

## The effect of golden flaxseed and by-product addition in beef patties: physicochemical properties and sensory acceptance

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### Abstract

Aiming to improve the nutritional profile of meat products, the present work evaluated the addition effect of different golden flaxseed blends and by-products on the physicochemical composition and sensory acceptance of beef patties. Beef patties were added with golden flaxseed blends (oil and/or flour and/or seed), varying from 2.5% to 5%, plus a control formulation, totalling nine formulations. Raw and cooked products were evaluated in relations to proximate composition, energy, cholesterol, pH, water activity, colour and fatty acids content. Consumer study evaluated the acceptability of the formulated beef patties. Levels of ash, protein, fat, carbohydrate and energy increased after blends addition in patties; however, the moisture and cholesterol levels decreased. There was little variation in pH and water activity between the formulations. Higher blend contents increased lightness and yellowness, but decreased redness of beef patties. Golden flaxseed addition increased *n*-3 fatty acids content, mainly with the oil use. Higher flaxseed levels reduced attributed notes to flavour and texture attributes, but there were no changes to appearance and aroma. Blends addition of golden flaxseed and by-products improved beef patties nutritional profile, but higher levels of blends reduced their acceptability..

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### Keywords

*Linum usitatissimum* L.

Acceptability

Meat product

### Introduction

Meat and meat products are foods with high consumption rate around the world. However, several epidemiological studies have been associated with meat consumption such as the development of chronic cardiovascular diseases and cancers (Cross *et al.*, 2007; Kontogianni *et al.*, 2008; Kim *et al.*, 2013; Bovalino *et al.*, 2016). Some nutrients present in red meat were evidenced as being responsible for these associations. Examples of these nutrients are fat, cholesterol and saturated fatty acids (SFA) (Kim *et al.*, 2013). Besides, a possible formation of carcinogenic compounds such as heterocyclic amines, which are formed in meat cooked at high temperatures has also been elucidated (Cross *et al.*, 2010). Therefore, the reformulation of processed meat products could be one of the main strategies for new products development that promote benefits to

humans through its functional properties. For this, ingredients such as flours, oils and probiotics have already been evaluated by the scientific community (Elif Bilek and Turhan, 2009; Olmedilla-Alonso *et al.*, 2013; Alejandre *et al.*, 2016).

A food that is noteworthy for its use in reformulated meat products is flaxseed (*Linum usitatissimum* L.), since it is classified as a functional food. The most reported benefits on flaxseed consumption are due to high levels of  $\alpha$ -linolenic fatty acid C18:3 *n*-3 (23%), lignans (26 mg 100 g<sup>-1</sup>) and dietary fibre (28 g 100 g<sup>-1</sup>). Original flaxseed oil contains high levels of polyunsaturated fatty acids (PUFA) (73%) and monounsaturated fatty acid (MUFA) (18%). Also present are low levels of SFA (9%), which is beneficial for health. Flaxseed oil is the biggest known source of *n*-3 fatty acids, comprising 57% of total fatty acids (Morris *et al.*, 2007).

Many researches have shown that the addition

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of flaxseed and its derivatives as an ingredient could improve the nutritional profile of reformulated meat products. However, there may be some sensory acceptability reduction among the consumers, which limits flaxseed high amount addition in meat products (Pelser *et al.*, 2007; Elif Bilek and Turhan, 2009). A more favourable nutritional profile in meat was also verified when animals chow was supplemented with *n-3* fatty acids from flaxseed and derivatives. Moreover, using this meat as a raw material in meat products has been shown to be effective in improving fatty acid content (Hoz *et al.*, 2004; Santos *et al.*, 2008; Turner *et al.*, 2015). Despite the different strategies reported for flaxseed addition in meat products, no evaluations have been found on joint use of flaxseed derivatives such oil, flour and seed in beef patties.

The objective the present work was therefore to evaluate the addition effect of golden flaxseed blends and by-products in beef patties, in relation to physicochemical and sensorial properties, in order to improve the nutritional profile of the product, thereby making it healthier for human consumption.

