

## THE EFFECT OF SWISS CHARD POWDER AND STARTER CULTURES ON COLOUR DEVELOPMENT IN SMOKED PORK LOIN

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### ABSTRACT

The aim of this research is to determine the influence of starter cultures and Swiss chard powder, as an alternative naturally occurring nitrite source, on development of superficial and cross-section colour in smoked pork loin produced in industrial conditions. It has been confirmed that smoked pork loin where nitrite and starter culture have been added (group III) has the most favorable instrumental values L, a and b of the colour at surface and fresh cross-section. In group IV (Swiss chard powder and starter culture), the colour develops only on the periphery of the smoked pork loin, which is due to the low content of nitrite during all production stages. Nitrate-reducing bacteria present in starter cultures are reducing nitrates from Swiss chard powder to nitrites. Formed nitrites react rapidly with ascorbic acid and because of that do not have the ability to react with myoglobin to contribute to the development of a red colour in the middle part of the meat. The differences in the colour, observed in the instrumentally measured L, a and b-values at the surface and fresh cross-section between the groups of productions stages, are statistically significant ( $p < 0.05$ ).

**Key words:** nitrates, nitrites, pork loin, cure meat.

### INTRODUCTION

Nitrites are food additives that are widely used in the production of meat products, along with salt, sugar, ascorbate and polyphosphates (Goswami et al., 2014). They are mainly used to maintain the safety and quality of products (development of colour, flavour, as well as to prevent lipid oxidation) (Danev, 1999; Vuković, 2006; Vuković, 2012; Alahakoon et al., 2015).

When choosing meat products, the consumer initially notices the colour on the surface and/or the cross-section of the product. Based on his first visual impression, he decides which product to reach for. Numerous studies indicate that the colour of the fresh meat and the product is one of the most important indicators of product quality and successful conduct of technological operations (Bendall & Swatland, 1988; Mancini & Hunt, 2005; Lawrie & Ledward, 2006; Møller & Skibsted, 2007; Brewer, 2010).

The major carrier of colour in meat is the pigment myoglobin, which is found in the sarcoplasm of muscle fibers (Rede & Petrović, 1997; Mancini & Hunt, 2005; Gašperlin & Rajar 2005). Feiner (2006) states that the colour of fresh meat, mainly, depends on the presence of

muscle pigment, primarily on myoglobin (90% - 95%), then hemoglobin (2% - 5%) and small insignificant amounts of cytokines, flavins, cobalamins, etc.

Apart from myoglobin (Mb), which is present in meat, the use of nitrites and nitrates is also necessary for the development of a characteristic, typical, stable, reddish-brown colour in meat products. Andrée et al., (2010) point out that the use of nitrites has a significant effect on the development and stability of colour in meat products. Colour development takes place through a series of reactions until nitrosyl myoglobin (NOMb) is formed. The pigment is not stable in air and rapid discolouration is possible (Varnam & Sutherland, 1995). Upon heating, nitrosyl myoglobin (NOMb) is converted to nitrosyl hemochrome (NOMbCr), a stable pigment in heat-treated and cured meat (Shahidi & Pegg, 1993). Nitrites, which are added to the cure, aim to ensure the formation of nitrosyl myoglobin (NOMb), which carries the desired red colour of cured meat. The red colour in cured meat is formed by binding highly reactive nitrogen oxide (NO), produced by nitrite, to iron in the heme porphyrin ring of myoglobin (Møller & Skibsted, 2007; Bozkurt & Bayram, 2006; Laursen et al., 2008; Vuković, 2012). The mechanism of formation of nitrosyl myoglobin (NOMb) is complex and there are different data about how it is obtained.

In addition to meat, contemporary people consume meat products in their diet, as well. During the processing of meat and meat products, a reaction occurs between nitrites and meat proteins, and as a product of the reaction of nitrosation, many harmful carcinogenic compounds, such as *N*-nitrosamines are produced (Pearson & Dutson, 1990; Hui, 1992; Bošnjir et al., 2003). The toxicity and carcinogenicity of nitrosamines have been demonstrated and described in a number of published papers over the past years (Jakszyn et al., 2004). Numerous epidemiological studies suggest that nitrosamines are etiological agents for the development of various types of cancer in humans (Pegg & Shahidi, 2000).

Nitrates and nitrites are naturally present in some vegetables. Beets, broccoli, celery, cabbage, lettuce, radish, spinach, contain high concentration of nitrates (> 1000 mg/kg), while nitrites in fresh vegetables are present in very low concentrations (< 1 mg/kg) (Gassara et al., 2016). The juice and vegetable powders contain much higher concentration of nitrates compared to fresh vegetables (Eisinaite et al., 2016). Alternative natural sources of nitrates, present in high concentrations in some vegetables, as well as the starter cultures, can be used in the production of meat products. Ready products have acceptable sensory characteristics (Sebranek & Bacus 2007b.).

