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EFFECT OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* VAR. VITAMINNAJA) EXTRACT ON SPOILAGE OF PORK SAUSAGES

Andrea Mesárošová[1], Judita Lidíková[1], Alica Bobková[1], Miroslav Kročko[1], Andrea Mendelová[1], Tomáš Tóth[1], Šárka Nedomová[2], Daniela Vörösová[3], Marek Bobko[1]*

Address(es):
[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Science, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[2] Mendel University in Brno, Faculty of AgriSciences, Institute of Food Technology, Zemědělská 1752, 613 00 Brno-sever-Černá Pole, Czech Republic.
[3] Constantine the Philosopher University in Nitra, Faculty of Natural Sciences and Informatics, Institute of Economics and Management, Tr. A. Hlinku 1, 94901 Nitra, Slovakia.

*Corresponding author: marek.bobko@uniag.sk

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ABSTRACT

Lipid oxidation and microbial deterioration are the main reasons for spoilage of meat and meat product. The common way of fighting those processes is to add synthetic antioxidants and preservatives. However, the modern trend is to replace synthetic additives with natural alternatives, mainly of plant origin. Our study incorporated sea buckthorn extract variety Vitaminnaja into pork sausages in two concentrations – 3 and 5 mL.kg^{-1}. The prepared extract examination showed that total antioxidant capacity and total polyphenol content were measured and expressed at an average of 65.04 % of DPPH radical inhibition and 677.58 g GAE.kg^{-1}. During the TBARS evaluation, we observed a significant reduction of malondialdehyde created in experimental samples compared to the control group. The antimicrobial effect of sea buckthorn was observed in various previous studies. In our experiment, we could not confirm this ability in meat products. On the other hand, we did not detect any negative effect of the extract on the microbial load of sausages. We recommend sea buckthorn as a possible source of natural antioxidants for the meat industry. Further multi-disciplinary research is still, however, needed.

