appearing show that reactions are enhanced in presence of leucine, confirming a strong contribution of Maillard reaction. Specific products can also be detected via the formation of Strecker aldehyde. Intermediate compounds enable to discriminate the favored pathways and help understanding the global reaction scheme. Temperature has a strong catalytic effect while convection's impact is not directly visible. With these results, the reaction scheme can be confirmed. A multi-response stoichio-kinetic modelling combined with heat and mass transfer modelling will then be developed to identify the kinetic rate constants for each reaction. Then, it will provide an excellent tool to monitor the thermal reactions contributing to the generation of process-induced compounds in solid food.

Keywords: Kinetics, cereal product, solid, aroma compounds, health-related compounds

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Physiological and Morphological Research of Olive Leaves in Irrigated and Non-Irrigated Conditions

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ABSTRACT

Drought stress is the main cause of reduced plant growth .(Guerfel,M.,at al.,2007) showed that there was significent genetic differences for morphological and phyisological traits for drought tolerance in 12 Tunisian olive cultıvers..In our study 3 different known drought tolerant and sensitive olive *(Olea europaea L.),*cultivers, were examined Under irrigated and non-irrigated conditions. Important differences were recorded in the measurements of olive leaves as density and numbers of stoma and the proportional surface , trichome density, number and diameter of trichome peltates length , wich was collected homogenously during 2015-2016 years from the national collection garden of the Bornova Olive Research Institute.

Keywords: Olea euorpeae L., stoma, stress, trichome,

1. INTRODUCTION

Drought is the most common cause of stress and is one of the factors affecting plant growth. When compared with other fruit tree species, olive tree can tolerate by providing morphological and physiological adaptation to water deficiency in the soil (Bacelar et al 2007).Bosabalidis and kofidis (2002), in their study of the effects of two olive varieties (mastoidis and coroneiki) on the leaf anatomy of drought, an increase in the number of stomata per unit area was observed. In addition, both types showed a decrease in stoma width and length due to stress.

Olive tree under the stress of water photosynthesis by maintaining low levels of stomatal conductivity by reducing the growth of sprout stops (Tubeileh et al, 2004).Leaf is the organ most adapted to environmental conditions and factors, leaf anatomy reflects water stress factors more clearly than the root and stem (Marchi et al. 2008). Guerfel et al. (2007) found morphological and physiological differences in leaves of arid conditions in Chott.Mariem (semi-arid climate) collection garden of 12 olive cultivars grown in Tunisia. They found differences between varieties in terms of different parameters such as stoma density number and diameter of hairs.

In this study, the morphological status and differences of the irrigated and non-irrigated leaves of 3 different local olive varieties were determined.

2. MATERIALS AND METHODS

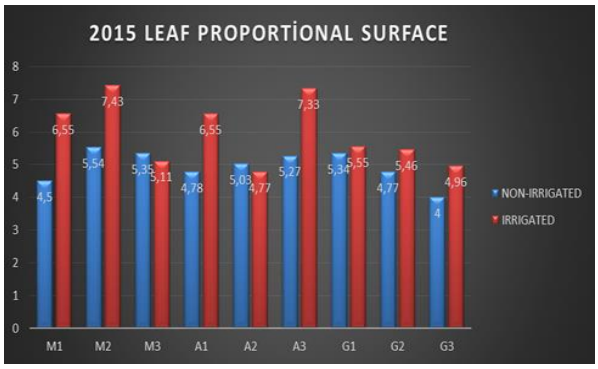
Plant material In this study, 3 different varieties of local olives (Olea europaea L.) (Ayvalık, Gemlik, Memecik) from the national collection garden of Bornova Olive Research Institute in August 2015-1016 were obtained homogeneously from 4 different directions of 3 different trees under irrigated and irrigated conditions. leaves were used.

Stomatal and trichome densities: In order to determine the density of stomata and hairs, 3 different trees of all kinds were randomly selected and samples were taken from the leaves in the middle part of one-year shoots from 4 directions. To calculate the trichome density, the molds were removed from the lower middle parts of the collected leaves with tape, the samples were placed on the slide and photographed under the microscope and the number of 1mm² leaf area was removed (Ennajeh et al. 2010).

The number of stomata, width and length of the leaf samples taken by applying a thin layer of polish in the lower middle part of the leaf are removed and examined under the microscope by gluing and photographed and the number of stomata in 1mm² leaf area and measurements are calculated (Ennajeh et al 2010).Measurement of leaf area; 30 mature leaf samples were taken from each variety and Licor LI3000A model leaf area was determined by the meter (Köksal et al., 2007).

3. RESULTS AND DISCUSSION

Morphological changes occurring in leaves under dry conditions are generally aimed at reducing the amount of water lost by transpiration. Determination of leaf area index is an important parameter for determining carbon exchange of leaves, amount of trapped and transmitted light. The lowest values in leaf area were seen in non-irrigated cultivars, while the highest values were found in irrigated cultivars.



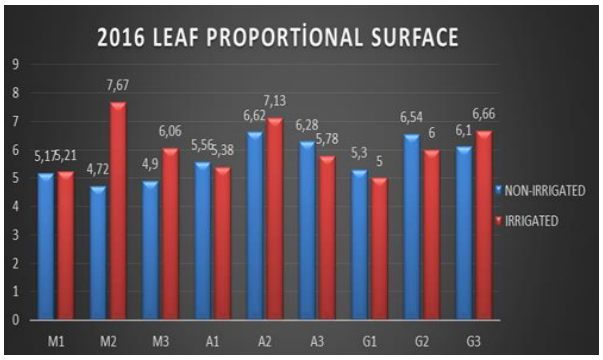


Fig.1. A, B. The measurement of leaf area of irrigated and non-irrigated varieties 2015 and 2016 as shown in the figure.

Trichoma (feathers) are usually located on the lower surface of the leaf and protect the stomata from wind by wrapping the pores, the large number of these structures are effective in limiting water loss from plants (Guerfel et al. 2007). In the study, a statistically significant difference was found between the irrigated and non-irrigated trees in terms of the number of feathers in 2015 (p value <0.001). There was a statistically significant effect on the number of hairs (p value = 0.0009). 2016 There was a statistically significant difference in the number of feathers between the irrigated and non-irrigated trees (p value <0.001). However, it was observed that the direction did not have a statistically significant effect on the number of hairs (p value = 0.12). There was a statistically significant difference between the difference (p value = 0.005). Olive leaves in the tricome diameter of the response to stress according to the research shows that the difference between the irrigated and non-irrigated varieties in the study of the differences between the varieties were observed.

Table 1.2015 irrigated and non-irrigated varieties and directions (S) South, (N) North, (E) east, (W) West (1mm²) differences in the number of trichomes (p value <0.001) (p value = 0.0009).

CULTIVARS	**MEMECİK**				**GEMLİK**				**AYVALİK**			
DIRECTIONS	S	N	E	W	S	N	E	W	S	N	E	W
NON-IRRIGATED	167	171	172	166	158	163	183	171	184	184	195	165
IRRIGATED	149	149	164	149	155	154	155	148	191	164	154	143

Table2.2016 differences in the number of tricomes between irrigated and non-irrigated varieties and directions directions (S) South, (N) North, (E) east, (W) West (p value <0.001).

CULTIVARS	MEMECİK				GEMLİK				AYVALİK			
DIRECTIONS	S	N	E	W	S	N	E	W	S	N	E	W
NON-IRRIGATED	181	175	172	173	184	185	175	176	174	176	180	170
IRRIGATED	145	151	154	145	129	137	153	131	146	147	166	165

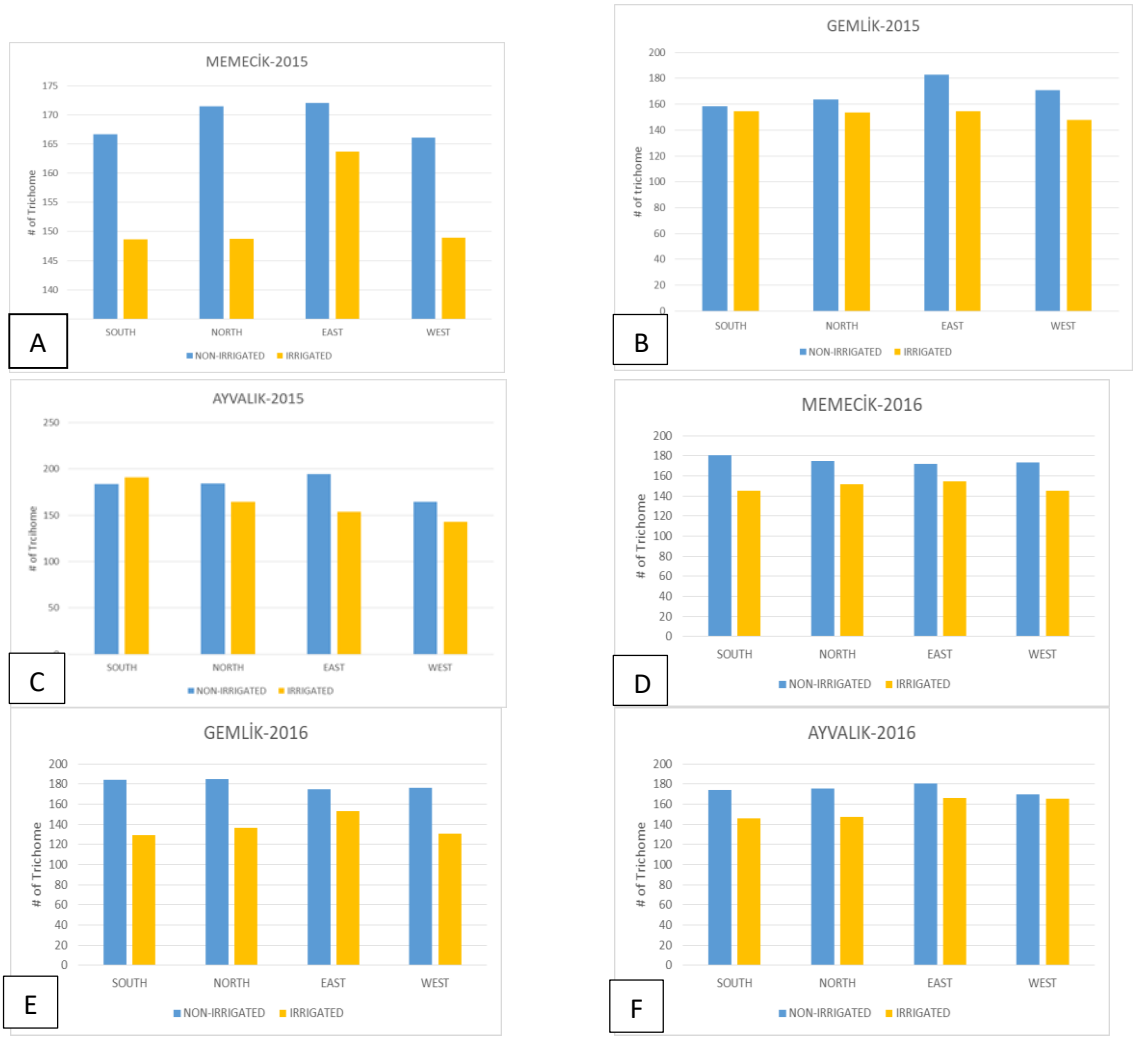


Fig.2. Irrigated and non-irrigated conditions and varieties grown in the direction of olive varieties (1mm²) differences in the number of trichome .

In water deficiency applications, they prevented excessive water loss by closing the stomata of olive trees. New leaves formed during the stress period provided better stomal control than the tolerance of old leaves (Fernandez et al. 1997).

There is a significant difference in stoma density between irrigated and non-irrigated trees in 2015 and 2016. It is thought that the number of stomata of the non-irrigated varieties is increased and this is seen as a result of adaptation.

Table3.2015 irrigated and non-irrigated varieties and directions (S) South, (N) North, (E) east, (W) West (1mm²) differences in the number of stomata.

CULTIVARS	GEMLİK				MEMECİK				AYVALİK			
DIRECTIONS	S	N	E	W	S	N	E	W	S	N	E	W
NON-IRRIGATED	452	483	513	533	403	439	406	413	530	468	468	596
IRRIGATED	396	344	425	374	496	432	512	435	529	454	468	455

Table4. 2016 irrigated and non-irrigated varieties and directions (S) South, (N) North, (E) east, (W) West (1mm²) differences in the number of stomata.

CULTIVARS	GEMLİK				MEMECİK				AYVALİK			
DIRECTIONS	S	N	E	W	S	N	E	W	S	N	E	W
NON-IRRIGATED	396	344	425	374	496	432	512	435	530	468	468	596
IRRIGATED	452	483	513	533	403	439	406	413	529	454	468	455

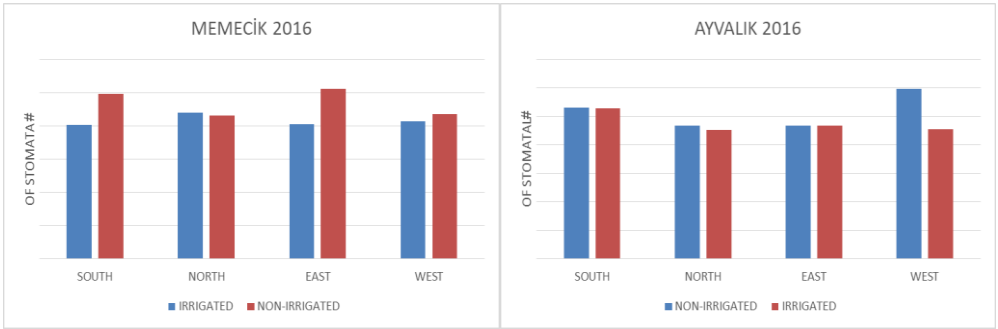


Fig.3. differences in the number of stomata (1mm²) of the leaves of olive varieties grown in irrigated and non-irrigated conditions.

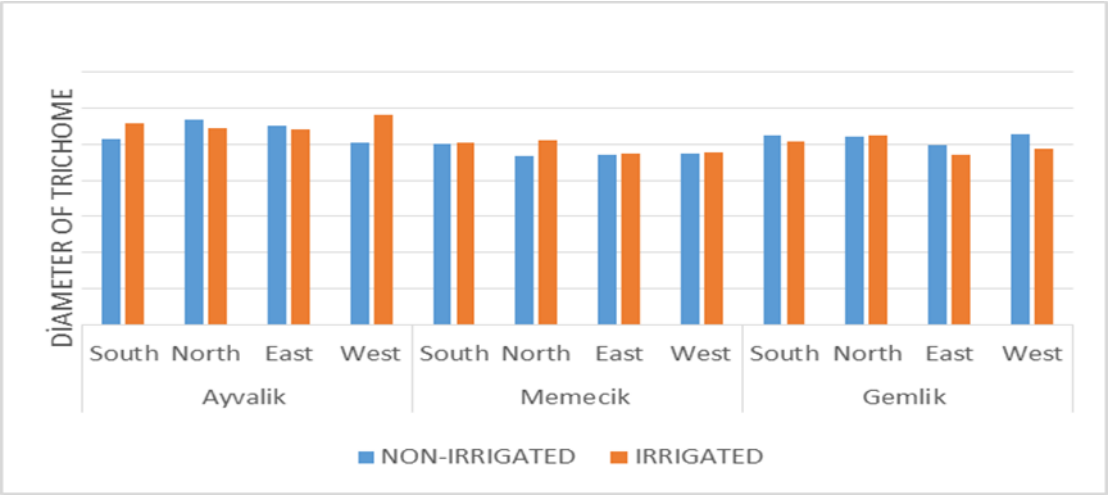


Fig.4. Differences of trichoma diameters (um) in the leaves of 3 varieties of olives with and without irrigation.

4. RESULTS

There were many important differences between the 3 native varieties investigated in both conditions. These variations seem to be related to their genetic background. During the summer months of the study, it will help to get an idea about the reactions and protection ability of each kind as non-irrigated varieties under dry conditions. As a result of the two years, there were differences between the irrigated and irrigated domestic varieties in the number of feathers. While the number of feathers increased in the non-irrigated condition in the Memecik variety, the Gemlik variety did not show much difference in the same conditions.

As a result, the number of hairs per unit leaf area of olive varieties exposed to drought stress was found to be statistically significant. Significant differences were also observed in stoma number measurements.

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AGROFOOD
20 - 21 June 2019, Istanbul

Bioactive Compounds in Water Extracts Prepared from Rosehip Containing Herbal Blends

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ABSTRACT

In the current study, infusions and decoctions were prepared from three types of rosehip-containing herbal blends (rosehip fruits – 1, hibiscus flowers and rosehip fruits – 2, and rosehip fruits, apple, pear, aerial parts of St. John's wort – 3). In order to define nutritional and caloric values of aqueous preparations, the phytochemical composition (lipids, proteins, and carbohydrates) was determined. The phenolic compounds and natural pigments (carotenoids and chlorophylls) were also assayed. The prepared extracts were also analysed for their antioxidant activities using DPPH and FRAP methods. The results showed that the decoctions were obtained in the highest extractive yields (38-45 % dw) compared to the infusions. The most significant content of uronic acids (27.8 mg/100 ml) and sugars - sucrose (29 mg/100 ml), glucose (89 mg/100 ml) and fructose (147 mg/ml) was found in the rosehip fruits (1) decoction. The highest values of carotenoids, chlorophylls, flavonoids, *p*-coumaric, ferulic and ellagic acids, as well as ascorbic and citric acids were found in herbal blend (3). The decoctions prepared from rosehip fruits (1) and herbal blend (3) possessed the highest antioxidant activity by DPPH (463 and 596 mM TE/250 ml) and FRAP (305 and 354 mM TE/250 ml) methods. The highest content of phenolic acids (chlorogenic, neochlorogenic, 2,3–dihydroxybenzoic, vanillic, caffeic, gallic), flavonoids (myricetin and kaempferol), and organic acids (malic, quinic, oxalic and α-ketoglutaric acids) were found in decoctions from herbal blend (2). The present study suggests that the decoction could be preferred as extraction methods of rosehip-containing blends since higher amounts of important constituents have been extracted.

Keywords: decoction, infusion, hibiscus, rosehip fruits, St. John's wort

AGROFOOD
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Pear Classfication Using Image Processing Techniques

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ABSTRACT

Pear is the common name for the plant species of *Pyrus* which are classified in the *Maloideae* subfamily of the *Rosaceae* family and the edible fruits of some of these species. The number of pear varieties in the world is over 5000 and in our country which has very different ecological conditions, it is reported that there are 640 pear varieties cultivated locally and suitable for each region. Pear which is preferred by consumers due to the taste, crispness, fragrance and aroma. It can be used in the production of puree, concentrate, nectar, clear pear juice, pulp fruit juice mixtures while it is usually consumed as fresh.

Efficiency, quality and automation studies in the agriculture and agriculture-based industry sector, which are among the prominent sectors of our country, become popular recently. Especially, in terms of quality and efficiency, automation is very important for food processing such as classification. Image processing applications which are defined as the creation of a new picture as a result of changing the properties and appearance of the image by processing as an input picture of real-life images are an important role in the food sector. In the image processing systems, the images obtained by the electronic camera, processed by the algorithms and the classification is done very quickly according to affecting on the quality properties of the fruits such as the stain, color and size. Moreover, the classification process which is carried out completely by people has many dissadvantages in terms of cost, time, more accurate results and practicality when it is compared to the analyzes made with many devices in the food industry.

In this study, photographs of pears which were purchased from a local market were obtained in a photo booth which provides a homogeneous distribution of light. In order to determine the

properties such as size, weight, stain ratios (%), stained area and diameter, which are the criteria for the classification of pears, Matlab (Licensed R2011) program has been formed according to the purpose of the application with various algorithms and pears were classificated according to the Turkish Standard 184. The diameters of the pears were between 75.28-198.78 mm, and the stain ratios (%) were between 0.18-4.26. In addition, the results obtained by converting the RGB (red, green, blue) values obtained by image processing method to L *, a *, b * and were compared with L *, a * and b * values obtained with Konica Minolta Chroma Meter (CR- 400, Japonya) and evaluated statistically. The L*, a* and b* values were determined as 56.06-73.08, -20.59- -2.86, 44.85-53.38 by an image processing based method using CIELAB color model and these values were determined as 60.06-75.93, -15.58- -1.13, 35-15- 46.96 by Konica Minolta Chroma Meter (CR- 400, Japan).

Key words: *Image Processing, MATLAB, Pear, Fruit Classification,*

AGROFOOD
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Non-O157 STEC Survival and Gastric Fluid Resistance During Storage of Commercially Available Frankfurters

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ABSTRACT

In this study survival and resistance to gastric fluid of the "big six" non-O157 STEC serogroups of O103 and O145 were evaluated during frankfurter storage. Commercially available frankfurters were obtained from grocery stores on the first day of their arrival to the store. Pathogens (O103 and O145) were individually inoculated onto frankfurters about 5±1 log cfu/g. Pathogen inoculated frankfurters and non-inoculated control group frankfurters were vacuum packed and stored at 4°C for 75 days. Viable counts of O103 and O145 decreased 0.94 log and 1.08 log respectively during storage. Simulated gastric fluid (SGF) resistance was evaluated by a static system and viable counts were obtained at 0, 30, 60 and 90 minutes of gastric fluid exposure (pH 1.5). SGF resistance of O103 decreased at 60th day of storage and the pathogen count decreased below the detection limit after exposure to SGF. Acid challenge experiments of O145 show that the SGF resistance of the pathogen decreased at 45 days of storage and decreased below the detection limit at 60 and 75 days of storage after SGF exposure. Frankfurter pH decreased from 6.74 to 5.13 during 75 days of storage. Results indicate that consumption of STEC contaminated frankfurters might poses a food safety risk that these pathogens could survive gastric acidity inoculated onto frankfurters.

Keywords: Acid resistance, STEC, frankfurter.

1. INTRODUCTION

Shigatoxigenic (Veratoxigenic) *Escherichia coli* (STEC) is one of the most important emerging foodborne pathogen groups of *E.coli*. These pathogens can cause disease in humans by the production of shiga-like toxins and play a part in serious outbreaks. Apart from commonly known strain *E.coli* O157:H7 other strains of STEC are called non-O157 STECs. Even though there are many studies related to STEC O157 behavior on foodstuffs, limited data is available on non-O157 STECs in the literature. According to CDC, non-O157 STEC's are responsible for a larger portion of STEC outbreaks that occur every year in the USA. Earlier laboratory practices were not able to identify non-O157 STEC strains therefore only recently the surveillance of these strains has been made possible. The main contamination source of these pathogens is cattle meat and its products (CDC, 2014).

Frankfurter type sausage is a Ready-to-eat (RTE) food product that has been widely consumed by people of all ages. Since the product is already cooked it is usually consumed without any additional cooking. But the product can be contaminated with pathogens after the heat treatment and therefore it is important to investigate the fate of the pathogens, especially which have low infectious dose such as STEC, during the storage of these products. Since the first barrier of defense in human body against pathogens is stomach acidity, we aimed to investigate the resistance of non-O157 strains to synthetic gastric fluid during frankfurter storage.

2. MATERIAL AND METHODS

2.1. Bacterial Culture Preparation and Inoculation of Frankfurters

Bacterial cultures of O103 and O145 were obtained from Department of Food Hygiene and Technology of Mustafa Kemal University Hatay/Turkey. Cultures were prepared from agar slants activated three times before inoculation. 18-hour cultures were used for inoculation individually for each strain. Cells were harvested by centrifugation (4000 rpm, 15 minutes) and washed with sterile saline solution. The cell pellet was transferred into 300 ml of sterile saline solution. This solution was used to inoculate frankfurters. Frankfurters were obtained from grocery stores on the first day of their arrival to the store.

