



# Effect of pre-fermented Swiss chard extract on the color stability of cured-cooked meat products

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## ABSTRACT

In response to growing consumer concerns over the safety of synthetic antioxidants and preservatives (e.g., nitrates and nitrites) in meat products, this study explores the feasibility of using Swiss chard extract (SCE) as a natural alternative for sodium nitrite in cured-cooked meat products. Three formulations of cured-cooked meat products were manufactured in a Serbian meat plant: a control sample (C) containing 95 ppm sodium nitrite, and two experimental batches with 3% (T3) and 6% (T6) SCE, respectively, without added nitrites. Instrumental color evaluation was conducted using the *CIE L\*a\*b\** system immediately after production and during 60 days of refrigerated storage. The extract-treated meat products exhibited color values comparable to the nitrite-containing control, with only slight differences in lightness and redness. These findings demonstrate that SCE can maintain color stability in cured-cooked meat products, highlighting its potential as a clean-label substitute for synthetic nitrites.

## 1. Introduction

Nitrites have been widely used in the meat industry due to their multifunctional technological effects: the stabilization of cured meat color, inhibition of pathogenic microorganisms (especially *Clostridium botulinum*), and antioxidative protection (Honikel, 2008, Shakil et al., 2022, Zhang et al., 2023). However, over the past decade, increased attention has been drawn to the potential health risks associated with nitrite intake. It is well known that nitrites, in the presence of secondary amines formed during processing or digestion, can lead to the formation of carcinogenic *N*-nitroso compounds (Vuković,

2020, Šojić et al., 2020, Yhang et al., 2023). As a result, regulatory frameworks have imposed stricter limits on the use of nitrites. Simultaneously, growing consumer demand for clean-label products has driven research into natural ingredients that can replace synthetic additives while preserving product safety and quality (Melios et al., 2024, Xie et al., 2023). Leafy vegetables rich in nitrates, such as celery, spinach, beetroot, and Swiss chard (*Beta vulgaris* subsp. *cicla*) are increasingly being considered as viable natural alternatives in cured meat systems (Jin et al., 2018, Iammarino et al., 2022, Luetic et al., 2023). These plant extracts, in their interactions with

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nitrate-reducing bacteria (e.g., *Staphylococcus carnosus*), can generate nitrites *in situ*, replicating the effect of traditional curing agents (Kim et al., 2019).

Moreover, these vegetables are sources of bioactive compounds, such as betalain pigments, vitamin C, and polyphenols, which could improve the color stability and oxidative resistance of meat products. Among leafy vegetables, Swiss chard is especially notable for its elevated nitrate concentrations, which have been reported to range from 200 to 1000 mg/kg fresh weight, depending on variety, growing conditions, and harvest season. Swiss chard extract (SCE) also contains flavonoids (2.4–3.0 mg/g), betacyanins, and ascorbic acid, which may support color retention, and antioxidative protection in cured meat products without synthetic nitrites (Iammariño et al., 2022).

The aim of this study was to evaluate the potential of pre-fermented SCE as a natural alternative to sodium nitrite in cured-cooked meat products. The experiment focused on instrumental color evaluation (*CIEL\**, *CIEa\**, *CIEb\**, and  $\Delta E$  values) over a defined storage period, comparing two concentrations of SCE (3% and 6%) with a conventional nitrite-containing control.

## 2. Materials and methods

### 2.1. Preparation of SCE

For the purposes of this study, a commercial plant-based extract derived from Swiss chard (MC-NRB®, Meat and Cracks, Germany) was used in

combination with a nitrate-reducing starter culture (M-CULTURE® NRC, Meat and Cracks, Germany). Two batches of the extract were prepared one day prior to use, following the manufacturer's instructions. In the first preparation (R1), 600 g of MC-NRB® and 150 g of M-CULTURE® NRC were mixed with 6 L of cold potable water in a clean plastic container until a homogeneous solution was achieved. In the second preparation (R2), 1200 g of MC-NRB® and 300 g of M-CULTURE® NRC were dissolved in 12 L of cold potable water. Both containers were covered and incubated at 25 °C for 24 hours to allow enzymatic reduction of nitrate to nitrite. After pre-fermentation, the solutions R1 and R2 were stored under refrigeration ( $4 \pm 1$  °C) and used as natural curing agents in meat formulations at levels of 3% and 6%, respectively.

