http://foodbulletin.net

FOOD BULLETIN
e-ISSN: 2979-9848

https://prensip.gen.tr

RESEARCH ARTICLE

Effect of Pomegranate Peel Extract as a Natural Antioxidant on Sausage Production from Rainbow Trout Fillet Waste

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ARTICLE INFO

Article History
Received: 19.12.2022
Accepted: 28.12.2022
First Published: 30.12.2022

Keywords
Antioxidant
Nutritional
Pomegranate
Sausage
Trout

ABSTRACT

Seafood is one of the leading meat products with high biological and nutritive value. Dried fermented products are called sausage, as a result of the maturation process under certain conditions by filling the material prepared in the form of dough into natural/artificial casings. Oxidation is one of the most important parameters affecting shelf life in seafood containing high levels of unsaturated fatty acids. With the knowledge of the harms of synthetic antioxidants, the plant and its parts from which natural antioxidants are obtained have come to the fore. In this study, sausage dough prepared from trout fillet waste was filled in artificial casings and fermented under specified conditions, to produce sausage. Prepared sausages were divided into 3 groups as the control group without additives, with natural additives (pomegranate peel extract) and artificial additives (ascorbic acid). The proximate, chemical and physical changes of sausages, which were heat treated, stored at +4 ºC were examined. According to the results, ATVB-N and TBA of all sausage groups, exceed the limit values on the 5th day of storage. It was determined that the shelf life of the sausage obtained in the study was limited to 5 days; however, the values of the sausage group with pomegranate peel extract were found to be lower than the other groups. As a result, although the shelf life of sausage groups was limited to 5 days, it was observed that pomegranate peel extract could be used instead of synthetic preservatives with its antioxidant and antimicrobial properties.

Please cite this paper as follows:

Çağlak, E., Kobya, O., Öğretmen, Ö. Y., Karslı, B., & Kara, B. (2022). Effect of pomegranate peel extract as a natural antioxidant on sausage production from rainbow trout fillet waste. *Food Bulletin*, *1*(1), 11-15. https://doi.org/10.29329/foodb.2022.495.03

Introduction

In terms of biological properties of meat structures, seafood is included in the group of foods that spoil quickly. However, when the values of its nutritional structure are revealed, it is included in the group of valuable foods in terms of human health and nutrition. In terms of these features, it is necessary to offer fishery products to the service of humanity by increasing the variety and rate of consumption in terms of both good preservation and nutritional benefits. Fermentation technology is one of the oldest methods to extend the preservation period of meat and to give meat different flavors and aroma properties. A combined process is formed by developing methods of preservation by adding drying and preservatives to the processing stages of meat products produced by fermentation (Sarıçoban, 2000).

In the last twenty-five years, aquaculture in the industrial sense has shown a great development all over the world and in Turkey. Among the aquaculture species, trout have an important proportion in this development. With the increasing amount of aquaculture, great developments have been observed in the consumption, export and processing methods of trout (Çağlak & Karslı, 2015). In the evaluation of the trout wastes generated in the processing industry, it is important both to provide alternative techniques and to create products based on

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ready-made meals such as sausage and meatballs, which are consumed with pleasure.

Oxidative balance is very important in maintaining the quality of products with high lipid content. Seafood and other foods can be spoiled in a short time by various decomposition reactions. Oxidation is the most important of these reactions. The most effective way to prevent or slow down oxidation is the use of antioxidants. Antioxidants prevent peroxide chain reactions, collect reactive oxygen species and inhibit lipid peroxidation (Sevim, 2011; Karabulut & Gülay, 2016; Kobya et al., 2019). Medicinal-aromatic plants are used to protect foods against microorganisms and oxidation with their antimicrobial and antioxidant properties (Deans & Ritchie, 1987; Özcan, 1998; Arslan & Kırca, 2006). The emergence of the side effects of synthetic preservatives used extensively in foods and the resistance of microorganisms have increased the importance of natural herbal preservatives (Nakipoğlu & Otan, 1992). During the production of pomegranate juice, the pulp consisting of pomegranate peel and seed is released. About 48 phenolic compounds were detected in the peel and other parts of the pomegranate, and about 30% of the anthocyanins were concentrated in the peel. In addition, it has antimutagenic, antioxidant and antimicrobial properties with its high molecular weight phenolics, tannins, complex polysaccharides, flavonoids and microelements (Fischer et al., 2011).

