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*Original scientific paper*

# Effect of Antioxidants on the Colour Stability of Fermented Sausage "Sucuk" Type

Danica Savanović[1], Slavica Grujić[1], Radoslav Grujić[2], Jovo Savanović[1]
[1]University of Banja Luka, Faculty of Technology, Banja Luka
[2]University of East Sarajevo, Faculty of Technology Zvornik

**Abstract:** **Purpose** - The aim of this study was to investigate effect of some antioxidants on the cross section color stability of dry-fermented beef sausage "sucuk" type, during 120 minute exposure of sausage slices to air, at the temperature of 20°C and 4°C.

**Design/methodology/approach** - Samples were produced in industrial conditions, seven experimental model samples with selected antioxidants added separately to each (sodium ascorbate, rosemary extract, green tea extract, ascorbic acid, ascorbyl palmitate, tocopherols and butylatedhydroxyanisole) and control sample, without antioxidants. Colour parameters, expressed as CIE L*, a* and b* values, total colour difference (ΔE), the kinetics of colour change, chemical composition and pH value were determined.

**Findings** - The cross section colour of all samples significantly changed ($p<0.05$), due to exposition of sausage cuts to air, but significantly smaller changes in colour ($p<0.05$) were observed in the samples kept at lower temperature. Dry-fermented beef sausage "sucuk" type, produced with selected antioxidants, can be sliced 120 minutes before serving and stored at the temperature of 4°C, and cross section colour changes are unrecognisable, but at the temperature of 20ºC colour changes are significantly greater. Addition of natural antioxidant, rosemary extract and green tea extract, have a positive effect on colour stability of tested sausage "sucuk" type.

**Originality/value -** This paper provides information on colour changes during exposition of dry sausage cuts to air and the findings may be interesting for entrepreneurs whose activity is connected with serving these products in restaurants, catering services or the households.

**Key words:** fermented sausage, colour stability, antioxidants

## Introduction

Dry-fermented sausage "sucuk" type is a very popular meat product in Turkey and in most Middle Eastern Countries and Europe (Bozkurt and Erkmen, 2002, Operta and Smajić, 2006). Manufacturing of the sucuk varies regionally, and different formulations exist. Ingredients used for its production usually are: beef, beef backfat, tail fat, salt, sugar, garlic, nitrite and/or nitrate and various spices (Yıldız-Turp and Serdaroglu, 2008).

One of the most important quality attributes of sucuk is colour, since it influences consumer acceptability (Bozkurt and Erkmen, 2002; Bozkurt and Bayram, 2006). Colour stability during storage and light time acting are very important quality attributes of meat products. Meat colour depends on the concentration and redox state of haem pigments in meat (Wójciak et al., 2011). In fermented sausages, the pigment responsible for the characteristic bright red colour of cured meat is the nitrosylmyoglobin ($MbFe^{II}NO$). The haem pigments in meat are the compounds that can be easily changed due to various physicochemical factors impact (Nam and Ahn, 2003, Rohlík et al., 2010) and they can be easily oxidized (Phung et al., 2013, Sorheim and Hoy, 2013). The oxidation of haem pigments and lipids cause the appearance of deterioration and colour changes at fermented sausages. During exposition of sausage cuts to light and oxygen, these reactions are accelerated (Hoz et al., 2004; Rohlik et al., 2010; Wojciak et al., 2012). Oxidative discoloration of cured meat products, transforms the nitrosylmyoglobin into nitrate and metmyoglobin ($MbFe^{3+}$). This process depends on partial oxygen pressure and myoglobin reducing systems in general and is related to the subsequent lipid oxidation (Wójciak et al., 2011; Parra et al., 2012). Lipid and pigment oxidation are the two main causes of quality deterioration limiting the quality and acceptability of meat and meat products. Lipid and pigment oxidation leads to discoloration, drip losses, off odor development, the production of potentially toxic compounds and modification of nutritional characteristics (Haile et al., 2013). Oxidation changes of myoglobin are critical to muscle-based food product appearance, because meat purchasing decisions are influenced by color more than any other quality factor and consumers use discoloration as an indicator of freshness and wholesomeness, and browning as an indicator of spoilage. According to Mancini and Hunt (2005), a 15% price reduction of retail meat is reported due to surface discoloration. Therefore, it is desirable to control oxidation processes and improve the quality of food products by addition of inhibitory substances (Gramza and Korczak, 2005).

The rate of lipid oxidation can be effectively controlled or minimized, by natural or synthetic antioxidants addition (Georgantelis et al., 2007; Mata et al., 2007; Grujić et al., 2009). Commonly used antioxidants in the meat products are: α-tocopherol, ascorbic acid, sodium ascorbate and ascorbyl palmitate (Georgantelis et al., 2007, Nam et al., 2006, Sorheim and Hoy, 2013). The most widely used synthetic antioxidants by the food industry are butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG), whose action represents the quick donation of a hydrogen atom to the free radical (Aranha and Jorge, 2012). The increasing consumer awareness and health consciousness, however, results in pressure to avoid synthetic additives use, which necessitates the use of natural additives to extend shelf life and/or improve product safety (Georgantelis et al., 2007; Carpenter et al, 2007; Velasco and Williams, 2011). Food products should meet the needs of consumers, and also, primarily, they should be safe. Therefore, we should agree on the opinion that "consumers expect both: a large selection of competitive (in terms of price), highly processed, convenience food products as well as fresh food with high nutrition values which is tasty and first-of-all safe" (Dabrowska, 2011). Sources of natural antioxidants usually are spices, herbs, teas, oils, seeds, cereals, cocoa shell, grains, fruits, vegetables (Mata et al., 2007; Karabacak and Bozkurt, 2008; Opara and Al-Ani, 2010; Maciel et al., 2011).

To investigate the treatment effects on colour and determine colour of meat and meat products it can be used one of the three major approaches i.e. using panelists (Visual inspection), colour measuring instrument (Instrumental) and measuring myoglobin concentration (Chemically). Colour standards are often used as reference material to carry out more objective colour analysis. Unfortunately, this method requires more specialized training of the observers and for this reason the use of colour measuring instruments such as the widely used L*a*b* colour space is recommended (Mancini and Hunt, 2005; Haile et al., 2013). This system is very effective for measuring color differences and tracking color changes during processing and storage (Wrolstad et al., 2005; Rohlík, et al., 2010; Haile et al. 2013).

