Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article OPEN ACCESS

Reduction of Lipid Oxidation without Compromising Color: the Role of Pomegranate Peel Extract in Pork Sausages

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Article Details:
Received: 2025-03-28
Accepted: 2025-05-20
Available online: 2025-05-31

DOI: https://doi.org/10.15414/ainhlq.2025.0015

The modern meat industry is under pressure from customers who demand clean-label products. Also, suspicion about the potentially harmful effects of synthetic antioxidants pushes the industry to develop natural antioxidant options for their product. Plants rich in polyphenols, like pomegranate, have been at the center of those attempts for several years. Our study incorporated pomegranate peel extract into experimental sausage to determine its potential to delay negative oxidative changes of lipids in meat products. To determine the antioxidant effect of extracts, the TBARS method was used. Addition of plant-based ingredients into the meat products could, however, alter sensory properties of the final product, such as color. The color of meat products is a highly important trait, as it is the first trait that is perceived by customers. To evaluate these potential changes of extract-enhanced sausages color was measured using a spectrophotometer Konica Minolta CM-2600d, and results were expressed as coordinates in CieLab color space. The negative control showed an increase in malondialdehyde levels of about 134% during the storage. On the other hand, treatments with peel extract addition saw a significantly lower increase in the same period. Regarding the color of final products, differences were observed only at the start of the storage period in lightness and yellowness parameters. At the end of the storage period, no significant differences were observed, therefore, we could argue that pomegranate peel extract has no negative effect on pork sausage color.

Keywords: pomegranate, pork sausage, lipid oxidation, natural antioxidant

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Introduction

Pomegranate (*Punica granatum* L.), a member of the Punicaceae family, is an ancient fruit species originally domesticated in the Middle East. Currently, major producers and exporters include India, Iran, China, Turkey, the United States, Spain, South Africa, Peru, Chile, and Argentina. Global pomegranate production is estimated at approximately 2 million tons annually, with the European Union representing a significant market for fresh fruit imports (Kalaycıoğlu and Erim, 2016). The edible portion of pomegranate comprises approximately 50% of the total fruit weight, with around 40% attributed to the arils (pulp) and 10% to the seeds, while the remaining 50% consists of the inedible peel (Li et al., 2005). The peel is particularly rich in phenolic compounds, minerals, and complex polysaccharides. In contrast, the pulp primarily consists of water (approximately 85%) and also contains sugars, pectin, organic acids, phenolics, and flavonoids, predominantly anthocyanins. Ellagitannins, especially punicalin, are the most prevalent phenolics, largely concentrated in the peel. These bioactive compounds have demonstrated anti-inflammatory and anti-tumor activities *in vitro* (Lu et al., 2007; Seeram et al., 2007).

During the processing and storage of meat products, undesirable alterations often occur, negatively affecting organoleptic qualities such as flavor, color, and protein integrity. These modifications can result in reduced sensory appeal and decreased consumer acceptance. The primary biochemical mechanisms responsible include lipid oxidation, proteolysis, and microbial proliferation. Previous research has identified pomegranate as a promising additive in meat products to mitigate these detrimental effects (Smaoui et al., 2019). Pomegranate by-products and their extracts, known for their potent bioactive properties, have been incorporated into meat, seafood, beverages, and bakery products to enhance quality, inhibit microbial growth, and delay spoilage (Salgado et al., 2012; Basiri et al., 2014). For instance, Naveena et al. (2008) reported that pomegranate peel extract significantly enhanced the oxidative stability of grilled chicken patties compared to the synthetic antioxidant butylated hydroxytoluene. Additionally, the incorporation of peel powder into cookies and biscuits improved their fiber, mineral, and antioxidant content, offering a more nutritious alternative to conventional baked goods (Ismail et al., 2014).

This work was aimed at the possibilities of utilizing pomegranate coproduct, peels, in the meat industry, like previously mentioned authors. Pomegranate fruit is rich in antioxidant compounds with strong effects. Peels are now considered waste, could be an interesting source of natural extracts. The meat industry is looking to replace commonly used synthetic antioxidants with natural counterparts. In our work, we are trying to objectively review the possibility of utilization of pomegranate peel extract in the meat industry. In our work, we observed experimental sausage with natural antioxidants for 21-day-long storage. We aimed to prove the protective abilities of the natural extract on fat oxidation and observe the potential color changes in pork sausages.

Material and Methodology

Extract Preparation

Pomegranate fruits were juiced using a manual press and then filtered using Whatman 4 grade filter paper (Chemlab, Barcelona, Spain). Peels (cut into small pieces) and seeds were cleaned from residues of pulp, dried for 48 hours at 60 ±1 °C, and ground into a fine powder (Grindomix, GM 200; Retsch GmbH, Germany). Next, an extract from plant powder was obtained according to Shirahigue et al. (2010).