2.2. Synthetic Gastric Fluid Experiments

Synthetic gastric fluid (SGF) was prepared according to Beumer et al (1999). For this protease peptone (8.3 g/liter; Difco), d-glucose (3.5 g/liter) NaCl (2.05 g/liter), KH_2PO_4 (0.6 g/liter), $CaCl_2$ (0.11 g/liter) and KCl (0.37 g/liter), ox bile (0.05 g/liter), lysozyme (0.10 g/liter), and pepsin (0.0133 g/liter) were mixed in deionized water. The pH of the SGF was adjusted to 1.5 with 1N HCl.

2.3. Microbiological Analysis

Microbiological analysis was conducted at days 0, 15, 30, 45, 60 and 75 of cold storage. STEC serogroups were enumerated on Sorbitol MacKonkey Agar plates after incubation at 35ºC for 24 hours (Dikici et al., 2015).

2.4. Total Acidity and pH Measurement

Total acidity calculations and pH measurements were also made at 0, 15, 30, 45, 60 and 75 days of storage. Acidity was expressed as lactic acid (AOAC, 2000) and pH was measured by a pH-meter.

2.5. Statistical Analysis

The numbers of bacteria are converted to $\log_{10}$ cfu/g. Then the data were subjected to Analysis of Variance (ANOVA). The means were separated by Fisher's least Square Differences method according to the General Linear Models (GLM) for a significance level of 0.05 (SAS,1999).

3. RESULTS AND DISCUSSION

STEC O103 and O145 were inoculated around 5.5 $\log_{10}$ cfu/g. Viable counts of STEC O103 did not change until the 60th day of storage whereas viable counts of STEC O145 decreased significantly at the 75th day of storage (Figure 1).

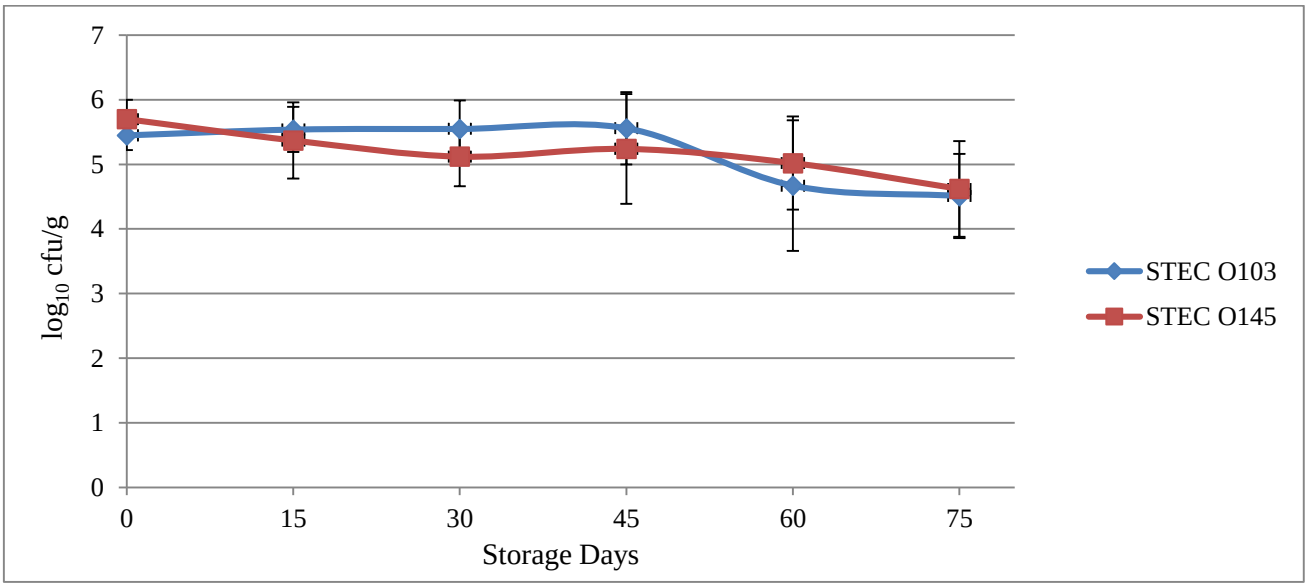

Figure 1: Population Changes of STEC O103 and O145 During Frankfurter Storage

Resistance of STEC O103 and O145 to synthetic gastric fluid was decreased at the 60th day of storage. The viable counts of pathogens decreased significantly after SGF exposure throughout storage but viable counts were still detectable until the 60th day of cold storage (Table 1, Figure 2 and Figure 3).

Table 1: Survival of STEC O103 and O145 During SGF Experiments of Frankfurters ($\log_{10}$ cfu/ g±SD)

Organism	Storage Days	Exposure Time (min.) 0	30	60	90
STEC O103	0	5.45 ± 0.23^{Ax}	3.86 ± 0.65^{Bx}	2.96 ± 1.07^{BCx}	2.53 ± 0.68^{Cx}
	15	5.54 ± 0.35^{Ax}	2.44 ± 0.68^{By}	2.56 ± 0.71^{Bx}	2.11 ± 0.42^{Bx}
	30	5.55 ± 0.44^{Ax}	3.10 ± 1.32^{Bx}	2.91 ± 1.01^{Bx}	2.64 ± 0.75^{Bx}
	45	5.56 ± 0.56^{Ax}	3.04 ± 0.99^{Bx}	2.73 ± 0.96^{Bx}	2.57 ± 0.89^{Bx}
	60	4.67 ± 1.01^{Axy}	2.09 ± 0.40^{By}	ND	ND
	75	4.51 ± 0.65^{y}	ND	ND	ND
STEC O145	0	5.70 ± 0.30^{Ax}	3.39 ± 1.08^{Bx}	2.74 ± 1.22^{Bx}	2.21 ± 0.82^{Bx}
	15	5.37 ± 0.59^{Axy}	2.47 ± 0.71^{Bx}	2.11 ± 0.30^{Bx}	ND
	30	5.12 ± 0.46^{Axy}	2.52 ± 0.75^{Bx}	2.27 ± 0.69^{Bx}	2.16 ± 0.63^{Bx}
	45	5.24 ± 0.85^{Axy}	2.60 ± 0.93^{Bx}	2.16 ± 0.64^{Bx}	1.84^{Bx}
	60	5.02 ± 0.72^{xy}	ND	ND	ND
	75	4.62 ± 0.74^{y}	ND	ND	ND

†$^{A.B.C.}$ Same letters indicate non-significant differences in the rows ($p>0.05$)
‡$^{x.y.z}$ Same letters indicate non-significant differences in the columns ($p>0.05$)
§ND: None detected

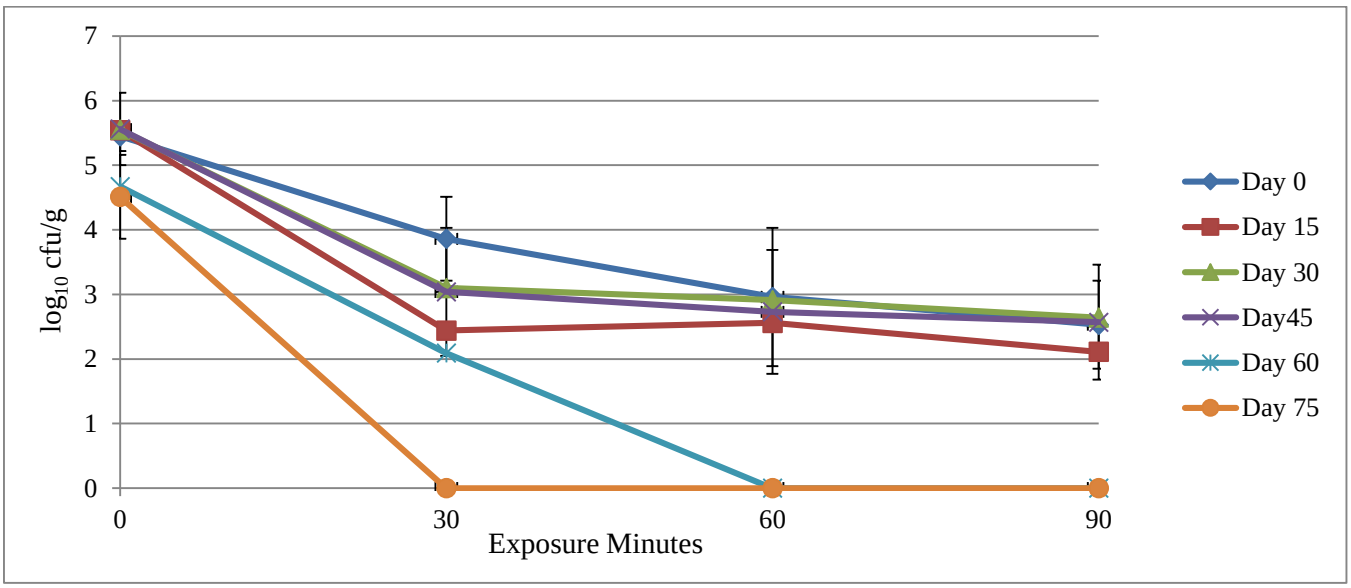

Figure 2: Viability Changes of STEC O103 During SGF Experiments

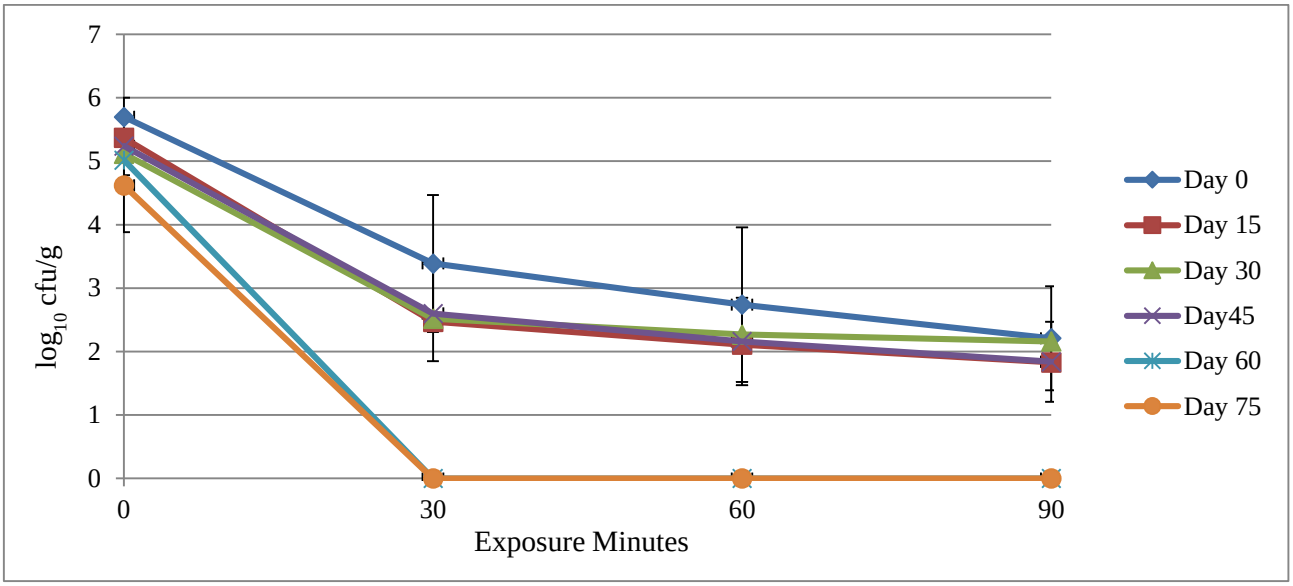

Figure 3: Viability Changes of STEC O145 During SGF Experiments

The pH of the frankfurters decreased gradually during storage (Figure 4). The sub-lethal pH environment that occurred during the storage might have helped the pathogens to protect their resistance to SGF. Sub-lethal pH values (4.5-5.5) are can result in an adaptation response of *E.coli* (Öztürk and Halkman, 2015).

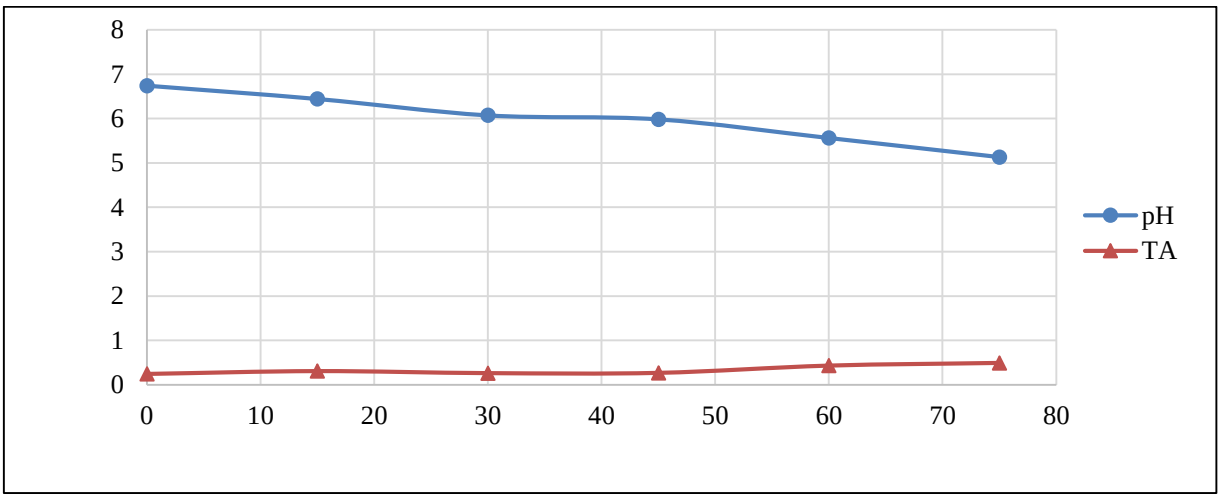

Figure 3: pH and Total Acidity Changes of Frankfurters During Storage

4. CONCLUSION

STEC is a group of acid resistant pathogens which have quite low infectious dose. The ready-to-eat products such as frankfurters pose a greater risk of infection caused by such pathogens. Frankfurters are usually consumed in cold salads without any additional cooking. Possible STEC contamination to frankfurters after the cooking process might cause a food safety risk considering the acid resistance of these pathogens especially on the early days of storage.

ACKNOWLEDGMENT

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AGROFOOD
20 - 21 June 2019, Istanbul

Evaluation of Nutritional Value and Antioxidant Potential of Biscuits Enriched with Selenium and Jeruslaem Artichoke Flour

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ABSTRACT

Jerusalem artichoke flour (JAF) is a rich source of functional dietary fiber (inulin) and polyphenols. In the current research butter biscuits were formulated from mixture of wheat flour with selenium content 0,01 mg/kg and JAF. Two types of flour from Jerusalem artichoke tubers were used: JAF 1 with high values of fructooligosacharides and sugars and low inulin content (7,09±0,25 g/100 g dw) and JAF 2 with low sugar and high inulin content (18,41±0,51 g/100 g dw) The percentage of substitution of the wheat flour with JAF was varied from 5 to 25 % but it was limited by the sensory characteristics of the biscuits. The carbohydrate content, selenium, the total phenols, flavonoids and antioxidant activity of JAF flours and the prepared biscuits were determined. It was found that after heat treatment the amount of polyphenols in butter biscuits containing 25% JAF2 flour was five times higher than that of the control sample (without JAF). The highest antioxidant activity demondtrated butter biscuits with 25% JAF2 - 458 mM TE/100 g (DPPH method) and 1865 mM TE/100 g (FRAP method). Consequently, the biological activity of the prepared biscuits with JAF2 is higher than the JAF1 batches. However, Selenium content in butter biscuit did not exceed the recommended levels of Se daily intake.Moreover, the formulation containing 25% JAF1 and 75% wheat flour, without added sugar showed the higher rate of acceptance, resulting a healthier alternative, with lower energy content, high inulin and fructooligosacharides as natural prebiotics than traditional wheat flour-based biscuits.

Keywords: biscuits, Jerusalem artichoke, inulin, selenium, wheat,

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Valorization and extraction of the essential oil from a food waste: lemon peels

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ABSTRACT

The purpose of this study is to valorize an agri-food waste, in this case lemon peels, by extracting the essential oil from its peel by steam distillation (SD) and microwave-assisted steam distillation. The optimal conditions for achieving the best extraction yields of the essential oil contained in this waste are determined.
Optimization has been realized by a parametric study. The operating parameters chosen are: the water vapour flow rate and the mass of the lemon peels.
The moisture content was calculated and found equivalent to 85%. The parametric study showed that the best essential oil yield after 3 hours of extraction was obtained with a lemon peels mass of 45g and a water vapour flow rate of 8g.min^{-1}. The same efficiency was achieved for microwave-assisted drive extraction after 6 minutes.
The analyses by CG made it possible to identify the constituents of the essential oil. The main constituent is d-limonene. In general, we can say that lemon essence is composed of 92 to 93% terpenes, of which d-limonene is the most abundant.
The antioxidant activity, determined by the DPPH test for oil extracted by steam distillation, has shown that the activity of this oil is important. The results obtained show the considerable potential of this plant as an antioxidant food additive.

Keywords: Antioxidant activity, Essential oil, Extraction, Food waste, Lemon peels.

1. INTRODUCTION

Current world production of Citrus is around 105 million tonnes (FAO, 2004). Citrus fruits are of considerable economic importance as a cash crop in many countries, as an export product in most countries and as a source of employment and economic activity, both in the agricultural sector and in various auxiliary branches (packaging, packaging, processing, transport, etc.).
Among the producing countries of the Mediterranean basin, the most important are: Spain and Italy, which together account for more than 50% of the region's production. In this group of citrus-growing countries, Algeria ranks 10th among producers.
Lemon is part of Algeria's vast plant heritage and is highly coveted. This aromatic, medicinal and condimentary fruit, belonging to the genus Citrus and the Rutaceae family, whose antibacterial properties (Caillet and Lacroix, 2007 and HIMED and all,2016), antifungal (Jazet Dongmoa, et al., 2002, and Hamdani, 2018 and antioxidant (HIMED and all.,2016) have been the subject of several studies.
Due to the increase in lemon production in recent years in Algeria and in view of the possible economic valorisation of its waste, we have proposed to study the extraction of the essential oil contained in the peel of this fruit considered as waste from the food industry (production of juice and jam) among others.

2. MATÉRIAL AND MÉTHODS

2.1. Vegetable material

The lemons were purchased in February 2019 at the local market in Bab Ezzouar (Algiers) in a single batch and identified at the National Institute of Agronomy of Algiers as being *Citrus Limonia*. The extraction tests were carried out on a laboratory scale. The yield of essential oil was estimated in relation to dry vegetable matter using the following relationship:

$$Y_{EO}(\%) = \frac{m_{EO}}{m_d} * 100 \qquad (1)$$

with : m_{EO} : mass of essential oil (g) ;
$m_{d:}$ mass of dry vegetable matter (g) ;
Y_{EO} (%): yields of essential oil.

The plant's H moisture content was determined by the Dean and Starck method (AOCS OFFICIAL Method, 1993) (xylene distillation) (Gueoguiev E, 1980).

2.2. Experimental installation

The water vapour training and microwave-assisted water vapour training (Figure 1) were carried out at the laboratory scale using the experimental device which includes a two-litre capacity balloon used to produce water vapour. This balloon is topped by a glass column containing the plant material and connected to a refrigerant. The steam formed in the balloon passes through the column containing the plant and carries the essential oil. The distillate (mixture of essential oil and water) is recovered in a Florentine vase. The separation of the essential oil from the water is done after condensation.

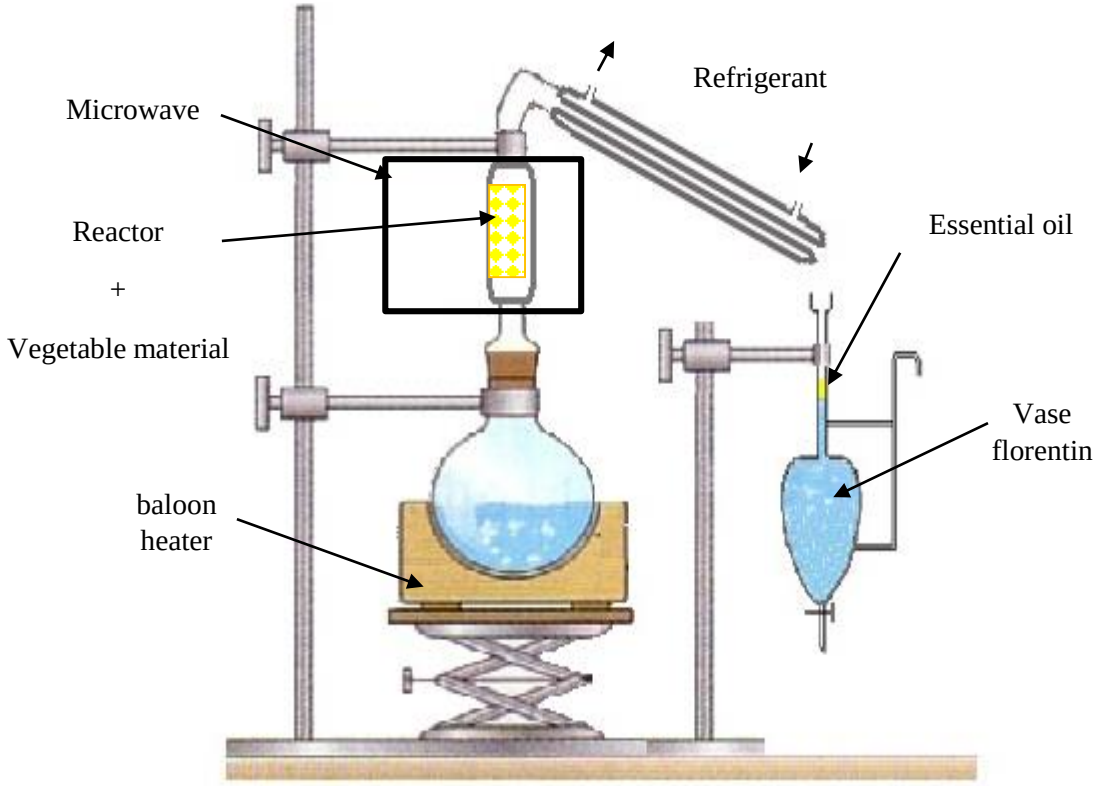

Figure 1. Experimental device for microwave-assisted steam training.

2.3.Antioxidant activity

The radical-scavenging activity (RSA) of an extract can be expressed as the percentage of DPPH (2,2-diphenyl-1-picrylhydrazyl) reduced by a given amount of extract. DPPH is a stable highly coloured free radical that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a colourless hydrazine (DPPH-H).

The antioxidant activity of essential oils obtained by the three processes was determined using a DPPH radical-scavenging activity assay method described by Mimica- Dukic, Bozin, Sokovic and Simin (2004). The essential oils samples were appropriately diluted with ethanol, then, an aliquot (25µL) was added to 975 µL of a 60µM DPPH solution in ethanol. The mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The Inhibition of free radical DPPH in percent (I%) was calculated as follow:

$$I\ (\%) = \frac{(\text{initial absorbance} - \text{final absorbance})}{\text{initial absorbance}} * 100 \qquad (2)$$

The initial absorbance and final absorbance are the absorbance values of DPPH at time zero and after 30 min, respectively. Antioxidant tests were performed at least three times, and the mean values were reported.

3. RESULTS AND DISCUSSION

3.1. Influence of the mass of plant material

The results of the study of the influence of the mass of vegetable matter on the yield of essential oil at constant flow rate (8 g/min) for 3 hours are shown in Figure 2, which shows that the best

yield is obtained for a mass of 45 g. On the other hand, with regard to the maximum mass (55g), the low yield is probably due to the settling of the plant material in the bed. For the minimum mass (25g) and the average mass (35g), the low efficiency obtained is explained by the creation of preferential paths due to a large dispersion of the bed and therefore a high porosity.

