Low-fat beef patties with cold-pressed oils optimized by mixture design

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Summary
Partial animal fat replacement by cold-pressed grape seed oil (GSO) and pomegranate seed oil (PSO) on low-fat beef patties was studied using simplex-centroid mixture design. Linear and quadratic model equations were developed in order to associate the independent variables, namely, animal fat, GSO and PSO, and the responses measured during the 12 days storage. The model for fat loss showed that increase in PSO significantly decreased fat loss ($p < 0.05$). However, increase in animal fat and GSO significantly increased fat loss ($p < 0.05$). The oils had notable preventive effect against the drop of pH ($p < 0.05$) during the 6 days storage. The oils prevented early lipid oxidation where the highest thiobarbituric acid values belonged to the samples containing high amounts of animal fat ($p < 0.05$). Cold-pressed oils showed neither preventive nor promoting effect on the growth of the monitored microorganisms. While GSO and PSO have a strong aromatic profile, none of the patties were rejected by the panelists. The results suggested that partial replacement of animal fat in beef patties by cold-pressed oils is possible.

Keywords
meat products; optimization; fat reduction; seed oil

Dietary fat intake is known to correlate with health problems such as diabetes, cardiometabolic diseases and also coronary heart disease mortality [1]. While the consumers are aware of their daily fat consumption and tend towards low-fat meat products, they still have expectations for the overall quality of the product to match that of its full-fat counterpart [2]. Therefore, development of low-fat meat products is a big challenge for manufacturers, according to the significant role of animal fat in physico-chemical and sensorial properties of the products.

Researchers have investigated the effects of different additives in the production of low-fat meat products to reach the optimized recipe. It is inherently essential to select an oily and/or fibrous substitute for this purpose and, consequently, poppy seed paste [2], fish oil [3], oat flour [4] and rye bran [5] are the examples of fat replacers used in significant studies. In addition to these, vegetable oils such as maize [6] or sunflower [7] oils were directly used in the production of different meat products instead of animal fat. Vegetable oils are used not only for fat reduction but also for development of novel meat products containing natural antioxidants and unsaturated fatty acids [8].

Cold-pressed oils containing great numbers of valuable bioactive substances could be obtained from seeds or fruits of different plants, and they are the alternatives to common seed oils due to the cold-pressing procedure that does not include chemical or heat treatments [9, 10]. Respecting this, several researchers have pointed out that cold-pressed oils could be used to improve the nutritional, microbiological and physico-chemical characteristics of food products [11–13]. However, to the best of our knowledge, there has been no work on the use of cold-pressed oils in the production of beef patties.

If there is a product composed of several components or ingredients, and the significant properties of the product depend on the relative proportions of those, mixture design approach could be used for the design of experiment that allows the researchers to find out the importance of ingredient interactions [14, 15]. This methodology was successfully used in the studies on different food

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systems including meat products [14, 16].

The objectives of the present study were to produce low-fat beef patty by replacing animal fat with grape seed oil (GSO) and pomegranate seed oil (PSO) up to 50%, and to determine the effects of these ingredients and their interactions on some physico-chemical, microbiological and sensorial properties of the product throughout the self-life.

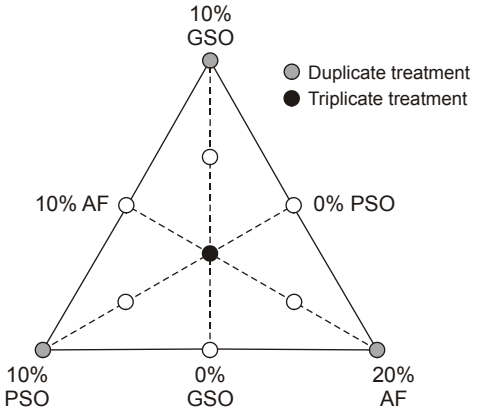

Fig. 1. Simplex design plot in amounts of the independent variables.

GSO – grape seed oil, PSO – pomegranate seed oil, AF – animal fat.

Tab. 1. Mixture composition of grape seed oil, pomegranate seed oil and animal fat.

Sample	Actual values [g·kg^{-1}]		
	GSO (x_1)	PSO (x_2)	AF (x_3)
1	50.0	50.0	100.0
2	0.0	100.0	100.0
3	16.7	66.7	116.7
4	100.0	0.0	100.0
5	33.3	33.3	133.3
6	50.0	0.0	150.0
7	16.7	16.7	166.7
8	100.0	0.0	100.0
9	33.3	33.3	133.3
10	0.0	0.0	200.0
11	0.0	100.0	100.0
12	33.3	33.3	133.3
13	0.0	0.0	200.0
14	0.0	50.0	150.0
15	66.7	16.7	116.7

Samples with duplicate treatment: 2, 11; 4, 8 and 10, 13. Samples with triplicate treatment: 5, 9, 12.
GSO – grape seed oil, PSO – pomegranate seed oil, AF – animal fat.

MATERIALS AND METHODS

Experimental design

Animal fat was partially replaced by GSO and PSO to reduce the fat content of the beef patty, using 3rd degree – three components simplex-centroid mixture design. The sum of three ingredients was 20 g fat-oil per 100 g of patty dough. While there are some efforts reported in the literature for the complete replacement of animal fat in the recipe, most of the results stated remarkable restrictions according to the deterioration in sensorial properties because of the use of excess vegetable oil. Therefore, the maximum substitution level for cold-pressed oil was selected to reach a replacement of up to 50% of animal fat [17, 18]. For this purpose, the mixture design with the constraints GSO (x_1) + PSO (x_2) + animal fat (x_3) = 20% (w/w) was used with the component ranges as GSO (0–10%), PSO (0–10%) and animal fat (10–20%). Simplex design plot in amounts of the independent variables is given in Fig. 1, which presents 10 main treatment points with 5 duplicate and/or triplicate treatments. Tab. 1 shows 15 treatment points provided by mixture design obtained using statistical software Minitab 17.1.0 (Minitab, State College, Pennsylvania, USA). Duplicate treatments were carried out at all design points (n = 2).

