

## Antioxidant Activity of Pomegranate Seed Oil in Fermented Sausage Enriched with Omega-3 Fatty Acids

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### Abstract

Oxidation of fatty acids reduces the quality of sausage over time. The use of natural antioxidants in sausage formulations is a good way to reduce the oxidation of fatty acids. In this study, changes in the microbial count, physicochemical properties, sensorial and textural characteristics were measured in the fermented sausage after the replacement of canola oil with pomegranate seed oil "PSO", omega-3 oil "O-3O" and a combination of PSO and O-3O during ripening. A significant decrease in water activity up to 89% was detected in PSO, O-3O, and their combinations 30 days after ripening, which indicated better conditions for compatibility of beneficial microorganisms in the new formulations. The increase in the lactic acid bacteria in these conditions confirmed this and a significant increase ( $P<0.05$ ) in the bacterial count, 6.5 Log CFU/g and 6.6 Log CFU/g respectively in PSO and O-3O, compared to control (6.4 Log CFU/g) was observed. The abundance of unsaturated fatty acids was observed at a higher level in PSO, and the fatty acid profile of sausage improved in terms of nutrition. PSO treatment contained high levels of PUFA such as punicic acid (29.03%) and to a lesser extent oleic acid (25.59%) and linoleic acid (16.05%) compared to control. Also, the reduction of lipid oxidation (24.35 % less MDA content) in PSO treatment caused an increase in product acceptance score compared to O-3O. The addition of PSO did not make a difference in the quality of sausages compared to control and prevented the reduction of its quality in samples containing O-3O. These results showed that PSO has the potential to be used in fermented sausage to produce healthier products.

**Keywords:** Fermented Sausage, Healthy Food, Omega-3 Oil, Pomegranate Seed Oil.

## Introduction

Red meat plays a significant role in providing essential amino acids, micronutrients, and vitamins (1). However, meat and its products are susceptible to quality deterioration due to lipid and protein oxidation and microbial growth (2).

Inoculation of sausages with suitable fermentation strains like lactic acid bacteria (LAB) is one of the oldest methods for food preservation. This process improves the nutritional value and the quality of meat products, inhibits the growth of pathogenic bacteria, enhances product shelf life, and resulting in safer final products (3). It is well known that the LAB increase acidity of the medium during the fermentation process in sausages resulted in promoting protein gelation, decreasing in water holding capacity, the drying speed, and the hardness of sausages. Besides, they decompose nitrite and reduce the formation of nitrosamines from the reaction of residual nitric oxide with secondary amines (4).

Although young people enjoy some of the properties of sausages, such as desirable taste that comes from animal fats, salts, a great variety of additives, and spices, however, the number of people that have a concern for their diet is increasing every day (5). Recently changes in formulations of meat products have been carried out by substitution of animal fats by other lipid sources in order to decrease the content of saturated fatty acids and cholesterol besides increasing the polyunsaturated fatty acid (PUFA) fraction (6-7).

In several studies with the use of olive oil, linseed oil, cottonseed oil, and soy oil, essential fatty acids such as oleic and linoleic acid levels increased, and cholesterol content reduced. At the same

time, the sensorial characteristics comprising of texture and color were comparable with those of commercial products (8).

Nonetheless, the unsaturated fatty acids accelerated lipid oxidation. Lipid oxidation is one of the major causes of the deterioration of meat derivatives' appearance and undesirable changes in flavor, texture, and nutritional value. Various synthetic antioxidants are currently used in the meat industry to reduce fatty acid oxidation (9).

However, consumers concern about the safety of synthetic antioxidants. This concern pressed the food industry to find natural sources (10). Natural antioxidants extracted from the plant have the advantage of being easily embraced by consumers, as they are considered natural. Various plant sources such as fruits, vegetables, herbs, and spices are extracted and successfully used to reduce lipid oxidation (11). Pomegranate (*Punica granatum* L.), a member of the Punicaceae family, is a well-known fruit with various health benefits. Juice, peel, and seeds of pomegranate contain several antioxidants such as steroids, flavonoids, polyphenols, saponins, alkaloids, triterpenoids, and vitamin C. Therapeutic properties of pomegranate extract as anticancer and anti-inflammatory were reported in many studies (12).

The pomegranate seed oil has several beneficial properties such as substantial amounts of antioxidants, unsaturated fatty acids, essential minerals, proteins, and phytochemical compositions (13).

The aim of this study was to evaluate the effect of replacement of canola oil with pomegranate seed oil and omega-3 oil on the main quality characteristics of the fermented sausage at 1, 15, and 30 days after ripening.

## Materials and Methods

### Preparation of starter cultures

The lyophilized *Lactobacillus plantarum* and *Pediococcus pentosaceus* were purchased from the Iranian Research Organization for Science and Technology (IROST). The lyophilized powder was added to the liquid medium and incubated at 30°C for 18h. Then, the medium was centrifuged at 10000g at 4°C, and the pellet washed three times with sterile saline solution (0.9% NaCl). This stage was repeated twice. Finally, the turbidity of bacterial suspension determined using the McFarland standards. The initial value of log 6 CFU/g was determined for the rest of the fermentation period (14).