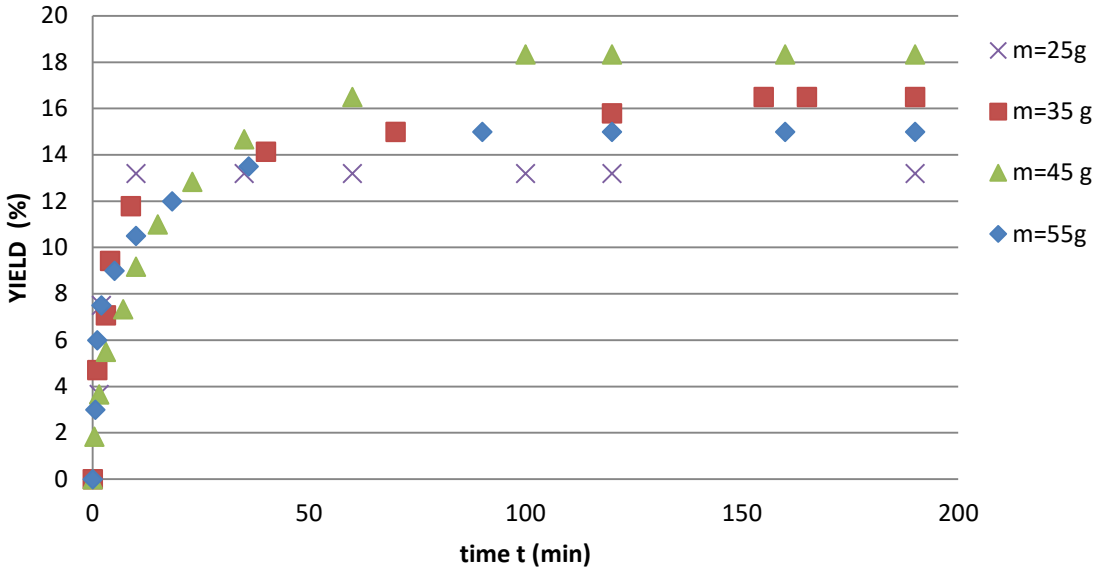

Figure 2 : Influence of the mass of vegetable matter on the yield of essential oil (Q = 8 g/min et t = 3 hours)

3. 2. Influence of the flow of water vapor and the power of the microwave

In order to determine the influence of the water vapour flow and the power of the microwave on the essential oil yield, tests at different water vapour flows were carried out and shown in Figure 3. We can see that for a mass and time set at 3 hours, the maximum extracted essential oil is obtained for the test with an average flow equal to 8 g/min and that with a power of 500W with a much shorter time which is 11 min instead of 100 min. For the flow rate of 2 g/min, the essential oil yield is the lowest. This could be due to the fact that the water vapour is not sufficient, so part of the essential oil is not carried away. Concerning the 10 g/min flow rate, the decrease in efficiency could be caused by the creation of preferential paths.

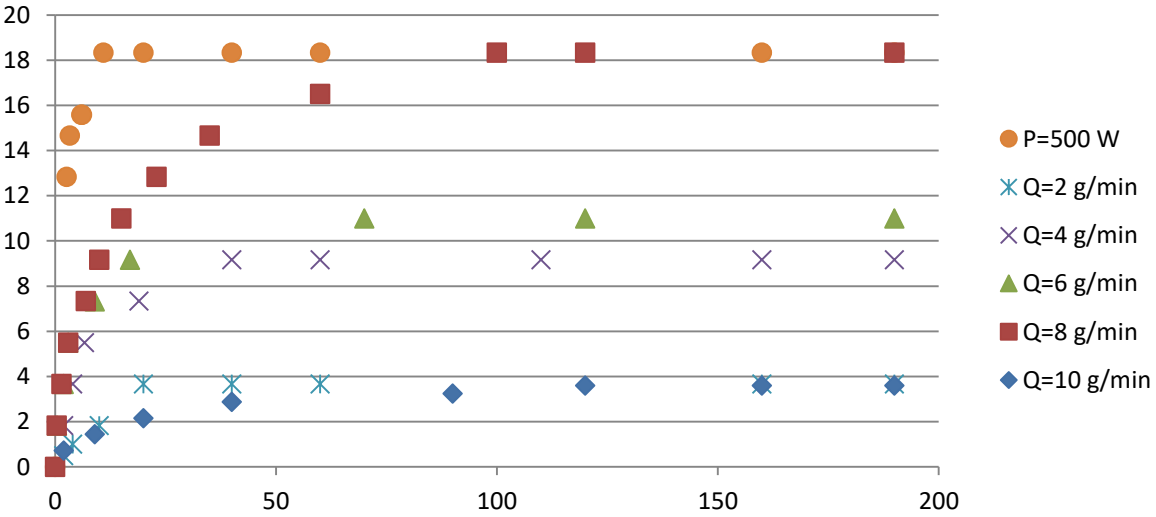

Figure 3 : Influence of the flow of water vapor and microwave power on the yield of essential oil (m = 45g and t = 3 hours)

3.3. Chromatographic analysis

The recovered essential oil was analyzed by GC/MS to identify the chemical composition.

Table 1 summarizes the results obtained.
The results show that the essential oil consists of 23 aromatic compounds. It appears from the data that limonene represents the majority compound. 95,48 %. Five compounds are present with a significant content: α-pinene 0.53%, sabinene 0.49%, β-myrcene 1.87%, Linalol 0.30% and decanal 0.19%.

Table 1 : Chemical composition of the essential oil of lemon rind extracted by steam distillation

Compounds		IR Calculated	SD
1	Pinene<Alpha->	926	0,53
2	Sabinene	961	0,49
3	Myrcene<Beta->	988	1,87
4	Phellandrene<Alpha->	1001	0,17
5	Limonene	1030	95,48
6	Ocimene<(E)-Beta->	1048	0,02
7	Terpinene<Gamma->	1103	0,03
8	Terpinolene	1120	0,02
9	Linalool	1125	0,30
10	Citronellal	1167	0,03
11	Terpin-4-ol	1191	0,06
12	Terpineol<Alpha->	1203	0,06
13	Nerol	1237	0,03
14	Neral	1268	0,03
15	Geraniol	1271	0,01
16	Geranial	1284	0,06
17	Caryophellene<E->	1391	0,02
18	Humulene<Alpha->	1450	0,03
19	Valencene	1488	0,05
20	Octanol<N->	1102	0,05
21	Nonanal<N->	1126	0,01
22	Decanal	1210	0,19
23	Citronellyl Acetate	1342	0,02

3.4. Antioxidant activity

The results obtained by a DPPH essay (Figure 4) show a good activity for oils obtained by steam distillation (SD).
The antioxidant activity of the essential oil extracted by SD increases gradually and reaches 62.97% for a concentration of 1000 mg.L-1. This oil is less active than BHA (91.5%) and BHT (93.1%) which are non-natural food additives.
These data show that lemon peel essential oil obtained by SD has an above average antioxidant capacity, and could be used as an antioxidant food additive.

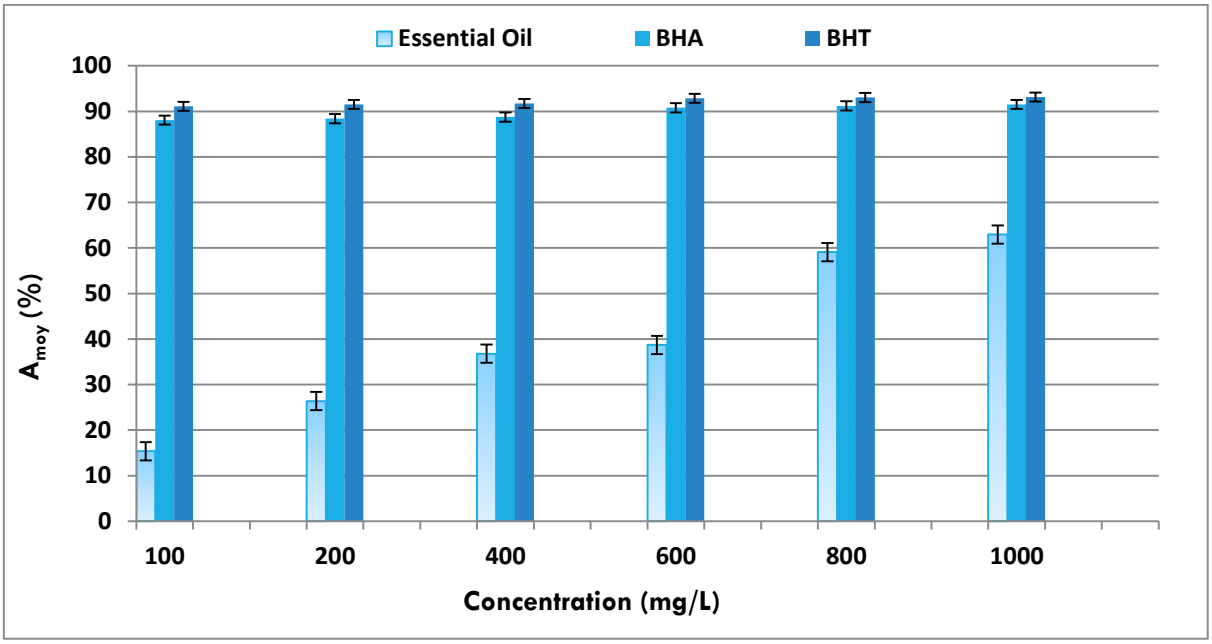

Figure 4 : Anti-oxidant activity of *Citrus Limonia* obtained by SD

4. CONCLUSION

The objective of this work was to optimize the operating conditions for the steam-driven extraction of essential oil from lemon peel, namely, the mass of plant material and the water vapour flow rate.

From the study carried out on these parameters on the essential oil yield, we were able to conclude that the optimal conditions are : Mass of plant material = 45 g and water vapour flow rate = **8 g/min** and power of the microwaves **= 500 W.**

The chromatographic analysis of the essential oil made it possible to identify 23 components. The main constituent is limonene.

The antioxidant activity, determined by the DPPH test for the oil extracted by steam distillation, showed that the activity of this oil is good. The results obtained show the considerable potential of this plant as an antioxidant food additive.

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AGROFOOD
20 - 21 June 2019, Istanbul

Evaluation of biological activities of jujube honey from three regions of Algeria

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ABSTRACT

Honey is a natural food, known for thousands of years, highly appreciated for its gustatory, nutritional and therapeutic qualities; it's been enjoying a renewed interest in recent years. In 2006, world production amounted to 1.3 million tonnes/year while in Algeria it was around 4000 tonnes/year. The low local production and the lack of knowledge of honey composition and its different biological values allow a disinterest of local honeys in favour of foreign honeys, so, it seems essential to us to be interested in everything related to its origin, its composition as well as some biological values.

The objective of our work is the evaluation of antimicrobial activity, by the disc method.
We have chosen a type of monofloral honey, widely consumed by Algerians for its highly prized therapeutic effects, named Jujubier "Sedra" honey. Our results showed that the honey tested partly meets the required international standards, and that it has good antimicrobial.

Keywords: Antimicrobial activity, Food waste, Jujube honey.

1. INTRODUCTION

Honey is well known by all and has been widely consumed by all populations since prehistoric times and the highest antiquity, at least for 13,000 years, as evidenced by Spanish cave paintings. It is one of the oldest foods in the world. It has had a long lifespan in the history of human food and has long been the only sweetener (Megherbi, 2006).

Honey is an all-natural product that contains no additives or conservator. It arrives on our table as the bees made it and as the beekeeper conscientiously harvested it.

It has been associated with various symbolic meanings in different cultures. In the history of the various known civilizations, it has always had a mythical value symbolizing life, health and purity (Dudouit, 1999).

The quality of the various honeys consumed worldwide depends on many physical-chemical, climatic and biological factors. They have different colours, smells and consistencies depending on their nature and origins.

To this end, we considered it imperative to establish the physico-chemical characteristics and biological values of honeys from three different regions of the country and compare the results obtained with European or international standards (Vienot de VAUBLANC ,2014).

For this work, we have chosen a type of mono floral honey, with very popular and widely consumed therapeutic effects, it is Jujubier honey "Sedra". This choice followed a preliminary survey of beekeepers in the capital.

2. MATERIALS AND METHODS

2.1.Evaluation of antimicrobial activity

The evaluation of the antimicrobial activity of honey was carried out by the disc method, which consists in highlighting a possible antimicrobial activity of honey by placing it in the presence of the tested germs. It is the most commonly used method in microbiology laboratories for antibiotic susceptibility testing, the only difference is the replacement of antibiotics by honey. Table I.1 shows the characteristics of the strains used.

Table I.1: Characteristics of the different strains used.

Reference strains			ATCC	Family
Bacteria	Gram +	*Escherichia coli*	4157	Entreobacterian
	Gram -	*Staphyloccocus aureus*	6538	Staphlococaceae

The MH Muller Hinton medium is melted in a water bath at 95°C and allowed to cool.

Aseptically the first layer of the media is poured into 9cm diameter kneading boxes, cooled and solidified (about 30min) on the bench.

from young culture (18 to 24 hours for bacteria and 48 hours for yeasts) make bacterial suspensions that are deposited in 5ml of sterile physiological water, we shake with a vortex, then a first reading of the suspension concentration is taken using a spectrometer at a wavelength of 620 nm by estimating the transmittance T between 22 and 32% or the optic density DO (0.2 to 0.3) for bacteria except for Staphylococcus aureus which must be between 20 and 30% (0.3 to 0.4). The values between the intervals below correspond to an optimal concentration of 107 - 108 germs/mL.

The two MH media are melted in a water bath and allowed to cool slightly, a 50ml test sample is poured into a vial, 200 µl of the bacterial suspension is added using a propette into the vial and then stirred manually and quickly 4ml of each seeded medium is applied to the surface of the first layer by rotating the can in a similar manner to obtain a uniform surface, and the can is

allowed to cool and solidify on the benchtop.
The discs are applied with sterile forceps and placed on the surface of the second layer of the agar, a drop of honey is applied to each disc with a sterile spatula. It is allowed to diffuse for 30 minutes then incubated at 37°C for 24 hours for bacteria and 48 hours for yeasts.
If the reading of the discs reveals the presence of a clear area around the disc, this indicates the presence of inhibitory activity.

If there is no clear area around the disc, this indicates the absence of inhibitory activity.
according to the Meena and Sethi scale (1994). and Ela et al. (1996) the inhibition activities are:

- Highly inhibitory when the diameter of ZI ≥ 28 mm;
- Moderately inhibitory when the diameter of ZI ≤ 28 mm;
- Slightly inhibitory when 10 mm ≤ the diameter of ZI ≤ 16 mm;
- Non-inhibiting when the diameter of ZI ≤ 10 mm.

2.2. Determination of the minimum inhibitory concentration

Minimum inhibitory concentrations are defined as the last or lowest concentration of an antimicrobial agent that can visibly inhibit the growth of a microorganism after 24 hours of incubation for bacteria and 48 hours for yeasts.
A suspension of 104 germs is prepared from a 24-hour microbial culture for bacteria and 48 hours for yeasts;
The MH and SAB media are melted at 95°C in a water bath; 200ml of each MH and SAB medium is prepared with 1ml of tween 80;
A series of dilutions of each honey (from the three regions) ranging from 2 to 0.03% are prepared in the appropriate media. The dilutions are carried out as follows:
1ml of honey in 50ml of medium in a first bottle in order to have a 2% dilution;
Half of the first dilution is poured into a second vial and readjusted to 50ml with the medium to obtain a 1% dilution; the same procedure is followed until the last dilution of 0.03% is obtained;
The dilutions are poured into petri dishes, at the rate of two boxes of kneaded kneads for each dilution, and the agar is allowed to harden;
The boxes are seeded by spot using a micro syringe at a rate of 1 to 2 µl of microbial suspension of 10 4germes/ml;
The positive control boxes containing the MH medium are prepared with tween 80 without honey and incubated at 37°C for 24 hours.
Read the IJC for which there is no culture visible to the naked eye, the presence of one or two colonies is not taken into consideration.

3. RESULTS AND DISCUSSION

The results obtained for the qualitative evaluation are shown in Table II and Figure 1.

Table II: The diameters of the inhibition zones of the different strains (in mm) by the disc method.

Reference strains			Results					
			Honey Bechar		Honey el Menia		Honey Biskra	
Bacteria	Gram +	*Escherichia-Coli*	Ø=30	Ø=28	Ø=31	Ø=30	Ø=38	Ø=31
	Gram -	*Staphyloccocus Aureus*	Ø=9	Ø=9	Ø=9	Ø=9	Ø=12	Ø=13

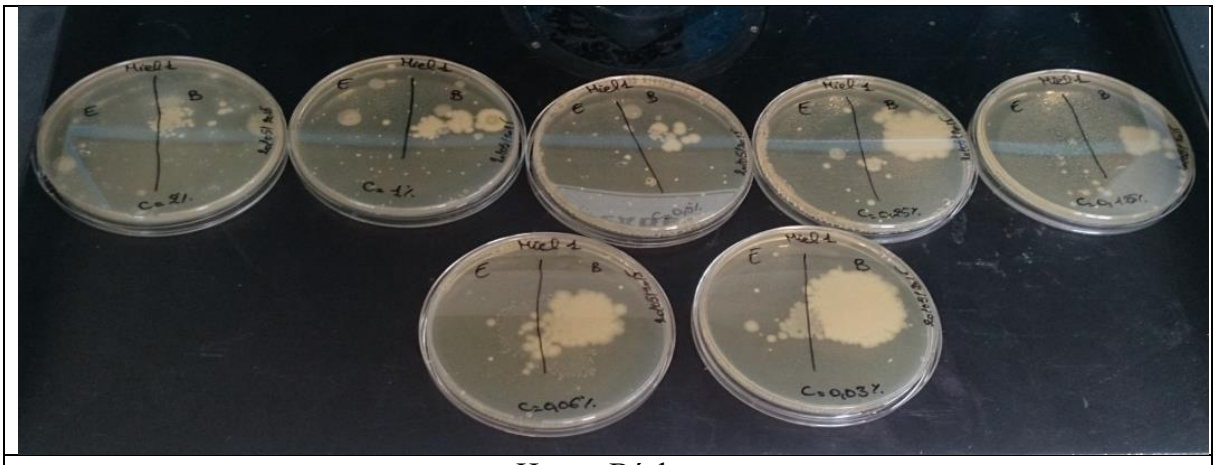
Figure 1. Aromatogram of the different strains (original, 2015)

According to the results obtained, our honeys have an antimicrobial activity, and act differently on the strains tested.

- Thus, Escherichia coli bacteria are the most sensitive to our honeys with inhibition zones varying between 28 and 40mm.
- On the other hand, the Staphyloccocus aureus bacterium is not sensitive to honeys from the Béchar and el Ménia regions.

3.1. Determination of the minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC) of our honeys with respect to the germs tested, we have only selected strains with a high antimicrobial activity which is Escherichia-coli



Honey Béchar

Figure 2 Determination of the minimum inhibitory concentration of Escherichia-coli (original,2015).

The results obtained for the MIC for the different Honey are shown in the following tables:

- Honey of Béchar

Table III: MIC of the fractions of Béchar Honey on bacterial strains.

Honey concentration (%)	**2**	**1**	**0,5**	**0,25**	**0,125**	**0,006**	**0,003**	**MIC**
Escherichia-coli	+	+	+	+	+	+	+	> 2%

- El Menia Honey

Table IV: MIC of the fractions of El Ménia Honey on bacterial strains.

Honey concentration (%)	**2**	**1**	**0,5**	**0,25**	**0,125**	**0,006**	**0,003**	**MIC**
Escherichia-coli	+	+	+	+	+	+	+	> 2%

- Biskra Honey

Table V: MICs of Biskra Honey fractions on bacterial strains

Honey concentration (%)	**2**	**1 %**	**0,5%**	**0,25%**	**0,125%**	**0,006 %**	**0,003 %**	**MIC**
Escherichia-coli	+	+	+	+	+	+	+	> 2%

It should be noted that the MIC is very high in all types of honey is observed on both strains tested, it is >2%, so this bacteria is not sensitive to honeys at low concentrations.

4. CONCLUSION

The results obtained from the evaluation of antimicrobial activity by the diffusion method showed that our honeys do not exhibit inhibition against Escherichia-Coli, which has been shown to be resistant. Results confirmed by the quantitative analysis, namely the minimum inhibitory concentration (MIC).

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AGROFOOD
20 - 21 June 2019, Istanbul

Mannooligosaccharide production by mannanase enzyme application from coffee extract

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The role of mannanases in the detergent, paper, pulp, and feed industry is well established and recently they have found application field in the food technologies such as fruit maseration, gum modification, production of instant coffee and prebiotics etc. Mannanases are mostly extracellular enzymes hydrolysing the 1,4-β-D-mannosidic linkages in mannans, galactomannans, glucomannans and galactoglucomannans. Extracts obtained from coffee beans contain high amounts of mannan. This makes coffee extracts an important source in the production of mannooligosaccharides (MOS), which are known to have prebiotic properties. In this study, different concentrations of mannanase enzyme obtained from microbial production were applied to coffee extracts and MOS production was performed. In order to produce mannanase enzyme, *Aspergillus sojae* fermentation was performed in bioreactor (30 L). The effect of different enzyme amounts (5-10 ml), temperature (40-60°C) and time (30-90 min) on MOS production by Mannanaz treatment (maximum 25 U / g enzyme) was performed using Box-Behnken Response Surface Method. The total amount of MOS (Mannobiose, Mannotriose, Mannotetrose, Mannopentose and Mannohexose) was analyzed by HPLC as a result of 15 trials using different temperature, time and enzyme concentration in coffee extract. The highest total MOS content was found to be 26815.95 ppm at 60°C, 10 ml of enzyme and 60 min. On the other hand, the lowest MOS production was found to be 21082.60 ppm at 40°C, 10 ml enzyme and 60 min. The results showed that coffee extract could be a potential source of MOS production. It has been observed that higher amounts of MOS production can be achieved by optimizing mannanase enzyme treatment conditions.
NOTE: This research was supported by the Scientific and Research Council of Turkey (TUBITAK) [Grant no: 115O051].

Keywords: Mannanese, coffee extract, mannaoolisaccharides, fermentation

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Climate Change and Agriculture

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ABSTRACT

In particular, the activities carried out by the people who gained momentum with the industrial revolution (fossil fuel use, deforestation, faulty agricultural practices) caused a significant increase in greenhouse gas emissions such as methane and carbon dioxide in the course of their losses. Global climate change is caused by the changes in the biosphere resulting from global warming, which will cause climate change across the world. Global warming and global climate change, which have become even more effective since 1980; Besides the socio-economic and political impacts such as the reduction of water resources, food shortage, energy shortage, drought, desertification and migration, the disruption of natural vegetation, ecosystems, species and gene resources, negatively affect the systems which are the basic elements of biological diversity. The change in marine and land ecosystems causes changes in the biological structures of these ecosystems. Due to heating in the seas and fresh waters, the living species here lose their productivity or migrate to cooler waters. For example, a 20% reduction in the productivity of salmon fish has been identified, and migration has been shown to threaten the future of other organisms that depend on these species in terms of nutrients. Terrestrial ecosystems and biodiversity that are part of these ecosystems are also at serious risk. Especially in tropical and mid-generation forests, plant and animal species will be seriously damaged, the adaptation process will be adversely affected and new species may be suitable for new conditions.

