# Pumpkin seed oil as a partial animal fat replacer in bologna-type sausages

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Running title: Pumpkin seed oil as a partial animal fat replacer

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**Abstract**

Beef fat was replaced with cold press pumpkin seed oil (0%, 5%, 15% and 20%) in the production of bologna-type sausages. pH, water-holding capacity, jelly-fat separation, emulsion stability and viscosity values were determined in meat batters. Thiobarbituric acid reactive substances (TBARS), color, and textural characteristics (TPA, shear test, penetration test) were determined in end-product at 1, 7, 14, 21, and 28 days of storage at 4℃. The pH values were varied between 6.06 and 6.08. With the increase in the level of pumpkin seed oil in meat batters, there was a significant increase in water-holding capacity, jelly-fat separation and viscosity values ($P<0.05$) while a significant decrease in emulsion stability ($P<0.05$). TBARS values of sausages were found to be significantly higher than in the control group ($P<0.05$), and this trend continued during storage. Increasing of pumpkin seed oil level were caused a significant increase in L* and b* values while a decrease in a* value ($P<0.05$). Hardness, adhesiveness and chewiness values were significantly reduced whereas cohesiveness and resilience values increased ($P<0.05$). Maximum shear force and work of shear was significantly decreased as the level of pumpkin seed oil increased ($P<0.05$). Hardness, work of penetration and the resistance during the withdrawal of the probe values (penetration tests) increased significantly with the increase in the level of pumpkin seed oil ($P<0.05$). These results indicate that pumpkin seed oil has potential to be use as a replacement of animal-based fats in the production of bologna-type sausages.

**Keywords:** Bologna type sausage, pumpkin seed oil, texture, TBARS

## Introduction

In the world, around 250 different types of emulsified meat products are being produced (Gökalp et al., 2010), majority of which have high fat content. For example, the animal fat content in emulsified meat products such as frankfurters and bologna type sausages ranges between 20% and 30% (Kang et al., 2016). In such products, fat that is part of meat enters emulsion formula, and also it can be added externally as an ingredient. The amount of fat that would be included in the formula varies depending on the fat content of the meat used as a main ingredient. Generally, 18-20% fat addition is sufficient for meats with normal fat content, whereas 16-18% of fat addition would be enough when fatty meats are used in the formula (Gökalp et al., 2010). The fat in the formula reduces the cost of production, and plays significant roles in the formation of many functional properties such as the development of sensorial and textural properties by imparting of a certain taste and flavour and by giving softer and more palatability (Choi et al., 2010; Chen et al., 2015). When its level in the formula is reduced, these essential effects of fats are eliminated, and the product becomes harder, more rubbery, drier and darker, thereby becoming less acceptable (Şişik et al., 2012). There is a need for some changes in product formulations in order to eliminate such negative effects.

Emulsified meat products are easily produced and consumed, and their prices are relatively low. However, the effects of emulsified meat products on health are still in debate, as the increased consumption of saturated fatty acids has been associated with detrimental health outcomes such as increasing the cholesterol levels and triggering the formation of coronary heart diseases. Therefore, there has been an increasing interest in development of emulsified meat products with reduced-cholesterol and animal fat content (Jimenez-Colmenero et al., 2010). Having cholesterol-free property, vegetable oils contain high levels of unsaturated fatty acids, tocopherols, ß-carotene, sterols, A and D provitamines (Jimenez-Colmenero et al., 2010; Zhuang et al., 2016). Therefore, the use of vegetable oils in the productions of emulsified meat products has become widespread and several studies have been conducted to analyse the quality parameters of emulsified meat products containing higher vegetable oil (Choi et al., 2009; Özvural and Vural, 2008; Pappa et al., 2000).

One of the vegetable oil that could be possibly used for the production of emulsified meat is pumpkin seed oil. The high nutritional value of pumpkin seed and its oil lately led to an increasing popularity. Preventing the growth of the prostate (Gossel-Williams et al., 2006) and decreasing diabetes by promoting hypoglycemic activity can be given as examples of health promoting effects of the pumpkin seed oil (Zuhair et al., 2000). Such benefits provided by pumpkin seed oil could be associated with a peculiar composition rich in mono- and poly-unsaturated fatty acids, minerals, vitamins, pyrazine derivatives, phytosterols, pigments and phenolic compounds (Nederal et al., 2014). Pumpkin seed oil has already been very commonly used in food applications; for example, it has been used as a cooking oil, salad oil and for margarine production (El-Adawy and Khaled, 2001).

No studies have been conducted to test the potential of pumpkin seed oil as an animal fat replacer in the production of emulsified meat products. Therefore, the aim of the present study was to investigate the possibilities of replacing of the animal fat with pumpkin seed oil in various levels during bologna-type sausage production and quality criterion of the product.

## Materials and Methods

### Extraction of pumpkin seed oil

The pumpkin seed called "Ürgüp Sivrisi" belonging to the *Cucurbita pepo* species was used in the study. Pumpkin seeds were purchased from Bahtiyar Dogan Food Co. (Nevşehir, Turkey). After being sun-dried, seeds were packed with polyethylene bag and stored at 15℃. Oil extraction and characteristic properties of the oil was previously defined by Aktaş et al. (2018).