### Preparation of Fermented Beef Sausage

Beef sausage was prepared using the following ingredients. As much as 1000±1 g (73.5%) of beef meat was ground first and then inserted sequentially with 15.5±0.01% canola oil (15), 5.5±0.01% ice, 1.8±0.01% salt, 3.7±0.01% special spices for sausages (include red pepper, garlic, paprika, nutmeg, black pepper), and log 6.17±0.06 CFU/g of *L. plantarum* and *P. pentosaceus*. Transparent pomegranate seed oil (PSO) was prepared from Saveh Anar Co, Saveh, Iran, and omega-3 fish oil (O-3O) was purchased from Sayadan Javan Jask Co. Jask, Iran. The O-3O and PSO and their combination in equal proportions were used as substitutes for the canola oil. The dough was mixed and stuffed into a synthetic polyamide casing. The sausages were then separated into pieces with a diameter of 30mm and a length of 10 cm with thread. The product was fermented at 35°C for 48 h and relative humidity (RH) of 95% to reach its pH to 5.3. To dry the fermented sausages and reduce the moisture, the samples were incubated for 24 hours at 25°C and RH 65%. Then, sausages were

packaged and stored under vacuum at refrigerator temperature (4°C) until the end of the experiment (30 days). All, physicochemical and microbiological analyses were done at 1, 15, and 30 days during the ripening process according to the research of Hu et al. (2008) (16).

### Microbiological Analysis

Briefly, the fermented sausage was weighed as much as 5 g at 1, 15, and 30 days during the ripening process and put into 45mL of 0.1% peptone water (PW) and homogenized. Three dilutions (1:10, 1:100, and 1:1000) were prepared using PW and an aliquot of 0.1 mL of each dilution was spread on MRS agar. Plates were incubated at 37°C for 48h. Representative colonies were count and, the total population of lactic acid bacteria was determined as previously described by Hu et al. (2008) (16).

### Physicochemical Analysis (pH, water content and color determination)

pH measurements were performed according to the AOAC (2005) (17) method. Five-gram samples were homogenized with 50ml of boiled distilled water free of carbon dioxide and thoroughly mixed for 30 seconds. The pH was measured with a digital pH meter (Mettler Toledo 320-s, Shanghai, China). The moisture percentage was calculated by the difference in weight of sausage weight loss experimented by the sample (5 g) maintained in an oven (Memmert, UL 60) at 105°C until constant weight according to the AOAC recommended method (18). The water activity system measured water activity levels following National Iranian Standard No. 9657 using a Thermoconstanter Novasina TH200 water activity meter (Axair Ltd., Switzerland) at 25°C. The color measurements of samples were measured using a colorimeter (Model TC-Pa G, Beijing optical Instruments Factory, Beijing, China). The samples were cut

into a slice of 0.5mm thickness before determination.  $L^*$ ,  $a^*$ , and  $b^*$  indices were determined which indicate lightness, redness or greenness, and yellowness or blueness, respectively (19). Colour readings were taken at three points on the central part of the cut surface of the two slices of three sausages.

#### Determination of free fatty acids

Free fatty acids were extracted using a solution containing 1mL hexane and 0.5 mL methoxide transesterification solution (30 mL sodium methoxide in 60 mL methanol). Briefly, 5 g sausage and 1 mL of extract solution were mixed and stirred continuously for 1 minute. Then 5mL hydrogen sulfate was added, and the upper phase was transferred to a new tube. Then 2–3 g sodium sulfate was added, and the cocktail was stirred and incubated for 20-30 minutes at room temperature. The samples were centrifuged at 3200 g for 15 minutes. The liquid phase containing methyl ester was used for individual free fatty acids measurement. The analysis was performed using an Agilent chromatography (6890, UK) equipped with the split injector and flame ionization detector in a capillary column (m, 250  $\mu$ m i.d., 0.2  $\mu$ m film, BPX-70 thickness 120). Nitrogen was the carrier gas. The oven temperature was set to 198°C and equilibrated for 6 minutes. The oven temperature was then increased to 180°C for 10 minutes and raised again to 210°C. The temperature of the regulator and the detector were set to 250°C and 280°C, respectively. The fatty acids were determined relatively based on the previously described method (20).

#### Measurement of peroxide index value

The peroxide index value was determined according to the Khlasi et al. (2012) (21) method with three replications. Five g sample was weighed

and heated in a water bath at 60°C for 3 min in a glass stoppered Erlenmeyer flask to melt the fat. Then, it was thoroughly agitated for 3 min with 30 mL acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper to remove meat particles. Further, 0.5 mL of saturated potassium iodide solution was added to the filtrate. Then 30 mL of distilled water was added to the solution. The titration was allowed to run against a standard solution of sodium thiosulfate (25 g/L) until the solution turns to light yellow. Then 5.5 mL of 1% starch was added to change the color to dark blue. The titration process continues until the blue turns to light color. Then, the peroxide index value was calculated and expressed as milliequivalent  $O_2/100$  g of sample.