## Materials and methods

### Ingredients

Beef chuck (55 kg) and shoulder clod (55 kg) cuts were obtained from local butcher. The other ingredients were obtained from regional suppliers. The flaxseed flour was prepared from the golden flaxseed that were milled in a laboratory blender (04245-20 model, Waring Commercial®, Stamford, Connecticut, USA).

The proximate composition (g 100 g<sup>-1</sup>), energy (kcal 100 g<sup>-1</sup>) and fatty acid composition (g 100 g<sup>-1</sup> product) of golden flaxseed seed/flour, oil and beef meat used in the beef patties were determined in below.

Golden flaxseed (seed/flour; oil) contained, respectively: moisture (4.30; 0.06), ash (3.77; 0.02), protein (23.14; 0), fat (35.62; 99.92), carbohydrate (33.17; 0), energy (542.05; 899.28), C12:0 (0.01; 0.02), C14:0 (0.03; 0.05), C15:0 (0.01; 0.02), C16:0 (1.93; 5.49), C16:1 *n-7* (0.05; 0.09), C17:0 (0.02; 0.06), C17:1 *n-7* (0.02; 0.04), C18:0 (1.44; 3.86), C18:1 *n-9* (8.70; 21.31), C18:2 *n-6* (5.14; 17.41), C18:2 *n-6t* (0.02; 0.05), C18:3 *n-3* (16.28; 46.15), C18:3 *n-3t* (0.08; 0.19), C20:0 (0.06; 0.19), C20:1 *n-9* (0.06; 0.17), C22:0 (0.06; 0.17), C24:0 (0.05; 0.11), PUFA/SAF ratio (5.93; 6.38) and *n-6/n-3* ratio (0.32; 0.38).

The beef contained: moisture (75.28), ash (0.99), protein (20.78), fat (2.96), carbohydrate (0), energy (110.11), C14:0 (0.07), C15:0 (0.01), C16:0 (0.58), C16:1 *n-7* (0.06), C17:0 (0.04), C17:1 *n-7* (0.02), C18:0 (0.57), C18:1 *n-9* (0.98), C18:1 *n-9t* (0.08), C18:2 *n-6* (0.07), C18:2 *n-6t* (0.01), C18:3 *n-3* (0.03), C18:3 *n-3t* (0.03), C18:4 *n-3* (0.01), C20:0 (0.01), C22:5 *n-3* (0.01), PUFA/SAF ratio (0.09) and *n-6/n-3* ratio (1.40).

Ingredients and blend levels of oil and/or flour and/or seed golden flaxseed used in beef patties formulations were selected after preliminary testing (Table 1). The statistical design was completely randomised, containing nine treatments and three replicates.

### Beef patties processing and cooking

Meat was ground in a meat grinder (C.A.F.®, São Paulo, Brazil) with a 3 mm disk and separated into nine formulations. Each formulation was homogenised in a mixer (Super Cutter Sire®, São Paulo, Brazil) for approximately 3 min. Ingredients were added in the following order: half of ice, seasonings (onion and garlic powder), sodium erythorbate and the rest of ice, salt, carrageenan, maltodextrin, palm oil and the corresponding levels of oil and/or flour and/or seed

Table 1. Beef patty formulations with different additions of golden flaxseed blends.

| Ingredient (%)        | F1    | F2    | F3    | F4    | F5    | F6    | F7    | F8    | F9    |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Beef                  | 75.85 | 68.35 | 70.85 | 70.85 | 70.85 | 60.85 | 65.85 | 65.85 | 65.85 |
| Ice flake             | 15.0  | 15.0  | 15.0  | 15.0  | 15.0  | 15.0  | 15.0  | 15.0  | 15.0  |
| Palm oil              | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   |
| Maltodextrin          | 1.8   | 1.8   | 1.8   | 1.8   | 1.8   | 1.8   | 1.8   | 1.8   | 1.8   |
| Salt                  | 1.5   | 1.5   | 1.5   | 1.5   | 1.5   | 1.5   | 1.5   | 1.5   | 1.5   |
| Carrageenan           | 0.2   | 0.2   | 0.2   | 0.2   | 0.2   | 0.2   | 0.2   | 0.2   | 0.2   |
| Onion powder          | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   |
| Garlic powder         | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   |
| Sodium erythorbate    | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  |
| Golden flaxseed oil   | -     | 2.5   | 2.5   | -     | 2.5   | 5.0   | 5.0   | -     | 5.0   |
| Golden flaxseed flour | -     | 2.5   | -     | 2.5   | 2.5   | 5.0   | 5.0   | 5.0   | -     |
| Golden flaxseed seed  | -     | 2.5   | 2.5   | 2.5   | -     | 5.0   | -     | 5.0   | 5.0   |