### Preparing of the bologna-type sausage batters and processing

Meat and beef fat used in sausage production were purchased from Kavdırlar Meat (Nevşehir, Turkey). For this purpose, 24 hours post-mortem *Longissimus thoracis et lumbarum* muscles were obtained from 3-year-old beef carcasses. The meats were frozen at -20℃ and stored at this temperature until being used. Prior to production, meat and beef fat used in sausage production were subjected to proximate analysis (71.93%±0.24 moisture, 19.70%±0.36 protein, 6.92%±0.26 fat, 1.00%±0.01 ash, pH: 5.52%±0.01; 16.76%±0.36 moisture, 4.74%±0.36 protein, 77.63%±0.61 fat and 0.18%±0.01 ash, respectively). Bologna-type sausages were produced to ensure 16% protein, 24% fat, and 60% moisture content in the final product. Beef fat was replaced with pumpkin seed oil in the levels 0%, 5%, 15% and 30% to form meat emulsions. Preparation of meat emulsions were based on the method by Aktaş and Gençcelep (2006). Accordingly, meat and beef fats being kept at -20℃ were placed into a refrigerator before starting production, partially thawed at refrigerator temperature (4℃) and ground in meat grinder. Meat were then placed into cutter (CTR10, Arı Makine, Istanbul) and chopped for 20-30 seconds. At the end of the duration, nitrite curing salt mixture (E-250; 99.5% NaCl+0.5% $NaNO_2$) (Alfasol, Istanbul) was added at 2.5% and mixed for 20-30 seconds. Half of the water to be enter the formulation was added to the medium in ice-form and the mixture were mixed until the temperature reached to 3℃. Afterwards, 2.00% sausage-spice mixture (Alfasol, Istanbul), 0.25% polyphosphate (Alfasol, Istanbul), 3.00% starch and the rest of the ice were added and mixed at high speed until 8℃. After mixing was completed, fat/oil was added to the system and emulsification was continued until the temperature reached 12℃. Finally, 0.01% hickory smoke (D402V, Dalgety Food Ingredients) was added to the system and cutter was rotated at low speed for a few rounds. The resulting emulsion batters were stuffed into 85 mm diameter cellulose casings by using a hydraulic filling machine (HD 15/40, Arı Makine, Istanbul, Turkey) and then crimped (Arı Makine, Istanbul, Turkey). The sausages were heated to an internal temperature of 72℃ in a 90℃ water bath, then cooled under tap water and stored at 5°C. This processing was carried out in duplicate for each formulation (treatment) at different occasions. All analyses were carried out in triplicate for each formulation.

**Physicochemical analyses of sausage batters**

In order to determine the pH value in sausage batters, 10 g of batters were weighed and blended with 90 ml of distilled water, then pH values were determined by using pH metre (PL-700PV, Taipei, Taiwan). The pH meter was calibrated with buffer solutions at pH 4.0 and 7.0 prior to measurements.

Jelly-fat separation, emulsion stability and water-holding capacity in emulsion batters were determined based on the method of Aktaş and Gençcelep (2006).

Emulsion batters prepared for viscosity determination were placed in equal amounts into jars (73x58 mm) without leaving any gaps. Viscosity values have been observed in a rotating-type viscometer (Alpha Series, Fungilab, Spain) with R7 spindle and at 1 rpm by taking 1 reading at every 30 seconds.

**Colour measurement**

The color intensities of the sausage samples during storage period (1, 7, 14, 21, 28$^{th}$ days) were measured using a Minolta (CR-400, Minolta Co, Osaka, Japan) colorimeter with an 8 mm aperture, a D65 illuminant, a 2° closely matches CIE 1931 standard observer. The instrument was first standardized using a white plate (C: Y = 85.4, x = 0.3176, y = 0.3255). Sausage samples were kept at room temperature for 45 min after being removed from the refrigerator. At the end of the period, cellulose casings were cut and color measurements performed. During the color measurements, the background used was always the same, a white surface. The color values were expressed in terms of the L* value (lightness), a* value (redness), and b* value (yellowness) according to the system of the International Commission of Illumination (CIE).

**Thiobarbituric acid reactive substances analyses**

TBARS analysis in sausages during the storage period (1, 7, 14, 21, 28$^{th}$ days) were determined based on Lemon (1975).

**Texture analyses**

Textural characteristics of the samples were defined by using TA.XT-Plus Texture Analysis (Stable Micro Systems, Godalming, Surrey, UK) device. Texture Profile Analysis (TPA) and shear force (by using Warner Bratzler "V" blade) tests were performed by using Texture Analysis device. Textural measurements took place on the 1, 7, 14, 21, 28$^{th}$ days of the 28-day storage period. For the TPA tests performed on sausages throughout the storage period, discs with 3 cm diameters were obtained from 1.5 cm thick samples by using cylindrical probe. Samples were compressed with P/100 probe in two consecutive cycles at 30% of their original height with 5s interval between cycles. TPA tests took place under 1 mm/s pre-test speed, 5 mm/s test speed, and 5 mm/s post-test speed, trigger type-auto/force 20 g. Hardness, cohesiveness, springiness, adhesiveness, gumminess, chewiness and resilience values were recorded under the specified test conditions.