Beef patty preparation

The traditional recipe was used for the sample production as explained by Yilmaz [5] and Yilmaz and Demirci [19] with slight modifications. Grounded veal meat (portion from semimembranosus muscle of 2 years Simmental breed at 24 h post mortem, with maximum 1.5% fat) obtained from local market in Tekirdag, Turkey was mixed with seasonings (black pepper 0.3%, red pepper 0.2% and cumin 0.5%), onion 5%, salt 2% and breadcrumbs 7%. The mixture was kneaded for 15 min and the homogenous dough was divided into 15 portions (each was 320 g). Partial fat substitution was done using two commercial cold-pressed oils (GSO and PSO) obtained from Neva (Istanbul, Turkey). GSO, PSO and animal fat were added according to the recipe described in Tab. 1 to reach the final 20% fat/oil content of each batch. Each sample portion was kneaded for additional 5 min to obtain homogenous dough. The samples were put in separate stainless steel vessels and stored in a refrigerator (at +4 °C) for one night. Then the samples were shaped into patties of a diameter of 7 cm and a weight of 55–60 g. Finally, the patties were put in polystyrene trays, wrapped separately with polyethylene film and

stored at +4 °C. Analyses were performed at days 1 (same day), 6 and 12.

Physico-chemical properties

The diameters of raw and cooked beef patties were measured for the determination of shrinkage according to the method described by Modi et al. [20]. The shrinkage (S) percentages were calculated with the following equation:

$$S = \frac{d_R - d_C}{d_R} \times 100 \quad (1)$$

where d_R is diameter of raw sample and d_C is diameter of cooked sample.

Fat loss was determined by measuring the crude lipid content of the raw and cooked beef patties according to AOAC Method No. 960.39 [21]. Additionally, the weights of raw and cooked samples were measured to evaluate weight loss percentages of the patties. Both the fat loss (F) and weight loss (W) percentages were calculated using the equations below [22].

$$F = \frac{f_R - f_C}{f_R} \times 100 \quad (2)$$

where f_R is fat content of raw sample and f_C is fat content of cooked sample.

$$W = \frac{w_R - w_C}{w_R} \times 100 \quad (3)$$

where w_R is weight of raw sample and w_C is weight of cooked sample.

pH value of each beef patty was measured according to AOAC Method No. 981.12 [21].

Thiobarbituric acid (TBA) reactive substances analysis was carried out according to the method described by Tarladgis et al. [23]. Absorbance measurements were done using UV-Vis spectrophotometer (UV-Vis 1208, Shimadzu, Kyoto, Japan) at 538 nm and the results were expressed as milligrams of malonaldehyde (MA) per kilogram of sample.

Colour

Colour measurement of the beef patties was performed using Hunter-Lab tristimulus colorimeter (D25LT; Hunter Associates Laboratory, Reston, Virginia, USA). One patty was put in standard Petri dish, immediately after taking it out from the refrigerator, and L^*, a^*, b^* colour measurement was done by the colorimeter. The average of the results obtained from six replicate measurements was used in calculations.

Microbiological analysis

All microbiological analysis were performed according to the Bacteriological Analytical Manual (BAM) released by FDA where the analysis of aerobic plate counts, coliform group bacteria counts, *Salmonella* as well as yeasts and moulds counts were determined according to the relevant Chapters 3, 4, 5 and 18 of BAM, respectively [24].

Plate count agar (PCA; Merck, Darmstadt, Germany) was used for the determination of aerobic plate counts, with incubation at 35 °C for 48 h.

The coliform group bacteria counts were determined using violet red bile agar (VRBA, Merck) after the incubation at 35 °C for 24 h.

Salmonella analysis was done as follows: Selenite cystine broth (Merck) was used for enrichment of 25 g sample at 35 °C for 24 h. After the enrichment step, the culture was streaked onto bismuth sulfite agar (Merck) and incubated again at 35 °C for 24 h. The typical colonies selected due to the macroscopic appearances were subjected to subsequent biochemical tests using Triple sugar iron and Lysine iron agar (Merck) slants.

In addition to these, the yeasts and moulds counts were determined using potato dextrose agar (PDA, Merck) with an incubation at 25 °C for 5 days.

Sensorial analysis

The sensorial analysis of the samples was performed on the first day of the study. The beef patties were cooked to achieve an internal temperature of 72 °C according to the procedure described by Velioglu et al. [22]. The samples were cooled at room temperature for 5 min. Sensory evaluation was conducted by a group of 8 semi-trained panelists. The samples were evaluated for appearance, hardness, juiciness, aroma and overall acceptability. A nine-point hedonic scale (1 – dislike extremely, 9 – like extremely) was used for sensorial analysis. Each sample was coded with a randomly selected 2-digit number.

Statistical analysis

All analyses were done in duplicate. The data obtained from the treatments were subjected to analysis of variance and Duncan Multiple Test was used to find out the differences between the mean values using the software PASW Statistics 18.0.0 (IBM, New York City, New York, USA). Analysis of mixture design was done using statistical software Minitab 17.1.0 and quadratic polynomial equations were fitted to data. The general equation is given below:

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (4)$$

where y is dependent variable (fat loss, weight loss,

Tab. 2. Model equations for fat loss, weight loss, shrinkage and overall acceptability of beef patties.

