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Research article

Use of grape seed extract as a natural antioxidant additive in dry-cured pork neck technology

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Abstract: *The grape seeds, which are the post-production waste of juices and wine production, are a rich source of biologically active compounds. The polyphenol compounds present in seeds can be used in the technology of new products of animal origin as a source of natural antioxidants. The effect of three levels (0.1, 0.2 and 0.5%) 40% (v/v) ethanol extract of grape seed (GSE) quality of dry-cured pork neck was tested. Meat product colour, pH value, water activity, free fatty acid (FFA) content and TBARS index were evaluated. The obtained results indicate that the addition of the extract decreased oxidation intensity dry-cured neck during ripening. The treatment with higher concentrations of extracts showed greater lipid stability than that of the lot with 0.1%. The three levels of GSE extract did not influence pH, water activity and colour significantly. This study indicates that the GSE extract was an effective inhibitor of lipid hydrolysis, and at an additive concentration of at least 0.2% limited the oxidative processes occurring in meats during ripening. Therefore, it may be used in dry-cured pork neck to improve the oxidative stability of meat products for the consumers.*

Keywords: *grape seed extrac, dry-cured neck, oxidative stability.*

Introduction

Dry-cured meats have a long production period. The first stage is meat fermentation which lasts for about three weeks and is carried out at a temperature of about 15°C. Then meat products are subjected to at least a monthly ripening at a lower temperature (about 4°C). The ripening time depends on the mass of the meat element and a speed of biophysicochemical changes that occur in the meat in this process. Microbial growth, lipid oxidation and colour are important factors to shelf-life and consequently for the consumer acceptance of dry-cured pork meat products. Oxidative processes in fermented meat products during long-time ripening and storage lead to the degradation of colour pigments, lipids and proteins that, in turn, can contribute to the deterioration in flavour, texture, colour and nutritional value of meat [1]. Antioxidants are added to meat products during processing to delay oxidation [2]. In an attempt to control this process, food

industries usually use commercial antioxidants such as sodium ascorbate. However there is also an alternative – it is possibility of using post-production waste as effective natural antioxidants in the fermented meats technology [3, 4].

The grape seeds, which are the by-product of juices and wine production, are a rich source of biologically active compounds [5, 6]. They exhibit a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and inhibit oxidative stress [1, 7].

According to Shi et al. [8] these compounds are able to trap and quench free radicals, and their antioxidant potentials have been shown to be four to five times higher than that of vitamin C or E. Lorenzo et al. [3] showed that the polyphenols content in grape seed extract could be in high concentration, about 373 mg gallic acid equivalent (GAE) per g of extract. Guendez et al. [9] have proven that grape seeds are rich in phenolic compounds. They reported that total content varied from 55.1 to 964 mg per 100 g of seeds, the average being 380 mg per 100 g dry mass. The most abundant polyphenol was catechin, accounting for 49.8% of total content, followed by epicatechin (26.0%) and proanthocyanidins B_1 and B_2 (10.9%) [10]. The use of natural antioxidants in meat technology is common [2, 3, 4, 11, 12]. Ribas-Agusti et al. [13] evaluated the content of phenolic compounds present in the grape seed extract and their contents in the matured sausage during storage. They found that time did not significantly reduce the concentration of phenolic compounds in sausages. It can therefore be presumed that throughout the shelf life of meat products, the antioxidant compounds present in the grape seed extract will show their beneficial effect.

Due to the fact that there are no scientific publications that deal with the problem of using grape seed extract (GSE) as an effective source of antioxidant substances in dry-cured pork neck technology, the present study was undertaken. Dry-cured neck is a meat product made of deboned whole muscle running from the neck to the fifth thoracic vertebra of pork and consists mainly of the muscles of the neck (*Longissimus cervicis*) and part of the *Longissimus dorsi* muscle. Neck muscles are a retail cut of the pork carcass with a high fat content, which directly affects the palatability of meat products made from them. However, it is difficult to use in the meat industry, because it is heterogeneous and has a varied content of fat and its different distribution. It is a great difficulty to determine how the fat fractions are distributed within a piece of meat, without prior cut open. These technological problems make the neck extremely rarely used in the whole meat technology of fermentation.

The aim of the study was to determine the effect of addition of grape seed extract on colour and oxidative stability of dry-cured neck during 2-months of ripening period. The effect of addition of natural antioxidants present in the 40% (v/v) ethanol extract of three levels (0.1, 0.2 and 0.5%) and sodium ascorbate (0.1%) and without antioxidant additives was compared.