Shear force (maximum force of the curve) and the work of shearing (the area under the curve) values of the samples (3 cm high and 1.7 cm diameter) were estimated with a Warner-Bratzler blade attached to the same texture analyser. Shear test was performed under 1.5 mm/s pre-test speed, 1.5 mm/s test speed, 10 mm/s post-test speed, trigger type-auto/force 40 g, and 25 mm shear distance conditions.

For the penetration test, 3 cm thickness-samples were cut from sausage samples. Penetration test was performed under 1 mm/s pre-test speed, 2 mm/s test speed, 2 mm/s post-test speed, trigger type-auto/force 5 g and 15 mm penetration distance conditions. Penetration hardness (maximum force of the curve), work of penetration (the area under the curve) and the resistance during the withdrawal of the probe (the negative area of the curve) values were obtained by using P/2 penetration probe.

## Statistical analyses

In the study, two independent batches of each sausage formulations were prepared in a completely randomized design. All measurements were triplicated for independently prepared batches. Data were represented as mean±standard error. Analysis of variance (ANOVA) was performed to evaluate the statistical significance of the sausage formulation and storage period, using SPSS 22 for Windows (SPSS Inc., Chicago, IL, USA). The data were analyzed using the general linear model (GLM) procedure, in which treatment groups and storage period were assigned as fixed effects and replications as a random effect. Duncan's multiple range tests was used to see whether mean differences were statistically significant at 0.05 significance level.

## Results and Discussion

### Physicochemical analyses of sausage batters

pH, viscosity, jelly-fat separation, emulsion stability and water-holding capacity values obtained from sausage batters are shown in Table 1. There was no statistically significant difference observed between the pH values of emulsion batters prepared by using different levels of pumpkin seed oil and the pH values of the control group. This agrees with previous studies that showed no significant differences in the pH values of the emulsified meat products prepared with various ratios of vegetable oils (Choi et al., 2010, 2013, 2014, 2015; Pappa et al., 2000).

Viscosity values of emulsion batters prepared using pumpkin seed oil ($P<0.05$) were significantly higher than those prepared with beef fat (Table 1). The increase in the level of pumpkin seed oil led to an increase in viscosity values of meat batters. Various studies conducted with different vegetable oils also reported that vegetable oils increases the viscosity values of the emulsion batters (Choi et al., 2010, 2014). Viscosity of emulsion is an extent of the fluidity of the emulsion and, an important quality criterion for the emulsion technology to impart a particular texture to the product. Formation of an extremely viscous structure in meat emulsions is not desirable. Because high levels of viscosity in emulsion batters can lead to the formation of various air pockets, oil and gelatine vesicles during filling and cooking, consequently leading to textural defects.

Use of pumpkin seed oil in replace of beef fat also led to significant increases ($P<0.05$) in the amount of jelly-fat separation. The lowest jelly-fat separation was observed in control group while the highest total expressible fluid (TEF) (%) was observed in emulsion batters prepared by using 30% pumpkin seed oil (Table 1). TEF express the mixture of fat and water separated from the supernatant, and is used as a measure of the possibility of exudates during the storage period (Fernandez-Lopez et al., 2020). Therefore, lower TEF values mean higher emulsion stability. The emulsion stabilities of the batters prepared by using beef fat were significantly higher than those the batters prepared with pumpkin seed oil ($P<0.05$, Table 1). It is also possible to state that vegetable oils have a lower melting point than animal fats and may adversely affect emulsion stability. The finding observed in beef fat may be related to the fact that some of the fat in the emulsion remains as undisrupted fat cells, thereby causing muscle proteins to retain more water.

Water-holding capacities of the sausage samples are given in Table 1. Pumpkin seed oil up to the level of 15% did not make any statistically significant difference in water-holding capacity ($P>0.05$); however, raising the incorporation level to 30% caused a statistically significant difference ($P<0.05$). A study conducted by Alvarez et al. (2011) to determine the effects of canola-olive oil, rice bran and walnut on sausages reported that use of canola-olive oil increases the water-holding capacity. Water-holding capacity is defined as the ability of meat to bind the internal and added water during the application of any force (Gökalp et al., 2010). It is a characteristic properties listed among the important traits in emulsified meat products, as a large of the composition of emulsified meat products are comprised of water. Higher water-holding capacity indicates that the product has a better water-holding capacity. Holding water within the structure is highly important due to economic and technological reasons and water leaving the structure can cause losses in taste, flavour and juiciness. Moreover, it has been reported that water-holding capacity in emulsified meat products such as sausages has an important effect on consumer acceptance (Liu et al., 2019).