Starter cultures used in meat products are defined as microorganisms that are added directly to the meat, in order to improve the viability, safety and acceptance of the product by the consumers. The use of starter cultures improves the development of colour, texture, aroma and flavour of the product (Toldrá, 2010).

The aim of this paper is to determine the development of a characteristic reddish-pink colour in smoked pork loin, which is dry salted and produced in industrial conditions by applying the starter culture BactoFerm Rosa and Swiss chard powder.

## **MATERIALS AND METHODS**

### **Materials**

Smoked pork loin, dry-salted, produced in industrial conditions is used in the analysis. The origin of the raw material frozen pork loin is from Spain (Costa Food Meat, Sl.). As an alternative to nitrite salt in the production of smoked pork loin, the following is used: Swiss chard powder (from Naturex, France) in combination with the starter culture BactoFerm Rosa, produced by Chr. Hansen, Denmark. Ascorbic acid and acerola powder, which is used as alternative source for ascorbic acid are manufactured by PI-1 (Bulgaria). Table salt is produced

by Solana (Bosnia and Herzegovina), dextrose is purchased by ADM Amylum, Bulgaria) and nitrite salt is obtained from Alkaloid, N. Macedonia.

The experiment is conducted in the meat processing factory "Fi-Sa Commerce", Skopje. Their usual technological way of producing smoked pork loin is taken as a basis. Four groups of smoked pork loin are produced, as follows:

- I group: table salt, dextrose and ascorbic acid;
- II group (control): nitrite salt, dextrose and ascorbic acid;
- III group: nitrite salt, dextrose, ascorbic acid and the starter culture BactoFerm Rosa;
- IV group: Swiss chard powder, dextrose, acerola powder and the starter culture BactoFerm Rosa.

Table 1 shows the composition of the raw material of the individual groups of smoked pork loin.

Table 1. Raw material composition of the individual groups of smoked pork loin

| Components                     | Groups of smoked pork loin |            |            |            |
|--------------------------------|----------------------------|------------|------------|------------|
|                                | I<br>%                     | II<br>%    | III<br>%   | IV<br>%    |
| Frozen pork loin               | 96.95                      | 96.95      | 96.93      | 96.55      |
| Nitrite salt                   |                            | 2.5        | 2.5        |            |
| Table salt                     | 2.5                        |            |            | 2.5        |
| Dextrose                       | 0.5                        | 0.5        | 0.5        | 0.5        |
| Ascorbic acid                  | 0.05                       | 0.05       | 0.05       |            |
| Acerola powder                 |                            |            |            | 0.2        |
| Starter culture BactoFerm Rosa |                            |            | 0.025      | 0.025      |
| Swiss chard powder             |                            |            |            | 0.25       |
| <b>Total</b>                   | <b>100</b>                 | <b>100</b> | <b>100</b> | <b>100</b> |

A frozen outer part of the pork loin (*m. longissimus dorsi*) is used, from which the bones, the connective and the adipose tissue were previously removed. The raw material is thawed by dry defrosting. The raw material used for the production of smoked pork loin was carefully chosen. Its pH value ranges from 5.7 to 6.2. in order to eliminate and avoid the negative consequences of using pale, soft, watery meat. Then the pieces are shaped, so that the product would have a characteristic shape with finely cut edges and incisions, and the residual blood is removed. After shaping the pieces, the next technological operation is dry curing. The previously measured mixture for dry curing is evenly applied and rubbed on the surface of the pieces of pork loin. The salted pieces are left for 14 days in a dark room, with a constant mode of calm cooling, with a temperature of 0 – 4 °C and a relative humidity of 85 to 90%.

The next step is the heat treatment, which took place with the following mode: heating to a temperature of 45 °C in the chamber, lasting for 15 minutes; drying to a temperature of 68 °C in the chamber and 45 °C in the central part of the meat product for a period of 2 hours and 30 minutes; smoking at a temperature of 70 °C in a chamber for 1 hour; roasting at a temperature of 80 °C in the chamber and 70 °C in the central part of the meat product for a period of 1.5 hours; curing at a temperature of 85 °C in the chamber and 74 °C in the central part of the meat product for a period of 30 minutes and blowing in the final stage.

## Methods

The instrumental analysis of the colour and the nitrite content is performed with a certain frequency, as follows: raw material, 0 day (12 hours after salting), 7<sup>th</sup> day (salting), 14<sup>th</sup> day

(salting) and after the heat treatment (ready product). One piece of pork loin is taken from each group for the appropriate frequency, on which all analyses are performed.

The pH-value is determined with a portable pH-meter (Ebro PHT 810 pH) with a prickly, glass gel-filled electrode for direct determination of pH in the center of the muscle. Before the start of the measurement, the pH-meter is standardized using buffers with pH = 4.00 and pH = 7.00. On each piece of pork loin, the pH-value is measured in nine places: three places in the middle and three places at both ends of the piece. After each measurement the electrode is washed with distilled water and dried with paper.