Fermented sausages are generally eaten cold. Before serving in the restaurants, catering services and in the household they are usually cut into thin slices. However, during the exposition of sausage cuts to air, sensory characteristics are changing, especially cross section colour. The aim of this study was to investigate effect of some antioxidants on the cross section colour stability of dry-fermented beef sausage "sucuk" type during 120 minute exposure of sausage slices to air, at the temperature of 20°C and 4°C, using instrumental colour analysis.

## Materials and methods

### *Sausage formulation and procesing*

Dry-fermented beef sausage "sucuk" type was used in the experiment as a model-product. Samples were produced in industrial conditions, according to the producer's specification. The following ingredients were used (quantity shown in descending order): beef, beef fat, salt, dextrose, starter culture, spices and preservative E 250. Seven model samples are produced with adding of different antioxidants (sodium ascorbate, rosemary extract, green tea extract, ascorbic acid, ascorbyl palmitate, tocopherols and butylatedhydroxyanisole), in the recommended concentrations (Table 1). The eighth model sample was produced without antioxidants, and used as a control sample. Frozen beef meat and beef fat, sliced into smaller pieces using the machine for frozen meat cutting (Farm Power, Type: SM 501, Slovakia), were used for the production of sausage model samples. Required amounts of meat and fat were weighed on an electronic platformer scale (Level, Type: Sigma 5ND12/600, Slovenia), and the required amount of additives and spices were weighed on an electronic scale (Level, Type: GAMMA PTE-N, Slovenia). Ingredients were chopped in cutter (Seydelmann, Type: DC 8 206 K, Germany).

**Table 1.** Type and quantity of antioxidants used for producing dry-fermented beef sausage "sucuk" type

| Sample code | Additive commercial name and producer | Additive ingredients | Quantity in product (%) |
|---|---|---|---|
| 1 | Miocolor VS, IREKS AROMA, Croatia | E 301, organic acids, dextrose | 0.30 |
| 2 | GUARDIAN Rosemary Extract 09, DANISCO, Denmark | Natural rosemary extract, phenolic diterpenes, E 433, E 1520 | 0.08 |
| 3 | GUARDIAN Green Tea Extract 20S, DANISCO, Denmark | Natural green tea extract, catechins, salt | 0.03 |
| 4 | Miocolor, IREKS AROMA, Croatia | E 300, organic acids, dextrose | 0.30 |
| 5 | GRINDOX ASCORBYL PALMITATE, DANISCO, Denmark | E 304 | 0.05 |
| 6 | GRINDOX 539, DANISCO, Denmark | Repeseed oil, E 304, E 306, E 322 | 0.075 |
| 7 | GRINDOX 105, DANISCO, Denmark | E 320, repeseed oil | 0.03 |
| 8 | - | - | - |

### *Packing and storage of samples*

Prepared sausage batter was filled into artificial collagen casings (43 mm diameter), on filling machine (Handtmann, Type: HF 622, Germany) and the sausages, approximately mass of 760g, were closed on both sides with metal clips (Poly Clip, Type: FCA 3430-18, Germany). The sausages were fermented and ripened in climate chambers (Mauting, Czech Republic) for 19 days during which the temperature declined from 25°C to 17°C and the relative humidity dropped from 90% to 77%. Technological parameters of the process during the ripening period are shown in Table 2. After the ripening process, the sausages with an approximate mass of 530g were individually vacuum packaged, using packaging machines (Multivac, Type: R 230, Germany). Multilayer film made of polyamide (PA) and polyethylene (PE), the structure of PA / PE / PA / PE, in which the thickness of the lower sheet was 200 μm and the upper film 100 μm, was used for sausage packaging. The samples were stored in vacuum packaging, at the temperature of 4°C, under usual storage conditions as the same kind of product. The shelf life of this product is six months and all analyses were performed during sixth months of storage, before the deadline.

**Table 2.** The dry-fermented beef sausage "sucuk" type ripening conditions

| Time (days) | Temperature (°C) | Relative humidity (%) | Smoking (hour) |
|---|---|---|---|
| 1 | 25 | 90 | - |
| 2 | 25 | 90 | 8 |
| 3 | 22 | 85 | 8 |
| 4 | 20 | 85 | - |
| 5 | 20 | 83 | - |
| 6 | 20 | 83 | - |
| 7-8 | 18 | 81 | - |
| 9-15 | 18 | 79 | - |
| 16-17 | 18 | 77 | - |
| 18-19 | 17 | 77 | - |

### *Instrumental colour measurement*

Instrumental color measurement was performed on the cross section of dry-fermented beef sausage "sucuk" type, during the exposition of sausage cuts (or sausage slices) to air, at the temperature of 20°C and 4°C. The sausages were cut in slices, approximately 20 mm thick. Measurements were carried out immediately after cutting and after 30, 60, 90 and 120 minutes of exposure to air. Colour measurements were performed using Spectrophotometer CM-2600d (KONICA MINOLTA SENSING INC, 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 with respect to the white calibration plate. Colour parameters, expressed as CIE L*, a* and b* values, were determined as indicators of lightness, redness and yellowness. Five measurements were taken on the three cross sections of two sausages from each treatment. The mean of 30 measurements was recorded for each colour parameter. Total colour difference (ΔE) expresses the stability of colour, and it was calculated according to the following formula (Szmańko et al., 2006; Wójciak et al., 2012):

$$\Delta E = ((L_o^* - L_x^*)^2 + (a_o^* - a_x^*)^2 + (b_o^* - b_x^*)^2)^{1/2}$$

where:

$\Delta E_X$ (means $\Delta E_{30}$ or $\Delta E_{60}$ or $\Delta E_{90}$ or $\Delta E_{120}$) – colour stability of dry-fermented beef sausage "sucuk" type after 30, 60, 90 or 120 minutes of sausage slices exposure to air;

$L_o^*$, $a_o^*$, $b_o^*$ – values of colour parameters L*, a*, b* measured on the cross section of dry- fermented beef sausage "sucuk" type, immediately after cutting the sausage;

$L_x^*$, $a_x^*$, $b_x^*$ – values of colour parameters L*, a*, b* measured on the cross section of dry-fermented beef sausage "sucuk" type, after 30, 60, 90 or 120 minutes of sausage slices exposure to air.

