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## Replacement of Beef Fat in Meatball with Oleogels (Black Cumin Seed Oil/Sunflower Oil)

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**ABSTRACT:** Meat products contain fatty acids, especially saturated fatty acids, which cause adverse health effects. The effect of fats on meat products is not only concerned with health, but also about the product's sensorial or textural properties. The research aimed to develop a new, healthier meatball formula in which the fat is substituted by oleogel gelled by carnauba wax and made with sunflower oil and black seed oil mixture. The effect of substituting animal fat with oleogel on color values, cooking parameters, lipid oxidation, and the textural properties of meatballs were determined. The effect of oleogel type on the TBA values of 50 and 75% substituted samples was statistically significant ( $p < 0.05$ ), and TBA values of the samples with oleogel substituted were higher than others at the end of the storage. The effect of substitution rates on the texture profile of meatball samples was found to be statistically significant ( $p < 0.05$ ). The oleogel (25%) added group scored significantly ( $p < 0.05$ ) higher than the control group in appearance, flavor, texture, juiciness, oiliness, and overall acceptability, and was not found difference between the treatment groups.

**Keywords:** Black seed oil, Meatball, Oleogel, Sunflower oil, TBA

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## INTRODUCTION

Meat and meat products contain saturated fatty acids, which may contribute to cardiovascular disease. In recent years, consumer demand for healthy products has been increased. So studies on the substitution of fats with healthier lipids in meat products (meatballs, pates, sausages, etc.) have increased rapidly (Kouzounis et al., 2017). The substitution of animal fats increases the concentration of unsaturated fatty acids while decreasing the concentration of saturated fatty acids in meat products. This is beneficial in terms of disease prevention (Dominguez et al., 2017). Animal fat has a technological, physicochemical, sensorial, and textural impact on the product in addition to its health benefits (Dominguez et al., 2017; Fagundes De Oliveira et al., 2017).

Black seed (*Nigella sativa*) and black seed oil have traditionally been used to treat various diseases. (Burits and Bucar, 2000). Black seed contains essential oils (Piras et al., 2013) as well as bioactive compounds such as phenolics, which have antioxidant properties (Burits and Bucar, 2000). Several studies have found that black seed has antioxidative properties. The majority of the studies substituted vegetable oils for fats, resulting in higher lipid oxidation levels due to the unsaturated fatty acid composition of the vegetable oils (Delgado-Pando et al., 2011). Thus, the combination of sunflower oil and black seed oil improves the antioxidant capacity of the oil, which would be used to produce oleogel.

New techniques for gelation of edible oils have been developed in order to form solid-like properties in the oil without altering its chemical structure. Oleogels have applications in the food, cosmetics, and pharmaceutical industries. (Co and Marangoni, 2012). Carnauba wax is extracted from the leaf and petiole of the *Copernicia cerifera* palm tree. During the production of carnauba wax, the leaves of the trees are cut, dried, and beaten until the wax is in powder form (Koonce and Brown, 1941).

The aim of the study was to determine the effect of substituting animal fat with an oleogel (sunflower/black cumin seed oil mixture) structured by carnauba wax on color values, cooking parameters, lipid oxidation, textural and sensorial properties.

## MATERIALS AND METHODS

Sunflower oil and black cumin seed oil were purchased from a local market in Afyonkarahisar. Car-

nauba wax obtained was from a trader in İstanbul. The Brown Swiss bulls were slaughtered at a commercial slaughterhouse. Samples of the thin flank of the carcasses were obtained 24 h post mortem from a local butcher. The flank was dissected into muscle and fat. Muscle and fat were comminuted separately at 0°C through a 6 and 3 mm plate, respectively, (Mateka EPA 22T, İstanbul, Turkey) and transported in cold conditions to the Food Science and Technology laboratory of Afyonkarahisar University of Health Sciences Department of Nutrition and Dietetics in 10 min. They were held in a refrigerator until the preparation of the meatballs. Other chemicals, butylated-hydroxyanisole, hexane, isopropanol, tetra methoxy propane, and thiobarbituric acid was purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Oleogel preparation