Parameter	Model	R^2
Fat loss [%]	$y = 6.46x_1 - 0.87x_2 + 2.45x_3 - 0.11x_1x_2 - 0.41x_1x_3 + 0.10x_2x_3$	0.82
Weight loss [%]	$y = 2.66x_1 + 0.64x_2 + 1.04x_3 + 0.06x_1x_2 - 0.16x_1x_3 + 0.05x_2x_3$	0.79
Shrinkage [%]	$y = -0.88x_1 + 1.22x_2 + 0.96x_3 - 0.04x_1x_2 + 0.15x_1x_3 - 0.06x_2x_3$	0.92
Overall acceptability	$y = 0.13x_1 + 0.48x_2 + 0.37x_3 + 0.02x_1x_2 + 0.003x_1x_3 - 0.03x_2x_3$	0.88

R^2 – coefficient of determination.

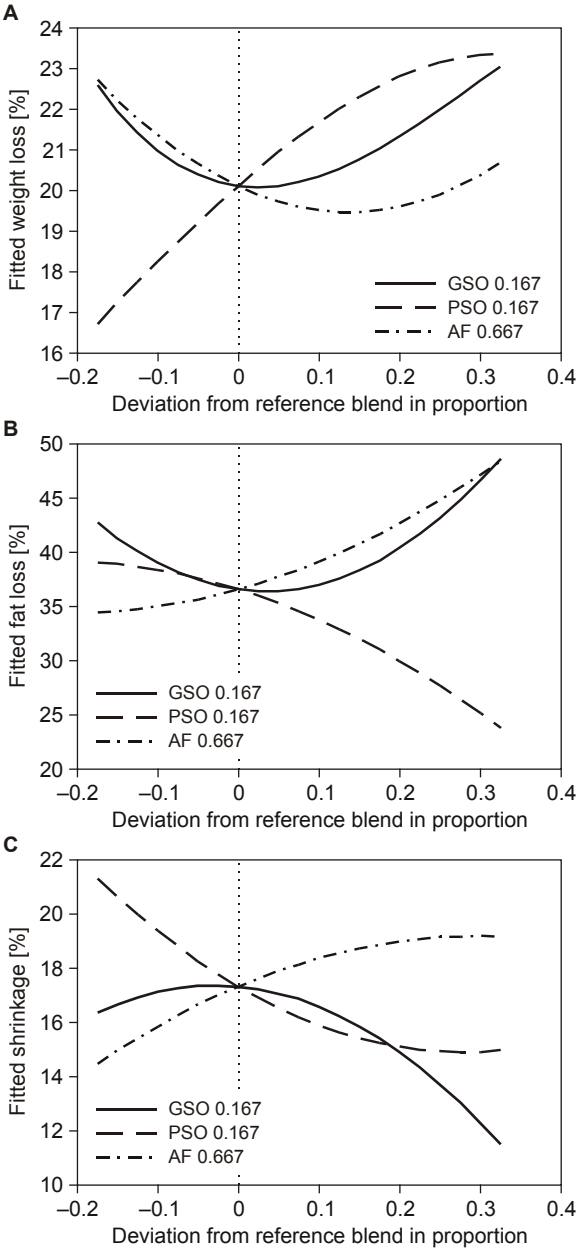

Fig. 2. Response trace plots for weight loss, fat loss and shrinkage versus proportion of ingredients.

A – weight loss, B – fat loss, C – shrinkage.
GSO – grape seed oil, PSO – pomegranate seed oil, AF – animal fat.

shrinkage, overall acceptance, rise in TBA, drop in pH); x_1, x_2 and x_3 are the coded independent variables that indicate proportions of GSO, PSO and animal fat, respectively. β_1, β_2, β_3, β_{12}, β_{13}, β_{23} are regression coefficients for the linear and quadratic coefficients.

The final interpretation of the models was assessed by drawing Cox response trace plots.

RESULTS AND DISCUSSION

Physico-chemical properties

Fat loss, weight loss and shrinkage

Fat loss, weight loss and shrinkage are considered as important quality characteristics of beef patty not only by consumers but also by industrial companies producing this product [22]. Several studies focused on the effects of ingredients on these characteristics and production of healthier meat products with low shrinkage and high oil/fat/moisture retention was aimed in previous studies [2, 20, 25]. In the present study, all three parameters were well explained by the quadratic model equations obtained from mixture design approach and are given in Tab. 2. Model equations had insignificant lack of fit ($p > 0.05$) which proved their high prediction capability. Our results showed that increasing GSO proportion decreased the fat loss until the point of inflection as seen in Fig. 2A. After this point, an increasing effect on fat loss was recorded, which could be recognized form the model equation in Tab. 2 where GSO has a regression coefficient of +6.46. However, the preventing effect of PSO on fat loss was continuous at all levels (Fig. 2A), while an increasing animal fat proportion significantly increased the fat loss. Similar result was reported previously, indicating that decreasing the fat content from 20% to 5% significantly decreased fat loss of meatballs [25]. While both GSO and PSO are cold-pressed seed oils, the difference in their behaviours regarding fat retention of patties may be due to the

better protein-fat interaction with PSO. Similarly, SINGH et al. [26] reported comparative effect of canola oil and linseed oil on the cooking yield of chicken patties.

The term weight loss involves not only fat loss but also moisture loss of the patty. As seen in Fig. 2B, GSO, PSO and animal fat showed increasing effect on weight loss after a certain level. However, this effect was observed for PSO at all levels while the others had prevented weight loss until a certain point. However, it should be taken into consideration that the increasing effect of PSO on weight loss was not at same level with the increasing effect of animal fat on fat loss, which could be deduced from the scales of Fig. 2A and Fig. 2B. As a conclusion, it could be conceived that PSO was less effective than GSO and animal fat on moisture retention of the product. Our results agree with those of DAS and RAJKUMAR [27] who stated that the increasing levels of vegetable oil addition resulted in low product yield after cooking of patties.

