www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 637.52:66.022.33:547.915
Original research paper
DOI: 10.5937/ffr0-53899

EFFECTS OF NATURAL COLOURANTS AND PULSED ELECTRIC FIELD TECHNOLOGY ON THE QUALITY PARAMETERS OF NITRITE-FREE BACON

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Abstract: Replacement of nitrites in cured meat products, such as bacon chops, challenges maintaining the characteristic pink colour and other quality attributes. This study evaluates the effectiveness of natural colourants: betanin, red yeast (*Monascus*) rice extract, and roselle (*Hibiscus sabdariffa*) extract, as alternatives to nitrites for colour enhancement in nitrite-free bacon. Pulsed electric field (PEF) pre-treatment was applied to samples before immersing them in brine with colourant. Bacon loins were treated with brines containing different concentrations of these colourants, then vacuum packaged, tumbled, and stored at 4°C until further testing. Instrumental colour parameters ($L*$, $a*$, $b*$, hue angle, chroma, and cured colour ratio) were measured for both uncooked and cooked samples. Results showed that uncooked bacon treated with 0.05% betanin exhibited similar redness ($a*$) and cured colour ratios to nitrite-treated controls, indicating that betanin has the potential as a feasible colourant. Red yeast rice extract at 0.10% also enhanced redness but increased yellowness ($b*$), affecting the overall colour. Upon cooking, $a*$ values of colorant-treated bacon were comparable with the nitrite-treated sample. However, they all displayed significantly (P<0.05) higher $b*$, hue angle, and chroma values, and decreased $L*$ and cured colour ratio values. While the applied PEF treatment did not affect the instrumental colour of the uncooked and cooked samples, it was beneficial for reducing lipid oxidation on uncooked bacon with betanin and roselle extracts. These findings suggest that while natural colourants can mimic the colour effects of nitrites in uncooked bacon, additional strategies are required to improve colour stability.

Key words: *cured meat, colour stability, lipid oxidation, nitrite alternatives*

INTRODUCTION

Nitrites are food additives used in processed meats to control the growth of pathogenic bacteria such as *Clostridium botulinum* and *Listeria monocytogenes*. They also fix the stable pink colour of cured meats and prevent lipid oxidation, which influences their distinct flavour (Pegg & Honikel, 2015). However, due to health issues associated with the use of nitrites in meat products, the removal of these additives can lead to changes in the quality and safety

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characteristics of these products (Molina, Frias-Celayeta, Bolton & Botinestean, 2024; Pöhnl, 2016; Zhang et al., 2023). In terms of visual attributes, meat products without nitrites will not exhibit the desired distinct pink colour typical to cured meats. In this regard, a suitable colourant for these meat product matrices is sought to be used in formulation to give this function to compensate for nitrite removal. Due to their chemical structures, many natural colourants also possess antioxidant properties. The incorporation of these nitrite alternatives presents challenges, as the structural integrity of the muscles remains intact, complicating the incorporation of nitrite alternatives. To overcome these challenges, we propose the application of a pulsed electric field (PEF). This technology was explored for its potential application as a pre-treatment for meat curing (Guo et al., 2024). This technology has been investigated by several researchers to improve curing and marinating efficiency (McDonnell, Allen, Chardonnereau, Arimi & Botinestean, 2014; Wang, Tao, Chen, Dong, Xiong & Li, 2024). It is important to look at the possible impacts of this technology on the quality parameters of nitrite-free meat applications. This study aims to determine the effect of selected colourants and PEF application on the instrumental colour and lipid oxidation parameters of bacon chops. Betanin, red yeast rice extract containing pigment from *Monascus purpureus,* and roselle flower (*Hibiscus sabdariffa)* powder extract were tested as colourants for uncooked and cooked bacon in this study.

MATERIALS AND METHODS

Production of treated bacon

Two whole rindless and boneless pork loins were used in the production of bacon. Each loin was cut into 16 chops (2.54 cm thick) for the different treatments. For each treatment, two adjacent loin chops were weighed, and 15% of the weight was calculated for brine incorporation. The brine was prepared by dissolving pure dried vacuum (PDV) salt, sodium ascorbate, nitrite curing salt (for nitrite-treated samples), and colourant (for colourant-treated samples) in cold water (4 °C). The colourant additives used in this study are liquid red beet extract, red yeast (*Monascus*) rice extract in powder form and roselle extract in powder form.

