Effect of incorporation of the plant extracts in natural casing on the color of fermented sausages

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fermented sausages, plant extracts, natural casing, color. In this study, the effect of incorporation of the plant extracts in a natural casing on the color and sensory attributes in fermented sausages, "sucuk" type, was evaluated. The sausages were produced in industrial conditions, stuffed into the pretreated natural casings, vacuum packaged, and stored at 4°C for six months. Five groups of sausages were tested, C1 (natural casing without treatment), C2 (treated with 6% (v/v) ethanol), C3 (treated with ascorbic acid), A (treated with ethanol extract of *Aronia melanocarpa*), and D (treated with ethanol extract of *Cornus mas*).

Based on the results, it was concluded that the use of Aronia extract had an effect on the color of the surface. During storage, there was an increase in lightness (L^*) and the proportion of red color (a^*), and also a slight increase in the proportion of yellow color (b^*). There were no major changes in the color parameters on the cross-section of the sausage.

Sensory testing of the sausage samples found that there was no difference between the tested samples in terms of external appearance, while the results of the "different from control" - DFC test showed that the A sample was visually more acceptable and had a nicer surface color.

INTRODUCTION

The share of fermented sausages production takes a significant place as one of the most complex areas in modern meat science and technology. The complexity of this production bases on the fact that fermented sausages are produced from biologically active materials, and the ripening process itself goes without the influence of high temperature with the goal of getting a high-quality product (Medić et al., 2009).

Among the factors that significantly affect the ripening process and the conversion of the filling into a final product can be the choice of the casing. The function of the casing is not only protection from the external environment and ensuring a certain shape, but also the regulation of the conversion of the contents of the filling into the desired product - sausage (Čavlek, 2001).

Hydrolytic and oxidative changes of lipids are closely related to the permeability of the casing. Beside microbiological growth, lipid oxidation of fatty acids is the basis of spoilage of fermented sausages. It directly affects the color, aroma, texture, nutritional properties, and sustainability of sausages, all of which are related to the permeability of the casing (Pidcock et al., 2002). On the other hand, as previously emphasized, the antioxidant or antimicrobial properties of the casing

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can be enhanced when combined with organic acids, plant extracts, and essential oils (Georgantelis et al., 2007; Kanatt et al., 2008; Ouattara et al., 2000). Previous research has shown that essential oils have efficacy as antimicrobial and antioxidant compounds (Prakash et al, 2015).

Chokeberry (*Aronia melanocarpa*) is the subject of numerous studies due to its high content of phenolic compounds, antioxidant properties, and potential positive impact on health. Ripe *A. melanocarpa* berries contain various compound types: anthocyanins, procyanidins, and flavonols (quercetin glycosides). These polyphenolic components of fruits make them a valuable material that can use as food or food supplements dedicated to protection from oxidative stress (Battino et al., 2009; Gralec et al., 2019).

Cornelian cherry dogwood (*Cornus mas*) is a valuable source of active ingredients, such as phenolic compounds, vitamin C, iridoids, flavonoids, and anthocyanins. Plant extracts and substances derived from *Cornus mas* L. show strong antibacterial, antioxidant, and tonic properties, effectively preventing the development of inflammation in living organisms (Nizioł-Łukaszewska et al., 2018).

Plant extracts with antimicrobial and antioxidant activity would satisfy today's consumers in search of healthy food without using chemical preservatives. By adding plant extracts to the casings, an active edible material for packaging with optimal properties was obtained, which contributes to preserving the stability of the quality of sausages and effectively protects

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against unwanted oxidative and microbiological changes (Velemir, 2022).

The chemical reactions that lead to the creation of the characteristic color are very complex and largely depend on the pH value, pigment concentration, distribution of brine salt, and ripening conditions (Savanović, 2011). Therefore, the color of the surface, as an integral part of the appearance of sausages, is very relevant to consumer acceptability. This study evaluates the effect of incorporating plant extracts of chokeberry and Cornelian cherry into the natural casing on the color and sensory characteristics of fermented sausages of the "sucuk" type. This research aimed to determine the influence of natural bioactive compounds incorporated in a natural casing on the sensory quality, i.e., the color of the sausage.

MATERIALS AND METHODS

Extraction of plant extracts

For the preparation of extracts, fruits were washed and the petioles and the seeds were removed. For ethanol extract, 40 g of fruits were homogenized with 160 mL 80% (v/v) ethanol, first on a Polytron PT 3100 homogenizer, for 10 minutes at 8000 rpm, then in an ultrasonic bath for 30 minutes, and 30 minutes on a magnetic stirrer. The mixture was filtered, and the obtained filtrate

was evaporated to a dry residue, first on a vacuum evaporator and then in a dry sterilizer at 50 °C.