Shrinkage of a meat product is related with the interaction between proteins, fat and water in the food matrix. As reported by previous studies, shrinkage is effected by oil/fat and moisture contents of the product [4, 22, 25]. In the present study, increasing animal fat levels increased the reduction in diameter of the patties, while GSO and PSO showed different behaviours at different addition levels (Fig. 2C). Also, as it was proven by the model equation in Tab. 2, GSO showed significantly decreasing effect on shrinkage while the regression coefficient of PSO was positive (+1.22). This finding may be related to the interactions between oils, fat and water in the product.

pH

While PSO, GSO and animal fat contain different fatty acids, they show particular acidic characteristics [28, 29, 30]. Tab. 3 shows the initial (first day) pH values of the samples and the significant volatility ($p < 0.05$) between these values of raw patty doughs could be explained by the different acidic properties of vegetable oils and animal fat. The pH values ranged between 5.50 and 5.91 where the lower pH values generally belonged to higher vegetable oil contents. Tab. 3 also

Tab. 3. Initial measurements and changes in pH, thiobarbituric acid and colour values.

Sample	pH	Drop in pH [%]		TBA [mg·kg^{-1}]	Rise in TBA [%]		L^*	Rise in L^* [%]		a^*	Drop in a^* [%]		b^*	Drop in b^* [%]	
		Day 6	Day 12		Day 6	Day 12		Day 6	Day 12		Day 6	Day 12		Day 6	Day 12
1	5.91 ± 0.01^{a}	7.9	20.5	1.01 ± 0.00^{e}	51.2	141.9	37.34 ± 0.01^{h}	0.1	6.2	9.69 ± 0.08^{bc}	39.0	42.3	12.31 ± 0.02^{c}	9.7	12.8
2	5.75 ± 0.01^{gh}	13.0	19.3	0.98 ± 0.00^{gh}	42.2	114.7	37.96 ± 0.07^{e}	0.2	7.3	8.63 ± 0.07^{h}	24.1	34.3	12.06 ± 0.04^{d}	9.0	9.9
3	5.50 ± 0.01^{i}	11.0	18.9	0.99 ± 0.00^{efgh}	52.0	144.8	39.54 ± 0.01^{b}	1.4	3.2	10.08 ± 0.05^{a}	35.6	41.6	12.55 ± 0.01^{b}	7.8	10.9
4	5.76 ± 0.00^{def}	12.4	17.7	1.00 ± 0.01^{efg}	29.8	89.8	36.94 ± 0.02^{j}	2.6	10.6	9.35 ± 0.03^{de}	35.7	37.8	11.54 ± 0.01^{f}	12.5	14.2
5	5.77 ± 0.01^{de}	15.1	19.6	1.01 ± 0.00^{ef}	58.1	159.2	38.27 ± 0.04^{d}	0.9	6.3	10.06 ± 0.04^{a}	39.8	40.9	12.23 ± 0.01^{c}	10.1	12.5
6	5.78 ± 0.00^{c}	15.0	19.9	1.05 ± 0.01^{d}	46.0	135.1	37.39 ± 0.03^{gh}	1.4	12.0	9.88 ± 0.03^{ab}	40.6	43.8	12.12 ± 0.04^{d}	10.9	12.3
7	5.81 ± 0.00^{b}	14.1	19.3	1.06 ± 0.00^{d}	68.1	187.4	37.02 ± 0.07^{ij}	3.0	9.4	9.14 ± 0.09^{ef}	36.9	40.7	11.52 ± 0.06^{f}	2.6	7.4
8	5.73 ± 0.00^{i}	12.4	17.3	1.05 ± 0.00^{d}	33.0	95.5	38.26 ± 0.07^{d}	3.0	6.8	9.17 ± 0.01^{ef}	35.1	41.5	12.11 ± 0.02^{d}	14.9	18.1
9	5.80 ± 0.00^{b}	13.8	20.3	0.99 ± 0.01^{fgh}	60.9	167.1	39.96 ± 0.02^{a}	0.7	1.8	9.34 ± 0.03^{de}	37.3	39.9	12.65 ± 0.02^{a}	9.2	15.4
10	5.77 ± 0.00^{cd}	13.2	17.7	1.26 ± 0.00^{a}	66.0	180.6	38.47 ± 0.05^{c}	2.4	8.7	8.04 ± 0.21^{i}	36.3	33.6	11.60 ± 0.07^{f}	0.6	8.7
11	5.75 ± 0.00^{fgh}	11.7	19.3	0.98 ± 0.00^{h}	45.0	123.4	36.43 ± 0.03^{k}	3.7	10.0	8.89 ± 0.02^{g}	25.0	36.3	11.53 ± 0.00^{f}	3.3	5.7
12	5.76 ± 0.01^{efg}	13.6	20.1	1.08 ± 0.02^{c}	62.0	167.3	37.88 ± 0.02^{e}	2.7	7.5	8.99 ± 0.02^{fg}	34.8	38.8	12.09 ± 0.02^{d}	10.2	11.6
13	5.78 ± 0.00^{c}	13.7	17.8	1.19 ± 0.00^{b}	71.0	192.4	37.11 ± 0.03^{i}	4.7	11.5	9.52 ± 0.04^{cd}	37.1	43.1	11.50 ± 0.04^{f}	2.6	7.9
14	5.76 ± 0.01^{efg}	13.9	19.6	1.05 ± 0.01^{d}	60.9	165.5	37.56 ± 0.03^{f}	3.3	8.8	9.33 ± 0.13^{de}	37.5	41.2	11.53 ± 0.02^{f}	0.1	2.9
15	5.74 ± 0.00^{hi}	12.7	19.2	0.99 ± 0.01^{fgh}	40.8	118.2	37.47 ± 0.03^{fg}	5.7	7.8	9.61 ± 0.06^{c}	38.2	43.9	11.87 ± 0.03^{e}	3.0	13.3

pH and thiobarbituric acid (TBA) values are given as mean value ± standard error. Different letters in the column indicate significant differences ($p < 0.05$).

