A bioprotective culture shows positive effects

The addition of *L. sakei* to cevapi results in better sensory characteristics

The effects of the bioprotective culture B-2 SafePro (*Lactobacillus sakei*) on pH, weight loss, degree of lipid oxidation and sensory characteristics of cevapi were investigated. It was established that adding B-2 SafePro significantly reduces pH, increases weight loss and improves sensory characteristics of cevapi. The bioprotective culture does not significantly effect on lipid oxidation.

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Fig. 1: Cevapi is very popular barbecue product all over the Balkan countries.

Cevapi production is dating since the Ottoman expansion over the Balkan countries territories. They are very popular barbecue products of grilled meat all over the Balkan countries, which are made from minced beef, pork or mutton (lamb) and various spices in addition (Fig. 1).

They are fast food products widely accepted by many consumers. Cevapi, made of minced meat, have short shelf life (max. 72 h) compared to meat in pieces. Minced meat is prone to deterioration faster than meat in pieces due to a wider area exposed to the external environment. Storage of cevapi, under aerobic conditions, enables growth of bacteria from the genus of *Pseudomonas* which causes changes in their texture, colour, smell and taste (Gill, 1986).

In order to prevent the rapid deterioration of meat and meat products, many chemical additives (polyphosphates, nitrite) are used. Their use is technologically justified due to ensuring products of desirable sensory characteristics, as well as due to inhibitory effects on the growth and development of pathogenic microflora. Long-term consumption of food products containing additives of chemical origin may result in a range of health disorders in humans. Phosphate and polyphosphate mixtures may induce cytotoxic effects and gastriointestinal problems. Nitrites are toxic, carcinogenic and mutagenic additives.

The alternative to chemical additives is bioconservation. This means the usage of selected lactic acid bacteria with capacity to control the growth of undesarable microflora in meat and meat products (Holzapfel et al., 1995; Abbe et al., 1995). Lactic acid bacteria are gram-positive facultative anaerobic bacteria of the geni *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Streptococcus* (Phumkhachorn and Rattanachaikunsopon, 2010). In general, these bacteria produce lactic acid as a final product during the glycolysis process (Kroll et al., 1989; Engesser and Hammes, 1994; Hammes, 1996) and have found application in manufacturing of a huge variety of meat products (Daly and Davis, 1998). Starter cultures in meat are defined as products that contain live microorganisms, which grow and develop in meat products due to their own metabolic activity (Hammes, 1996).

Lactic acid bacteria have an important role in production and preserving of food which is healthy, safe and sensory acceptable. These bacteria have the ability to convert carbohydrates into lactic acid. As a result of this

Control and standard had the same base

Tab. 1: Composition of cevapi (%)

Raw materials	Standard	B-2 SafePro
Beef (70% fresh and 30% frozen)	65	65
Chicken gut (frozen)	13	13
Textured soy	10	10
Fresh onion	8	8
Soy flour	4	4
Additives		
Salt	1.8	1.8
Polyphosphates	0.3	0.3
Mixture of reductive agents	0.1	–
Bombal	0.3	–
Soy isolate	1	1
Ground black pepper	0.45	0.45
B-2 SafePro	–	0.25

Source: Belichovska et al.

The raw material was chilled

Tab. 2: pH and temperature of the raw material before grinding

Raw material	pH	Temperature (°C)
Fresh beef	5.89	6.9
Frozen beef	6.1	-1.6
Frozen chicken gut	5.6	-1.2
Textured soy	6.52	4.6

Source: Belichovska et al. FleischWirtschaft International 2/2016

The control showed lower pH-values

Tab. 3: pH of cevapi three and seven days after production

Time (days)	Standard			B-2 SafePro			Significance
	M	S	C	M	S	C	
pH_3	6.14[a]	0.03	0.01	6.05[b]	0.10	0.01	***
pH_7	6.22[a]	0.09	0.01	5.78[b]	0.10	0.02	***

[ab] –Means in rows with different superscripts are significantly different (P< 0.05)
M= mean; S= standard deviation; C= coefficient of variation; n= 6

Source: Belichovska et al. FleischWirtschaft International 2/2016

process, pH value reduction, inhibition of undesirable microorganisms growth, protein coagulation, reduction of water binding capacity and desired red colour (by favouring the reaction between nitrogen monoxide and myoglobin) is obtained in meat products. The proteolytic activity of lactic acid bacteria contributes to a desired flavour development, throughout release of amines and peptides (Verpaelatse, 1994; Molly et al., 1997). The bioprotective cultures transform the primary food products into food that is safe and delicious with a longer shelf life. Lactobacilli induce the decarboxylation of amino acids by creating biogenic amines that have a negative impact on human health. Such activity is not detected in *L. sakei*, while some strains like *L. curvatus* have the potential to produce biogenic amines (Hammes and Knauf, 1994; Straub et al., 1995).