Keywords: meat product, pork, oxidation, lipid, microbiology

INTRODUCTION

The main goal of the meat industries is to provide meat and meat products with excellent sensory properties, such as flavor and aroma, for prolonged storage periods at the lowest possible cost **(Garrine *et al.*, 2021)**. When huge volumes of meat began to be exported long distances (e.g., from Australia to the UK), scientific interest in meat microbiology surged, and this trend continued in the 1950s with the advent of supermarkets. Meat spoilage is not always obvious, and consumers would agree that the key qualitative criteria for meat rejection would be extreme discoloration, strong off-odors, and slime development. In general, spoiling is a subjective judgment made by the customer, which can be impacted by cultural and economic reasons, the individual's sensory acuity, and the degree of the change **(Nychas *et al.*, 2008)**.
The potential for microbiological spoilage varies greatly with the category of meat product. Spoilage potential can also vary within a meat category as formulations are varied. Careful consideration must be given to the specific formulation parameters when establishing product shelf life and spoilage potential or troubleshooting a problem **(Cerveny *et al.*, 2009)**. Meats are subject to spoilage by a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, yeasts, and molds **(Jay *et al.*, 2005)**. Perishable raw salted and salted-cured products such as fresh pork sausage, Polish sausage, Italian sausage, bratwurst, chorizo, uncooked hams, bacon, and corned beef are common in the USA and elsewhere. The type of packaging used for these products is a major determinant of shelf life. Fresh sausage bulk-packaged into trays or sold in edible casings has a very short shelf life, typically 7–21 days. The predominant spoilage microflora for these products is the psychrotrophic pseudomonads, molds, and yeasts. On the other hand, fresh sausage products sold in oxygen-impermeable casings generally have a longer shelf life (about 4 weeks). The predominant spoilage microflora in these products is the lactic acid bacteria **(Cerveny *et al.*, 2009)**.
The other key factor responsible for the acceptability and quality of meat products and the loss of their flavour and taste is oxidation. Muscle tissues have a high concentration of unsaturated lipids, heme pigments, metal catalysts, and oxidizing agents, making the meat susceptible to oxidative deterioration. Discoloration, off-flavour development, hazardous chemical generation, short shelf-life, and nutritional and drip losses indicate oxidative deterioration in meat and meat products. In addition to nutritional degradation, lipid/ protein oxidation produces cytotoxic and genotoxic chemicals that damage human health **(Amoli *et al.*, 2021; Domínguez *et al.*, 2021)**.
Due to their health benefits and growing consumer awareness, international markets have seen a tremendous increase in demand for dietary supplements supplemented with bioactive components, such as meat and meat products, over the previous 70 years. Meat is a staple meal rich in critical nutrients such as high-quality proteins, vitamins, bioactive substances, carbohydrates, minerals, and colors, and contains varied amounts of storage (triacylglycerols) and structural (phospholipids) lipids depending on muscle type **(Cheng *et al.*, 2020)**. Synthetic antioxidants such as butylated hydroxytoluene (BHT) were widely employed to delay, retard, or prevent lipid oxidation by scavenging chain-carrying peroxyl radicals or reducing free radical production. However, due to concerns about the safety of these synthetic compounds, extensive research is being conducted to identify novel and naturally occurring compounds that can delay oxidative degradation of lipids, improve quality, and maintain the nutritional value of foods **(de Ciriano *et al.*, 2010)**. Because natural antioxidants are more acceptable to consumers than synthetic antioxidants, they have better application potential in the meat sector. However, applying plant extracts, herbs, spices, and essential oils with antioxidant benefits is still a long way off due to a lack of evidence on their effects on various meat products **(Kumar *et al.*, 2015b)**. Sea buckthorn (Hippophae rhamnoides L.) berries are an excellent source of bioactive chemicals. Buckthorn's fruits are high in polyphenols and vitamins and contain a high concentration of quercetin and flavonols in diverse forms **(Guliyev et al., 2004)** as nutraceuticals are used to treat skin changes caused by radiation, burns, mouth irritation, and gastric ulcers. Reduced cholesterol levels in blood plasma, prevention of platelet aggregation, and control of immunological function may all positively impact human health **(Zadernowski *et al.*, 2003)**.
Our study aimed to incorporate natural extract from sea buckthorn of variety Vitaminnaja into soft raw meat product to improve its oxidative stability, reduce microbiological spoilage and therefore prolong overall shelf-life. We believe that our obtained data will benefit the meat industry's effort to utilize plant-based antioxidants in their products and shift toward the modern clean-label products customers' demand.

MATERIAL AND METHODS

Extract preparation

Sea buckthorn berries (*Hippophae rhamnoides* var. Vitaminnaja) were obtained from the Botanical Garden of SUA in Nitra. Berries were harvested, lyophilized, and used to prepare extract according to the methodology used by **Shirahigue *et al.* (2010).**

Total antioxidant capacity (TAC)

To evaluate the antioxidant potential of sea buckthorn extract, DPPH radical method was used as suggested by the authors **Demianová *et al.* (2021).**

Total Polyphenols Content (TPC)

Total polyphenol content was measured using the method published by **Bobková *et al.* (2021)** using the Folin-Ciocalteu assay. The final concentration is expressed as equivalents of gallic acid as g GAE.kg^{-1} of dry matter.

Meat product preparation

Sausage preparation was carried out according to the recipe from guidelines by **Šedivý (2022).** Meat for the preparation of the control and experimental sausages (shoulder and loin) were purchased at a local butchery. Antioxidant addition into sausage groups was as follows: Control-0 – group without added antioxidant; Control-C – ascorbic acid addition 0.5 g.kg^{-1}; Experiment-3 – sea buckthorn extract addition 3 ml.kg^{-1}; Experiment-5 – sea buckthorn extract addition 5 ml.kg^{-1}.

pH values of meat products

A piercing probe tabletop pH meter (Orion StarTM A211 tabletop pH meter, Beijing, China) was used to determine the pH of the prepared products. Calibration solutions (Hamilton AG Bonaduz, Bonaduz, Switzerland, with pH values of 4, 7, and 10) were used to calibrate the pH meter. The pH was measured after the meat products reached the room temperature.