Key Words: Biodiversity Climate change, , Ecosystem Global warming, , Greenhouse effect

AGROFOOD
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Effects of mannanase enzyme treatment on yield, beta carotene content and turbidity in the carrot juice production

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The mannanases have been used in the food industry for fruit maseration in order to increase extract yield and juice quality. Mannanases are well known extracellular enzymes hydrolysing the 1,4-β-D-mannosidic linkages in mannans, galactomannans, glucomannans and galactoglucomannans in cell wall. It can be used for enzymatic liquefaction in carrot juice that soluble compounds such as sugars and beta carotene can easily release. In this study, the effects of mannanase enzyme treatment in the carrot juice production on the juice yield, the amount of ß-Carotene and turbidity were investigated. In order to produce mannanases, *Aspergillus sojae* fermentation were performed in bioreactor (30 L). Viscozyme L and Pectinex Ultra SP-L, the most used maseration enzymes in fruit juice industry, was donated from Novozyme. Carrots were firstly squeezed with a juicer to produce pulp and carrot juice. At the begining of the extraction without enzyme treatment, the yield in carrot juice was determined as 33.90%. The effect of enzyme concentrations on yield (Viscozyme L, Pectinex Ultra SP-L and mannanase) were optimized by Box-Behnken Response Surface Method. As a result of the maceration process, fruit juice yields were increased. The highest yield (58,51%) was obtained from the combination of 0.15 g / kg Mannanase and 0.3 g / kg Viscozyme. Thus, the addition of 5, 10 and 15 ml mannanases individually (175 U / ml enzyme) was yielded 46.60%, 52.10% and 56.71%, respectively. Results showed that the usage of microbial mannanase enzyme in carrot juice can be increased the extraction yield in maceration process. Moreover, the total beta-carotene concentrations of carrot juice was increased by using mannanase enzyme in maseration. The highest beta carotene concentration in carrot juice was found to be 2,75 mg/100 g. Thus, the mannanase enzyme treatment was improved the turbidity in carrot juice. Results showed that microbial mannanase without using mix enzyme cocktails can be successfully improved the juice yield, beta carotene content and turbidity.
NOTE: This research was supported by the Scientific and Research Council of Turkey (TUBITAK) [Grant no: 115O051].

Keywords: Mannanese, carrot juice, beta carotene, turbidity

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Determination of Total Phenolic, Antioxidant, Sugar Content, Minerals andPomological Characteristics of Some Banana Varieties and Local Types

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ABSTRACT
In this study, some pomological and phytochemical properties of 'Dwarf Cavendish', 'Grand Nain', 'Erdemli Yerli', 'Erdemli Küllü' and 'Alata Azman' banana varieties and local types, cultivated in Alata Horticultural Research Institute's genetic parcel, were assessed. Samples were examined for total phenol, antioxidant, reducing sugar, minerals, color (L, a, b, Hue angle, Chroma), finger weight, finger length, finger diameter, peel thickness, total soluble solid, maturity index, pH and acidity. The significance of differences between means were obtained by Tukey's test, ($p < 0.05$). The results showed that finger weight, peer thickness and finger diameter values of 'Erdemli Küllü' and 'Erdemli Yerli' bananas were statistically higher values. However, in terms of finger length, 'Dwarf Cavendish', 'Grand Nain' were more preferable. There was no statistically difference between samples in respect to ratio of total soluble solid to acidity. Chroma values of 'Erdemli Küllü' and 'Dwarf Cavendish' bananas were lower than the other banana samples. Fructose and glucose contents of 'Erdemli Yerli' were found to be superior to other samples. Moreover, organic acid content was found to be an important selection criteria between Erdemli local types and other bananas varieties. Another important result obtained from the research was that 'Grand Nain' and 'Dwarf Cavendish' had more mineral contents. Furthermore, 'Alata Azman' cultivars was prominent in terms of total phenol content and antioxidants, which was concluded as a significant result by virtue of the importance of these phytochemicals.

Keywords: Banana, Fruit color, Minerals, Total antioxidant, Total phenolic

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Green Manure Used Purpose Cowpea (*Vigna Sinensis* L.) and Soybean (*Gylcine Max*. L. Merr.) Yield Exposed Organic Strawberry Production, Quality, Leaf Effects on Soil Fertility and Nutrient Content Features

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ABSTRACT

This study was carried out in Çukurova University Yumurtalık Vocational School on the organic farmland. Albion strawberry variety was used in the experiment. In addition to green manure and green manure, the effects of poultry manure applications on yield, fruit quality, leaf nutrient content and soil fertility of organic strawberry production were investigated. The study was carried out between 2015-2018. Soybean (S) (*Gylcine max*. L. Merr.) and cowpea (B) (*Vigna sinensis* L) were used as green manure (GM). The green fertilization parcels were divided into two and one part of the green manure was added to the fertilizer of the manure (PM (+)) and the other part was not applied (PM (-)). Green fertilization has not been done in the control parcel. Considering all applications, the total yield obtained in the first year of the experiment ranged from 177.93 to 372.10 g / plant. After the green fertilization, the yield values of poultry manure supplied with green manure were effective. In the first year the highest total yield was obtained from poultry manure and fertilized cowpea applications. In the second and third year, the highest total yield was obtained from soybean + PM grape (389.91-394.91 g / plant). As a result; It has been shown that green fertilizer plants can be used in organic strawberry cultivation because of the positive effect on fruit quality and soil fertility.

Keywords: Organic Agriculture, Green Fertilizer, Poultry manure, Strawberry,

AGROFOOD
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Characteristic of Listeria monocytogenes, isolated from the meat of different species of animals, Kazakhstan

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ABSTRACT

A descriptive study was conducted to isolate, identify, and characterize Listeria strains isolated from animal meat produced in various regions of Kazakhstan. A total of 50 samples of meat of various types (beef, lamb, pork, horsemeat and chicken) were analyzed. The most significant for public health pathogen Listeria monocytogenes was detected in the studied meat samples. The resulting Listeria monocytogenes cultures were tested for antimicrobial resistance to 14 antibiotics. The isolated culture of Listeria monocytogenes was resistant to 6 antibiotics: ampicillin (10 µg / disc), cefadroxil (30 µg / disc), tetracycline (30 µg / disc), amoxicillin (10 µg / disc), cefixime (5 µg / disc), polymyxin-B (100 µg / disc).

Keywords: food safety, meat, listeria, antibiotic resistance

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INTRODUCTION

As a result of the emergence of emergent pathogens, the risks of food-related diseases have increased significantly, especially in people with weakened immunity (children, the elderly, cancer patients, etc.). Listeriosis is considered one and such emergent infections. Listeria monocytogenes is the causative agent of listeriosis in humans and animals. Currently, the disease is considered one of the most significant food infections in the world. The main factors of listeriosis transmission are milk and dairy products, meat of animals and poultry, vegetables and seafood [1,2,3,4,5,6]. Listeria monocytogenes can be transmitted through contaminated food at any stage of their production and processing. The main role is played by dairy products, unpasteurized or poorly pasteurized milk, cheese, butter and ice cream. Food products of animal origin are also contaminated by listeria, a pathogen is found in boiled sausages and sausages, raw and smoked meat products, in semi-finished products.

In the Republic of Kazakhstan (RK), listeriosis is subject to registration since 2002, from the moment the order No. 946/326 "On prevention of listeriosis in the Republic of Kazakhstan" was issued, the Ministry of Health of the Republic of Kazakhstan and the Ministry of Agriculture of the Republic of Kazakhstan.

According to Musayeva A.K. et al., in permanently not free for listeriosis farms of the Almaty region with cattle and sheep&goats, listeriosis is found in 10–30% of the animals studied. In the Kazakh Scientific Research Veterinary Institute from 10 samples provided from farms in the Almaty region of Kazakhstan in 2009, the causative agent of listeriosis was isolated in two cases (from 7 months old calf, 2-year-old cow), in 2011 - in two cases (from 3-year cows, one-year-old sheep); in 2014 - in three cases (from 6 months old calf, sheep, 8 months old lamb); and in 2015 - in two cases (from a cow and a bull), and in 2019 - ten cases (from calves, lambs and pigs). As a result of our own research, 50 samples of meat from different types of animals (beef, lamb, pork, horsemeat and chicken) produced in different regions of Kazakhstan(Almaty, West Kazakhstan and North Kazakhstan regions) in 8 samples (beef 3 samples, pork 4 samples and lamb 1 sample) Listeria was found.

Therefore, the purpose of the research was to study the properties of Listeria strains isolated from the meat of various animal species.

MATERIALS AND METHODS

Diagnostic preparations, nutrient media and reagents: nutrient media (MPB (meat-peptone broth), MPA (meat-peptone agar), agar Palcam for identification of Listeria, blood agar, Muller-Hinton agar, dye solutions (Gram stains), Hiss medium, sterile distilled water, saline.

The study of the biological properties of the isolated microorganisms conducted in accordance with the approved orders and kit instructions.

The following research methods were used: bacteriological, biochemical. In total, 50 samples of meat of various types of animals (beef, lamb, pork, horse meat and chicken) were selected for research. Meat samples were taken in the markets in accordance with GOST R 51447-99 (ISO 3100-1-91).

Microbiological studies of meat were carried out according to GOST R 54354-2011 "Meat and meat products. General requirements and methods of microbiological analysis".

Sampling was carried out from whole carcass in the amount of 50 g. Then from each meat sample from the depth of the muscle fibers were taken pieces of 1x1 cm. Samples were ground with scissors and grinded in a mortar to a homogeneous state. A portion of the meat was placed in a test tube with pre-prepared MPB and shaken. The resulting suspension was sown on Petri dishes with MPA. Petri dishes with cultures were placed in a thermostat for 24 hours at 37 ° C. After 24 hours of cultivation, smears were made from the grown material and Gram-stained. To do this, a fixed smear was stained with gentian violet carbolic solution for 1-2 minutes. For 1 minute, the smear was treated with Lugol's solution, decolorized with alcohol, 10-20 seconds,

washed with water. Then stained the smear with an aqueous solution of Pfeiffer fuchsin for 1-2 minutes.

Antibiotic resistance determination was carried out by disk-diffusion method using paper discs with antibiotics and the method of serial dilutions of an antibiotic in a dense medium (Agar Muller-Hinton) (MUK4.2 1890-04 of the Ministry of Health of the Russian Federation, 2004). The results were interpreted according to the instructions for the disks. The sensitivity to antibiotics was evaluated by the diameter of the zone of growth inhibition, on the basis of which Listeria was characterized as sensitive, moderately sensitive or resistant. Preparations for the determination of antibiotic resistance were chosen taking into account the spectrum of the antimicrobial activity, as well as those available and often used in veterinary practice. Also taken into account the natural resistance of Listeria to antibiotics. After incubation for 20 hours, the diameter of the zone of inhibition of growth of the microorganism was measured and the selected microorganisms were classified as sensitive, moderately sensitive or resistant to antibiotics.

To determine sensitivity to antibiotics, Listeria's daily broth culture, not contaminated by extraneous microflora, was used. In work used standard paper disks with antibiotics: amikacin (30 μg / disc), ampicillin (10 μg / disc), cefadroxyl (30 μg / disc), norfloxacin (10 μg / disc), clindamycin (2 μg / disc), tetracycline (30 μg / disc), ciprofloxacin (5 μg / disc), amoxicillin (10 μg / disc), tobramycin (10 μg / disc), lomefloxacin (10 μg / disc), nitillin (30 μg / disc), ofloxacin (5 μg / disk), cefixime (5 μg / disk), polymyxin B (100 μg / disk).

In sterile Petri dishes with a diameter of 100 mm were sterilely poured 25 cm^3 of MPA. Bacterial suspension (daily broth culture) in an amount of 0.1 cm^3 was applied to the surface of the agar and equally distributed with a spatula, after which disks with various antibiotics were applied with sterile forceps. In each dish, 7 antibiotics were tested. After disc application, Petri dishes were incubated at 37 ° C for 18-20 hours upside down. Evaluation of the results was carried out by the presence of zones of growth inhibition of microorganisms around the disks. Lack of growth of the test organism at a distance of more than the absence of growth of test organism at a distance of more than 15 mm from the disk with antibiotic indicated the sensitivity of the culture to this antibiotic [7, 8]. If the test microorganism developed in close proximity to the disk with an antibiotic, then the microorganism was assessed as resistant to the antibiotic. The diameter of growth inhibition zones, taking into account the diameter of the disc itself, is measured with an accuracy of 1 mm.

RESEARCH RESULTS

Cultural and morphological properties. Colonies of Listeria on a dense nutrient medium looked small, more often isolated, with magnificent growth up, their edges rise steeply, the top of the colony is some indented or rounded, the edge of the colonies is even, the surface is rough with a white sparkling and bluish shade (S-shape), figure 1.

Microscopy of smears obtained from the culture - small gram-positive rods with rounded ends, arranged in pairs, Figure 2.

Figure 1 - Growth of L.monocytogenes on MPA

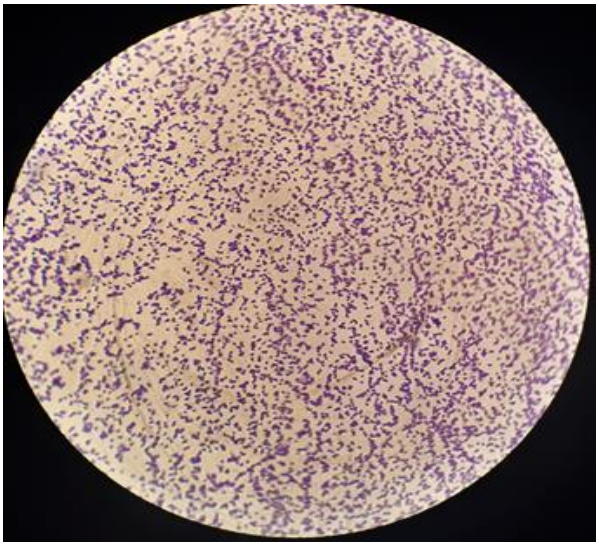

Figure 2 - L. monocytogenes in a gram-stained smear

After 24 hours, with the appearance of continuous growth of colonies, by bacteriological loop were subcultured on Palkam selective diagnostic medium. After 24 hours of incubation on selective Pamcam medium, abundant growth of small, grayish-green or olive-green colonies with a black halo, 0.5-1.0 mm in diameter, was observed. After 48 hours, colonies with a diameter of 1.0-2.0 mm acquired a green color with deep centers surrounded by a black halo. When a continuous growth of colonies of Listeria was made by the bacteriological loop were subcultured from areas of greatest medium blackening with strokes into 2-3 Petri dishes with selective differential-diagnostic medium to obtain isolated colonies, Figure 3.

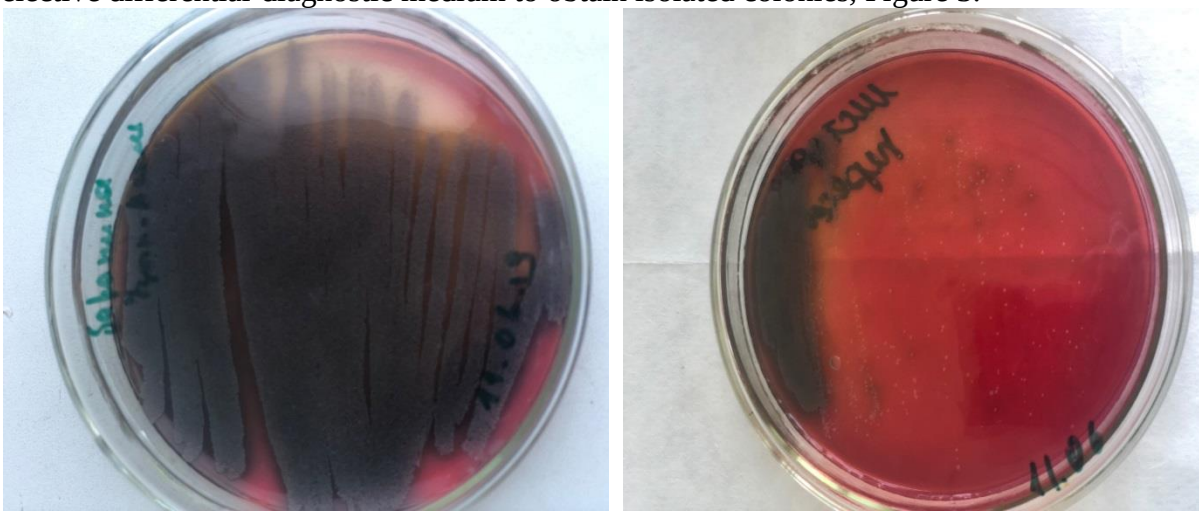

Figure 3 - Listeria Growth on Palcam Selective Medium

Biochemical properties. The enzymatic activity of freshly isolated Listeria strains was characterized by expressed catalase activity (Listeria split hydrogen peroxide with the formation of O_2), the fermentation of glucose, maltose, mannitol. Bacteria did not ferment lactose, arabinose, dulcite, inulin, sorbitol, did not form indole and hydrogen sulfide, did not liquefy gelatin, did not reduce nitrates to nitrites, Figure 4.

Figure 4 - Biochemical properties of L. monocytogenes (1-test for catalase activity, 2-sowing of daily cultures on Hiss media: left to right: 1- lactose, 2- beckons, 3- maltose and 4 -glucose)

Antibiotic sensitivity. The sensitivity to 14 antibacterial preparations of 8 strains of listeria isolated from meat was studied. The research results are summarized in the table 1.

Table 1 - The sensitivity of the isolated culture of Listeria from meat (beef, pork, lamb)

№	Antibiotic	Contents in a disk, mkg	Growth inhibition zone,mm	Sensitivity
1	Amikacin AK30	30	22	S
2	Ampicillin AP10	10	-	R
3	Cefadroxil CFR30	30	-	R
4	Norfloxacin NX10	10	23	S
5	Clindamycin CD2	2	10	MS
6	Tetracycline TE30	30	-	R
7	Ciprofloxacin CFL5	5	20	S
8	Amoxicillin AX10	10	-	R
9	Tobramycin TOB10	10	11	MS
10	Lomefloxacin LOM10	10	22	S
11	Nitillin NET30	30	15	MS
12	Oflosacin OF5	5	19	S
13	Cefixime CFM5	5	-	R
14	Polymyxin –B PB100U	100	-	R
Note: S - sensitive; MS - moderately sensitive; R - resistant.				

From table 1 it is clear that L. monocytogenes was sensitive to the fluoroquinolone antibiotics (norfloxacin (10 µg / disc), lomefloxacin (10 µg / disc), ofloxacin (5 µg / disc) and

aminoglycosia (amikacin (30 μg / disc)), moderately sensitive to clindamycin (2 μg / disc), tobramycin (10 μg / disc), nitillin (30 μg / disc). Listeria showed resistance to ampicillin (10 μg / disc), cefadroxil (30 μg / disc), tetracycline (30 μg / disc), amoxicillin (10 μg / disc), cefixime (5 μg / disc) and polymyxin B (100 μg / disc).

Conclusion. All isolated Listeria strains had typical cultural-morphological and biochemical properties (growth pattern, pathogen morphology, biochemical properties). Listeria resistance to 6 antibiotics was established: ampicillin, cefadroxil, tetracycline, amoxicillin, cefixime, polymyxin B.

The presence of Listeria in meat samples, the presence of antibiotic resistance in strains isolated from meat samples indicates a high risk to the health of people from meat products sold on the markets.

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AGROFOOD
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The Parasitism Performance of *Trichogramma pintoi* in Stored Materials

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ABSTRACT

In this study, it was aimed to determine the parasite capacity of *Trichogramma pintoi* in the biological struggle of *Ephestia kuehniella*. The experiments were carried out 26±1°C temperature, 65-70% relative humidity, 16:8 hour light:dark period. In the study, crackers were placed in small boxes (5 cm length, 9 cm width), 25 non-parasitized *E. kuehniella* eggs were placed on both the upper and lower parts of these crackers. Then to the side of these boxes is the last stage of the pupa period *T. pintoi* are attached to the papers which are glued with arabic gum. Experiments carried out 15 replications. *T.pintoi* was placed in different numbers (15, 20 and 25) in each box. According to the experiments, *E. kuehniella* eggs at the upper part of the box were more parasitized than those in the lower. The highest parasitic performance was obtained in experiments in which 20 and 25 *T. pintoi* (18,86 ±2,55 and 18,33±2,13 units).

Keywords: Biological control, *E. kuehniella, T. pintoi*

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A Functional Food Waste: Pea Pod

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ABSTRACT

Food waste is an important subject worldwide as substantial amount of food that should have been eaten ends up as waste. The production of food waste covers all the food cycle: from agriculture to industrial manufacturing and processing, retail and household. Up to 42% of food waste is produced by households, 39% losses occur in the food manufacturing, 14% is related to food sector (ready to eat food, catering and restaurants), while 5% is lost along distribution chain. Peas *Pisum sativum* L. are consumed after a simple industrial process, in which the pod is removed to prepare the seed as frozen, canned or fresh food. Peas are widely consumed, and are grown all over the world. World production rate of peas was approximately 8.3 million tons/year. During this process, a fibre-rich pod is removed. The large quantities of by-products generated during the processing of plant food involve an economic and environmental problem due to their high volumes and elimination costs. Nowadays, they are considered a promising source of functional compounds. Some nutritional benefits are related to the reduced digestibility of pea starch and the content of dietary fiber of peas, mainly located in their pod fraction. Therefore, these by-products could be added to different foods to provide these beneficial properties. The aim of this study is to provide information about composition and functional properties of pea pods.

Keywords: pea, pea pod, *Pisum sativum* L., fiber

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Effect of Aging on Taste and Smell Perception

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ABSTRACT

Aging is a morphofunctional involution affecting major physiological systems. A gradual loss in taste and smell perception is a part of the aging process. Although some losses may occur earlier, most individuals begin to suffer chemosensory decrements by the age of 60 and more losses occur over the age of 70. The classification of taste and smell loss includes ageusia (no taste sensation), hypogeusia (decreased taste sensation), dysgeusia (distorted taste sensation), anosmia (no sensation of smell), hyposmia (decreased sensation of smell), and dysosmia (distorted smell sensation). The reduction of these senses can cause loss of appetite, food monotony, decreased dietary intake and malnutrition. These losses result not only from anatomic changes that occur during normal aging but also from certain diseases; pharmacological and surgical interventions, radiation, and environmental pollutants. The design of foods for the elderly which could compensate for taste and smell losses and meet nutritional needs presents new challenges and opportunities for the food industry. The aim of this study is to provide an overview about the effect of aging on taste and smell perception.

Keywords: aging, elderly, smell perception, taste perception

AGROFOOD
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Effects of furfural concentration on ethanol production

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Ethanol is a renewable energy source with high efficiency and low environmental impact. Therefore, it can be used as fuel in different proportions with fuel oil. It is also used in food additives, beverage production and table chemicals. Ethanol has been recently produced from lignocellulosic sources. However, in the pre-treatment of lignocelluloses, inhibitory substances such as furans (HMF, furfural), weak acids and phenolic compounds that may adversely affect the microbial growth are formed. Furans directly inhibited alcohol dehydrogenase (ADH), pyruvate dehydrogenase (PDH) and aldehyde dehydrogenase (ALDH). The aim of this study was to determine the toleration limit of furfural concentration for immobilized microbial growth in ethanol fermentation. Therefore, the effect of different furfural concentarations (0.1, 0.5, 2, 4, 6, 8, 10 g / L) on ethanol fermentation has been performed. *Saccharomyces cerevisiae* (ATCC 36858) was firstly immobilized in calcium alginate and then fermentations was started at (temperature) and (time) in bioreactor (1.5 L). Ethanol were analysed by HPLC (ThermoScientific Ultimate 3000, USA). Results showed that the highest ethanol yield was found to be 36.57% in the lowest furfural concentration. On the other hand, it was observed that the ethanol yield was decreased with the increase of furfural concentrations. The lowest ethanol yield was 9.29% in the highest furfural concentration. Thus, furfural in the medium has been found to adversely affect the growth of *S.cerevisiae* even it is immobilized. But, it could grow in the lowest furfural concenrations.
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Keywords: Ethanol, inhibitory effect, furfural, fermentation

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The effects of mannanase activity on viscosity in different gums

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Mannanases are extracellular enzymes that hydrolyze 1,4-β-D mannozidic bonds in mannan, galactomannan, glucomannan and galactoglucomannans. It can be used to reduce the viscosity of foods that is important for gum modification. Thus, it is used as stabilizer and thickening agent in foods. Locust bean gum and guar gum have a wide range of use from food to textile as thickening agent. In this study, the effect of mannanase enzyme on the locust bean gum and guar gum was investigated. For this purpose, Box-Behnken Response Surface Method was created for both gum. Sugar, viscosity and mannooligosaccharide (MOS) analyzes were performed in the samples obtained during the study. Viscosity was determined by Brookfield Viscometer using appropriate spindle. As a result of the viscosity analysis made in the samples of locust bean and guar gum, while the maximum values were 437.73 mPas and 1302.73 mPas, the minimum ones were 1.00 mPas and 1.77 mPas, respectively. ANOVA analysis revealed that there is a statistically significant difference between the viscosities of the prepared solutions and their viscosities before and after the processes. When all these data were analyzed, a significant decrease in viscosity was observed on the gums with the use of mannanase enzyme. Results showed that mannanase enzyme can be used for gum modification for increasing the usage areas in food industry.
NOTE: This research was supported by the Scientific and Research Council of Turkey (TUBITAK) [Grant no: 115O051].