One of the reasons why lactic acid bacteria are selected, is their production of bacteriocin (small ribosomal antimicrobial peptides or proteins) (De Vuyst et al., 2004). The antimicrobial activity of bacteriocins against *E. coli* and *Salmonella* is detected in fermented sausage (Stevens et al. 1991). Most important for the meat industry is the bacteriocin of *L. sakei* (sakacin) because of its high competitiveness to undesirable microorganisms. Montel et al. (1995) and Sanz et al. (1998) have isolated and determined few exopeptidases in *L. sakei*, emphasising the proteolitic activity role of this bacteria in meat products.

The meat industry from developed European countries has already successfully substituted the chemical additives by bioprotective cultures and spices in context of a healthier food production. However, in Macedonia the usage of bioprotective cultures in meat products have not been implemented yet.

The aim of this investigation was to determine the impact of bioprotective culture of *L.sakei* on certain characteristics of cevapi produced under industrial conditions.

Production of cevapi

The cevapi for the tests were produced under industrial conditions. The composition of cevapi mixture is shown in Table 1. A mixture of reductive agents consisting of E 300 (ascorbic acid), E 316 (sodium eritorbate) and E 330 (citric acid) was used. Bombal containes E 262 (sodium acetate), E 331 (sodium citrate), E 315 (isoascorbic acid) and E 316 (sodium erythorbate). The bioprotective culture B-2 SafePro (Chr. Hansen, Denmark) was used as a commercial product. The product containes the freeze dried form of the lacto-acid homofermentative bacteria *Lactobacillus sakei*. The bioprotective culture was packed in aluminum-foil-pouches, each of 25 g weight. It was dissolved in 0.5 l distilled water and the solution with a concentration of 0.25% (10^7 cfu / g) was added to the mixture of cevapi.

Two variants of cevapi were produced:

- Bombal and mixture of reductive substances (standard)
- Cevapi with the bioprotective culture B-2 SafePro (control)

The temperature and pH value of the raw materials were measured by a pH-meter (Mettler Toledo MP 120). The results are shown in Table 2. Beef, chicken gut and fresh onions were minced in a grinder (CFS Auto Grind, Germany) using a plate with a hole diameter of 5.5 mm.

The minced raw materials were put into a vacuum bowl chopper (CFS CutMaster, Germany) and chopped at 220 min^{-1} at the beginning. Then the remaining components were added by the following order: textured soy, polyphosphate, salt, ground black pepper, soy isolate and soy flour. The mixture was

The control lost more weight

Tab. 4: Grilling weight loss of cevapi, 3 and 7 days after production

Time (days)	Standard			B-2 SafePro			Significance
	M	S	C	M	S	C	
Three days after production (%)	12.46[a]	2.56	0.20	15.20[b]	2.49	0.16	***
Seven days after production (%)	12.70[a]	3.34	0.27	17.10[b]	2.80	0.17	***

[ab]–Means in rows with different superscripts are significantly different (P< 0.05)
M= mean; S= standard deviation; C= coefficient of variation; n= 6

Source: Belichovska et al. FleischWirtschaft International 2/2016

The effect of the reductive agents was limited

Tab. 5: Average TBARS (mg MA / kg product) of cevapi, seven and 90 days after production

Time (days)	Standard			B-2 SafePro			Significance
		S	C		S	C	
7 days after production	0.50[a]	0.19	0.38	0.52[a]	0.23	0.44	ns
90 days after production	1.03[a]	0.13	0.13	0.87[a]	0.09	0.10	ns

[ab]–Means in rows with different superscripts are significantly different (P< 0.05)
M= mean; S= standard deviation; C= coefficient of variation; n= 6

Source: Belichovska et al. FleischWirtschaft International 2/2016

Fig. 2: External appearance and colour of raw, thermally not processed cevapi, three days after production R\B\A – Standard (left), R\L – B-2 SafePro (right)

Fig. 3:. External appearance and colour of raw, thermaly not processed cevapi, seven days after production R\B\A – Standard (left), R\L – B-2 SafePro (right)

chopped at 550 min^{-1} for two minutes. Ten kilogram from the obtained homogeneous mixture were transferred into two separate plastic containers. The mixture of each container was separately mixed in a meat mixing machine (Henneken, Germany), Bombal and a mixture of reductive agents were added to the first container, and B-2 SafePro was added to the mixture of the other container. After this procedure, the mixture was shaped into cevapi using a machine (Lakidis, Greece). The cevapi were stored at a temperature of 0–4 °C after the production.

Measuring methods

The pH value of cevapi was measured by a pH meter (pH-540 GLP, "WTW", Germany). The electrode and the temperature probe were directly inserted into six aliquots of each group of raw, thermally not processed cevapi. The mean value was calculated out of six measurements. The measurements were conducted at the third and seventh day after the production.