Experimental

Manufacture of dry-cured pork necks

The study was carried out on pork meat cuts of Polish White Large breed. Necks (*M. longissimus cervicis*) were excised at 24-h post-mortem from half-carcasses chilled at 4°C. At 72-h post-mortem, each piece of neck was divided into 3 equal parts and meat underwent curing using a surface massage with curing mixture at 30 g kg^{-1} of meat. The curing mixture of composition: 56% pickling salt, 43.5% sea salt and 0.5% sodium V nitrate. Antioxidant additives were applied together with curing salts, and then five experimental variants were obtained:

GSE-1 (with 0.1% grape seed extract for mass of meat)
GSE-2 (with 0.2% grape seed extract for mass of meat)
GSE-5 (with 0.5% grape seed extract for mass of meat)
ASC-1 (with 0.1% sodium ascorbate for mass of meat)
CON (without antioxidant added).

These additives were applied by rubbing into the meat surface for about 3 minutes. Subsequently all batches were then kept at 0±1°C for 72 h to allow to diffuse. After then the neck portions were hung in a fermentation chamber under controlled conditions for 21 days. The conditions were: temperature at 16±1°C, relative humidity 75±5% and 30% air circulation. Following fermentation and drying, the necks were vacuum-packed into polyethylene barrier bags and after that they ripened in a refrigerator at 4±1°C for 2 months. Product was tested twice: immediately after fermentation (before ripening) and after 2-months of ripening (after ripening). Three independent experimental trials were conducted, and all determinations were performed in triplicate.

GSE preparation

The GSE was prepared on the basis of own unpublished research, and the seeds were obtained from a local wine producer from Poland as post-production waste. Grape seeds were washed under a stream of water and dried at 40±5°C, then milled. The milled seeds and a 40% ethanol solution were in a ratio of 1 to 7. The slurry was shaken for 2 hours on a laboratory shaker at 150 rpm min^{-1}. The water bath temperature was 50±10°C. Subsequently all was pre-filtered through a paper filter, next the extract was separated thoroughly in a centrifuge at 5000×g for 15 minutes. The supernatant was transferred to a round bottom flask and concentrated in a vacuum evaporator at a water bath temperature of 50-60°C and a pressure of 100-175 hPa. When 90% of the solvent was evaporated then the extract was sealed tightly in conical flask and placed in a refrigerator. The antioxidant potential of the obtained extract was evaluated using the DPPH test. The extract portions (0.1, 0.2 or 0.5 g per 100 g of meat) were diluted immediately before use with distilled water to a volume of 2 mL.

Methods

The antioxidant activity of grape seed extract

The free radical-scavenging activity indicating the antioxidant activity of the grape seed extract was determined colorimetrically as a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay. This assay was performed by using a method described by Park et al. [14], which is based on spectrophotometric analysis of the bleaching of a purple-colored methanolic solution of DPPH after neutralization by antioxidants in the samples. A volume of 1 mL of 0.2 mM DPPH dissolved in methanol was added to 200 μL supernatant and 800 μL distilled water. The mixture was vortexed and placed in a dark room for 30 min. A tube containing 1 mL of methanol and 1 mL of 0.2 mM DPPH dissolved in methanol was used as a control, whereas methanol alone was used as a blank. The absorbance of the solution was measured at 517 nm. The scavenging activity of the sample against DPPH radical was calculated by the following equation:

$$\% \text{ inhibition of DPPH} = (\text{Abs control} - \text{Abs sample}) / (\text{Abs control} \times 100)$$

The physicochemical parameters of dry-cured pork neck

The pH value of dry-cured pork neck was determined by ISO 2917:1999 [15] with digital pH meter equipped with a pH electrode dedicated to measurements of active acidity. The measurement of water activity of meat product was carried out at 20°C, using a LabMaster-aw Novasina instrument standardised with equipment humidity sources.

Thiobarbituric acid reactive substances (TBARS) of dry-cured pork neck was determined as per the method described by Pikul et al. [16]. TBARS assay is based on spectrophotometric analysis of the pink complex formed after the reaction of malondialdehyde (MDA) with 2-thiobarbituric acid. The results were expressed in mg MDA per kg of sample, and it was calculated by the following equation:

$$\text{TBARS} = \text{Abs at } 532 \text{ nm} \times 5.5 \text{ (conversion factor)}$$