Determination of Total Antioxidant Capacity (TAC)

Total Antioxidant Capacity of the extract was measured using the radical-scavenging method by Brand-Williams et al. (1995) with DPPH radical – 2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany).

Pork Sausage Preparation

Shoulder and loin cuts used for the preparation of both control and experimental sausage samples were obtained from a local butcher. The meat was cut into smaller portions, ground using a Mincer – 450 (ggm Gastro; Ochtrup, Germany), and subsequently mixed with water and additional ingredients. The resulting meat batter was divided into equal portions, to prepare negative control (Cont-0) and experimental groups with 3 and 5 $ml.kg^{-1}$ of peel extract (Peel-3 and Peel-5). The prepared mixture was then stuffed into pork casings, thermally processed until reaching a core temperature of 70 ±1 °C for a minimum duration of 10 minutes, followed by cooling, vacuum packaging, and storage at 4 °C for the duration of the experimental period.

Color Measurement

Cross-sectional surfaces of the experimental sausages were used for color evaluation. Color parameters were expressed in the CIELab color space, where L* denotes lightness (ranging from 0 = black to 100 = white), a* represents the red-green axis (positive values indicating red and negative values green), and b* indicates the yellow-blue axis (positive values indicating yellow and negative values blue). A spectrophotometer (Konica Minolta CM-2600d, Osaka, Japan) equipped with a D65 light source, a 10° standard observer, and an 8 mm aperture was used for measurements. Due to the matte surface characteristics of meat products, the spectrophotometer was operated in the Specular Component Included (SCI) mode.

Oxidative Stability

Lipid oxidation was evaluated following the thiobarbituric acid reactive substances (TBARS) method as described by Jurčaga et al. (2021).

Statistical Analysis

To perform statistical analysis, XLSTAT software was used (XLSTAT Addinsoft, statistical and data analysis solution, 2021, New York, NY, USA). ANOVA analysis with the Duncan test was used to compare the results of individual measurements.

Results and Discussion

Total Antioxidant Capacity (TAC) results

The antioxidant potential of pomegranate peel extract was evaluated by the radical scavenging method with DPPH radical. DPPH is a purple-coloured stable free radical with an absorption band at 517 nm. It is reduced to 2, 2-diphenyl-1-picrylhydrazine (yellow coloured) by accepting an electron or hydrogen radical from an antioxidant (Hseu et al., 2008). Results showed that the prepared extract reached a relatively high percentage of radical inhibition, 87.45 ±3.63. Malviya et al. (2013) demonstrated that the total antioxidant capacity (TAC) of pomegranate is influenced by the choice of extraction solvent. Their study showed that the extract obtained using 70% ethanol exhibited a high antioxidant activity, with approximately 76% inhibition in the DPPH assay. They also noted that extracts prepared with 30% ethanol and methanol yielded comparable antioxidant capacities, with no statistically significant differences between them. Similar results of various antioxidant potential of pomegranate extract depending on solvent and concentration was observed by More and Arya (2020). Furthermore, Jayaprakasha and Rao (2000) reported the use of pomegranate peel extract for evaluating its antioxidant efficacy, including lipid peroxidation inhibition, hydroxyl radical scavenging activity, and prevention of low-density lipoprotein (LDL) oxidation. In a separate study, the methanolic extract derived from the peels exhibited antioxidant activities of 83% and 81% at a concentration of 50 ppm, as assessed using the β-carotene-linoleate and DPPH assay systems, respectively (Murthy et al., 2002). From those results is clear that methanol is the best solvent to prepare the most antioxidant potent extract. However, methanol extract is not suitable for usage in food product due to health risks.

Color Determination Results

Color is one of the most critical quality factors in cured meat products that determines consumer acceptance (Kim et al., 2017). Color measurement was carried out in the fresh state, on day 1 and at the end of the storage period – day 21. Statistically significant ($p \leq 0.05$) differences among pork sausage groups were observed only on Day 1. In the lightness (L*) parameter, we observed that the addition of pomegranate peel extract caused a lowering of the L* value, darkening of the final product. Similarly, we observed a lowering of yellowness value (b*) with increased addition of peel extract. In the case of redness value (a*), we did not observe any significant changes among groups. In the work of Gutiérrez-Pacheco et al. (2021) authors claimed that the sausages exhibited a moderate darkening, which may be attributed to the inherent coloration of pomegranate juice and powder.

Table 1 Results of color analysis ($\bar{x}$ ±s.d.)

