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EFFECT OF AMELANCHIER EXTRACT ON LIPID OXIDATION AND SENSORY FEATURES OF PORK SAUSAGES

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ABSTRACT

The meat industry in recent years leaning towards the replacement of synthetic antioxidants, such as BHT, for natural, mostly plant-based alternatives. For such purpose, polyphenol-rich plant species are selected and researched. Amelanchier *(Amelanchier alnifolia)* fruit seems to be an interesting option for adding to meat products to increase oxidative stability. In our study, we incorporate 3 and 5 ml of Amelanchier extract into pork sausage and observed changes in pH, color, sensory, and oxidative stability for 21 days period of vacuum storage. During storage period we measured pH values, color (expressed as cieLab coordinates), malondialdehyde concentration. Also, sensory evaluation was conducted. We compared those experimental meat products with sausages without any antioxidant and commercially used synthetic vitamin C. In our study we did not observe negative effects of Amelanchier extract addition compared to control groups in terms of pH, color and sensory properties. Regarding oxidative stability, Amelanchier extract was able to retard oxidation processes at comparable rate as vitamin C. Amelanchier therefore shows promising possibilities for food industry. However, further study of this natural resource is still needed.

Keywords: pork, meat product, lipid oxidation, sensory quality, antioxidant, Amelanchier

INTRODUCTION

The meat industry in the 21st century is facing many challenges. Creating safe, nutritional valuable, tasty, and healthy products is the highest priority of all manufacturers **(Estévez, 2017)**. Meat plays a significant role in human development and is an integral part of a healthy and balanced humans' diet because of its nutritional composition. The current meat industry is trying to sum up the role and importance of meat in a balanced human diet and research some of the pejorative convictions about meat consumption. The growing occurrence of certain chronic diseases, such as cancer or cardiovascular diseases, motivated extensive research of food believed to be associated with the rising incidence of such diseases. Despite being nutritionally rich, meat consumption is often connected to a higher risk of previously mentioned diseases **(Pereira & Vincente, 2013)**. Lipid oxidation creates unpleasant flavors and supports color changes of muscle food during storage, even in a cooler or fridge. Besides that, compounds possibly causing severe health problems are also created and stored in those products **(Lorenzo & Gómez, 2012)**.

For over a decade, natural oxidation gained more and more attention from the broad public and food manufacturers. Generally, customers will choose food without additives. If that is not possible, he is more likely to select the one with natural additives than with synthetic ones **(Carocho *et al.*, 2015).** The new, attractive, and unconventional fruit of small fruit with considerable potential is Amelanchier (*Amelanchier* spp., *Rosaceae* family). Amelanchier includes about 25 types of bushes and small deciduous trees. It's native to North America. However, Slovakia offers favorable weather conditions for its growth and cultivation **(Gajdošová & Libiaková, 2015)**.

Amelanchier leaves and fruits are a rich resource of nutrients, antioxidants, and other compounds that may have a protective effect on living organisms **(Męczarska *et al.*, 2017)**. Research of the chemical composition of Amelanchier fruit revealed water to be the major component (82 – 84 %), followed by carbohydrates (15 – 20 %). Fruits contain low levels of proteins and fats, enough dietary fiber, and a relatively high amount of potassium, iron, magnesium, and phosphorus. The Vitamin composition of Amelanchier includes vitamin C, thiamine, riboflavin, pantothenic acid, vitamin B6, folate, vitamin A and vitamin E. Phenolic compounds, especially anthocyanins, are the main functional components of Amelanchier fruits **(Mazza & Cottrell, 2008)**. Fruits of Amelanchier are a rich source of phenols for potential usage in the food industry. In recent years there is an increasing demand of customers for food without synthetic antioxidants, such as BHT. Producers, however, still need antioxidants for elongating the shelf life of their products. One of the best resources of naturally occurring antioxidants are phenols of various fruits **(De Souza *et al.*, 2019)**.

As mentioned, the meat industry faces a great challenge to fulfill customer demand for products synthetic addition-free products. On the other hand, meat products with high-fat content are susceptible to oxidation changes. Therefore, antioxidant addition is necessary. Antioxidants from natural sources, such as plants becoming more and more interesting for the industry. Experiments were conducted with several potential plant sources and various versions. In our study, we present another option as a source, Amelanchier, in the form of extract. We believe this plant offers suitable properties for meat products and should be considered in the future for further studies and, potentially, for commercial use.

