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THE INFLUENCE OF TWO STARTER CULTURES ON SOME QUALITY PROPERTIES OF MACEDONIAN TRADITIONAL SAUSAGE

Monika Stojanova*, Olga Najdenovska, Zlatko Pejkovski

Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food - Skopje, Republic of North Macedonia

*Corresponding author: stojanova.monika@yahoo.com

ABSTRACT

Sausages belong to the widest range of meat products available in a wide variety of species and with various commercial names. The aim of this paper is to monitor the influence of two starter cultures on the color and lipid oxidation of industrially produced Macedonian traditional sausage. The research covered three variants: Variant 1: Control variant (conventionally produced Macedonian traditional sausages using nitrite salt and powdered acerola); Variant 2: Macedonian traditional sausages where the basic formulation was enriched by addition of starter culture CS-300 (*Staphylococcus carnosus ssp. utilis* + *Staphylococcus carnosus*) in combination with Swiss chard powder and powdered acerola; Variant 3: Macedonian traditional sausages where the basic formulation was enriched by addition of starter cultures CS-300 (*Staphylococcus carnosus ssp. utilis* + *Staphylococcus carnosus*) and BLC-78 (*Pediococcus acidilactici* + *Staphylococcus carnosus*) in combination with Swiss chard powder and powdered acerola. The lightness of the color (*L**) continuously decreases in the control variant, resulting in a loss of color in the specified time interval. This phenomenon is not observed in the samples from variant 2 and variant 3. On the 4th day of production, variant 2 and variant 3 have statistically significantly differences ($p<0.05$) for redness value, compared to the control variant. From the aspect of retaining the values for redness (*a**) and the yellowness (*b**), better effect showed the starter culture CS-300. Thus, the samples of this variant showed better values for total color change (ΔE) and color saturation (C). The lowest TBA value was determined in the variant 2, and the highest TBA value was determined in the variant 3. According to the obtained results, with the use of the starter culture CS-300 good quality of the sausages is achieved. At the same time a safe product is obtained where the use of nitrite salt is completely eliminated.

Keywords: traditional sausages, starter cultures, color.

INTRODUCTION

The processing of meat in meat products records its first beginnings in the Phoenicians, and hence it extends all over the world. According to numerous written data, the first meat preparations have been started with the salting of the meat, and later, other means of conservation began to be applied (Hammes and Hertel, 1998; Čavlek, 2001).

The various properties of sausages, including their overall quality, are conditioned, above all, by the type and quality of the meat, but also by the added additives, spices, preservatives, and the applied technological procedures in the production (Feiner, 2006; Honikel, 2008; Leroy et al., 2006).

One of the most types of sausages that are produced in the Republic of Macedonia is the traditional *Vevcanski* sausage, whose formulation is 1400 years old. Traditional food is considered as a legacy that is transmitted by generations, and consumers expect specific sensory properties and high quality food. At the same time, traditional food must be safe from the microbiological and chemical-physical aspect, without uncontrolled processing and without the presence of preservatives.

The color science (colorimetry) has been developed because of the need for an objective evaluation of color characteristics, which can not be achieved solely with human perception of color, that is, because of the need for color to be quantified and expressed in numerical values. The color of the objects does not depend only on the characteristics of the subject itself, but also depends on the light that illuminates the subject, as well as the condition in which the observer is located, because the weary eye has a reduced sensitivity to the color (Đurišić et al. 2007).

There are many color definitions, and according to SRPS ISO standard (SRPS EN ISO 5492: 2012), color is a feeling caused by the stimulation of the retina of light beams at different wavelengths. MacDougall (2002) defines color as a combination of visually understood information contained in the light reflected by the sample.

In sausages and other meat products, the desired color is achieved by adding nitrites and other chemicals, and in a natural way it is obtained by adding appropriate starter cultures (Janssens et al., 2012; Maksimović et al., 2015).

Lipid oxidation is the cause of the most significant chemical changes in food, especially in meat and meat products, which can result in numerous harmful effects on consumer health. The oxidation of unsaturated fatty acids begins with the binding of hydrogen to carbon near the unsaturated bond. As a result, fatty acid decomposition and its degradation occurs to unstable peroxides, which, at the end of the reaction, pass into stable oxidative products. The most toxic are: aldehydes (malonaldehyde, hydroxoneone, hydroxyhexanal), ketones, epoxies, alcohols and other organic molecules. The presence of any of these components causes distortion of the taste and smell of the final product, as well as numerous diseases in the consumer. Malonaldehyde (1,3 dicarbonyl aldehyde, $C_3H_4O_2$) is a very reactive component and is usually found in the enolone form.