Oleogel was prepared according to the method of Lim et al., (2017) and Ögütçü and Yilmaz (2015) with slight differences. Three different oleogel were prepared with sunflower oil, the second one used 90% sunflower oil (OG) and 10% black cumin seed oil mixture (OG10), and the last one was 80% sunflower oil and 20% black cumin seed oil mixture (OG20). Oleogels were prepared by the addition of carnauba wax to the sunflower oil and sunflower black cumin seed oil mixture at a ratio of 7.5% (w/v). This concentration was selected because of the appearance and texture of the oleogels (only sensorial evaluation was made by the researcher, below this percentage, the oleogel mixture was more fluid, and above this concentration the color of the oleogel was darker in color). After adding the oil and wax, the mixture heated to 82 °C and held at this temperature for 5 minutes after having a clear appearance. The oleogel mixture was allowed to cool to room temperature and stored at 4 °C at refrigerator overnight.

### Meatball preparation

Beef fat added a ratio of 25% of meat, and salt was added to the mixture at 2 %. The minced meat and fat were kneaded by hand for 5 min. The meatball dough was cut into 25 gram pieces and rounded, then put onto plates covered with cling film and stored in the refrigerator (4±2 °C) for 6 days. After the preparation of the control group, other groups were prepared in the same manner, except for fat addition. The fat was substituted by oleogels (OG, OG10, OG20) at a ratio of 25, 50 and 75% of fat content in each group. Meatball samples were prepared in two replications.

### Instrumental color determination

A colorimeter(X-Rite (Ci6X)) was used to determine the CIE color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) of the meatball samples. Before determining the values, the colorimeter was calibrated with white and black plaque after calibration readings were taken from three different points of the samples.

### Lipid oxidation

#### Thiobarbituric acid (TBA) Analysis

TBA values were determined according to the method of (Pikul et al., 1989). 10g of meatball was mixed with 35 ml of 4 % perchloric acid and 1 ml BHA added to the mixture. The mixture was homogenized at 13800 rpm for 1 minute. After homogenization, the slurry was filtered and washed with 5 ml distilled water. The filtrate was filled to 50 ml with perchloric acid. The filtrate (5ml) and 5 ml of TBA (0.02 M) were mixed and heated at 80 °C for 1 hour. After heating, the mixture was cooled to room temperature for 10 min, and the absorbance of samples was read at 532 nm wavelength. The results were given from the calibration curve, which was prepared by the tetramethoxypropane (TMP). Results were given per mg malondialdehyde (MDA)/ kg meatball sample.

#### Conjugated dienes analysis

A 0.5 g meatball sample was suspended and homogenized with 5 ml distilled water. 0.5 ml aliquot was mixed with 5 ml of extraction solution (3:1, hexane: isopropanol) for 1 minute. After extraction, the solution was centrifuged at 2000g for 5 min. The supernatant of the samples was measured at 223 nm wavelength, and the result was given as micromole per mg meat sample (Juntachote et al., 2007).

### Cooking parameters

#### Cooking yield (CY)

The cooking yield of the meatballs was determined by the ratio of the weight of cooked meatballs to the weight of raw meatballs. The results were expressed by (%) of the initial weight. (Murphy et al., 1975).

#### Moisture retention (MR)

Moisture retention was determined as the amount of moisture in the sample of 100g cooked meatballs. The equation of (El-Magoli et al., 1996) was used to determine this;

$$MR(\%): (CY(\%) * \text{Moisture of Cooked meatball}) / 100$$

### Fat retention (FR)

Fat retention was determined by the equation of (Murphy et al., 1975).

$$FR(\%): (\text{Fat content of cooked meatball}) * (\text{Cooked meatball weight}) / (\text{Fat content of uncooked meatball}) * (\text{Uncooked meatball weight}) * 100$$

### Diameter reduction (DR)

The diameter of the meatballs was calculated using a manual caliper before cooking and after cooking. Diameter reduction was calculated according to the equation of

$$DR: (\text{Uncooked meatball diameter} - \text{Cooked meatball diameter}) / \text{Uncooked meatball diameter} * 100$$

(Kilincceker and Yilmaz, 2019)

### Texture profile analysis

Texture profile analysis was conducted with an analyzer (TA-HD Plus, Stable Micro Systems, UK) with a 25 kg load cell (Bourne, 1978). The meatballs were put into a cylindrical container. The height of the samples was 2 cm, and the analysis was done at room temperature. The probe was 10 mm above the meatball, and the test speed was 5 mm/sec. The meatball was compressed twice at 50% by the P/36R probe. Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience of the samples were determined by the software program of the instrument.