To determine the nitrite content, samples are taken from each piece from the middle part and from both ends, with a total amount of 500 g, which was nicely homogenized with a homogenizing mixer and then the nitrite content in the meat and the meat products is determined, according to the method ISO 2918 : 1975.

Instrumental colour analysis is measured on the surface and on the fresh cross-section, using a colourimeter Dr. Lange. Before the start of the measurement, it is calibrated with a black and white calibration plate, according to the standard procedure of the manufacturer. The colour characteristics are expressed in three coordinates L, a and b, through which the colour of the samples is defined: L - the value describes how light the sample is (+ L - lighter; - L - darker). The values a and b describe the colour shade: + a - more red (less green), - a - more green (less red), + b - more yellow (less blue), - b - more blue (less yellow).

The data are statistically processed using the software package for statistical data processing STATISTICA (data analysis software system), version 8.0. (StatSoft, Inc., 2007). The differences between the mean values of the measured nitrite contents and the measured L, a and b - surface values and fresh cross-section between the groups and the production phases are tested using a two-way analysis of variance (Two-way- ANOVA: Tukey`s HSD post hoc). All mean values in the survey are presented as mean and standard deviation ( $\bar{x} \pm SD$ ).

## RESULTS AND DISCUSSION

Measuring pH is a direct way to obtain information about meat quality. For meat processing it is best to use meat that has a pH value of 5.7 to 6.3 (Müller, 1989). The results shown in Table 2 indicate that the average pH of the raw material (pork loin) is 5.75. This eliminates the negative consequences of using pale, soft, watery meat when producing smoked pork loin.

Table 2. Descriptive statistical representation of the measured pH value of the raw material

|           | <b>n</b> | $\bar{x}$ | <b>SD</b> | <b>SE</b> |
|-----------|----------|-----------|-----------|-----------|
| <b>pH</b> | 144      | 5.75      | 0.59      | 0.004     |

n-number of repetitions,  $\bar{x}$  - mean value, SD standard deviation, SE – standard error

Table 3. Descriptive statistical indicator of instrumentally measured colour at the surface and fresh cross-section of the raw material

|          | <b>Colour on the surface</b> |           |           |           | <b>Colour of the fresh cross-section</b> |           |           |           |      |
|----------|------------------------------|-----------|-----------|-----------|--|-----------|-----------|-----------|------|
|          | <b>n</b>                     | $\bar{x}$ | <b>SD</b> | <b>SE</b> | <b>n</b>                                 | $\bar{x}$ | <b>SD</b> | <b>SE</b> |      |
| <b>L</b> | 45                           | 52.75     | 2.48      | 0.37      | <b>L</b>                                 | 45        | 48.19     | 5.54      | 0.83 |
| <b>a</b> | 45                           | 4.64      | 0.71      | 0.11      | <b>a</b>                                 | 45        | 2.99      | 1.56      | 0.23 |
| <b>b</b> | 45                           | 11.65     | 1.08      | 0.16      | <b>b</b>                                 | 45        | 6.01      | 0.82      | 0.12 |

n-number of repetitions,  $\bar{x}$  - mean value, SD standard deviation, SE – standard error

Brewer and al., (2001) state that in pork, the measured L-value on a fresh cross-section is an indicator for determining the appearance of pale, soft and watery meat. If the L-value

interval of fresh cross-section in fresh pork loin (*m. longissimus dorsi*) is 43 - 50, then the meat is reddish-brown, as opposed to the L-value greater than 50, when the meat is pale (Tomović, 2013). As can be seen from Table 3, the average L-value of the fresh cross-section of thawed pork loin (*m. longissimus dorsi*) is 48.19, which indicates that the raw material used is reddish-pink in colour. From the other results shown in Table 3, it can be seen the average a-value (share of red colour) of the surface is 4.64, while on the fresh cross-section it is 2.99. The average b-value (yellow share) of the surface is 11.65, while on the fresh cross-section is 6.01.