Free Fatty Acids (FFA value) of dry-cured meat was determined as per the method described by Koniecko [17], and with slight modifications by Malik and Sharma [18]. Exactly 10 g of ground meat sample was blended for 2 min. with 60 mL of chloroform in the presence of about 10 g anhydrous sodium sulphate. Then it was filtered through filter paper, yielding exactly 40 ml of chloroform extract. Half of the filtrate was dried in an oven determine the fat weight. The other half of the filtrate was collected into a conical flask. About 5 drops of 0.2% phenolphthalein indicator was added to the filtrate, which was titrated against 0.1 M alcoholic potassium hydroxide to get the pink colour end point. The quantity of potassium hydroxide consumed during titration was recorded. FFA as percent of oleic acid was calculated as follows:

$$\text{FFA} = (2{,}82 \times \text{mL 0.1 M alcoholic KOH}) / \text{weight of fat (g)}$$

The colour evaluation

The colour of dry-cured neck was measured using an X-Rite Colour 8200 spectrophotometer. The instrumental conditions were 13 mm port size,

illuminant D65 and 10° standard observer. Colour results were determined in the CIE L*a*b* scale [19] and lightness (L*), redness (a*) and yellowness (b*) were calculated. The X-Rite's white and black standards were used to calibrate the spectrophotometer. The measurements were performed at room temperature at six different locations of freshly cut surface of meat slices with thickness of 10 mm. The total colour difference (ΔE*) was determined for each sample according to the equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2},$$

where: ΔL*, Δa* and Δb* are changes in time.

Statistical analysis

For the statistical analysis collected data were analysed by two-way analysis of variance (ANOVA). Post hoc comparisons were run by Tukey's test for pairwise comparisons (KyPlot ver 2.0; Kyens Lab Inc., Tokyo, Japan). Differences among groups were determined as statistically significant at a level of $P \leq 0.05$. All results are expressed as means ± standard deviation.

Results and discussion

The antioxidant activity of grape seed extract

For measuring the potentially antioxidant properties of extract prepared by the authors, the DPPH assay was used. The radical-scavenging activity was expressed as the percentage of DPPH inhibition (65.4%±0,06). The concentrated in the evaporator had a high antioxidant potential, which pretends to be used in pork neck technology.

The physicochemical parameters of dry-cured pork neck

The all pH values ranged between 5.42 and 5.76 in dry-cured neck (Table 1). There were no significant ($P > 0.05$) differences between pH values of neck at the beginning of experiment (before ripening) and at the end (after ripening) independently of kind of antioxidant additives which was used. The naturally fermented meat products are generally characterized by low acidity with a final pH ranging from 5.2 to 6.4 as in Italy, Greece and Spain [20].

Table 1. Physicochemical properties of dry-cured neck (mean ± SD)

	time	*GSE-1*	*GSE-2*	*GSE-5*	*ASC-1*	*CON*
pH	BR	5.78^{aA} ±0.12	5.80^{aA} ±0.09	5.75^{aA} ±0.06	5.65^{aA} ±0.10	5.72^{aA} ±0.09
	AR	5.53^{aB} ±0.09	5.41^{aB} ±0.06	5.39^{aB} ±0.10	5.42^{aB} ±0.07	5.40^{aB} ±0.08
aw	BR	0.880^{aA} ±0.003	0.881^{aA} ±0.002	0.879^{aA} ±0.004	0.880^{aA} ±0.002	0.882^{aA} ±0.003
	AR	0.839^{aB} ±0.004	0.840^{aB} ±0.003	0.842^{aB} ±0.005	0.838^{aB} ±0.006	0.843^{aB} ±0.005

Explanatory notes: BR – before ripening; A – after ripening; aw – water activity; means followed by common small letters (additives) and means followed by common capital letters (time) are not significantly different ($P > 0.05$).

There were no significant (P > 0.05) differences between the water activities of neck with antioxidant compared with control at the beginning of the experiment. There was also no difference (P > 0.05) between samples at the end of the ripening period. Water activity of all study samples systematically decreased during ripening in terms of 0.88 to 0.84. The behavior of this parameter corresponds to the course described by Virgili et al. [21] for dry-cured hams. Probably, the high organic acids concentration (low pH value) speed up the water diffusion during ripening caused the decrease in water content and water activity.

