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Application of Spice Freon Extracts in the Processing of "Hamburgski" Sausage

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Running title: **Replacement of Encapsulated Oleoresins with Spice Freon Extracts**

Abstract
Cooked sausages are under high risk of microbial cross-contamination and physical impurities which come from ground spices. On the other hand, they lose their quality attributes during refrigerated storage. The aim of this work was to study the replacement of encapsulated oleoresins of black pepper (*Piper nigrum* L.), and nutmeg (*Myristica fragrans*) with their aliquots of tetrafluoroethane extracts on quality and safety of a typical representative of cooked sausages Hamburg salami. The proximate composition, sensory properties, colour characteristics, acid value, peroxide value, TBARS, free amino nitrogen, protein carbonyls, pH, residual nitrites and total plate count of aerobic mesophilic microorganisms of Hamburg salami were determined. The replacement of encapsulated oleoresins of black pepper and nutmeg with tetrafluoroethane extracts in Hamburg salami effectively saves the sensory scores for colour of cut surface, taste and odour, and the L*, a* and b* values. It did not significantly affect the change trends of proximate composition, appearance, structure, texture, juiciness, acid value, free amino nitrogen, pH and residual nitrites content during 28 days storage of the sausages at 0 - 4°C. In the same time, in comparison with control samples this replacement reduces the POV approx. 1.5 times; TBARS about 2.1 times; protein carbonyls nearly 1.2 times; and total plate count of aerobic mesophilic microorganisms roughly 1.1 times.

Practical applications
The black pepper and nutmeg tetrafluoroethane extracts can be used in the composition of the cooked sausages such as Hamburg salami. They show strong antioxidant activity with beneficial effect on lipid and protein oxidation, colour stability, some of sensory attributes such as taste, odour, and colour of cut surface during 28 days storage at 0 - 4ºC. In general, results suggest that black pepper and nutmeg tetrafluoroethane extracts can be successfully applied to decrease the risk of microbiological hazard in cooked sausages.

Key words: black pepper extract, nutmeg extract, cooked sausages, quality, safety

Introduction

Many advantages such as: high concentration of the organoleptic compounds; guaranteed high quality of the flavour and aroma; better storage stability; economical consumption rate; retention of food properties during prolonged storage; occupation of smaller volumes and comparatively easy transport are reasons to look for application of aromatic products such as extracts (Shaikh et al., 2006). Basic compounds, determining the antioxidant and antimicrobial activity of the spice extracts are phenolic compounds and terpenes, as well as their derivatives. The antioxidant capacity of extracts of spices is due to their ability to deactivate free radicals (Zheng et al., 2009), metal chelating activity with variable valences (Andjelkovic et al., 2006) and/or reduction of the active singlet oxygen (Mukai et al., 2005). The antimicrobial effect of the spice extracts is due to their distribution as active ingredients in the aqueous phase of membrane cell structure which differ with lipophilic character (Sikkema et al., 1994). They can also attack the double phospholipids layer of microorganism cell membranes (Arques et al., 2008), by means of hydrophobic and hydrogen bonding with the membrane proteins, followed by separation of lipid bilayer (Juven et al., 1994). Extracts of spices cause disturbances in membrane permeability as inhibit the membrane enzymes (Burt et al., 2007), and destroy them (Caccioni et al., 2000); violate electron tran-sport systems and by many ways cause interference in the cell wall (Tassou et al., 2000). Last but not least they are responsible for disorders of the genetic material of bacteria (Lanciotti et al., 2004) and the formation of hydroperoxides causing oxidation of unsaturated fatty acids (Proestos et al., 2008; Skoci-busic et al., 2006). The objective of this study was to assess the quality and safety of representative of cooked sausages Hamburg salami produced with addition of tetrafluoroethane spice extracts of black pepper and nutmeg in comparison with their encapsulated oleoresins.

