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Sea buckthorn (*Hippophae rhamnoides* Hergo) as potential natural antioxidant for meat industry

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Abstract

Modern consumers are highly susceptible to synthetic additives, and looking for "clean label" products. For the meat industry, the application of antioxidant additives is necessary. Therefore, producers are exploring the natural sources of those compounds. The present work aimed to explore sea buckthorn as such a source. To evaluate the oxidative protection ability of extract, the TBARS method was used. Parameters such as pH, colour, and sensory quality were observed to compare experimental and control sausages. Total phenolic content, total antioxidant capacity, and polyphenol profile were determined to examine the extract. After 21 days of storage, the extract's protective ability against lipid oxidation processes was observed. No significant differences between experimental and control samples regarding instrumental parameters were observed. Sea buckthorn berry extract has shown promising results for application in meat products. Lipid protective ability in samples was concentration-dependent. However, in higher concentrations, sensory parameters were affected.

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Introduction

One of the most pressing issues faced by meat factories is to offer meat and meat products of pleasant flavour and colour. Freshness and attractiveness must also be maintained throughout the shelf life of meat and meat products (Hadidi *et al.*, 2022). Efforts are mainly aimed at delaying lipid oxidation, which is the main factor in the deterioration of the sensory quality of meat and meat products. Among the results of lipid oxidation in meat and meat products are discoloration, development of rancid flavours, deterioration of nutrient values, and formation of harmful toxic compounds (Amoli *et al.*, 2021). High concentrations of unsaturated lipids, haem pigments, metal catalysts, and oxidising agents in muscle tissues make the meat prone to oxidative degradation (Domínguez *et al.*, 2019). A way to combat those changes caused by lipid oxidation is the addition of antioxidants, mainly into meat products. Antioxidants are hydrogen atom donors, and therefore can retard or prevent oxidative reactions. Most of the antioxidants used in animal products, such as meat, are synthetic. However, the current trend in the food industry is to replace synthetic additives with more natural components (Lorenzo *et al.*, 2019). To combat the challenges of oxidative instability of lipids and proteins in meat, it is necessary to explore natural alternatives, including plant-derived antioxidants, for their low cost and health benefits (Munekata *et al.*, 2020).

Sea buckthorn (*Hippophae rhamnoides* Hergo) is a shrub species belonging to the family Elaeagnaceae. Higher altitudes and climates of Europe and Asia create suitable habitats for the species (Papuc *et al.*, 2008). It is a hardy plant, and drought- and cold-resistant. It has vigorous vegetative reproduction, and a robust and complex root system with nitrogen-fixing nodules. Currently, it is domesticated in several regions of the world due to its nutritional and medicinal properties (Suryakumar and Gupta, 2021). The buckthorn plant has been used

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extensively, for hundreds of years, in the traditional oriental system of medicine as a treatment for asthma, skin diseases, gastric ulcers, and lung disorders. Modern research supports conventional buckthorn uses, and this plant has recently been reported with a broad spectrum of pharmacological effects. Proven effects include antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective, and tissue repair properties (Saggu *et al.*, 2007; Upadhyay *et al.*, 2011; Xing *et al.*, 2022). Buckthorn berries are typically orange-yellow in colour. They are an abundant source of beneficial components such as vitamins (C and E), carotenoids (β-carotene, lycopene, lutein, and zeaxanthin), flavonoids (isorhamnetin, quercetin, isorhamnet-in-3-beta-D-glucoside; isorhamnetin-3-beta-D-glucosaminide; kaempferol, *etc*.), organic acids, amino acids, and micro- and macronutrients (Kallio *et al.*, 2002). The vast demand for products with added value and functional food leads producers and manufacturers to enrich their food products with plant-based extracts. The rich spectrum of bioactive compounds found in sea buckthorn has inspired researchers to investigate the application of its various parts and extracts in foods, with anticipation of promoting the shelf life of food products. Previous studies have shown that sea buckthorn can inhibit oxidation in meat products. This plant has a high content of bioactive compounds, including antioxidants, phytosterols, essential fatty acids, and amino acids, as well as vitamins C, K, and E (Jaśniewska and Diowksz, 2021).