Table 4. Comparative display of nitrite content (mg/kg) among groups and production phases

|                  | n | Production phase                  |                                  |                                   |   |
|------------------|---|-----------------------------------|----------------------------------|-----------------------------------|---|
|                  |   | 0 day<br>(12 hours after salting) | 7 <sup>th</sup> day<br>(salting) | 14 <sup>th</sup> day<br>(salting) | ready product<br>(after heat treatment) |
| <b>I group</b>   | 6 | 0.03 ± 0.02 <sup>a1</sup>         | 0.19 ± 0.02 <sup>a1</sup>        | 0.39 ± 0.03 <sup>a1</sup>         | 0.39 ± 0.02 <sup>a1</sup>               |
| <b>II group</b>  | 6 | 66.79 ± 0.53 <sup>b1</sup>        | 6.44 ± 0.32 <sup>b2</sup>        | 1.80 ± 0.14 <sup>b23</sup>        | 1.09 ± 0.03 <sup>b234</sup>             |
| <b>III group</b> | 6 | 71.77 ± 0.48 <sup>bc1</sup>       | 12.90 ± 0.10 <sup>bc2</sup>      | 4.89 ± 0.08 <sup>bc23</sup>       | 1.37 ± 0.03 <sup>c234</sup>             |
| <b>IV group</b>  | 6 | 0.68 ± 0.18 <sup>d1</sup>         | 0.75 ± 0.16 <sup>d1</sup>        | 0.19 ± 0.04 <sup>a2</sup>         | 0.39 ± 0.05 <sup>a1</sup>               |

Different letters (a-d) of indexes of the values in the columns indicate a statistically significant difference ( $p < 0.05$ )

Different numbers (1-4) of indexes of the values in the rows indicate a statistically significant difference ( $p < 0.05$ )

From Table 4 it can be seen that 12 hours after salting, the highest nitrite content is determined at 71.77 mg/kg in group III, followed by group II with 66.79 mg/kg nitrite content, as opposed to group I in which 12 hours after salting, the lowest nitrite content of 0.03 mg/kg is determined, while in group IV a nitrite content of 0.68 mg/kg is found. The differences in nitrite content that occur among the groups and the production phases are statistically significant ( $p < 0.05$ ). Undoubtedly, group I has the lowest values of nitrites because it is salted with table salt. It is also understandable that groups II and III have the highest amount of nitrite because in these two groups the initial nitrite input is 148 mg/kg. In group IV, nitrates present in Swiss chard powder with the help of nitrate-reducing bacteria present in the starter culture are reduced to nitrites, but nitrites react rapidly with the ascorbic acid from the acerola powder and therefore their amount is very low during all production stages, as it is pointed out by Feiner (2006). The moisture present on the surface of the meat is sufficient to cause a reaction with nitrite, which results in the formation of nitrogen oxides NO and NO<sub>2</sub>, thus inhibiting the formation of N-nitrosamines through the mechanisms of competition with nitro-stable precursors (amines) with the means for nitrosation. Products formed by the reaction between nitrites and ascorbic acid are not only responsible for the nitrosation reaction, but also for the loss of nitrites during drying and frying of meat (Chow & Hong, 2002).

Table 5. Comparative display of instrumentally measured L-value on the surface among the groups and production phases of smoked pork loin

|                  | n  | Production phase                  |                                  |                                   |   |
|------------------|----|-----------------------------------|----------------------------------|-----------------------------------|---|
|                  |    | 0 day<br>(12 hours after salting) | 7 <sup>th</sup> day<br>(salting) | 14 <sup>th</sup> day<br>(salting) | ready product<br>(after heat treatment) |
| <b>I group</b>   | 30 | 35.94 ± 3.36 <sup>a1</sup>        | 37.48 ± 4.19 <sup>a1</sup>       | 40.92 ± 4.65 <sup>a2</sup>        | 38.85 ± 4.90 <sup>a1</sup>              |
| <b>II group</b>  | 30 | 38.64 ± 0.86 <sup>a1</sup>        | 42.11 ± 1.65 <sup>b1</sup>       | 39.17 ± 7.02 <sup>a1</sup>        | 40.19 ± 1.50 <sup>a1</sup>              |
| <b>III group</b> | 30 | 39.84 ± 1.81 <sup>b1</sup>        | 41.02 ± 2.28 <sup>a1</sup>       | 41.63 ± 2.21 <sup>a1</sup>        | 37.99 ± 2.51 <sup>a1</sup>              |
| <b>IV group</b>  | 30 | 39.52 ± 3.33 <sup>c1</sup>        | 40.11 ± 5.30 <sup>a1</sup>       | 42.24 ± 7.23 <sup>a1</sup>        | 36.85 ± 4.53 <sup>a1</sup>              |

Different letters (a-d) of indexes of the values in the columns indicate a statistically significant difference ( $p < 0.05$ )

Different numbers (1-4) of indexes of the values in the rows indicate a statistically significant difference ( $p < 0.05$ )

The results of the instrumentally measured L-value on the surface are shown in Table 5 and the results from the fresh cross-section are shown in Table 8. From Table 5 it can be concluded that the highest L-value on the surface (42.24) is in IV group of the 14<sup>th</sup> day of salting, while the lowest value is observed in group I (35.94) on the day 0 (12 hours after salting). The differences observed in the L-value on the surface among the groups and the production phases themselves are statistically significant ( $p < 0.05$ ). As for the fresh cross-section (Table 8) during the production phases, the lowest L-value is found in group III (41.15) on the 14<sup>th</sup> day after salting, while the highest L-value is observed in group IV after the heat treatment (72.18). The differences observed in the L-value on the surface among the groups and the production phases themselves are statistically significant ( $p < 0.05$ ).