Materials and Methods

Materials

Meat raw materials

Beef and pork carcasses were obtained from Holstein calves Friesian breed and Duroc x Landrace crossbred pigs, resp. in slaughterhouse Papex Ltd. (Peshtera, Bulgaria). The temperature in the thickest muscles of the delivered chilled beef or pork was not more than 7°C. The chilled beef or pork was frozen overnight at minus 10°C. The next day, the frozen meat was annealed to minus 4°C. In this stage the pH value of beef and pork was 6.15 and 6.18, respectively. The cooled to about 1°C back fat was purchased from slaughterhouse Papex Ltd. (Peshtera, Bulgaria). The back fat was obtained from Duroc x Landrace crossbred pigs. The back fat was frozen overnight at minus 18°C, and after 24 h was annealed to - 8°C. The pH value of annealed back fat before it use was 6.18.

Casings

The artificial polyamide casings Ø60 were obtained from Assenova krepost AD (Asenovgrad, Bulgaria).

Additives and ingredients

Nitrite (E 250) salt (4% $NaNO_2$), was purchased from BBT Ltd., Sofia, Bulgaria. Soy proteins concentrate Solpro 510 A, the hermetically sealed packed encapsulated black pepper (*Piper nigrum* L.) and encapsulated nutmeg (*Myristica fragrans*) were supplied by IP Ltd (Plovdiv, Bulgaria). The samples processed with natural ground spices were labelled as control samples C. The auxiliary mixed preparation for meat cutting Herokal F was purchased by Eurocompact Ltd. (Sofia, Bulgaria).

Spice extracts

The tetrafluoroethane extracts of black pepper (*Piper nigrum* L.) and nutmeg (*Myristica fragrans*) were derived from the department of Heat Engineering of University of Food Technologies (Plovdiv, Bulga-ria). As ground raw materials for extraction were used the same spices supplied by Kressona Ltd. (Bu-zovrad, Bulgaria). The spice extraction was performed with non-polar food grade liquefied gas tetrafluoroethane (CAS number 811-97-2) (Ne-nov, 2006). The extract was obtained in laboratory extractor (Nenov et al., 2008) under following conditions: temperature 20-25ºC, pressure 0.57-0.65 MPa, extraction time 50-70 min (Nenov et al., 2014). The samples processed with tetrafluoroethane spice ex-tracts were labelled as samples E.

Sausage preparation

For the purpose of the study was used sausage Hamburgski produced in the Meat processing plant Borex Ltd. (Malo Konare village, municipality Pazardzhik, Bulgaria) by the following formulation of the filling mass (Table 1). Unfrozen to - 2°C beef was cut in cutter machine to obtain a homogeneous, thick, fluid, solder filling mass. During the meat cut-ting the nitrite salt, spices, and refrigerated water/ flake ice were added. After cutting into pieces the pork semi-fat meat and back fat were frozen for a night to - 8°C. So frozen pork and back fat were added to the beef homogenate

and meat cutting was continued until the structure with fatty meat and back fat particle sizes with up to 4 mm. The prepared fil-ling mass with temperature not higher than 13°C was tightly filled in artificial polyamide casings Ø 60. Sausages were shaped of batons with a length 18-20 cm, which were clipping bilaterally. The filled sausages were placed on stainless profiles and with their help were arranged chequer wise on trolleys. The sausages were cooked to 72°C, and after that were cooled with water and then with air to the 4°C. The sausages were packed in PVC boxes and stored at 0-4°C.

Chemicals and reagents

Malondialdehyde, 2-thiobarbituric acid, trichloroacetic acid, chloroform, methanol, and other reagents and chemicals were supplied by Merck KGaA (Darmstadt, Germany).

Analysis

Sample preparations

Sampling was carried out following the Official Methods of Analysis of AOAC International **(**Latimer, 2012). Samples were packed into aluminium foil bags and were stored 24 h at 0 - 4°C.

Proximate composition

The analysis of proximate composition of cooked sausages was performed according to Dzudie et al. (2002). The moisture, protein, fat, sugars and ash were determined. The content of fibbers was calculated as a difference between 100 and sum of the rest components, in %.

Sensory analyses

Samples were evaluated by 7 assessors selected from staff members of the Department of Meat & Fish Technology of UFT (Plovdiv, Bulgaria) taking into account their habits, acquaintance with the sausage to be analysed, their sensitivity and ability to reproduce the evaluations were made. The analyses were performed by descriptions of Cáceres et al. (2004). The degustation was carried out using non-structured nine scaled hedonic scale. The assessors evaluated appearance, colour, taste, odour, texture and juiciness (0: dislike extremely and 9: like extremely).