In this study, sausages obtained from rainbow trout (*Oncorhynchus mykiss* W.) fillet wastes were divided into three different groups: Control (without antioxidant), natural (with pomegranate peel extract as a natural antioxidant and synthetic (with ascorbic acid as a synthetic antioxidant. Chemical, physical and proximate changes during storage were examined, So, the effects of the production of sausage from trout waste and the use of natural antioxidants were revealed.

Materials and Methods

Rainbow trout were obtained from the aquaculture facility in Trabzon, Turkey and were brought to the Recep Tayyip Erdoğan University, Faculty of Fisheries, Processing and Feed Technology Laboratory in insulated tanks and in a water-ice mixture. Fish over 1 kg were used in the study. In sausage making; The fish were filleted and minced with the addition of appropriate tail fat. The minced fish were kept in refrigerator conditions for 12 hours, covered with stretch film, in order to remove the excessive water. All materials used in sausage production were sterilized at 121.1 °C for 15 minutes. Sausage dough was formed with 100% mixture of fish meat, antioxidant, salt, red pepper, black pepper, cumin, ginger, allspice, sugar, garlic, coriander, tallow and wheat starch. Three sausage groups were formed: Control group (without antioxidants), Synthetic group (containing ascorbic acid) and Natural group (containing pomegranate peel extract). Pomegranate peel extract was obtained as a result of keeping it in a shaking water bath for 24 hours in 5% cold water. Sausage dough was filled into natural sausage casings and heat treatment was applied at 70 °C for 120 minutes. After the heat treatment, the products were cooled in the water-ice mixture for 20-minute and left to the drying/maturation process in outdoor conditions. The ripened products were vacuum packed and stored at 3 °C.

Dry matter, crude protein, crude fat and crude ash analyzes during storage were carried out according to AOAC (1990) and the results were given as % (Çağlak et al., 2015). TVB-N analysis according to Lücke-Geidel method (İnal, 1992; Varlık et al., 1993), thiobarbutiric acid (TBA) analysis according to Tarladgis et al. (1960)'s method (Smith et al., 1992; Varlık et al., 1993) and for pH measurement Curran et al. (1980) according to the method applied. Color analysis was performed with a Konica Minolta (CR-10, Japan) color measuring device (Karsli et al., 2021). Water activity values were carried out at 20 ºC using a water activity device (Aqua Lab 4TE, USA). Statistical comparison of the groups was made by one-way ANOVA with using JMP 5.0.1 (SAS Institute Inc, NC, USA). Tukey's studentized range with a significance level at $p<0.05$ was subjected to determine significance of differences (Sümbüloğlu & Sümbüloğlu, 2000).

Results and Discussion

Chemical and Physical Analysis Results

The chemical and physical analysis results obtained in the present study are shown in Table 1. TVB-N value was 20.41mg/100 g for fresh trout. TVB-N value, which increased during the storage period, was found the highest in the synthetic (33.08 mg/100 g) and the lowest in the natural (28.00 mg/100g) on the first day of storage. TVB-N values of control, synthetic and natural groups were found to be 44.40, 41.63 and 35.90 mg/100 g on the 5th day, respectively. In terms of these values determined on the 5th day of storage, it was determined that the TVB-N value of all groups exceeded the consumable limit value (35 mg/100 g) suggested by Kietzmann et al. (1969). It was observed that the pomegranate peel extract added as a natural additive was suppressive in the increase of the TVB-N value. The differences between and within the groups were significant ($p<0.05$). Çağlak and Karslı (2015) stated that the TVB-N value in fresh trout was 14.08 mg/100 g and it increased depending on the storage period. Karsli et al. (2021) stated that the TVB-N value of vacuum packaged rainbow trout fillets treated with black cumin oil was 53.9 mg/100 g at the end of the 16-day refrigerated storage. Abou-Taleb et al. (2022) stated that the TVB-N values of fish sausage treated with different plant extract varied between 14.22-20.12 mg/100 g. Nitrogen compounds are important in determining the quality of fish meat. These compounds increase due to post-mortem deterioration (Gökoğlu, 2002). During the present study, TVB-N increased depending on the storage time. It was observed that similar results were obtained in other studies as well.

