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# Functional and quality attributes of beef burgers fortified by brown linseed powder

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Submission Date: November 26th, 2021; Acceptance Date: December 29th, 2021; Publication Date: January 10th, 2022

**Please cite this article as:** Basiri S., Yousefi M.H., Shekarforoush S.S. Functional and quality attributes of beef burgers fortified by brown linseed powder. *Functional Foods in Health and Disease* 2022; 12(1): 1-11. DOI: <a href="https://www.doi.org/10.31989/ffhd.v12i1.855">https://www.doi.org/10.31989/ffhd.v12i1.855</a>

# ABSTRACT

**Background:** Beef burgers are a popular food all over the world. However, the high amounts of fat, low fiber, and frying process can cause concerns for consumers. In this study, the ability to replace some components of the burger formulation with brown linseed powder as a natural fiber has been investigated.

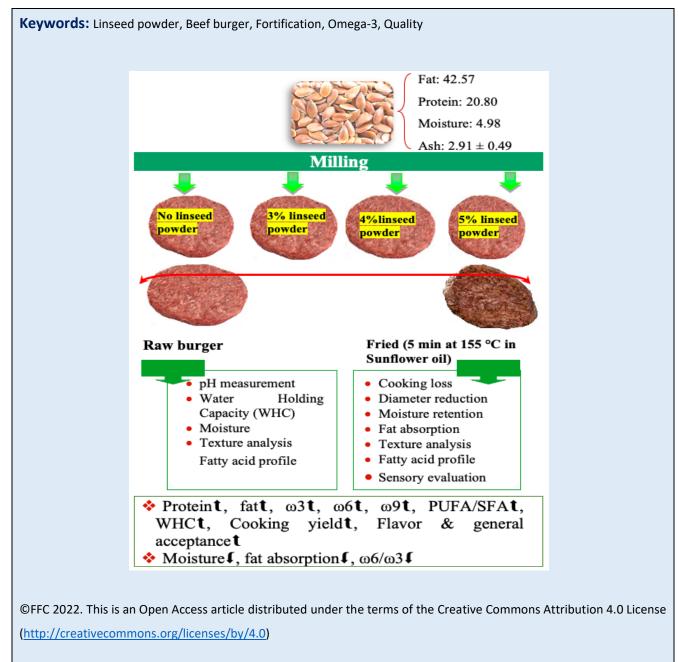
**Methods:** The brown linseed at different concentrations (3, 4, and 5%) was added to the burger formulation, and its effect on the chemical, physical, nutritional, and sensory properties of raw and fried burgers were analyzed.

**Results:** Linseed powder increased the protein and fat content and decreased the moisture of the raw burgers and fat absorption after frying. It also increased the  $\omega$ 3,  $\omega$ 6, and  $\omega$ 9 level; increased the PUFA/SFA ratio; and reduced the  $\omega$ 6: $\omega$ 3 fraction. Improving the cooking yield and water holding capacity were other benefits of this fortification. The linseed did not change the texture and sensory properties of burgers, but it improved the flavor and general acceptance.

**Conclusions:** Adding the linseed powder to the beef burger formulation is a good way for improving the yield and simultaneously improving the physicochemical property and nutritional value of the beef burger.



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# **INTRODUCTION**

Although the use of ready-to-eat and fast foods is favored worldwide, the high content of fat and low dietary fibers is the disadvantages of such foods. These diets seem to increase the likelihood of disorders such as obesity and colon cancer [1-2]. Reduction of the undesirable compounds or their replacement with useful ones and improving the production and consumption process are the best means to reduce the complications associated with these food products. Previously, various natural compounds, such as lemon albedo [3], oat fiber [4], whey protein [4], pea fiber [5], aloe vera [6], and quince seed gum [7], have been used to improve the quality of meat products.

Recently, linseed (*Linum usitatissimum*) has drawn much attention in the food industry as a functional food. This oilseed comes in two varieties: brown and golden. Both are rich in fiber, omega-3 fatty acids, and phenolic compounds [8]. However, the brown one has a higher amount of fiber and more antioxidant property [9].