Pulsed electric field (PEF) was applied as pre-treatment to loin chops, according to the method of (McDonnell et al., 2014) with modifications. The experiment was conducted at the National Prepared Consumer Foods Centre (NPCFC) at Teagasc Ashtown Food Research Centre, Dublin, Ireland. Two pieces of loin chops (242.4±28.7 g for each piece) were loaded into the 20x10x10 cm batch cell (DIL-HVP10 Mod, Quackenbruck, Germany). One litre of water (0.427 mS/cm) was added to the cell to suspend the meat for the PEF treatment. The cell had a constant electrode gap of 20 cm, and a 1 kV/cm field strength. The pulse width, frequency and energy input used were 9 µs, 100 Hz and 7 kJ respectively. The samples were drained and then immersed in the prepared brine and allowed to mature. Non-PEF-treated loin chops were injected with the prepared brine.

The injected loin chops were placed in vacuum packages and tumbled for 10 minutes using a multi-tumbler system (Inject Star, Germany) at 2.6 rpm, 4 °C. The samples were matured at 4 °C chiller for 6 days. One set of the treated samples was analysed for quality parameters.

Table 1.
Treatments applied to uncooked and cooked bacon

Sample code	Pre-treatment	Level of additive/colourant incorporation (w/w)
PC (positive control)	No PEF	0.012% $NaNO_2$
NF (nitrite-free)	No PEF	None
NF 0.05 BR	No PEF	0.05% beet red extract (betanin)
NF 0.10 BR	No PEF	0.10% beet red extract (betanin)
NF 0.20 BR	No PEF	0.20% beet red extract (betanin)
NF PEF 0.20 BR	PEF	0.20% beet red extract (betanin)
NF 0.05 MN	No PEF	0.05% red yeast (*Monascus*) rice extract
NF 0.10 MN	No PEF	0.10% red yeast (*Monascus*) rice extract
NF 0.20 MN	No PEF	0.20% red yeast (*Monascus*) rice extract
NF PEF 0.20 MN	PEF	0.20% red yeast (*Monascus*) rice extract
NF 0.05 RS	No PEF	0.05% roselle extract
NF 0.10 RS	No PEF	0.10% roselle extract
NF 0.20 RS	No PEF	0.20% roselle extract
NF PEF 0.20 RS	PEF	0.20% roselle extract

The other set of treated loin chops was cooked in a hot-water bath until the core temperature reached 75 °C. After cooking, the samples were rapidly cooled and stored in the chiller (4 °C) overnight until analysis. Table 1 shows the different treatments applied to the meat samples prepared in this study.

Analysis of quality parameters

Instrumental colour was measured with an Ultrascan XE Spectrophotometer (Hunter Associates Laboratory, Inc., USA) using illuminant D65, 8 ° viewing angle and 8 mm port size. L^* (lightness), a^* (redness) and b^* (yellowness) were recorded, and hue angle (Arctan b^*/a^*) and chroma ($(a^{*2} + b^{*2})^{-1/2}$) were calculated accordingly. The ratio of % reflectance at 570 nm and 650 nm wavelengths was also recorded and accounted for the calculation of the cured colour ratio, according to King et al. (2023). The pH was measured using a Hanna Professional portable pH meter (HI 98163, Hanna Instruments Ltd., U.K.). Warner-Bratzler shear force values of PEF and non-PEF treated cooked samples with 0.20% incorporation of colorants were measured using a V-shaped shear blade, attached to an Instron Universal testing machine (Model 3342 Ltd., High Wycombe, U.K.), using a 500 N load cell at a crosshead speed of 50 mm/min according to the method of Wheeler, Shackelford & Koohmaraie (1996). Force values (N) were calculated as the average of five cores, which were representative of the sample. Thiobarbituric acid reactive substances (TBARS) were quantified according to the method used by Alirezalu et al. (2021) to determine the degree of lipid oxidation. The results were expressed as mg malonaldehyde (MDA)/kg sample.