### Sensorial analysis

Sensorial characteristics of the samples were assessed by 12-member semi-trained panel who are students at the Department of Nutrition and Dietetics in Afyonkarahisar Health Sciences University. The panelists have a mean age of 20 years who regularly consumed meatballs. Meatballs were grilled in an electric oven at 180 °C for 15 minutes. Between the samples, bread and water were provided to the panelists to cleanse the palate. 9-point scale was used for the evaluation of the samples which "9" indicates the highest acceptability and "1" is the lowest. Appearance, odor, flavor, texture, juiciness and overall acceptance were evaluated (Gokalp et al., 1999).

### Statistical analysis

A software program was used to perform analysis of variance (ANOVA) on the obtained data (SPSS 20). The Shapiro-Wilk test was used to determine the normality of the data distribution (Shapiro and Wilk, 1965). The data is given in the form of a mean value

and a standard deviation. If the distribution was normal, the difference in means was determined using the Tukey Test, and if the distribution was not normal, the difference in means was determined using the Dunnett's T3 test. All analyses were performed in triplicate; color, TBA, and conjugated dienes analysis were performed on days 0, 2, 4, and 6, and cooking parameters and texture profile analysis of cooked meatballs were performed on day 0.

## RESULTS

### Instrumental determination of color

The storage time had no statistically significant impact on the  $L^*$  and  $a^*$  values of all samples ( $p > 0.05$ ) (Table 1). Except for the 25% and 75% substituted OG20 and control groups, the effect of the storage time was statistically significant ( $p < 0.05$ ) when the  $b^*$  value results were examined. The effect of substitution rates on the  $L^*$  values of OG meatball samples on days 0, 4, and 6 and OG10 samples on days 2, 4, and 6 was found to be statistically significant ( $p < 0.05$ ), while the OG20 group samples showed no significant difference.

The effect of storage time, rate of substitution, and type of oleogel on the  $a^*$  values of meatball samples was determined to be statistically insignificant. Red color is associated with meat/meat product quality in customer preferences. Since no dye or spice was applied to the meatball formula in this study, there has been no masking effect of the different lipid sources in meatball samples in the manner of an  $a^*$  value.

Different findings were published by Kouzounis et al., (2017) in a similar study, and they concluded that the fat sources can affect the product's instrumental color parameters. By replacing the fats in frankfurters with organogels, Barbut et al. (2016) found that the lightness of the frankfurters was reduced. They also said that replacing fat with organogels had hardly effect on redness and yellowness values. According to Wolfer et al., (2018), the processing of frankfurter style sausages using oleogels instead of pork meat has a lower  $L^*$  value than the control group, and the cross-section color of the sausages in the control group was redder.

### Thiobarbituric acid (TBA) analysis

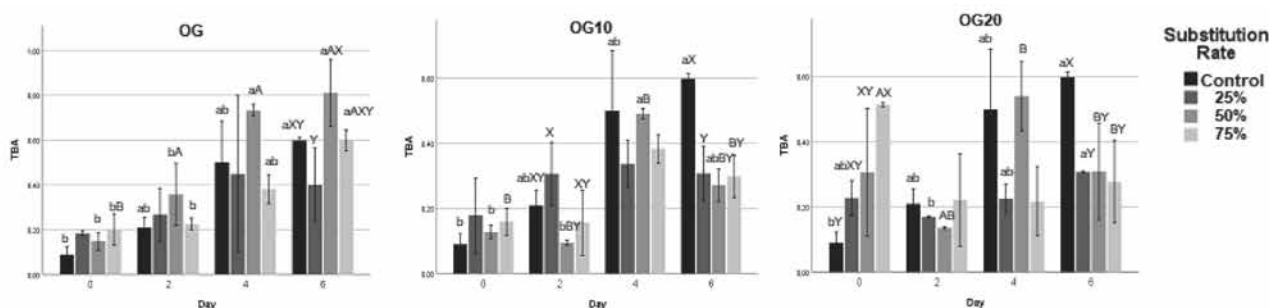
The oxidation of lipids in meat and meat products is an essential quality characteristic. The deterioration of lipids not only result in an unpleasant taste, but it also affects the texture, color, and shelf life of the product (Poyato et al., 2015).