Colour characteristics

The colour of sausages was measured using a chromameter Konica Minolta, model CR 410 (Konica Minolta Sensing, Inc., Tokyo, Japan) and expressed as the Hunter *L** (lightness), *a** (redness), *b** (yellowness) values as Pietrasik (1999) was described.

Total lipids extraction

The extraction of total lipids from the sausages was carried out by the Bligh & Dyer method with some modification (Güntersperger & Escher, 1994). A hundred g of sample containing (or adjusted to contain) 75 g water (as determined by oven drying separate aliquots) was homogenized with 100 mL chloroform and 200 mL methanol (monophasic system). The solution was rehomogenized with 100 mL chloroform, following which 100 mL of either distilled water or weak salt solution (0.88% NaCl) was added. After filtration, the lower (chloroform) phase from final biphasic system was collected. Lipid content was determined gravimetrically after evaporating a measured aliquot of the combined chloroform phase to dryness under nitrogen.

Acid value

The AV of the tested samples was used to determine the degree of hydrolytic rancidity of sausage lipids. AV of the extracted lipids was determined according EN ISO 660:2009-10 procedure (Wang, 2010).

Peroxide value

POV of the studied lipids is determined by standard ISO 3960 iodometric method (Latimer, 2012).

TBARS

TBARS were determined by the method proposed by Newburg & Concon (1980) using double beam uv-vis spectrophotometer Camspec M 550 (Cambridge, UK) with a small adaptation (Bakalivanova & Kaloyanov, 2014).

Free amino nitrogen

The free amino nitrogen was determined by Ninhyd-rin assay (Bryce et al. 2010).

Protein carbonyls

The protein carbonyls were determined by DNPH method (Armenteros et al., 2009).

pH

The pH of the sample slurry was measured using a Microsyst MS 2004 pH meter with a glass pH electrode (Sensorex Co., Garden Grove, CA, USA). The measurement was carried out in triplicate as was shown by Zhang et al. (2007).

Residual nitrites

The residual nitrites were determined by a method described by Galán-Vidal et al. (2014).

Preparation of serial dilution for microbiological analysis

The serial decimal dilutions (up to 10^{-6}) were prepared as described by Harrigan (1998).

Determination of total plate count of aerobic mesophilic microorganisms

The total plate count of aerobic mesophilic microorganisms was carried out using the pour-plate methods as described by Harrigan (1998).

Statistical analysis

The obtained data, excluding FAME analysis, were analyzed by SPSS 11.0 software (SPSS Inc., Chica-go, Illinois, USA). Nine repetitions (n = 9) for each sample were carried out. Data were processed by the ANOVA method with a $p < 0.05$ (Tang et al., 2015). Duncan's multiple comparison test (SPSS) with a significant difference set at $p \geq 0.05$ was used to compare sample means. Significant differences between means less than 0.05 were considered statistically significant (Broadhurst & Kell, 2006).

Results

Proximate composition of Hamburg salami

The results (Table 2) show that were not found significant ($p < 0.05$) differences between parameters of proximate composition of samples C and E during 28 days of sausages storage. This means that replacement of encapsulated oleoresins with their tetrafluoroethane spice extracts did not influenced on the Hamburg salami proximate composition.

Sensory evaluated scores of Hamburg salami

Sensory evaluated scores (Table 3) about appearance, structure texture and juiciness were not significantly ($p < 0.05$) changed during 28 days of sausage storage neither at samples C and samples E. In contrast, the colour of the cut surface, the taste and odour of the samples E were retained during the 28 days of the sausage refrigerated storage. For comparison, sensory scores for colour of cut surface, taste and odour of the samples C indicate lasting tendency to-wards significant reduction.

Colour characteristics of Hamburg salami

Colour characteristics (Table 4) of samples E were not significantly ($p < 0.05$) changed during 28 days of sausage refrigerated storage (0 – 4°C). Contrary, the L* and a* values of samples C significantly ($p < 0.05$) decrease, but b* value significantly ($p < 0.05$) increases after 28 days of refrigerated storage.