#### Determination of thiobarbituric acid reactive substances (TBARS)

Assay of TBARS, according to the method of Egan et al. (1981) (22) was carried out to measure malondialdehyde (MDA) in the sample. Briefly, 10 grams of sample was homogenized with 50 mL of distilled water. The mixture was distilled with 5.47 mL water, 5.2 mL 4 N hydrochloric acid, anti-foaming, and anti-boiling materials in the Erlenmeyer distiller. Then, 5 mL of distilled material and 5 mL of TBARS reagent were mixed and incubated in boiling water for 35 minutes. The samples were cooled for 10 minutes after being boiled for 35 minutes, and their optical density will be read in 1 cm cells in front of the control at a wavelength of 538 nm.

The absorbance of the supernatant was measured at 538 nm using a spectrophotometer. The TBARS value was calculated using a standard curve of MDA. The units were expressed as mg MDA/kg sample.

### Sensory evaluation

The samples were submitted to a sensory evaluation in order to determine whether differences existed between the control sausage and samples oil substituted with PSO and O-3O and their combination based on scores on a 5-point hedonic scale (Scale: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely). A panel of 15 persons with experience in sensorial evaluation (trained staff) and 50 volunteers evaluated the sensory properties of the fermented sausages. Panelists were given a white plastic plate containing four samples of sausages coded with four numbers. The panelists evaluated color, taste, texture, and overall acceptance of samples. After the evaluation, means values were calculated for each parameter.

### Statistical analysis and visualization

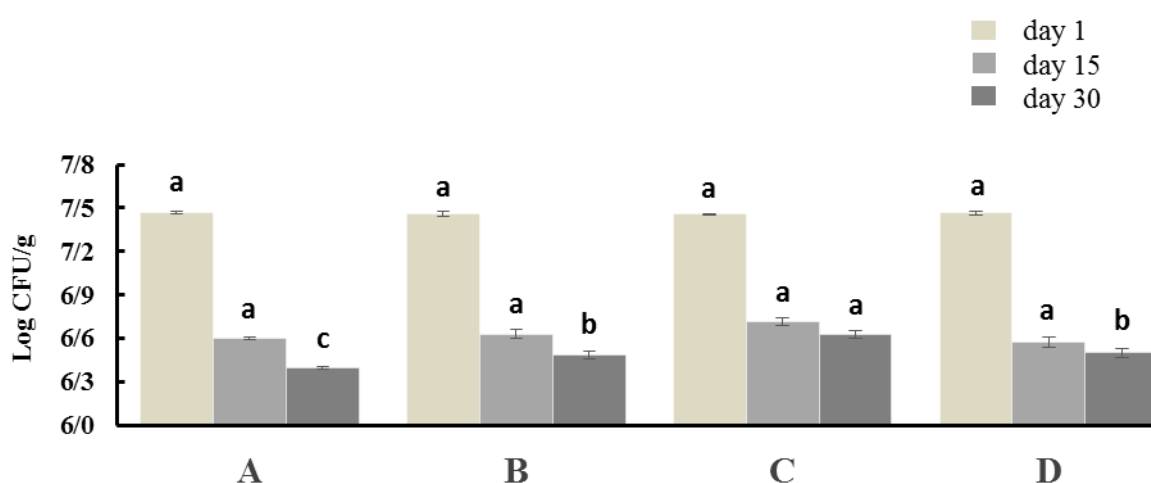
Statistical analysis was performed using one-way ANOVA in SPSS

software version 21 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to determine any significant difference between mean values at the 0.05 level. Graphs were then also created using Excel software (Microsoft Inc., USA).

## Results and discussion

### Microbiological analyses

In the production of fermented sausage, a sufficient quantity of lactic acid bacteria and Micrococcaceae are necessary for the adequate development of the fermentation process. These microorganisms improve the nutritional value of meat and inhibit the growth of pathogenic bacteria resulting in a safe product (23-24). In the current study, the bacterial count of *L. plantarum* and *P. pentosaceus* significantly decreased during the ripening of control and treated sausages (Figure 1).



**Figure 1.** Total bacterial colonies on MRS agar evaluated during the ripening time. A) Control with canola oil, B) PSO, C) O-3O, D) PSO and O-3O. Same letters represent non-significant difference according to Duncan's Multiple Range Test ( $P < 0.05$ ).

Further, no difference was observed in the development of LAB when PSO, O-3O, and their combination were substituted with canola oil 15 days of storage, which is consistent with the previous study on the production of sausages with inulin, corn oil, and linseed (25- 26). However, when storage time reached 30 days, a significant increase ( $P<0.05$ ) in the bacterial count, 6.5 Log CFU/g and 6.6 Log CFU/g respectively in PSO and O-3O, compared to control (6.4 Log CFU/g) was observed. This data indicates that in the presence of O-3O and PSO, the lactic acid bacteria are dominated microbiota of fermented sausages. The results were consistent with those of Hu et al. (2008) (16) and Xu et al. (2010) (27) that showed at the end of the fermentation process, lactic acid bacteria become the predominant species of fermented sausages, although they have been reduced over time due to reduced available substrate levels, their metabolites remain.