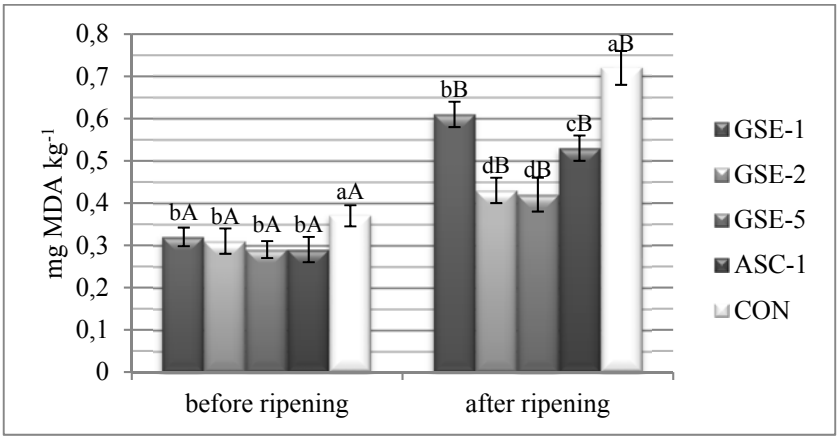

Figure 1. TBARS index in dry-cured pork neck during ripening at 4ºC.
Explanatory note: Means followed by common small letters (additives) and means followed by common capital letters (time) are not significantly different (P > 0.05)

The progression of lipid oxidation during the ripening of neck was evaluated by thiobarbituric acid reactive substances (TBARS), as shown in Figure 1. The oxidation process has progressed in all samples over time. The ripening pork neck produced with antioxidant additives (GSEs and ASC-1) was characterized by increased oxidative stability during storage as compared to the CON one. All antioxidants inhibited unfavourable fat metabolism with similar efficacy. Before ripening, samples GSE-5 and ASC-1 revealed the concentration of compounds reacting with 2-thiobarbituric acid lower by approximately 22% than in the CON one. Instead, the difference in TBARS between control and other samples (GSE-1 and GSE-2) was only 16% at the beginning of the experiment. The TBARS index after two-month of neck ripening has almost doubled (P ≤ 0.05). A 20-40% difference in TBARS index was found between samples containing antioxidant additive and a control sample. The TBARS value after ripening in necks GSE-2 and GSE-5 were lower (0.42 mg MDA kg^{-1}) than the ASC-1 (0.53 mg MDA kg^{-1}), while in the control, this value was as high as 0.72 mg MDA kg^{-1}. Wang et al. [11] noticed similar (0.38 mg MDA kg^{-1}) TBARS index in the dry-cured bacon with vine seed extract addition. Very similar to the results have been also described by Lorenzo et al. [3] who used grape seed extract in chorizo production. Addition of

the extract affected the reduction of the amount of secondary fat oxidation products.

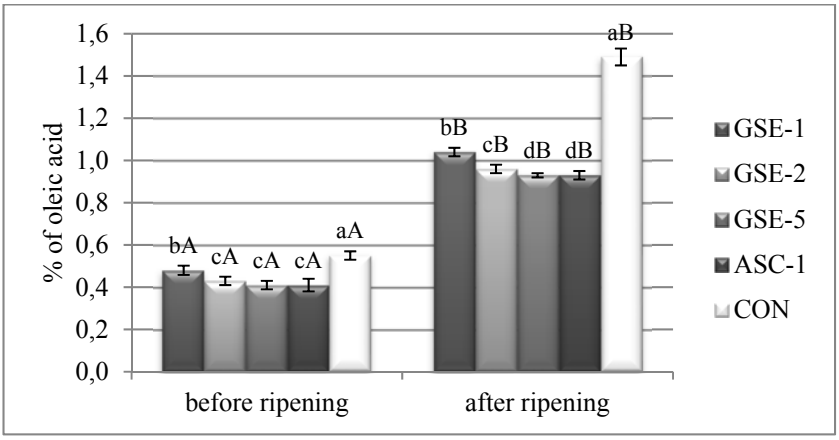

Figure 2. Free Fatty Acids content in dry-cured pork neck during ripening at 4ºC. **Explanatory note:** Means followed by common small letters (additives) and means followed by common capital letters (time) are not significantly different ($P > 0.05$)

There was a statistically significant ($P \leq 0.05$) effect of addition of grape seed extract on lipid hydrolysis inhibition (Figure 2). In the GSE-2 and GSE-5 samples, a lower (0.41% of oleic acid) concentration of free fatty acids (FFA) was found, which was similar to that obtained in the ASC sample. Before starting the 2-month ripening, the free fatty acid content in GSE-2, GSE-5 and ASC-1 samples was 22-25% lower and 36-38% lower after ripening as compared to the CON sample. The sample with the lowest extract addition (GSE-1) was showed a lower concentration of free fatty acids by about 13-30% compared to control. The hydrolysis process has progressed in all samples during ripening. The FFA content after two-month of neck ripening has doubled, and in control sample it was tripled ($P \leq 0.05$).