Hydrolytic rancidity determined by acid value

The degree of lipolysis in Hamburg salami not de-pendent on replacement of the encapsulated oleore-sins with aliquots of their extracts. Neither the eighth day nor twenty-eighth day of refrigeration of Ham-burg salami were not found significant ($p \geq 0.05$) differences between AV values (Table 5). At the same time, continuous increases (with ≈ 65%) in AV during the 28 days storage of Hamburg salami was found (Table 5). Comparatively low levels of AV (not higher than 0.29 mg $KOH.g^{-1}$ lipids) determined in samples C and E on the 28^{th} day of the storage (Table 5) show that the lipolysis is just in the beginning.

Oxidative rancidity of lipids

Primary lipid peroxidation products determined by peroxide value

The concentrations of lipid hydro peroxides in filling mass were found relatively low 2.04 meq $O_2.kg^{-1}$ lipids. This means that the meat raw materials were fresh and deep lipid oxidative processes have not been initiated. After 28 days of storage (0 – 4°C) POV increase 3.45 times in samples C, and only 2.25 times in samples E (Table 5). The replacement of the encapsulated oleoresin with their aliquots tetrafluoroethane extracts achieving reductions of lipid hydro peroxides approximately 1.5 times.

Secondary lipid peroxidation products determined by TBARS

Changes to the secondary products of lipid peroxidation, established by TBARS, demonstrated the same tendency. In the filling mass TBARS were found very low – 0.071 mg $MDA.kg^{-1}$. This shows that the meat raw materials have been completely fresh and the lipids peroxidation has not been developed. In the end of experiment (after 28 days of storage at 0 – 4°C) TBARS in samples C were 2.15 times higher compare to TBARS in samples E, and 3.85 times higher than the initially established levels. TBARS significantly ($p < 0.05$) increased during storage of chilled sausages. The replacement of encapsulated spice oleoresins with their tetrafluoroethane extracts contributes to significant reduction of secondary lipid peroxidation products (Table 5).

Proteolysis determined by free amino nitrogen

Changes established for free amino nitrogen (FAN) are identical to those found for the acid value (Table 5). After 28 days of refrigerated storage FAN increases by approximately 70 % was determined.

Protein oxidation determined by protein carbonyls

Changes of protein carbonyls are in good agreement with those observed in POV and TBARS (Table 5). The protein oxidation almost

absent (0.37 nmol.mg^{-1} protein) in the filling mass. After 28 days of refrigerated storage protein carbonyls in samples E decreased by 16.5% compared to samples C.

pH value

For the studied period (28 days, 0 – 4°C) there were no significant ($p \geq 0.05$) differences in pH values in samples C and E (Table 6). The replacement of encapsulated spice oleoresins with their tetrafluoroethane extracts did not influenced on the Hamburg salami pH.

Residual nitrites

The replacement of encapsulated spice oleoresins (samples C) with their tetrafluoroethane extracts (samples E) did not influenced on the residual nitrites in Hamburg salami, too (Table 6). Compared to the first day, the residual nitrites in the two samples decrease significantly ($p < 0.05$) around two times after 28 days of storage.

Total plate count of aerobic microorganisms

For the studied period (28 days, 0 – 4°C) the total plate count of aerobic mesophilic micro-organisms (TPC) increases more than twice ($p < 0.05$) in both studied samples (C and E) (Table 6).