#### Physicochemical analyses

##### pH value

The evaluation of pH value showed that all fermented sausages were similar to that of the control. However, the pH value decreased 15 days of storage with no significant difference between treatments and control, which is consistent with the previous report (28) (Figure 2a).

The rate of pH decrease increased during storage time and on day 30 a significant difference was detected between pH of O-3O (5.03) and control (5.11). The decrease in pH on the last day compared to the first day in PSO, O-3O and their combination was 3%, 4%, and 3%, respectively which was more than control (2%) and was consistent

with the microbial count. Our results show that LAB survive in a formulation containing O-3O and PSO, thus maintaining the proper pH of the sausage and helping to retain its nutrients (24).

##### Moisture content and water activity

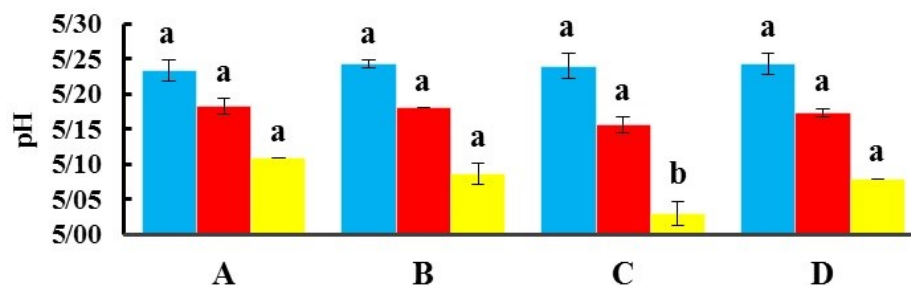
Fat content and the differences in the chemical composition (such as protein:fat: water ratio) are usually associated with an increase in the hardness and chewiness of fermented sausages (25). Our experiment results showed that moisture content was not significantly ( $P<0.05$ ) different among sausages in control and treatment groups 1 and 15 days after ripening. However, 30 days after ripening, the water content in O-3O (55.14%) was in contrast to the samples containing PSO, their combination and control which significantly was higher and showed a range from 55.24% to 55.37 % (Figure 2b). This data suggesting a faster drying process in O-3O treatment and also indicates that the substituted PSO and not O-3O alone is comparable with the control and may generate a barrier avoiding the water loss during the fermented process. The sausage water activity in days 1 and 15 in control was statistically ( $P<0.05$ ) different from the treatment groups, indicating that the substitution of oil content affected the water activity of the products. This was more pronounced when the storage was extended for 30 days in which the water activity decreased to 89% in PSO, O-3O, and their combinations (Figure 2c).

This may suggest that the use of substitutes based on PSO and O-3Os can decrease the water activity of products and prevent unpleasant appearance disallowing the growth of microorganisms, with a consequent increase in their shelf life. Soyer et al. (2005) (29) showed that the decrease in water activity is correlated with pH

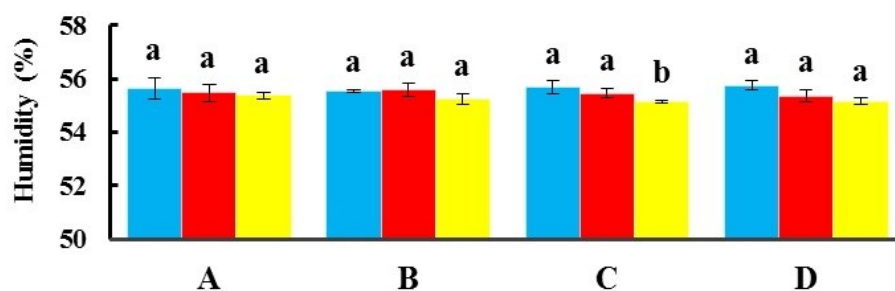
decline. As we have seen here, the slope of pH reduction in the treatments was

higher than the control and corresponded to the reduction of water activity.