The dry extracts prepared in this way were stored in a dark place until the moment of use. The concentrations of used solutions were determined experimentally. The prepared dry extract of *A. melanocarpa* diluted to 45 mg/mL, and the extract of *C. mas* diluted to 22.5 mg/mL in 6% (v/v) ethanol and used for casing treatment. The duration of submersion was 24h for all casings.

Sucuk preparation

Domestic fermented sausages, sucuk, were produced traditionally under industrial conditions. The dough consisted of beef meat 80%, beef fat 16%, nitrite salt 2.5%, spices: black pepper 0.3%, white pepper 0.15%, garlic granules 0.35%, additive (mixture of dextrose, salt, sucrose, ascorbic acid, preservative E252 (2.5%) 0.6%, starter culture 0.01%. After grinding and mixing in the cutter, the sausage emulsion was filled into natural beef casings with the addition of all components. For this study, five samples of domestic fermented sausages: C1-the control (natural casing without treatment); C2 (natural casing treated with 6% (v/v) ethanol), C3 (natural casing treated with ethanol

extract of A. *melanocarpa*), and D (natural casing treated with ethanol extract of C. *mas*) were made. The sausages were first tempered (18-20°C) for 8h, then smoked (beech wood) for three days (20-23°C, 92-95% RH), and finally left for fermentation (ripening) at 16-18°C for eight days. After production, sausages were vacuum-packed and stored in a cooling chamber at 4°C until sampling (usual storage conditions for this kind of product). Analyses of sausages were carried out after production and after three and six months of storage.

Determination of color

Color measurements were performed using a Spectrophotometer CM-2600d (Konica Minolta, Japan), with 8 mm port size, illuminant D65, and a 10° standard observer, in a room with fluorescent lighting and after standardization of the instrument concerning the white calibration plate. Color parameters, expressed as CIE L^* , a^* , and b^* values, were determined as lightness, redness, and yellowness indicators. The sausage surface color was measured as described by Stajić et al. (2014).

Sensory analysis

The sensory quality of fermented sausages (external appearance and condition of casing, cross-sectional appearance, and the color of cross-section) was examined according to the method of quantitative descriptive analysis (ISO 6564:1985) to identify the quality level of selected product properties. The external appearance and color of the sausage casings were also examined using the "difference-from-control" test (Whelan, 2017).

Statistical analysis

Statistical analysis of the obtained results was performed using Microsoft Excel 2013 software package and the IBM SPSS Statistics 22.0 (Armonk, NY, United States). Results were presented as mean values of individual measurements ± standard deviations. The significance of differences between arithmetic means was determined and expressed with 95% probability (Tukey's test).

RESULTS AND DISCUSSION

The color of the surface is an essential indicator of quality, and as an integral part of the appearance of the sausage, it is very relevant in terms of consumer acceptability (Stajić et al., 2017). Table 1. shows the mean values of changes in instrumental color indicators determined on the surface of the casing of the examined samples, after ripening (0) and during storage (3 and 6 months).

From the shown results, it can be seen that after ripening, the average lightness values (L^*) determined on the surface of the casing did not differ significantly (p>0.05), ranged from 39.21 for the A sample to 41.34 for the D sample. During storage, a similar trend occurs in most samples, where first there is an increase in L^* values in all samples (40.37-43.23), and then a decrease (39.99-41.59). Only for the A sample, the L^* value increased during the whole storage period (from 39.21 to 42.25).

Stajić et al. (2017) confirm that after ripening and during storage of Sremska sausage (domestic pork sausage), there were no significant deviations in the light parameter L^* . Lower L^* values were reported Ozturk et al. (2021) for the analysis of sucuk.

Hromiš (2015) points out that in sausage with a layer of chitosan and oregano oil, the L^* parameter of the surface changed over time, gradually decreasing, while the combination of chitosan and cumin oil led to a significant increase in the brightness, with a more pronounced change and a more stable surface color. The cause may be the nature of the essential oil and the used concentration.

The average values of the proportion of red color (a^*) on the casing surface ranged from 3.70 (sample C1), to 5.07 (sample D). During storage a similar change can be observed in most samples (except for the A sample) value first increases and then slightly decreases. For the A sample, a^* value increases during the whole storage period.