Statistical analysis

The data were analysed using SPSS software version 29.0.1.0 (IBM SPSS Statistics). The average values were reported and compared using Tukey's test. Analysis of variance (ANOVA) was done to determine the statistical differences among the treatments. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The pH values of uncooked cured bacon ranged from 5.36 – 5.66 regardless of the addition of colourants, the level of incorporation, and PEF application. This is in line with Honikel (2008) who stated that this is in the usual pH range for the curing of meat to take place. Nitrite-treated bacon (PC) exhibited pink colouring while nitrite-free and colourant-free bacon (NF) showed a more brownish colour during storage. This can be seen in the significant difference ($P<0.05$) in a^* value, hue angle and cured colour ratio between these two samples (Fig. 1). This difference is attributed to the formation of nitrosomyoglobin pigment which gives the pink colour to the uncooked meat while the colour of the untreated meat is due to metmyoglobin predominantly in the meat (Pegg & Honikel, 2015). Similarly, significantly higher a^*, hue angle, cured colour ratio and lower b^* ($P<0.05$) were observed on cooked bacon with nitrite than the one without and with no colourant added. This is expected and attributable to the formation of nitrosylhemochrome pigment on the cured cooked meat. All three proposed colourant additives at 0.05% level in nitrite-free uncooked samples resulted in a^* parameter values comparable with the nitrite-treated sample. Considering hue angle and cured colour ratio values, the incorporation of beet red extract resulted in similar colour parameters to the control sample.

A higher level of incorporation of beet red extract and red yeast rice extract also increased a^* of nitrite-free uncooked bacon. A^* values of uncooked bacon with 0.05% red yeast (*Monascus*) rice extract were not significantly different ($P>0.05$) from uncooked nitrite-treated bacon. A^* values also increased with the increase in the level of incorporation. However, b^* values were significantly higher ($P<0.05$) leading to a brownish appearance. Huang et al. (2020) suggested the combination of *Monascus* and beet red to have a more stable red colour. On the other hand, increasing levels of roselle extract decrease a^*, b^*, chroma and cured colour ratio while increasing hue angle indicates increased brownness.

The effect of adding beet red, red rice yeast extract and roselle extract has varying impacts on the colour of bacon. These differences in the colour of bacon can be attributed to the properties of pigments and the affecting intrinsic factors in the matrix. Beet red extract is from beetroot that naturally contains betalains. This is a group of nitrogenous pigments responsible for red colour and mostly stable at pH 5-6, the pH range of the uncooked cured meat (Domínguez et al., 2020). The pigments responsible for the colour of red yeast extract are metabolites produced by *Monascus purpureus* during fermentation rubropunctamine and monascorubramine which do not lose pigmentation at pH 3-8 (Gong et al., 2023). On the other hand, the main pigments in roselle extract are anthocyanins, which contribute to the deep red colour, but are sensitive to pH. Organic acids such as oxalic, malic and fumaric acids are also abundant in this extract (Jabeur et al., 2017). This might be responsible for the decreased redness of meat when the level of incorporation was increased. The incorporation of the colourants (beet red extract, red yeast rice extract and roselle extract) at 0.05% level on cooked bacon resulted in a similar a^* as of the nitrite-treated sample. However, all other instrumental colour parameters were significantly different (P<0.05); L^* and cured colour ratio values were lower, while b^*, hue angle and chroma values were higher (Fig. 1). However, beet red extract contributed to the colour similar to fresh cured meat, the colour was faded when heated as shown in the instrumental colour of treated meat with 0.10-0.20% level of incorporation. Significantly higher (P<0.05) hue angle and b^* values of cooked bacon with beet red compared to uncooked counterpart imply that the resulting colour leaned towards increased yellowness and less red/pink. This indicates the degradation of pigments in the colourant during heat treatment at 75 °C. Similar results were also observed by Dias et al. (2020) on cooked ham with beet red and hibiscus extracts. Both betalains and anthocyanins are sensitive to thermal degradation thus suitable techniques should be applied to stabilize them in cooked meat applications. Samples with red yeast rice extract exhibited increasing a^* upon increasing the level of incorporation. This showed more pigment