of golden flaxseed. Formulations were packed in low density polyethylene (LDPE) plastic bags and stored in freezer (0°C to -1°C) for approximately 1 h. Meat dough was moulded in units of 110 g (10 cm in diameter) in a manual burger modeler (Müller®, São Paulo, Brazil). All formulations were frozen (-18°C) throughout the experiment.

Frozen beef patties were grilled on an electric plate, containing grill on upper and lower sides (George Foreman® jumbo size, Beachwood, New Jersey, USA) and heated to 200°C (8 to 10 min). Internal beef patties temperature was controlled by digital thermometer (B 345 model, Micronal®, São Paulo, Brazil) with coupled thermocouple, until reaching 75°C.

#### Physicochemical characterisation

Moisture analyses (drying at 105°C to constant weight), ash (muffle at 550°C) and protein (Kjeldahl; factor of 6.25) were determined according to AOAC (1990). Fat content was evaluated by Bligh and Dyer (1959) method. Carbohydrate was estimated by subtracting 100 from obtained values for moisture, ash, protein and fat. Energy content (kcal) was calculated based on Atwater values (or combustion heat) to fat (9 kcal g<sup>-1</sup>), protein (4.02 kcal g<sup>-1</sup>) and carbohydrate (3.87 kcal g<sup>-1</sup>) (Atwater and Woods, 1896). All analyses were performed in triplicate.

To determination the fatty acid composition, lipids were extracted from golden flaxseed (seed/flour and oil), beef meat and beef patties (Bligh and Dyer, 1959). Subsequently, methyl esters were saponified and esterified in triplicate (Hartman and Lago, 1973). SFA, MUFA and PUFA profiles were determined using capillary gas chromatograph (6850 Series GC System, Agilent®, Santa Clara, California, USA) containing Agilent DB-23 capillary column (50% cyanopropyl-methylpolysiloxane), dimensions 60 m; Ø int: 0.25 mm and 0.25 µm film. Chromatograph operating conditions were: column flow of 1.00 mL min<sup>-1</sup>, linear velocity 24 cm sec<sup>-1</sup>, detector temperature 280°C, injector temperature 250°C and oven temperature 110°C - 5 min, 110-215°C (5°C min<sup>-1</sup>), 215°C - 24 min. Helium gas (White Martins®, Campinas, São Paulo, Brazil) was used as the entrainment gas, and an aliquot of 1 µL of the samples was injected into the apparatus. Fatty acid identification was performed by comparing sample peak retention times with peak retention times of standard peaks. The following individual fatty acid standards were purchased (Merck®, Darmstadt, Hessen, Germany): SFA (7:0 a 24:0); MUFA (C16:1 *n*-7, C17:1 *n*-7, C18:1 *n*-9, C18:4 *n*-3, C20:1 *n*-9, C22:5 *n*-3); PUFA (C18:2 *n*-6, C18:3 *n*-3, C22:5 *n*-3)

and *trans* fatty acids (C18:1 *trans* 9, C18:2 *trans* 6, C18:2 *trans* 9, *trans* 12 and C18:3 *trans*). Results were obtained in area percentage (%) and converted to g 100 g<sup>-1</sup>.