Larger $\Delta E_X$ value means larger colour changes what is equal to smaller colour stability.

Changes in colour L*, a* and b* and ΔE values were investingated using the following zero and first order kinetics, as shown in equations (Kumar et al., 2012):

$$C = C_0 \pm k_0 t$$

$$\ln C = \ln C_1 \pm k_1 t$$

where C is the measured value of response, $C_0$ is the initial value of the corresponding response, t is the storage time and $K_0$, $K_1$ are the reaction rate constants for zero and first order respectively. Whereas (+) and (-) signs represent the increase and decrease in the corresponding response.

### *Chemical composition*

Moisture content (drying at 105°C to constant mass), fat content (according to the Soxhlet method) and protein content (according to the Kjeldahl method) were determined according to AOAC procedures

(A.O.A.C., 2006). Analysis of chemical composition was carried out in Laboratory for food analysis at the Faculty of Technology, University of Banja Luka, Bosnia and Herzegovina. All analyses were carried out with 6 replicates (6 sausages from each treatment).

### *pH measurement*

pH values were measured using a pH meter (Testo 252, Lenzkirch, Germany) equipped with a stab glass electrode (Testo pH Electrodes, Typ 01-06, Germany) for the direct measurement of pH in meat and meat products. Before and during the values reading, calibration of the pH meter was realized using standard buffer solution (pH buffer calibration was 7.02 and 4.00 to 20°C). Measurements were carried out with 6 replicates (6 sausages from each treatment).

### *Statistical analysis*

Statistical analysis of the results was performed using the software package Minitab 14. The data obtained for instrumental colour measurement were statistically analysed by a two-factor factorial arrangement. The factors were storage time (0, 30, 60, 90, 120 minutes of sausage slices exposure to air) and type of antioxidants used for producing sausage model samples. Kinetic data was analysed by using linear regression. The level of significance $p<0.05$ was used for all comparisons (Komić, 2000; Lovrić et al., 2006).

## Results and discussion

### *Colour changes of sliced sausages, at the temperature of 20°C*

Colour is one of the most important quality attributes of sucuk since it affects overall quality. The results of color parameters, expressed as CIE L*, a*, b* values, measured on the cross section of dry-fermented beef sausage "sucuk" type immediately after cutting of the sausages and after 30, 60, 90 and 120 minutes of sausage slices exposure to air, at the temperature of 20°C (or during 120 min storage sliced samples at the temperature of 20°C) are shown in Figure 1, 2, 3. Color (L*, a*, b*) values were affected by ripening time ($p<0.05$) and the addition of antioxidants into the sausage batter ($p<0.05$).

Values for color parameter L* (lightness), color parameter a* (redness) and colour parameter b*(yellowness) on the cross section of all model samples decreased significantly ($p<0.05$) during 120 minutes, at the temperature of 20°C (Figure 1, 2, 3). Similarly, Rohlík et al. (2010) found that the lightness L* of the dried sausages, which had been exposed to the light, gradually decreased. They reported that the decrease of lightness L* was affected by the drying of the sample, which obviously led to a darker colour due to a higher concentration of haem pigments. Hoz et al. (2004) examined the color changes of dry fermented Spanish sausage at three different analysis time periods (freshly cut sausages, 4 and 24 h after cutting), at the room temperature. They noted a clear influence of air exposure after cutting (0, 4, 24 h) for L* value, and the a*- and b*-values were almost constant (no significant differences were found). This observation may be explained by the loss of superficial water and oxidation processes.

The analysis of the colour stability of dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 20°C revealed that the cross section colour of all samples changed, due to the exposition of sausage cuts to air (Table 3). In all tested samples, the smallest color change was observed after 30 minutes, and the total colour difference ($\Delta E$) increased during storage time. The value of $\Delta E$ represents relative color changes that an observer might report for the materials after treatment or between time periods. Thus, $\Delta E$ is more meaningful than the individual L*, a* and b* values.

According to International Lighting Commission (CIE), the total colour difference (ΔE) between 0 and 2 is unrecognisable, and from 2 to 3.5 recognisable by an inexperienced observer, whereas the value exceeding 3.5 constitutes a significant colour deviation for the observer (Wójciak et al., 2012). The criterion classified total colour differences (ΔE) relevantly to the human perception of colours. Thus total colour differences of sample 4, produced with ascorbic acid, after 30 minutes, were unrecognisable ($\Delta E_{30}$=1.91), total colour differences of samples 1, 2, 3, 6, 7, 8, after 30 minutes were recognisable by an inexperienced observer, and total colour differences of sample 5, produced with ascorbyl palmitate, were significant colour deviation for the observer ($\Delta E_{30}$=4.75) (Table 3). During further time period, after 60, 90 and 120 minutes of sausage slices exposure to air, larger changes of ΔE values were recorded which constitutes a significant colour deviation for the observer. In all tested samples, the largest color change was observed after 120 minutes. The smallest colour changes and the largest colour stability, after 120 minutes were observed in the sample 4, produced with ascorbic acid ($\Delta E_{120}$=5.93), as was expected because ascorbic acid is commonly used as antioxidant in the beef sausages production. During 120 minutes of sausage slices exposure to air, at the temperature of 20°C, slightly smaller colour stability than the sample 4 had sample 3, produced with plant extract of green tea ($\Delta E_{120}$=6.64). Green tea extract contains phenolic compounds that have antioxidant effects. Phenolic compounds could be a major determinant of antioxidant potentials of foods and could therefore be a natural source of antioxidants. Bozkurt (2006) reported that addition of green tea extract (Thymbra spicata oil) in Turkish dry-fermented sausage (sucuk) reduced TBARS, putrescine, histamine and tyramine formation significantly. Aranha and Jorge (2012) reported that the ethanolic extract of oregano has antioxidant potential, and can be used as an alternative to the use of synthetic antioxidants, which can be considered toxic.

Larger colour changes and smaller colour stability, after 120 minutes were observed in samples 8, 7, 1, 6, 2, and the largest colour changes and the smallest colour stability, after 120 minutes were observed in sample 5, produced with ascorbyl palmitate ($\Delta E_{120}$=9.09). The differences in the measured L*, a*, b* values on the cross sections of sausage model samples and different colour stability during the exposition of sausage cuts to air can be connected to the presence of various antioxidants and their activity in the tested samples.