The weight loss (shrinkage) during cevapi grilling was determined by six aliquots from each group, three and seven days after their production. The difference in weight of cevapi before and after grilling expressed as a percentage of cevapi weight before grilling is defined as "shrinkage" of thermaly processed cevapi.

The degree of lipid oxidation in cevapi was determined by the TBARS (Thibarbituric Acid Reactive Substances) test according to the method of Tarladgis et al. (1960), modified by Shahidi et al. (1983 and 1987). The determination was performed seven days after the production (the products were stored at temperature of 0–4 °C) as well as after 90 days (the products were kept at temperature of –18 °C). The TBARS value was determined in grilled cevapi (stored overnight in a refrigerator at a temperature of 0–4 °C).

The investigation of sensory characteristics was performed three and seven days after production. This evaluation was performed according to the score-pointing method (Radovanović and Popov-Rajlić, 2000). The sensory evaluation included five features such as: appearance, cross section appearance, texture, smell and taste. For each of the forgoing properties a defined coefficient of importance had been established: external appearance – 1; cross section appearance – 4; texture – 3; colour – 3; smell - 4 and taste – 5. Evaluated properties were valued on a scale of 1 to 5 (1 – bad; 2 – weak; 3 – good; 4 – very good and 5 – excellent). The mark of each feature was multiplied by the coefficient of importance, and the resulting value was presented in the table. The entered values were collected and divided by the sum of the coefficients (20). The resulting value represented the average mark i.e a general quality assessment of the investigated cevapi. Moreover, the percentage of the maximum possible quality was calculated, which represents the assesed average value (AAV) against the maximal score (5) (AAV / 5 x 100).

Statistical analysis of data obtained from the research was done by model of variance

The control showed better sensory scores

Tab. 6: Sensory evaluation of cevapi, three and seven days after production

Sensory characteristics selected	CI	Standard				B-2 SafePro			
		3 days		7 days		3 days		7 days	
		S	C	S	C	S	C	S	C
External appearance	1	3.7	3.7	3.8	3.8	4.0	4.0	4.5	4.5
Cross section appearance	4	4.0	16	3.6	14	4.2	16.8	4.3	17.2
Texture	3	3.9	11.7	4.0	12	4.2	12.6	4.6	13.8
Color	3	3.8	11.4	3.6	11	3.9	11.7	4.5	13.5
Smell	4	3.9	15.6	3.6	14	4.3	17.2	4.6	18.4
Taste	5	3.9	19.5	3.6	18	4.1	20.5	4.5	22.5
Total CI	20								
Pointed mean value			3.895		3.7		4.14		4.495
% from maximum possible quality			77.8		73		82.8		89.9

CI= Coefficient of importance; S= Score; C= Corrected score

Source: Belichovska et al.

analysis (ANOVA) using the statistical package SPSS.

Grilling loss increases while pH decreases

The data presented in Table 3 show that cevapi with the bioprotective culture B-2 SafePro manifest a significantly (P<0.05) lower pH three or seven days after the production. For burgers, in which the bioprotective culture F-SC-111 Bactoferm (*L.sakei*) was added, the pH (6.2) measured the very same day after its production was the same as the reference product. However, three days after production, pH of burgers with *L.sakei* was evidently lower (5.5) compared to the reference group (6.1) (Erkes, 2011). In products with B-2 SafePro, carbohydrates are converted into lactic acid, which results in an obvious reduction of the pH value (Jelle, 1991). Acidification of meat products, which arises due to proliferation of the bacteria, has multiple positive effects including reduction of pH and providing hygienic stability of the meat products (Verplaetse, 1994; Molly et al., 1997).

Cevapi with B-2 SafePro have significantly (P <0.05) higher grilling weight loss three and seven days after their production (Tab. 4). A negative correlation (R= –0.357) between pH and grilling weight loss of cevapi has been found (P<0.01). These results confirm the findings of Kraft (1992), that grilling weight loss increases with pH decrease.

Lipolysis was not limited at frozen storage

It can be noted (Tab. 5) that TBARS of cevapi was low (<0.60) seven days after storage (0–4 °C), but 90 days after frozen storage (-18 °C) evidently higher (>0.80). There was no significant difference in TBARS, seven and 90 days after storage (P>0.05). According to Jelle (1991) *Lactobacillus sakei* limits the animal origin fat lipolysis and reduces the proteolytical activity. It also does not produce biogenic amines, H_2O_2 and bacteriocin. However, this conclusion was not confirmed in this research. Thus, 90 days after production in products with *L. sakei* lipid oxidation was 0.87 mg MA/kg of cevapi. It is assumed that in this study *L. sakei* did not limit the fat lipolysis because the products were stored at –18 °C.