Linseed contains both soluble and insoluble (cellulose and lignin) dietary fiber. The soluble fiber fraction, the mucilage gum, is composed of L-galactose, D-xylose, Larabinose, L-rhamnose, D-galacturonic acid, and Dglucose [10-11]. Linseed mucilage has a good waterholding capacity and weak gel-like properties [12]. It also can act as an anti-arrhythmic, anti-inflammatory, and vascular-protective agent and has the potential to reduce the risk of cardiovascular diseases and some hormonedependent cancers [13]. Flaxseed can be regarded as a functional food, which means the food or food ingredients may run physiological benefits and help in preventing and/or treating diseases [14]

Despite various studies on the use of linseed oil in burgers and meat products [15-16], there is little data on using linseed powder in this group of meat products [17]. The main goal of the present study was to evaluate the impact of brown linseed powder in the formulation, physicochemical, and nutritional quality of beef burgers to produce a functional product.

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## MATERIALS AND METHODS

*Linseed analysis:* Brown linseed (Giahine, Iran) was purchased from a local market in Shiraz, Iran. The seeds were cleaned and then milled and sieved (pore size 0.64 mm<sup>2</sup>). The fat [18], protein [19], ash [20], crude fiber [21] and moisture [22] of linseed powder was determined.

**Preparation of Beef Burgers:** According to Table 1, beef burgers were formulated to contain 0 (Control), 3, 4, and 5% linseed powder. The ingredients were mixed and ground by the grinder (Kenwood MG450, UK) and formed in a size of 100 grams using a forming machine, shaped between two sheets of paper, packed in four, and frozen overnight at  $-20^{\circ}$ C. Four replicates from each treatment group were made on different days from the same batch of ingredients. The samples defrosted before the analysis. For preparing the fried beef burgers, samples were defrosted and then pan-fried for 5 min at 155°C with sunflower oil. Each raw and fried sample was evaluated 3 times (n = 4×3) for chemical, physical, and sensory properties.

	Treatment groups				
Ingredients	Control	3% linseed powder	4% linseed powder	5% linseed powder	
Lean meat	64	64	64	64	
Fresh onion paste	20	20	20	20	
Gluten	2	2	2	2	
Salt	1	1	1	1	
Spices	1	1	1	1	
Phosphate	0.5	0.5	0.5	0.5	
Wheat flour	1.5	0.5	0	0	
Toasted flour	2	0	0	0	
Textured soy protein	8	8	7.5	6.5	
Linseed powder	0	3	4	5	

## Table 1. Formulation of different beef burgers

# **Raw sample Analysis**

**Proximate composition:** The soxhlet [18] and Kjeldahl methods [18] were used for measuring fat and protein

content, respectively. The ash content [20] and the dry matter of burgers were analyzed by the gravimetric method [22].

*pH measurement:* The pH value was measured by immersing the pH electrode (CG 824, Germany) into homogenates of beef burgers in distilled water (1:9) [6].

Water Holding Capacity (WHC): The centrifuge method was used to assess The WHC of raw burgers [23]. Briefly, burgers were mixed with water. After centrifugation, the WHC (%) was determined by comparing the volume of supernatant with the volume of added water per unit weight of the sample.

**Texture analysis**: A texture profile analysis (TPA) was done using a CT3 texture analyzer (Brookfield, USA), TA41 cylindrical probe (6 mm Diameter, 35 mm Length), and software Texture Pro CTV 1. 3. The test, return, and pretest were done at the same speed of 2 mm/s. The deformation value was 3 mm and the trigger was 6.8 g. Cubic samples ( $1 \times 3 \times 3$  cm) were cut and subjected to texture analysis at 3 points. Four parameters of hardness (g), cohesiveness (g/s), adhesiveness (MJ), and springiness (mm) were evaluated at each sample.

*Fatty acid composition:* Total lipids were extracted [24] and methylated [25]. The fatty acid was analyzed using the Agilent 7890 gas chromatography coupled with an Agilent 5977A mass spectroscopy detector (GC-MS) system. An Agilent DB-225MS column (30 m, 0.25 mm ID, 0.25  $\mu$ m 7in) was used for the GC system (J & W Scientific, USA). The temperature of the oven held at 120°C for 1 min, then 10°C/min to 180°C for 5 minutes, and lastly, increased by 3° C/min to 220°C. The run time was 25.33 min, injected volume was 1.0  $\mu$ L, carrier gas was helium at a pressure of 15.475 psi, and split ratio was 30:1. An ionization energy (EI) of 70 eV was used for the mass spectroscopy detector with a scan range of 35-500 amu and a scan rate of 1.562 s<sup>-1</sup>. The mass spectra compared to the NIST05a.L Mass Spectral Library 2.0.

