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Address
Macedonian Veterinary Review, Lazar Pop Trajkov 5/7, 1000 Skopje, Republic of Macedonia
Tel: ++389 2 3240 700; Fax: ++ 389 2 3114 619; e-mail: macvetrev@fvm.ukim.edu.mk; URL: www.macvetrev.mk

CONTENT

the range from 5 to 250 µg/kg. The LODs and LOQs were estimated at concentration levels with S/N ratio of at least 3:1 and 10:1, respectively. Accuracy was tested spiking blank bovine liver at two concentration levels, 10 and 50 µg/kg (n=5).

Results: The linearity determination revealed regression coefficients values higher than 0.99 for all pesticides tested.The estimated LODs and LOQs were lower than 10 µg/kg, with exception of diazinone, for which, LOQ value of 12.4 µg/kg was obtained. However all values estimated are lower than the established maximum residue levels for liver. Recoveries obtained were in the range 71.9 -115.9 % and 70.2 – 98.8 %, for spiked levels of 10 µg/kg and 50 µg/kg, respectively. The determined precision for all pesticides within the method scope was lower than 20%. Validation parameters estimated are in line with the respective legislative requirements, proving that the method is suitable for performing official pesticides control.

Conclusion: It was presented that QuChERS sample preparation, with some modifications, may be successfully applied for pesticides determination in liver samples with UHPLC-Tandem mass spectrometry. In accordance with the legislation requirements, the method proposed may be used for pesticide analysis within the Monitoring program for residues in animal products.

P20

Aflatoxins occurrence in peanuts and products containing peanuts

Katerina Davcheva*,
Biljana Stojanovska-Dimzoska,
Basak Kucukcakan, Zehra Hajrulai-Musliu,
Elizabeta Dimitrieska-Stojkovic, Risto Uzunov,
Aleksandra Miza, Dean Jankuloski

Food Institute, Faculty of Veterinary Medicine,
Ss. Cyril and Methodius University
Lazar Pop Trajkov 5-7, Skopje, Macedonia

Introduction: Aflatoxins are highly toxic, cancer causing metabolites of certain strains of the fungi *Aspergillus flavus*and *A. parasiticus*, that cause immune-system suppression, growth retardation, liver disease and death in both humans and domestic animals throughout the world. They are a group of closely related compounds with small differences in chemical composition. Aflatoxins of concern are designated B1, B2, G1,G2 and are usually found together in various foods and feeds in various proportions, of which aflatoxin B1 is considered the most prevalent and the most potent form. In accordance to EU Commission Regulation (165/2010) groundnuts (peanuts) intended for direct human consumption or use as an ingredient in foodstuffs, should comprise maximum concentration of 2.0 µg/kg of AFB1 i.e. 4.0 µg/kg of sum of B1, B2, G1 and G2.

Material and methods: HPLC method with fluorescence detection was used for investigation of quantitative determination of aflatoxins in peanuts and products containing peanuts, after their clean-up on immunoaffinity columns. This method is in accordance with ISO 16050 and AOAC Official Method 991.31. In duration of 21 consecutive months (2013, October – 2015, June), 38 samples of peanuts and products containing peanuts were analyzed. Among them: 19 samples raw peanuts, 7 samples roasted peanuts, 6 samples salty sticks with peanut butter, 4 samples peanut flips, 1 sample peanut paste and 1 sample peanut skins. Limit of detection (LOD) is 0.005 µg/kg.

Results: Aflatoxins concentration was below LOD in 26 (68.42%) samples: in 21 out of 26 peanuts samples and in 5 out of 12 products containing peanuts.Aflatoxins were detected in 12 (31.58%) samples, in a concentration range between 3.21 µg/kg and 61.42 µg/kg. Aflatoxins concentration detected was: 3.21 µg/kg, 10.6 µg/kg, 12.8 µg/kg and 14.2 µg/kg in 4 samples raw peanuts; 2.12µg/kg in a roasted peanuts sample; 61.42 µg/kg in a peanut paste; 4.98 µg/kg and 5.7µg/kg in 2 samples salty sticks with peanut butter; 16.83 µg/kg, 21.11 µg/kg, 22.68 µg/kg and 37.2 µg/kg in 4 samples peanut flips. Ten of the samples exceeded maximum limit of aflatoxins concentration i.e. 26.31% of total investigated samples.

Conclusion: Relative high percent of aflatoxin occurrence in investigated samples points out that further examinations on this topic are necessary. Thus it is highly recommended to increase the monitoring of aflatoxins in commodities.Beside this, proper agricultural and agronomic practices have to be imposed to reduce susceptibility and exposure of commodities to fungal invasion during pre-harvest, storage and processing periods.

P21

Comparison of extraction methods in HPLC-FD analysis of ochratoxin-A in swine kidney

Basak Kucukcakan*, Zehra Hajrulai-Musliu,
Biljana Stojanovska-Dimzoska,
Elizabeta Dimitrieska-Stojkovic,
Risto Uzunov, Katerina Davceva

Food Institute, Faculty of Veterinary Medicine, University
Ss "Cyril and Methodius" Lazar Pop Trajkov 5-7, Skopje,
R. Macedonia

Introduction: OchratoxinA (OTA) is amycotoxin which is produced by fungi *Asperillus* and *Penicillium* species. OTA is found in foodcommoditiesand several animal products. The toxin is known as etiologic agent of Balkan Endemic Nephropathy disease. OTA was classified in Group 2B as a possible carcinogen in humans by The International Agency for Research on Cancer. The aim of this study was to make comparison between three extraction methods (immune-affinity column extraction (IAC), liquid-liquid extraction (LLE) and solid phase

extraction (SPE), prior to HPLC-FD analysis of swine kidney.