Table 6. Comparative display of instrumentally measured a-value on the surface among the groups and production phases of smoked pork loin

|                  | n  | Production phase                  |                                  |                                   |   |
|------------------|----|-----------------------------------|----------------------------------|-----------------------------------|---|
|                  |    | 0 day<br>(12 hours after salting) | 7 <sup>th</sup> day<br>(salting) | 14 <sup>th</sup> day<br>(salting) | ready product<br>(after heat treatment) |
| <b>I group</b>   | 30 | 3.20 ± 0.71 <sup>a1</sup>         | 3.23 ± 1.30 <sup>a1</sup>        | 2.52 ± 1.07 <sup>a1</sup>         | 17.65 ± 1.23 <sup>a2</sup>              |
| <b>II group</b>  | 30 | 6.20 ± 0.46 <sup>b1</sup>         | 6.01 ± 0.64 <sup>b1</sup>        | 3.25 ± 0.32 <sup>a2</sup>         | 17.10 ± 0.64 <sup>a23</sup>             |
| <b>III group</b> | 30 | 5.24 ± 0.42 <sup>bc1</sup>        | 5.14 ± 0.69 <sup>bc1</sup>       | 3.42 ± 0.76 <sup>b2</sup>         | 19.29 ± 0.92 <sup>b23</sup>             |
| <b>IV group</b>  | 30 | 3.41 ± 1.31 <sup>a1</sup>         | 3.10 ± 1.31 <sup>a1</sup>        | 3.18 ± 0.90 <sup>a1</sup>         | 18.01 ± 1.78 <sup>a2</sup>              |

Different letters (a-d) of indexes of the values in the columns indicate a statistically significant difference ( $p < 0.05$ )

Different numbers (1-4) of indexes of the values in the rows indicate a statistically significant difference ( $p < 0.05$ )

Table 7. Comparative display of instrumentally measured b-value at the surface among the groups and production phases of smoked pork loin

|                  | n  | Production phase                  |                                  |                                   |   |
|------------------|----|-----------------------------------|----------------------------------|-----------------------------------|---|
|                  |    | 0 day<br>(12 hours after salting) | 7 <sup>th</sup> day<br>(salting) | 14 <sup>th</sup> day<br>(salting) | ready product<br>(after heat treatment) |
| <b>I group</b>   | 30 | 3.31 ± 1.26 <sup>a1</sup>         | 5.02 ± 1.14 <sup>a2</sup>        | 6.01 ± 1.05 <sup>a3</sup>         | 26.08 ± 2.46 <sup>a34</sup>             |
| <b>II group</b>  | 30 | 2.29 ± 0.57 <sup>a1</sup>         | 3.44 ± 0.51 <sup>b2</sup>        | 3.47 ± 0.69 <sup>b3</sup>         | 23.64 ± 1.29 <sup>b4</sup>              |
| <b>III group</b> | 30 | 2.42 ± 0.53 <sup>a1</sup>         | 2.87 ± 0.89 <sup>c1</sup>        | 3.52 ± 1.02 <sup>bc1</sup>        | 24.54 ± 1.69 <sup>c2</sup>              |
| <b>IV group</b>  | 30 | 4.64 ± 0.97 <sup>b1</sup>         | 4.64 ± 1.14 <sup>a1</sup>        | 4.86 ± 1.52 <sup>d1</sup>         | 23.43 ± 1.99 <sup>bd2</sup>             |

Different letters (a-d) of indexes of the values in the columns indicate a statistically significant difference ( $p < 0.05$ )

Different numbers (1-4) of indexes of the values in the rows indicate a statistically significant difference ( $p < 0.05$ )

Table 8. Comparative display of instrumentally measured L-value on the fresh cross-section among the groups and production phases in smoked pork loin

|                  | n  | Production phase                  |                                  |                                   |   |
|------------------|----|-----------------------------------|----------------------------------|-----------------------------------|---|
|                  |    | 0 day<br>(12 hours after salting) | 7 <sup>th</sup> day<br>(salting) | 14 <sup>th</sup> day<br>(salting) | ready product<br>(after heat treatment) |
| <b>I group</b>   | 30 | 48.19 ± 1.67 <sup>a1</sup>        | 42.50 ± 2.15 <sup>a2</sup>       | 42.61 ± 2.39 <sup>a23</sup>       | 66.02 ± 5.14 <sup>a234</sup>            |
| <b>II group</b>  | 30 | 47.28 ± 1.53 <sup>a1</sup>        | 46.36 ± 3.95 <sup>b1</sup>       | 41.85 ± 1.58 <sup>a2</sup>        | 65.83 ± 4.66 <sup>a23</sup>             |
| <b>III group</b> | 30 | 49.47 ± 2.52 <sup>a1</sup>        | 41.34 ± 3.89 <sup>a2</sup>       | 41.15 ± 2.45 <sup>a23</sup>       | 64.34 ± 4.05 <sup>a234</sup>            |
| <b>IV group</b>  | 30 | 48.72 ± 2.85 <sup>a1</sup>        | 43.09 ± 2.33 <sup>a2</sup>       | 43.96 ± 1.77 <sup>a3</sup>        | 72.18 ± 3.09 <sup>b24</sup>             |