Cholesterol composition was evaluated according to Al-Hasani *et al.* (1993) in triplicate. Sample was saponified with alcoholic KOH (Merck®, Darmstadt Hessen, Germany) and non-saponifiable fraction was extracted with hexane (Merck®, Darmstadt, Hessen, Germany) and concentrated extract was injected into a non-derivatised gas chromatograph (5890 Series II GC, Hewlett Packard®, Palo Alto, California, USA) with FID detector and injector Split 1:100 containing HP-5 capillary column. Chromatograph operating conditions were: column temperature 160-270°C (10°C min<sup>-1</sup>), detector temperature 270°C and injector temperature 250°C. Helium gas (White Martins®, Campinas, São Paulo, Brazil): 1 mL min<sup>-1</sup>, H<sub>2</sub> gas (White Martins®, Campinas, São Paulo, Brazil): 20 mL min<sup>-1</sup>, N<sub>2</sub> gas (White Martins®, Campinas, São Paulo, Brazil) - auxiliary: 30 mL min<sup>-1</sup> and synthetic air: 300 mL min<sup>-1</sup>. Cholesterol identification was performed by comparing samples retention time with injected standard and quantification through corresponding areas of peaks by internal standardisation. Cholesterol and 5- $\alpha$ -cholestane standards (Merck®, Darmstadt, Hessen, Germany) were compared, establishing by standard cholesterol curve that was used to analyse cholesterol in mg 100 g<sup>-1</sup> of sample.

Water activity ( $a_w$ ) was determined using  $a_w$  meter (AquaLab Series 3 TE, Decagon®, Sockburn, Christchurch, New Zealand) at 25°C. Samples pH was measured using a digital potentiometer (MP125 pH Meter, Mettler Toledo®, Columbus, Ohio, USA). Both analyses were performed in triplicate.

Colour determination was performed using Colorquest II colorimeter (Hunter-Lab®, USA) according to CIE  $L^*$  (lightness)  $a^*$  (redness) and  $b^*$  (yellowness) system. Apparatus was previously calibrated, operating with illuminant D65, 10° observer angle, in RSEX (specular reflectance excluded) mode. Six beef patties of each formulation were randomly selected for colour determination. Colour parameters were evaluated at three different points for each beef patties.

#### Consumer study

For conducting the sensory test, beef patties were cooked as previously described. All samples were evaluated by means of an acceptance test using a nine-point hedonic scale, with extremes ranging from dislike extremely (1) to like extremely (9) (Meilgaard *et al.*, 1999). The evaluated attributes were related to appearance, aroma, flavour and texture. Overall

acceptance and purchase intent questions were evaluated by means of two specific points: "yes" (liked and would buy) and "no" (did not like and would not buy) (Moskowitz, 1994).

Participated in sensory analyses were 50 untrained volunteer subjects who were beef patties usual consumers. Consumers aged between 18 to 60 years old and were recruited among students and staff of Universidade Estadual de Campinas, São Paulo, Brazil. Each sample was served to consumers in white plates coded with randomly selected 3-digit numbers in monadic form and using balanced design (Macfie *et al.*, 1989). Sensory evaluations were performed by consumers under fluorescence lighting. After consuming each sample, consumer was instructed to drink water for palate cleansing. Samples were evaluated in triplicate in separate session.

#### Statistical analysis

The results were analysed using analysis of variance (ANOVA). The means were compared by Tukey's test at 5% significance level ( $p \leq 0.05$ ), with support the Statgraphics® Plus, 5.1 version (Warrenton, Virginia, USA).

## Results and discussion

#### Physicochemical characterisation

The proximate composition, energy value, cholesterol, pH and  $a_w$  of beef patties formulated with golden flaxseed blends are shown in Table 2.

Golden flaxseed addition decreased the moisture contents in raw and cooked beef patties ( $p < 0.05$ ), mainly in formulation with 5% of seed, flour and oil (F6). Despite of this, there was an increase in the ash, carbohydrates and energy contents in these products, which occurred with greater relevance in formulations containing flaxseed flour and seed. Similar effects were verified by Elif Bilek and Turhan (2009) and Turhan *et al.* (2005; 2007), who evaluated beef patties added with flaxseed flour and beef burgers containing hazelnut pellicle and beef patties with wet okara, respectively. Protein content increased for raw and cooked formulations of F4, F7, F8 and F9, which could be explained by the higher flaxseed protein content (23.1%) when compared to beef (20.8%). Similarly, a lipid increase was also observed for all raw and cooked formulations added with flaxseed derivatives. These formulations with flaxseed oil addition are highlighted, since they present a high fat content (99.9%) as compared to beef (3.0%). Moreover, fat has higher amount of energy (9 kcal g<sup>-1</sup>) when compared to protein (4.02 kcal g<sup>-1</sup>) and carbohydrate (3.87 kcal g<sup>-1</sup>) (Atwater and

Woods, 1896). Thus, there was a significant increase ( $p < 0.05$ ) in energy value of formulations containing higher flaxseed oil contents (F6, F7 and F9).