Discussion

Because of its relatively short shelf life, cooked sausages undergo sensory, chemical and microbiological changes, accompanied by rancidity and deterioration of colour, flavour and safety (Hayes et al., 2011; Gradinarska et al., 2012). As a result of cutting and cooking, in the presence of heat, metal-proteins and non-heme iron, free fatty acids can interact with oxygen (Cáceres et al., 2004). The nutmeg essential oil extract preserves the cooked sausages flavour for longer time (Šojić et al., 2015). In support of our results Bulambaeva et al. (2014) and Pietrasik (1999) confirm the colour delaying and prolonged sensory characteristics preservation of the sausage. During storage of cooked sausages the hydrolytic and oxidative processes significantly intensified, causing more rapid deterioration and development of rancidity (Jayawardana et al., 2011). Changes in colour properties of Hamburg salami directly related to the oxidation (Zhang et al., 2007). The reduction of the a* value in the control samples C is likely due to reaction of the pigments with derivatives of lipid oxidation (Zhang et al., 2016). More precisely, it comes to oxidation of red oxymyoglobin in metmyoglobin, which gives the sausage cut surface unattractive greyish brown colour (Bulambaeva et al., 2014). Meat products produced with the addition of nitrite change their intense red colour due to the oxidation, characterized by conversion of nitrosomyoglobin in metmyoglobin (Feng et al., 2016) and release after the cooking of nitrosohemochromogen. This is likely reason for the changes determined by us regarding the content of residual nitrites. Obviously application of examined extracts of black pepper and nutmeg delay the transformation of the red colour of the sausages by increasing the a* value due to the delayed formation of metmyoglobin (Suman & Joseph, 2013). Manifesting antioxidant action these extracts contribute for inhibition of the nitrosopigment's oxidation in the cooked sausages (Zhang et al., 2007). The generated free fatty acids as a result of the ongoing lipolysis are mentioned as source of lipid oxidation (Wang et al., 2010). The amount of those free fatty acids influence on the sausage sensory characteristics (Bulambaeva et al., 2014). The oxidation of meat lipids being intensified during cooking and refrigeration storage of the sausages (Šojić et al., 2015) and causes rancidity and formation of volatile compounds with unpleasant smell and toxic action, which shortening the shelf-life (Cáceres et al., 2004; Proestos et al., 2008; Soladoye et al., 2015). Like us Hayes et al., (2011) and Šojić et al., (2015) report on reduction of POV and MDA after use of the extracts of black pepper and nutmeg. Zarai et al., (2013) explain this phenomenon by a radical-inhibiting activity of the black pepper extract containing β-caryophyllene, limonene, terpinolene and p-cymene (Stoyanova et al., 2006). The heat treatment assist denaturation, oxidation and polymerization of the proteins (Soladoye et al., 2015). The ongoing hydrolysis and oxidation increase free amino nitrogen and protein carbonyls content (Bulambaeva et al., 2014; Serikkaisai et al., 2014). At the time of the sausage storage is reported additional accumulation of protein carbonyls - closely associated with the occurrence of nonheme iron and lipid oxidation development. The oxidation of unsaturated fatty acids and oxymyoglobin into metmyoglobin leads to the formation of free radicals that initiate the meat proteins oxidation (Suman & Joseph, 2013). The accumulation of protein carbonyls has been associated with negative changes in the sausage colour and consistency during its storage (Soladoye et al., 2015). Like us Dzudie et al. (2002) and Bulambaeva et al. (2014) believe that the addition of spice extracts in the cooked sausage has no influence on pH changes during its refrigerated

storage. Our results for microbial growth suppression were confirmed by Sachindra et al. (2005) and Bostan & Isinmahan (2011), too. Zarai et al. (2013) and Zhang et al. (2016) suggested that the reason is antimicrobial activity of the black pepper extract, reach of monoterpene compounds such as: sabinene, β-pinene, limonene, borneol, 1,8-cineole, linalool and β-caryophyllene. This compounds were found effective against the development of gram positive bacteria (*Staphylococcus epidermis, Staphy-lococcus aureus, Staphylococcus xylosus, Bacillus subtilis*), as well as gram negative bacteria (*Escherichia coli, Klebsiella pneumonia, Salmonella enterica*).

Conclusions

The replacement of encapsulated oleoresins from black pepper and nutmeg with aliquot parts of their tetrafluoroethane extracts during Hamburg salami production can be used successfully to improve the quality and to minimise the risk of microbial hazard during processing and 28 days storage at 0-4°C.

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Table 1: Formulations of the filling mass of Hamburg salami processed with encapsulated natural spices (C) or tetrafluoroethane spice extracts (E).