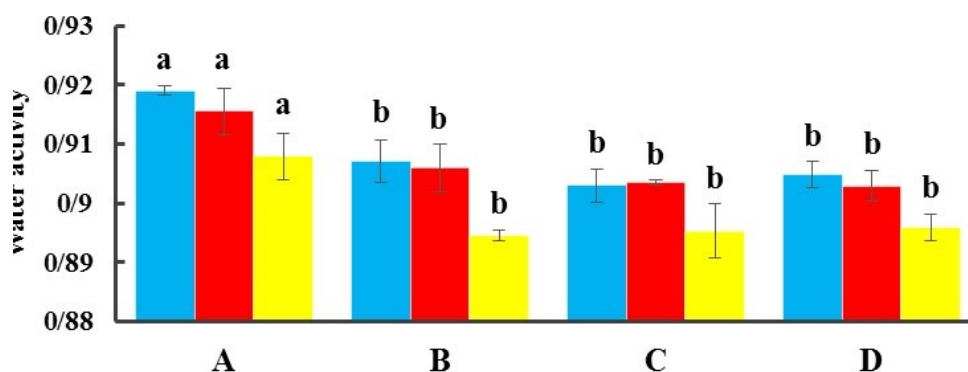
**a**



**b**



**c**



**Figure 2.** Physicochemical analyses of fermented sausages evaluated during the ripening time: a: pH value; b: humidity; c: water activity. A) Control with canola oil, B) PSO, C) O-3O, D) PSO and O-3O during the ripening time. Same letters represent non-significant difference according to Duncan's Multiple Range Test ( $P < 0.05$ ).

### Color texture

Regarding color parameters including  $L^*$ ,  $a^*$ , and  $b^*$ , the results showed no

significant differences for luminosity ( $L^*$ ) in day 1 and when the days extended to 15 days (Table 1).

**Table 1.** Effect of substitution of canola oil on color factors of fermented sausages during the ripening time.

Time	Treatment	Luminosity ( $L^*$ )	Redness ( $a^*$ )	Yellowness ( $b^*$ )
day 1	A	58.89±0.13 <sup>a</sup>	14.65±0.04 <sup>a</sup>	6.60±0.3 <sup>a</sup>
	B	59.01±0.11 <sup>a</sup>	14.47±0.09 <sup>a</sup>	6.55±0.14 <sup>a</sup>
	C	58.38±0.23 <sup>a</sup>	14.50±0.05 <sup>a</sup>	6.53±0.22 <sup>a</sup>
	D	58.64±0.44 <sup>a</sup>	14.60±0.30 <sup>a</sup>	6.77±0.19 <sup>a</sup>
day 15	A	57.51±0.19 <sup>a</sup>	15.78±0.21 <sup>b</sup>	6.88±0.05 <sup>ab</sup>
	B	57.68±0.26 <sup>a</sup>	15.12±0.13 <sup>c</sup>	6.71±0.12 <sup>b</sup>
	C	56.97±0.13 <sup>a</sup>	16.98±0.17 <sup>a</sup>	7.02±0.09 <sup>a</sup>
	D	57.30±0.02 <sup>a</sup>	16.65±0.23 <sup>a</sup>	6.83±0.10 <sup>ab</sup>
day 30	A	53.79±0.31 <sup>c</sup>	17.77±0.11 <sup>b</sup>	8.47±0.10 <sup>a</sup>
	B	55.72±0.24 <sup>a</sup>	16.23±0.16 <sup>c</sup>	7.34±0.16 <sup>c</sup>
	C	52.21±0.31 <sup>d</sup>	19.45±0.35 <sup>a</sup>	9.19±0.13 <sup>a</sup>
	D	54.79±0.25 <sup>b</sup>	17.84±0.16 <sup>b</sup>	8.71±0.26 <sup>a</sup>

A) Control with canola oil, B) pomegranate seed oil, C) omega-3, and D) pomegranate seed oil and omega-3. Values are the means (averaged from three replicates) ±SD.

However, the values of  $L^*$  were influenced and significantly decreased 30 days after ripening in fermented sausage with O-3O (52.21) compared to control (53.79). Further, the highest values of  $L^*$  were observed for PSO (55.72) and combination of it with O-3O (54.79). Similar results have been reported in other studies in which the replacement of pork backfat by olive oil and linseed oil has led to the production of lighter-colored sausages (30- 31). The redness ( $a^*$ ) values were not affected in day 1 in treatments compared to control. However, 15 and 30 days after ripening, the substituted oils (O-3O and combination of O-3O and PSO) showed an increase in redness compared to the control, indicating a similar approach based on the relationship between product color and standard oil content as previously reported (32). The yellowness ( $b^*$ ) was strongly affected and increased ( $P<0.05$ ) by the replacement of canola

oil with O-3O and its combination with PSO (Table 1).

This result may influence consumer acceptability, and show some treatments are more similar to control sausages in terms of the final color. These findings also are consistent with the previous report that a fat replacement by olive and soybean oil resulted in lighter and yellower fermented sausages (33-34).