Various authors have conducted experiments with adding sea buckthorn to meat products. Mäkinen *et al.* (2020) experimented with adding sea buckthorn extract from leaves to marinades and experimental sausages. Wagh and Chatli (2017) incorporated methanolic extract powder into raw ground pork meat. Papuc *et al.* (2008) used sea buckthorn fruit extract to inhibit lipid and protein oxidation of frozen carp meat. All authors reported some positive and negative effects of sea buckthorn on the quality parameters of meat products. Those effect depends on form of extract and extraction reagent.

The present work aimed to use ethanolic sea buckthorn extract as a natural antioxidant for the meat industry. Pork sausage was chosen as the model meat product due to its homogenous consistency and relatively high-fat content. Those properties make sausages a good meat product for observing lipid oxidation changes over time, and for observing other properties such as colour, pH, and sensory characteristics. We aimed to prove the protective abilities of sea buckthorn extract on fat oxidation. Points of interest are also the mechanical properties of the final product, and potential customers sensory acceptance of these novel and innovative products.

Materials and methods

Sea buckthorn fruit samples were obtained from the botanical garden at the Slovak University of Agriculture in Nitra. Experimental meat product manufacturing and examination of all its properties were completed twice due to the validation and comparison of obtained results from each experiment. Each test of extracts and meat product samples was carried out in triplicates. All processing and experimental work were carried out at the Institute of Food Sciences of the Slovak University of Agriculture in Nitra.

Extract preparation

Sea buckthorn berries were collected, frozen, and lyophilised. The preparation of the extract was carried out according to Shirahigue *et al.* (2010). The extract was then refrigerated 4 ± 1°C until further analyses.

Total antioxidant capacity (TAC)

Total antioxidant capacity was measured using the radical-scavenging method according to Demianová *et al.* (2021) using DPPH radical (2,2-diphenyl-1-picrylhydrazyl, $C_{18}H_{12}N_5O_6$; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany).

Total phenolic content (TPC)

Total phenolic content was measured according to Lachman *et al.* (2011), using the modified Folin-Ciocalteu spectrophotometric assay. A double-beam UV-VIS spectrophotometer (T80 UV/VIS Spectrometer; PG Instruments, Ltd., Lutterworth, United Kingdom) was used for the measurement. Results were expressed as milligrams of gallic acid equivalents (GAE) in kilograms of dry fruit dry matter.

Moisture

The moisture percentage in the powdered sample was measured using a KERN DAB 100-3 moisture analyser (KERN & SOHN GmbH, Balingen, Germany) Drying was performed at 110°C.

High-performance liquid chromatography

The determination and quantification of polyphenols in the extract used in our experiment were conducted according to Gabriele *et al.* (2018) using high-performance liquid chromatography with a diode array detector (HPLC DAD).

Sausage preparation

Sausage preparation followed the recipe described by Šedivý (2022). The meat used to prepare the control and experimental sausages (shoulder and loin) were purchased from a local butchery. The meat was sliced into smaller pieces, minced using a mincer (Gastro, Ochtrupand, Germany), and mixed with water and other ingredients. Before filling, the meat batter was separated into four parts, and fortified with antioxidants. A negative control group (Con-0) was made without the addition of antioxidants, Control C group (Con-C) was made with the addition of ascorbic acid in concentration 0.5 g/kg of the final product, and experimental groups (Exp-3 and Exp-5) were made with 3 and 5 mL/kg, respectively. Final products were heat-treated to a core temperature of 70 ± 1°C for 10 min, chilled, vacuum-packed, and refrigerated at 4°C until further analyses.

pH

To determine the pH of the final meat product, control, and experimental groups, a piercing probe benchtop pH meter (Orion Star™ A211 Benchtop pH Meter, Beijing, China) was used. The pH meter was calibrated using calibration solutions (Hamilton AG Bonaduz, Bonaduz, Switzerland; with pH values of 4, 7, and 10). As recommended, calibration was performed at a room temperature of 21 ± 1°C. Sausage samples were taken out of the refrigerator, and let to temper to room temperature before conducting the measurement.