Keywords: Mannanese, locust bean gum, guar gum, viscosity, fermentation

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The effects of Methylamine treatments on some soil physical and chemical parameters of pepper under drought stress

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ABSTRACT

The decrease in water resources has reached to a level that negatively affects the sustainable life as well as the damage caused to the environment. The study was conducted to determine effects of a new synthesis of methylamine (MA) on some soil physical and chemical parameters in pepper under drought stress. There were 4 irrigation levels [full irrigation (100%) (I0), 80% (I1), 60% (I2) and 40% (I3)] and two methylamine (MA) treatments (0, 2.5 mM). Irrigation levels and MA treatments significantly affected soil physical and chemical parameters except for pH and Na content. MA treatments increased the organic matter, P, K, Mg, Fe, Cu, Mn and Zn content of soil cultivated pepper. In conclusion, MA applications could improve some soil physical and chemical parameters in pepper under drought stress.

Key words: irrigation, pepper, methylamine, soil, physical, chemical

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Investigation of the Use of Thyme (Thymus Vulgaris) Extract and Rosemary (Rosmarinus officinalis) Extract as a Natural Preservative

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ABSTRACT

In order to prevent microbiological spoilage in foods, some ingredients are added to the food. The inclusion of antimicrobials may pose a risk to public health. However, the use of these substances is directly effective in prolonging the shelf life since it will provide the microbiological balance of the foods.

In this study, the availability of thyme and rosemary extracts as a natural preservative was investigated. First of all, the extracts of the plants with the maceration technique were added to the tomato paste and liquid extracts in certain proportions by enriching the active ingredients. Accelerated stability studies were performed.

As a result, a 2% protection effect is provided for thyme when added individually. However, rosemary alone did not show a sufficiently sluggish effect. Protective activity was provided by the addition of two (0.5% + 0.5%) to 1.0%. Further studies are needed because they cause partial odor and taste changes.

Keywords: natural preservatives, thyme, rosemary.

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A scale-up processing with different microparticle agent for mannanase fermentation in bioreactor

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Mannanases one of the most important enzyme for industry and they are produced in particularly with *Aspergillus sojae*. In this study, large-scaled mannanase production was performed at 30ºC, 15 lpm aeration rate and 204 rpm stirring speed in the bioreactor (30L). Recombinant *Aspergillus sojae* (ATCC 11906) was used for enzyme production by using fed-batch strategy in this study. Fermentation media was contained glucose (40 g/l fermentable sugar), 0,4% (w/v) yeast extract, 0,05% (w/v) magnesium sulphate and 0,1% dipotassium hydrogen phosphate and different concentrations of magnesium silicate (1, 5, 10 g/L) and aluminium oxide (5, 10, 15 g/L) as a migroparticle agent. According to the results, the maximum mannanase activity was found to be 117,04 U/mL in aluminium oxide (5 g/L) microparticle agent for the glucose medium. In addition, mannanase activities at the different microparticle agent concentrations was found to be 3,27 U/mL (10 g/L aluminium oxide), 15,79 U/mL (15 g/L alumunium oxide) and 70 U/mL (1 g/L magnesium silicate), 24,06 U/mL (5 g/L magnesium silicate), 6,97 U/mL (10 g/L magnesium silicate) respectively. In all the experiments performed, the low and high enzyme activity has been demonstrated that the enzyme production can be carried out successfully with the addition of microparticle in the 30L bioreactor and the enzyme activity values can be increased with the appropriate parameters.
NOTE: This research was supported by the Scientific and Research Council of Turkey (TUBITAK) [Grant no: 115O051].

Keywords: Alumunium oxide, Scale-up, Mannanase, Magnesium silicate, Microparticle

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The effect of different phenol concentrations on inulinase production

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Lignocellulosic wastes have been recently used as a carbon source in many fermentation processes. However, during the hydrolysis process of lignocellulosic biomass, several inhibitors (phenols, furans, weak acids etc.) are formed and released at the fermentation medium. These inhibitors mostly arise from lignin, hemicellulose and cellulose. Phenols originated from lignin hydrolysis and they have negative effect on cell membrane function. Inulinase have a significant value in the food industry. Microbial inulinases catalayzed the hydrolysis of inulin and their end-products can be used as a prebiotics. Inulinases were produced by many microorganisms but especially used of *Aspergillus niger*. The aim of this study was to investigate the effect of phenol compound on inulinase activity produced from *Aspergillus niger* A42 (ATCC 204447). Inulinase fermentations were performed at 30ºC and 250 rpm in the erlenmayer flasks with 150mL working volume. Fermentation medium was included %1 yeast extract (as a nitrogen sourses), %10 sugar (sucrose as a fermentable sugar), %3 inoculum size in different phenol concentrations (0, 0.1, 0.5, 0.75, 1.0, 5.0 g/L). Results showed that when the phenol concentrations increased in the fermentation medium, the inulinase activity was reduced. According to the results, inulinase activities (U/mL) were found to be 202.95, 117.39, 63.35, 54.82, 56.47, 12.57, respectively. Thus, lignocellulosic materials can be used in inulinase productions without detoxification process, when the hydrolysate contains in low concentrations of phenol.

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Keywords: Inulinase, inhibitor, phenol, fermentation

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Mannooligosaccharides production from different gums using mannanase

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Locust bean and guar gum can be used in the production of mannooligosaccharides (MOS) which is well know to have prebiotic effects. The both gum subtances are rich in point of mannans, galactomannans, glucomannans and galactoglucomannans. These compounds can be hydrolyzed using mannanases by hydrolysing the 1,4-β-D-mannosidic linkages for MOS production. In this study, different concentrations of mannanase enzyme obtained from microbial production were applied to different gums. In order to produce mannanase enzyme, *Aspergillus sojae* fermentation was performed in bioreactor (30 L). The effect of different enzyme amounts (5-10 ml), temperature (40-60°C) and locust bean gum solution (0.5-2 %) on MOS production by Mannanaz treatment (maximum 25 U / g enzyme) was performed using Box-Behnken Response Surface Method for locust bean and guar gums. The total amount of MOS (Mannobiose, Mannotriose, Mannotetrose, Mannopentose and Mannohexose) was analyzed by HPLC. While the highest MOS concentrations (21838,03 mg/L) in locust bean gum were obtained from 2% of gum solution, 7.5 ml of enzyme loading and 60°C, it was found to be 24957.70 mg/L for guar gum in %1.25 of gum solution, 5 ml of enzyme loading and 40°C. The results showed that locust bean and guar gums could be a potential source of MOS production. It has been observed that higher amounts of MOS production can be achieved by optimizing mannanase enzyme treatment conditions.
NOTE: This research was supported by the Scientific and Research Council of Turkey (TUBITAK) [Grant no: 115O051].

Keywords: Mannanase, locust bean gum, guar gum, mannooligosaccharid

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Mannanase Production by Solid State Fermentation (SSF) Using Recombinant *Aspergillus sojae* AsT3 (ATCC 11906)

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Solid state fermentation (SSF) has been widely performed for the production of value-added products such as biofuels, enzymes, organic acids, polysaccharides and drugs in biotechnological processes. Mannanases are well used for the detergent, paper, pulp, and feed industry. These group of enzymes are hydrolysing the 1,4-β-D-mannosidic linkages in mannans, galactomannans, glucomannans and galactoglucomannans. The main advantage of the solid state fermentation is a lower energy requirement to produce a product. Lignocellulosic biomass (LCB) is the most abundant, cheap and recently used raw material in the world for the production of enzymes. In this study, different lignocellulosic carbon sources such as wheat bran, rye bran, barley hulls and oat hulls were used as carbon sources in different inoculation rates (3,5, and 7 of %). Fermentations were performed in flasks at 30°C for 7 days. The mannanase enzyme activities were found be 136.6 U/mg for the oat hulls, 177.8 U/mg for the barley hulls, 126.2 U/mg for the rye bran and 274.2 U/mg for the wheat bran. The highest results for every carbon sources were obtained from 5% of inoculation rate. Results showed that mannanase enzyme can be produced from lignocellulosic materials by recombinant *Aspergillus sojae* in solid state fermentation.

Keywords: Solid state fermentation (SSF), mannanase, carbon source, recombinant *Aspergillus sojae*

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Heterologous Expression of *Aspergillus niger* Pectinase Gene in *Pichia pastoris* and its characterization

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ABSTRACT

Pectinase is one of the most widely used enzymes in different fields of food industry for different purposes, such as clarification of fruit juice, extraction of vegetable oil and saccharification of agricultural substrates. The aim of this study is the recombinant production of polygalacturonase, one of the most commonly used commercial pectinases.

In this study, *A. niger* pectinase was produced extracellularly using *P. pastoris* host system. Within the scope of the study, codon-optimized and non-codon-optimized pectinase genes were transferred to *P. pastoris* X33 strain and expressed under the regulation of methanol-inducible *AOX1* and ethanol-inducible *ADH3* promoter. Protein production studies were carried out under the shake-flask conditions and ethanol was used for the *ADH3* promoter and methanol was used for the *AOX1* promoter as a carbon source.

As a result of the study, the promoter and codon combination which exhibited the highest production level was determined as the *ADH3* and codon-optimized pectinase. The best producer clone was cultured in 400 mL media in 2L shake-flask and the enzyme was purified by his-tag method. The optimum working conditions of the purified enyzme was 50°C and pH 5.0, and after incubation at 60°C for 1-hour, enzyme activity was maintained at 60% level. Future studies will focus on large scale production of his-tag free enzyme with high-cell density fermentation.

Keywords: *Aspergillus niger*, Codon-optimization, Pectinase, *Pichia pastoris*, Protein purification

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Investigation of Antifungal Activity of Yeasts Isolated from Orange Fruits against *Penicillium digitatum*

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ABSTRACT

There are significant losses in the production of fresh fruits and vegetables every year due to fungal plant pathogen. In recent years, many scientific studies have been conducted on the negative effects of chemical fungicides on the environment, the development of resistance in plant pathogen fungi and on the harm to human health. In addition to these studies, interest in the use of antagonist microorganisms and their metabolites as biofungicides has increased. Antagonist yeasts are found in many different areas such as fruit surface, fruit inside and soil and have an important place in protecting plants from plant pathogen fungi before harvest.

In this study, antifungal activity of yeasts isolated from different oranges fruits against *Penicillium digitatum* mold which has a significant negative effect on citrus products is investigated. 8 different yeasts were isolated from the oranges using the antibiotic mixed medium. Pichia kudriavzevii was determined as the result of the molecular characterization of the yeast with the highest activity, when the isolated yeasts were tested at different concentrations in the microplate against P. digitatum mold. In vitro antifungal tests showed that *Pichia kudriavzevii* was an effective antagonist yeast. It is thought that *Pichia kudriavzevii* may be used as biofungicide in its pre-harvest stage.

Keywords: *Pichia kudriavzevii , Penicillium digitatum,* antagonist microorganism, orange

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Determination of basic kinetic parameters of strains *Streptococcus thermophilus* isolated from naturally fermented dairy products

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ABSTRACT

Mathematical modeling of the kinetics of growth and acidification for strains *Streptococcus theromophilus* T1, *Streptococcus theromophilus* T2, *Streptococcus theromophilus* T3 and *Streptococcus theromophilus* T4 has been conducted. The strains are cultivated in reconstituted dry milk and basic kinetic parameters are determined. Models based on the logistic curve have been used for modeling of growth kinetics. Parameters that indicate the influence of cumulative lactic acid on cells as well as on its own synthesis have been included in the models. According to the data obtained, the maximum specific growth rates (μ_m) of the strains tested are in the range of 0.133 to 0.301 h-1. For strains *Sreptococcus thermophilus* T2 and *Sttreptococcus thermophilus* T3, higher values of the parameter **c** is observed (2.93 and 1.950) compared to the other strains tested (1,864 and 1,894). This indicates that a moderate process of lactic acid biosynthesis and less post-acidification are expected for strains T2 and T3. This is confirmed by the fact that for these strains the parameter δ of the Weibull model has values of 2.26 and 2.11, respectively, compared to strains T1 and T4, where this parameter has values of 3.13 and 4.14. From the mathematical modeling, it has been determined that the T3 strain cells show the highest sensitivity (lowest resistance) to the lactic acid accumulation compared to the other strains tested.

Keywords: kinetics, mathematical modeling.

1. INTRODUCTION

Streptococcus thermophilus is a Gram-positive, non-pathogenic, facultative anaerobic lactic acid bacteria (LAB) (Dan T. *et al*., 2018). This species can be used alone or in combination with other LABs for the production of dairy products such as cheese and yogurt (Fatih O. *et al.*, 2015). Industrial strains of LAB can be isolated from different milk environments, including fermented milk, cheese, and kefir (Ayad, E.H.E. *et al.*, 2000). The consumption of products containing lactic acid bacteria helps to protect the digestive system from disorders associated with imbalances in gastrointestinal micro flora (O'Brien et al., 1999). In Bulgaria, yogurt is a major fermented dairy product. Therefore, this study investigated the fermentation characteristics of milk that had been fermented by strains *S. thermophilus* isolated from traditional fermented dairy products collected from the Rhodope Mountains in Bulgaria.
A key requirement for the implementation of LAB strains for the production of different dairy products is industrial cultivation. The strains should develop well in the food matrix and maintain a high concentration of active cells in the finished food products during both production and storage (Padmavathi T. *et al.* 2018). This result prompted the need to cultivate the newly isolated strains *Streptococcus thermophilus* in reconstituted skim milk powder and under static conditions. Mathematical modeling of growth kinetics was also conducted. It was known that an increase in the concentration of lactic acid in the medium resulted in a certain inhibiting action on the growth of lactobacilli and that this sensitivity to lactic acid was strain-specific (Bouguettoucha, *et al*., 2011; Gordeev L. *et al*. 2017). For this reason, the selected mathematical models included parameters characterizing the effect of lactic acid on lactobacilli. When studying and comparing the kinetics of development in individual strains in the same cultivation environment and under the same conditions, it is necessary to start the process with the same concentration of active cells and the same initial acidity. Because this is challenging, the biomass and titratable acidity in mathematical models were presented in a non-dimensional form (Tishin V.B., Golovinskaya O.V, 2015; Tishin V.B., A.V. Fedorov 2016). The main advantage of the mathematical modeling is the precise prediction of numerous parameters simultaneously and the relatively high correlation coefficient compared to the experimental data.

2. MATERIAL AND METHODS

2.1. Microorganisms

Four strains of lactic acid bacteria of the species *Streptococcus thermophilus* were isolated and identified from naturally fermented lactic acid products (yoghurt, cheese, etc.) collected from the Rhodope Mountains in Bulgaria: *Streptococcus thermophilus* T1 *Streptococcus thermophilus* T2, *Streptococcus thermophilus* T3 and *Streptococcus thermophilus* T4.

2.4 Cultural medium

M17 agar base. Composition (g/dm^3): peptic digest of animal tissue- 5; papaic digest of soyaben meal- 5; yeast extract-2.5; beef extract- 5; ascorbic acid- 0.5; magnesium sulphate- 0.25; Lactose- 5; agar- 10; pH- 7,1. Sterilization -20 minutes at 121°C.

2.5 Cultivation of the strains tested

Cultivation of the tested strains was carried out under static conditions in sterile, reconstituted (12% dry matter) dry skim milk at 42 ± 1 °C.

2.6 Determination of the number of active viable cells

The number of viable cells was determined by the tenfold dilution method according to ISO 7889: 2005.

2.7 Determination of titratable acidity

The titratable acidity values of each sample were determined after mixing 10 mL sample with 20 mL of distilled water and titrating with 0.1 N NaOH containing 1% phenolphthalein as an indicator to an end point of faint pink color.

2.8 Modeling the kinetics of the fermentation process

Models based on the logistic curve have been used for modeling of growth kinetics (Model 1 and Model 2). The models contain parameters that indicate the influence of cumulative lactic acid on cells as well as on its own synthesis. The Weibull model has also been used (Bouguettoucha *et al.*, 2011; Tishin VB, Golovinskaya O.B, 2015; Tishin V.B., A.V. Fedorov 2016; Gordeev L *et al.*, 2017). Identification of the model parameters was performed by minimizing the error between the experimental and calculated model data by the method of least squares and using the fourth line of the algorithm of Runge-Kutta (Kostov, 2015).

$$\frac{dX_b}{d\tau} = \mu_{max}\left(1 - \frac{P_b}{P_{bm}}\right)^c X_b \qquad \text{Model 1}$$

$$\frac{dX_b}{d\tau} = \mu_{max}\left(1 - \frac{X_b}{X_{bm}}\right)^n X_b \qquad \text{Model 2}$$

$$K_T = a - be^{-(q_p\tau)^\delta} \qquad \text{Weibull}$$

where: μmax is the maximum specific growth rate, h^{-1}; X_b, P_b, X_{bm} and P_{bm} are the biomass, the amount of lactic acid, the final concentration of biomass and lactic acid in a dimensionless form; c - a parameter measuring the inhibitory effect of the accumulated product (lactic acid) on cell development; n- coefficient that measures the influence of lactic acid on the cells, respectively showing the resistance of the cells to the increasing concentration of the product; K_T - titratable acidity in dimensionless form; a - maximum value of titratable acidity in a dimensionless form; b - a coefficient equal to the difference between the maximum and the initial titratable acidity in a dimensionless form; q_P - specific rate of acid formation, h^{-1}; δ - an indicator defining the change in the shape of the curve or the change in the rate of accumulation of lactic acid over time; τ - time of cultivation, h^{-1}.

3. RESULTS AND DISCUSSION

Identification of the model parameters and comparison of the experimental data with the selected models were made. The data from these studies is presented in Figures 1 to 8 and Tables 1 and 2.

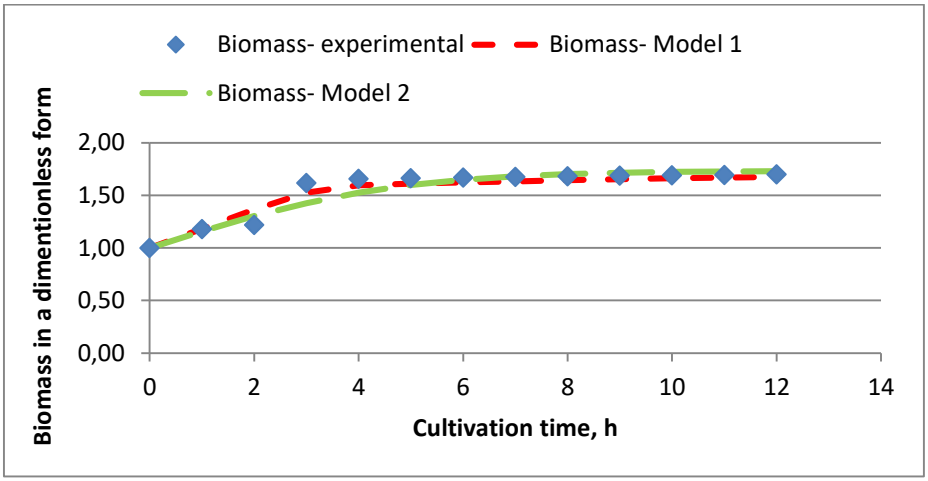

Figure 1. Comparison of experimental data with the selected Models for *St. thermophilus* T1

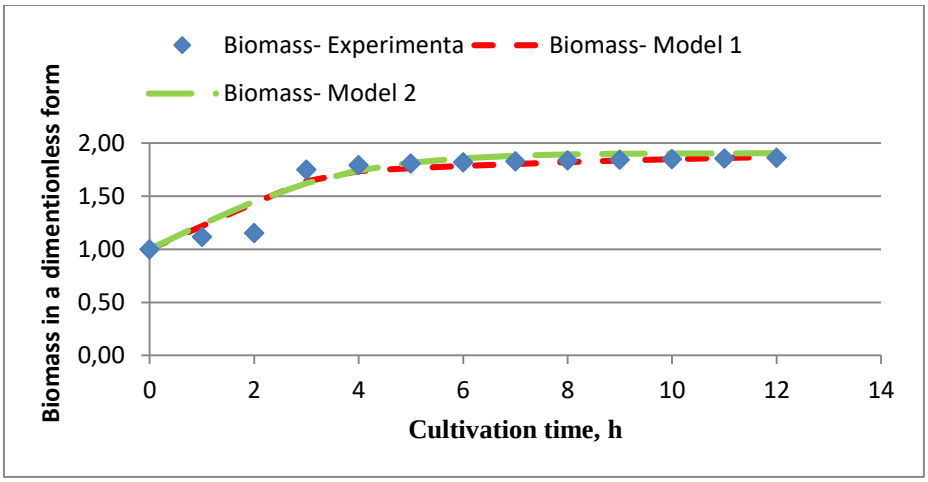

Figure 2. Comparison of experimental data with the selected Models for *St. thermophilus* T2

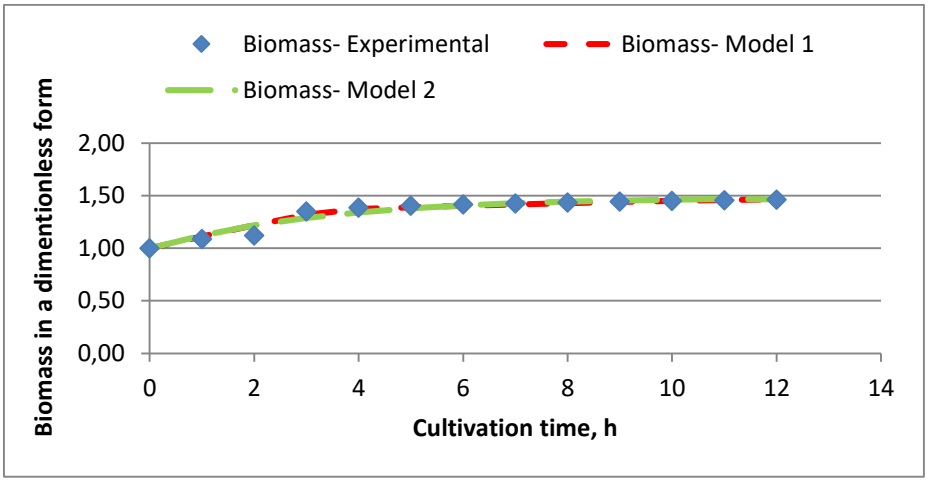

Figure 3. Comparison of experimental data with the selected Models for *St. thermophilus* T3