From the aspect of full realization of this potential, research on the influence of starter cultures and their metabolites on the quality of foodstuffs is of great importance, and in order to become their obligatory part of the regular, industrial production processes (Bhat et al., 2012; Casaburi et al., 2007). The use of starter cultures in the food industry is a substitute for many chemical additives (including additives containing natural components), which contributes to the creation of new and attractive products containing less chemical preservatives (Arihara, 2006; Demeyer et al., 2000).

The aim of this paper is to monitor the influence of two starter cultures on instrumental values for color and its stability on the surface of industrially produced *Macedonian traditional sausage*, as well as the lipid oxidation.

MATERIAL AND METHODS

As a material for work was used *Macedonian traditional sausage* produced in industrial conditions in the meat industry "Soleta" in Skopje. As a basis for the production of this product was taken the traditional formulation of *Vevcanski* sausage, modified for industrial use. Pork meat (I category) and dorsal bacon were used in the ratio 75: 25%. Water was added in an amount of 150 g/kg mixture. Then additives, spices and starter cultures were added to the mixture. The following starter cultures were used: CS-300 *(Staphylococcus carnosus ssp. utilis*) and BLC-78 (*Pediococcus acidilactici* + *Staphylococcus carnosus*).

The research in this paper covered three variants:

- *Variant 1*: Control variant (conventionally produced *Macedonian traditional sausages* using nitrite salt and powdered acerola);
- *Variant 2*: Macedonian traditional sausages where the basic formulation was enriched by the addition of starter culture CS-300 in combination with powder Swiss chard (as a substitute for nitrite salt) and powdered acerola;
- *Variant 3*: Macedonian traditional sausages where the basic formulation was enriched by the addition of starter cultures CS-300 and BLC-78 in combination with powder Swiss chard (as a substitute for nitrite salt) and powdered acerola.

The meat and bacon were ground to pieces of 8 mm. Then all spices and starter cultures were added, according to the determined formulation. The aim of adding Swiss chard powder is to provide a natural source of nitrates that the added starter cultures will convert into nitrites with which it is expected to achieve better results compared to the control variant where nitrite salt is added during production, and the only source nitrates is the leek that is part of the basic formulation. In this way, not only nitrite salt is completely excluded from use, thus eliminating its adverse effects on the health of consumers, but also improvements in the quality of sausages have been achieved. The meat, together with the added spices and starter cultures, was mechanically mixed in a stirrer. Then, the mixture was left to stand for 48 hours in a refrigerator at a temperature of 1-3 °C.

After leaving the mixture, it was accessed to machine filling of the sausages, where during the filling of the mixture of each variant, detailed washing and cleaning of the filler was carried out. Sausages were then thermally treated according to a program that was created according to the needs and modification of the basic formulation.

For measuring the instrumental parameter color, randomly selected three *Macedonian traditional sausages* from each variant separately were taken, and the measurement was done on the surface of the samples. This parameter is determined on the finished product on the 4th and 30th day of production.
For determining this parameter, the colorimeter Dr Lange, spectro color, was used. Before each series of measurements, the instrument was calibrated using a white calibration plate CR-A43, according to the standard procedure of the production instructions. The color characteristics are expressed according to CIE L^* a^* b^* (CIE, 1976), which is based on three coordinates that define the color of the samples: L^* (lightness), a^* (redness (+a^*) or green (-a^*)) and b^* (yellowness (+b^*) or blue (-b^*)). The measured values L^* a^* b^* were read directly from the colorimeter, and based on these three values, the following color parameters are calculated using the appropriate mathematical relations:

Total color change (ΔE):
The total color change (ΔE) is calculated in relation to the standard sample, which determines the influence of a factor (in this study the influence of starter cultures) on the characteristics and color quality.

$$\Delta E = \sqrt{(L_0^* - L^*) + (a_0^* - a^*) + (b_0^* - b^*)^2}$$

where: L_0^*, a_0^* и b_0^* - parameters of the standard (the control variant 1 was taken as the reference value in this study);
L^*, a^* и b^* - sample parameters (variants 2 and 3)

Color Saturation (C):*
Color Saturation (C*) is a measure of the degree of color purity. In the center of the coordinate system is 0 and increases with the distance of the color from the center to the peripheral parts. It is calculated on the basis of parameters a^* and b^*