MATERIAL AND METHODS

Extract preparation

The extraction of Amelanchier berries was carried out according to **Shirahigue *et al.* (2010)**. Dried and homogenized *Amelanchier alnifolia* fruits (20 g) were mixed with 100 mL of 80% ethanol in a shaker and let rest for 24 hours in the dark at room temperature. The supernatants were vacuum filtered and concentrated in a vacuum rotary evaporator at 65 °C until total solvent evaporation. The residues were dissolved in water to a final volume of 50 mL.

Total polyphenol content

Amelanchier extract was prepared and tested for polyphenol content and antioxidant activity. Total phenolic content was determined by Folin–Ciocalteu reagent method according to **Fu *et al.* (2011)**. The content was expressed as grams of Gallic acid equivalents (GAE) in kilogram of wet fruit weight.

Total Antioxidant activity

Total Antioxidant Capacity was measured using the radical-scavenging method by **Brand-Williams (1995)** with DPPH radical - 2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$, Sigma-Aldrich).

Sausage preparation

We used same meat product and recipe as in our previous work **Jurčaga *et al.* (2021)**. Ingredients for sausage preparation were homogenized and mixed together.

This meat batter was divided into groups and incorporated with selected antioxidants. All products were heat treated to 70 °C in core for 10 minutes.

pH measurement

Measurement of pH was carried out using calibrated Orion Star™ A211 Benchtop pH meter (China) with piercing probe.

Color determination

All sausages were sliced, and color measurement was conducted on cut surface using spectrophotometer Konica Minolta CM-2600d (Japan). D65 light source and a 10° observer, with an 8 mm diameter port were used. Setting Specular Component Included (SCI) option setting was used due to matt character of samples. Results are expressed as a* (redness), b*(yellowness) and L* (lightness) coordinates.

Sensory evaluation

A sensory panel of evaluators performed the sensory evaluation of products on the 21st day after preparing, at the end of the storage period. All samples were heated before evaluating. Five sensory parameters were observed: appearance (surface and on a cut), color, aroma, consistency, and taste. Every parameter was evaluated on a 5-point scale where 5 is best, and 1 is the worst rate of selected parameter. Together, the product can obtain 25 points at best. The sensory panel consisted of 10 evaluators of both genders aged from 25 to 60 years. All evaluators are from the Department of Technology and Quality of Animal Products.

Oxidative stability

Oxidative stability was measured using the previously published TBARS methodology in **Jurčaga *et al.* (2021)** using UV spectrophotometry. Final results were calculated using a calibration curve and expressed as the quantity of malondialdehyde (MDA) (mg) present in 1 kg of sample.

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 analyzed 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

To examine properties of Amelanchier extract, two characteristics were observed. The DPPH scavenging activity and Total Polyphenol Content (TPC) were measured first day after the preparation. TAC results of Amelanchier extract were determined as 89.89 ± 0.12 % of DPPH radical inhibition. Total polyphenol content count of selected extract was measured 5.93 ± 0.12 g GAE. kg^{-1}.

pH measurement

The value of pH affects many properties during food processing. For example, protein properties as denaturizing, gelification, enzymatic activities, the growth and mortality of microorganisms, the germinating or inactivation of bacterial spores and various chemical reactions, such as Maillard reaction (Stippl et al., 2004). The pH of most food products varies between 3.5 and 7. The pH has an important effect on pigments (e.g., chlorophyll, carotenoids, anthocyanins, etc.) responsible for the color of fruits, vegetables, and meat. Thus, knowledge of pH is necessary to produce safe, high-quality, and value-added products (Andrés-Bello et al., 2013).
Measurement of pH was performed on the 1st, 7th, 14th, and 21st day of storage at 4 °C. Results from Table 2 show that without any additional antioxidant (Con-0) pH decreased over the first 14 days in the control group. After that subtle increase was observed. This could be explained by initial decomposition changes in meat products. With the addition of vitamin (Con-0), the group shows the lowest pH value at the end of the storage period. Both groups with additional content of Amelanchier (Ext-3 and Ext-5) show very similar progress of pH change. Values of pH of those groups after the initial drop stay stable for the entire observation duration. Also, both experimental groups showed similar results to the Con-C group, statistically varying from the 7th day measurement.