The minimal temperature for bacterial growth is +2 °C. The temperature of –18 °C inactivated the growth and development of *L. sakei*, but did not inactivate the bacterial enzymes. As a result of the lipolytic action of bacterial enzymes, the lipid oxidation 90 days after storage of the products at –18 °C was not prevented. High concentration of malonaldehyde (1.03 mg/kg) 90 days after production was detected in standard products, despite the presence of reductive agents. This can be explained by the fact that the reductive agents used were soluble in water, so their antioxidant effect was limited in the lipid phase.

The test cevapi showed better sensory results

Table 6 presents the results of the sensory analysis of cevapi three (Fig. 2) and seven days (Fig. 3) after their production. Besides the average grades given by panellists adjusted averages are presented. It is known that ordinary average grades are not a real indicator of the general (total) quality of the product, because all characteristics evaluated have no equal importance to total quality, so a correction (C) by appropriate coefficients of importance (CI) was required. Taste and smell, without doubt, have a significant share in the overall assessment. The external appearance and colour were assessed in fresh (raw, thermaly not processed products), while the other sensory characteristics were assessed in grilled cevapi.

It is noticeable that cevapi with B-2 SafePro were better evaluated. On the basis of the score, which is expressed through the pointed mean value and though the percentage from maximum possible quality, it could be noticed that these values were higher in cevapi with B-2 SafePro.

The results presented in Table 7 indicate that three days after production the cevapi with B-2 SafePro had a significantly (P <0.05) better texture. In other sensory attributes the differences between groups were statistically insignificant. Seven days after production (Tab. 8) cevapi with B-2 SafePro had significantly (P<0.05) higher scores of all investigated sensory characteristics.

In the cevapi in which the bioprotective culture was added the microorganisms initiated a chain of reactions which induced a desirable improvement of the senso-

Three days after production the control scored better

Tab. 7: Comparative overview of basic statistical parameters of sensory properties of cevapi, three days after production

Sensory characteristics selected	Standard			B-2 SafePro			Significance
	M	S	C	M	S	C	
External appearance	3.73[a]	0.93	0.25	3.92[a]	0.97	0.24	ns
Cross section appearance	4.05[a]	0.74	0.18	4.00[a]	0.75	0.17	ns
Texture	3.92[a]	0.79	0.2	4.22[b]	0.85	0.2	***
Colour	3.81[a]	0.91	0.24	3.95[a]	0.91	0.23	ns
Smell	3.92[a]	0.95	0.24	4.27[a]	0.73	0.17	ns
Taste	3.92[a]	1.01	0.26	4.11[a]	0.73	0.18	ns

[ab]– Means in rows with different superscripts are significantly different (P< 0.05)
M= mean; S= standard deviation; C= coefficient of variation; n= 6

Source: Belichovska et al.

After seven days the control scored better too

Tab. 8: Comparative overview of basic statistical parameters of sensory properties of cevapi, seven days after production

Sensory characteristics selected	Standard			B-2 SafePro			Significance
		S	C		S	C	
External appearance	3.82[a]	0.92	0.24	4.55[b]	0.56	0.12	***
Cross section appearance	3.61[a]	0.75	0.21	4.30[b]	0.69	0.16	***
Texture	4.00[a]	0.9	0.22	4.61[b]	0.56	0.12	***
Colour	3.64[a]	0.82	0.22	4.48[b]	0.67	0.15	***
Smell	3.61[a]	0.75	0.21	4.58[b]	0.56	0.12	***
Taste	3.64[a]	0.70	0.19	4.52[b]	0.75	0.16	***

[ab]– Means in rows with different superscripts are significantly different (P< 0.05)
M= mean; S= standard deviation; C= coefficient of variation; n= 6

Source: Belichovska et al.

ry characteristics (Verplaetse, 1994). The bioprotective culture reduced the water binding ability of the meat product obtaining the desirable texture (Nishimura et al., 1988; Kato et al., 1989). During the maturation process of cevapi myoglobin is denatured to miochromogen of nitrogen monoxide. This process increased the colour stability because nitrogen monoxide slowly deliberates from the chemgroup (Demeyer, 1992). It was noted that the formation of a stable colour, desired texture and flavour was obtained in hamburgers with a bioprotective culture (Erkes, 2011).

Conclusion

The addition of the bioprotective culture B-2 SafePro (*L. sakei*) to cevapi significantly reduces the pH and consequently increases the grilling weight loss of this meat product. *L. sakei* does not limit the fat lipolysis in cevapi after 90 days storage at –18 °C. By addition of *L. sakei* meat products with superior sensory characteristics compared to the standard products are obtained.

References
Literature references can be requested from the corresponding author or the editorial office, respectively.

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