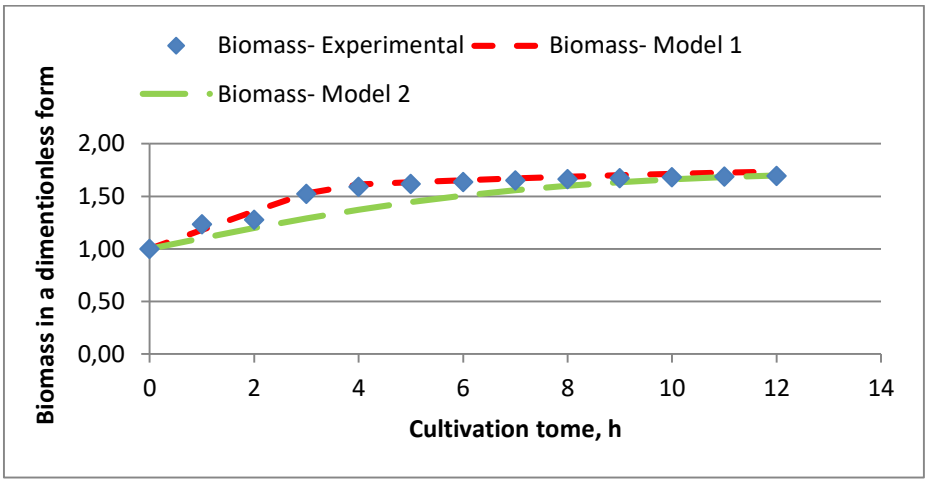

Figure 4. Comparison of experimental data with the selected Models for *St. thermophilus* T4

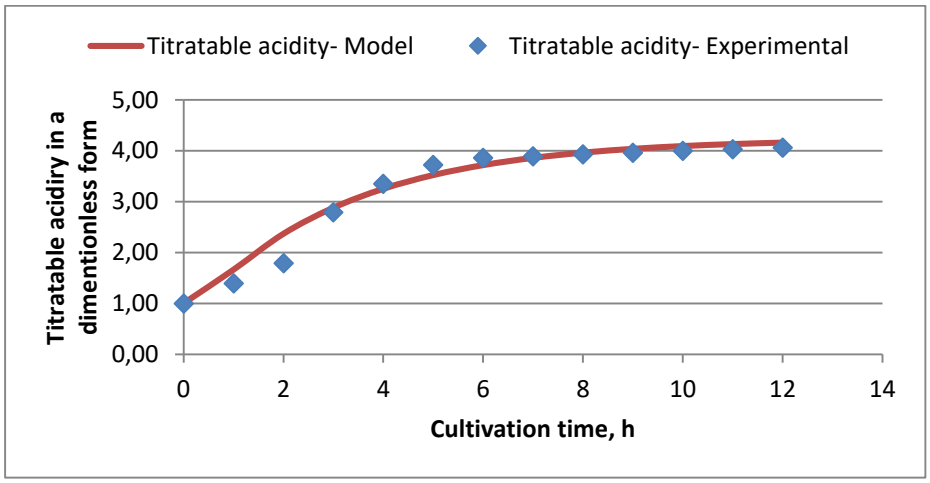

Figure 5. Comparison of experimental data with those of the Weibull model for *St.. thermophilus* T1

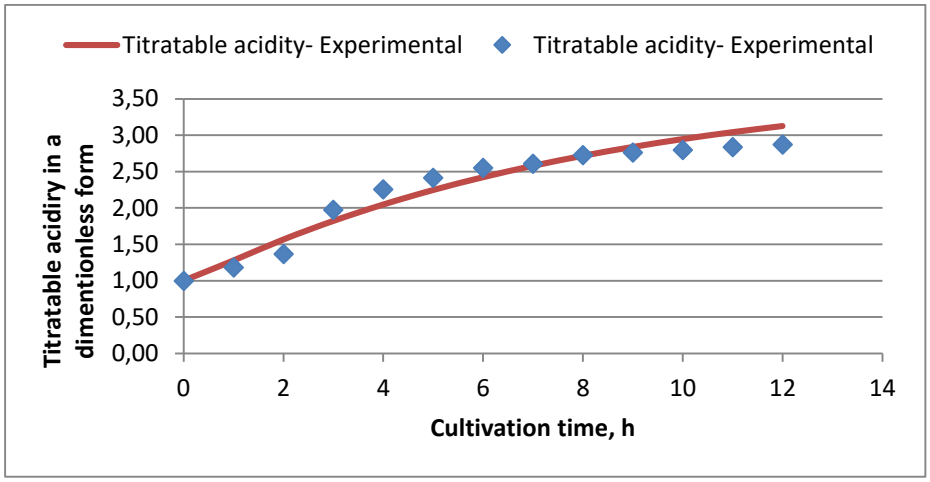

Figure 6. Comparison of experimental data with those of the Weibull model for *St. thermophilus* T2

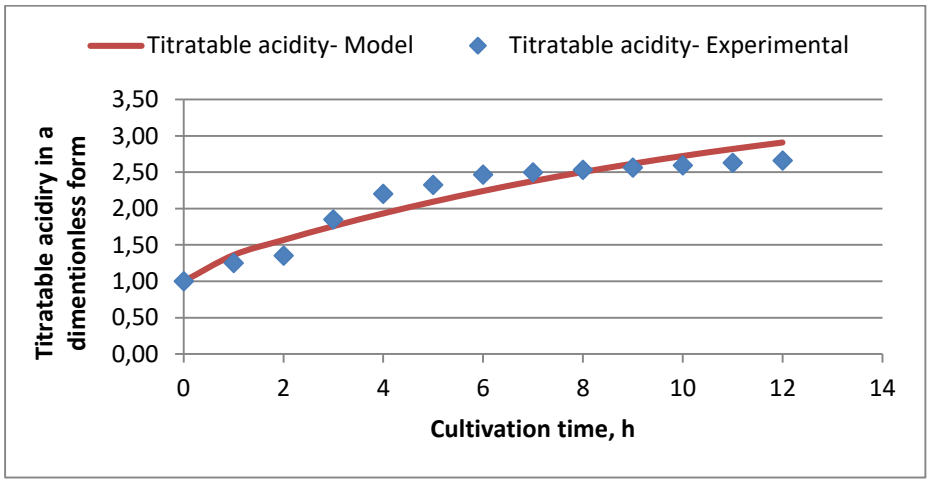

Figure 7. Comparison of experimental data with those of the Weibull model for *St. thermophilus* T3

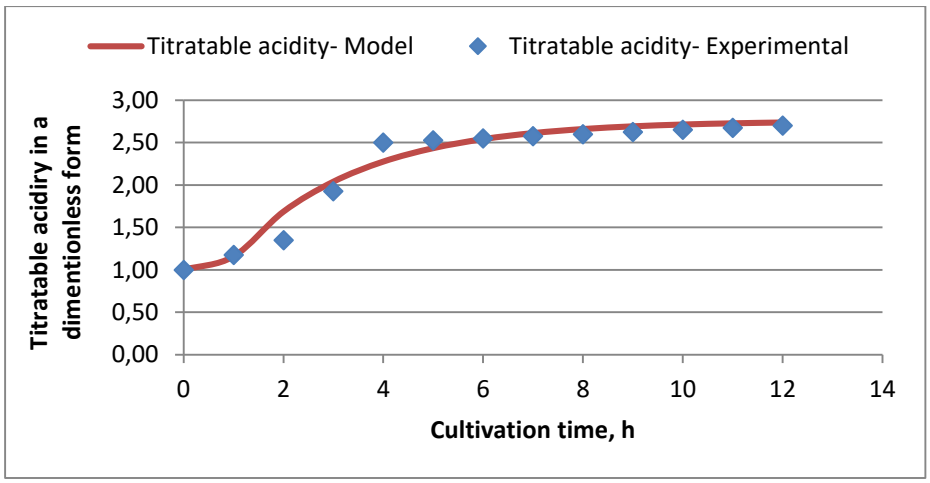

Figure 8. Comparison of experimental data with those of the Weibull model for *St. thermophilus* T4

It is clear from the figures that the results of the mathematical models are very well coordinated with the experimental results. This is confirmed by the relatively high correlation coefficients

and the low values of the identification error "e". R^2 varies from 0.7140 to 0.9587 and "e" from 0.04 to 1.29 (Table 1 and Table 2).

Table 1. Kinetic parameters in Models 1 and 2 for the tested strains *Streptococcus thermophilus*

S. thermophilus Strain	Mathematical models							
	Model 1				Model 2			
	μ_m, h^{-1}	c	R^2	e	μ_m, h^{-1}	n	R^2	e
T1	0,265	1,864	0,9544	0,059	0,209	0,950	0,8984	0,06
T2	0,301	2,393	0,8817	0,076	0,290	0,998	0,8684	0,08
T3	0,143	1,950	0,9531	0,040	0,295	1,295	0,9342	0,04
T4	0,226	1,894	0,9743	0,064	0,133	0,944	0,7140	1,29

From the results presented in Table 1, it is evident that according to logistic Model 1, the highest maximum specific growth rate has strain *Streptococcus theromophilus* T2 μ_m=0.301 h^{-1}, followed by strain *Streptococcus theromophilus* T1 and strain *Streptococcus theromophilus* T4 having a maximum specific growth rates of 0.265 h^{-1} and 0.226 h^{-1}, respectively. According to this model, *Streptococcus theromophilus* T3 has the lowest maximum specific growth rate of 0.143 h^{-1}.

For strains *Streptococcus thermophilus* T2 and *Streptococcus thermophilus* T3, higher values of the parameter "c" - 2.93 and 1.950 were observed compared to the other strains tested (1,864 and 1,894). This indicates that for strains T2 and T3 there will be a greater impact of the accumulated lactic acid on its own synthesis and a moderate process of lactic acid biosynthesis is expected. Strains T1 and T4 hold near values of parameter "c", indicating that these strains would be expected to have a similar acidification process.

It is interesting to clarify the influence of cumulative lactic acid on both the maximum specific growth rate and the biomass. For this purpose, the modeling of kinetics is performed in logistic model 2 which contains the parameter "n" indicating the influence of lactic acid on the biomass itself or the susceptibility of the microbial cells to the accumulated lactic acid. Data obtained from logistic model 2 is reported in Table 1. It is apparent from the table that according to this model, strains *St. thermophilus* T3 and *St. thermophilus* T2 have the highest values of maximum specific growth rates of 0.295 and 0.290 h^{-1}, respectively. The other two studied strains *St. thermophilus* T1 and *St. thermophilus* T4 showed lower maximum growth rates of respectively 0.209 and 0.133 h^{-1}. From the data presented in Table 1, it can be seen that the highest value of parameter "n" was observed for strain *St. thermophilus* T3- 1,295. This indicates that the cells of this strain exhibit the highest sensitivity (least resistance) to the lactic acid that accumulates. This is essential for maintaining a high titer of active cells in the products during storage. Therefore strain *St. thermophilus* T3 should be included in symbiotic combinations with *Lactobacillus bulgaricus* strains that have a less pronounced acidic forming ability, which will ensure that the desired ratio between the two species in the symbiotic culture is maintained.

For the remaining, newly isolated strains of *St. thermophilus* T1, *St. thermophilus* T2 and St.thermophilus T4, close values of "n", 0.950, 0.998 and 0.944, respectively, were observed. From these values, it can be concluded that the cells of these strains will have a higher resistance (less sensitivity) to the increasing concentration of lactic acid.

Another important process characteristic is the acidification kinetics of these newly isolated strains. Therefore, a mathematical modeling of the kinetics of acidification with the Weibull model was performed. An identification of the model parameters has been made and their values are reflected in Table 2. In Table 3 are presented the explicit forms of the Weibull model for the studied strains.

Table 2. Kinetic parameters in the Weibull model for the tested strains *Streptococcus thermophilus*

S. thermophilus	Weibul model parameters					
Strain	a,	b,	q_p, h^{-1}	δ	R^2	e
T1	4,23	3,54	0,102	3,13	0,9587	0,28
T2	3,77	2,82	0,054	2,26	0,9488	0,19
T3	3,92	2,78	0,040	2,11	0,9115	0,16
T4	2,76	2,39	0,098	4,10	0,9544	0,17

From the data presented in Table 2 it can be seen that higher rates of acid formation (0.102 and 0.098 h^{-1}) were observed for strains *St. thermophilus* T1 and *St. thermophilus* T4 compared to strains *St. thermophilus* T2 and *St. thermophilus* T3, where the values are 0.054 and 0.040 h^{-1}, respectively.

Table 3. Weibull's mathematical model in explicit form

S. thermophilus Strain	Explicit models
T1	$K_T = 4{,}23 - 3{,}54e^{-(0{,}102\tau)^{3{,}13}}$
T2	$K_T = 3{,}77 - 2{,}82e^{-(0{,}054\tau)^{2{,}26}}$
T3	$K_T = 3{,}92 - 2{,}78e^{-(0{,}040\tau)^{2{,}11}}$
T4	$K_T = 2{,}76 - 2{,}39e^{-(0{,}098\tau)^{4{,}10}}$

An analogous trend is also observed in the values of the parameter "δ", which characterizes the intensity of the acidification process as a whole. For strains *St. thermophilus* T1 and *St. thermophilus* T4, significantly higher values of this parameter (3,13 and 4,10) were observed compared to the other two strains studied *St. thermophilus* T2 and *St. thermophilus* T3 (2.26 and 2,11). It can be concluded from this that for strains *St. thermophilus* T1 and *St. thermophilus* T4 a more intensive acidification process will occur compared to strains *St. thermophilus* T2 and *St. thermophilus* T3. The results obtained from the Weibull model totally correlate with those obtained from logistic model 1, showing the influence of lactic acid accumulation both on the maximum specific growth rate and on its own synthesis.

4. CONCLUSION

The cells of strain T3 exhibit the highest sensitivity (least resistance) to the lactic acid that accumulates. This is essential for maintaining a high titer of active cells in the products during

storage. Therefore strain *Streptococcus thermophilus* T3 should be included in symbiotic combinations with *Lactobacillus bulgaricus* strains that have a less pronounced acidic forming ability, which will ensure that the desired ratio between the two species in the symbiotic culture is maintained.
The intense acidification process of strains *Streptococcus thermophilus* T1 and T4 makes these strains suitable for products in which only starter culture used is *Streptococcus thermophilus*, e.g., mozzarella. The high acidity would lead to the formation of an elastic structure and a pleasantly expressed cream flavor and aroma.

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Impacts of Acidification and High-Pressure Treatment on Sensory Characteristics of Licorice Root Sherbet

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ABSTRACT

Licorice root sherbet (LRS) is a traditional beverage exclusive to Southeastern and Eastern Anatolia of Turkey. In this study, an acidified form of LRS (ALRS) as a new licorice beverage was developed to improve its taste for a wider audience. Therefore, the major purpose of this study was to determine the effects of the acidification process on sensory characteristics of LRS developed by descriptive sensory evaluation technique (Spectrum™ method). The secondary aim was to investigate the effect of HP (High Pressure; 450 MPa-5 min) treatment as a non-thermal preservation technology on volatile compounds of the LRS and ALRS by Gas Chromatography-Olfactory (GC-O). Results showed that wet cardboard, pine/resin, grassy, rubbery, fresh potato, earthy, caramel, sweet, sour, bitter and astringent were the characteristic flavors and taste lexicons developed for LRS. After acidification process, the intensities of basic tastes (sweet, bitter and astringent) and after tastes (sweet and bitter) in LRS significantly decreased ($P<0.05$). However, the flavor terms except for pine/resin and caramel did not change significantly ($P>0.05$). In total, 42 aroma active compounds were found, and 13 of these aroma compounds were in all sherbet samples (LRS, ALRS, HP-treated LRS, and HP-treated ALRS) regarding GC-O analysis. These flavors included hexanal (grass), (Z)-4-heptanal (oxidized oil), 2-acetyl-1-pyrrole (popcorn), 1-octen-3-ol (fungus), acetophenone (powdered pepper/concrete), 2-ethyl-3,5-dimethyl pyrazine (dusty/dirty), nonanal (oxidized oil), (E, Z)-2,6-nonadienal (cucumber), (E)-2-nonenal (straw/hay), 2-isobutyl-3-methoxypyrazine (dusty/dirty), α-terpineol (plant/herb), (E,E)-2,4-nonadienal (oxidized oil) and γ-nonalactone (perfume/sugar). However, the application of high pressure did not have a large effect on most of the aroma active compounds.

Keywords: Licorice root sherbet, acidification, HP technology, descriptive sensory analysis, GC-O.

AGROFOOD
20 - 21 June 2019, Istanbul

Water Kefir

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ABSTRACT

Water kefir is a dairy-free fermented beverage made with water kefir grains which is composed of polysaccharides and microorganisms. In this study, pH, turbidity, Brix values and L*, a*, b* color parameters of water kefir prepared by different types of sugar solutions were determined during fermentation. Sugar solutions (6.5% w/v) were prepared by dissolving two different types of sugars (plain white sugar (WS) and brown sugar (BS)) in two different sources of water (boiled tap water (TW) and bottled water (BW)). The effect of adding a slice of lemon to sugar solution was also investigated. Water kefir fermentation processes were followed for 5 days and the investigated parameters were measured on each day. While the initial pH values of water kefir liquor was around 6.1 for the samples with lemon and around 3.1 for the samples without lemon, it reduced to a value between 2.7 and 3.2 for all samples at the end of fermentation. The initial values of pH (around 6.1) were larger for the samples without lemon compared to the samples with lemon (around 3.1). The relative decrease of pH values for the samples without lemon was higher and at the end of fermentation, all samples had a pH value between 2.7 and 3.2. At the end of fermentation, turbidity values of samples with lemon were similar to each other and higher than the turbidity of samples without lemon. The sugar type and lemon were found to be important factors affecting pH and turbidity whereas the source of water was not. The lowest Brix value belongs to the kefir prepared using tap water and lemon (5.6%). The sugar type was determined as an ineffective factor on the Brix. A reduction was noted in L*, a* and b* values of all samples at the end of fermentation. The a* (1.9-2.2) and b* (1.8-2.4) values of the water kefir samples prepared with tap water containing lemon were the lowest irrespective of the type of sugar.

Keywords: Brown sugar, Color, pH, Turbidity, Water kefir

AGROFOOD
20 - 21 June 2019, Istanbul

Physicochemical and Sensory Characterization of Bread Produced from Different Dough Formulations by *Kluyveromyces lactis*

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ABSTRACT

Several studies have focused on the increase in the nutritional value of bread. Fortification of dough with whey improves bread protein quality, but this may not be suitable for people with lactose intolerance. From this perspective, present study aimed to investigate the potential of *Kluyveromyces lactis* which is beneficial yeast naturally present in milk and can use lactose as carbon source owing to enzyme *β*-galactosidase, to be used as baker's yeast. For this purpose lean and enriched dough samples were prepared and used in bread making. While lean dough contained flour, water, salt and yeast, the enriched doughs were prepared by the addition of 10 % lactose, 10 % sucrose and 13.35 % whey. The prepared doughs were subjected to final fermentation for one hour following mass fermentation for two hours and baked in an oven preheated to 175°C' for 15 minutes. Results showed that the acidity, pH, protein content and color properties of breads prepared from the whey powder fortified dough with *Kluyveromyces lactis* showed similar characteristics to those produced by *Saccharomyces cerevisiae*. On the other hand, better numerical values in terms of specific volume, color, texture, weight loss and moisture content were obtained as compared to *Saccharomyces cerevisiae*. It was seen that, lactose and whey powder fortified breads produced by using *Kluyveromyces lactis* possess acceptable quality in terms of shell color, shell structure, crumb pore structure, elasticity, aroma and general acceptability criterions according to sensory evaluation results made by panelists.

Keywords: Fortified bread, *Kluyveromyces lactis*, Lactose, *Saccharomyces cerevisiae*, Whey.

Acknowledgment: This study was funded by Ordu University (TF 1412)

AGROFOOD
20 - 21 June 2019, Istanbul

Genetic diversity evaluation of amylolytic lactic acid bacteria (LAB), isolated from 20 traditional Bulgarian sourdoughs using multiplex PCR, genotyping and 16S rDNA analysis

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ABSTRACT

Using highly discriminatory multiplex PCR analysis, phylogenetically related species were successfully distinguished in combination with RAPD-PCR analysis. Thirty-five isolates from 20 traditional Bulgarian sourdoughs were identified by partial sequence analysis of the 16S rDNA. Different species of LAB - *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus* and *Weissella*, were presented as a component of microflora in the processes of dough fermentation. The objective of this work was to investigate the structure and diversity of LAB communities in traditional Bulgarian sourdoughs. 16S rDNA sequencing, multiplex PCR assay and RAPD-PCR were applied for the identification and genotyping of the LAB. The methods were used for comparative analysis of the accuracy of identification of LAB. A multiplex PCR assay was performed with the *recA* gene-based primers published by Torriani, 2001. RAPD-PCR analysis with primer P4 was performed as described by De Angelis, 2001 The PCR conditions for primer M13 were according to Siragusa, 2009. The combined RAPD profiles were subjected to cluster analysis by UPGMA. The results have revealed that *Lactobacillus brevis and Lactobacillus plantarum* dominated, while *Lactobacillus sakei and Lactobacillus curvatus* were isolated only from a limited number of samples. Multiplex PCR analysis was found to be a rapid method for identifying isolates at the species level. The fingerprints obtained by RAPD-PCR were used to identify clusters of closely related species. It is noteworthy that this analysis also confirmed the genetic similarity between *L. plantarum* and *L. brevis* and distinguished *L. sakei* from them. It was observed intra-species diversity between species.

Keywords: 16S ribosomal DNA, RAPD analysis, sourdough, lactic acid bacteria

AGROFOOD
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Comparison of endophytic colonization of Bulgarian variety of tobacco by enthomopathogenic fungi - *Beauveria bassiana* and *Beauveria brongniartii*

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ABSTRACT

In the modern breeding programs, the application and utilization of endophytic potential of microorganism is an opportunity to reduce damage from different pests and viruses on tobacco plants. In the present study, 48 plants of 56 day seedlings of oriental tobacco (Krumovgrad 58) plants treated with 2 strains 538 and 730 of the entomopathogenic fungi *Beauveria bassiana* and a strain 646 of *Beauveria brongniartii*. Two different inoculation techniques were applied by spraying the leaves and by directly placing the inoculant in the soil near the root of the plants. In order to compare the effectiveness of the colonization techniques of different tobacco tissues roots, stems and leaves, samples were taken for analysis on 7, 21 and 28 days after inoculation. Results have proven that all three strains of *Beauveria* endophyticaly colonize different tobacco tissues within 28 days after inoculation. The outcomes of the present study show the potential of *B. bassiana* and *B. brongniartii* to use for prevention and protection of tobacco plants.

Key words: *Beauveria bassiana, Beauveria brongniartii, Nicotiana tabaccum, endophyte*

1. INTRODUCTION

Tobacco production is of great importance for the Bulgarian economy. Unlike other agricultural crops, tobacco occupies relatively small areas, and the products obtained from it have a great economic significance (Dimitrov *et al*., 2005). One of the main reasons for reducing tobacco crops is damage and attack from various diseases, pests, weeds and parasites. In addition to the application of various pesticides and herbicides today, a number of effective and more environmentally friendly methods for combating tobacco diseases are sought (Dimitrov, 2003). Trends in plant breeding require a more profound understanding of the interactions between different components of the ecosystem and the use of this knowledge to applying new strategies in diseases and pests control (Vega *et al*., 2009). A key role in this relatively new approach to plant protection involves entomopathogenic fungi. Those microorganisms are usually endophytes which means that they exist inside plant tissue without causing any symptoms to the infected plant (Russo *et al*., 2015). This microorganisms can produce a series of chitinases, some of which act synergistically with proteases degrading the chitin shell of insects (Fan *et. al*., 2013). One of the best studied and commonly used endophyte fungi are *B. bassiana* and *B. brongniartii*. Different techniques for introducing these endophytes in plants and soils have being conducted. As a result colonization rate of fungi has been evaluated. Considering the fact that endophytes have an antagonistic effect on specific insects and plant pathogens, the ultimate goal is to use them as a biological control agent against certain pests (Vega *et al*., 2009).

In addition to pest and disease control, a positive role of four *B. bassiana* strains has been published to decrease Zucchini yellow mosaic virus (ZYMV) infection in pumpkins (Jaber and Salem, 2014) has also been demonstrated. *B. bassiana* and *B. brongniartii* are effective against *Paraproba pendula* larvae (Tajuddin *et al*., 2010), *Stachys affinis* and the adults of *Tenebrio molitor* (L.), *Ceratitis capitate* and *Bactrocera oleae* (Goble *et al*., 2014; Konstantopoulou and Mazomenos, 2005). *B. brongniartii* produces secondary metabolites by which it kills the larvae of *Dendrolimus. tabulaeformis* (Fan *et al*., 2013).