Table 1 pH of sausage samples during storage

Group	Day 1	Day 7	Day 14	Day 21
Con-0	6.33 ± 0.02^{a}	6.26 ± 0.01^{a}	6.23 ± 0.04^{a}	6.28 ± 0.01^{a}
Con-C	6.27 ± 0.02^{a}	6.15 ± 0.02^{c}	6.16 ± 0.02^{a}	6.15 ± 0.03^{b}
Ext-3	6.30 ± 0.02^{a}	6.22 ± 0.03^{ab}	6.18 ± 0.01^{a}	6.20 ± 0.04^{b}
Ext-5	6.29 ± 0.02^{a}	6.20 ± 0.02^{bc}	6.21 ± 0.02^{a}	6.17 ± 0.02^{b}

Note: Con-0 – negative control, no antioxidant; Con-C – 0.5 $g.kg^{-1}$ vit. C addition; Ext-3 – 3$ml.kg^{-1}$ Amelanchier extract addition; Ext-5 – 5 $ml.kg^{-1}$ Amelanchier extract addition. Values are expressed as pH ± S.D.; a,b,c as upper index represent a statistically significant differences between samples in column.

Color determination

Appearance determines how consumers perceive quality and significantly influences purchasing behavior. In the study of four European countries the most important product characteristic which consumers base their quality evaluations on are the appearance attributes: fat content and color (Resurreccion, 2004).
Color measurement was carried out for every group on 1st, 7th, 14th, and 21st days of storage. No significant visible change of color occurred during the observation period. When compared coordinates of color determination, all results are closely distributed in one color shade. Lightness (L*) varied only 4.91 points among all samples. Similarly, coordinates a* and b* show minimal variations of 1.63 and 4.73, respectively.
It has been shown that treatment with some natural sources can cause changes in the color of meat or meat products. Karre et al. (2013) stated that plum, grape seed extract, pine bark extract, and rosemary extract have all caused color changes when used in some meat or poultry products. This could be perceived as a negative trait, primarily if the redness of chicken meat increased, due to the reddish product could be perceived as undercooked. On the other hand, extracts of oregano or pomegranate do not show any significant color deviation. Devatkal et al. (2010) observed no negative significant effect on the color of goat patties. In our study, we proved that Amelanchier products do not carry negative changes of product color as well.

Sensory evaluation

We did not observe any statistical difference by comparing average scores for all experimental parameters (Appearance, Color, Aroma, Consistency, and Taste) (α = 0,05). However, it is important to note that both experimental groups with Amelanchier extract (with 3 and 5 mL) obtained a lower score in all parameters, except taste, compared to control groups. Also, overall acceptability by panelists was highest for Con-0, then Con-C, followed by Ext-3 and Ext-5 group samples. Graphic visualization of sensory evaluation is shown in Figure 1. In the past, a major obstacle to the use of natural plant extracts in foods has been the imposition of undesirable flavors and odors. However, technological developments have enabled food ingredient manufacturers to produce extracts with non-interfering sensory characteristics while maintaining antioxidant properties (McBride et al., 2007). In our study, we did not observe the appearance of any unpleasant flavors. Moreover, the experimental group with 3 ml extract addition obtained a higher score than the Con-0 group.