**Colour**

The L*, a* and b* values obtained from sausages produced with different levels of pumpkin seed oil are shown in Table 2. An increase in the level of pumpkin seed oil resulted in a statistically significant ($P<0.05$) increase in the L* and b* values. a* value, on the other hand, was found to decrease significantly ($P<0.05$). The level of pumpkin seed oil x storage period interaction was found to had a significant effect on L*, a* and b* values ($P<0.05$, Figure 2). During the storage period, the highest L * and b * values were determined in sausages produced using 30% pumpkin seed oil, while the lowest L * and b * values were determined in sausages belonging to the control group (Figure 2). Figure 2 also indicated that, the lowest a* values of sausages were observed in sausages produced with 30% pumpkin seed oil, while the highest a* value were observed in control group. Colour formation and colour stability are among the important quality characteristics in meat products. Due to consumer attitudes, colour is being deemed as an important quality indicator in meat and meat products. As product-specific pinkish red colour are required in emulsified meat products, determination of colour values is among the important quality characteristics analyses. The increase in the L* value with the increase in pumpkin seed oil level was attributed to the fact that vegetable oil is better and smaller dispersed than animal fat. As vegetable oil disperses and distributes much better than animal fat, light falling onto the surface is much more broken, thereby increasing the effect of lightness. Also, smaller oil globules possess greater surface area, which could cause more light reflection. Similar findings were determined by Kang et al. (2016), Tan et al. (2001), and Youssef and Barbut (2009, 2010, 2011), who investigated the effects of soy oil, canola oil and palm oil on the color of frankfurters. The increase in the pumpkin seed oil level led to a reduction in the a* values of the sausages. The decreases in a* value was at most 6.95%. The decreased redness caused by pumpkin seed oil level was probably due to the distribution of oil phase into the matrix of myofibrillar proteins that causes an increase in the surface of the fat particles. This result was in good accordance with findings of Ambrosiadis et al. (1996) who reported a lower in the redness values of cooked meat emulsion containing vegetable oils. The increase that occurs in b* value may be associated with the yellow colour of the pumpkin seed oil (Aktas et al., 2018). A similar result was also observed in meat emulsions containing canola oil (Youssef and Barbut, 2009).

**TBARS**

TBARS values were significantly increased with increasing levels of pumpkin seed oil ($P<0.05$). The level of pumpkin seed oil x storage period interaction was found to have a significant effect on TBARS values ($P<0.05$, Figure 1). As Figure 1 indicates, for a 28-day storage period at 4℃, the lowest TBARS values of the sausages were determined in control group while the highest values were determined in sausages produced with 30% pumpkin seed oil. Oxidative stability is one of the important quality criterion in processed meat products. During the 28-days storage period, TBARS values of samples containing animal fat were significantly lower than those of samples containing pumpkin seed oil. With the increase in pumpkin seed oil level, TBARS values were shifted toward higher values. This increase in TBARS values could be attributed to the fact that pumpkin seed oil contains a significant amount of unsaturated fatty acid. Pumpkin seed oil contains around 81% unsaturated fatty acid

(Aktas et al., 2018). Although an increase in the TBARS values were observed, all of the obtained results were below the acceptable upper limit in cooked cured meat products. Ockerman (1985) reported that TBARS value of 1 mg malonaldehyde/kg sample is considered to be rancid that product would be unacceptable. These findings agree with earlier reports use of vegetable oils in emulsified meat products have been found to lead to a reduction in oxidative stability (Choi et al., 2010; Kılıç and Özer, 2019; Pappa et al., 2000).

**Texture**

Pumpkin seed oil caused a reduction in hardness, adhesiveness, chewiness values but an increase in cohesiveness and resilience values (Table 2). Except for sausages produced with 30% pumpkin seed oil, no significant changes were observed in springiness values ($P$>0.05). The level of pumpkin seed oil x storage period interaction was found to have significant effects on all measured textural properties, except for cohesiveness values ($P$<0.05, Figure 3). Textural structure of emulsified meat products is affected by emulsion parameters, and characteristics of meat-fat and additives used in the formulation. The texture of a good-quality sausage should not be loose, too hard or too soft. There should be no fat and jelly pockets on the section surface and it should be easily sliceable (Gökalp et al., 2010). Lower hardness values obtained from sausages produced with pumpkin seed oil (compared to the control group, the decreases in hardness was at most 5.06%) could be attributed to the fact that pumpkin seed oil containing monounsaturated fatty acid have lower hardness values at high temperatures. Also, the texture of animal fat is hard and solid. Wang et al. (2018) reported that animal fat is a tissue and the adipocyte structure remains intact after production and, causing an increase in the hardness of the structure. Luruena-Martinez et al. (2004) conducted a study on frankfurters prepared with olive oil and carob/xanthan gum addition instead of animal fat and concluded that samples with olive oil had lower hardness values. The result of the research conducted by using sunflower oil indicated that hardness value was detected to be decreased with the use of vegetable oil

(Choi et al., 2015). In this study, the tendency observed in cohesiveness, adhesiveness and springiness values was also observed in a study conducted by Jimenez-Colmenero et al. (2010) using olive oil. The lower cohesiveness value determined in the control group might be perceived as an indication that the sausages in this group have a more easily broken structure.