Oxidative stability of meat products

The oxidative stability of the raw-cooked product was based on measurements of the malondialdehyde (MDA) concentration by thiobarbiturate test using a 2-thiobarbituric acid (TBA) solution as described by **Jurčaga *et al.* (2022).**

Microbiological examination of meat products

The microbiological examination was carried out by using a simple dilution method. Microbial analysis was performed during storage on the 7th, 14th, and 21st days. We focused on the following microorganisms with conditions as listed:

- *Enterococcus* sp. - Slanetz and Bartley agar (Merc KGaA, Darmstadt, Germany), 3 days at 37 °C ± 1 °C,
- *Lactobacillus* sp. - De Man, Rogosa and Sharpe (MRS) agar (Merc KGaA, Darmstadt, Germany), 5 days, 37 °C ± 1 °C.
- Psychrotrophic bacteria - Plate count agar (PCA) (Merc KGaA, Darmstadt, Germany), 10 days, 6.5 ± 1 °C.

To determine the colony-forming unit, the plate counting method was used and subsequently calculated, and expressed as log cfu·g^{-1}.

Statistical analysis

Statistical analysis was performed using XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France, 2017). To compare the results of the individual analysed groups, ANOVA analysis with Duncan test was used. For all the tests, the level of signification α was set to 0.05.

RESULTS AND DISCUSSION

Extract examination

Tkacs *et al.* (2019) examined the antioxidant potential of sea buckthorn. Their results showed that antioxidant properties can vary depending on the methodology used and the variety of plants. During our analysis, we measured the total antioxidant capacity of our extract to 65.04 ± 2.94% of DPPH radical inhibition. Sea buckthorn is rich in polyphenols **(Criste *et al.*, 2020)**. **Mendelová *et al.* (2016)** claimed that polyphenol content is highly dependent on genotype. In addition, the method of preparation of the extract is also important. **Kreps *et al.*, (2021)** proved that extracts prepared from 70% ethanol reached a higher TPC content than those prepared from 96% ethanol. Our prepared 80% ethanol extract achieved average polyphenol content 677.58 ± 3.38 g GAE.kg^{-1}.

pH

Values of pH could be important indicators of meat product spoilage. Especially the spoilage caused by microorganisms such as *Lactobacillus* sp. due to their ability to produce lactic acid and decrease the pH of final products. Comparing the individual days, we observed a minimal change in pH values among groups. Only on the last day of storage (day 21), we observed that experimental sausages with extract addition reached significantly ($p \leq 0.05$) higher pH values than control groups with ascorbic acid or without any antioxidant at all. Regarding pH changes in time, we observed very little variability. In the Control-C group, we observed increased pH values after 7 days of storage. The extract-5 group undergoes changes in pH during the first weeks of storage. On the other hand, at the end of the storage period, the pH values of this experimental group were practically identical. The results of the analysis are shown in the Table 1.

Table 1 pH values of sausage samples during storage *(mean ± S.D.)*

Sample	Day 1	Day 7	Day 14	Day 21
Control-0	6.46 ± 0.03^{aA}	6.43 ± 0.06^{aA}	6.44 ± 0.05^{aA}	6.39 ± 0.04^{bA}
Control-C	6.34 ± 0.02^{aB}	6.41 ± 0.03^{aA}	6.40 ± 0.01^{aA}	6.39 ± 0.01^{bA}
Extract-3	6.47 ± 0.02^{aA}	6.49 ± 0.03^{aA}	6.47 ± 0.01^{aA}	6.47 ± 0.02^{aA}
Extract-5	6.45 ± 0.02^{aAB}	6.48 ± 0.01^{aA}	6.42 ± 0.03^{aB}	6.46 ± 0.01^{aAB}

Note: Control-0 – group without added antioxidant; Control-C – ascorbic acid addition 0,5 g.kg^{-1}; Experiment-3 – sea buckthorn extract addition 3 ml.kg^{-1}; Experiment-5 – sea buckthorn extract addition 5 ml.kg^{-1}; a, b = groups within a column with different superscripts differ significantly at $p \leq 0.05$, one-way ANOVA; A, B = groups within a row with different superscripts differ significantly at $p \leq 0.05$, one-way ANOVA