presents the pH drop percentages during the storage period between the days 1–6 and 1–12. The effects of GSO, PSO and animal fat on the drop of pH value could be seen in Fig. 3. In the first half of the storage period, GSO and PSO had notable preventive effect against the drop of pH. Contrary to this, the increase of animal fat proportion in the product significantly ($p < 0.05$) decreased the pH. The mathematical equation (Eq. 5) obtained from the analysis of mixture design indicates that animal fat had a promoting effect on pH drop in the first 6 days of the storage. Eq. 6 and Fig. 3B present the change in pH of the patties during the entire storage period.

$$y = -0.72x_1 - 0.17x_2 + 0.66x_3 - 0.14x_1x_2 + 0.13x_1x_3 + 0.07x_2x_3 \quad (5)$$

$$y = 0.07x_1 + 0.78x_2 + 0.89x_3 + 0.06x_1x_2 + 0.08x_1x_3 + 0.02x_2x_3 \quad (6)$$

Animal fat lost the aforementioned negative effect, while an opposite effect was observed with PSO. It could be concluded that the preventive effect of PSO on pH drop decreased during the long-term storage (12 days). Consequently, GSO was the most effective ingredient among all, showing a significant positive effect in terms of preventing the pH drop. Our findings match the literature data verifying the fact that the main cause of the pH drop of meat products is lactic acid formation due to the bacterial breakdown of saccharides [19, 31]. The oxidative deterioration of fat and oil has a subsidiary effect and also decreases pH during the storage period. As a conclusion, the preventive effect of GSO and PSO could be due to the antimicrobial and antioxidant properties, which were proven in previous studies [29, 32]. The results of the present study showed that the models for pH drop in both parts of the storage period were quadratic and were highly promising with satisfactory R^2 values (0.81 and 0.86) for the first half and for the entire storage period, respectively. Additionally, lack-of-fit values of both model equations (Eq. 5 and Eq. 6) were higher than the tabulated values, which means that there was no lack of fit ($p > 0.05$) between the experimental results and the model equations.

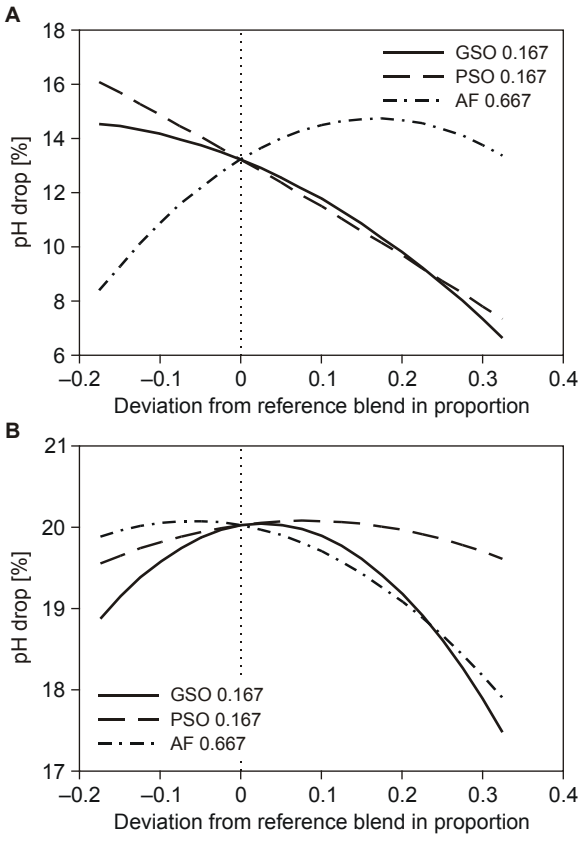

Fig. 3. Response trace plots for drop in pH versus proportion of ingredients.

A – drop in pH between days 1–6, B – drop in pH between days 1–12.
GSO – grape seed oil, PSO – pomegranate seed oil, AF – animal fat.

Thiobarbituric acid analysis

The lipid oxidation in meat products is generally evaluated using TBA analysis. Tab. 3 shows the TBA data obtained on the first day of the cold storage, which indicates that the TBA values were affected by cold-pressed oil addition ($p < 0.05$). The highest TBA values belonged to the samples with highest animal fat contents (7, 10 and 13). This finding proved that the cold-pressed oils prevented early lipid oxidation just after the mixing and maturing step of the patties. Similar results were reported by Fernandez-Lopez et al. [33] who observed the antioxidant effect of natural extracts in early stage of the storage period of meatballs. The TBA values of the raw patties on the first day were different from the findings reported by other researchers. Yilmaz and Demirci [19] determined the initial TBA values of raw meatballs to range from 0.180 mg·kg^{-1} to 0.424 mg·kg^{-1}. Ilyasoglu [34] reported that the TBA value of raw meatball was approximately 2.5 mg·kg^{-1} (expressed as MA). Additionally, another study by Gecgel [30] indicated that the TBA value of the meatball samples after one-week storage was 0.45 mg·kg^{-1}. In this manner, the TBA values between 0.980–1.264 mg·kg^{-1} could be considered as reasonable.

The long-term antioxidant effect of cold-pressed oils could be seen from the Tab. 3 and Fig. 4. As expected, the samples containing more

animal fat were highly at risk of oxidation compared to the samples containing more cold-pressed oil. While both seed oils showed significant preventive effect on lipid oxidation, GSO was more effective than PSO throughout the storage period. Oxidative stability of oils is related to the content of unsaturated fatty acid, which means the higher the polyunsaturated fatty acid content, the lower the oxidative stability [29]. It could be concluded that GSO has a stronger protective effect than PSO on the increase of TBA value (Fig. 4) because it contains more linoleic acid (C18:2) instead of pucinic acid (C18:3).