Colour determination

A portable spectrophotometer Konica Minolta CM-2600d (Konica Minolta, Osaka, Japan) was used to determine the instrumental colour of meat products in six repetitions. Before the measurement, sausages were sliced perpendicularly, and only the inside of the products was used for the colour measurement, not the intestine covering the surface. The spectrophotometer parameters was a D65 light source, and a 10° observer with an 8 mm diameter aperture size. For colour analysis, SCI (Specular Component Included) setting was chosen due to the matte surface properties of meat products. The results represent coordinates in the colour interface of CIELab. Coordinate L* represents the clarity (L = 0 is black, and L* = 100 is colourless). Coordinate a* represents the shade of red and green(a* > 0 indicates red colour, and a* < 0 indicates green colour). Coordinate b* represents the shade of blue and yellow (b* > 0 indicates yellow colour, and b* < 0 indicates blue colour).

Oxidative stability

Oxidative stability using 2-thiobarbituric acid reactive substances (TBARS) method was carried out according to Jurčaga *et al.* (2022). The final value of absorbance was analysed at a wavelength of 532 nm (T80 UV/VIS Spectrometer, PG Instruments, Ltd., Lutterworth, UK). Subsequently, the final concentration of malondialdehyde (MDA) was calculated from absorbance levels, and was expressed as mg of MDA per 1 kg of the final product (mg/kg).

Sensory evaluation

The 5-point scale with a detailed description of point values was used to evaluate sausage samples' sensory properties. Sensory evaluation was performed under controlled conditions on the 7th, 14th, and 21st day of storage. The sensory panel consisted of six evaluators of both genders from 23 to 45 years of age. Evaluators were selected from the Department of Technology and Quality of Animal Products, and all were experienced with animal product evaluations. The evaluators were trained before the first day of analysis. The sensory analysis focused on the following descriptors: appearance (both on the surface and a cut), colour, aroma, consistency, and taste.

Statistical analysis

To perform statistical analysis, XLSTAT software was used (XLSTAT Addinsoft, Statistical and Data Analysis Solution, New York, NY, USA). Analysis of variance (ANOVA) with Duncan's multiple range test was used to compare the results of individual measurements.

Results

Dry matter content, total antioxidant capacity (TAC), and total phenolic content (TPC)

The dry matter (DM) content in raw fruit sea buckthorn was measured in the range of 13 - 13.7%.

Piłat and Zadernowski (2019) reported that the amount of DM in sea buckthorn fresh fruit was 12.89 ± 3.00% DM.

To better understand the function of the extract in the meat product, we determined the TAC, TPC, and the profile of individual polyphenols in the extracts. Tkacz *et al.* (2019) observed the antioxidant potential of sea buckthorn. Their results showed that antioxidant properties might vary, given the methodology and variety. Their DPPH analysis showed 94% of inhibition. The extract used in our study showed lower inhibition ability of 67.78 ± 1.80%.

Polyphenolic quantification

The extract used for the enrichment of sausages was subjected to HPLC-DAD analysis. Several polyphenolic compounds were quantified, and are listed in Table 1. The most dominant component was rutin, followed by chlorogenic acid and kaempferol. The least represented compounds were quercetin and myricetin.

Table 1. Phenolic compounds and their retention times (min), wavelengths (nm), and concentrations (mg/kg DW ± S.D.).