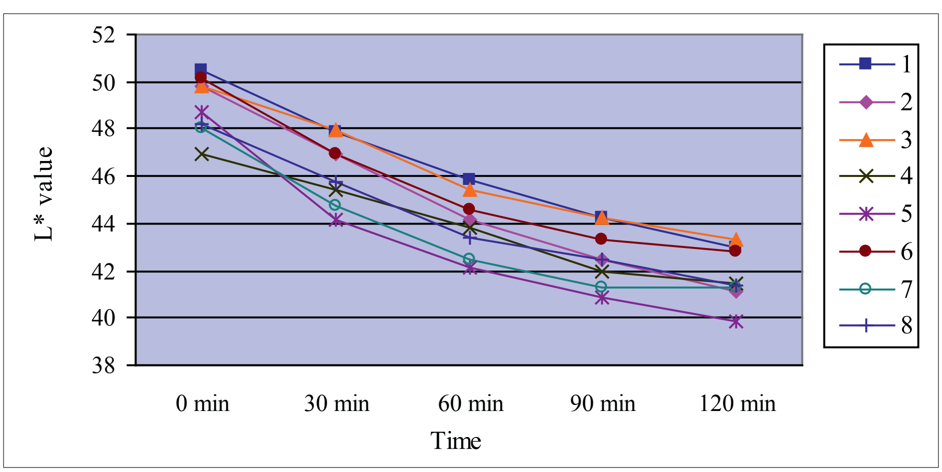


**Figure 1.** Changes L*- values in dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 20°C

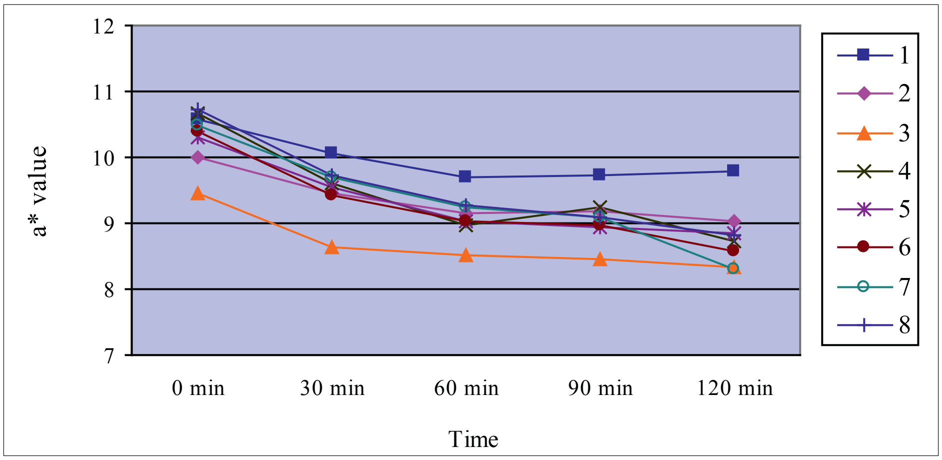


**Figure 2.** Changes a* - values in dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 20°C

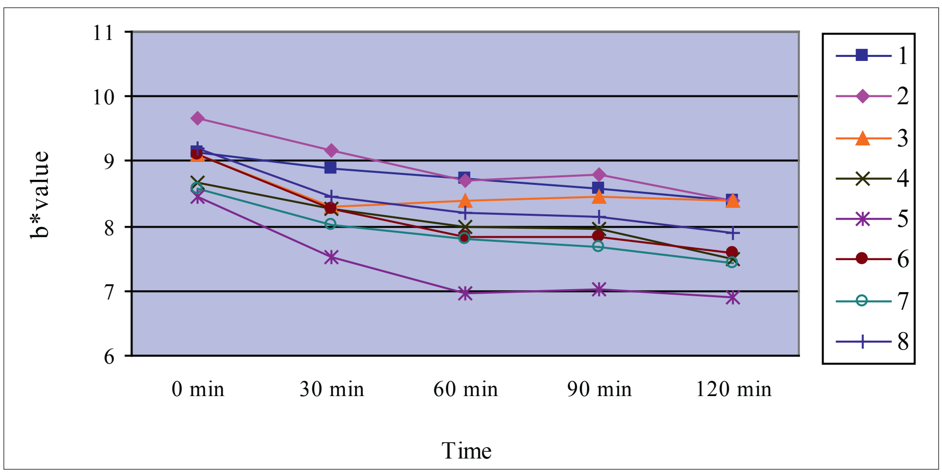


**Figure 3.** Changes b* - values in dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 20°C

**Table 3.** Colour stability of dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 20°C

| Sample code | $\Delta E_{30}$ | $\Delta E_{60}$ | $\Delta E_{90}$ | $\Delta E_{120}$ |
|---|---|---|---|---|
| 1 | 2,60 | 4,66 | 6,30 | 7,51 |
| 2 | 2,94 | 5,78 | 7,45 | 8,82 |
| 3 | 2,23 | 4,60 | 5,76 | 6,64 |
| 4 | 1,91 | 3,68 | 5,24 | 5,93 |
| 5 | 4,75 | 6,91 | 8,08 | 9,09 |
| 6 | 3,45 | 5,91 | 7,07 | 7,77 |
| 7 | 3,44 | 5,80 | 6,94 | 7,21 |
| 8 | 2,77 | 5,08 | 6,09 | 7,20 |

### *Colour changes of sliced sausages, at the temperature of 4°C*

The results of color parameters, expressed as CIE L*, a*, b* values, measured on the cross section of dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 4°C are shown in Figure 4, 5, 6. Lightness (L* value) and redness (a* value) on the cross section of all model samples were affected by storage time ($p<0.05$) and the addition of antioxidants into the sausage batter ($p<0.05$) and decreased significantly ($p<0.05$) during the storage period. Yellowness (y* value) was affected by the addition of antioxidants into the sausage batter ($p<0.05$) and storage time had no effect on the share of yellow colour of tested samples ($p>0.05$), during 120 minutes of sausage slices exposure to air, at the temperature of 4°C.