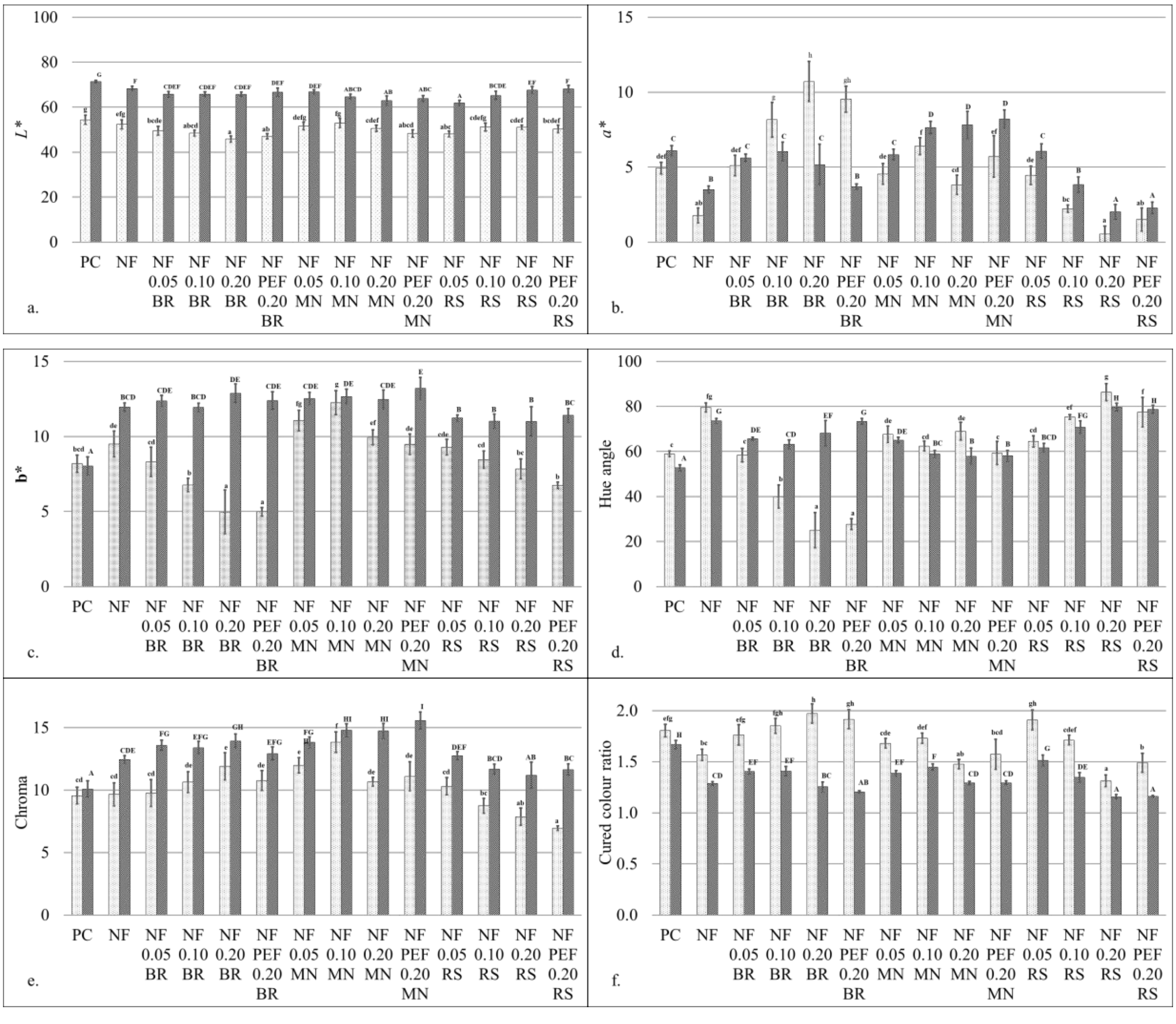

Figure 1. Instrumental colour parameters of different treated uncooked ▥ and cooked □ bacon. (PC-positive control; NF-nitrite-free; PEF-pulsed electric field treated; BR-beet red; MN-red yeast (*Monascus*) rice extract; RS-roselle extract). Mean values (n = 6) with the same letter for the same bar colour are not significantly different (P>0.05)

thermal stability than the other tested colourants.

PEF treatment did not affect the instrumental colour of the uncooked and cooked samples at 0.20% level of colourant incorporation. Data showed that there was no significant difference (P>0.05) in the values of instrumental colour values of PEF and non-PEF treated samples at the same level of added colourant. Likewise, shear force values applied to cooked samples with and without nitrite and on nitrite-free samples with 0.20% beet red, red yeast rice and roselle extracts were also not affected by the application of PEF on bacon (Fig. 2).