Ingredients	Control sample	Experem. sample
1) Beef (topside) prime quality, kg	60.000	60.000
2) Pork (semi fat) second quality, kg	20.000	20.000
3) Back fat (firm), kg	20.000	20.000
4) Nitrite salt (4% $NaNO_2$), kg	2.000	2.000
5) Soy protein concentrate SOLPRO 510 A, kg	4.000	4.000
6) Auxiliary mixed preparation for meat cutting HEROKAL-F, kg	0.400	0.400
7) Encapsulated black pepper, kg	0.400	-
8) Encapsulated nutmeg, kg	0.100	-
9) Black pepper essential oil extract (104 g) dissolved in sunflower oil (296 g), kg	-	0.400
10) Nutmeg essential oil extract (23 g) dissolved in sunflower oil (77 g), kg	-	0.100
11) Water + Flake ice, kg	20.000	20.000
12) Artificial polyamide sausage casings Ø 60, m	100.00	100.00

Table 2: Changes of the proximate composition of Hamburg salami processed with encapsulated natural spices (C) or tetrafluoroethane spice extracts (E).

Parameters, %	1st day of sausages making	9th day of storage at 0 - 4°C		28th day of storage at 0 - 4°C	
		Samples C	Samples E	Samples C	Samples E
Water	57.32 ± 0.38 [a]	57.10 ± 0.36 [a]	57.01 ± 0.34 [a]	57.12 ± 0.37 [a]	57.07 ± 0.35 [a]
Protein	17.03 ± 0.29 [a]	17.10 ± 0.21 [a]	17.22 ± 0.23 [a]	17.19 ± 0.23 [a]	17.20 ± 0.27 [a]
Lipids	22.04 ± 0.85 [a]	22.10 ± 0.87 [a]	22.11 ± 0.88 [a]	22.15 ± 0.89 [a]	22.21 ± 0.81 [a]
Sugars	1.01 ± 0.12 [a]	0.92 ± 0.10 [a]	1.03 ± 0.13 [a]	0.98 ± 0.11 [a]	1.02 ± 0.12 [a]
Ash	1.74 ± 0.16 [a]	1.83 ± 0.19 [a]	1.84 ± 0.17 [a]	1.85 ± 0.20 [a]	1.82 ± 0.16 [a]
Fibres	1.03 ± 0.11 [a]	1.04 ± 0.12 [a]	0.92 ± 0.12 [a]	1.05 ± 0.13 [a]	0.99 ± 0.14 [a]

Data were expressed as Mean ± SD (n = 9).

Table 3: Changes of the sensory evaluated scores of Hamburg salami processed with encapsulated natural spices (C) or tetrafluoroethane spice extracts (E).

Parameters	1st day of sausages making	9th day of storage at 0 - 4°C		28th day of storage at 0 - 4°C	
		Samples C	Samples E	Samples C	Samples E
Appearance	9.00 ± 0.00 [a]	8.96 ± 0.03 [a]	8.98 ± 0.02 [a]	8.95 ± 0.03 [a]	8.97 ± 0.03 [a]
Colour of the cut surface	9.00 ± 0.00 [c]	8.21 ± 0.13 [b]	8.89 ± 0.11 [c]	7.94 ± 0.17 [a]	8.83 ± 0.14 [c]
Structure of the cut surface	8.94 ± 0.05 [c]	8.95 ± 0.04 [c]	8.96 ± 0.05 [c]	8.91 ± 0.04 [c]	8.93 ± 0.06 [c]
Taste	9.00 ± 0.00 [c]	8.70 ± 0.06 [b]	8.90 ± 0.10 [c]	7.95 ± 0.08 [a]	8.88 ± 0.09 [c]
Odour	9.00 ± 0.00 [c]	8.75 ± 0.08 [b]	8.95 ± 0.05 [c]	7.92 ± 0.09 [a]	8.90 ± 0.06 [c]
Texture	9.00 ± 0.00 [d]	8.98 ± 0.02 [d]	8.99 ± 0.01 [d]	8.85± 0.03 [d]	8.88 ± 0.05 [d]
Juiciness	9.00 ± 0.00 [a]	8.94 ± 0.07 [a]	8.97 ± 0.05 [a]	8.90 ± 0.06 [a]	8.95 ± 0.05 [a]

Data were expressed as Mean ± SD (n = 9).
[a, b, c] - different letters in one row indicated that values of the means are significantly different (p < 0.05).*

Table 4. Effect of replacement of encapsulated natural spices (C) with tetrafluoroethane spice extracts (E) on the colour characteristics of Hamburg salami.