Comparing instrumental measured colour parameters during 120 minutes of sausage slices exposure to air at 20°C and 4°C, it can be observed that the changes L*, a*, b* values were significantly less ($p<0.05$) in samples stored at a lower temperature (4°C), from which it can be seen that storage temperature affects the cut color change of fermented beef sausage. These results were in agreement with those reported in literature, where a decrease of redness a* occurred in slices of dried sausages exposed to air and the most remarkable changes were observed with the slices exposed to the light at 25°C (Rohlík et al., 2010). They explained that the higher temperature and light conditions accelerated the oxidation reactions of haem pigments and therefore caused the discoloration of the samples.

The analysis of the colour stability of dry-fermented beef sausage "sucuk" during 120 minutes of sausage slices exposure to air, at the temperature of 4°C revealed that the cross section colour of all samples changed, but these changes were significantly less ($p<0.05$) than colour changes of sausage slices exposed to air, at the temperature of 20°C. In all tested samples, the total colour difference (ΔE) increased during storage time. After 30 and 60 minutes of sausage slices exposure to air, at the temperature of 4ºC, the total colour difference (ΔE), in most tested samples was unrecognisable ($\Delta E<2$), except sample 7 ($\Delta E_{60}$=2.27). After 90 and 120 minutes of sausage slices exposure to air, at the temperature of 4ºC, the smallest colour change and the largest colour stability were observed in sample 2, produced with the addition of rosemary extract ($\Delta E_{90}$=0.40; $\Delta E_{120}$=0.64). Larger total colour difference after 90 and 120 minutes was observed in samples 1, 4, 5, 3, 8 but according to International Lighting Commission (CIE), the total colour difference of these samples was unrecognisable ($\Delta E<2$) (Wójciak et al., 2012). After 90 and 120 minutes, sample 6, produced with ascorbyl palmitate and tocopherols and sample 7, produced with butylated hydroxyanisole, had the lowest colour stability (Table 4). Based on these results, it can be seen that sample 2, produced with the addition of rosemary extract, had a greater color stability, during 120 minutes of sausage slices exposure to air, at the temperature of 4°C, than samples 1 and 4, produced with standard antioxidant sodium ascorbate and ascorbic acid, which justifies the use of rosemary extracts as antioxidants in the production of fermented beef sausages. Rosemary (Rosmarinus officinalis) extracts contain antioxidant compounds, the most active being phenolic diterpenes that break free radical chain reactions by hydrogen atom donation (Georgantelis et al., 2007).

Meat products should be safe, stable within the stipulated time period and the corresponding sensory properties. According to the consumer, color is an important parameter of the meat product quality, because it reflects the composition of the product, the freshness of used raw materials and the proper storage conditions. Undesirable color of the meat product is associated with poorer product quality. Product composition, storage time, and duration of exposure to air after threading, have a significant affect on the color change of meat product (Rohlík et al., 2010; Haile et al., 2013). Many chemical reactions can take place affecting the colour of the meat product. The most important chemical reactions that take place are lipid oxidations which in fact affect the oxidation of haem pigments whose red colour turns into brown (Rohlík et al., 2010; Georgantelis et al., 2007; Parra et al., 2012; Haile et al., 2013).

The zero and first order kinetic models were fitted in order to describe the reaction rate as a function of storage time and predict the colour of dry-fermented beef sausage "sucuk" type during sausage slices exposure to air. The L*, a*, b* and ΔE values were used to determine the rate of change in colour. Kinetic parameters from zero and first order reactions of colour (L*, a*, b* and ΔE) are shown in Table 5, 6, 7, 8.

**Table 4.** Colour stability of dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 4°C

| Sample code | $\Delta E_{30}$ | $\Delta E_{60}$ | $\Delta E_{90}$ | $\Delta E_{120}$ |
|---|---|---|---|---|
| 1 | 0,64 | 0,72 | 0,66 | 0,92 |
| 2 | 0,23 | 0,54 | 0,40 | 0,64 |
| 3 | 0,48 | 1,07 | 1,42 | 1,65 |
| 4 | 0,54 | 0,62 | 1,02 | 1,52 |
| 5 | 0,62 | 0,99 | 1,37 | 1,59 |
| 6 | 1,03 | 1,15 | 2,07 | 2,83 |
| 7 | 1,01 | 2,27 | 2,18 | 3,76 |
| 8 | 0,41 | 0,76 | 1,55 | 1,94 |