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Hue angle (h):
The hue angle (h) is calculated on the basis of the parameters a^* and b^*, and determines the value of the angle under which the corresponding color is located (point A, B, C), counting with respect to the + a^* axis of the coordinate system .

$$h = \tan^{-1}(b^*/a^*)$$

Degree of lipid oxidation (TBA test)
The degree of lipid oxidation (TBA test) was determined in the finished product on 42nd day of production according to the method of Tarladgis et al. (1960), modified by Shahidi et al. (1987). This method is based on the reaction of the aldehydes, formed as secondary products in the oxidation of lipids with thiobarbital acid, whereby the product produces complex compounds with pink staining. By measuring the absorbance of the obtained complex, the extent of lipid oxidation is directly determined. The values from the distillate were reading on the spectrophotometer (Jenway 6305 UV-VIS) at a wavelength of 532 nm, which are then multiplied by a factor of 8.1, which are converted into TBA numbers, defined as the concentration of malonaldehyde in mg/kg (mg MDA/kg).
Presented data are statistically processed with ANOVA test in SPSS package.

RESULTS AND DISCUSSION
The results of the instrumentally measured values for the color of the sausage surface, L^*, a^* and b^*, as well as the values for ΔE, C and h are shown in Table 1 and Table 2.

Table 1. Average values of instrumental color analysis on the surface of sausages

Sample	4th day of production			30th day of production		
	*L**	*a**	*b**	*L**	*a**	*b**
Variant 1 (control)	52,911[a] ± 0,95	17,642[a] ± 0,26	29,907[a] ± 0,52	51,468[a] ± 1,30	16,276[a] ± 0,93	30,999[a] ± 0,53
Variant 2 (CS-300)	38,301[b] ± 6,23	36,678[b] ± 1,48	65,855[b] ± 2,48	52,330[a] ± 2,20	19.773[a] ± 0,93	31,980[a] ± 2,65
Variant 3 (CS-300 + BLC-78)	39,543[b] ± 1,50	34,410[c] ± 1,14	67,880[c] ± 2,05	52,781[a] ± 1,70	17,695[a] ± 0,58	31,060[a] ± 1,21

[a, b, c] – the values for *L**, *a**, *b** per days marked with different letters have a statistically significant difference between the examined variants ($p<0.05$)

Table 2. Average values of instrumental color analysis on the surface of sausages

Sample	4th day of production			30th day of production		
	ΔE	C	h	ΔE	C	h
Variant 1 (control)	Reference	34,72[a] ± 0,01	59,45[a] ± 0,08	Reference	35,01[a] ± 0,01	62,29[a] ± 0,01
Variant 2 (CS-300)	43,16	75,38[b] ± 0,01	60,88[b] ± 0,01	3,79	37,60[b] ± 0,01	58,27[b] ± 0,01
Variant 3 (CS-300 + BLC-78)	43,50	76,10[c] ± 0,01	63,11[c] ± 0,01	1,93	35,75[c] ± 0,01	59,94[c] ± 0,01

[a, b, c] – the values for *L**, *a**, *b** per days marked with different letters have a statistically significant difference between the examined variants ($p<0.05$)

Lightness values (L*)

According to the data from Table 1, can be noted that with the highest value for *L**, i.e. with the brightest color on the 4th day of production, are characterized the sausages from the control variant (52.911), while on the 30th day of production, i.e. in the course of storage, a decrease in this value is noted up to 51.468. On the 4th day of the production, the sausages of variant 2 are characterized by the lowest value for the color light, which is 38.301, but already on the 30th day of the production, this variant shows a significant increase in value (52.330), indicating the fact during storage, the number and activity of the present microflora is reduced, but the sausages do not receive dark color, but it becomes brighter and does not lose the attractiveness, that is approaches the color of the sausages from the control variant.

A similar trend to increase the *L** value is also determined in the samples of variant 3, where from 39.543 on 4th day of production, the value reaches up to 52.781 on 30th day of the production. On 4th day of the production, a statistically significant difference ($p<0.05$) was found in variant 2 and variant 3 in relation to the control variant. Between variant 2 and variant 3 there is no statistically significant difference ($p>0.05$). On 30th day of production, there are no statistically significant differences between the investigated variants.