The aforementioned properties of *B. bassiana* and *B. brongniartii* are the reason for their artificial introduction into various economically important crop plants. The endophyte nature of insect pathogenic fungi in economically important plants such as *Vicia faba* plants (Jaber and Enkerli, 2016) has been proven. The study of C-sources utilization of isolates, collected from different regions of Bulgaria, has been studied and is compared, which included their phenotypic characterization and differentiation on their biochemical profiles. Each fungal isolate has been shown to exhibit a different specific biochemical profile, but sucrose, maltose and trehalose are assimilated to a higher degree than esculin, arabinose and dulcitol (Cafora *et al.*, 2016)

In tobacco seedlings, foliar treatment results in 100% colonization of the leaves seven days after inoculation and decreases at the 28^{th} day after inoculation. In maize, wheat and soybean, significant differences ($p < 0.001$) in endophyte colonization between different foliar, root and seed inoculation techniques (Russo *et al*., 2015) have been observed. Besides specifying the best inoculation technique for a given crop, another important aspect for maximally effective use of enthomopathogenic fungi is to determine the length of colonization of the fungus in the

tissues. In a banana, *B. bassiana* was able to colonize the plant tissues for 4 months after the tissue-cultured plants were immersed in a spore suspension (Akello *et al*., 2009).
The purpose of the study was to determine the ability of two *B. bassiana* strains (538 and 730) and a strain of *B. brongniartii* (646) to colonize different parts of the tobacco up to 28 th days after inoculation and to compare the effectiveness of colonized leafy and soil inoculation of *Nicotiana tabacco* plants.

2. MATERIALS AND METHODS

The research was conducted at the Tobacco and Tobacco Products Institute - Markovo and Agraricultural University – Plovdiv.

1. Plant material

The present study was conducted with 56 day seedlings from oriental tobacco (variety Krumovgrad 58, botanical classification: *N. tabacum*, Basma). The vegetation period, from planting to mass flowering, is 70-80 days. Each pot contains 400 g of peat mixture. All pot plants were watered with 50 mL of spring water in the pads to avoid inoculum loss in the soil-treated plants.

2. Fungal isolates

The fungal isolates were provided by prof. Slavimira Draganova Agricultural Academy – Bulgaria, Institute of Soil Science, Agrotechnologies and Plant Protection (ISSAPP). The strains 538 and 730 of *B. bassiana* (Bals.) Vuill Moniliaceae family, order *Moniliales*, class *Deuteromycetes*, were isolated from larvae of the *Coleoptera* family *Chrysomelidae spp*.
Strain 646 strain of *B. brongniartii* was isolated from *Coleoptera* species (*Hylurgops palliatus* Gyll.) of the family *Curculionidae*. Fungal cultures, starting from dry conidia, were grown on Sabouraud's dextrose agar in dark at 22 ° C.

3. Morphological evaluation of the isolates

The fungal isolates were cultured on YEA plate (0.5 g yeast extract, 10 g glucose, 20 g agar and 1000 ml distilled water) and were maintained in an incubation chamber at 25°C. After an 8-day period, the macroscopic characteristics of each colony were described through the observation of the following parameters: growth rate considering the colony diameter, aspect and color of conidial and reverse masses, and exudate production.

4. Molecular identification

DNA was isolated with HiPurA™ Fungal DNA Purification Kit (HiMedia). The control of purity and concentrations of genomic DNA was conducted by electrophoresis in an agarose gel. ITS1-5.8-ITS2 region of the nuclear ribosomal DNA was amplified with ITS1 and ITS4 universal primers (White *et al*., 1990). PCR analysis was performed in 20 µl reaction final volumes containing 1 µl (30-50 ng) of DNA and a reaction mixture containing PCR buffer, solution of dNTPs, $MgCl_2$, 1 µl of 10 µM primers and 0.25 µl of 5 U / µl of Red-Taq DNA polymerase (Canvax, Spain). The amplification reaction conditions consisted of 2 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 2 min at 72°C with a final extension of 5 min at 72°C. Expected amplicons of 500 bp are excised from the gel and purified with a Gel isolation kit (Exgene Cells SV, mini, Gene All, U.K.). PCR products were separated on 1%

agarose gel stained with SafeView (NBS Biologicals, UK) at 100 V for 50 minutes using a VWR Mini Electrophoreis System for gel visualization. Gene Ruler 1 kb plus (Bioneer, South Korea) is used as a molecular marker. The resulting sequences were analyzed with BLAST software (www.ncbi.nlm.nih.gov/BLAST/) and compared with nucleotide sequences in the gene bank database www.ncbi.nlm.nih.gov).

5. Conidial suspension

Conidia were obtained from cultures grown on YEA after incubation for 10 days at 25°C in darkness. Conidia were harvested with glass cell scrapers and placed in test tubes containing 0.01% (v/v) Tween 80 (polyoxyethylene sorbitan monolaurate) (Merck® KGaA, USA). Suspensions were vortexed for 2 min, filtered through four layers of sterile muslin, and adjusted to 1×10^8 conidia ml–1 (Gurulingappa *et al*., 2010) after cell counting by camera. Conidial viability was assessed before every experiment (Goettel and Inglis, 1997). This germination test was repeated for each stock suspension to maintain the constancy of the viability assessments. In all cases, the average viability of the conidia was over 90% for isolate 538, 95% for isolate 646, and 98% for isolate 730.

6. Inoculation techniques

Soil inoculation was performed by using a total of 36 plants with a 10 mL a conidia suspension obtained from each of examined strains with a concentration of 1×10^8 and was placed in the soil in close proximity to the plant roots (Figure 1). Control plants were free of inoculum treatment. The foliar treatment was performed with 10 mL conidia suspension with concentration of 1×10^8. Aluminum foil was also placed to prevent inoculation from the soil and the roots of the plants. Isolations of leaf-treated plants were removed 12 hours after inoculation (Figure 1).

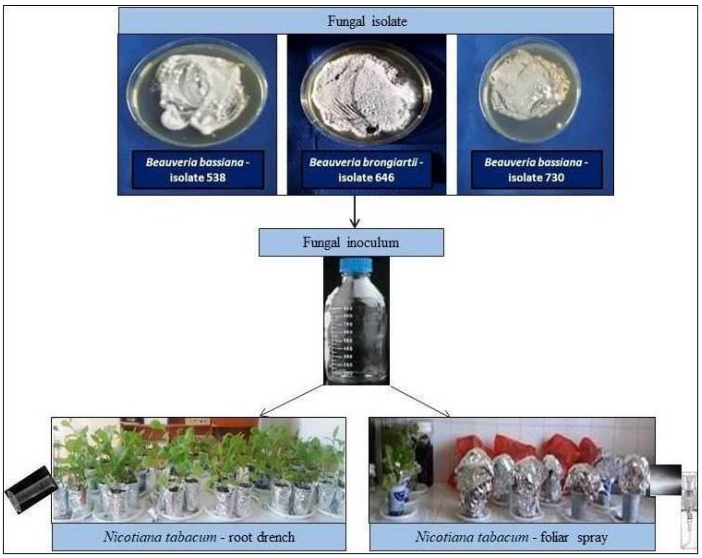

Figure 1. Different inoculation techniques –soil drench and foliar spray with strains 730, 646 and 538.

7. Endophytic activity evaluation

On the 7th, 21st and 28th days post tobacco inoculation, samples of treated plants were taken to detect the presence of *B. bassiana* and *B. brongniartii* by inoculation of leaf, stem, and root explants of YEA medium. Two whole plants (i.e., root, stem and leaf) treated with the 3

different strains and 3 control plants were taken from the soil drench tobacco. Plants were removed from the soil and washed with dH_2O. Prior to introducing the explants into an *in vitro* medium, surface sterilization of the leaves, stems and roots was performed for 3 minutes in 0.008% Tween 80 w/v, 3 min in sodium hypochlorite NaOCl solution, 1 min in 70% ethanol and three times rinsed with sterile dH_2O for 50 s. To control the quality of antiseptic inoculation an antibiotic broth was made to the last washing sterile dH_2O (used in sterilization of the explants) to control the sterilization performed and to prove that the grown colonies of the fungus in the explants placed were not due to the surface layer of the plants. Six leaf disks of approximately 1 cm^3 were incubated in culture medium with antibiotics added of concentration 0.02 g ampicillin, streptomycin and tetracycline. The presence of *Beauveria* fungi was recorded 10 day post incubation at 27 ° C in the dark.

8. Statistical processing of the results obtained

Isolation frequency (IF) and the degree of colonization (CR) of the *Beauveria* strains are calculated using the following formula:

Isolation frequency (IF) = Ni / Nt × 100

Colonization coefficient (CR) = Nc / Nt × 100

Where Ni is the number of segments from which a fungi has been isolated; Nc is the total number of segments from which mushroom fungi were isolated from a sample and Nt is the total number of segments from which strains 538 and 730 of *B. bassiana* and strain 646 of *B. brongniartii* were isolated (Sun, 2011; Russo *et al*., 2015).

3. RESULTS AND DISCUSSION

Morphological evaluation and molecular identification of the f isolates

The extensive overlap in conidia shape and dimensions among *Beauveria* species has limited their utility as key taxonomic structures (De Hoog, 1972; Parsa *et al*., 2013). Isolates could be divided into B. bassiana and B. brongniartii based on conidial dimensions; isolates with conidia longer than 3 μm were classified as B. brongniartii, isolates with shorter, spherical conidia were B. bassiana. On YEA *B. bassiana* grows slowly as a white mould with dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells, resulting in a long zig-zag extension. The fungi is characterized morphologically by globular to subglobular conidia. Although was strain 646 is determined morphologically as *Beauveria brongniartii* by its ellipsoidal conidia.

After processing the sequencing results and performing BLAST analysis with available data in GenBank the strain species identity was determined. Based on 18S gene sequences were compared with available in the database for genera *Beauveria*. Nucletide sequence of 500 bp PCR fragments were used to define genetic similarity of the isolates with Mega 7.0 program by using neighbour-joining analysis (Kumar *et al*., 2016). After analysis, similarity between *B. bassiana* and *B. brongniartii* was very high. (Figure 2). Isolate 538 showed the high percent similarity with *B. bassiana* MG642849.1 (Vu *et al*., 2019; Mukawa *et al*., 2011). 646 strain was established as *B. brongniartii*. Significant isolation-by-distance relationship was found (r = 0.33). Neighbor-joining analysis results showed that all the studied populations were divided into two discrete genetic groups with significant separation insignificant separation between

two forms of *Beauveria fungi*. The sequencing of the ITS1-5.8S-ITS2 rDNA regions also showed the insignificant separation of the two strains 730 and 538 of *B. bassiana*.

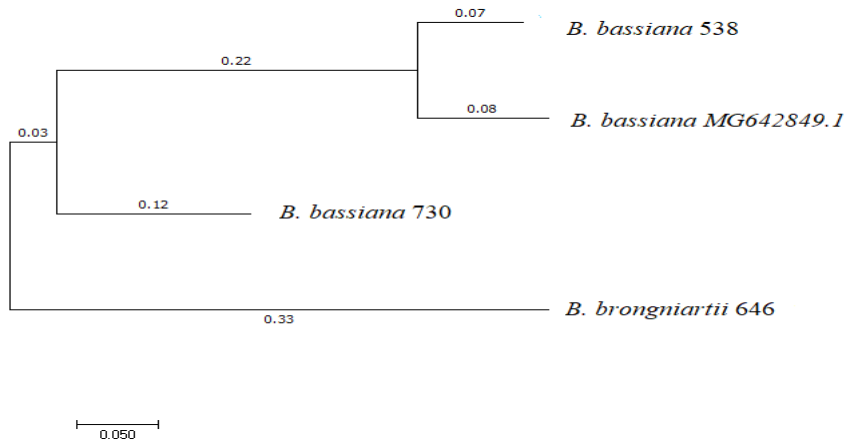

Figure 2. Phylogenetic tree derived from neighbor-joining analysis depicting members of the *Beauveria* clades and a reference species. Branch lengths are proportional to the number of nucleotide differences. The marker bar denotes relative branch lengths.

Investigation of the frequency of fungal colonization

The frequency of colonization of the three isolates of insect pathogenic fungi (538, 646 and 730) was studied in a total of 36 soil treated plants and 12 leaf spraying plants. *B. bassiana* and *B. brongniartii* have not been applied to control plants.

On the seventh day after treatment of tobacco plants with 1 x 10^8 spore suspensions of *B. bassiana* isolates 538, 730 and isolate 646 of *B. brongniartii*, using the direct inoculation technique in the soil, the highest colonization rate was recorded in *B. bassiana* strain 538 - 16.6%, followed by strain 730 - 15.3%. *B. brongniartii* isolate 646 has the lower colonization rate of tobacco roots around 7.14% (Figure 4). The endophytic colonization of leaf explants on YEA media were relatively low - from 6.6 % to 16.6 %. Significantly high colonization of steam was recorded with root drench with isolate 538 – 41.66 %. Compared to this result when leaves were plated on YEA medium the amount of the inoculum was low - 6.6

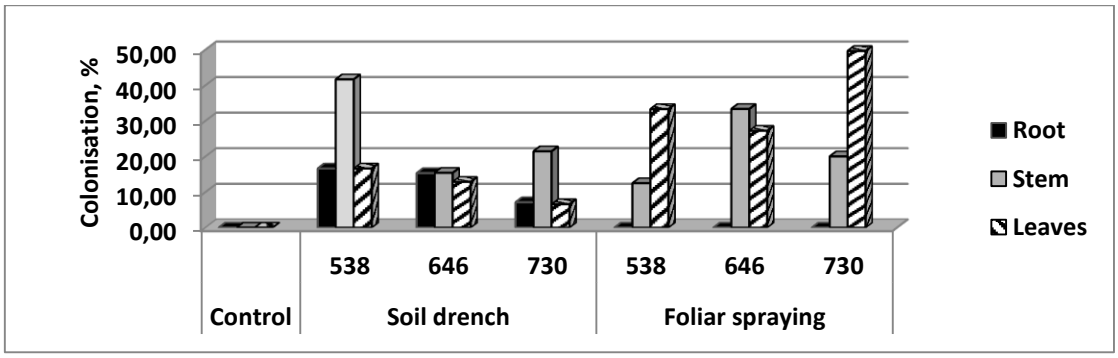

Figure 4. Recovery percentage of *Beauveria* isolates colonization of tobacco plants by soil drench and foliar spraying on 7th day post inoculation.

In the first week after foliar spraying of the aerial parts of the plants, high colonization values of the leaves were found 27,20 - 49,65% and less of the stems 12,48 % - 33,33%. As a result of

soil isolation during leaf inoculation, there is no development of the fungus in the roots of the test plants.

On day 7 after soil drench, the highest colonization rate shows *B. bassiana* isolate 538, and *B. bassiana* isolate 730 (Figure 4).

On 21 days after the spore suspension was introduced into the soil, was observed an increase in the colonization rate of the fungal isolates of the three strains in the tobacco stems and leaves (Figure 5). Similar results are also found with foliar treatment. There was a slight increase in stem and leaf colonization percentages in all strains tested. In order to better elucidate the vertical movement of mushroom endophytes, samples were taken from the lower and young upper leaves. It is particularly interesting that the old leaves have a higher colonization than the upper leaves. Results determined three times higher allocation of spores of isolate 538 and isolate 730 to the root in leaf-spaying treated plants on 21 DAT. In contract, when *B. brongniartii* isolate 646 was applied by leaf spraying, the root colonization was the two times lower compare to soil-inoculated plants.

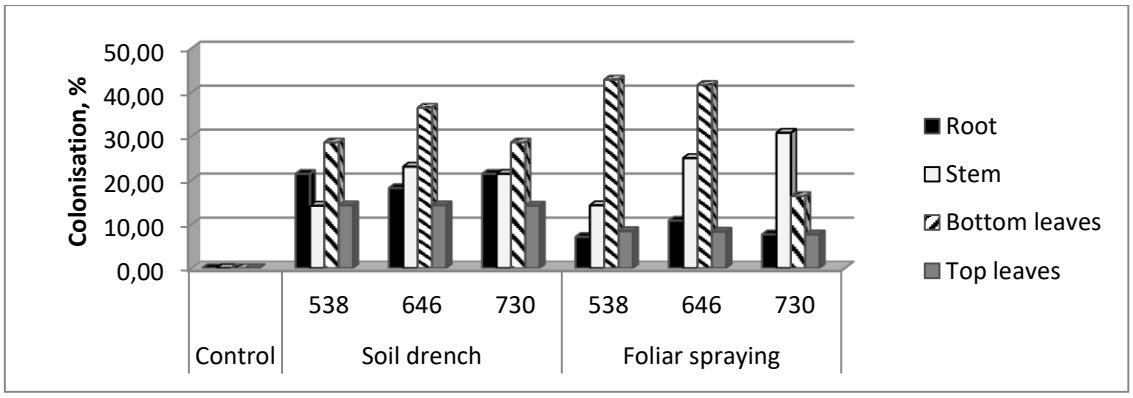

Figure 5. Recovery percentage of *Beauveria* isolates colonization of tobacco plants by soil drench and foliar spraying method on 21st day post inoculation.

On the 28th day after treatment, colonization was reduced in both inoculum delivery techniques as compared to the 21-day colonization rate (Figure 6). In soil treatment, *B. brongniartii* isolate 646 has the highest value. Foliar spraying of the plants with the highest activity with *B. bassiana* isolate 538. In leaf treatment, the colonization factor is higher on the lower leaf and decreases in the study of the upper young leaves.

Control plants are pure from colonization by *Beauveria bassiana* and *Beauveria brongniartii*.

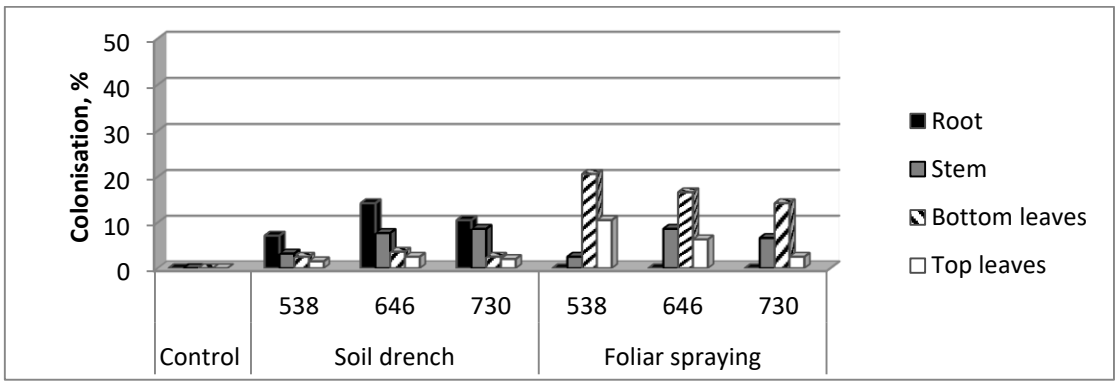

Figure 6. Recovery percentage of *Beauveria* isolates colonization of tobacco plants by soil drech and foliar spraying on 28th day post inoculation.

Calculation of a colonization factor (CR)

Figure 7 presents the results of calculating a colonization factor (CR). On day 7 after soil introduction of inoculum from *B. bassiana* and *B. brongniartii*, isolate 538 was the most effective, followed by 646, and lowest value was determined at isolate 730. In foliar treatment, highest rates of colonization were recorded when isolate 730 was applied and lowest when isolate 538 was used. *B. brongniartii* 646 is equally effective in soil drench application and foliar spraying inoculation till 28 day after treatment.

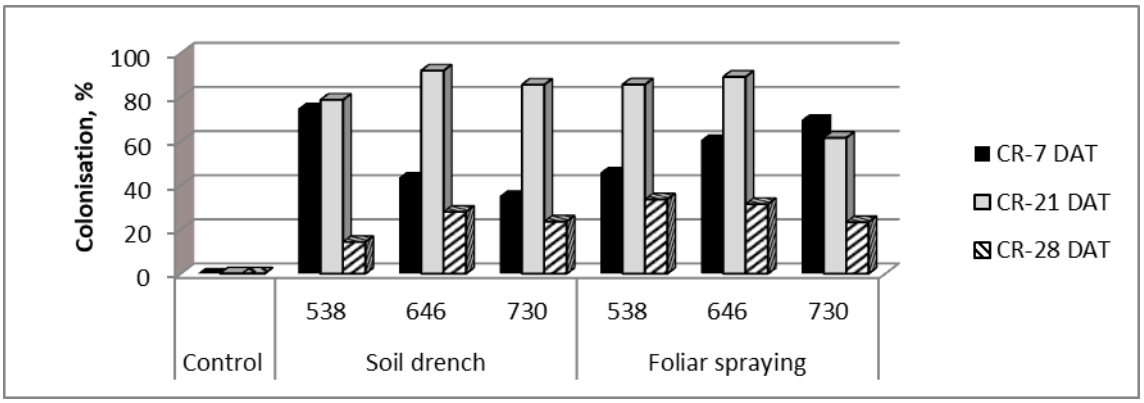

Figure 7. Recovery percentage of CR of *Beauveria* fungi by different inoculation technique at 7, 21 and 28 days after treatment.

At the second and fourth weeks after treatment, the three isolates showed similar endophytic activity in both inoculum modes. On day 21, *B. brongniartii* 646 has with the highest colonization coefficient of 92.01% for soil treatment and 89.16% for leaf spraying. Analogous to the data in Figure 7, at 28 days after the application of the fungal isolates, a significant reduction in colonization of tobacco plants was observed, the lowest being in soil treatment with strain 538 was 14.32%.

The present study found that *B. bassiana* and *B. brongniartii* successfully colonized tobacco plants. This result is similar to previous studies conducted with tobacco, corn soybean (Russo *et al*., 2015), opium (Quesada-Moraga *et al*., 2006) and tomatoes (Ownley *et al*., 2004). When different inoculation techniques were applied to several plants, alterations were observed and the date of the highest rate of colonization was recorded. In wheat, the highest percentage of colonization was achieved by leaf treatment on 14 DAT, and in root immersion and seed inoculation, the highest colonization was obtained on the seven day. In maize, the highest rate of colonization was achieved by foliar treatment on days 7 and 14 DAT (Russo *et al*., 2015).

According to Russo *et al*., (2015) data from the method of inoculation of tobacco plants with *B. bassiana*, the highest percentage of colonization was achieved by foliar treatment and the highest rate of colonization was recorded on 7 DAT. In contrast to the data obtained by Russo, current experiment shows relatively uniform rates of colonization with both applied techniques, with the exception that the leaf-treated plants with *Beauveria* was established root infection only on 21 day. The highest activity was recorded on the 7th day after inoculation, and in the present study, the highest colonization rates were 21 days after inoculation. Possible explanation is the activity of those fungi isolates, the type of inoculation and climatic conditions in the country. In support of this explanation is the result observed in two of the applied strains of *B. bassiana*. As a result of soil drench application of strain 538 on tobacco Krumovgrad 58

the percentage of the colonization rates were with similar values on 7th and 21st day. In leaf treated plants with strain 730 again the highest colonization rate was recorded on 7 DAT. Only in strain 646, distinguished by sequencing analysis as *B. brongniartii*, the highest colonization rate was found at 21st day for both inoculation methods. The results obtained by Russo *et al*., (2015) and current results demonstrated the tendency to reduce the colonization rate after the 21st day of treatment. In current experiment the endophyte activity of the fungus on day 28 was greatly reduced.
Most studies in other crops tend to reduce the fungal colonization of the various tissues of the plant over time (Greenfield *et al*., 2016), although vertical transmission of *B. bassiana* to the generations of endophyte colonized mother plants (Quesada-Moraga *et al*., 2014).