Phenolic compound	Retention time	Wavelength	Concentration
Rutin	12.468	265	39.71 ± 0.38
Quercetin	18.694	372	0.82 ± 0.02
Kaempferol	19.897	372	9.61 ± 0.34
Myricetin	16.982	372	1.50 ± 0.24
Caffeic acid	7.841	325	7.11 ± 0.34
Ferulic acid	12.627	310	2.28 ± 0.44
Orientin	11.006	320	3.00 ± 0.36
Chlorogenic acid	6.330	320	20.86 ± 0.14

pH

All sample values were monitored throughout the storage period and measured on the 1st, 7th, 14th, and 21st days. On the first day, a minimal variation of pH values was observed among the groups. After seven days, a drop in pH was observed. This drop was significantly more prevalent in samples with an ascorbic acid treatment, and higher in the negative control. However, a lower drop was observed in the experimental group Exp-3. This trend continued even after another week of storage. However, on the final day of the experiment (21st day), we observed a slight increase in pH values. This could be explained by the exhaustion of added antioxidants and/or by decomposing protein components of meat products and the production of basic compounds. Only the control group with ascorbic acid addition showed significantly lower values than all other groups. All measured values of pH are listed in Table 2.

Table 2. pH values of sausage samples during storage (pH value ± S.D.).

Sample	Day 1	Day 7	Day 14	Day 21
Con-0	6.34 ± 0.03^{A}	6.28 ± 0.01^{A}	6.29 ± 0.02^{A}	6.30 ± 0.04^{A}
Con-C	6.30 ± 0.02^{A}	6.17 ± 0.02^{B}	6.17 ± 0.02^{C}	6.27 ± 0.01^{B}
Exp-3	6.34 ± 0.05^{A}	6.28 ± 0.04^{A}	6.26 ± 0.01^{AB}	6.31 ± 0.01^{A}
Exp-5	6.34 ± 0.02^{A}	6.25 ± 0.04^{AB}	6.23 ± 0.02^{B}	6.30 ± 0.03^{A}

Uppercase superscripts indicate statistically significant differences (ANOVA; Duncan's multiple range test; $\alpha = 0.05$) between samples in the column; Con-0: negative control; Con-C: control with 0.5 g/kg ascorbic acid; Exp-3: 3 mL/kg sea buckthorn extract addition; and Exp-5: 5 mL/kg sea buckthorn extract.

Colour determination

Colour determination of sausage samples was conducted on the 1st, 7th, 14th, and 21st days of storage. Lightness (L*) and yellowness (b*) were measured without significant differences. The only significant difference ($\alpha = 0.05$) was observed on the first day in the redness parameter (a*) between the negative control and the control with ascorbic acid. After a

week of storage, values of redness (a*) and yellowness (b*) showed no differences among groups. The only difference occurred in lightness (L*) between the negative control group, which was the brightest sample, and the experimental group (Exp-5) with 5 mL/kg sea buckthorn extract as the darkest one. This could be explained by the darker colour of the added extract. Lightness values showed a significant difference between the Con-C (ascorbic acid group) and the Exp-5 group, which showed the most prevalent darkening. Also, the Exp-5 group showed higher values in yellowness than other samples. Day 14 observation further proved no changes in redness values among samples. At the end of the storage period, redness (a*) and yellowness (b*) showed no statistical difference among all groups. Lightness values (L*) proved that only the Exp-5 group reached the darkest (deepest) colour. This darkening could be explained by the darker colour of the extract and its higher concentration than in other experimental groups. All measured CIELab values of samples are presented in Table 3.

Table 3. Colour values of sausage samples during storage (colour value ± S.D.).