Salejda *et al.* (2017) reported different results compared to ours. The authors stated that the addition of sea buckthorn significantly influenced the pH values of pork sausages. The acidity of the final products was increasing along with a growing content of the plant additive in the recipe. In our study, we were not able to replicate those results. Higher pH values of experimental sausage samples were observed only on the last day of storage. **Najgebauer-Lejko *et al.* (2021)** added fruit extract, including sea buckthorn puree, into the probiotic yogurts. Authors reported lower pH values in samples with sea buckthorn addition. The authors stated that sea buckthorn fruit puree addition resulted in higher acidity of fresh probiotic yogurts compared to other treatments, but these differences were insignificant after storage. This treatment also produced yogurts with the lowest pH directly after production. **Kumar *et al.* (2015b)** used sea buckthorn extract in pork patties storage and did not report significant differences among groups on individual days of storage.

Oxidative stability

As mentioned above, lipid oxidation is a cause of deterioration of meat products due to the creation of aldehydes causing negative flavours and aromas. In our study, no significant differences were observed in MDA created among sausage sample groups on the first day and even after one week of storage. After fourteen days, differences started to become significant, and we confirmed that ascorbic acid and sea buckthorn addition have lipid protective ability in sample products. At the end of the storage period, differences became even more visible, with Control-0 samples reaching the highest MDA levels as expected. On the other hand, the lowest recorded MDA values were measured in Extract-5 samples, but differences among groups with added antioxidants were not significant. The results of the TBARS assay are presented in Table 2.

Table 2 Oxidative stability results of pork sausages (mg MDA.kg^{-1} ± S.D.)

Sample	Day 1	Day 7	Day 14	Day 21
Control-0	0.120 ± 0.001^{aC}	0.132 ± 0.008^{aC}	0.180 ± 0.016^{aB}	0.252 ± 0.029^{aA}
Control-C	0.114 ± 0.008^{aC}	0.128 ± 0.005^{aC}	0.147 ± 0.006^{bB}	0.180 ± 0.007^{bA}
Extract-3	0.116 ± 0.006^{aC}	0.137 ± 0.005^{aB}	0.150 ± 0.012^{bB}	0.186 ± 0.007^{bA}
Extract-5	0.113 ± 0.004^{aD}	0.126 ± 0.005^{aC}	0.145 ± 0.004^{bB}	0.175 ± 0.006^{bA}

Note: Control-0 – group without added antioxidant; Control-C – ascorbic acid addition 0,5 g.kg^{-1}; Experiment-3 – sea buckthorn extract addition 3 ml.kg^{-1}; Experiment-5 – sea buckthorn extract addition 5 ml.kg^{-1}; a, b = groups within a column with different superscripts differ significantly at $p \leq 0.05$, one-way ANOVA; A, B = groups within a row with different superscripts differ significantly at $p \leq 0.05$, one-way ANOVA

Püssa *et al.* (2008) utilized the addition of dried sea buckthorn residue after juicing to the mechanically deboned chicken meat and observed its oxidation rate. The authors evaluate that the lipid protective effect of sea buckthorn was stronger in raw chicken meat. A 1% addition of sea buckthorn powder significantly reduced MDA creation in samples after a 6-day storage period. Similarly, **Salejda *et al.* (2017)** incorporated sea buckthorn extract into pork sausage stored for 28 days in 1.5 and 3% addition. The authors confirmed that extract addition reduced MDA creation in sausage samples. The authors, however, observed that at the end of the storage period (day 28), the experimental group with 1.5% addition reached lower MDA levels than the 3% addition. This contradicts our finding, as we observed that the antioxidant effect of natural extract is concentration-dependent. **Bobko *et al.* (2019)** used sea buckthorn oil in raw, cooked meat products stored for 10 days. The authors reported improvement in the oxidative stability of experimental sausage samples compared to control samples without antioxidant addition. Several authors reported similar positive results of various fruit extracts such as black currant, grape, or bearberry **(Jurčaga *et al.*, 2021; Garrido *et al.*, 2011; Carpenter *et al.*, 2007).**