### 2.2. Preparation of cured-cooked meat products

Pasteurized canned meat products in the type of cooked ham were produced in an industrial meat processing facility (Zlatiborac LLC., Mačkat, Serbia) based on the formulations shown in Table 1. Three batches of 200 kg each were prepared: a control batch (C) and two treatment batches (T3 and T6). All batches were produced on the same day from pork ham of identical origin and quality. For the control batch (C), the curing brine contained 1.9% nitrite curing salt, resulting in approximately 95 mg/kg of sodium nitrite in the final product. In contrast, brines for the T3 and T6 batches were prepared with the same concentration (1.9%) of regular table salt,

**Table 1.** Composition of cured-cooked meat formulations (%)

| Ingredient             | C (Control) | T3 (3% R1) | T6 (6% R2) |
|------------------------|-------------|------------|------------|
| Pork ham meat          | 72.00       | 72.00      | 72.00      |
| Water                  | 23.95       | 20.95      | 17.95      |
| Table salt             | –           | 1.90       | 1.90       |
| Nitrite curing salt    | 1.90        | –          | –          |
| Phosphate blend        | 0.50        | 0.50       | 0.50       |
| Starch                 | 1.00        | 1.00       | 1.00       |
| Sugar                  | 0.20        | 0.20       | 0.20       |
| Carrageenan            | 0.40        | 0.40       | 0.40       |
| Sodium isoascorbate    | 0.05        | 0.05       | 0.05       |
| Extract R1 (fermented) | –           | 3.00       | –          |
| Extract R2 (fermented) | –           | –          | 6.00       |

–, indicates the absence of the respective ingredient in the formulation.

without added nitrite. Brines were prepared using a brine mixer (Metalquimia, Spain), which was thoroughly cleaned and sanitized between each preparation. The injection sequence followed the order T3 → T6 → C, with the injector cleaned between batches to prevent cross-contamination. Following brine injection and mechanical tenderization, the meat batters from each batch were transferred into vacuum tumblers. Prior to tumbling, batch T3 was supplemented with fermented extract R1 (3%), and batch T6 with extract R2 (6%). Tumbling was performed for 2 hours under vacuum, after which the meat batters were stuffed into polyamide casings and rested at  $4 \pm 1$  °C for 24 hours. Thermal processing was carried out in the same chamber for all batches until a core temperature of 72 °C was reached. The products were then cooled and stabilized at  $4 \pm 1$  °C for 48 hours.

### 2.3. Instrumental color determination

The color of cured-cooked meat pieces was measured on the surface of the fresh product for each tested variant individually, with a total of 12 measurements per sample. Color was determined using a Minolta Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) under D65 illumination, with a standard 2° observer angle and an 8 mm aperture on the measuring head. Prior to measurement, the instrument was warmed up in accordance with the manufacturer's instructions and calibrated using the standard procedure. Color characteristics were expressed in the CIE system (CIE, 1976), through:  $CIEL^*$  value, indicating lightness (black–white axis), and  $CIEa^*$  value, indicating the proportion of red color (red–green spectrum). The total color difference ( $\Delta E$ ) between treatment samples (T) and the control sample (C) on day 0 was calculated as follows (Šojić *et al.*, 2020):

$$\Delta E = \sqrt[3]{(L_T^* - L_C^*)^2 + (a_T^* - a_C^*)^2 + (b_T^* - b_C^*)^2};$$

### 2.4. Statistical Analysis

The experiment was designed as a  $3 \times 5$  factorial with three treatments (C, T3, T6) and five storage times (0, 15, 30, 45, and 60 days) as fixed factors. Color parameters ( $CIEL^*$ ,  $CIEa^*$ ,  $CIEb^*$ ,  $\Delta E$ ) were analyzed by two-way analysis of variance (ANOVA), and differences were tested at the 5% significance level ( $P < 0.05$ ) using Fisher's Least Significant Difference (LSD) test for post hoc com-

parisons. Statistical analyses were conducted using STATISTICA software, version 12.0 (StatSoft Inc., Tulsa, OK, USA).