Sample	L* (D65)	a* (D65)	b* (D65)
Day 1			
Con-0	67.41 ± 0.30^{A}	14.14 ± 0.74^{B}	22.72 ± 0.89^{A}
Con-C	66.21 ± 0.94^{A}	15.40 ± 0.31^{A}	22.77 ± 0.68^{A}
Exp-3	67.10 ± 0.12^{A}	14.86 ± 0.25^{AB}	23.22 ± 0.50^{A}
Exp-5	67.62 ± 0.90^{A}	14.77 ± 0.20^{AB}	23.14 ± 0.38^{A}
Day 7			
Con-0	69.01 ± 0.65^{A}	14.34 ± 0.47^{A}	23.08 ± 0.22^{A}
Con-C	67.96 ± 1.03^{AB}	15.46 ± 0.65^{A}	24.01 ± 0.70^{A}
Exp-3	66.48 ± 0.50^{AB}	15.34 ± 0.27^{A}	23.53 ± 0.88^{A}
Exp-5	67.20 ± 0.69^{B}	14.89 ± 0.39^{A}	23.69 ± 0.45^{A}
Day 14			
Con-0	68.22 ± 1.78^{AB}	14.50 ± 0.08^{A}	22.09 ± 0.23^{B}
Con-C	69.67 ± 0.97^{A}	14.66 ± 0.09^{A}	22.50 ± 0.32^{B}
Exp-3	68.31 ± 0.77^{AB}	15.63 ± 0.58^{A}	22.49 ± 0.21^{B}
Exp-5	66.79 ± 0.66^{B}	15.19 ± 0.80^{A}	23.32 ± 0.37^{A}
Day 21			
Con-0	69.02 ± 0.82^{A}	14.94 ± 0.62^{A}	21.79 ± 0.37^{A}
Con-C	68.13 ± 0.53^{A}	15.20 ± 0.48^{A}	22.23 ± 0.36^{A}
Exp-3	68.58 ± 1.13^{A}	15.47 ± 0.74^{A}	22.16 ± 0.31^{A}
Exp-5	65.78 ± 0.73^{B}	15.27 ± 1.47^{A}	22.31 ± 1.50^{A}

Uppercase superscripts indicate statistically significant differences (ANOVA; Duncan's multiple range test; $\alpha = 0.05$) between samples in similar columns within one storage period; Con-0: negative control; Con-C: control with 0.5 g/kg ascorbic acid; Exp-3: 3 mL/kg sea buckthorn extract addition; and Exp-5: 5 mL/kg sea buckthorn extract.

Oxidative stability

Oxidative stability analysis of sausage samples was performed on the 1st, 7th, 14th, and 21st day of refrigerated storage. Results are expressed as mg of MDA in kg of the final product. After one week of storage, we observed a significant increase in MDA formation in all samples. This increase was steady; no significant difference occurred among the groups. After another week, a significant increase in MDA was observed in all groups, except the Exp-5 group with 5 mL/kg extract addition. In group comparison, both Con-C and Exp-5 groups showed significantly lower MDA values than the negative control (Con-0) and the experimental group with the lower extract addition (Exp-3). At the end of the observation period, another increase in MDA levels was observed in all groups. Among groups, the negative control showed significantly higher MDA concentration than

every other group. The percentage increase in MDA concentration in negative control samples was around 100%. The control with the ascorbic acid and Exp-3 groups showed a comparable increase of 63 and 66%, respectively. The lowest growth, just 56%, was observed in Exp-5 samples with 5 mL/kg addition of sea buck-thorn extract. All measured values of MDA concentration are listed in Table 4.

Table 4. Measured values of MDA in sausage samples during storage (mg/kg ± S.D.).

Sample	Day 1	Day 7	Day 14	Day 21
Con-0	0.158 ± 0.002^{Ad}	0.209 ± 0.004^{Ac}	0.253 ± 0.007^{Ab}	0.317 ± 0.022^{Aa}
Con-C	0.163 ± 0.006^{Ad}	0.208 ± 0.005^{Ac}	0.232 ± 0.003^{Bb}	0.266 ± 0.003^{Ba}
Exp-3	0.166 ± 0.006^{Ad}	0.205 ± 0.006^{Ac}	0.246 ± 0.006^{Ab}	0.276 ± 0.001^{Ba}
Exp-5	0.166 ± 0.010^{Ac}	0.219 ± 0.018^{Ab}	0.233 ± 0.004^{Bb}	0.260 ± 0.005^{Ba}

Uppercase superscripts indicate statistically significant differences (ANOVA; Duncan's multiple range test; $\alpha = 0.05$) between samples in similar columns; lowercase superscripts indicate statistically significant differences (ANOVA; Duncan's multiple range test; $\alpha = 0.05$) between samples in similar rows; Con-0: negative control; Con-C: control with 0.5 g/kg ascorbic acid; Exp-3: 3 mL/kg sea buckthorn extract addition; and Exp-5: 5 mL/kg sea buckthorn extract.