Microbiological examination

On the seventh day of storage, the growth of psychrotrophic microorganisms was detected in all groups except the control group with the addition of ascorbic acid (Control-C). On the contrary, representatives of the *Lactobacillus* were observed only in the Control-0 and Control-C groups. In the later stages of observation, the growth of psychrotrophic microorganisms and representatives of the *Lactobacillus* sp. was also detected in all groups of meat products. It was also observed that the number of these microorganisms increases with the length of the storage period. During the storage period, no representatives of *Enterococcus* sp. were detected in the analysed samples. The results of microbiological analysis are shown in Table 3.

Table 3 Results of microbial examination of pork sausage samples (log cfu·g^{-1})

Sample	Microorganisms group	Day 7	Day 14	Day 21
Control-0	*Enterococcus* sp.	ND	ND	ND
	Lactobacillus sp.	2.11	2.30	3.60
	Psychrotrophic bacteria	3.30	4.75	5.30
Control-C	*Enterococcus* sp.	ND	ND	ND
	Lactobacillus sp.	3.47	1.00	3.60
	Psychrotrophic bacteria	ND	4.40	5.32
Extract-3	*Enterococcus* sp.	ND	ND	ND
	Lactobacillus sp.	ND	2.20	3.10
	Psychrotrophic bacteria	2.88	5.28	6.09
Extract-5	*Enterococcus* sp.	ND	ND	ND
	Lactobacillus sp.	ND	2.20	3.15
	Psychrotrophic bacteria	2.79	4.55	5.88

Note: Control-0 – group without added antioxidant; Control-C – ascorbic acid addition 0,5 g.kg^{-1}; Experiment-3 – sea buckthorn extract addition 3 ml.kg^{-1}; Experiment-5 – sea buckthorn extract addition 5 ml.kg^{-1}.

Nowak *et al.*, (2022) performed *in vitro* testing of multiple fruit juices and their antimicrobial properties, including sea buckthorn juice. The results distinctly show that higher antimicrobial activity was achieved against Gram-positive strains, than against Gram-negative. Therefore, antimicrobial potential of sea buckthorn fruit was confirmed. **Wagh and Chatli (2017)** used sea buckthorn extract as an additive to ground pork stored at -18 ± 1°C for 9 days. In their work, the authors observed that all three used extract concentrations (0.1, 0.2 and 0.3%) were statistically effective in reducing the microbial load of raw minced pork. The total plate count was significantly ($P \leq 0.05$) higher in the negative control samples than in all experimental products. The same trend was observed by the authors in the cultivation of psychrotrophic microorganisms. Conversely, **Kumar *et al.* (2015a)** in their work reported an increase in total plate count in pork patties after the addition of 0.3% sea buckthorn extract and stored at 4 ± 1 °C. In our work we could not demonstrably confirm antimicrobial effect of sea buckthorn extract in pork sausages. On the other hand, we did not observe negative effect of extract addition on microbial load. Therefore, we can state that sea buckthorn extract does not pose microbial hazard for raw cooked meat product – pork sausages.

CONCLUSION

In our study, we incorporate Vitaminnaja sea buckthorn extract into pork sausage and observe its effect on oxidative and microbial spoilage of meat products. We were able to confirm that sea buckthorn was able to retard lipid oxidation in experimental sausage samples compared to control without antioxidants. On the other hand, we did not observe the antimicrobial effect of sea buckthorn reported by various authors. However, our study proved that sea buckthorn extract does not pose an antimicrobial threat to a final product. Sea buckthorn extract addition improves oxidative stability, slowing oxidative deterioration and not affecting microbial spoilage of meat products. Therefore, it could be an interesting natural alternative to commercially used antioxidants in the meat industry. Further study of the topic is, however, still recommended.

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