## 3. Results and discussion

Color is one of the most important quality indicators of meat and meat products (Škaljac, 2014). Instrumental color measurements are presented in Table 2. At the beginning of storage, lightness ( $CIEL^*$ ) values ranged from 73.77 in treatment T6 to 75.46 in the control sample (C). No statistically significant differences ( $P > 0.05$ ) were observed between the control and the treatment groups (T3 and T6). During the 60-day storage period, a general trend of decreasing  $CIEL^*$  values was observed in all three formulations (C, T3, and T6). However, at the end of storage, the differences in  $CIEL^*$  remained statistically insignificant ( $P > 0.05$ ), indicating that the addition of SCE did not adversely affect the lightness of the product during storage.

At the beginning of the storage period, the yellowness component ( $CIEb^*$ ) ranged from 6.83 in treatment T6 to 8.38 in the control sample (C). Statistically significant differences ( $P < 0.05$ ) were observed between T6 and the other two samples (K and T3). However, throughout the 60-day storage period,  $CIEb^*$  values remained stable and did not change significantly over time ( $P > 0.05$ ). At the end of the storage,  $CIEb^*$  values in both T3 and T6 were significantly higher than in the control ( $P < 0.05$ ), likely due to the presence of anthocyanins and other pigment compounds derived from Swiss chard and their interactions with meat proteins and brine components during curing and thermal treatment (Kim *et al.*, 2019).

The total color difference ( $\Delta E$ ) values, calculated relative to the control on day 0, ranged from 2.03 (C – day 15) to 5.82 (T6 – day 60). According to the scale proposed by Pogorzelska *et al.* (2018), these differences correspond to “noticeable” to “distinct” visual changes. On day 0,  $\Delta E$  values were 2.39 for T3 and 3.57 for T6, suggesting only very slight to noticeable differences compared to the control. Similar findings were reported by Sindelar *et al.* (2007), who investigated the use of various concentrations of celery extract as a total replacement for nitrites in cooked ham.

With regard to the two most important color parameters ( $CIEL^*$  and  $CIEa^*$ ), the use of SCE at optimal concentrations appears to produce effects comparable to conventional sodium nitrite (Kim *et*

**Table 2.** Instrumental color parameters of cured-cooked meat products

| Time (day) | CIEL* value              |                           |                           |
|------------|--------------------------|---------------------------|---------------------------|
|            | C                        | T3                        | T6                        |
| 0          | 75.46±1.52 <sup>Aa</sup> | 74.38±1.20 <sup>Aa</sup>  | 73.77±2.62 <sup>Aa</sup>  |
| 15         | 75.46±1.88 <sup>Aa</sup> | 73.26±2.38 <sup>Ba</sup>  | 73.79±1.66 <sup>Aba</sup> |
| 30         | 71.05±1.05 <sup>Ab</sup> | 71.54±0.96 <sup>Ab</sup>  | 70.73±2.05 <sup>Ab</sup>  |
| 45         | 72.36±1.44 <sup>Ab</sup> | 71.37±1.81 <sup>ABb</sup> | 70.71±1.30 <sup>Bb</sup>  |
| 60         | 71.38±1.07 <sup>Ab</sup> | 71.09±1.28 <sup>Ab</sup>  | 70.29±2.24 <sup>Ab</sup>  |

| Time (day) | CIEa* value               |                           |                          |
|------------|---------------------------|---------------------------|--------------------------|
|            | C                         | T3                        | T6                       |
| 0          | 10.37±0.87 <sup>Aa</sup>  | 10.13±0.42 <sup>Ab</sup>  | 9.78±1.25 <sup>Aa</sup>  |
| 15         | 10.39±1.26 <sup>Aba</sup> | 10.71±0.88 <sup>Aa</sup>  | 9.76±0.61 <sup>Ba</sup>  |
| 30         | 10.98±0.87 <sup>Aa</sup>  | 10.02±0.55 <sup>Bb</sup>  | 10.07±0.94 <sup>Ba</sup> |
| 45         | 11.17±0.72 <sup>Aa</sup>  | 10.81±0.88 <sup>Aa</sup>  | 10.26±1.78 <sup>Aa</sup> |
| 60         | 10.28±0.66 <sup>Aa</sup>  | 10.29±0.71 <sup>Aab</sup> | 10.35±1.06 <sup>Aa</sup> |