There was an increase in TBA values, which is expected depending on time (Figure 1). However, in some groups, the increase was not linear, and there were occasional increases and decreases. This may be due to the instability of the oxidation product compounds.

On the first day, the differences in TBA values between the control group and the samples prepared using different substitution rates and oleogel added samples were statistically significant, except the OG10 oleogel samples ( $p < 0.05$ ). The TBA values were changed to 0.15-0.20, 0.13-0.18, and 0.23-0.51 for the OG, OG10, and OG20 groups, respectively, while the control group was 0.09 mg MDA/kg meat sample. When the effect of substitution rates on TBA values of the same oleogel added samples was evaluated at the end of storage, statistically significant differences ( $p < 0.05$ ) were found. TBA values of meatballs prepared with different substitution rates and with OG10 (0.27-0.31 mg MDA/kg meat) and OG20 (0.28-0.31 mg MDA/kg meat) type oleogels were found to be statistically lower on the last day of storage than the control (0.60 mg MDA/kg meat) group, in which there was no statistically significant difference between the substitution rates. The effect of oleogel type on the TBA values of 50 and 75 % substituted samples was statistically significant, and the TBA values of OG substituted samples were higher at the end of storage than the others. This decrease may be related to the antioxidant compounds found in black cumin seed oil.

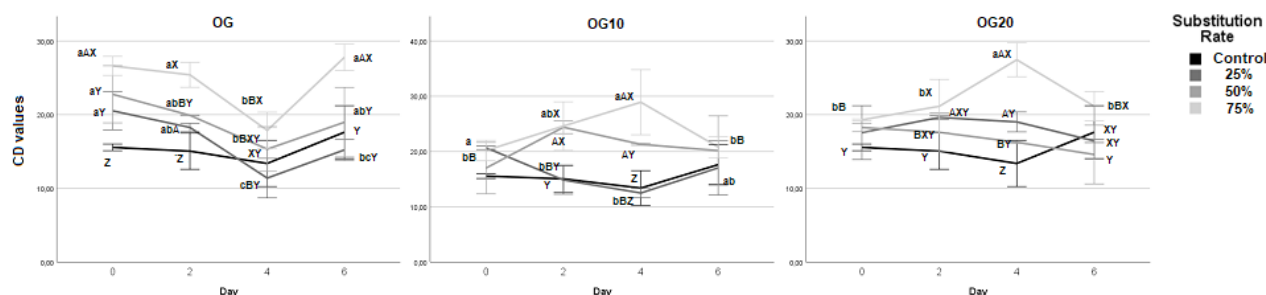
Malonaldehyde loss may be caused by interactions with other molecules (amino acids, proteins) or intermolecular reactions (polymerization) (Jamora and Rhee, 2002). Yilmaz and Ögütçü (2015) concluded that the rate of oxidation in cookies could be influenced by the solid fat content, storage conditions, processing conditions, and ingredients with antioxidant activity. Delgado-Pando et al. (2011) concluded that formulation and storage time had an impact on the TBA values of healthy frankfurters with fat replacers. Gómez-Estaca et al., (2019) showed that ethylcellulose oleogel substituted products have the highest initial TBA values, which increases during storage. They suggested that the degree of lipid oxidation was related to the oleogels' process parameters. According to da Silva et al. (2019), the TBA values of modified sausages were significantly lower as compared to the control groups. Poyato et al. (2015) concluded that replacing fat in burger patties with gelled carrageenan-containing emulsion results in substantially lower TBA values as compared to the control.





**Figure 1.** TBA values of meatball samples whose fats were substituted with oleogels.

<sup>a-c</sup>: Different superscript lowercase letters show differences between the storage days with respect to the same substitution rates and same oleogel types ( $p < 0.05$ ). <sup>A-D</sup>: Different superscript uppercase letters show differences between the oleogel type with respect to the same storage day and substitution rate ( $p < 0.05$ ). <sup>X-Z</sup>: Different superscript uppercase letters show differences between the substitution rates with respect to the same storage day and oleogel type ( $p < 0.05$ ). OG: Oleogel prepared by sunflower oil, OG 10: Oleogel prepared by mixing black cumin seed oil:sunflower oil (10:90), OG 20: Oleogel prepared by mixing black cumin seed oil:sunflower oil (20:80).