Sensory evaluation

Sensory analysis of sausage samples was conducted on the 7th, 14th, and 21st day of storage. The first day after production was omitted for practical reasons; customers cannot purchase such products in stores the day after production. Repeated sensory evaluation helped us to observe changes among groups over time. Day 7 (Figure 1) evaluation did not show significant ($\alpha = 0.05$) differences among groups in any descriptor. However, the group with the ascorbic acid had the highest overall taste and consistency score. Furthermore, the group with 3 mL/kg addition obtained the highest score regarding colour, appearance, and aroma parameters. Results suggested that sea buckthorn could enhance multiple parameters, and be accepted or even preferred by customers. Day 14 (Figure 1) evaluation did not prove any significant ($\alpha = 0.05$) difference among sausage samples. Same as the week before, the average best score in the taste parameter was observed in the sample with ascorbic acid (Con-C). Experimental samples with 3 mL/kg sea buckthorn extract addition again obtained a higher score in appearance and colour parameters. However, this group obtained the lowest score in aroma, which is surprising after positive marks on day 7. The last sensory evaluation of the experiment took place on the 21st day of storage (Figure 1). This should represent the sensory of sausages nearing their expiration date. As for the whole duration, we did not observe significant ($\alpha = 0.05$) differences among sausage samples. At this time, the best average score in taste was obtained by the negative control group. The experimental group with 3 mL/kg sea buckthorn extract addition (Exp-3) obtained the highest colour, aroma, and consistency scores. In addition, the Exp-3 group obtained the second-best score in taste. Therefore, this treatment showed a high potential for utilisation in the meat industry. On the other hand, the experimental group with the addition of more concentrated extract (Exp-5) was penalised in taste parameter, given that the evaluators reported a bitter aftertaste in this late stage of storage. Also, in other parameters, the Exp-5 group obtained a lower average score than the Exp-3 group.

Discussion

Dry matter content, total antioxidant capacity (TAC), and total phenolic content (TPC)

Sea buckthorn is rich in polyphenols (Criste *et al.*, 2020). Our extract yielded an average TPC of 628.78 ± 4.34 mg GAE/kg. Mendelová *et al.* (2016) claimed that the content of polyphenols is genotype-dependent. Furthermore, the method of extract preparation is essential too. Kreps *et al.* (2021) proved that extracts prepared from 70% ethanol yielded a higher content of TPC than those prepared from 96% ethanol.

Phenolic quantification

Our results were comparable to the findings of other authors examining the phenolic profile of sea buckthorn. A similar profile of phenolic compounds was reported in the work of authors Rösch *et al.*