Shear results are given as two parameters: maximum shear force and work of shear. Maximum shear force is defined as the force to cut the sample while work of shear is defined as the work needed to move the blade through the sample. As Table 3 indicates, the lowest maximum shear force and work of shearing values were determined in sausages produced with 30% pumpkin seed oil, while the highest maximum shear force and work of shearing values were found in control group sausages. Similar maximum shear force and work of shearing values were obtained from sausages produced with 15% and 30% pumpkin seed oil ($P$>0.05), while control group and 5% pumpkin seed oil containing sausages given statistically significant differences ($P$<0.05). Warner-Bratzler shear force is obtained by the maximum force required to shear the samples while TPA hardness is defined as the maximum force required to compress the sample (Bourne, 2002). Warner Bratzler shear force results generally provide information about the tenderness of meats. The results of Warner Bratzler shear indicated that the sausages containing pumpkin seed oil were tender than the control group.

The results of penetration analyses are given as hardness of penetration, work of penetration and resistance during the withdrawal of the probe (Table 3). The lowest penetration hardness values were observed in control group sausages, while the highest penetration hardness value was observed in sausages produced with 15% pumpkin seed oil. Significant differences were obtained between the penetration hardness values of sausages produced by using different levels of pumpkin seed oil ($P$<0,05). The trends determined in penetration hardness values were also determined for the value of work of penetration. The lowest resistance during the withdrawal of the probe value were observed in sausages produced with

15% pumpkin seed oil, and the highest resistance during the withdrawal of the probe value were observed in sausages produced with 30% pumpkin seed oil. Similar resistance during the withdrawal of the probe values were obtained in control group and in sausages produced with 5% pumpkin seed oil, but there were statistically significant differences between the resistance during the withdrawal of the probe values of sausages produced with other levels of pumpkin seed oil ($P$<0,05). This might be attributed to the interaction between oil and proteins. Shi et al. (2014) noted that oil could act as filler in the matrix of gels in meat products by placing the cavities of the protein gel matrix and restricting the matrix against movement, thereby having ability to alter gel-forming capacity in meat products.

## Conclusions

It has been determined that the use of pumpkin seed oil in certain levels as a replacement of animal fat had significant effects on the bologna-type sausage emulsion batters and on the characteristics of the final product. Sausages prepared with pumpkin seed oil were somewhat softer than sausages prepared with beef fat. Colours of the sausages with pumpkin seed oil were lighter and less red than the produced with beef fat. Although TBARS values of the sausages with pumpkin seed oil were higher than the sausages with the beef fat during the storage period, this increase remained below the acceptable upper limit. The obtained results lead to the conclusion that the pre-emulsified pumpkin seed oil would be yield more positive results on the physicochemical and textural characteristics and also that more researches are needed on this issue.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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**Author Contributions**

Conceptualization: Aktaş N. Formal analysis: Uzlaşır T, Gerçekaslan, KE. Methodology: Aktaş N, Uzlaşır T, Gerçekaslan KE. Validation: Uzlaşır, T. Investigation: Aktaş N, Uzlaşır T, Gerçekaslan KE. Writing - original draft: Aktaş N, Uzlaşır T. Writing - review & editing: Aktaş N.

**Ethics approval**

This article does not require IRB/IACUC approval because there are no human and animal participants.