Values for redness (a*)

From the data in Table 1, can be seen that the highest value for redness was measured in the sausages of variant 2 (36.678) on 4th day of the production. During storage, the number of microorganisms decreases, thereby reducing their activity. As a result, sausages have a tendency to decrease the intensity of red. However, on the 30th day of production, the most intense red color (19.773) was measured in the sausages of variant 2. In the sausages of variant 3, where two starter cultures were applied, the value for the intensity of redness, from 34.410 on the 4th day of production is reduced to 17.695 on the 30th day of production. The lowest average value for *a** (17,642) on the 4th day of production was measured in the sausages from the control variant, which

slightly decreased to 30th day (16.276), which is also the lowest value compared to the other variants.
On the 4th day of production, variant 2 and variant 3 have statistically significantly differences ($p<0.05$) compared to the control variant. There is a statistically significant difference ($p<0.05$) between variant 2 and variant 3. On the 30th day of production, there are no statistically significant differences between the examined variants ($p>0.05$).

Value for yellowness (b*)
Regarding the yellowness, the highest value on the 4th day of production was measured in sausages from variant 3 (67.880), which during the storage, on the 30th day is reduced to 31.060. Similar values were also measured in the sausages of variant 2, where from 65.855 on the 4th day of production, *b** is reduced to 31.980 on the 30th day of production, which is also the highest value in the analyzed time interval, indicating that sausages of this variant show the greatest tendency to retain color during storage. In the control variant, on the 4th day of production, *b** has a value of 29.907, while on the 30th day it is 30.999, which is at the same time the lowest value. According to the obtained values, on the 4th day of production, statistically significant difference ($p<0.05$) is found in variants 2 and 3 compared to the control variant, as well as between variant 2 and variant 3. On the 30th day of production, there is no statistically significant differences between the examined variants ($p>0.05$).

Total color change values (ΔE)
In order to determine the total color change of the surface area of the sausages from varieties 2 and 3 where starter cultures are applied, the control variant (variant 1) is taken as a reference trial because it is produced according to the traditional recipe of *Vevcanski* sausage using nitrite salt, modified for industrial applications. According to the obtained calculations, on 4th day of the production, a greater total color change is observed in the samples of variant 3 (43.50), compared with the samples of variant 2 (43.16). On 30th day of the production, a greater color change is observed in variant 2 (3.78) compared to variant 3 (1.93). Based on these data, it can be concluded that during the storage, in the sausages of variant 3, the activity and the presence of starter cultures and in general the present microflora decreases, which directly affects the decrease in the intensity of the color, i.e. the approximation to the color of the control variant, where this and other quality properties are achieved as a result of the added nitrite salt. On the other hand, sausages of variant 2 indicate greater color consistency during storage.

Color saturation values (C*)
According to the data shown in Table 2, sausages of variant 3 (76.10) on 4th day of the production are characterized with the highest color saturation, and this value decreases to 35.75 on the 30th day of production. Sausages of variant 2 on 4th day of the production show a slightly lower color saturation value (75.38) compared to the sausages of variant 3, but, on the contrary, on the 30th day of production, the sausages of variant 2 have the highest a color saturation value of 37.60. The lowest values for this parameter are characterized in the sausages from the control variant, where on the 4th day of production a value of 34.72 was obtained, while on the 30th day there was a minimal increase in this value to 35.01. According to the statistical analysis data, it can be noticed that on day 4th and 30th day of the production there is a statistically significant difference ($p<0.05$) in variants 2 and 3 compared to the control variant, as well as between variant 2 and variant 3.

Hue angle values (h)

On 4th day of the production, the highest value for the hue angle (*h*) on the sausage surface was calculated for the samples of variant 3 (63.11), while on the 30th day of the production the samples of variant 3 are characterized with the lowest value for this parameter (59.94). Sausages of variant 1, on 4th day of production, have the lowest value for this parameter (59.45), which increases to 62.29 on 30th day of production. Sausages from variant 2 are characterized by the most constant value for the hue angle that is 60.88 on the 4th day of production and 58.27 on the 30th day of production. A statistically significant difference ($p<0.05$) is determined between variants 2 and 3 compared to control variant, as well as between variant 2 and variant 3, on the 4th and on the 30th day of the production.