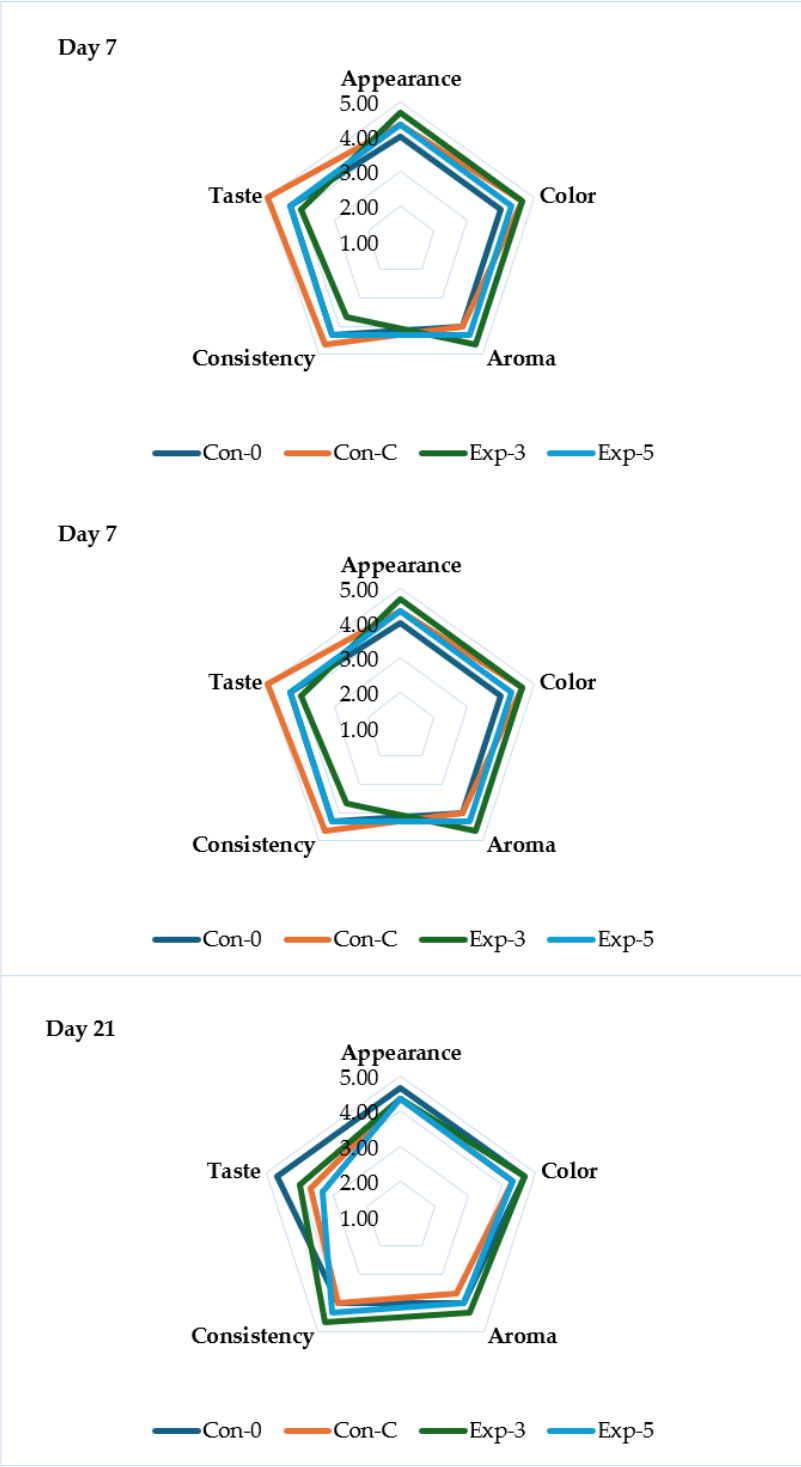

Figure 1. Results of sensory evaluation on 7th, 14th and 21st day of storage. Con-0: negative control; Con-C: control with 0.5 g/kg ascorbic acid; Exp-3: 3 mL/kg sea buckthorn extract addition; and Exp-5: 5 mL/kg sea buckthorn extract.

(2003) and Olas (2016), who identified three major flavonols in sea buckthorn samples (isorhamnetin, quercetin, and kaempferol), two of which also occurred in our samples. Regarding phenolic acids, we confirmed the presence of caffeic and ferulic acids, as Arimboor *et al.* (2008) reported. The complex profile of polyphenolic compounds could vary among sea buckthorn varieties (Sytařová *et al.*, 2020).

pH

Regarding pH, our results contradicted Salejda *et al.* (2017). In their work, the authors reported a significant increase in the acidity of pork sausage samples with a sea buckthorn extract. Also, the acidity increase was dependent on the extract concentration. Similarly, Kumar *et al.* (2015) reported lower pH values in minced pork patties treated with sea buckthorn extract than in negative control samples. Our research did not confirm the authors' findings. Diverse results could be explained by the extract itself, mainly by different extractors or buckthorn varieties used.

Colour

Colour is an essential quality factor for animal products. Different techniques can be used to achieve better colour of the product. Sea buckthorn has not been used as such since its colour improvement ability is proven to be very limited (Ben-Mahmoud *et al.*, 2014). However, another research described that buckthorn could change animal product colours depending on its carotenoid composition. As previously described, sea buckthorn fruit contains significant concentrations of carotenoids (Pop *et al.*, 2014). Kozhakhiyeva *et al.* (2018) incorporated sea buckthorn extract powder in manufacturing horse meat, and reported significant colour changes compared to the traditional preparation method. Also, the darkening of pork sausages was reported in other studies. Sea buckthorn extract addition significantly affected the colour parameters of sausages expressed by CIELab parameters. The application of 3% extract to the recipe of sausages caused a significant reduction in lightness (L*). This effect was also observed in the raw sausage samples after 28 days of chill storage (Salejda *et al.*, 2014; 2017).