**Figure 2.** CD values of meatball samples whose fats were substituted with oleogels.

<sup>a-c</sup>: Different superscript lowercase letters show differences between the storage days with respect to the same substitution rates and same oleogel types ( $p < 0.05$ ). <sup>A-D</sup>: Different superscript uppercase letters show differences between the oleogel type with respect to the same storage day and substitution rate ( $p < 0.05$ ). <sup>X-Z</sup>: Different superscript uppercase letters show differences between the substitution rates with respect to the same storage day and oleogel type ( $p < 0.05$ ). OG: Oleogel prepared by sunflower oil, OG 10: Oleogel prepared by mixing black cumin seed oil:sunflower oil (10:90), OG 20: Oleogel prepared by mixing black cumin seed oil:sunflower oil (20:80).

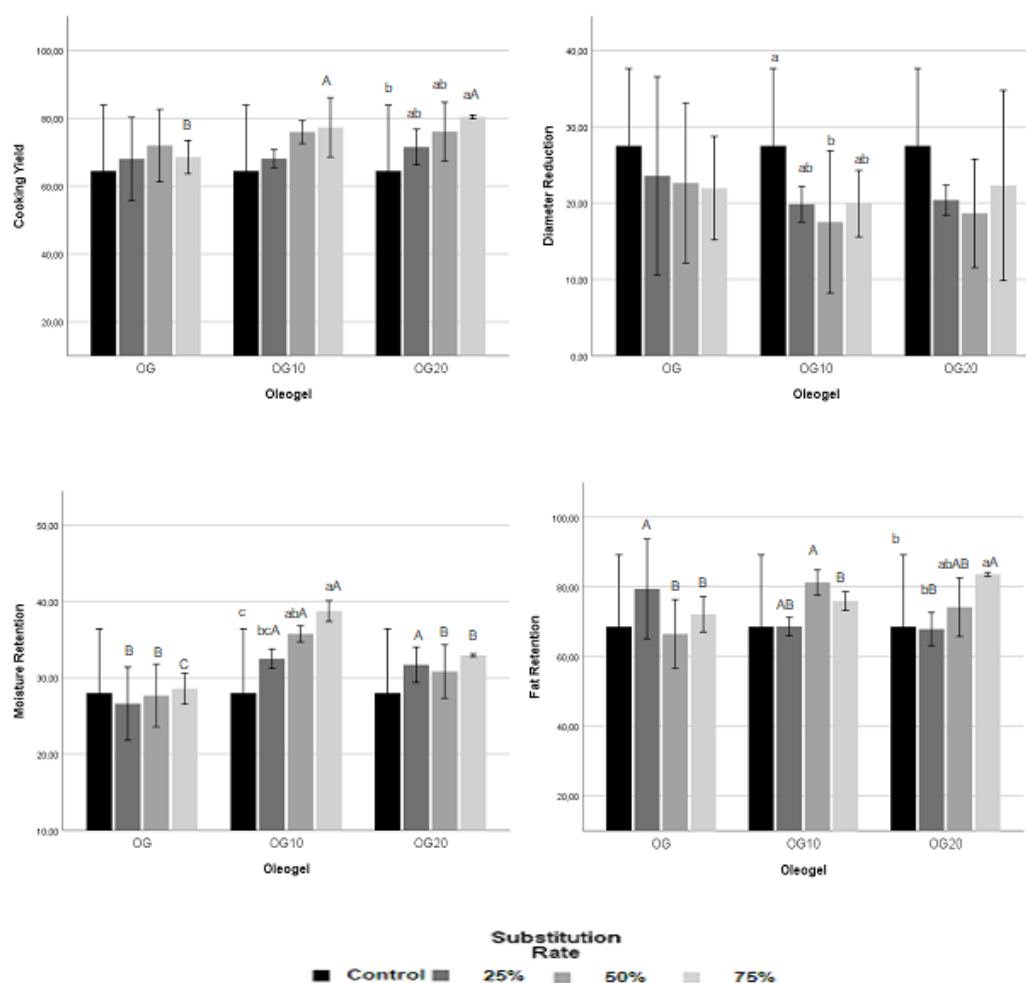
### Conjugated dienes analysis

Figure 2 shows the findings of the conjugated dienes analysis. According to the results, the CD values of meatballs were statistically ( $p < 0.05$ ) different in meatball samples made with different substitution rates in the same oleogel type groups at the beginning of storage. At the beginning of storage, the OG and OG20 groups had the highest conjugated diene values with a substitution ratio of 75% and the OG10 group had a substitution ratio of 25%.