The model equations obtained from mixture design with independent variables (GSO, x_1; PSO, x_2 and animal fat, x_3) and the related response (rise in TBA in percent, y) for the patty samples are given below:

$$y = -0.16x_1 - 2.60x_2 + 3.45x_3 + \\ + 0.60x_1x_2 - 0.03x_1x_3 + 0.34x_2x_3 \quad (7)$$

$$y = -1.34x_1 - 6.12x_2 + 9.41x_3 + \\ + 1.52x_1x_2 + 0.09x_1x_3 + 0.85x_2x_3 \quad (8)$$

Eq. 7 has good prediction capability (R^2, 0.94) and an insignificant lack-of-fit value ($p > 0.05$). It shows the negative effect of GSO and PSO on the percentual increase in TBA value between days 1 and 6. The model equation (Eq. 8) and the experimental results are in good agreement with an insignificant lack of fit ($p > 0.05$). Also, the high value of coefficient of determination (R^2, 0.95) stated a high prediction capability. The pattern of the antioxidant effect due to the addition of cold-pressed oils was the same during the entire storage period (Fig. 4B) with a slight decrease in their protective features. Similarly, SHAH et al. [35] reported that the antioxidant effect of natural additives on meat products continued through the storage period.

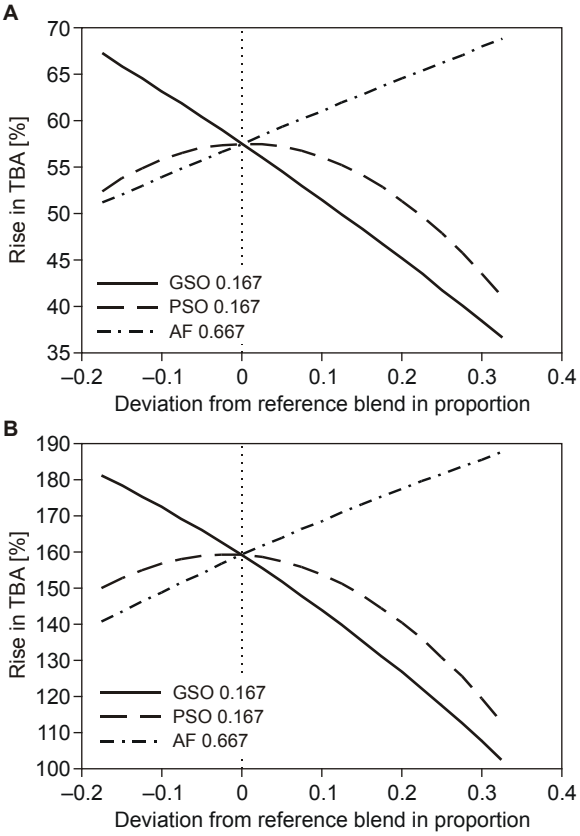

Fig. 4. Response trace plots for rise in thiobarbituric acid versus proportion of ingredients.

A – rise in thiobarbituric acid (TBA) between days 1–6, B – rise in TBA between days 1–12.
GSO – grape seed oil, PSO – pomegranate seed oil, AF – animal fat.

Colour

Lightness (L^*), redness (a^*) and yellowness (b^*) values of the samples are given in Tab. 3 according to CIE system. While some significant ($p < 0.05$) differences were observed in the colour values of the samples, there was not an accurate correlation between the cold-pressed oil addition and initial colour scores for the first day of the storage period. It might have been due to the dominant effect of the spices, such as red pepper, on the colour of the product at the beginning of storage, and due to a low level of cold-pressed oil addition (0–10%) in the present study. In spite of this, higher a^* values were determined for samples 3, 1 and 6 which contained more cold-pressed oil than the others. Further, it was observed that the higher the cold-pressed oil addition, the more the yellow coloured product, such as in case of samples 2, 3, 6 and 8. Similar results were reported by MUGUERZA et al. [36] who used olive oil as a fat replacer in frankfurters and obtained a more yellow product. No significant change was observed for the L^* and b^* values of the samples in the first half of the storage period, which is the reason why a model equation could not be derived for those parameters. On the contrary, cold-pressed oil addition had a dramatic effect on the red colour of the products between days 1 and 6. Eq. 9 states that the quadratic model fitted well for the relation between proportional amounts of oils/fat and a drop in redness of the sample. Fig. 5 shows that PSO had a preventing effect on the drop of redness, while the effect of GSO followed it.

$$y = 0.93x_1 - 1.25x_2 + 1.83x_3 + \\ + 0.29x_1x_2 + 0.08x_1x_3 + 0.19x_2x_3 \quad (9)$$

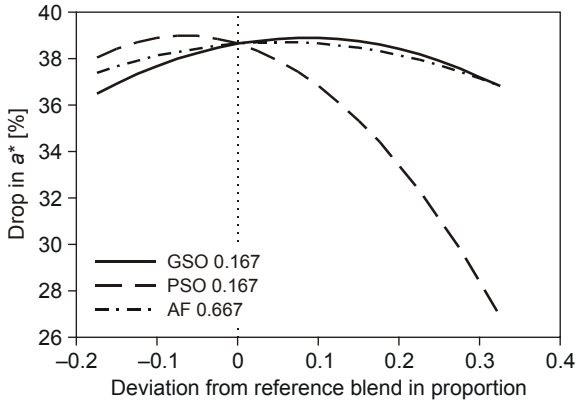

Fig. 5. Response trace plot for drop in *a** (redness) between days 1-6 versus proportion of ingredients.

GSO – grape seed oil, PSO – pomegranate seed oil, AF – animal fat.