Similar results were also found by Arroyo et al. (2015) on PEF- and non-PEF-treated turkey meat. PEF treatment may not have caused significant physical disruptions in meat fibres to affect the shear force value parameter. The use of nitrite on uncooked and cooked bacon resulted in low TBARS values, indicating its antioxidant function on cured meat. On the other hand, nitrite-free samples, with or without the added colourants showed significantly higher levels (P<0.05) of TBARS values, except for PEF-treated uncooked bacon with 0.20% beet red and roselle extracts (Figure 3). The application of PEF and incorporation of beet red and roselle extracts resulted in lower TBARS values than the non-PEF counterpart and were not significantly different (P>0.05) from the nitrite-treated uncooked bacon. Electroporation may have caused partial disruption of cell membranes causing migration of pro-oxidants and exposing unsaturated fatty acids to oxidation (Guo et al., 2024; McDonnell, Allen, Chardonnereau, Arimi & Lyng, 2014). Several research studies have shown the potential of PEF to increase meat lipid oxidation (Faridnia et al., 2015; Kantono, Hamid, Ma, Oey & Farouk, 2021; Ma et al., 2016); but other research findings also showed that the application of PEF did not have an effect lipid oxidation on treated meats (Arroyo et al., 2015; Mungure et al., 2023). Cropotova et al. (2021)

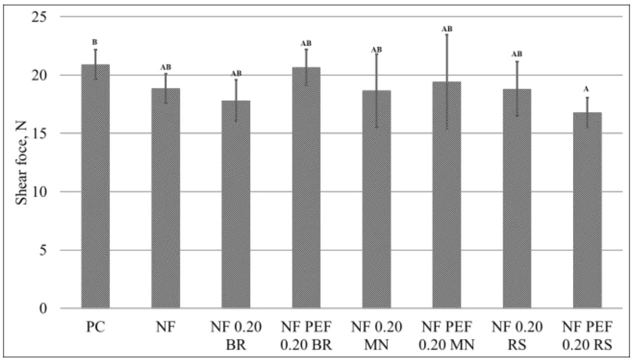

Figure 2. Shear force values of treated cooked bacon. (PC-positive control; NF-nitrite-free; PEF-pulsed electric field treated; BR-beet red; MN-red yeast (*Monascus*) rice extract; RS-roselle extract). Mean values (n = 5) with the same letter at the same bar colour are not significantly different (P>0.05)

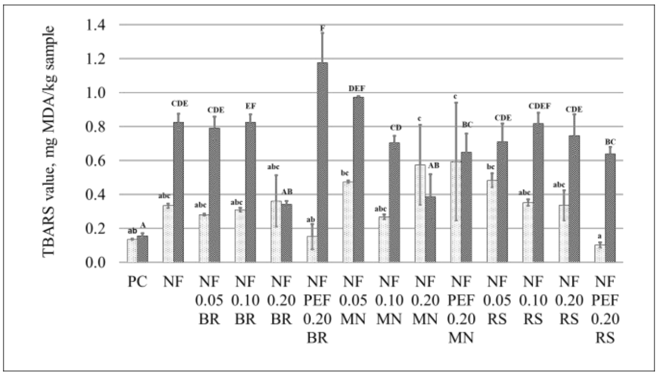

Figure 3. TBARS values of different treated uncooked ▥ and cooked □ bacon. (PC-positive control; NF-nitrite-free; PEF-pulsed electric field treated; BR-beet red; MN-red yeast (*Monascus*) rice extract; RS-roselle extract). Mean values (n = 4) with the same letter at the same bar colour are not significantly different (P>0.05)

demonstrated that PEF treatment can have both pro-oxidant and antioxidant effects on TBARS values in meat samples. The antioxidant effect of PEF is attributed to its ability to inactivate pro-oxidant endogenous enzymes, as explained by Zhao, Yang and Zhang (2012).

In this study, the presence of colourants with antioxidant properties may have also helped to minimize oxidation by reacting with radicals. It is also important to note that the MDA contents on the uncooked samples are still below the critical threshold of 1.0-2.0 mg MDA/kg for detectable off-flavour on meats (Ma et al., 2016). In this regard, PEF might have potential application as a pre-treatment on pork loins to have a synergistic effect with the antioxidant capacity of natural colourants being added.