Conjugated diene measurement shows the oxidative stability of the fats or oils in the product which formed by the triplet oxygen or singlet oxygen (Akhtar et al., 2018). Juntachote et al. (2007) concluded that storage period reduced the conjugated diene value, but our result shows the opposite. Except for OG10 and OG20 added samples with a substitution

rate of 75%, conjugated diene values decreased until the fourth day and increased on the sixth day in this study. Conjugated diene values in these two groups rise until the fourth day of storage, then rise again on the sixth day. This situation has arisen as a result of an increase in the amount of unsaturated fatty acids. According to Juntachote et al. (2007), the decomposition of the conjugated dienehydroperoxides increases the TBA values of the samples. The samples with the lowest TBA values had the highest conjugated diene values in the study. This can be explained by the fact that the compounds present in black cumin seed oil inhibit the formation of TBA substances.





**Figure 3.** Cooking parameters of meatball samples whose fats were substituted with oleogels

a-c: Different superscript lowercase letters show differences between the substitution rates ( $p < 0.05$ ). A-D: Different superscript uppercase letters show differences between the oleogel types ( $p < 0.05$ ). OG: Oleogel prepared by sunflower oil, OG 10: Oleogel prepared by mixing black cumin seed oil:sunflower oil (10:90), OG 20: Oleogel prepared by mixing black cumin seed oil:sunflower oil (20:80).

### Cooking parameters

Figure 3 shows the cooking parameters of meatball samples prepared with fat substitution by different oleogels at different substitution rates. Except for 75%, there was no significant difference observed between the oleogel types in terms of different substitution rates. Furthermore, no significant difference was observed between the control group and the fats substituted at a 25% ratio groups. However, meatball fats substituted at 50% and 75% had significantly ( $p < 0.05$ ) higher cooking yields than the control and 25% substituted classes.

Figure 3 shows that the diameter reduction of the meatball samples ranged from 17.51 to 23.55%. The effect of substitution rate was significantly ( $p < 0.05$ ) lower than the diameters of the meatballs only in the OG10 added samples.

By increasing the substitution rate in samples where fats were substituted with OG10, the moisture retention of the meatball samples was increased. In terms of moisture retention, there was a significant ( $p < 0.05$ ) difference between the oleogel types with different substitution rates of samples. The oleogel type impacted fat retention in all substitution rates, and substitution rates impacted fat retention values in the OG10 and OG20 samples.

Barbut et al. (2016) reported that oleogels in meat products reduced cooking losses, and da Silva et al. (2019) reported that high pork back fat reduced cooking losses in sausages. This may be due to the presence of organogel in the matrix, which causes large fat globules in the matrix to help minimize liquid losses. Moghtadaei et al. (2018) showed that increasing the replacement amount of oleogels in burgers made



with different replacement ratios reduced cooking loss. According to Fagundes De Oliveira et al. (2017), reformulated burgers with pork skin and canola oil gels have lower diameter reduction and cooking loss values.

### Texture profile analysis

The effect of substitution rates on the hardness, springiness, cohesiveness, gumminess, chewiness and resilience values of the meatball samples was found to be statistically significant ( $p < 0.05$ ) (Table 2). As compared to the control group, the hardness, springiness, cohesiveness, gumminess, chewiness, and resilience values of all fat substituted samples decreased. This is to be predicted because animal fats have an effect not only on health but also on the texture properties of the food. The effect of oleogel type on the cohesiveness and resilience values in all substitution rates was found to be statistically insignificant ( $p > 0.05$ ).