### Peroxides index and TBARS

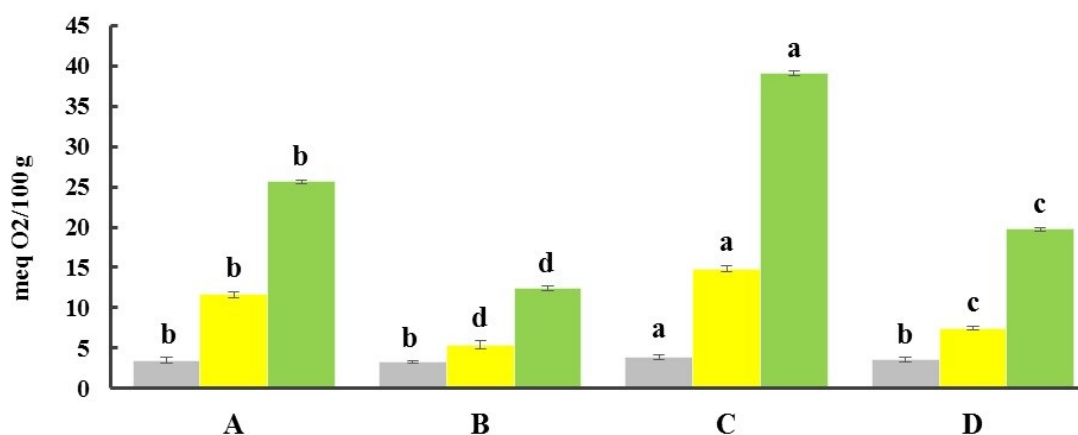
The peroxide value is the first evidence that an oil sample has undergone primary oxidation, a phenomenon observed in oxidative stress. Malondialdehyde (MDA) also is an organic compound known as a marker for oxidative stress determined using TBARS assay (35). In this study, Lipid oxidation was followed through the analysis of TBARS and peroxides. On the first day, the peroxide index showed a significantly higher value (3.90 meq O<sub>2</sub>/100g sample) in the



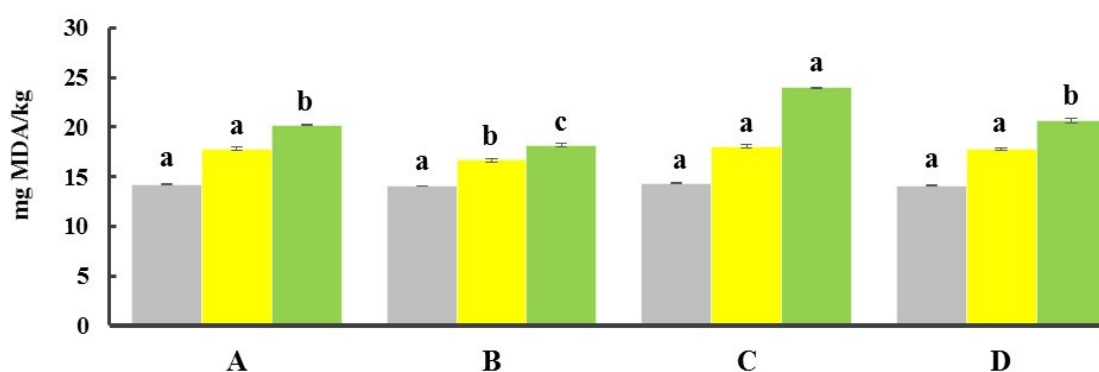
sausage that canola oil substituted with O-3O while the TBARS indicated no significant change compared to control sausage (3.46 meq O<sub>2</sub>/100 g sample) (Figure 3a ). Further, both lipid oxidation factors reduced significantly in

the sausage that its oil was substituted with PSO and showed peroxides indices of 3.28 and 12.45 meq O<sub>2</sub>/100 g sample (Figure 3a) and MDA content of 16.67 and 18.14 mg/kg (Figure 3b) 15 and 30 days ripening respectively.

**a**



**b**



**Figure 3.** Sausage lipid oxidation indices evaluated during ripening time: a: peroxide value; b: MDA content. A) Control with canola oil, B) PSO, C) O-3O, and D) PSO and O-3O during the ripening time. Same letters represent non-significant difference according to Duncan's Multiple Range Test ( $P < 0.05$ ).

Thus, it could be concluded that replacing canola oil with PSO shows fewer signs of oxidation. Protective effects of PSO on animal body tissues antioxidant-oxidant status due to its antioxidant properties were demonstrated (36). Therefore, adding this powerful antioxidant to food is beneficial for human health. Decreases in the peroxide

values in sausages containing more than 750ppm of pomegranate peel extract have recently been reported (37).

#### Fatty acid profile

The fatty acid composition of controlled and treated sausages is shown in Table 2.

**Table 2.** Fatty acid profile of fermented sausages.

Fatty acid	Concentration (w/w)			
	Treatment			
	A	B	C	D
Myristic acid	2.26±0.02	2.16±0.14	5.28±0.13	5.76±0.29
Palmitic acid	10.87±0.64	6.50±0.25	7.44±0.34	8.15±0.65
Palmitoleic acid	4.31±0.30	4.77±0.09	2.74±0.07	3.04±0.13
Oleic acid	33.85±1.21	25.59±0.09	11.72±0.75	16.15±0.91
Linoleic acid	20.54±0.98	16.05±0.44	26.29±1.30	17.71±0.76
Linolenic acid	14.57±0.70	-	3.92±0.12	-
Ecosanoic acid	7.94±0.23	9.74±0.62	2.94±0.13	6.95±0.128
Punicic- Linolenic acid	-	29.03±1.02	-	21.28±1.05
Behenic acid	5.62±0.39	6.07±0.18	3.84±0.17	4.77±0.23
Arachidonic acid	-	-	20.75±1.10	8.47±0.30
Eicosapentaenoic acid (EPA)	-	-	5.88±0.16	3.04±0.14
Docosahexaenoic acid (DHA)	-	-	9.81±0.72	4.34±0.41

A) Control with canola oil, B) pomegranate seed oil, C) omega-3, and D) pomegranate seed oil and omega-3. Values are the means (averaged from three replicates) ±SD.