CONCLUSION

Betanin from red beet, red yeast (*Monascus*) rice extract and roselle extract contributed to enhancing the redness of nitrite-free uncooked bacon. Beet red extract showed promising results as a colouring agent for uncooked meat product applications. Its antioxidant capacity and stability during heat treatment should be taken into account, as most of the natural pigments responsible for the red colour are sensitive to heat and light. A^* parameter values of colourant-treated bacon were comparable with the nitrite-treated sample upon cooking. However, they all displayed significantly ($P<0.05$) higher b^*, hue angle, and chroma values, and decreased L^* and cured colour ratio values.

While the use of PEF did not affect the instrumental colour of uncooked and cooked samples, it was beneficial in reducing the level of lipid oxidation particularly in the samples with beet red extract and roselle extract. Our further research will explore the optimization of the synergistic effect of colourant formulations and PEF on developing new formulations of nitrite-free cured meats with acceptable colour and overall quality.

AUTHOR CONTRIBUTIONS

Conceptualization, J.R.G.M. and C.B.; writing—original draft preparation, J.R.G.M.; writing—review and editing, J.R.G.M., C.B., J.M.F.-C. and D.J.B.; supervision, C.B. and J.M.F.-C.; project administration, C.B. and D.J.B.; funding acquisition, C.B., J.M.F.-C. and D.J.B. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, J.R.G.M., upon reasonable request.

ACKNOWLEDGMENTS

This research is funded by the Department of Agriculture, Food, and the Marine (DAFM) Ireland, Funding Agency Reference Number 2021R426, Project “Novel Nitrate/Nitrite Reduced and Nitrate/Nitrite Free Meats: Safety, Quality and Shelf-life”, and the Teagasc Walsh Scholarships Programme.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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UTICAJ PRIRODNIH SREDSTAVA ZA BOJENJE I TEHNOLOGIJE PULSNOG ELEKTRIČNOG POLJA NA PARAMETRE KVALITETA SLANINE BEZ DODATOG NITRITA

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Sažetak: Zamena nitrita u proizvodima od mesa, kao što je slanina, predstavlja izazov u održavanju karakteristične ružičaste boje i drugih kvalitativnih osobina. Ova studija procenjuje efikasnost prirodnih boja: betanina, ekstrakta crvenog kvasca *(Monascus)* i ekstrakta hibiskusa *(Hibiscus sabdariffa)*, kao alternativa nitritima za poboljšanje boje u slanini bez nitrita. Predtretman pulsnim električnim poljem (PEF) primenjen je na uzorke pre nego što su uronjeni u salamuru sa bojom. Komadi slanine su tretirani uzorcima salamure koji su sadržali različite koncentracije ovih boja, zatim su vakuumski pakovani, i čuvani na 4 °C do daljeg testiranja. Instrumentalni parametri boje (L*, a*, b*, ugao nijanse, hroma i odnos boje) su mereni za sirove i kuvane uzorke. Rezultati su pokazali da je sirova slanina tretirana sa 0,05% betanina imala sličnu crvenu nijansu (a*) i odnos boje kao kontrolni uzorci tretirani nitritima, što ukazuje da betanin ima potencijal kao boja izvodljiva u praktičnim uslovima. Ekstrakt crvenog kvasca u koncentraciji od 0,10% takođe je povećao crvenu nijansu, ali je povećao i udeo žutog tona (b*), što je uticalo na ukupnu boju. Nakon kuvanja, vrednosti crvenog tona (a*) slanine tretirane prirodnim bojama bile su uporedive sa uzorkom koji je tretiran nitritima. Međutim, svi su pokazali značajno ($P<0,05$) veće vrednosti žutog tona b*, ugla nijanse i hrome, i smanjene vrednosti svetline L* i odnosa boje. Iako primenjeni PEF tretman nije uticao na instrumentalnu boju sirovih i kuvanih uzoraka, bio je koristan za smanjenje oksidacije lipida kod sirove slanine sa betaninom i ekstraktom hibiskusa. Ovi rezultati sugerišu da prirodne boje mogu imitirati efekte boje nitrita u sirovoj slanini, ali su potrebne dodatne strategije za poboljšanje stabilnosti boje.

Ključne reči: *salamureno meso, stabilnost boje, lipidna oksidacija, zamene za nitrite*

Received: *03 Ocober 2024/* ***Received in revised form:*** *11 November 2024/* ***Accepted:*** *13 November2024*

Available online: *December 2024*

This work was reported at the 5th International Congress "Food Technology, Quality and Safety (FoodTech 2024)" held in Novi Sad, Serbia, October 16-18, 2024 organized by the Institute of Food Technology in Novi Sad.