Reduction in cholesterol content was more relevant to raw (25.5% to 12.7%) and cooked (23.1% to 13.4%) beef patties containing higher flaxseed contents (F6, F7, F8 and F9) ( $p < 0.05$ ). This difference might have occurred due to lower beef amount. These results corroborate with Kayaardi and Gök (2004), who studied olive oil addition in sucuk; and Marquez *et al.* (1989) who evaluated peanut oil effect on beef frankfurters. The favourable results for the reduction of cholesterol in beef patties are in line with the recommendation for lower daily cholesterol consumption (300 mg) (USDA and HHS, 2010). The intent is to reduce chronic non-communicable diseases related to high cholesterol consumption, such as cardiovascular diseases (Larsson *et al.*, 2012). Cooking promoted moisture loss in beef patties, as also shown by other studies (Serdaroğlu, 2006; Turhan *et al.*, 2007; Elif Bilek and Turhan, 2009).

There was little variability between formulations on pH levels. F6 which contained the highest concentrations of golden flaxseed derivatives, presented the lowest pH ( $p < 0.05$ ). After cooking, there was an increase in pH for all products. Lower pH levels were observed in cooked formulations F1, F2, F3 and F4, which had the lowest flaxseed contents. The pH results for beef patties are close to normal meat, which ranges from 5.5 to 6.4 (Warriss, 2000; Viljoena *et al.*, 2002). Thereby, golden flaxseed blends addition did not promote the beef patties protein denaturation. Higher  $a_w$  levels in the raw samples were verified for F7 as compared to control. In cooked beef patties, the highest  $a_w$  levels were found in F7, F8 and F9. There was no significant difference between the other raw and cooked formulations ( $p > 0.05$ ), and there was little variation in  $a_w$  values between the samples. The  $a_w$  values of raw and cooked formulations remained within the recommended normal levels for meats (0.95 - 1.00). These values allowed for most microorganism's development (Banwart, 2004), and making meat products highly perishable. Similar results were verified by Passos and Kuaye (2002) who studied different hamburgers cooking times.

The instrumental colour results are shown in Table 3. The golden flax seed addition reduced the lightness of raw beef patties. The oil presence increased  $L^*$  values in general ( $p < 0.05$ ), since it had a yellowish appearance. Similar results were verified by Dreeling *et al.* (2002) and Jeong *et al.* (2007) evaluating hamburgers containing different fat levels. The addition of golden flaxseed and by-products in

Table 2. Proximate composition, energy value, cholesterol, pH and water activity of beef patties formulated with different additions of golden flaxseed blends.