#### **Fried sample Analysis**

*Diameter reduction (Shrinkage):* The caliber of each sample was measured before and after frying using a

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digital caliper (Insizer, code: 1112-150) at 3 randomly chosen points. Diameter reduction (%) was determined as follows [26]:

Shrinkage (%) = [(Raw burger diameter - Fried burger diameter) / Raw burger diameter)] × 100.

*Cooking loss:* Cooking loss percent of the samples was measured after weighing raw and fried hamburgers based on the following equation [27]:

Cooking loss (%) = [(Fried weight - Raw weight) / Raw weight] × 100

*Moisture retention:* Moisture retention (%) was measured by dividing the raw burgers moisture content into fried burgers moisture content and multiplies it by 100 [6].

*Fat absorption:* The fat content of the fried samples was also assessed using the soxhlet method. The fat absorption amount (%) was calculated by subtracting the fat percentage of raw burgers from the fried ones [6]. The TPA and fatty acid profile of fried burgers was determined according to the same methods of raw burgers.

Sensory evaluation: The beef burgers were presented as whole, fried, and warm to 12 trained panelists (6 men and 6 women aged 20–40) in a random order using digital codes to examine the taste, color, texture, odor, and general acceptance. The panelists were the staff of Safir meat company (Shiraz, Iran) and passed training sessions before the formal sensory evaluations. Samples were presented to panelists on a white plastic plate under natural light and evaluated on a four-point scale in which a score of 4 meant "excellent" and a score of 1 meant "unacceptable." Tap water was provided to rinse the mouth (to cleanse the palate) of the panelists after each sample tested. **Statistical analyses:** Each parameter of raw and fried burgers was analyzed separately using the Analyses of Variance (ANOVA) test. Mean values ± standard errors (SE) were reported for each case. The analysis was performed using the SPSS package (SPSS 16 for Windows, SPSS Inc, Chicago, IL, USA) with Duncan's multiple range tests for mean comparisons. The Pearson correlation method was used for quantitative data. The P-values less than 0.05 (P < 0.05) were considered significant.

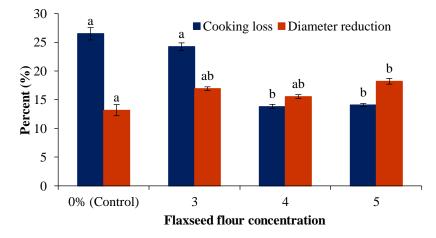
#### **RESULTS AND DISCUSSION**

**Proximate composition of linseed meal:** Mean percent moisture, protein, fat, crude fiber, and ash values of linseed powder were  $4.98 \pm 0.27$ ,  $20.80 \pm 0.46$ ,  $42.57 \pm 2.07$ ,  $25.20 \pm 1.56$ , and  $2.91 \pm 0.49$ , respectively. The results of the chemical analysis were consistent with others [28-29].

*pH measurement:* There was no significant difference in the pH of raw burgers with different concentrations (data not shown) of the linseed with the control (P > 0.05). The lowest pH was in the control group (6.52 ± 0.03). Increasing the linseed powder concentration did not affect the final pH of the product significantly (P > 0.05). Similar results were seen in the meat patties that contained linseed powder [30].

**Diameter reduction (shrinkage):** The addition of linseed powder significantly increased the diameter reduction percent of the burgers (P < 0.05) (Fig. 1). Protein denaturation and fat and water loss during cooking are the main causes of diameter reduction in beef burgers. There was a positive correlation between the fat content and the diameter reduction percent of beef burgers (P =0.001, r = 0.46). Serdaroglu and Degirmencioglu (2004) also reported that meatball shrinkage decreased by reducing the fat level [31]. The WHC of the linseed seemed to have a preventive effect on shrinkage, which is consistent with that of Soltanizadeh and Ghiasi-Esfahani (2015) [6].