The prediction capability of the model equation (Eq. 9) from the amounts of and interactions between oils and fat was reliable, with R^2 of 0.89, and had an insignificant ($p > 0.05$) lack-of-fit value. While the colour alteration in meat products during cold storage is related with pigment oxidation, it is coherent that the best-fitted model equation is for redness value of products [37]. This finding agreed with KHODDAMI et al. [28] and ABBASI et al. [38] who remarked that PSO could act as an antioxidant agent due to its high content of pucinic acid and phenolic compounds, which contributed to the preventing effect of PSO on the loss of red colour of the products. Nevertheless, the results obtained for day 12 showed that the cold-pressed oils were not influential in the present long-term storage conditions. It could be concluded that the colour of the samples was lost due to high microbial growth that was obviously observed with a warmed-over flavour. So, none of the mixture design models were fitted between the change in colour values and oils/fat addition for days 1–12.

Microbiological quality

Raw burger produced from minced meat is generally accepted as poor in microbiological quality. As shown in Tab. 4, the initial microbial load of the samples was different. However, there was no accurate correlation between the sample composition and the bacterial contamination. The initial aerobic plate counts were between 5.6 log CFU·g^{-1} and 7.8 log CFU·g^{-1}, and they continued to rise during the storage period. GSO was reported as effective against the growth of *Escherichia coli* and *Staphylococcus aureus* in vacuum-packaged hot smoked sea bream, while it was less effective on *Listeria monocytogenes* [39]. Similarly, antimicrobial effect of GSO was investigated using agar disk-diffusion method against ten species of microorganisms and it was found that GSO is more effective than fuji apple seeds oil and mulberry seeds oil. Additionally, the researchers reported that the effect of natural product may change due to species variation [40]. In another study, PSO was found to be effective against the

Tab. 4. Results of microbiological analysis.

Sample	Aerobic plate counts [log CFU·g^{-1}]			Coliforms [log CFU·g^{-1}]			Yeasts and moulds [log CFU·g^{-1}]		
	Day 1	Day 6	Day 12	Day 1	Day 6	Day 12	Day 1	Day 6	Day 12
1	7.0	7.0	8.4	4.8	4.9	5.6	4.2	5.1	5.5
2	6.7	6.8	8.0	4.4	5.3	5.6	4.7	5.6	5.9
3	6.1	7.0	7.6	4.8	5.0	5.3	4.6	5.3	5.7
4	6.3	7.2	7.3	4.2	4.6	5.0	4.6	5.5	5.8
5	7.2	7.6	8.3	4.8	5.8	6.6	4.1	5.0	5.7
6	5.9	6.9	7.0	4.0	5.7	6.0	4.6	5.6	6.7
7	6.6	6.8	7.8	3.8	5.5	6.3	4.6	5.8	6.7
8	6.3	6.9	7.0	4.0	4.6	6.4	4.3	5.5	5.8
9	6.7	7.0	8.3	4.9	5.0	6.9	3.9	4.1	5.6
10	7.0	7.8	8.6	4.0	4.8	6.0	4.4	5.4	6.8
11	5.6	6.4	7.9	4.4	5.0	6.8	4.4	5.3	5.9
12	7.2	7.3	8.3	3.5	5.0	6.8	4.2	5.0	5.7
13	7.8	8.1	8.7	4.3	4.7	6.0	4.6	5.7	6.5
14	7.7	7.9	8.0	4.7	5.0	5.9	4.7	5.3	5.7
15	6.2	6.7	7.8	4.8	6.3	6.8	4.6	5.8	6.8

Tab. 5. Results of sensorial analysis.

Sample	Appearance	Hardness	Juiciness	Aroma	Overall acceptability
1	6.88 ± 0.64	6.38 ± 0.68	5.75 ± 0.70	5.25 ± 0.75^{abcde}	6.00 ± 0.46^{abc}
2	7.13 ± 0.40	5.50 ± 0.42	6.00 ± 0.54	4.63 ± 0.57^{cde}	5.50 ± 0.60^{bc}
3	6.75 ± 0.45	6.00 ± 0.54	5.75 ± 0.59	5.00 ± 0.38^{bcde}	5.75 ± 0.41^{abc}
4	6.88 ± 0.40	5.63 ± 0.63	6.00 ± 0.71	5.50 ± 0.50^{abcde}	5.75 ± 0.56^{abc}
5	6.75 ± 0.31	4.88 ± 0.69	6.63 ± 0.38	5.50 ± 0.54^{abcde}	6.13 ± 0.35^{abc}
6	7.63 ± 0.18	5.75 ± 0.53	6.50 ± 0.50	6.13 ± 0.52^{abc}	6.63 ± 0.38^{ab}
7	7.25 ± 0.25	5.50 ± 0.60	6.63 ± 0.46	5.88 ± 0.72^{abcd}	6.38 ± 0.53^{abc}
8	7.25 ± 0.31	5.63 ± 0.46	5.75 ± 0.84	4.25 ± 0.68^{de}	4.75 ± 0.56^{c}
9	7.00 ± 0.42	4.88 ± 0.58	6.13 ± 0.48	5.50 ± 0.46^{abcde}	5.63 ± 0.46^{bc}
10	7.63 ± 0.38	5.88 ± 0.55	6.00 ± 0.60	6.75 ± 0.53^{ab}	7.38 ± 0.38^{a}
11	6.63 ± 0.38	4.63 ± 0.75	6.25 ± 0.62	3.88 ± 0.44^{e}	5.50 ± 0.54^{bc}
12	6.88 ± 0.44	5.75 ± 0.49	5.50 ± 0.76	5.88 ± 0.40^{abcd}	5.75 ± 0.65^{abc}
13	7.25 ± 0.25	5.75 ± 0.59	6.63 ± 0.50	6.88 ± 0.44^{a}	7.38 ± 0.32^{a}
14	6.75 ± 0.37	5.13 ± 0.61	6.88 ± 0.35	5.50 ± 0.68^{abcde}	5.88 ± 0.61^{abc}
15	6.50 ± 0.38	4.50 ± 0.46	5.75 ± 0.68	3.88 ± 0.61^{e}	5.63 ± 0.57^{bc}

The results are given as mean value ± standard error. Different letters in the column indicate significant differences ($p < 0.05$).

growth of *Micrococcus luteus* and *Salmonella* Enteritidis, but its inhibition effect was weaker than that of pomegranate seed extract for the inhibition of *Bacillus cereus* and *E. coli* [41].