Parameters	1st day of sausages making	9th day of storage at 0 - 4°C		28th day of storage at 0 - 4°C	
		Samples C	Samples E	Samples C	Samples E
Lightness (L*)	56.12 ± 0.21 [c]	55.47± 0.16 [b]	56.06 ± 0.14 [c]	54.03 ± 0.17 [a]	56.07 ± 0.15 [c]
Red colour (a*)	15.28 ± 0.19 [c]	14.65 ± 0.11 [b]	15.22 ± 0.13 [c]	13.99 ± 0.13 [a]	15.20 ± 0.17 [c]
Yellow colour (b*)	9.84 ± 0.15 [a]	9.90 ± 0.17 [b]	9.81 ± 0.18 [a]	10.07 ± 0.19 [c]	9.79 ± 0.11 [a]

Data were expressed as Mean ± SD (n = 9).
[a, b, c] - different letters in one row indicated that values of the means are significantly different (p < 0.05).*

Table 5. Effect of replacement of encapsulated natural spices (C) with tetrafluoroethane spice extracts (E) on the hydrolytically and oxidative stability of Hamburg salami.

Parameters	1st day of sausages making	9th day of storage at 0 - 4°C		28th day of storage at 0 - 4°C	
		Samples C	Samples E	Samples C	Samples E
Acid value, mg KOH.g^{-1}	0.10 ± 0.02 [a]	0.17 ± 0.03 [b]	0.18 ± 0.02 [b]	0.29 ± 0.04 [c]	0.28 ± 0.03 [c]
Peroxide value, meq O_2.kg^{-1}	2.04 ± 0.15 [a]	4.16 ± 0.17 [c]	3.51 ± 0.18 [b]	7.05 ± 0.19 [e]	4.59 ± 0.18 [d]
TBARS, mg MDA.kg^{-1}	0.071± 0.01 [a]	0.130± 0.02 [c]	0.094± 0.02 [b]	0.273± 0.03 [d]	0.127± 0.02 [c]
Free amino nitrogen, mg.100 g^{-1}	5.74 ± 0.14 [a]	11.87 ± 0.18 [b]	11.84 ± 0.16 [b]	18.89 ± 0.14 [c]	18.83 ± 0.19 [c]
Protein carbonyls, nmol.mg^{-1} protein	0.37 ± 0.05 [a]	0.89 ± 0.06 [c]	0.74 ± 0.07 [b]	2.12 ± 0.07 [e]	1.77 ± 0.09 [d]

Data were expressed as Mean ± SD (n = 9).
[a, b, c, d, e] - different letters in one row indicated that values of the means are significantly different (p < 0.05).*

Table 6. Effect of replacement of encapsulated natural spices (C) with tetrafluoroethane spice extracts (E) on the pH, residual nitrites, and total aerobic count of mesophilic microorganisms in Hamburg salami.

Parameters	1st day of sausages making	9th day of storage at 0 - 4°C		28th day of storage at 0 - 4°C	
		Samples C	Samples E	Samples C	Samples E
pH	6.82 ± 0.19 [a]	6.77± 0.14 [a]	6.79 ± 0.18 [a]	6.63 ± 0.16 [a]	6.77 ± 0.17 [a]
Residual nitrites, mg.kg^{-1}	61.22 ± 1.43[a]	49.1 ± 1.39 [b]	48.9 ± 1.28 [b]	27.8 ± 1.34 [c]	26.5 ± 1.27 [c]
TPC,$\log_{10}$ cfu.g^{-1}	2.32 ± 0.48 [a]	3.27 ± 0.26 [b]	3.11 ± 0.34 [b]	6.23 ± 0.37 [d]	5.68 ± 0.25 [c]

Data were expressed as Mean ± SD (n = 9).
[a, b, c] - different letters in one row indicated that values of the means are significantly different (p < 0.05).*