**Cooking Loss:** The results of a cooking loss for different burgers show in Figure 1. Adding the linseed powder to burgers improved the cooking yield (decreased cooking loss) at all levels, which was dose-dependent (P < 0.05). This result supported the findings reported by Soltanizadeh and Ghiasi-Esfahani (2015) which used aloe vera in the beef burgers [6]. There was a significant negative correlation between the WHC and cooking loss of samples (P = 0.003, r = -0.82). The linseed can produce mucilaginous compounds that trap high amounts of water, prevent water loss, and subsequent weight loss during frying.



**Figure 1.** The Cooking Loss (%) and diameter reduction (%) of beef burgers with different linseed powder concentrations and in control. Different letters indicate a statistical significant difference (P < 0.05) (n= 4×3). Error bars indicates the standard error.

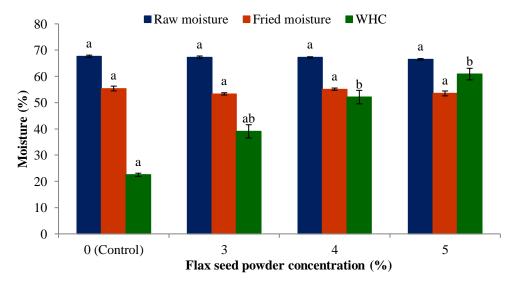
**Moisture content:** The moisture content of raw and fried beef burgers shows in Figure 2. The moisture content of all treatments was lower than that of the control group (P > 0.05). The moisture content of beef burgers decreased significantly after frying (P < 0.05), but there was no significant difference between groups (P > 0.05). The moisture retention percent was not statistically different between groups (P > 0.05). As the fat level of raw beef burgers increased, the moisture percentage decreased (P = 0.002, r = -0.44). Similar results were reported by Turhan et al. (2007) by using hazelnut pellicle in burgers [32].

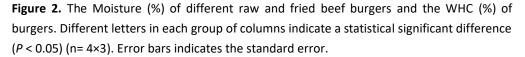
**WHC:** The WHC of the meat, defined as its ability to hold all or part of its own and added water, is one of the most important indexes for both processors and consumers

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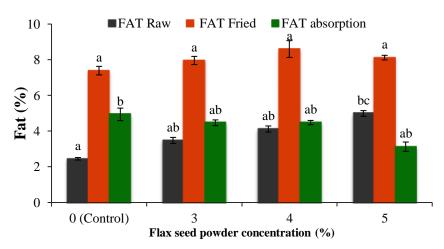
[23]. The WHC (%) in different groups of raw beef burgers is present in Figure 2. The control group had the lowest WHC (P < 0.05). Adding the linseed powder increased the WHC of beef burgers significantly (P < 0.05). Similar results were observed using quince seed gum to the lowfat beef burgers [7].

Myofibrillar protein (principally actin and myosin) plays a crucial role in the texture and WHC of meat products [33]. The linseed gum facilitates the myofibrillar protein unfolding before aggregation and makes a gel with fine pore size which retains water in myofibrillar structures by capillary forces and increases the WHC [34]. No significant differences were observed in WHC among the different burger formulas after frying.





**Fat content and fat absorption amount:** Adding linseed powder increased the fat content of raw burgers (P < 0.05) due to the linseed fat content (41.2%). Frying increased the fat of all beef burgers, but there were no significant differences between groups (P > 0.05) (Fig. 3). Similar results showed by Bilek and Turhan (2009) [30]. By increasing the linseed concentration, the fat absorption percent decreased, which was statistically significant in the sample with 5% linseed powder (P < 0.05) (Fig. 3). By adding linseed, the hydrophilic part of the fibers make gel in contact with the water and result in greater WHC and lower fat absorption. During frying, because of water loss (evaporation), dry matter and total fat content increase. Therefore, by increasing the water holding capacity, the fat absorption amount will decrease during frying.



**Figure 3.** Fat content (%) of different raw and fried beef burgers and their fat absorption (%). Different letters in each group of columns indicate a statistical significant difference (P < 0.05) (n= 4×3). Error bars indicates the standard error.