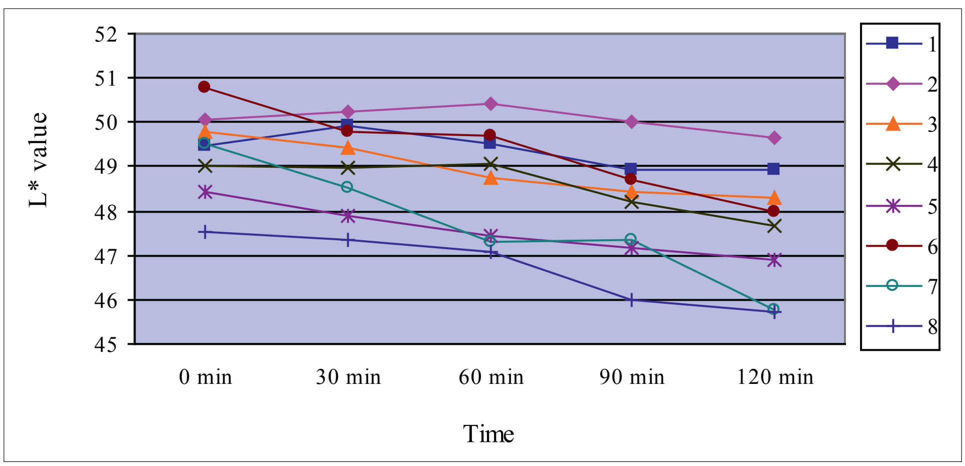


**Figure 4.** Changes L*- values in dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 4°C

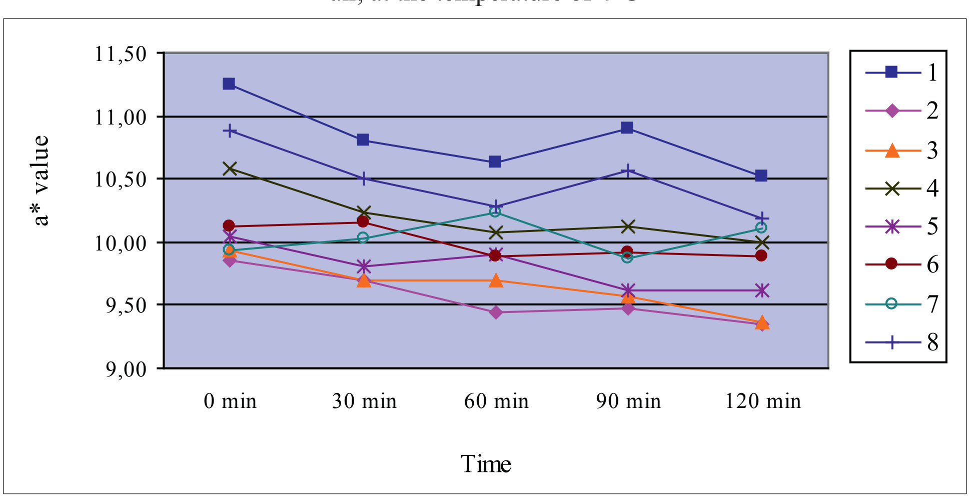


**Figure 5.** Changes a*- values in dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 4°C

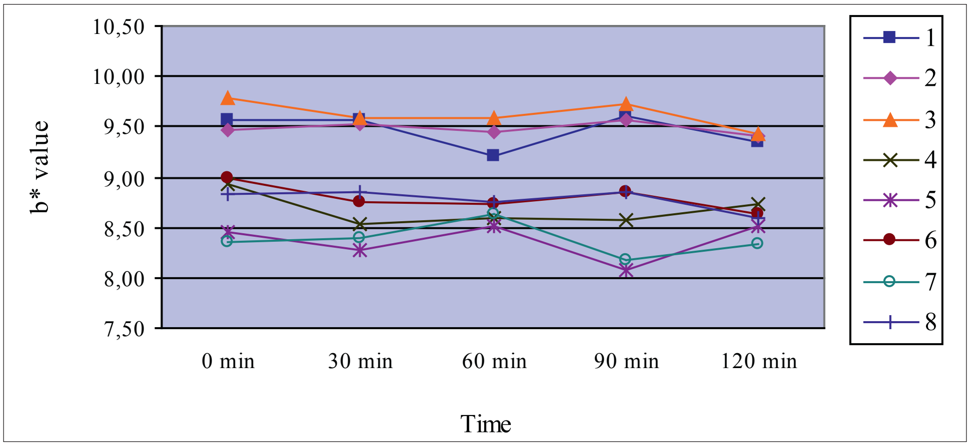


**Figure 6.** Changes b* - values in dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air, at the temperature of 4°C

**Table 5.** Kinetic parameters from zero and first order reactions of colour for L* value

| Re-sponse | Sam-ple | Tempera-ture (ºC) | Zero order | | | First order | | |
|---|---|---|---|---|---|---|---|---|
| | | | $K_0$±SE | $C_0$±SE | $R^2$ | $K_1$±SE | $C_1$±SE | $R^2$ |
| L* | 1 | 20 | -0.061800±0.004917 | 49.9980±0.3613 | 0.981 | -0.001329±0.000088 | 50.0509±0.0065 | 0.987 |
| | 2 | 20 | -0.072800±0.006837 | 49.2580±0.5024 | 0.974 | -0.001611±0.000128 | 49.3284±0.0094 | 0.981 |
| | 3 | 20 | -0.055733±0.006291 | 49.4820±0.4623 | 0.963 | -0.001201±0.000125 | 49.5187±0.0092 | 0.968 |
| | 4 | 20 | -0.048000±0.004270 | 46.8140±0.3138 | 0.977 | -0.001089±0.000093 | 46.8509±0.0068 | 0.979 |
| | 5 | 20 | -0.069700±0.013830 | 47.3320±1.0170 | 0.894 | -0.001588±0.000286 | 47.3430±0.0210 | 0.911 |
| | 6 | 20 | -0.061300±0.010520 | 49.2360±0.7732 | 0.919 | -0.001329±0.000213 | 49.2545±0.0156 | 0.929 |
| | 7 | 20 | -0.056670±0.012560 | 46.9600±0.9229 | 0.872 | -0.001282±0.000272 | 46.9592±0.0200 | 0.881 |
| | 8 | 20 | -0.056467±0.006984 | 47.6220±0.5132 | 0.956 | -0.001267±0.000142 | 47.6551±0.0104 | 0.964 |
| | 1 | 4 | -0.006833±0.003360 | 49.7660±0.2469 | 0.580 | -0.000139±0.000068 | 49.7664±0.0050 | 0.581 |
| | 2 | 4 | -0.003400±0.002770 | 50.2740±0.2035 | 0.334 | -0.000068±0.000055 | 50.2746±0.0041 | 0.336 |
| | 3 | 4 | -0.013400±0.001668 | 49.7480±0.1225 | 0.956 | -0.000273±0.000034 | 49.7500±0.0025 | 0.956 |
| | 4 | 4 | -0.011767±0.003510 | 49.2880±0.2579 | 0.789 | -0.000243±0.000073 | 49.2924±0.0053 | 0.789 |
| | 5 | 4 | -0.012533±0.001153 | 48.3240±0.0848 | 0.975 | -0.000263±0.000023 | 48.3260±0.0017 | 0.977 |
| | 6 | 4 | -0.022200±0.002507 | 50.7200±0.1842 | 0.963 | -0.000450±0.000051 | 50.7296±0.0037 | 0.963 |
| | 7 | 4 | -0.028933±0.004284 | 49.4300±0.3148 | 0.938 | -0.000607±0.000091 | 49.4454±0.0067 | 0.937 |
| | 8 | 4 | -0.016533±0.002947 | 47.7220±0.2165 | 0.913 | -0.000354±0.000064 | 47.7290±0.0047 | 0.912 |

$K_0$, $K_1$ - the reaction rate constants, $C_0$ - the initial response, $R^2$ – coefficient of determination, SE - standard error