In the studies of Elías and Carrascosa (2010) for determining the color of the Portuguese traditional sausage *Paio do Alentejo*, the following color parameters have been obtained: *L** (43.1), *a** (16.4) and *b** (13.2). According to this recipe, the color is achieved as a result of the added nitrates in the form of $NaNO_3$ (0.039%) and KNO_3 (0.008%), and nitrites in the form of KNO_2 (0.0076%). Compared with the values of the control variant of the *Macedonian traditional sausage*, the Portuguese sausages show a lower color intensity for all three parameters.

Škaljac (2014) points out that sausages produced without the addition of starter cultures, in traditional conditions, were darker at the end of the production process ($p<0.05$) compared to sausages in which starter cultures were applied ($p<0.05$), where a higher pH value was also noted. The research conducted with the addition of starter cultures in the production of *Macedonian traditional sausage*, completely coincides with the literature data from the aspect of lightness. Namely, in sausages from both variants in which starter cultures are added, color illumination is observed during storage, compared with the control variant, where the value for the lightness decreases, i.e. the sausages have a darker color.

According to El Adab et al. (2014), the development of color in fermented sausages is largely conditioned by the duration of ripening of sausages rather than by added starter cultures. The authors point out that *L**, *a** and *b** values are usually reduced during the maturation process in the event that starter cultures are not added.

Table 3. Average TBA values (mg MDA/kg) in sausages

Sample	n	42nd day of production
		$\bar{x}$ ± SD
Variant 1 (control)	2	0,16[a] ± 0,01
Variant 2 (CS-300)	2	0,12[a] ± 0,04
Variant 3 (CS-300 + BLC-78)	2	0,21[a] ± 0,05

[a] – TBA values marked with same letters have no statistically significant difference between the examined variants ($p>0.05$)

According to the results shown in Table 3 for the determined TBA values on 42nd day of the production, it can be seen that the sausages from the variant 3 are characterized with the highest content of malaldehyde (0,21 mg MDA/kg). The lowest average TBA value was determined in sausages from the variant 2 (0,12 mg MDA/kg), while sausages from the control variant have an average TBA value of 0,16 mg MDA/kg. However, this minimal lipid oxidation did not change the sensory properties of any of the variants, because to induce a change in taste or odor, the minimum TBA value should be 0,6 mg MDA/kg. On the other hand, if the values are equal to or higher than 1 mg MDA/kg, in this case the product may cause certain health disorders among the consumers. According to the obtained results, no statistically significant differences were found between the three examined variants.

Ferarri and Torres (2002) in their studies have determined the degree of oxidation of lipids by the TBA test and the possible relationship with other parameters that define the quality properties of sausages from the Brazilian market "sacolões". The authors have proven that the mean TBA value was 0,44 mg MDA/kg. Lorenzo et al. (2000) points out that in sausages of the type "Botilho" the

average TBA value is 0,96 mg MDA/kg, while in sausages "Androlla" is much higher and it is 4,05 mg MDA/kg. On the other hand, literary data show that in smoked pig bacon, poultry products, as well as in burgers (Wang et al., 1995), TBA values are many times higher than those obtained in this research. Greene and Cumuze (1981) found that higher TBA values of 0,6 mg MDA/kg cause changes in the sensory properties of sausages, and according to this criteria, the authors point out that 16,70 % of the sausages in the markets have inadequate sensory properties, while 11,10 % of sausages have high critical TBA values.

CONCLUSION

According to the presented data for the instrumental measurement of the color of the surface of *Macedonian traditional sausage* from all three variants, on the 4th and the 30th day of production, can be concluded that in all samples there is a tendency of reduction of the color values over the course of storage. The lightness of the color (*L**) continuously decreases in the control variant, resulting in a loss of color in the specified time interval. This phenomenon is not observed in the samples of variants 2 and 3, and contrary to this, these samples show an increase in the values for lightness.

From the aspect of retaining the values for redness (*a**) and the yellowness (*b**), a better effect showed a starter culture that was added to variant 2 (CS-300 - *Staphylococcus carnosus spp. utilis* and *Staphylococcus carnosus*) Thus, the samples of this variant showed better values for total color change (ΔE) and color saturation (C).

On the other hand, the lowest TBA value was determined in the variant 2 (CS-300), and the highest TBA value was determined in the variant 3 (CS-300 + BLC-78).

Starter cultures have a positive influence on the color and oxidation of lipids in the industrially produced *Macedonian traditional sausage*. According to the obtained results, with the use of the starter culture CS-300 *(Staphylococcus carnosus ssp. utilis)* good quality of the sausages is achieved. At the same time a safe product is obtained where the use of nitrite salt is completely eliminated.

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