**Protein and ash percent:** Adding 3, 4, and 5% linseed powder to raw beef burger increased the protein content significantly (19.51  $\pm$  0.25, 20.19  $\pm$  0.15, and 20.61  $\pm$  0.25 respectively) compared to the control group (16.20  $\pm$  0.45) (P < 0.05). There was no significant difference in the amount of ash in the treated groups (P > 0.05), while the ash in the control group was significantly less than the others (data not shown).

*Texture analysis:* The TPA data of unruptured raw and fried burgers presents in Table 2. Linseed powder did not

change the texture parameters of raw burgers significantly (P > 0.05). There was a negative correlation between cohesiveness and adhesiveness of uncooked burgers (P = 0.00, r = -0.51).

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The frying process increased the hardness of all groups (P < 0.05), while the adhesiveness, cohesiveness, and springiness were not affected by frying (P > 0.05). In fried, linseed-contained burgers, the hardness and adhesiveness was decreased compared to the control, which may be due to the gel production and higher WHC [35].

Burgers	Texture		Treatments		
	characteristics	0% (Control)	3% linseed powder	4% linseed powder	5% linseed powder
Raw	Hardness	84.29 ± 5.59 <sup>aA</sup>	81.73 ± 4.68 <sup>aA</sup>	74.25 ± 2.74 <sup>aA</sup>	81.94 ± 2.27 <sup>aA</sup>
	Cohesiveness	0.68 ± 0.05 <sup>aA</sup>	$0.70 \pm 0.03^{aA}$	0.74 ± 0.03 <sup>aA</sup>	0.67 ± 0.05 <sup>aA</sup>
	Adhesiveness	0.57 ± 0.02 <sup>aA</sup>	0.58 ±0.02 <sup>aA</sup>	0.57 ± 0.01 <sup>aA</sup>	$0.60 \pm 0.05^{aA}$
	Spiringiness	2.61 ± 0.03 <sup>bcA</sup>	2.63 ± 0.02 <sup>cA</sup>	2.69 ± 0.01 <sup>cA</sup>	2.44 ± 0.02ªA
Fried	Hardness	520.96 ± 41.97 <sup>aB</sup>	490.41 ± 14.13 <sup>aB</sup>	479.62 ± 20.00 bA	484.50 ± 14.68 <sup>aB</sup>
	Cohesiveness	0.86 ± 0.02 <sup>aA</sup>	$0.81 \pm 0.02^{aA}$	0.84 ± 0.01 <sup>aA</sup>	$0.81 \pm 0.02^{aA}$
	Adhesiveness	0.41 ± 0.01 <sup>aA</sup>	$0.45 \pm 0.02^{aA}$	0.41 ± 0.02 <sup>aA</sup>	0.38 ± 0.01 <sup>aA</sup>
	Spiringiness	2.68 ± 0.03 <sup>aA</sup>	2.71 ± 0.01 <sup>aA</sup>	2.70 ± 0.03 <sup>aA</sup>	2.66 ± 0.05 <sup>aA</sup>

Table 2. Mean ± SE TPA parameters of raw and fried burgers with different concentrations of linseed powder

Values are Mean  $\pm$  Standard Error. The different uppercase letters indicate significant differences between the raw and fried samples (*P* < 0.05). The different lowercase letters indicate significant differences in the row (*P* < 0.05).

Fatty acid composition: The fatty acid profile of raw and fried burgers is presented in Table 3. The amount of saturated fatty acid (SFA) increased by adding linseed powder, which was significant at 4% and 5% concentrations. The predominant SFA of burgers was palmitic acid (16:0), stearic acid (18:0), and myristic acid (14:0), respectively. There was no significant difference in the MUFA content of different groups (P > 0.05). Adding linseed powder increased the PUFA,  $\omega$ 3 ( $\alpha$ -Linolenic acid and Eicosatrienoic acid), and  $\omega 9$  (Oleic acid) content of burgers, significantly (P < 0.05). This increase was concentration-dependent, and the highest amount was in burgers with 5% linseed powder (P < 0.05). The  $\omega$ 6 (Linoleic acid and Arachidonic acid) content of burgers increased by adding linseed powder, which was only significant in the 5% linseed powder group.