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## FIGURE CAPTIONS

**Figure 1.** Pumpkin seed oil x storage period interaction effects on TBARS values

**Figure 2.** Pumpkin seed oil x storage period interaction effects on L*, a* and b* values

**Figure 3.** Pumpkin seed oil x storage period interaction effects on TPA values

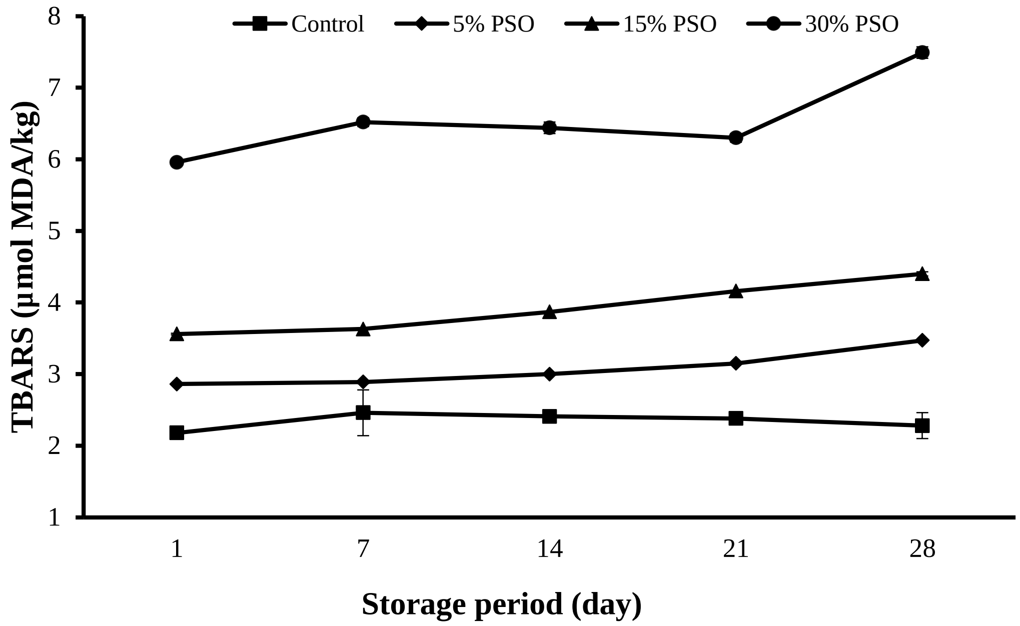


**Figure 1.** Pumpkin seed oil x storage period interaction effects on TBARS values. TBARS, thiobarbituric acid reactive substances; PSO, pumpkin seed oil.