4. CONCLUSIONS

All three tested strains of *Beauveria* (646, 730 and 538) exhibit endophyte nature in the tobacco. Present study observed differences in efficacy among the two inoculation techniques over time. Both applied techniques with the three strains under examination have been found to colonize the different parts of the plants. When root immersion was used, the highest percentage of colonization of tobacco was detected at 21st day for the all the *Beauveria* strains applied. Leaf treatment with fungal strains 538 confirmed the highest percentage of colonization of tobacco on 7 day till 21 day after colonization. For strain 730 the highest percentage was recorded on 7 day post innoculation. There was no difference in colonization efficiency when applying the two different inoculation techniques. The strains of *B. bassiana* and *B. brongniartii* have been shown to be preserved in the different parts of the tobacco until 28 DAT, but the percentage of the inoculum decreases on the 28 DAT. Foliar-spray treated plants also showed roots colonization by the entomogenous fungi and it was recorded only on 21 DAT. The percentage of colonization of the leaves and stems decreases again on 28 DAT. For both techniques, the colonization rate of the lower leaves was higher than the percentage of the colonization on the upper leaves for all three strains tested. Plant colonization does not affect the normal physiological development of tobacco.

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AGROFOOD
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Effects of Probiotic Administration on *Helicobacter pylori* Eradication

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ABSTRACT

Helicobacter pylori is a Gram negative, microaerophilic, flagellated pathogenic bacteria usually responsible for dyspepsia, gastritis and gastric ulcers. It is also related to the gastric cancer, according to the International Agency for Research on Cancer (IARC) *H. pylori* is "group 1 carcinogen (Carcinogenic to humans)" and it is estimated that 60% of gastric cancer result from *H. pylori* infection. Additionally, more than half of the world population host *H. pylori* in their gastric epithelial cells. Currently, the treatment of infection require several antibiotics including tinidazole, metronidazole, clarithromycin and amoxicillin plus a proton pump inhibitor (PPI). Antibiotic treatment leads to some adverse effects such as nausea, vomiting, diarrhea, dysbiosis of intestinal microbiota as well as increase in antibiotic resistance. In addition to antibiotic therapy probiotic supplementation appear to have an antagonistic activity against *H. pylori* colonization also reduce antibiotic related adverse effects. These beneficial properties of probiotics are originated from their ability to produce antimicrobial compounds such as organic acids, bacteriocins and peroxides besides, their role in immunomodulation. Based on these features some probiotic strains of *Bifidobacterium*, *Lactobacillus*, *Bacillus* and *Saccharomyces* can prevent or reduce *H. pylori* colonization. Antagonistic activity of probiotics against *H. pylori* is strain specific and many researches show that *L. casei, L. johnsonii, L. plantarum, L. acidophilus, L. salivarius, L. reuteri, L. rhamnosus, B. longum, B. infantis,* and *S. boulardii* have promising results. In this study, we reviewed the available literature for the effect of probiotics on *H. pylori* eradication.

Keywords: Eradication, gastric ulcers, *Helicobacter pylori*, probiotics

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The Effect of Organic Fertilizer (Compost) Doses on Physical Properties of Pomegranate Fruit

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ABSTRACT

This work; It was carried out at Koruklu Talat Demirören Research Station between the years 2012-2016 under Harran Plain conditions. The aim of the study was to determine the effects of the composting of the pomegranate on the physical properties of pomegranate fruit. The experiment was designed to have 3 replications according to the randomized block design. Organic (compost) fertilizer (0 g N-tree-1, 150 g N-tree-1, 300 g N-tree-1, 450 g N-tree-1, 600 g N-tree-1) were applied in the experiment.

In order to determine the effects of organic fertilizer application on the average pomegranate fruit weight, fruit width and length and shell thickness, some pomological measurements were made on pomegranate fruit. According to this;

According to the subjects average fruit weight ranged between 351-381 g. The highest pomegranate weight on a three-year average was found to be 377 g. The width of the fruit ranged from 82.5 to 95.9 mm. The three-year average highest pomegranate width was measured as 89.5 mm. Fruit length values ranged from 74.2 to 84.9 mm. The highest pomegranate height was found to be 81.9 mm on a three-year average. The thickness of the crust ranged from 2,8 to 3,7 mm, while it was measured as 3,4 mm on average.

Keywords: Compost, Harran Plain, Organic Fertilizer, Pomegranate.

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Evaluation of Quality and Emulsion Stability of A Fat-Reduced Beef Burger Prepared With an Olive Oil Oleogel

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ABSTRACT

In this work, fat-reduced functional beef burgers with olive oil oleogel based-emulsion (OOE) was produced and then, changes in quality characteristics of vacuum-packed fat-reduced functional beef burgers which was stored at 4 ℃ during 7 days were determined. Burgers were produced with different replacement levels (33, 66, 100%) of OOE by animal fat. In addition, peapod powder at the rate of 1% was added into OOE incorporated groups to improve textural, sensorial and cooking characteristics. A 51% and 35% reduction of the total fat and energy content with an increment of the unsaturated fatty acids (65%), and a significant decrease in saturated fat (44%) were achieved in the product with the highest level of substitution. OOE treatments showed significantly lower TBARS compared to control during the storage ($P<0.05$). The control group had lower cooking yields values and higher fat content ($P<0.05$). The replacement of animal fat with OOE in functional beef burger resulted in lower hardness, chewiness and springiness values during the storage ($P<0.05$). The control and OOE incorporation group contain 33% OOE and 1% peapod powder had higher acceptability scores at the end of the storage ($P<0.05$).

Keywords: oleogel, olive oil, emulsion, fat-reduced functional burger, shelf-life

AGROFOOD
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Kinetic improvement of bioactive compounds extraction from red grape (*Vitis vinifera* Moldova*)* pomace by ultrasonic treatment

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Abstract

Grape pomace from a red grape variety (*Vitis vinifera* Moldova) cultivated in the north eastern region of Romania has been studied as a valuable source for the extraction of total monomeric anthocyanin (TMA) and total phenolic content (TPC). The effects of extraction solvent (2-propanol and methanol), solvent concentration, temperature, and extraction time, as well as the influence of the frequency selected in the ultrasonic treatment were investigated. Response surface methodology was implemented via a Box–Behnken design to optimize the extraction. The optimal parameters for achieving the highest concentration of TMA (7.727 mg/g) were a concentration of 51% of 2-propanol, 27.5 kHz ultrasonic frequency, a temperature of 61.7 °C and 32.6 min extraction time. For the purpose of reaching the maximum extraction of TPC (62.487 mg/g) the optimal conditions were a concentration of 50% of 2-propanol, 24.7 kHz ultrasonic frequency, temperature of 50 °C, and 29.6 min extraction time.

Keywords: grape pomace, extraction, anthocyanin, phenolic content, ultrasound

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Combined enzyme-assisted extraction – ultrasound-assisted extraction of pectin from *Malus domestica* 'Fălticeni' apple pomace

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ABSTRACT

Pectins are structural heteropolysaccharides generally extracted from citrus peel or apple pomace, that have diverse food applications such as thickening, gelling and stabilizing agents, and fat replacers. Due to the rising demand for pectin use in the food industry, and also the expansion of its use to numerous non-food applications, pectin market finds itself in a continuous grow. As a result, recent studies focused on the use of different side streams as new sources for obtaining pectins with diverse compositions and functional properties. This work subscribes to this trend of research in this field, as its purpose was the capitalizations of *Malus domestica* 'Fălticeni' apple pomace as pectin source. A combined enzyme-assisted extraction – ultrasound assisted extraction process was applied to obtain pectin from this plant source. First, dried apple pomace with particle sizes of 125-200 µm was mixed with distilled water acidified to a pH of 4.5 in a solid-to-liquid ratio of 1:15 (w/v), then cellulase was added to a mixture in a dose of 7.5 mg cellulase/g apple pomace, and the extraction was conducted at a temperature of 47 °C for 20 h under constant shaking (200 rpm). Enzyme-assisted extraction was followed by an ultrasound extraction at different amplitudes (20, 60, and 100%) and extraction time (10, 20, and 30 min). Box–Behnken response surface methodology was employed for the optimization of process parameters in order to simultaneously achieve the highest pectin yield and the best physicochemical properties of the product.

Keywords: apple, enzyme, extraction, pectin, ultrasound.

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Some Important Health Benefits of D-Allulose (D-Psicose)

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ABSTRACT

D-allulose, also known as D-psicose has very important beneficial effects on human health. It has a caloric value of 0.39 kcal, which is roughly equivalent to 10% of the caloric value of sucrose and suppresses the elevation of after-meal blood glucose levels. It also helps to treat and prevent diabetes, inhibits the storage of abdominal fat, and even has anticavity properties. Toxicity tests have shown that D-allulose is safe as a food material. D-allulose was given Generally Recognized as Safe status by the US Food and Drug Administration in August 2011 (GRN No. 400). D-Allulose also inhibits hepatic fatty acid synthase by controlling adipose tissue deposition. It has been reported to exhibit a variety of interesting physiological activities in humans, including anti-diabetic, anti-obesity, and anti-arteriosclerosis activities. D-psicose is poorly absorbed in the digestive tract which might make it useful as an aid to weight reduction. It can also reduce fever. Neither any abnormal effects nor clinical problems caused by the continuous ingestion of D-allulose were found.

Keywords: Allulose, Psicose, Health, Natural, Rare sugar

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Texture Profile Analysis of Quince Waste Emulsion

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ABSTRACT

In this study quince fruit waste (QW) after juice removal used in meat emulsion for evaluation purposes. Quince waste was obtained on the laboratory by squeezing fruit and removing its juice. Removal waste was dried in 40°C and grinded. For the emulsion, oil in water emulsions formed with quince waste of 4 different concentration (0.5%, %1, %2 and %3) and chicken breast. TPA analysis applied to the emulsion samples before and after the heat treatment (80°C, 30 min.) Quince waste adding had positive effect on hardness value of emulsion. Generally adding guince waste on emulsion is increased adhesiveness value, but had no effect on cohesiveness value. Gummines value increased for row emulsions, but decreased for cooked emulsion when quince waste added on it.

Keywords: Food waste, quince, emulsion, TPA analyse.

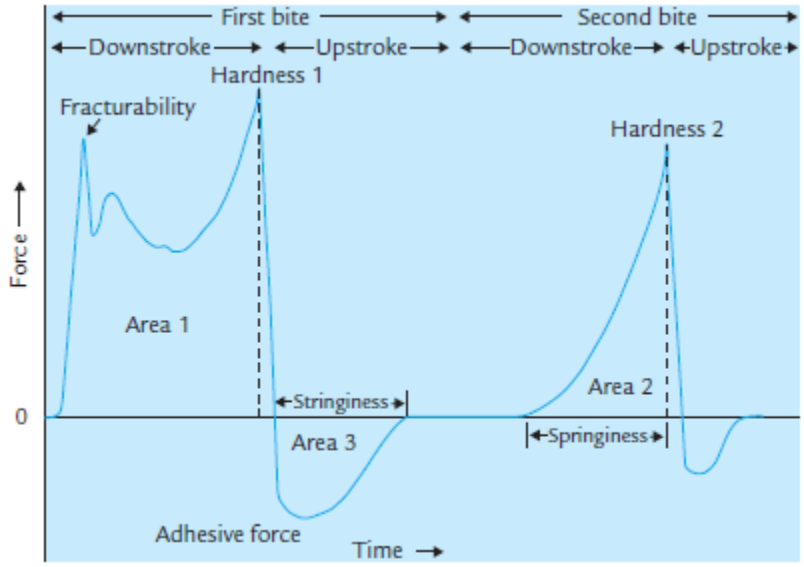
5. INTRODUCTION

Food wastes cause adverse environmental problems, not only in terms of food scarcity, but also environmental impacts that prevent food availability, particularly inefficient use of water and land in some parts of the world with limited food resources and unnecessary release of greenhouse gases (Salihoglu et al., 2017). In food factories, large amounts of food waste are produced as a result of product processing, and most of these wastes are disposed of or converted to low-value products such as animal feed using simple technologies.

Turkey is one of the prominent quince growers (Kaya et al., 2007). It's difficult to consume quince fruit as fresh and juicy. Also the tendency of the quince turning to brown is a limiting factor in the long-term storage of this fruit (Yin et al, 2017). So this fruit generally consumed as quince jam and quince juice and its waste used as animal feed. Since the fruit and vegetable wastes contains many precious bioactive compounds and disposal of them causes many environmental problems, it's so essential to reuse this food wastes in production.

Texture is defined as the properties of a food product covering all the rheological and structural properties that can be perceived by mechanical, tactile, and possibly visual and auditory receptors (Lundberg vd, 2014).

Figure 1: A generalized texture profile analysis (TPA) curve (Bourne, 2002)



The parameters obtained according to the general TPA graph are as follows;
Hardness: peak of initial compression.
Cohesiveness: the ratio of the positive force areas under the first and second compressions (Area2/Area1).
Adhesiveness: the negative force area of the first bite (Area3) represented the work necessary to pull the compressing plunger away from the sample.
Springiness: the distance that the food recovered its height during the time that elapsed between the end of the first bite and the start of the second bite.
Gumminess is defined as the product of hardness X cohesiveness. It is the energy required to disintegrate a semisolid food to a state of readiness for swallowing.

6. MATERIAL AND METHODS

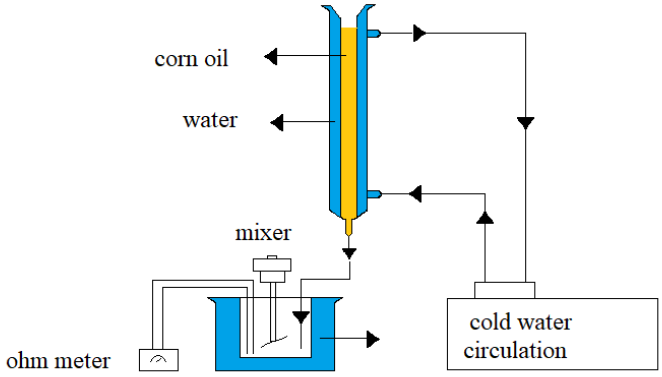
6.1. Material

The quince fruit was obtained in a local market and the chicken breast used in emulsion was also purchased in local market in Turkey. The fresh fruit was squeezed in a kitchen type fruit juicer and then fruit juice removed. The fruit waste was kept at in a water bath for 5 min at 80C in order to avoid browning and then washed several times under tap water. Drained quince waste was dried in 80 C for 20 hours, then grinded and saved in a dark color bottle.

2.9 Methods

The emulsion forming: A suspension formed by homogenizing 100 mL 0.4 M NaCl and 25 g chicken in a homogenizator at 13000 rpm. For treatment samples %0.5, %1, %2 and %3 QW added on suspension. In order to obtain emulsion; 12.5 g suspension and 37.5 g NaCl solution homogenized with 50 mL corn oil. During the homogenization, corn oil added to emulsion till the emulsion breaking point. Figure 2 represents emulsion producing system.

Figure 2: Emulsion system used in study



TPA analyse: TPA analysis applied on both row and cooked emulsions. Approximately 40 g of the prepared emulsions were placed in a glass jar. The textural properties of the samples were stretched to 50% of the original sample height using a 36 mm diameter cylindrical probe and using a TPA device (TA.XT Plus, Texture Technologies Corp., UK). In the analysis where the two compaction intervals were 5 s, the force-time deformation curves were obtained with a 30 kg load cell and 5 g trigger force at a speed of 1 mm/s. For the calculation of TPA values forces against time were used (Saricaoglu and Turhan, 2013).

7. RESULTS AND DISCUSSION

Analyse results of raw QW emulsions was shown on table 1. For the hardness of raw emulsions, QW emulsions have higher hardness value than the control sample and the highest hardness value was determined in 247.2 g with 1% QW added emulsion. Hardness decreases with moisture and fat contetnt and increases with pectin content (Singh et al., 2013). Quince fruit contain high amount of pectin (Fiorentino et al., 2008) and the addition of QW decreased moisture and fat ratio of emulsions, so hardness is increased. The spiringiness value of samples changed between 0.94-0.96 mm. The sample has the highest springiness value is 0.5% QW added emulsion which has the lowest concentration. The addition of QW more than this ratio had no effect on the springiness value and these emulsions were not statistically different with the control emulsion. Adding QW to the raw emulsions causes decrease in adhesiveness of the emulsions and statistically there is no difference in QW added emulsions. Since the adhesiveness indicates minus area and it means the work needed for removing of sample and plunger (Uslu et al., 2010) adding QW is increased required force. For the cohesion parameters of raw emulsions; the addition of QW to the raw emulsion did not affect the cohesiveness value and there was no significant difference between the treatment samples and the control emulsion binding values ($p > 0.05$). The gumminess value of treatment emulsions changed between 108.5-137.3 g and emulsions positively affected by adding QW. All the treatment samples have higher gumminess than control emulsion. Gummines is also positively correlated with pectin content (Singh et al., 2013) so adding QW increased gumminess value of samples.

Table 1: Texture profile of row quince waste emulsion samples

	ROW EMULSION				
	Control	%0.5	%1	%2	%3
HARDNESS (G)	163,8±9,15^{c}	215,3±3,4^{ab}	247,2±28,9^{a}	219,3±18,5^{ab}	189,2±29^{bc}
SPRINGINESS (MM)	0,94±0,01^{b}	0,96±0,01^{a}	0,94±0,01^{b}	0,94±0,01^{b}	0,95±0,01^{b}

ADHESIVENESS (G.S)	-55,9±3.7[a]	-80,1±11,1[b]	-95,1±12,4[b]	-75,1±12,7[ab]	-78,2±14,8[b]
COHESIVENESS (DIAMENSIONLESS)	0,56±0,01[a]	0,55±0.01[a]	0,55±0.01[a]	0,56±0,00[a]	0,55±0,01[a]
GUMMINESS (G)	93,5±4,2[c]	116,4±4.5[ab]	137,3±16,5[a]	122,8±10,1[ab]	108,5±19,3[bc]

Table 2 indicates the analyse results of cooked QW emulsions. Addition of 0.5% QW to the emulsion is resulted in a decrease in hardness value for cooked emulsions. Addition of 1%, 2%, 3% QW is satistically not affected the hardness value. For cooked emulsions, the highest adhesiveness value was determined with -213.3 g.s in 2% QW sample. Adhesion values of 0.5% and 1% samples were not statistically different from the control sample but adding 2% and 3%QW to the emulsion causes incease in adhesiveness for cooked emulsions. Adding QW has no effect on cohesiveness value. Gumminess of cooked emulsions was changed between 183.1 and 261.9 g. Although adding QW numerically reduced gummines value of cooked emulsions, only 0.5% QW emulsion decreased gumminess statistically.

Table 2: Texture profile of cooked quince waste emulsion samples

	COOKED EMULSION				
	Control	%0.5	%1	%2	%3
HARDNESS (G)	480.3±41,9[a]	354,5±27,29[b]	540,9±27,57 [a]	495,2±50,93[a]	473,7±18,9 [a]
SPRINGINESS (MM)	0,95±0,01[b]	0,95±0,01[b]	0,90±0,05[b]	1,01±0,01[a]	0,93±0,01[b]
ADHESIVENESS (G.S)	-107,4±20,4[a]	-111,7±11,4[a]	-137,2±33,1[ab]	-213,3±4,3[c]	-161,3±10,2[b]
COHESIVENESS (DIAMENSIONLESS)	0,54±0,01[a]	0,51±0,01[a]	0,48±0,07[a]	0,51±0,02[a]	0,48±0,00[a]
GUMMINESS (G)	261,9±27,5[a]	183,1±12,1[b]	235,5±31,3[a]	252,4±37,3[a]	223,3±9,3[ab]

Generally, hardness, adhesiveness and gumminess values of cooked emulsions are higher than raw emulsions, but cohesiveness value is lower. This differences caused by the effects of heat treatment. Heat treatment causes the degradation of pectic polysaccharides that provide cell adhesion in the cell wall (Tosh and Yada, 2010). It also causes decrease in moisture and fat content. For these reasons cooked emulsions have different TPA profile.

8. CONCLUSION

Quince production waste is generally discharced or evaluated as animal feed. In this study its used in meat emulsion after obtaining quince waste. Emulsion texture profile is generally positively affected

From quince waste. Adding quince waste on meat emulsion caused increase in hardness and adhesiveness values of emulsions. Only cohesiveness value not effected by the addition of

quince waste. Quince waste can be used such type of emulsions, it promotes texture profile and also it provides cheap additive and it can be used as a fat replacer.

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Dietary Fiber Composition of Tomato, Quince and Grapefruit Waste

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ABSTRACT

The aim of this study is determine dietary fiber content of some food wastes. Large quantities of plant origin wastes are obtained with farming, processing, distribution and sales. These wastes contain bioactive compounds such as polyphenols and essential oils, as well as dietary fibers that provide economic benefits for the food, cosmetic and pharmaceutical industries. Fiber-rich by-products are very valuable products for food operators, especially because they are rich in bioactive compounds and dietary fiber, especially because consumers prefer natural supplements. Dietary fibers are classified as soluble or insoluble depending on whether they form a solution with water. Soluble dietary fibers include pectic substances, gums, mucilages and certain hemicelluloses, while cellulose, other types of hemicelluloses and lignin are included in the insoluble fraction. Quince and grapefruit wastes were obtained in the laboratory by squeezing quince fruit and removing their juice. Tomato waste was purcashed in a tomato paste factory as a residue of tomato paste manufacturing. Total dietary fiber (TDF) and insoluble dietary fiber (IDF) contents of samples were determined by following AOAC-991.43 (AOAC, 2000) and AACC-32-07 (AACC, 2004) standard methods. According to the results of the dietary fiber analysis, grapefruit has the highest percentage of the TDF with 90.34%. IDF ratio of this sample was determined as 83.31%. TDF amount of quince waste was found to be 87.90% and IDF was 82.68%. While tomato pulp has the lowest TDF ratio (63.76%) compared to other samples, also it is found that tomato waste has the lowest IDF ratio (57.66%).

Keywords: Dietary fiber, food waste, grapefruit, quince, tomato

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Energy Requirements and Sustainability in Food Industry

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ABSTRACT

Food and energy terms are crucial for human survival. The abundance of both is affected by the human population and sector demands. Rapid population growth increases the need for food, especially in developing countries. Thus, the food industry needs more energy to produce more food products. According to the energy consumption reports, generally, industry is the second largest energy consuming sector (%28 of total energy consumption) after housing (%30 of total energy consumption). Feasibility studies in Turkey showed that the food industry has nearly %10 of the energy consumption of the industrial consumption. Furthermore, 20% of the energy consumed in the food industry can be reduced. Until the last 20 years, production quality and competitiveness were the two most important objectives in the food manufacturers. Contrary to this idea, energy efficiency and sustainability terms become an important consideration in food production processes today. Thus, improving product quality and competitiveness alone are not sufficient to assure the success of a new technology. Nowadays, scientists, manufacturers and governments are focusing on the energy requirements of the food industry and also the sustainability of the production steps. Food industry is heavily dependent on the fossil fuels and it consist lots of energy needed processes such as thermal processes. Drying, pasteurization, sterilization, blanching and other food processing processes consumes too much energy. In the last decade, there are lots of studies on the hybrid drying and other novel technologies to reduce energy requirements of these processes. To illustrate, the bread making process requires 6.12 MJ/kg energy while 5.33 MJ/kg of energy is required for biscuit production process. For baking, meat, cheese and other food manufacturing processes, the highest energy consuming steps are generally drying, roasting, extraction processes etc. Specific Energy Consumption (SEC (MJ/kg)), Specific Moisture Extraction Rate (SMER (kg/kWh)) and Moisture Extraction Rate (MER (kg/h)) values are used to evaluate the energy consumption of these processes especially drying processes in food industry. The scope of the study is to compile the energy requirements of the main food industry processes, to evaluate the energy requirement data and to gain a better understanding of the applicability of low energy consumption methods in food processes by comparing energy consumption of the processes.

Keywords: energy requirement, sustainability, food industry, energy saving technologies, drying.

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