Although we could not state any numerical equation comprising the relation between aerobic plate counts and the proportion of the oils/fat, our results indicated that the lowest aerobic plate counts were during the storage period in samples containing higher amounts of cold-pressed oils (samples 2, 3, 4, 11), which agreed with the findings of previous studies. Nevertheless, aerobic plate counts at the end of the storage period (12th day) were higher than 7.0 log CFU·g^{-1}, which indiciated spoilage of this type of product [19].

The counts of coliforms on the first day were between 3.5 log CFU·g^{-1} and 4.9 log CFU·g^{-1}. No effect of cold-pressed oils on the growth of coliforms was observed, as could be recognized by comparison of the results for samples containing high amounts of oil (samples 2, 4, 8 and 11) with those for samples containing high animal fat (samples 10 and 13). Results in Tab. 4 demonstrate that the rise in coliform counts continued independently from the proportion of cold-pressed oil in the sample during the storage period. This might be due to the low antimicrobial effect of cold-pressed oils against coliforms. Karaman et al. [42] investigated the antimicrobial effect of the by-products obtained from GSO and PSO production and they reported that the by-product of GSO showed no antibacterial activity against pathogenic bacteria, while the PSO by-product had preventive effect depending on the content. In another study, Alvarez et al. [43] found that dried extract of pomegranate was less effective against *E. coli* than the other natural extracts they used in their study. While no data were available on the antimicrobial effect of cold-pressed oils against coliforms, our results show that these oils have no remarkable effect on coliforms. Correspondingly, all samples were *Salmonella*-positive at all stages of the storage period (data not shown), which indicated that the cold-pressed oils showed no preventive effect on growth of *Salmonella*.

Counts of yeasts and moulds in the patties were between 3.9 log CFU·g^{-1} and 4.7 log CFU·g^{-1} at the initial stage of storage. While it could not be verified by the mixture design approach with a model equation, it is conceivable that the cold-pressed oils showed a synergistic effect against yeasts and moulds in samples 1, 5, 9 and 12. Sagdic et al. [44] stated that grape pomace extract was effective against yeasts and moulds if used at a level of 5–10% in the beef patties. However, in the present study, cold-pressed oils were used instead of extracts and that is probably why we could not observe a notable effect of GSO when it was solely used in the recipe (Samples 2 and 11).

Sensorial quality

Tab. 5 presents the results of the sensory analysis, which indicate that reducing the fat content and adding cold-pressed oil did not affect appearance, hardness and juiciness of the products ($p > 0.05$). Aroma and overall acceptability scores changed significantly due to the oil addition ($p < 0.05$), where the highest scores were obtained

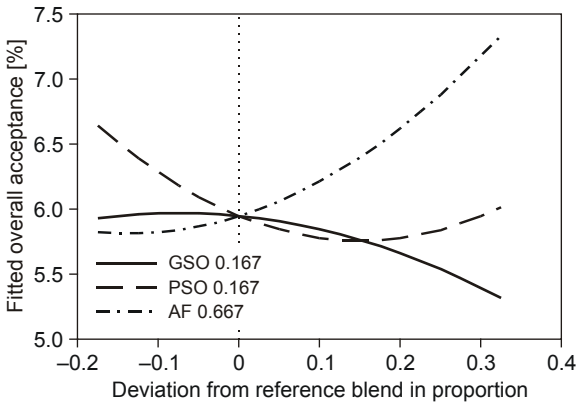

Fig. 6. Response trace plot for overall acceptability score versus proportion of ingredients.

GSO – grape seed oil, PSO – pomegranate seed oil, AF – animal fat.

for samples containing only animal fat (10 and 13). The model equation in Tab. 2 was adequate to describe the effects of ingredients on overall acceptability of the product and it had a good prediction capability (R^2, 0.88) with an insignificant lack-of-fit value ($p > 0.05$). Fig. 6 shows the effects of oils and fat on the overall acceptability score. As expected, increasing animal fat content increased the score, while the effects of oils were opposite, in particular at high proportions. This effect might have been due to the strong aromatic profile of GSO and PSO, which were produced without a refining process [45]. GSO was less effective than PSO at low levels but this situation changed at high levels. This might have been due to the taste and aroma perceptions of the panelists, since some of the panelists stated that the sour aroma of PSO appealed to their taste even at high levels. Our findings agree with the literature reporting that the decreasing fat levels resulted in low overall acceptance score in meat products [25].

Conclusion

Scientific literature provides almost no information on the effect of cold-pressed oils on the quality characteristics of patties. According to the results of the present study, it is concluded that GSO and PSO produced by the cold pressing technique could be used for the production of low-fat beef patties and could help to prevent lipid oxidation during short-term storage without affecting the microbial or physico-chemical properties. While the highest overall acceptability scores were recorded for the samples with high contents of animal fat, none of the samples were rejected or found as intolerable by the panelists. Mixture design approach was found successful in determining the optimal proportions of the ingredients and for investigating the interactions between them. High prediction capabilities of the model equations proved the success of the present study. The models developed in the present study suggest that the use of GSO and PSO, or mixture of them, in the beef patty production instead of animal fat up to 50% is possible. Our results would be useful for professionals in meat industry who develop healthier products by fat reduction while keeping the sensorial quality at acceptable level.

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Received 29 October 2015; 1st revised 7 February 2016; accepted 9 February 2016; published online 15 February 2016.