Using a chitosan coating with additives (oregano and cumin essential oils) during the production of fermented sausages, Hromiš (2015) did not observe a change in the proportion of red color during storage, and the values ranged from 7 to 8. Slightly higher values for the proportion of red color in traditional sucuk during storage were reported by Ozturk et al. (2021). The values of the proportion of yellow color b^* after ripening ranged from 2.58 in sample C1 to 3.73 in sample D. A statistically significant difference (p<0.05) was present between the samples. During three months of storage, the value of b^* increases (3.85-6.63) in all samples, but during further storage, this value varies slightly (4.39-6.69).

Slightly lower b^* values on the surface of fermented sausages are reported by Ozturk et al. (2021). Hromiš (2015) indicates that the color parameter b^* of the outer surface did not change during storage in any of the investigated groups of chitosan-coated sausages, and the values ranged from 6 to 7.5.

Table 2. shows the mean values of changes in instrumental indicators determined on the crosssection of the examined samples, after ripening (0) and during storage (3 and 6 months). After ripening, the average values of lightness (L^*), determined on the cross-section, did not differ significantly (p>0.05) and ranged from 48.47 for sample D to 50.28 for sample C2.

After three months of storage, there was an increase in the value of L^* , except for sample C3. During further storage, these values were higher in all samples. After six months, sample C2 had the highest value of 54.24, and the lowest value found in sample A (51.46).

Lešić et al. (2020) believe that the color of dry fermented sausages depends on several factors, such as the composition of the filling, the amount and type of applied spices and additives, and technological procedures. Krol et al. (2017) indicate that the use of alginate resulted in slightly higher lightness values (53.81-56.77), while Bozkurt (2006) states that using green tea extracts and essential oil of *Thymbra spicata* in sucuk dough does not change the color parameter L^* . For most authors, the value of the L^* parameter was lower, ranging from 26 to 42 (Hromiš, 2015; Lešić et al., 2020)

Table 1. Average values of L^{*}, a^* and $b^* \pm$ SD measured on the casing surface of the tested sausage samples after ripening (0) and during storage (3 and 6 months)

	L*			a*			b*		
	0	3	6	0	3	6	0	3	6
C1	41.27 ^{aA*}	42.36 ^{abA}	41.59ª ^A	3.70ªA	5.86ªB	5.14 ^{abB}	2.58ªA	5.28 ^{abB}	5.93 ^{abB}
	±1.34	±1.00	±0.66	±0.50	±0.31	±0.77	±0.45	±0.66	±0.58
C2	41.00 ^{a.A}	43.22 ^{bB}	39.99 ª ^A	4.72 ^{abA}	6.34 ^{abB}	6.28 ^{bcB}	3.41 ^{abA}	6.13 ^{bB}	5.70 ^{abB}
C2	±1.02	±0.64	±1.23	±0.48	±0.53	±0.50	±0.48	±1.16	±1.02
C3	40.00ªA	43.23 ^{bA}	41.14ª ^A	3.73ªA	7.10 ^{bB}	6.60 ^{cB}	3.50 ^{abA}	6.63 ^{bB}	6.69ªB
C3	±3.14	±1.38	±0.65	±0.48	±0.89	±0.99	±0.81	±1.24	±0.98
	39.21ª ^A	40.37 ^{aAB}	42.25 ^{aB}	3.84ª ^A	5.33ªB	7.13℃	2.77 ^{abA}	3.85ªB	6.95ª [℃]
Α	±2.00	±1.75	±1.17	±0.18	±0.38	±0.68	±0.35	±0.57	±0.87
D	41.34ª ^A	42.48 ^{abA}	41.57ª ^A	5.07 ^{bA}	5.76ªA	4.85ªA	3.73 ^{bA}	5.28 ^{abB}	4.39 ^{bAB}
	±0.53	±1.45	±2.02	±1.00	±0.52	±0.74	±0.66	±0.49	±0.76

^{a-c} - mean values with different letters in the same column differ statically significantly with 95% probability (p < 0.05) ^{A-C} - mean values with different letters in the same raw differ statically significantly with 95% probability (p < 0.05) Demirok Soncu et al. (2018) analyzing sucuk with a coating of chitosan and essential oils did not observe a change in the L^* color value. Similar conclusions were reached by Hromiš (2015) in his work with chitosan coatings. The same author also explains that the decrease in the color light value (L^*) on the cross-section of fermented dry sausages is influenced by the decrease in water content, while the decrease in pH value, which leads to the cross-linking of myofibrils and the displacement of part of the water, leads to a greater reflection of light, and an increase in the L^* value.