By frying, despite a relative decrease in  $\omega$ 3 content, the linseed-contained burgers had higher  $\omega$ 3 content than the control group (P < 0.05), and the highest amount was in groups with 5% linseed powder. Our results were consistent with Bilek and Turhan (2009) [30]. The SFA, MUFA, and PUFA/SFA ratios were not significantly different between treatments and the control (P > 0.05). The  $\omega$ 6 and  $\omega$ 9 contents increased in the linseed-contained groups, but it was not significant (P > 0.05).

The significant factor in the fatty acid's usefulness is not their definite value, but the ratio between various fatty acids [36]. Adding the linseed powder to the raw burgers significantly increased the PUFA/SFA of burgers (P < 0.05). Other researchers have shown the same results [15, 37]. Increase PUFA/SFA ratio may decrease the incidence of cardiovascular disease [38].

Linseed powder also decreased the  $\omega 6:\omega 3$  ratio in raw and fried beef burgers significantly (P < 0.05), and burgers containing 5% linseed powder had the lowest ratio. The  $\omega 6$  and  $\omega 3$  are essential PUFAs that must be omitted from the diet. Today, the  $\omega 6:\omega 3$  ratio in the diet has increased to 15–20: [36], which increases the risk of chronic diseases. By reducing this ratio to 4:1, Simopoulos (2002) observed a reduction in the total mortality and rate of breast cancer [39]. In addition to all the positive effects of flaxseed, fried products such as hamburgers should be consumed cautiously.

**Table 3.** Mean ± SE of different fatty acids (gr/ 100 g burger) in the fat content of raw and fried control burgers with different concentrations of the linseed powder

		Treatments			
Burgers	Parameter	0% (Control)	3% linseed powder	4% linseed powder	5% linseed powder
Raw	SFA	0.83 ± 0.02 <sup>a</sup>	0.92 ± 0.04 <sup>ab</sup>	0.97 ± 0.05 <sup>bc</sup>	1.06 ± 0.02°
	MUFA	$0.10 \pm 0.00^{a}$	0.09 ± 0.02ª	0.09 ± 0.01ª	0.11 ±0.02ª
	PUFA	$0.55 \pm 0.06^{a}$	1.32 ± 0.08 <sup>b</sup>	1.78 ± 0.13 <sup>c</sup>	$2.43 \pm 0.02^{d}$
	ω3	$0.02 \pm 0.00^{a}$	$0.80 \pm 0.06^{b}$	$1.09 \pm 0.05^{b}$	1.49 ± 0.01 <sup>c</sup>
	ω6	$0.52 \pm 0.06^{a}$	0.53 ± 0.03ª	0.69 ± 0.08 <sup>ab</sup>	0.95 ± 0.01 <sup>b</sup>
	ω9	$0.87 \pm 0.03^{a}$	$1.06 \pm 0.01^{b}$	$1.19 \pm 0.11^{bc}$	1.36 ± 0.01 <sup>c</sup>
	PUFA/SFA	$0.66 \pm 0.09^{a}$	1.45 ± 0.15 <sup>b</sup>	1.86 ± 0.23 <sup>bc</sup>	2.29 ± 0.06 <sup>c</sup>
	ω 6/ ω3	22.44 ± 2.60 <sup>a</sup>	0.67 ± 0.02 <sup>b</sup>	0.64 ± 0.05 <sup>b</sup>	$0.63 \pm 0.00^{b}$
Fried	SFA	0.89 ± 0.34 <sup>a</sup>	1.40 ± 0.13ª	1.51 ± 0.07 <sup>a</sup>	1.36 ± 0.17 <sup>a</sup>
	MUFA	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$
	PUFA	2.78 ± 1.07 <sup>a</sup>	4.16 ± 0.15 <sup>ab</sup>	$4.50 \pm 0.02^{ab}$	$4.39 \pm 0.27^{ab}$
	ω3	$0.01 \pm 0.00^{a}$	$0.53 \pm 0.01^{b}$	0.78 ± 0.19 °	0.57 ± 0.33 <sup>c</sup>
	ω6	2.78 ± 1.07 <sup>a</sup>	3.89 ± 0.13 <sup>ab</sup>	3.72 ± 0.14 <sup>ab</sup>	3.82 ± 0.07 <sup>ab</sup>
	ω9	1.54 ± 0.57ª	2.19 ± 0.05 <sup>ab</sup>	$2.41 \pm 0.02^{ab}$	$2.20 \pm 0.01^{ab}$
	PUFA/SFA	3.14 ± 0.03 ª	3.01 ± 0.40 ª	2.99 ± 0.13 ª	3.31 ± 0.62 ª
	ω 6/ ω3	278.00 ± 107.00 ª	7.34 ± 0.38 <sup>b</sup>	4.98 ± 1.05 <sup>b</sup>	4.39 ± 0.23 <sup>b</sup>