**Table 6.** Kinetic parameters from zero and first order reactions of colour for a* value

| Response | Sample | Temperature (ºC) | Zero order | | | First order | | |
|---|---|---|---|---|---|---|---|---|
| | | | $K_0$±SE | $C_0$±SE | $R^2$ | $K_1$±SE | $C_1$±SE | $R^2$ |
| a* | 1 | 20 | -0.006267±0.002536 | 10.3480±0.1864 | 0.671 | -0.000619±0.000250 | 10.3438±0.0184 | 0.671 |
| | 2 | 20 | -0.007367±0.002138 | 9.8100±0.1571 | 0.798 | -0.000776±0.000220 | 9.8078±0.0162 | 0.805 |
| | 3 | 20 | -0.008167±0.002681 | 9.1720±0.1970 | 0.756 | -0.000923±0.000293 | 9.1671±0.0215 | 0.768 |
| | 4 | 20 | -0.014200±0.004328 | 10.2920±0.3180 | 0.782 | -0.001472±0.000436 | 10.2860±0.0320 | 0.792 |
| | 5 | 20 | -0.011800±0.002933 | 10.0460±0.2156 | 0.844 | -0.001241±0.000297 | 10.0435±0.0218 | 0.854 |
| | 6 | 20 | -0.013633±0.002941 | 10.1000±0.2161 | 0.878 | -0.001447±0.000297 | 10.1021±0.0213 | 0.893 |
| | 7 | 20 | -0.016367±0.002083 | 10.3420±0.1531 | 0.954 | -0.001750±0.000220 | 10.3662±0.0162 | 0.955 |
| | 8 | 20 | -0.014833±0.003072 | 10.4200±0.2257 | 0.886 | -0.001530±0.000290 | 10.4213±0.0213 | 0.903 |
| | 1 | 4 | -0.004533±0.002218 | 11.092±0.1630 | 0.582 | -0.000416±0.000204 | 11.0908±0.0150 | 0.583 |
| | 2 | 4 | -0.004100±0.000839 | 9.8080±0.0616 | 0.888 | -0.000427±0.000087 | 9.8085±0.0064 | 0.890 |
| | 3 | 4 | -0.004200±0.000702 | 9.9000±0.0516 | 0.923 | -0.000436±0.000073 | 9.9016±0.0054 | 0.922 |
| | 4 | 4 | -0.004300±0.001309 | 10.4660±0.0962 | 0.782 | -0.000418±0.000126 | 10.4652±0.0092 | 0.786 |
| | 5 | 4 | -0.003533±0.001028 | 10.0080±0.0756 | 0.797 | -0.000360±0.000104 | 10.0085±0.0077 | 0.798 |
| | 6 | 4 | -0.002300±0.000854 | 10.1320±0.0628 | 0.707 | -0.000230±0.000085 | 10.1320±0.0063 | 0.708 |
| | 7 | 4 | 0.000533±0.001677 | 10.0020±0.1233 | 0.033 | 0.000053±0.000167 | 10.0015±0.0123 | 0.032 |
| | 8 | 4 | -0.004500±0.002082 | 10.7560±0.1530 | 0.609 | -0.000427±0.000198 | 10.7553±0.0145 | 0.609 |

$K_0$, $K_1$ - the reaction rate constants, $C_0$ - the initial response, $R^2$ – coefficient of determination, SE - standard error

**Table 7.** Kinetic parameters from zero and first order reactions of colour for b* value

| Response | Sample | Temperature (ºC) | Zero order | | | First order | | |
|---|---|---|---|---|---|---|---|---|
| | | | $K_0$±SE | $C_0$±SE | $R^2$ | $K_1$±SE | $C_1$±SE | $R^2$ |
| b* | 1 | 20 | -0.006067± 0.000383 | 9.1140±0.0281 | 0.988 | -0.000692±0.000040 | 9.1173±0.0030 | 0.990 |
| | 2 | 20 | -0.009700±0.001918 | 9.5280±0.1409 | 0.895 | -0.001077±0.000208 | 9.5320±0.0153 | 0.900 |
| | 3 | 20 | -0.004267±0.003110 | 8.7800±0.2285 | 0.386 | -0.000486±0.000358 | 8.7712±0.0263 | 0.380 |
| | 4 | 20 | -0.008967±0.001183 | 8.6180±0.0870 | 0.950 | -0.001110±0.000146 | 8.6262±0.0107 | 0.951 |
| | 5 | 20 | -0.012000±0.003835 | 8.0940±0.2818 | 0.765 | -0.001580±0.000489 | 8.0832±0.0359 | 0.777 |
| | 6 | 20 | -0.011733±0.003000 | 8.8300±0.2205 | 0.836 | -0.001415±0.000342 | 8.8271±0.0252 | 0.851 |
| | 7 | 20 | -0.008800±0.001433 | 8.4280±0.1053 | 0.926 | -0.001105±0.000167 | 8.4315±0.0123 | 0.936 |
| | 8 | 20 | -0.009833±0.002253 | 8.9720±0.1655 | 0.864 | -0.001160±0.000250 | 8.9720±0.0183 | 0.877 |
| | 1 | 4 | -0.001367± 0.001988 | 9.5400±0.1461 | 0.136 | -0.000145±0.000211 | 9.5391±0.0155 | 0.135 |
| | 2 | 4 | -0.000233±0.000753 | 9.4940±0.0553 | 0.031 | -0.000025±0.000079 | 9.4940±0.0058 | 0.032 |
| | 3 | 4 | -0.001933±0.001283 | 9.7340±0.0943 | 0.431 | -0.000202±0.000133 | 9.7342±0.0098 | 0.432 |
| | 4 | 4 | -0.001267±0.001906 | 8.7480±0.1400 | 0.128 | -0.000143±0.000219 | 8.7453±0.0161 | 0.125 |
| | 5 | 4 | -0.000233±0.002331 | 8.3840±0.1713 | 0.003 | -0.000030±0.000280 | 8.3834±0.0206 | 0.004 |
| | 6 | 4 | -0.002033±0.001091 | 8.9180±0.0801 | 0.537 | -0.000231±0.000124 | 8.9178±0.0091 | 0.537 |
| | 7 | 4 | -0.000833±0.001909 | 8.4300±0.1402 | 0.060 | -0.000100±0.000227 | 8.4294±0.0166 | 0.061 |
| | 8 | 4 | -0.001700±0.000979 | 8.8800±0.0719 | 0.501 | -0.000195±0.000112 | 8.8807±0.0082 | 0.502 |

$K_0$, $K_1$ - the reaction rate constants, $C_0$ - the initial response, $R^2$ – coefficient of determination, SE - standard error