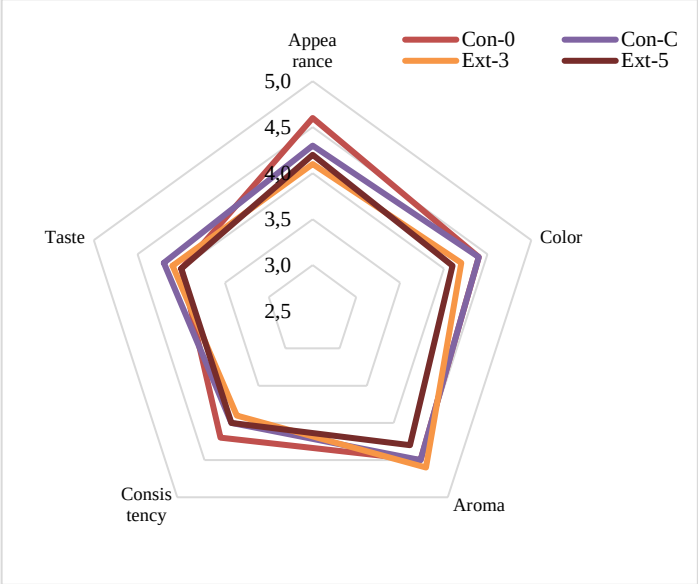

Figure 1 Visualization of sensory evaluation
Note: Con-0 – negative control, no antioxidant; Con-C – 0.5 g.kg^{-1} vit. C addition; Ext-3 – 3ml.kg^{-1} Amelanchier extract addition; Ext-5 – 5 ml.kg^{-1} Amelanchier extract addition.

Oxidative stability

Antioxidants are commonly used in meat products to reduce and prevent oxidative changes and preserve sensory properties. The most frequently used antioxidants are ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), nitrites, and nitrates. All of the mentioned have been involved in the etiology of the stomach, bowel, and food allergy (Ribeiro et al., 2019). In the context of those findings, the use of natural antioxidants appears as a good possibility to lower the consumption of synthetic additives. The general population widely consumes Plant-based antioxidants without showing signs of low toxicity, also carrying out functional activities beneficial to human health. Many authors reported favorable anti-oxidative properties of the variety of phenolic compounds present in fruit extracts, industrial residues, spices, and seeds (Shah et al., 2014; Qi et al., 2015; Lorenzo et al., 2018; Echegaray et al., 2018).
Oxidative stability is expressed as an amount of malondialdehyde (MDA) in mg per kg of product. In our experiment, in the first two measurements (Day 1 and Day 7) we did not observe any statistical difference in MDA produced in all groups. This is due to the vacuum package of sausages where the lack of oxygen available stopped extensive lipid oxidation. After 14 days, however, oxidation became more prevalent in the negative control (Con-0) compared to experimental groups with extract addition and group with vitamin C. At this point, we observe the statistical difference between negative control and all other groups. Those results are repeated at the end of the storage period, on the 21st day. The difference between negative control and other groups, with antioxidant addition, widen. On the other hand, we did not observe any significant difference among Experimental groups with Amelanchier extract and control with vitamin C. Our findings suggest Amelanchier extract to be as effective in meat products as vitamin C. Amount of MDA measured in all samples is listed in Table 2.

Table 2 Amount of MDA created in sausage groups during storage

Group	Day 1	Day 7	Day 14	Day 21
Con-0	0.110 ± 0.007^{a}	0.123 ± 0.012^{a}	0.137 ± 0.008^{a}	0.165 ± 0.017^{a}
Con-C	0.103 ± 0.005^{a}	0.113 ± 0.009^{a}	0.118 ± 0.006^{b}	0.128 ± 0.009^{b}
Ext-3	0.106 ± 0.004^{a}	0.118 ± 0.008^{a}	0.123 ± 0.010^{b}	0.131 ± 0.011^{b}
Ext-5	0.103 ± 0.002^{a}	0.116 ± 0.011^{a}	0.120 ± 0.006^{b}	0.129 ± 0.014^{b}

Note: Con-0 – negative control, no antioxidant; Con-C – 0.5 g.kg^{-1} vit. C addition; Ext-3 – 3ml.kg^{-1} Amelanchier extract addition; Ext-5 – 5 ml.kg^{-1} Amelanchier extract addition. Results are expressed as mg (MDA).kg^{-1} ± *S.D.*; a,b as upper index represent a statistically significant differences between samples in column.

CONCLUSION

In our experiment, Amelanchier (*Amelanchier alnifolia*) extract proved to be as effective as ascorbic acid in the storage of vacuum-sealed pork sausages at fridge temperatures. While maintaining the ani-oxidative properties, the extract did not negatively affect the sensory evaluation of sausages. Also, pH and color determination did not show impairment of pork sausages by Amelanchier extract addition. Therefore, Amelanchier should be considered in future discussions for food antioxidants in the meat industry. Before application in commercial use, detailed research from various fields, such as food chemistry or food technology, is necessary.

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