Different letters (a-d) of indexes of the values in the columns indicate a statistically significant difference ( $p < 0.05$ )

Different numbers (1-4) of indexes of the values in the rows indicate a statistically significant difference ( $p < 0.05$ )

Table 6 shows the results of the instrumentally measured a-value on the surface of the smoked pork loin during all production phases. On the surface, the highest a-value is observed in group III (19.29) after heat treatment (ready product), while the lowest a-value on the surface

(2.52) was observed in group I, 14 days after salting. The differences observed in the a-value on the surface between the groups and the production phases themselves are statistically significant ( $p < 0.05$ ). The highest a-value (7.36), and at the same time the reddest colour of fresh cross-section is found in group III after heat treatment (ready product), and the lowest value (1.93) is found in group I after heat treatment (ready product) (Table 9). Also, the differences observed in the a-value of the fresh cross-section between the groups and the production phases themselves are statistically significant ( $p < 0.05$ ). In all production phases, group III has a higher a-value because the use of nitrite salt in combination with the starter culture contributes to the development of the red colour, which is agreed with the data given in the literature (Krause et al., 2011, Tae-Kyung et al., 2019). It is clear that group I, which is produced without nitrite salt, only with table salt, has a low a-value on the surface and the fresh cross-section during all production phases. As can be seen from Table 4, the nitrite content of groups II, III and IV during the production phases is significantly reduced due to the reaction of the nitrite with the ascorbic acid. In order to develop a desirable colour in the ready product Wirth (1991) points out that the amounts of 20 to 50 mg of sodium nitrite per kg are sufficient. Added nitrite salt, and the initial nitrite input of 147 mg/kg in groups II and III contributes to the development of red colour. It can be seen from Table 4, the amount of nitrites during all production phases in group IV is very low. As can be seen from Figure 2 of the fresh cross-section of ready product in group IV, a red-pinkish colour is developed only on the periphery, while in the central part there is no red-pinkish colour.