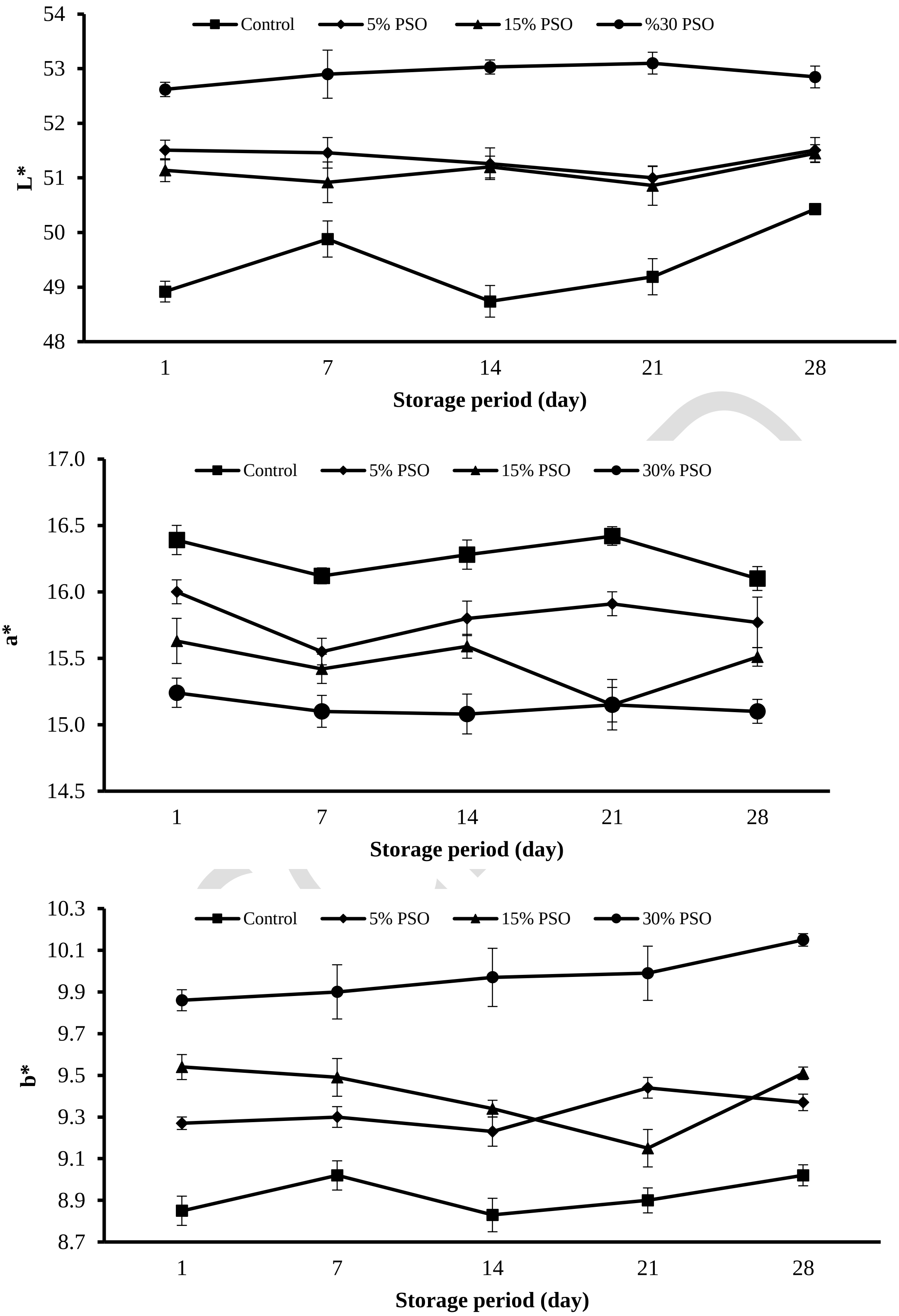


**Figure 2.** Pumpkin seed oil x storage period interaction effects on L*, a* and b* values. PSO, pumpkin seed oil.

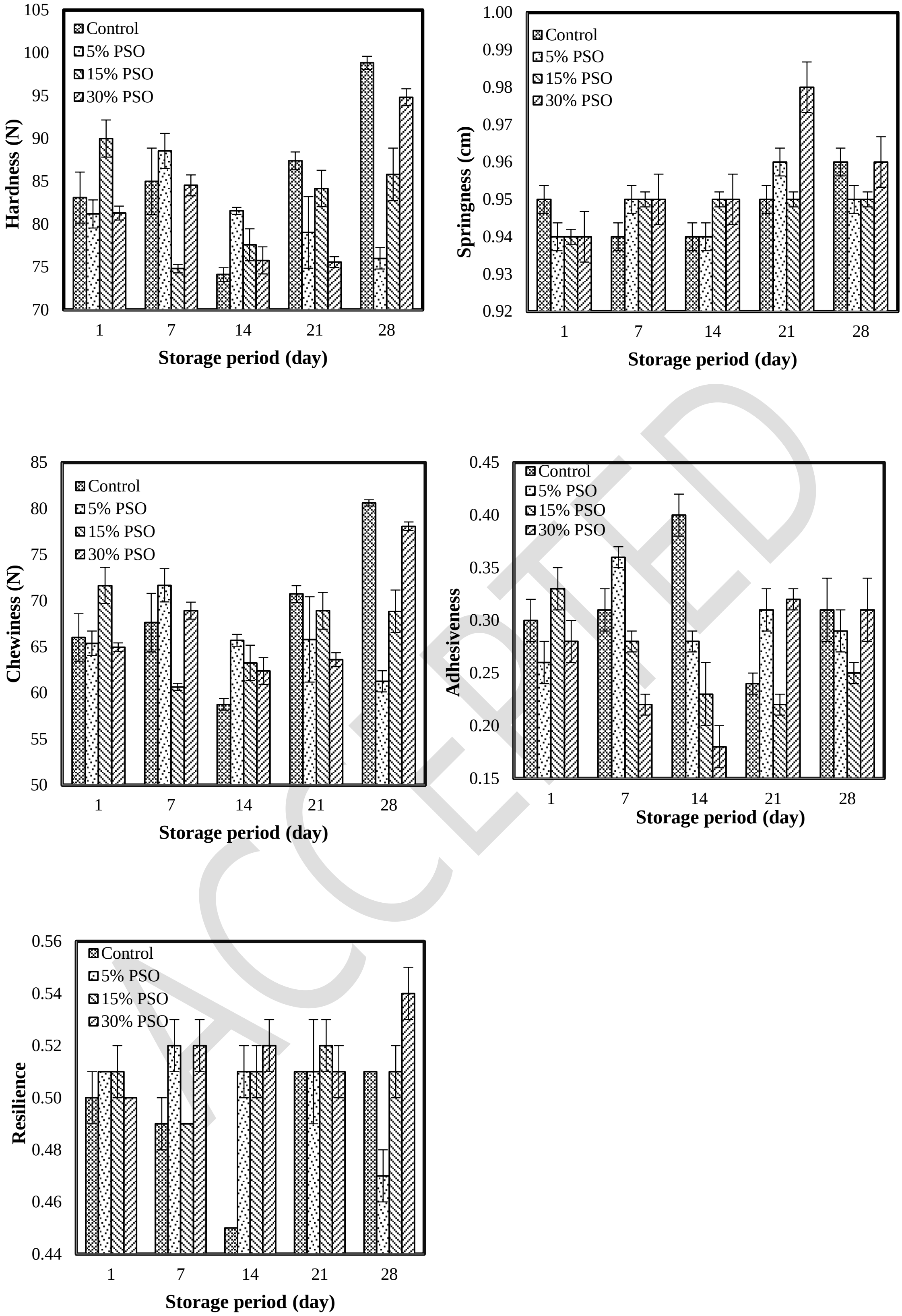


**Figure 3.** Pumpkin seed oil x storage period interaction effects on TPA values. PSO, pumpkin seed oil.

TABLE CAPTIONS

Table 1. **Physicochemical properties of bologna-type sausage produced with different levels of pumpkin seed oil.**

Table 2. **The values of TBARS, color and textural properties of bologna-type sausage produced with different levels of pumpkin seed oil.**

Table 3. **The results of WBS and penetration of bologna-type sausage produced with different levels of pumpkin seed oil.**