Group	Day 1			Group	Day 21		
	L*(D65)	a*(D65)	b*(D65)		L*(D65)	a*(D65)	b*(D65)
Cont-0	70.33 ±0.55^{a}	14.75 ±0.33^{a}	23.03 ±0.64^{a}	**Cont-0**	68.59 ±0.80^{a}	14.35 ±0.15^{a}	22.85 ±0.28^{a}
Peel-3	66.44 ±0.49^{b}	14.69 ±0.18^{a}	22.89 ±0.23^{a}	**Peel-3**	68.79 ±0.56^{a}	15.19 ±0.20^{a}	23.23 ±0.58^{a}
Peel-5	66.88 ±0.49^{b}	14.18 ±0.32^{a}	21.03 ±0.67^{b}	**Peel-5**	69.65 ±0.37^{a}	14.95 ±0.27^{a}	23.35 ±0.50^{a}

Notes: a,b as upper indices represent a statistically significant difference between samples in columns

Table 2 Results of lipid oxidation measurement ($\bar{x}$ ±s.d. mg MDA.kg^{-1})

Group	Day 1	Day 7	Day 14	Day 21
Cont-0	0.131 ±0.003^{a}	0.169 ±0.015^{a}	0.216 ±0.008^{a}	0.307 ±0.009^{a}
Peel-3	0.122 ±0.003^{a}	0.168 ±0.004^{a}	0.185 ±0.001^{b}	0.228 ±0.013^{b}
Peel-5	0.125 ±0.005^{a}	0.156 ±0.023^{a}	0.171 ±0.002^{b}	0.211 ±0.006^{b}

Note: a, b as upper indices represent a statistically significant difference between samples in columns

Additionally, pomegranate juice caused a slight but statistically significant reduction in the a* and b* values compared to the control. Similar results were observed in our study at the start of storage period – Day 1. Results of color determination are listed in Table 1.

Qin et al. (2013) reported that incorporating pomegranate peel into experimental pork patties resulted in increased redness (a*) and decreased lightness (L*) and yellowness (b*) values when compared to samples treated with BHT and the negative control. Similarly, Firuzi et al. (2018) monitored color changes in pork frankfurters during extended storage and observed a rise in L* values accompanied by a decline in both a* and b* values. In another study, Naveena et al. (2008) applied pomegranate rind powder extract as a curing agent in chicken meat and noted a decrease in L* value along with an increase in a* value relative to the untreated control. A decrease in lightness could be explained by the natural dark color of pomegranate peel extract, and therefore slightly lower the yellowness value in our case. In our study, we could not replicate the increased redness of pomegranate peel enhanced products as other abovementioned authors. As reported by Savadkoohi et al. (2014), variations in instrumental color measurements are considered perceptible to the human eye when the total color difference exceeds a value of 2. By this standard, we can argue that consumers would be able to differentiate between sausage groups only in the fresh state – at the Day 1.

Lipid Oxidation Results

Lipid oxidation significantly affects the sensory attributes of meat products – including taste, odor, texture, and color – as well as their stability throughout processing and storage, ultimately reducing shelf life (Shahamirian et al., 2019). Lipid oxidation of pork sausages was measured four times during the duration of the experiment. At the beginning of the experiment, as well as after the first week of storage, no significant difference among groups was observed. On Day 14 and Day 21, differences were observable between the two groups with added pomegranate peel extract and a control group without any added antioxidant (Table 2).

Qin et al. (2013) incorporated pomegranate seed ethanol extract (20 mg/100 g) into minced pork patties stored at 4 °C and observed a significant reduction in malondialdehyde (MDA) levels throughout the storage period. Similarly, Devatkal et al. (2012) reported a 40% decrease in MDA concentration in goat meat nuggets supplemented with 1% pomegranate peel extract compared to the negative control. Kaur et al. (2015) applied powdered pomegranate seed extract to chicken patties and also noted a significant decline in MDA levels relative to untreated samples. In another study, Firuzi et al. (2018) added pomegranate rind extract to frankfurters stored at 4 °C and found a marked reduction in MDA content in treated samples. Notably, the antioxidant effect of the rind extract surpassed that of the synthetic antioxidant butylated hydroxytoluene (BHT). The antioxidative potential of various pomegranate-derived extracts has been demonstrated in multiple meat matrices, including pork, ground beef, and chicken (Shan et al., 2009; Juneja et al., 2016; Bouarab-Chibane et al., 2017). The suppression of lipid oxidation in dry sausages by pomegranate peel extract supports the strong *in vitro* antioxidant activity observed in the extract itself, as well as the residual antioxidant activity detected within the meat products (Cava and Ladero, 2023).

Conclusions

In our study, we confirmed that pomegranate peel extract could significantly delay the lipid oxidation changes in meat products. Also, we observed that during the storage of meat products, pomegranate peel extract does not have a negative effect on the color of experimental meat products. Pomegranate peel, therefore, remains an interesting alternative as a source of antioxidants for the meat industry. Further studies in multiple departments, such as food technology, toxicology, or food safety, are still needed before introducing this option commercially.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical Statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This publication was funded by the Ministry of Education, Science, Research and Sport of the Slovak Republic via projects KEGA 001SPU-4/2023.

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