The different letters indicate significant differences between different treatments (P < 0.05). SFA: Saturated Fatty Acid; MUFA: Mono Unsaturated Fatty Acid; PUFA: Poly Unsaturated Fatty Acid.

Sensory properties: Changes in the sensory properties of beef burger with different concentrations of linseed powder are represented in Table 4. The addition of linseed (3, 4, and 5%) to burgers did not significantly change the color, texture or odor of the beef burgers (P > 0.05), which was similar to the results of Ghafouri-Oskuei et al. (2020) by adding 3% linseed and tomato powder to the sausage [40]. In a study conducted by Bilek and Turhan (2009), the addition of linseed powder reduced the sensory value of beef patties [30]. In our study, adding 4 and 5% linseed significantly improved the flavor and general acceptance of beef burgers (P < 0.05), which can attribute to the volatile compounds (mainly acetaldehyde, ethanol, hexanal, and 2-methylbutanal) in the linseed [41].

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Table 4. Mean ± SE sensory score of fried beef burger with different concentrations of linseed powder.

Burgers	Sensory attributes	Treatments			
		0% (Control)	3% linseed powder	4% linseed powder	5% linseed powder
Fried	Flavor	2.66 ± 0.07ª	2.74 ± 0.08 <sup>ab</sup>	$2.92 \pm 0.09^{b}$	2.98 ± 0.07 <sup>c</sup>
	Color	2.94 ± 0.08ª	$3.00 \pm 0.07^{a}$	2.82 ± 0.07ª	2.92 ± 0.10 <sup>a</sup>
	Odor	2.91 ± 0.11ª	2.91 ± 0.09ª	$3.04 \pm 0.08^{a}$	3.12 ± 0.12 <sup>a</sup>
	Texture	2.70 ± 0.10ª	2.77 ± 0.11ª	$2.80 \pm 0.08^{a}$	2.78 ± 0.09ª
	General acceptance	$2.81 \pm 0.10^{a}$	$2.84 \pm 0.07^{ab}$	3.16 ± 0.07 <sup>c</sup>	3.06 ± 0.09 <sup>bc</sup>

Values are Mean ± Standard Error. The different uppercase letters indicate significant differences between the raw and fried samples (P < 0.05). The different lowercase letters indicate significant differences in the row (P < 0.05). Sensory score rating: 1= Unacceptable; 2= Acceptable; 3=Good; 4=Excellent.

# CONCLUSION

The results indicated that adding brown linseed powder to the beef burgers improves most quality attributes. The high amounts of dietary fibers produce a gell with the water and consequently increase the WHC and cooking yield. Considering the fatty acid profile evaluation results, the high quantity of  $\omega$ 3 fatty acids improves the  $\omega$ 6/ $\omega$ 3 of fried burgers and proposes linseed as a functional ingredient to improve physicochemical and nutritional properties of beef burgers.

List Of Abbreviations: PUFA: Poly Unsaturated Fatty Acid, SFA: Saturated Fatty Acid, MUFA: Mono Unsaturated Fatty Acid, WHC: Water Holding Capacity, TPA: Texture Profile Analysis. Author's Contributions: S. Basiri and S.S. Shekarforoush designed and supervised the research. M.H. Yousefi performed the tests and collected data. S. Basiri performed statistical analyses and wrote the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments: The authors acknowledged the research affair of Shiraz University and the Safir Meat Products Company for technical assistance.

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**Competing Interests:** There is no competing interest.

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