TBA value of fresh trout was found to be 1.54 mg MDA/kg. This value was determined as 6.61 mg MDA/kg for the control, 5.76 mg MDA/kg for the synthetic group and 3.89 mg MDA/kg for the natural group on the 1st day of the sausages produced by applying heat treatment. In the present study, all groups exceeded the consumable limit value of 7-8 mg MDA/kg (Varlık et al. 1993) on the 5th day of storage. These values were found as 9.09 mg MDA/kg in control, 8.85 mg MDA/kg in synthetic and 7.96 mg MDA/kg in natural at the end of storage. Significant changes were detected both within and between groups during storage ($p<0.05$). According to these results, it was observed that application of pomegranate peel extract as a natural additive had a more protective effect on the fish sausage in terms of its antioxidant property. Dinçer et al. (2017) stated that TBA values in saithe (*Pollachius virens*) sausage increased depending on the storage period. Abou-Taleb et al. (2022) stated that the TBA values of fish sausage treated with different plant extract varied between 0.27-0.35 mg MA/kg. Oxidation occurs at a significant rate in these products with high unsaturated fat content. When the data of the present and previous studies were examined, it was found that there were similarities in terms of TBA changes during storage period.

The initial pH of fresh trout was determined as 6.18. The pH values of control, synthetic and natural sausages were determined as 6.28, 6.02 and 6.2, respectively, on the 1st day of storage after heat treatment. It was determined that these pH values tended to decrease in all groups during storage and were 5.96 for the control group, 5.88 for the synthetic additive group and 6.09 for the natural additive group on the 5th day of storage. There was no significant difference between the groups ($p>0.05$), however; a significant difference was observed in intragroup changes depending on the storage time was observed ($p<0.05$). Çağlak and Karslı (2015) stated that the pH values of marinade and brine injected rainbow trout stored at 3 ±1 ºC were in the range of 6.28 to 6.72. In other study, pH value of fermented *Chalcalburnus mossulensis* sausage decreased during storage (Arslan et al., 2001). Abou-Taleb et al. (2022) stated that the pH values of fish sausage treated with different plant extract varied between 7.84-8.37 during frozen storage at -18 °C for 120 days. Determining the pH value and keeping it constant is an important factor in maintaining product quality in foods (Banwart, 1987). It has been observed that the pH changes show a certain amount of decrease depending on the storage time, which is similar to the previous study data.

The water activity was measured as 0.9933 in fresh trout and this value decreased from the 1st day of storage. While the lowest water activity value was determined in the sausages with natural antioxidants on the 1st day of storage, the highest was determined in the control. At the end of the 5-day refrigerated storage, water activity values of the sausage product were found in the control (0.8631), natural (0.8781) and synthetic (0.8921) groups from the lowest to the highest, respectively. Karslı et al. (2021) stated that the water activity values of vacuum packaged rainbow trout fillets treated with black cumin oil trout were between 0.9877 and 0.9948. The change in water activity due to storage occurred due to drying. While there was no significant difference between the groups, the change in storage days was significant ($p<0.05$). In the present study, it is thought that this decrease in water activity is due to the decreasing amount of water due to the heat treatment applied in sausage production. These changes were found to be statistically significant within the group ($p<0.05$), but were insignificant among the groups.

The L*, a*, and b* values of fresh trout were 51.30, 26.00, and 28.80 respectively. L* (brightness) and a* (redness) values decreased significantly from the first day of storage as a result of the added spices and heat treatment ($p<0.05$). It was observed that the change in b* (yellowness) value remained at a more limited level. At the end of storage, the lowest L* value was observed in the control group (21.85), while the lowest a* (15.40) and b* (20.30) values were found in the group with natural antioxidants. The highest L*, a* and b* values were determined in synthetic (22.40), synthetic (15.75) and control (25.45) groups, respectively. Karsli et al. (2021) stated that the min-max values of L*, a* and *b of vacuum packaged rainbow trout fillets treated with black cumin were 18.80-46.25, 3.00-13.25 and 4.40-14.50, respectively. While the present study data is found to be compatible with other studies, it is a fact that feed additives have an effect on color in aquaculture products. It is thought that the color change in sausage products occurs depending on the drying process and additives. In this study, it was determined that the changes in L*, a*, b* (except for natural) values were significant during the study ($p<0.05$).