The colour evaluation

There was no effect of addition of grape seed extract (GSE) or sodium ascorbate (ASC) on the lightness (L*) of meat products during the first study period. Studies carried out after a two-month ripening showed differences between tests containing antioxidant additives (Table 2). In GSE samples, the lightness of the meat product was found to be almost 10% higher than the control sample, but these were not significant changes. This parameter was significantly ($P \leq .05$) modified by the storage period and decreased over time. The lightness of ripening neck produced without the antioxidant (CON) has changed by about 24% as compared to the values obtained immediately after the meat product has been manufactured.

Table 2. Colour parameters of dry-cured neck (mean ± SD)

	time	*GSE-1*	*GSE-2*	*GSE-5*	*ASC-1*	*CON*
L*	BR	50.0^{aA}±4.0	48.6^{aA}±3.6	46.7^{aA}±3.8	52.0^{aA}±4.4	51.6^{aA}±3.5
	AR	44.2^{aA}±3.9	42.4^{aA}±3.7	42.0^{aA}±2.5	40.6^{aB}±2.7	38.9^{aB}±2.5
a*	BR	11.7^{aA}±0.5	12.3^{aA}±0.5	12.8^{aA}± 0.5	9.1^{bA}±0.4	9.0^{bA}±0.4
	AR	14.2^{aB}±0.4	14.4^{aB}±0.4	15.1^{aB}±0.4	11.1^{bB}±0.6	10.8^{bB}±0.5
b*	BR	11.9^{aA}±0.7	11.7^{aA}±0.7	12.1^{aA}±0.6	13.0^{aA}±0.6	11.8^{aA}±0.8
	AR	13.1^{aA}±0.6	12.3^{aA}±0.8	12.6^{aA}±0.8	12.5^{aA}±0.6	12.8^{aA}±0.8
ΔE*		6.40	6.61	5.22	11.51	12,82

Explanatory notes: BR – before ripening; A – after ripening; L*, a*, b* and ΔE* – parameters of colour; means followed by common small letters (additives) and means followed by common capital letters (time) are not significantly different ($P > 0.05$).

The significant effect of the grape seed extract addition at a concentration above 0.2% on the color of meat was found. During ripening at 4°C, it was noticed that in necks with extract discoloration of meat was inhibited by reducing the loss of redness and increase of yellow. The value of redness of necks with extract was higher than the other variants by above 20%. Lowering the L* value while increasing the a* denotes a change in the colour of the meat product to dark red or brown. Dry-cured pork necks with grape seed extract (GSEs) were reddish than the others (CON, ASC-1) after ripening. The GSE samples was found to be higher ($P \leq .05$) by approximately 24-28% of the a* value as compared to other samples. These results indicate the antioxidant effect of the extract. It stabilizes the colour of the product by inhibiting the conversion of red myoglobin to brown metmyoglobin. The applied plant extract and storage duration did not modify the b* parameter. The yellow colour component of meat products ranged from 11.4 to 13.1. Analysis of the total colour change (ΔE*) of the meat product during storage showed that the colours of the CON and ASC-1 necks were the least stable. The ΔE* of control variant during two months of ripening was 12.8 units, indicating a large deviation of colour from the standard that was the pork neck immediately after production. In the GSE-1, GSE-2 and GSE-5 samples, the total colour change after ripening were the lowest (6.4, 6.6 and 5.2 units, respectively). These values indicate average colour deviations. Other authors have also noticed a change in the colour of matured meat product depending on time. Lorenzo et al. [3] recorded the dependence observed in this experiment for higher values of the a* parameter in the GSE additive sample. On the 50th day of the experiment, they noticed that the chorizo red colour parameter with GSE addition was about 3 units higher as compared to the control sample, but on the other hand, the yellow chromaticity and the lightness (L*) was higher by 5 units.

Summary

The results of the present study support the use of grape seed extract as an effective antioxidant in the technology of dry-cured pork neck. Among the three tested concentrations of grape seed extract, these higher concentrations showed the most potential as alternatives to commercial antioxidants, for increasing the quality

and extending the shelf-life of ripening neck. The addition of grape seed extract effectively inhibits the lipid hydrolysis processes in the dry-cured pork neck, reducing the free fatty acid content after two months of refrigerated storage. The grape seed extract, with efficacy similar to sodium ascorbate, counteracts unfavourable oxidative changes in the neck during the two months of refrigerated storage. Finally, it was found that the addition of grape seed extract to the dry-cured pork neck had a positive effect on the colour of the product. The colour on the cross-section was more red as compared to the other test variants, and the total colour change of the product during storage was the smallest. Results of the experiment demonstrate the effective antioxidant properties of natural substances found in the extract produced from grape seeds.

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