Kouzounis et al. (2017) found similar findings for the hardness, gumminess, and chewiness values of frankfurters made with pork lard or sunflower oil oleogel. They concluded that the hardness value of a lard-based frankfurter was higher than that of oleogel. They also concluded that any differences in these parameters could be attributed to the properties of oleo-

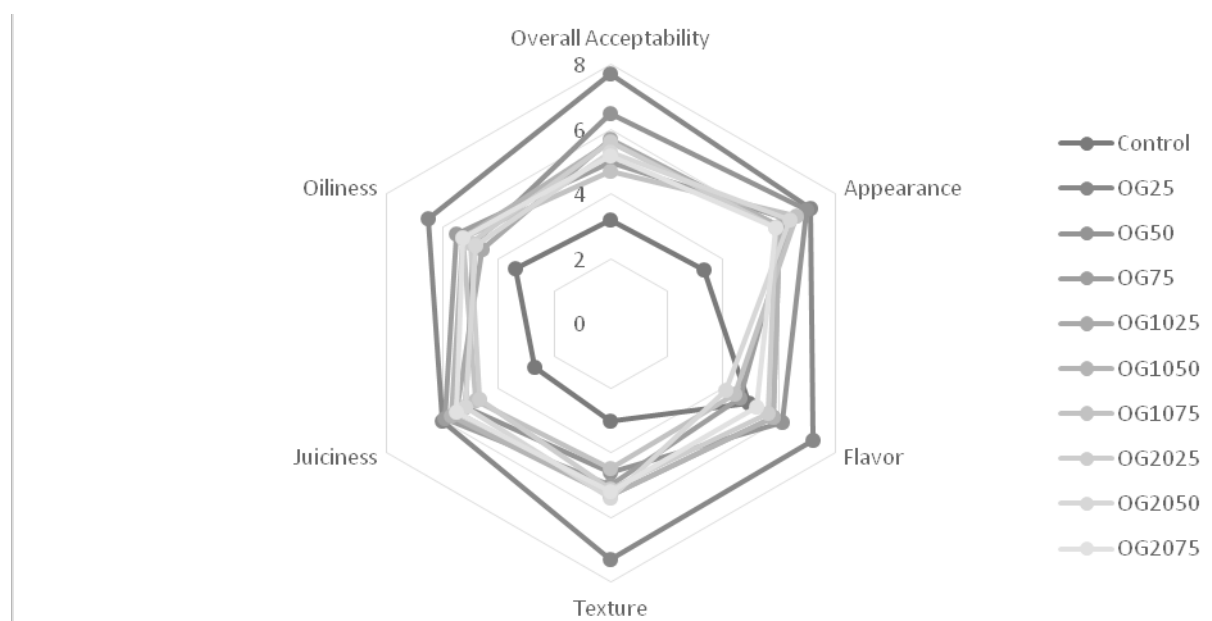
gel as well as its interaction with the meat dough. Barbut et al. (2016) showed that substituting canola oil or organogel for pork fat results in significantly lower hardness scores. They reached the conclusion that the difference may be due to the scale of fat globules.

### Sensory analysis results

Sensory analysis results (Figure 4) showed that the meatballs' color, taste, texture, juiciness, oiliness, and overall acceptability scores were significantly different.

They were similar in terms of appearance, taste, and oiliness ratings.

The OG25 group had the highest scores for appearance, flavor, texture, juiciness, oiliness, and overall acceptability and was significantly different from the control group, but there was no difference between the OG25 and the other groups. Although, the results showed that all values of the control group were the lowest except flavor. Fat content is important for both the textural and sensorial characteristics of the product, and also for human health. Because of the adverse affects of animal fats, healthy lipids may be substituted. As a consequence, this sensory analysis yields valuable results for developing new formulations and products for consumer acceptance.



**Figure 4.** The sensory analysis on the average scores of the meatball samples

OG25: Meatball fat substituted with OG at 25% level, OG50: Meatball fat substituted with OG at 50% level, OG75: Meatball fat substituted with OG at 75% level, OG1025: Meatball fat substituted with OG10 at 25% level, OG1050: Meatball fat substituted with OG10 at 50% level, OG1075: Meatball fat substituted with OG10 at 75% level, OG2025: Meatball fat substituted with OG20 at 25% level, OG2050: Meatball fat substituted with OG20 at 50% level, OG2075: Meatball fat substituted with OG20 at 75% level.

## CONCLUSIONS

TBA values of meatballs are reduced during storage when black seed oil is added to the oleogel mixture. These oleogel research must be conducted in greater depth and may be applicable to the development of new healthy goods. More research is re-

quired to determine the properties of oleogels made from sunflower oil and black seed oil. Oleogels can be made using a variety of gelling agents.

## CONFLICT OF INTEREST STATEMENT

None declared.

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