In control sausages, oleic acid (33.85%), linolenic acid (20.54%), and linoleic acid (14.5%) were higher than other fatty acids, which is consistent with other studies on fermented sausages (7). As expected, PSO treatment contained high levels of PUFA such as punicic acid (29.03%) and to a lesser extent oleic acid (25.59%) and linoleic acid (16.05%). The addition of O-3O oil instead of canola oil resulted in significant differences in the lipid profile of fermented sausages. In general, twelve fatty acids were profiled, among which a PUFA fatty acid, linoleic was detected (16.05-26.29%) in all treatments and control. Other PUFAs, omega-3 fatty acids, including docosahexaenoic acid

(DHA) and eicosapentaenoic acid (EPA), were detected only in treatments containing O-3O. The combination of O-3O and PSO led to changes in the amount of PUFAs so that punicic acid (21.28%), linoleic acid (17.71%), and oleic acid (16.15%) had the highest concentrations. Our data are consistent with previous studies (38-39, 9) showing that unsaturated fatty acids (USFAs) content of fermented sausages increases after the replacement of animal fats with rich sources of USFAs. Palmitic acid, the most common saturated fatty acid (SFA) found in animal and plant resources was reduced 25-40% in treatments compared to the control. There is a positive and dose-dependent relationship between the

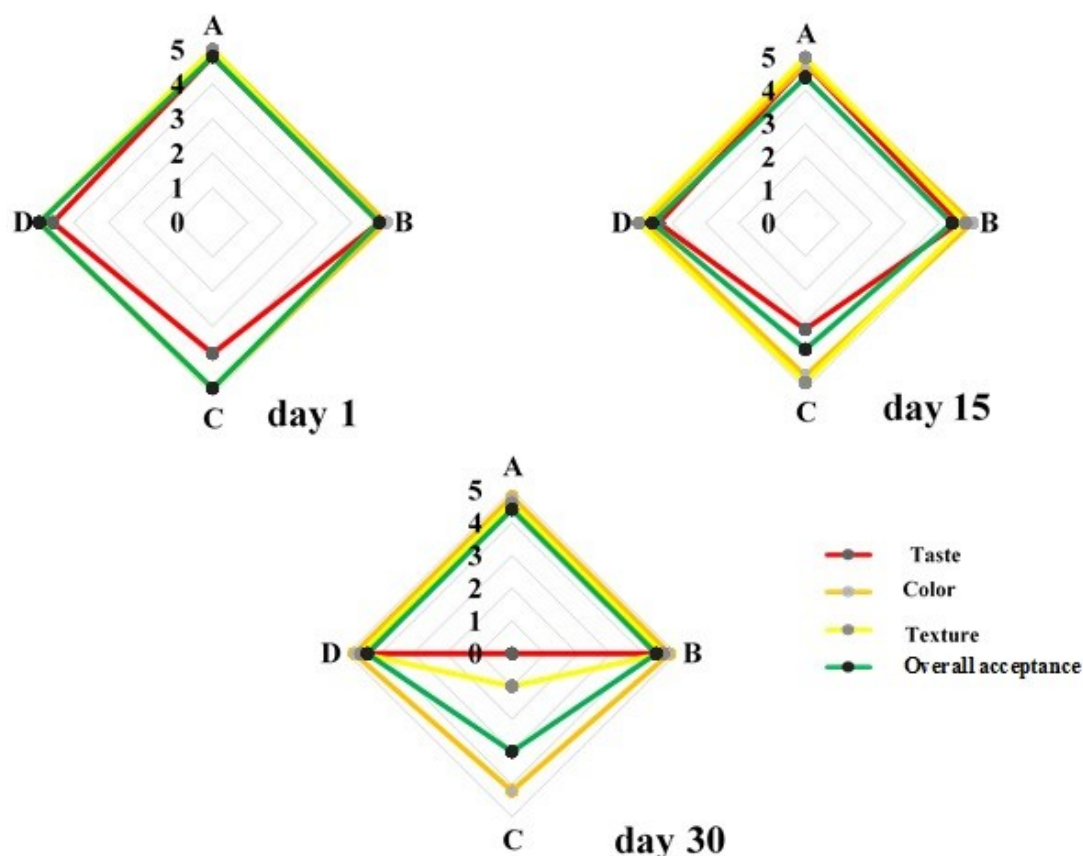
intake of SFAs and blood low density lipoprotein (LDL) cholesterol concentrations. Therefore, the production of healthy meat products is necessary to reduce SFA in the human diet and related diseases (40).