| Parameters                               | F1                         | F2                          | F3                         | F4                         | F5                         | F6                         | F7                         | F8                          | F9                         |
|--|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|
| <b>Raw patties</b>                       |                            |                             |                            |                            |                            |                            |                            |                             |                            |
| Moisture (g 100 g <sup>-1</sup> )        | 74.22 ± 0.04 <sup>a</sup>  | 67.28 ± 0.04 <sup>e</sup>   | 70.75 ± 0.14 <sup>b</sup>  | 68.87 ± 0.13 <sup>d</sup>  | 70.43 ± 0.06 <sup>c</sup>  | 60.81 ± 0.01 <sup>h</sup>  | 64.95 ± 0.03 <sup>g</sup>  | 65.90 ± 0.03 <sup>f</sup>   | 65.01 ± 0.01 <sup>g</sup>  |
| Ash (g 100 g <sup>-1</sup> )             | 1.61 ± 0.01 <sup>d</sup>   | 1.95 ± 0.08 <sup>b</sup>    | 1.77 ± 0.05 <sup>cd</sup>  | 1.97 ± 0.06 <sup>b</sup>   | 1.79 ± 0.04 <sup>cd</sup>  | 2.41 ± 0.03 <sup>a</sup>   | 1.98 ± 0.06 <sup>b</sup>   | 2.40 ± 0.01 <sup>a</sup>    | 1.98 ± 0.06 <sup>b</sup>   |
| Protein (g 100 g <sup>-1</sup> )         | 15.95 ± 0.02 <sup>c</sup>  | 15.40 ± 0.03 <sup>d</sup>   | 15.25 ± 0.18 <sup>d</sup>  | 16.32 ± 0.08 <sup>b</sup>  | 15.55 ± 0.08 <sup>d</sup>  | 16.08 ± 0.01 <sup>c</sup>  | 16.49 ± 0.01 <sup>a</sup>  | 16.42 ± 0.06 <sup>ab</sup>  | 16.47 ± 0.01 <sup>a</sup>  |
| Fat (g 100 g <sup>-1</sup> )             | 6.20 ± 0.05 <sup>g</sup>   | 11.40 ± 0.12 <sup>c</sup>   | 9.40 ± 0.04 <sup>e</sup>   | 8.89 ± 0.06 <sup>f</sup>   | 9.38 ± 0.07 <sup>c</sup>   | 15.38 ± 0.07 <sup>a</sup>  | 12.96 ± 0.10 <sup>b</sup>  | 9.83 ± 0.03 <sup>d</sup>    | 12.94 ± 0.08 <sup>b</sup>  |
| Carbohydrate (g 100 g <sup>-1</sup> )    | 2.02 ± 0.02 <sup>e</sup>   | 3.99 ± 0.02 <sup>b</sup>    | 2.83 ± 0.06 <sup>d</sup>   | 3.96 ± 0.05 <sup>b</sup>   | 2.84 ± 0.13 <sup>d</sup>   | 5.31 ± 0.10 <sup>a</sup>   | 3.62 ± 0.01 <sup>c</sup>   | 5.44 ± 0.09 <sup>a</sup>    | 3.59 ± 0.08 <sup>c</sup>   |
| Energy value (kcal 100 g <sup>-1</sup> ) | 127.71 ± 0.44 <sup>g</sup> | 179.71 ± 1.14 <sup>e</sup>  | 156.87 ± 0.55 <sup>f</sup> | 160.90 ± 1.08 <sup>e</sup> | 157.96 ± 0.44 <sup>f</sup> | 223.60 ± 0.22 <sup>a</sup> | 197.02 ± 0.89 <sup>b</sup> | 175.58 ± 0.05 <sup>d</sup>  | 196.61 ± 0.66 <sup>b</sup> |
| Cholesterol (mg 100 g <sup>-1</sup> )    | 19.69 ± 0.39 <sup>a</sup>  | 17.87 ± 0.20 <sup>ab</sup>  | 18.66 ± 0.90 <sup>ab</sup> | 18.78 ± 0.68 <sup>ab</sup> | 18.61 ± 0.98 <sup>ab</sup> | 14.66 ± 0.22 <sup>c</sup>  | 17.01 ± 0.61 <sup>b</sup>  | 17.25 ± 0.04 <sup>b</sup>   | 17.18 ± 0.64 <sup>b</sup>  |