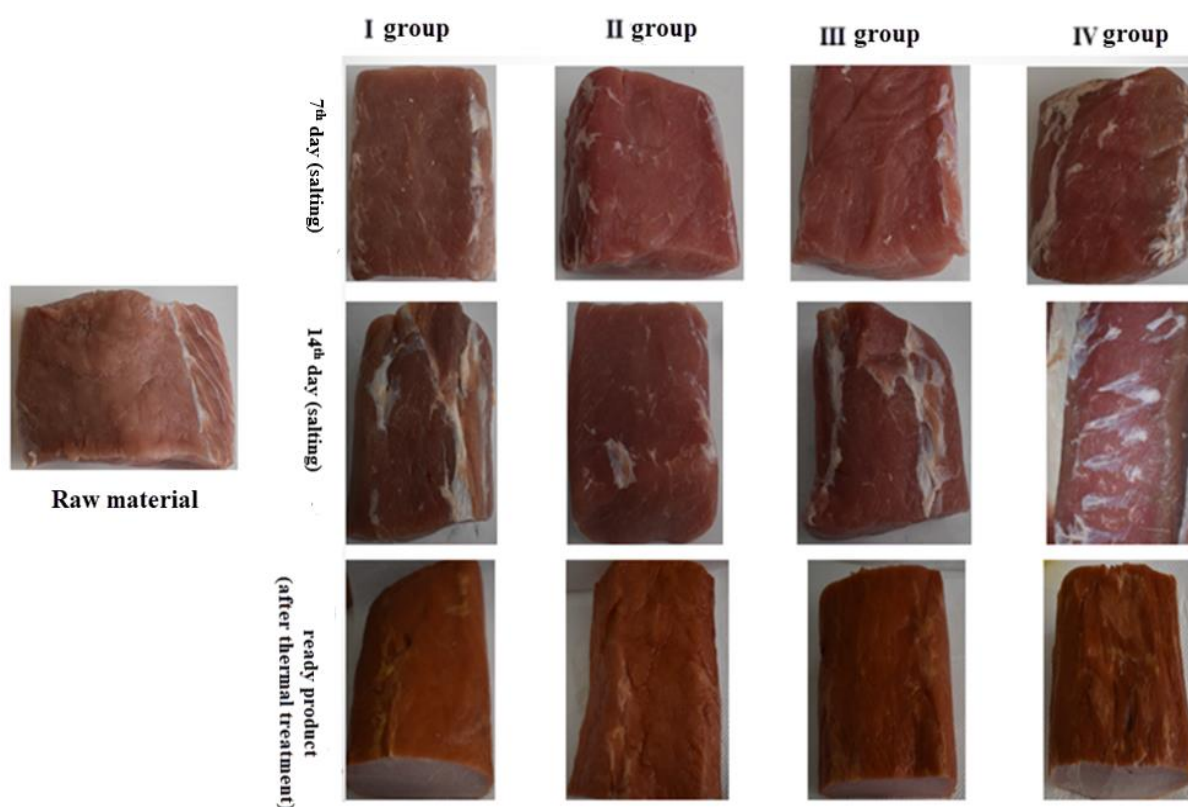


Figure 1. Display of colour development on the surface of the raw material, 7<sup>th</sup> day (salting), 14<sup>th</sup> day (salting), ready product (after heat treatment)

Mean values of instrumentally measured b-value at the surface of smoked pork loin are shown in Table 7. The highest b-value at the surface is shown in group I (26.08) after heat treatment (ready product), and the lowest (2.29) in group II on day 0 (12 hours after salting). As for the fresh cross-section (Table 10), the share of yellow colour is the highest in group IV

(7.46) after heat treatment (ready product), and the lowest in group II (4.18) on the 7<sup>th</sup> day of salting. The differences observed in the b-value at the surface, as well as at the cross-section among the groups and the production phases themselves are statistically significant ( $p < 0.05$ ) (Tables 7 and 10).

Table 9. Comparative display of instrumentally measured a-value on fresh cross-section among the groups and production phases in smoked pork loin

|                  | n  | Production phase                  |                                  |                                   |   |
|------------------|----|-----------------------------------|----------------------------------|-----------------------------------|---|
|                  |    | 0 day<br>(12 hours after salting) | 7 <sup>th</sup> day<br>(salting) | 14 <sup>th</sup> day<br>(salting) | ready product<br>(after heat treatment) |
| <b>I group</b>   | 30 | 2.74 ± 0.63 <sup>a1</sup>         | 2.47 ± 0.84 <sup>a1</sup>        | 2.03 ± 0.73 <sup>a1</sup>         | 1.93 ± 0.37 <sup>a1</sup>               |
| <b>II group</b>  | 30 | 5.14 ± 0.75 <sup>b1</sup>         | 4.73 ± 1.29 <sup>b1</sup>        | 4.65 ± 1.07 <sup>b1</sup>         | 5.32 ± 0.67 <sup>b1</sup>               |
| <b>III group</b> | 30 | 4.30 ± 0.74 <sup>bc1</sup>        | 5.63 ± 1.07 <sup>bc2</sup>       | 4.85 ± 1.33 <sup>bc1</sup>        | 7.36 ± 0.42 <sup>bc3</sup>              |
| <b>IV group</b>  | 30 | 2.31 ± 0.40 <sup>a1</sup>         | 2.94 ± 1.12 <sup>a1</sup>        | 2.54 ± 1.28 <sup>a1</sup>         | 2.11 ± 1.21 <sup>a1</sup>               |

Different letters (a-d) of indexes of the values in the columns indicate a statistically significant difference ( $p < 0.05$ )

Different numbers (1-4) of indexes of the values in the rows indicate a statistically significant difference ( $p < 0.05$ )

Table 10. Comparative display of the instrumentally measured b-value at fresh cross-section among the groups and production phases in smoked pork loin

|                  | n  | Production phase                  |                                  |                                   |   |
|------------------|----|-----------------------------------|----------------------------------|-----------------------------------|---|
|                  |    | 0 day<br>(12 hours after salting) | 7 <sup>th</sup> day<br>(salting) | 14 <sup>th</sup> day<br>(salting) | ready product<br>(after heat treatment) |
| <b>I group</b>   | 30 | 5.47 ± 0.62 <sup>a1</sup>         | 4.69 ± 1.08 <sup>a1</sup>        | 4.67 ± 0.69 <sup>a1</sup>         | 7.09 ± 1.86 <sup>a1</sup>               |
| <b>II group</b>  | 30 | 6.68 ± 7.43 <sup>a1</sup>         | 4.18 ± 1.01 <sup>a2</sup>        | 4.42 ± 1.08 <sup>a3</sup>         | 5.50 ± 0.47 <sup>a1</sup>               |
| <b>III group</b> | 30 | 6.41 ± 0.68 <sup>a1</sup>         | 4.19 ± 1.46 <sup>a2</sup>        | 4.58 ± 1.43 <sup>a1</sup>         | 5.64 ± 0.52 <sup>a1</sup>               |
| <b>IV group</b>  | 30 | 5.12 ± 0.80 <sup>a1</sup>         | 4.90 ± 0.88 <sup>a1</sup>        | 4.49 ± 1.06 <sup>a1</sup>         | 7.46 ± 1.22 <sup>a2</sup>               |