The values of the proportion of red color (a^*) in the final product did not differ significantly (p>0.05). The lowest value was measured for sample C3 (8.79), while the highest value had sample D (9.93). During storage, a similar decreasing trend was present in all samples, and the values ranged from 6.90 (D) to 7.36 (A).

The similar a^* values report Lešić et al. (2020), while many authors report higher values for the share of red color (Kamenik et al., 2012; Krol et al., 2017). In experiments with chitosan casings and the use of essential oils, there was no change in a^* value during storage (Demirok Sonku et al., 2018; Hromiš, 2015). Kurt (2016) found that the addition of grapefruit seed flour leads to a decrease in the proportion of red color in fermented sausages during the ripening period and during storage, probably due to the content of color pigments in the used additive.

Lešić et al. (2020) believe that a lower protein content leads to a decrease in a^* value due to the dilution of myoglobin, but the results of this research can also be related to the addition of hot pepper, which affects the decrease in the L^* value and the increase in a^* and b^* values. Other authors report a decrease in the proportion of red color during ripening as a result of nitrosomyoglobin denaturation (Bozkurt, 2006; Stajić et al., 2017). It is considered that the characteristic dark red color of the cross-section of fermented sausage mainly originates from red hot pepper, as well as smoke components, and the most likely reason for the decrease in the a^* value is the oxidation of red hot pepper components (Hromiš, 2015). Ozturk et al. (2021) believe that the decrease in the proportion of red color of sucuk produced with added lactic acid bacteria is most likely caused by lactic acid causing denaturation of myoglobin.

The values of the proportion of yellow color (b^*), after ripening ranged from 6.02, for sample C1 to 7.47, for sample D. During storage, the b^* value first increased (except for sample A). After 6 months, these values decreased in samples C2, C3, and D, and slightly increased in C1 and A.

Similar data reported Ozturk et al. (2021) for sucuk analysis. Slightly higher values reported Bozkurt (2006), who states that natural and artificial antioxidants addition to fermented sausages had no effect on the proportion of yellow color. During storage, the b^* value decreases, probably caused by a decrease in the content of oxymyoglobin, which contributes to the yellow color due to the microorganisms oxygen consumption (Bozkurt, 2006; Kurt, 2016). Other possible causes of the decrease in the b^* value may be the drying of the sausages, or the content of polyphenols added (Kurt, 2016). Hromiš (2015) believes that the cause of the decrease in the value of the b^* parameter may be an increase in the salt content, which occurs due to the decrease of the moisture content in fermented sausages.

The sensory analysis of the tested sausage samples included the evaluation of the external appearance and condition of the casing, the appearance of the cross-section, and the color of the cross-section after ripening and during storage.

	L*			a*			b*		
	0	3	6	0	3	6	0	3	6
C1	48.76ªA	51.67 ^{aB}	53.12 ^{abB}	9.06ªA	8.39 ^{abA}	7.09 ^{aB}	6.02ªA	7.58 ^{abB}	7.82 ^{abB}
CI	±0.87	±2.06	±2.62	±0.48	±0.57	±0.58	±0.83	±0.76	±1.09
C 2	50.28ªA	51.17 ^{abA}	54.24ª [₿]	9.06ªA	8.80ªA	7.09 ^{aB}	6.46 ^{abA}	8.76 ^{aB}	8.06 ^{bB}
C2	±3.54	±1.91	±0.65	±0.48	±0.57	±0.58	±0.70	±1.11	±0.52
C3	49.10 ^{aA}	48.99 ^{bA}	51.98 ^{abB}	8.79 ^{aA}	8.72ªA	6.97ªA	6.47 ^{abA}	7.75 ^{abB}	6.91 ^{acAB}
C3	±1.16	±1.91	±1.69	±0.70	±0.73	±0.51	±1.04	±1.15	±0.65
•	48.83ªA	50.20 ^{abAB}	52.08 ^{abB}	9.72ªA	7.74 ^{bB}	7.36 ^{aB}	6.99 ^{abA}	6.98 ^{bA}	7.16 ^{abcA}
Α	±1.35	±1.90	±1.80	±1.11	±0.65	±0.64	±1.16	±0.44	±0.75
P	48.47ªA	50.16 ^{abAB}	51.46 ^{bA}	9.93ªA	8.22 ^{abB}	6.90 ^{aC}	7.47 ^{bA}	7.65 ^{abA}	6.76 ^{cA}
D	±1.99	±1.77	±1.56	±1.16	±0.86	±0.51	±1.12	±1.11	±0.81