Table 1. Chemical and physical analysis results of trout sausages.

Groups	Day	TVB-N (mg/100 g)	TBA (mg MDA/kg)	pH	Water Activity	Color		
						L*	a*	b*
Fresh		20.41±0,99	1.54±0.01	6.18±0.00	0.9933±0.0009	51.30±0.14	26.00±1.27	28.80±0.98
Control	1	$28.86±0.99_{B}^{a}$	$6.61±0.7_{B}^{a}$	$6.28±0.02_{A}^{a}$	$0.9669±0.0012_{A}^{a}$	$31.80±0.00_{A}^{a}$	$15.60±0.25_{A}^{a}$	$25.20±0.00_{A}^{a}$
	5	$44.40±1.00_{A}^{b}$	$9.09±0.78_{A}^{b}$	$5.96±0.05_{A}^{b}$	$0.8631±0.0019_{A}^{b}$	$21.85±0.07_{A}^{b}$	$15.65±0.07_{A}^{a}$	$25.45±0.21_{A}^{a}$
Synthetic	1	$33.08±0.99_{A}^{a}$	$5.76±0.16_{B}^{a}$	$6.02±0.02_{A}^{a}$	$0.9629±0.0007_{A}^{a}$	$36.10±1.13_{B}^{a}$	$17.45±0.49_{B}^{a}$	$28.45±0.35_{B}^{a}$
	5	$41.63±1.13_{A}^{b}$	$8.85±0.24_{A}^{b}$	$5.88±0.02_{A}^{b}$	$0.8921±0.0006_{A}^{b}$	$22.40±0.42_{A}^{b}$	$15.75±0.07_{A}^{b}$	$23.75±0.21_{B}^{b}$
Natural	1	$28.00±0.00_{B}^{a}$	$3.89±0.29_{A}^{a}$	$6.20±0.00_{A}^{a}$	$0.9581±0.0002_{A}^{a}$	$25.35±0.35_{C}^{a}$	$13.60±0.00_{C}^{a}$	$22.20±0.14_{C}^{a}$
	5	$35.90±1.98_{B}^{b}$	$7.96±0.08_{B}^{b}$	$6.09±0.03_{A}^{b}$	$0.8781±0.0078_{A}^{b}$	$21.95±0.07_{A}^{b}$	$15.40±0.56_{A}^{b}$	$20.30±0.56_{C}^{b}$

Different lowercase letters (a,b,c…) in the same column represent within-group changes on different storage days ($p<0.05$). Different capital letters (A,B,C…) in the same column represent the variation between groups on the same storage days ($p<0.05$).

Proximate Composition Results

The proximate composition data obtained during storage are shown in Table 2. The amount of crude protein was found to be 18.74% in fresh trout. Depending on the increasing amount of dry matter, the amount of crude protein also increased and it was determined as 36.90%, 33.78% and 40.57% in the control, synthetic and natural groups, respectively, on the 5th day of storage. This change was found to be statistically significant ($p<0.05$). Çağlak and Karslı (2015) stated that the protein value of fresh rainbow trout was 16.83%. Arslan et al. (2001) stated that the protein values of sausage production from *Chalcalburnus mossulensis* ranged between 20.05% and 28.92%.

In the present study, the crude fat content of fresh trout was found to be 12.87%. As a result of the applied processes, this value increased during storage in parallel with the decrease in moisture content ($p<0.05$). At the end of the 5-day storage, the crude fat values of the control, synthetic and natural groups were determined as 20.61%, 22.85% and 20.82%, respectively. In a study, the fat values in sausage production from *C. mossulensis* ranged between 25.23% and 31.44% (Arslan et al., 2001). Berik and Kahraman (2010) found that fat value of mullet (*Mugil cephalus*) sausage was in the range of 2.43% to 24.34%. Our study data were found to be compatible with the literature in terms of the amount of fat increasing due to the decreasing amount of water in sausage products.