Different letters (a-d) of indexes of the values in the columns indicate a statistically significant difference ( $p < 0.05$ )

Different numbers (1-4) of indexes of the values in the rows indicate a statistically significant difference ( $p < 0.05$ )

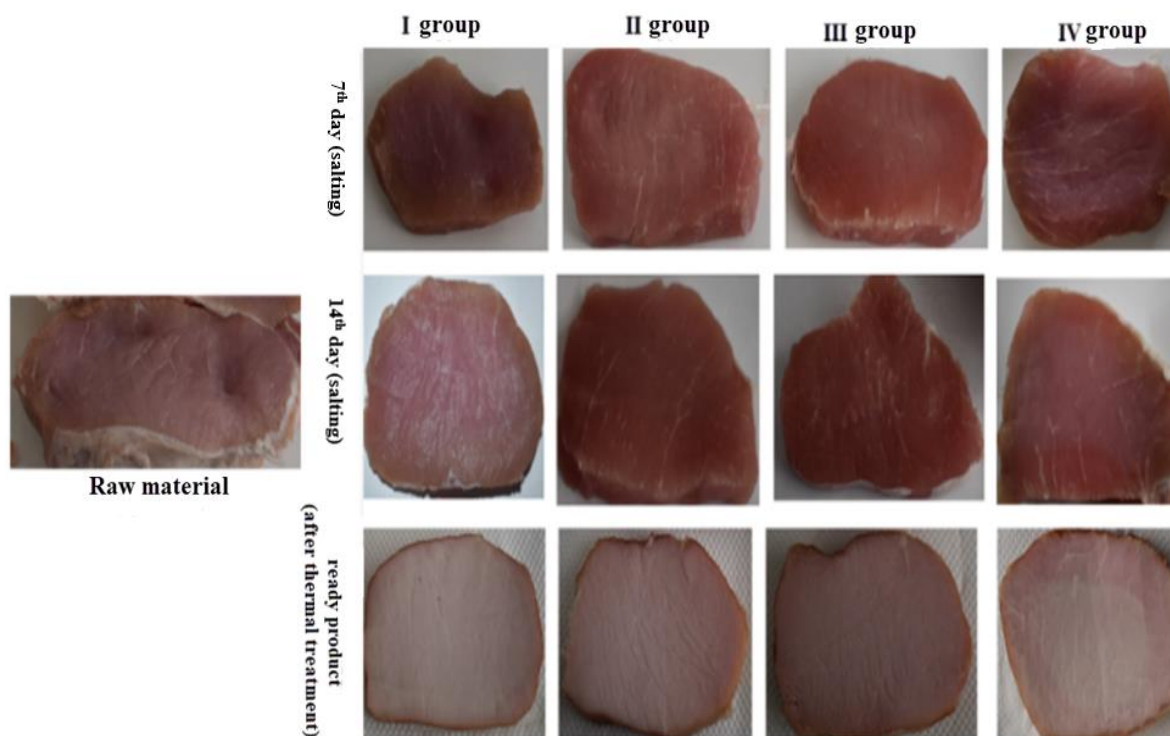




Figure 2. Display colour development at the fresh cross-section of the raw material, 7<sup>th</sup> day (salting), 14<sup>th</sup> day (salting), ready product (after heat treatment)

Tae-Kyung et al., (2019) have obtained wished for results from the use of chard powder, as a natural source of nitrites in the production of semi-dry pork loin. They used frozen chard powder, which was dissolved in water, and starter cultures were added. The liquid was incubated for 24 hours at a temperature of 37 °C, where nitrates were reduced to nitrites. A maximum of 40 % fermented chard was injected into the pieces of pork loin.

In European Union countries, unlike the United States and Asia, the use of pre-fermented cure which contains nitrates is not allowed.

## CONCLUSION

Based on the data obtained from this research, the following conclusions are drawn:

- The use of starter cultures favorably affects the instrumentally measured L, a, and b colour values in smoked pork loin from group III.
- Ascorbic acid, as a reducing agent, is not desirable to use in the production of semi-dry meat products that are dry salted (14 days).
- In order to achieve a desired effect from use of chard powder as a natural source of nitrites in combination with starter cultures in semi-dry meat products, it is necessary to work in direction of combined curing (dry and wet curing) or application of wet curing with previously converted nitrite by incubating the cure at 37 °C for 24 hours. However, it would be harder to apply this method in industry. In addition, in the countries of the European Union, pre-fermented cure which contains nitrates is not allowed. A better solution would be to activate the nitrate-reducing bacteria at a temperature of 40 °C for a period of 90 minutes before the heat treatment of the product.

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