#### Sensory evaluation

The sensory attributes evaluated by the trained panelists and the results are shown in the form of a radar charts in Figure 3. The analysis indicated no significant differences in overall acceptability in control fermented sausage and the sausage influenced by oil types one day after production. Although omega-3 oil or fish oil has

many benefits, it is less used in the food industry due to its bad smell and bad taste, and rapid oxidation, and is mostly used as a supplement. An odorless form of this oil is also expensive and not economical to use in the food industry. The overall acceptance score, taste, and texture of samples containing O-3O decreased over time and was lower than other treatments and control. Lipid oxidation is responsible for rancid flavors and sensory defects in sausage (41).

Hypersensitivity of O-3O to oxidation, which causes bad taste, is one of the factors reducing its acceptance. These results were consistent with the results for lipid oxidation factors (Figure 4).



**Figure 4.** Effect of substitution of canola oil on sensory quality of fermented sausages during the ripening time. A) Control with canola oil, B) PSO, C) O-3O, and D) PSO and O-3O.

The use of PSO instead of canola did not alter any of the sensory traits assessed for sausage. The addition of PSO also reduced the oxidation in sausages containing O-3O and maintained its quality compared to sausages containing O-3O alone. Adding different concentrations of pomegranate peel extract to sausages up to 750 ppm did not make a significant difference in terms of odor and taste (37).

According to the results of this study, the addition of PSO not only does not make a difference in the quality of sausages but also prevents the reduction of its quality in the sample containing O-3O.

### Conclusions

Although the effect of different concentrations of pomegranate peel powder (42) or extract (37) on sausage quality has been reported, to our knowledge this is the first report of the

effect of PSO on sausage quality. The lactic acid bacteria were dominant in the presence of O-3O and PSO, and their combination in fermented sausages, which contributes to the health of the product. One of the issues that emerge from our findings is that the use of PSO decreases the water activity and the signs of oxidation during the ripening process. This also preserves the quality of the sausage.

Due to the stability of unsaturated fatty acids in ripe sausages containing PSO, sausage production with this natural substance is promising for the production of healthy meat products. Of course, maintaining and stabilizing the quality and sensory indicators of sausages containing PSO is also an important parameter that was shown in this study.

### Conflict of interest

Authors declare that there exists no conflict of interest among them.

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## فعالیت آنتی اکسیدانی روغن هسته انار در سوسیس تخمیری غنی شده با اسیدهای

### چرب امگا ۳

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صفحه ۳۳-۴۸

### چکیده

اکسیداسیون اسیدهای چرب با گذشت زمان کیفیت سوسیس و کالباس را کاهش می دهد. استفاده از آنتی اکسیدان های طبیعی در فرمولاسیون سوسیس روش خوبی برای کاهش اکسیداسیون اسیدهای چرب است. در این مطالعه، تغییرات جمعیت میکروبی، خصوصیات فیزیکی-شیمیایی، خصوصیات حسی و بافتی سوسیس تخمیری پس از جایگزینی روغن کانولا با روغن هسته انار (PSO)، روغن امگا ۳ (O-3O) و ترکیب PSO و O-3O در طی زمان رسیدن اندازه گیری شد. کاهش معنی دار فعالیت آبی تا ۸۹٪ در PSO، O-3O و ترکیب آن ها ۳۰ روز پس از رسیدن مشاهده شد، که نشان دهنده شرایط بهتر برای سازگاری میکروارگانیسم های مفید در فرمولاسیون های جدید بود. افزایش باکتری های اسید لاکتیک در این شرایط این موضوع را تأیید کرد و افزایش معنی داری ( $P < 0.05$ ) در جمعیت باکتری، Log CFU/g برابر با ۶/۵ و ۶/۶ به ترتیب در PSO و O-3O در مقایسه با Log CFU/g برابر با ۶/۴ در کنترل، مشاهده شد. فراوانی اسیدهای چرب اشباع نشده در سطوح بالاتری در PSO مشاهده شد و مشخصات اسیدهای چرب سوسیس از نظر مواد مغذی بهبود یافت. تیمار با PSO حاوی مقادیر بالاتری از اسیدهای چرب اشباع نشده مانند پونیسیک اسید (۲۹/۰۳٪) و مقادیر کمتری از اولئیک اسید (۲۵/۵۹٪) و لینولئیک اسید (۱۶/۰۳٪) در مقایسه با کنترل بود. همچنین، کاهش اکسیداسیون لیپید (MDA ۲۴/۳۵٪ کمتر) در تیمار PSO باعث افزایش نمره پذیرش محصول در مقایسه با O-3O شد. افزودن PSO تفاوتی در کیفیت سوسیس ها نسبت به کنترل ایجاد نکرد و از کاهش کیفیت آن در نمونه های حاوی O-3O جلوگیری کرد. این نتایج نشان داد که PSO توانایی استفاده در سوسیس تخمیری برای تولید محصولات سالم تر را دارد.

واژه های کلیدی: روغن امگا ۳، روغن هسته انار، سوسیس تخمیری، غذای سالم.