Oxidative stability

Our findings proved the protective oxidation effect of sea buckthorn extract in sausages. The inhibition efficiency of natural extract in both concentrations was comparable to the efficiency of ascorbic acid in the control group. This effect could be observed in the latter storage stages, after 14 and 21 days. Authors Kozhakhiyeva *et al.* (2018) injected horse meat strips with brine to reach concentrations of 0.5 and 1.0% in the final product. After cooking, strips were cooled to 10 - 12°C and vacuum-packaged before sampling. The vacuum-packed samples were stored for 21 days at 0 - 4°C. They reported significant ($\alpha = 0.05$) reduction in primary lipid oxidation products (lipid hydroperoxides) of 24% in samples with 1.0% concentration. Also, the authors observed 17% reduction in MDA in the sample with an addition of 0.5% extract compared to the control. The reduction in secondary lipid oxidation products (TBARS) reached 53% in samples with 1.0% extract concentration, and 44% in samples with 0.5% extract concentration. Püssa *et al.* (2008) used ethanol and sea buckthorn extract to retard lipid oxidation of mechanically deboned meat. The 1% extract of sea buckthorn addition significantly reduced MDA formation in raw and cooked deboned meat samples. Salejda *et al.* (2017) used powdered buckthorn extract as an antioxidant for vacuum-packed pork sausages. The authors reported significantly improved oxidation stability of experimental samples compared to the negative control. It was observed that extract addition to meat-fatty batter caused higher protection against lipid oxidation in cooked sausages. The powder obtained from dried sea buckthorn berries (added in a concentration of 25 g/kg in the final product) showed strong lipid stabilisation during storage.

Our findings agreed with the results of other authors researching the topic. We proved that sea buckthorn, combined with vacuum packaging and refrigerated storage, could be a potent antioxidant suitable for meat products.

Sensory evaluation

Similar sensory evaluation results of pork sausages with sea buckthorn juice and oil addition were obtained by Bobko *et al.* (2019). The authors reported that during the evaluation of the individual sensory quality indicators after natural antioxidants addition, no significant differences ($\alpha = 0.05$) among individual groups were recorded. This indicated that their addition did not negatively affect the sensory quality of sausages. In evaluating the most crucial indicator of sensory quality (taste), authors recorded similar ratings in all experimental groups with the sea buckthorn addition compared to the negative control group. Salejda *et al.* (2014) incorporated sea buckthorn extract into the pork sausages. The authors described that sausage samples with 1.5% extract addition obtained better scores than the negative control group. On the other hand, the increase in sea buckthorn extract concentration in the recipe caused a decrease in colour acceptance by the panellists. This was also observed in our results. Improvement of

sensory attributes of food by adding sea buckthorn is not limited to meat products. Authors Ghendov-Moşanu (2019) reported improved sensory properties of cream cheese stored for ten days.

Conclusion

The present work focused on incorporating sea buckthorn berries extract into pork sausages to explore its potential utilisation as a natural antioxidant for the meat industry. Lipid protective ability was observed comparable to pure ascorbic acid in a concentration-dependent manner. No significant changes were observed among groups in values of pH. Higher concentration (5 mL/kg) caused darkening in meat products in the CIELab colour range. However, this change was not negatively reflected in the sensory analysis. For most of the panellists, the taste was the decisive marker. Panellists found it challenging to accept the bitterness of experimental samples, especially at the end of the storage period. This could be improved by altering sausage extract concentration or using a different extraction technique. Overall, sea buckthorn could be a promising plant-based antioxidant for the meat industry. However, before its practical use, further technological, toxicological, and sensorial studies need to be carried out.

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