Material and methods: The study was performed using OTA free swine kidney samples. IAC clean-up procedure was done according to study of Jorgensen K and Petersen A (2002) and LLE and SPE analysis were done according to the work ofMonaci et al. (2004). HPLC-FD method was validated in the respect of EU Decision (2002/657/EC) in our laboratory. The validation parameters were: specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision (repeatability and reproducibility).

Results: The linearity of the method at concentration range of 0.1 to 10 ng/ml was satisfactory with high value for coefficient of correlation (r^2>0.9999). The chromatogram obtained when IAC clean-up procedure was employed, was very clean, without matrix effect and retention time was between 5-6 minutes. Accuracy was evaluated by recovery levels which were 77.6 and 103%. Repeability (RSD_r) levels were 8.8 and 9.4%; reproducibility (RSD_R) levels were 3.24 and 8.8% for the concentrations at 1 and 5 ppb. LLE method with small amount of sample (2.5g) and extraction solvent (5ml) was resulted with low recovery (average 26%). LLE with high amount of sample (20g) and extraction solvent (100ml) gave the high recovery level as average 69.5-112%. LLE was resulted in a poor quality of clean chromatogram due to matrix effect. There was no acceptable recovery levels (0.9%) of SPE method.

Conclusion: The validation of the HPLC-FD method with previous IAC extraction procedure was achieved complying with the EU Decision (2002/657/EC). LLE technique was not adequate to provide pure chromatograms in compare to IAC clean-up procedure, but it was also efficient, with satisfactory validation parameters, rapid, less expensive and easy to perform as well. It should be extraction method of choice in analysis of OTA in kidney if the laboratory is dealing with big number of samples.

P22

Antioxidative properties and GC-MS analyses of Croatian native propolis for implementation in veterinary medicine

Kristina Starčević[1]*, Marijana Hranjec[2], Ivana Zorić[2], Diana Brozić[1], Tomislav Mašek[1], Lada Radin[1], Božo Radić[3], Jelena Šuran[1]

[1]University of Zagreb, Faculty of Veterinary Medicine Zagreb, Heinzelova 55, 10000 Zagreb
[2]University of Zagreb, Faculty of Chemical Engineering and Technology, Marulicevtrg 20, 10000 Zagreb
[3]Hedera d.o.o., 4.gardijske brigade 35, Kamen, 21000 Split

Introduction: The aim of this study was to evaluate antioxidative properties and chemical composition of Croatian native propolis (CNP) as a part of the project: „Intramammarypropolis formulation for prevention and treatment of mastitis in dairy ruminants". Propolis is collected by honey bees from various plant sources and its composition and properties depends on geographic region and extraction procedure. This extraction process should remove the inert material and preserve the polyphenolic (flavonoid and other phenolic compounds) fraction, which is considered to contribute more to the observed healing effects than the other propolis constituents. The influence of different extraction methods and solvents on total phenolic compounds, antioxidative capacity and chemical composition of CNP extracts were in focus of this study.

Material and methods: Three different methods of extraction were used: maceration, reflux, and microwave-assisted extraction (MAE), as well as three different aqueous solutions of ethanol. Total polyphenol content and antioxidant activities were evaluated for all CNP extracts using respectively Folin-Ciocalteu and 2,2-diPhenyl-1-PicrylHydrazyl (DPPH). The reduction of this radical by an antioxidant compound results in a decrease in absorbance and is proportional to the number of electrons absorbed, indicating the antiradical capacity of the substances in study. Afterwards, extracts were analysed by GC-MS to quantify chemical compounds responsible for differences in antioxidative capacity.

Results: Propolis extracts showed differences in antioxidative capacity determined by DPPH method with the best results for 2 hours, room temperature and ethanol/water ratio 70:30. Extracts also differed in quantity and composition of different compounds responsible for antioxidative properties and biological activity determined by GC-MS.

Conclusion: Extraction procedure as well as ratios of solvents has effects on composition of phenolic acids and esters as well as flavonoids and medicinal properties of propolis. All extracts of CNP showed respectable antioxidant activities in comparison to standards (trolox, vitamin C). These data should be considered when using propolis in veterinary medicine.

P23

Microbiological quality of soft, semi-hard and hard cheeses during the shelf-life

Josip Vrdoljak[1], Vesna Dobranić[2], Ivana Filipović[2], Nevijo Zdolec[2]*

[1]PUĐA D.O.O., Economic Zone Čaporice 133, 21240 Čaporice, Croatia
[2]University of Zagreb, Faculty of Veterinary Medicine, Department of Hygiene, Technology and Food Safety, Heinzelova 55, 10000 Zagreb, Croatia

Introduction: Cheeses as ready-to-eat food should be considered as potential source of foodborne pathogens, primarily *Listeria monocytogenes.* The aim of present study was to determine microbiological quality of soft, semi-hard and hard cheeses during the shelf-life, with particular reference to *L. monocytogenes.*

Material and methods: Five types of cheeses were sampled at different time-points during the cold storage