| Time (day) | CIEb* value             |                         |                         |
|------------|-------------------------|-------------------------|-------------------------|
|            | C                       | T3                      | T6                      |
| 0          | 6.83±0.98 <sup>Bb</sup> | 7.52±0.58 <sup>Ba</sup> | 8.38±0.98 <sup>Aa</sup> |
| 15         | 7.35±0.59 <sup>Ba</sup> | 7.46±0.47 <sup>Ba</sup> | 8.59±1.00 <sup>Aa</sup> |
| 30         | 6.37±0.59 <sup>Cb</sup> | 7.07±0.42 <sup>Ba</sup> | 8.34±0.57 <sup>Aa</sup> |
| 45         | 6.73±0.40 <sup>Bb</sup> | 7.23±0.46 <sup>Ba</sup> | 8.01±0.78 <sup>Aa</sup> |
| 60         | 6.28±0.68 <sup>Bb</sup> | 7.57±0.61 <sup>Aa</sup> | 7.89±0.85 <sup>Aa</sup> |

| Time (day) | ΔE value                |                          |                         |
|------------|-------------------------|--------------------------|-------------------------|
|            | C                       | T3                       | T6                      |
| 0          | -                       | 2.39±1.35 <sup>Ab</sup>  | 3.57±1.29 <sup>Ab</sup> |
| 15         | 2.03±0.85 <sup>Ab</sup> | 3.29±2.07 <sup>Aab</sup> | 3.51±1.52 <sup>Ab</sup> |
| 30         | 4.78±1.40 <sup>Aa</sup> | 4.13±1.27 <sup>Aa</sup>  | 5.37±2.05 <sup>Aa</sup> |
| 45         | 3.85±1.33 <sup>Ba</sup> | 4.38±1.52 <sup>ABa</sup> | 5.54±2.06 <sup>Aa</sup> |
| 60         | 4.39±1.83 <sup>Aa</sup> | 4.81±2.03 <sup>Aa</sup>  | 5.82±1.80 <sup>Aa</sup> |

Values are presented as means ( $\bar{X} \pm SD$ ). Values with different uppercase letters (A–C) within the same row differ significantly ( $P < 0.05$ ); values with different lowercase letters (a–c) within the same column differ significantly ( $P < 0.05$ ).

al., 2019; Schopfer et al., 2022). Kim et al. (2019) also observed that increasing concentrations of SCE led to enhanced redness, likely due to higher levels of residual nitrite in cooked meat samples. The most pronounced changes were observed on day 60, mainly due to a reduction in lightness (CIEL\*) for all three meat products. Despite these trends, the ΔE differences between treatments and the control were not statistically significant at any time point (P

> 0.05). Taken together, the results demonstrate that the addition of 3% fermented SCE maintains color properties close to the conventional nitrite-treated product. This supports its potential use as a natural alternative to synthetic curing agents—specifically sodium nitrite—in the production of cured-cooked meat products such as cooked ham, without compromising visual appearance at any stage of the storage period.

## 4. Conclusion

The results of this study demonstrate that the application of 3% pre-fermented SCE can effectively maintain the color stability of cured-cooked meat products, such as cooked ham, with no significant differences in lightness (*CIE L\**) and redness (*CIE a\**) compared to the nitrite-treated control. The


slightly elevated yellowness (*CIE b\**) observed in meat products containing SCE is attributed to natural pigments present in the extract. Overall, total color differences ( $\Delta E$ ) remained within acceptable sensory thresholds, supporting the feasibility of using SCE as a natural alternative to nitrite in the development of clean-label meat products.

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