**Table 1.** Physicochemical properties of bologna-type sausage produced with different levels of pumpkin seed oil.

| | Batter pH | WHC | Emulsion stability | | | Jelly-fat separation (%) | Viscosity (cp) |
|---|---|---|---|---|---|---|---|
| | | | TEF (%) | Fat released (%) | Water released (%) | | |
| Control | $6.06\pm0.01$ | $0.10\pm0.01^{a}$ | $0.65\pm0.03^{a}$ | $0.06\pm0.01^{a}$ | $0.59\pm0.03^{a}$ | $0.99\pm0.01^{a}$ | $2.92\times10^{6}\pm0.02^{a}$ |
| PSO (5%) | $6.07\pm0.01$ | $0.10\pm0.01^{a}$ | $0.70\pm0.03^{b}$ | $0.07\pm0.01^{b}$ | $0.64\pm0.03^{b}$ | $1.75\pm0.01^{b}$ | $3.26\times10^{6}\pm0.02^{b}$ |
| PSO (15%) | $6.08\pm0.01$ | $0.10\pm0.01^{a}$ | $0.79\pm0.01^{c}$ | $0.09\pm0.01^{c}$ | $0.70\pm0.01^{c}$ | $1.83\pm0.01^{c}$ | $3.76\times10^{6}\pm0.01^{c}$ |
| PSO (30%) | $6.08\pm0.01$ | $0.12\pm0.01^{b}$ | $0.80\pm0.01^{c}$ | $0.09\pm0.01^{c}$ | $0.71\pm0.01^{d}$ | $1.92\pm0.01^{d}$ | $3.78\times10^{6}\pm0.00^{c}$ |

[a-d] Means ± standard error in the same column, values in a column with the different superscript letters are significantly different (*P*<0.05). WHC, water-holding capacity; TEF, total expressible fluid; cp, centipoise; PSO, pumpkin seed oil.

**Table 2.** The values of TBARS, color and textural properties of bologna-type sausage produced with different levels of pumpkin seed oil.

| | | Control | PSO (5%) | PSO (15%) | PSO (30%) |
|---|---|---|---|---|---|
| TBARS (µmol MDA/ kg) | | 2.34±0.06$^{a}$ | 3.08±0.07$^{b}$ | 3.93±0.10$^{c}$ | 6.54±0.17$^{d}$ |
| L* | | 49.43±0.21$^{a}$ | 51.12±0.08$^{b}$ | 51.34±0.09$^{b}$ | 52.90±0.06$^{c}$ |
| a* | | 16.26±0.06$^{d}$ | 15.80±0.06$^{c}$ | 15.46±0.06$^{b}$ | 15.13±0.02$^{a}$ |
| b* | | 8.93±0.05$^{a}$ | 9.32±0.03$^{b}$ | 9.40±0.05$^{c}$ | 9.97±0.03$^{d}$ |
| TPA | | | | | |
| | Hardness (N) | 85.63±1.76$^{b}$ | 81.30±1.22$^{a}$ | 82.50±1.35$^{a}$ | 82.40±1.39$^{a}$ |
| | Cohesiveness | 0.84±0.01$^{a}$ | 0.86±0.01b$^{c}$ | 0.85±0.01$^{b}$ | 0.86±0.01$^{c}$ |
| | Springiness (cm) | 0.95±0.01$^{a}$ | 0.95±0.01$^{a}$ | 0.95±0.01$^{a}$ | 0.96±0.01$^{b}$ |
| | Adhesiveness | 0.31±0.01$^{b}$ | 0.30±0.01$^{b}$ | 0.26±0.01$^{a}$ | 0.26±0.01$^{a}$ |
| | Chewiness (N) | 68.75±1.54$^{b}$ | 66.00±1.16$^{a}$ | 66.66±1.07$^{ab}$ | 67.59±1.12$^{ab}$ |
| | Resilience | 0.49±0.01$^{a}$ | 0.51±0.01$^{b}$ | 0.51±0.01$^{b}$ | 0.52±0.01$^{c}$ |

$^{a-d}$ Means ± standard error in the same column, values in a row with the different superscript letters are significantly different (*P*<0.05). TBARS, Thiobarbituric acid reactive substances; PSO, pumpkin seed oil; TPA, texture profile analysis.

**Table 3.** The results of WBS and penetration of bologna-type sausage produced with different levels of pumpkin seed oil.

| | Results of WBS | | Results of Penetration | | |
|---|---|---|---|---|---|
| | Maximum shear force (N) | Work of shear (N s) | Penetration hardness (N) | Work of penetration (N s) | The resistance during the withdrawal of the probe (N s) |
| Control | 6.36±0.20[c] | 62.92±2.03[c] | 2.30±0.04[a] | 12.50±0.25[a] | 2.90±0.06[ab] |
| PSO (5%) | 5.66±0.13[b] | 55.15±1.24[b] | 2.40±0.05[b] | 12.64±0.20[a] | 2.94±0.11[ab] |
| PSO (15%) | 5.30±0.13[a] | 48.90±0.89[a] | 2.49±0.02[c] | 13.17±0.12[b] | 2.80±0.09[a] |
| PSO (30%) | 5.08±0.06[a] | 47.40±0.52[a] | 2.43±0.03[bc] | 13.01±0.15[b] | 3.00±0.12[b] |

[a-c] Means ± standard error in the same column, values in a column with the different superscript letters are significantly different (*P*<0.05). WBS, warner-bratzler shear; PSO, pumpkin seed oil.