Table 2. Average values of L^* , a^* and $b^* \pm SD$ measured on the cross-section of the tested sausage samples after ripening (0) and during storage (3 and 6 months)

^{a-c} - mean values with different letters in the same column differ statically significantly with 95% probability (p <0.05) ^{A-C} - mean values with different letters in the same raw differ statically significantly with 95% probability (p <0.05)

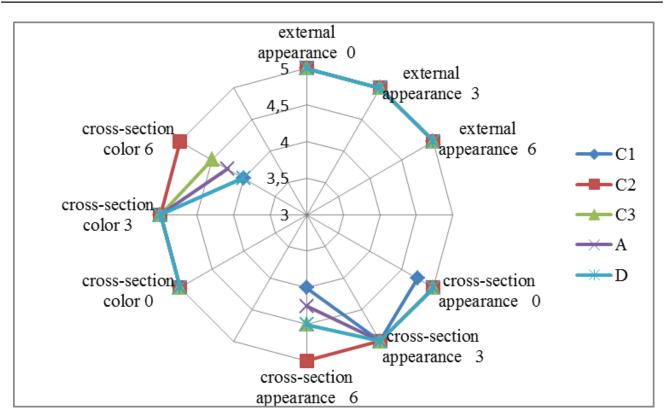


Figure 1. Sensory evaluations for the external appearance and condition of the casing, the cross-section appearance and the cross-section color after ripening and during storage.

The average scores for the analyzed sensory properties of the tested samples can see in Figure 1.

As can be seen from the presented results, the external appearance of all sausages during the entire test period had higher grades. The casing was slightly wrinkled, did not separate from the filling, and no deformations or damages present. The consequence of the more intense color of the extract used in the treatment is the more intense color of the surface of the casing in sample A.

Cross-section of the product after ripening had the appearance of a mosaic, the component parts were well connected, and there were no visible cracks inside the product, with the exception of sample C1, which had small cavities.

After three months of storage, there were no visible cracks on the cross-section of the product, and all samples received the highest rating.

Scores for cross-sectional appearance after six months of storage ranged from 4 to 5, due to the appearance of cracks on the cross-sectional surface.

The color of the cross-section after ripening and after three months of storage was appropriate for all samples, the pieces of meat were red, and the particles of fatty tissue were white.

The grades for the color of the cross-section after 6

months ranged from 4 to 5. The cross-section color of control sample C1 was uneven and changed due to the appearance of cracks in the central part. Changes in the central part, due to the appearance of cracks were also present in a smaller amount in other samples (C3, A, D). In sample C2 there were no changes in color, the surface was uniform.

Arslan et al. (2018) reported that the high concentration of chitosan applied to fermented sausages did not cause any negative changes, and there are no statistically significant differences in overall acceptability, and Hromiš (2015) also agrees with that. Demirok Soncu et al. (2018) believe that chitosan solution in combination with thyme or rosemary has no significant effect on sensory properties but can have a positive effect by preventing the surface growth of fungi in Turkish fermented sausages. Extracts of some plants can influence the improvement of the color of meat and meat products (Effenberger-Szmechtyk et al., 2020).

Using the difference-from-control (DFC) test, after production (0) and during storage, there was no difference between the samples in terms of the crosssection appearance and the cross-section color. Very little to moderate difference was observed in the A sample in terms of the color of the casing, which had a more intense red color and gave the product a more acceptable look. Zamuz et al. (2018) state that the addition of plant extracts can cause changes in the color of the samples. Krol et al. (2017) determined that with the use of alginate in the production of fermented sausages, the differences in color between the samples were minimal and not visually noticeable.

CONCLUSION

The use of the Aronia extract influenced color parameters. During storage, there was an increase in lightness (L^*) and the proportion of red color (a^*), and a slight increase was present in the proportion of yellow color (b^*). On the cross-section of sausages, there were no major changes in the parameters L^* and a^* , and there is a similar trend present in all samples.

Sensory testing of the sausage samples found that there was no difference between the tested samples in terms of external appearance. After 6 months of storage, changes are visible in the cross-section appearance and the color, in the form of cracks and minimal color changes.

The results of the "different from control" - DFC test showed that the A sample was visually more acceptable and had a nicer surface color, which was also confirmed by the results of the casing color testing.

Natural plant extracts represent a good alternative for improving the sensory quality and can affect the safety of fermented sausages. By adding plant extracts to the casings, it is possible to obtain an active edible packaging material with optimal properties.

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