**Table 8.** Kinetic parameters from zero and first order reactions of colour for ΔE

| Re-sponse | Sam-ple | Tempera-ture (ºC) | Zero order | | | First order | | |
|---|---|---|---|---|---|---|---|---|
| | | | $K_0$±SE | $C_0$±SE | $R^2$ | $K_1$±SE | $C_1$±SE | $R^2$ |
| ΔE | 1 | 20 | 0.062400±0.005210 | 0.4700±0.3829 | 0.980 | 0.011612±0.002181 | 2.0481±0.1792 | 0.934 |
| | 2 | 20 | 0.073833±0.007161 | 0.5680±0.5262 | 0.973 | 0.011832±0.002789 | 2.3800±0.2291 | 0.900 |
| | 3 | 20 | 0.056033±0.006622 | 0.4840±0.4866 | 0.960 | 0.011661±0.003220 | 1.8561±0.2646 | 0.868 |
| | 4 | 20 | 0.050633±0.004554 | 0.3140±0.3346 | 0.976 | 0.012507±0.002810 | 1.5045±0.2308 | 0.908 |
| | 5 | 20 | 0.071700±0.014560 | 1.4640±1.0700 | 0.890 | 0.007012±0.001419 | 4.1413±0.1166 | 0.924 |
| | 6 | 20 | 0.063870±0.011140 | 1.0080±0.8185 | 0.916 | 0.008716±0.002427 | 3.0090±0.1994 | 0.866 |
| | 7 | 20 | 0.059730±0.012310 | 1.0940±0.9045 | 0.887 | 0.007998±0.002596 | 3.0855±0.2133 | 0.826 |
| | 8 | 20 | 0.059067±0.007764 | 0.6840±0.5706 | 0.951 | 0.010157±0.002509 | 2.3268±0.2061 | 0.891 |
| | 1 | 4 | 0.006200±0.002239 | 0.2160±0.1645 | 0.719 | 0.003339±0.001854 | 0.5662±0.1524 | 0.618 |
| | 2 | 4 | 0.004833±0.001342 | 0.0720±0.0986 | 0.812 | 0.009234±0.004964 | 0.2113±0.4079 | 0.634 |
| | 3 | 4 | 0.014133±0.001327 | 0.0760±0.0975 | 0.974 | 0.013291±0.003552 | 0.3865±0.2918 | 0.875 |
| | 4 | 4 | 0.011733±0.001358 | 0.0360±0.0998 | 0.961 | 0.012008±0.001748 | 0.3449±0.1437 | 0.959 |
| | 5 | 4 | 0.013100±0.001334 | 0.1280±0.0980 | 0.970 | 0.010501±0.001683 | 0.4892±0.1383 | 0.951 |
| | 6 | 4 | 0.022333±0.002456 | 0.0760±0.1805 | 0.965 | 0.012066±0.002070 | 0.6566±0.1701 | 0.944 |
| | 7 | 4 | 0.028967±0.004345 | 0.1060±0.3193 | 0.937 | 0.013010±0.003661 | 0.7847±0.3008 | 0.863 |
| | 8 | 4 | 0.016733±0.001342 | -0.0720±0.0986 | 0.981 | 0.017919±0.002485 | 0.2566±0.2042 | 0.963 |

$K_0$, $K_1$ - the reaction rate constants, $C_0$ - the initial response, $R^2$ – coefficient of determination, SE - standard error

### *Moisture, fat and protein content*

The average chemical composition of the tested sausage samples is shown in Table 9. All samples were produced in the same conditions of industrial production and variations of moisture, fat and protein content between the samples were relatively small, as was expected. The content of the tested compounds in all samples was in accordance with the requirements defined by the Regulation on the quality of meat products ("Official Gazette of SFRJ", No. 29/74).

### *pH value*

Measured pH values of dry-fermented beef sausage "sucuk" type are shown in Table 9. pH value of the tested samples ranged from 5.02 (sample 4) to 5.39 (sample 3). According to the Turkish Standard Institute (Karabacak and Bozkurt, 2008), pH value for high quality sucuk should be in the range of 4.7–5.4. Based on the obtained results, it can be seen that the pH of all model samples was in the range of standard values.

**Table 9.** pH values, moisture, fat and protein content in dry-fermented beef sausage "sucuk" type

| Sample code | pH | Moisture (%) | Fat (%) | Proteins (%) |
|---|---|---|---|---|
| 1 | 5.13 | 24.90 | 47.90 | 23.00 |
| 2 | 5.22 | 25.30 | 47.30 | 23.40 |
| 3 | 5.39 | 26.80 | 45.20 | 24.00 |
| 4 | 5.02 | 24.50 | 49.10 | 21.70 |
| 5 | 5.10 | 23.70 | 51.10 | 20.70 |
| 6 | 5.10 | 24.40 | 49.20 | 22.30 |
| 7 | 5.17 | 24.40 | 49.20 | 22.50 |
| 8 | 5.17 | 25.80 | 46.80 | 23.20 |

## Conclusion

Different antioxidants were used in dry-fermented beef sausage "sucuk" type production and their affects on the colour stability of fermented sausage "sucuk" type were investigated. The analysis of the colour stability of dry-fermented beef sausage "sucuk" during 120 minutes of sausage slices exposure to air, at the temperature of 20°C nad 4°C revealed that the cross section colour of all samples significantly changed ($p<0.05$), due to the exposition of sausage cuts to air, but significantly smaller changes in colour ($p<0.05$) were observed in the samples kept at lower temperature. The differences in cross section colour of sausage model samples and different colour stability during the exposition of sausage cuts to air can be connected to the presence of various antioxidants and their activity in the tested samples. The results of this study indicated that the addition of natural antioxidant, rosemary extract and green tea extract, have a positive effect on colour stability of dry-fermented beef sausage "sucuk" type during 120 minutes of sausage slices exposure to air. This natural antioxidant prevents the change of cross section colour and could be used in dry-fermented beef sausage "sucuk" type production in order to increase the colour stability of fermented sausage "sucuk" type and provide product quality and safety. Based on these results, it can be concluded that dry-fermented beef sausage "sucuk" type, produced with selected antioxidants, can be sliced 120 minutes before serving and stored at the temperature of 4°C. Cross section colour changes of tested samples at the temperature of 4°C are unrecognisable, but at the temperature of 20ºC colour changes are significantly greater.

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