The dry matter ratio of fresh trout was determined as 32.74%. As the sausages dried and lost water during the storage period, the dry matter contents of all groups increased significantly($p<0.05$). On the 1st day of storage, the lowest dry matter was found in the control group, and the highest was found in the sausages with natural additives. On the 5th day of storage, the dry matter content was highest in the group with natural antioxidants (71.48%), followed by the control (68.59%) and synthetic (68.16%) groups, respectively. The increase in the amount of dry matter in sausage-like products is an expected situation due to the loss of water in both the applied heat treatment and the ripening processes. Karsli et al. (2021) determined the dry matter content of fresh rainbow trout to be 25%. In another study, it has been reported that the dry matter values of sausage made from *C. mossulensis* ranged between 46.90% and 61.88% during refrigerated storage (Arslan et al., 2001).

The amount of crude ash in fresh rainbow trout was determined as 1.04%This value increased significantly from the first day of the sausage products depending on the heat treatment ($p<0.05$). After five days of storage, the amount of crude ash was determined as 4.56%, 4.42% and 5.35% in the control, synthetic and natural groups, respectively. Similarly, Berik and Kahraman (2010) stated that the ash values, which was 1.11% in fresh mullet, increased in of mullet sausage groups and ranged between 2.36% and 5.03%.

Protein, fat, moisture and ash contents of seafood products can vary depending on various factors (age, gender, season, nutrition, etc.) (Borgstrom, 1961; Huss, 1988). When the nutritional composition changes are examined, it is observed that the data of this study are compatible with the studies conducted. While the amount of dry matter increased in the dried samples due to the sausage production process, parallel increases were observed in the amounts of fat, protein and ash. In addition, it should be known that additives used in sausage products are also effective in nutritional content. This situation is also consistent with other studies.

Table 2. Proximate composition results of trout sausages (%).

Groups	Day	Protein (%)	Fat (%)	Dry Matter (%)	Ash (%)
Fresh		18.74±0.67	12.87±1.11	32.74±0.02	1.04±0.04
Control	1	$19.55±1.06_{A}^{a}$	$16.01±1.04_{A}^{a}$	$42.78±0.08_{A}^{a}$	$3.13±0.01_{A}^{a}$
	5	$36.90±0.11_{A}^{b}$	$20.61±1.66_{A}^{b}$	$68.59±0.21_{A}^{b}$	$4.56±0,07_{A}^{b}$
Synthetic	1	$20.28±0.30_{A}^{a}$	$15.34±0.32_{A}^{a}$	$44.42±0.34_{B}^{a}$	$3.01±0.00_{A}^{a}$
	5	$33.78±1.06_{B}^{b}$	$22.85±0.02_{A}^{b}$	$68.16±0.02_{A}^{b}$	$4.42±0.07_{A}^{b}$
Natural	1	$19.69±0.96_{A}^{a}$	$15.48±0.18_{A}^{a}$	$45.26±0.13_{B}^{a}$	$3.09±0.13_{A}^{a}$
	5	$40.57±1.56_{C}^{b}$	$20.82±1.00_{A}^{b}$	$71.48±0.88_{B}^{b}$	$5.35±0.04_{B}^{b}$

Different lowercase letters (a,b,c…) in the same column represent within-group changes on different storage days ($p<0.05$). Different capital letters (A,B,C…) in the same column represent the variation between groups on the same storage days ($p<0.05$).

Conclusion

The results of the present study showed that the effects of the processing steps in sausage production such as added additives, heat treatment and drying on the proximate composition, chemical and physical parameters were important in sausage production. It is very important for human health that synthetic preservatives are replaced by natural preservatives. According to our study data, it was observed that the use of pomegranate peel extract as a natural preservative for sausages contributed significantly to the restriction of chemical changes. Based on these results, it has been determined that by-products can be used in sausage production in seafood products and the effect of natural additives may be important in maintaining the quality and increasing the shelf life of this type of product. In addition, it is important to carry out more comprehensive studies in terms of determining the shelf life of sausages that can be made from fish and by-products in order to be an alternative to sausages obtained from meat and chicken.

Conflict of Interest

The authors declare that there is no conflict of interest.

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