| pH                                       | 5.94 ± 0.01 <sup>ab</sup>  | 5.97 ± 0.01 <sup>ab</sup>   | 5.93 ± 0.01 <sup>b</sup>   | 5.92 ± 0.00 <sup>b</sup>   | 5.84 ± 0.01 <sup>cd</sup>  | 5.81 ± 0.01 <sup>d</sup>   | 5.85 ± 0.02 <sup>c</sup>   | 5.98 ± 0.03 <sup>a</sup>    | 5.92 ± 0.01 <sup>b</sup>   |
| a <sub>w</sub>                           | 0.97 ± 0.00 <sup>b</sup>   | 0.98 ± 0.00 <sup>ab</sup>   | 0.98 ± 0.00 <sup>ab</sup>  | 0.98 ± 0.00 <sup>ab</sup>  | 0.98 ± 0.00 <sup>ab</sup>  | 0.98 ± 0.00 <sup>ab</sup>  | 0.99 ± 0.00 <sup>a</sup>   | 0.98 ± 0.00 <sup>ab</sup>   | 0.98 ± 0.00 <sup>ab</sup>  |
| <b>Cooked patties</b>                    |                            |                             |                            |                            |                            |                            |                            |                             |                            |
| Moisture (g 100 g <sup>-1</sup> )        | 68.59 ± 0.00 <sup>a</sup>  | 60.19 ± 0.04 <sup>g</sup>   | 64.54 ± 0.08 <sup>c</sup>  | 64.16 ± 0.02 <sup>d</sup>  | 65.65 ± 0.04 <sup>b</sup>  | 55.15 ± 0.05 <sup>i</sup>  | 56.88 ± 0.08 <sup>h</sup>  | 62.01 ± 0.09 <sup>e</sup>   | 60.70 ± 0.07 <sup>f</sup>  |
| Ash (g 100 g <sup>-1</sup> )             | 2.07 ± 0.03 <sup>c</sup>   | 2.33 ± 0.03 <sup>b</sup>    | 2.14 ± 0.07 <sup>e</sup>   | 2.32 ± 0.13 <sup>b</sup>   | 2.11 ± 0.01 <sup>c</sup>   | 2.72 ± 0.08 <sup>a</sup>   | 2.30 ± 0.07 <sup>b</sup>   | 2.71 ± 0.16 <sup>a</sup>    | 2.28 ± 0.11 <sup>b</sup>   |
| Protein (g 100 g <sup>-1</sup> )         | 16.82 ± 0.03 <sup>b</sup>  | 16.61 ± 0.06 <sup>c</sup>   | 16.27 ± 0.09 <sup>d</sup>  | 16.98 ± 0.05 <sup>ab</sup> | 16.49 ± 0.07 <sup>cd</sup> | 16.71 ± 0.03 <sup>b</sup>  | 17.15 ± 0.07 <sup>a</sup>  | 17.13 ± 0.09 <sup>a</sup>   | 17.11 ± 0.10 <sup>a</sup>  |
| Fat (g 100 g <sup>-1</sup> )             | 7.07 ± 0.06 <sup>b</sup>   | 12.61 ± 0.25 <sup>d</sup>   | 10.42 ± 0.08 <sup>f</sup>  | 9.55 ± 0.06 <sup>g</sup>   | 10.32 ± 0.05 <sup>f</sup>  | 17.81 ± 0.06 <sup>a</sup>  | 15.69 ± 0.03 <sup>b</sup>  | 11.27 ± 0.02 <sup>e</sup>   | 14.39 ± 0.03 <sup>e</sup>  |
| Carbohydrate (g 100 g <sup>-1</sup> )    | 5.45 ± 0.12 <sup>e</sup>   | 8.26 ± 0.12 <sup>a</sup>    | 6.63 ± 0.12 <sup>d</sup>   | 6.99 ± 0.12 <sup>c</sup>   | 5.43 ± 0.12 <sup>c</sup>   | 7.61 ± 0.12 <sup>b</sup>   | 7.98 ± 0.12 <sup>ab</sup>  | 6.88 ± 0.12 <sup>cd</sup>   | 5.52 ± 0.12 <sup>e</sup>   |
| Energy value (kcal 100 g <sup>-1</sup> ) | 152.34 ± 0.19 <sup>d</sup> | 212.23 ± 0.34 <sup>b</sup>  | 184.84 ± 0.65 <sup>c</sup> | 181.26 ± 0.27 <sup>c</sup> | 180.18 ± 0.86 <sup>c</sup> | 256.91 ± 0.52 <sup>a</sup> | 241.04 ± 0.61 <sup>a</sup> | 196.92 ± 0.22 <sup>bc</sup> | 219.65 ± 0.36 <sup>b</sup> |
| Cholesterol (mg 100 g <sup>-1</sup> )    | 24.78 ± 0.83 <sup>a</sup>  | 21.27 ± 0.73 <sup>bed</sup> | 22.33 ± 0.92 <sup>bc</sup> | 24.84 ± 0.30 <sup>a</sup>  | 23.08 ± 0.25 <sup>ab</sup> | 19.07 ± 0.70 <sup>d</sup>  | 20.42 ± 0.60 <sup>cd</sup> | 20.31 ± 0.26 <sup>cd</sup>  | 21.46 ± 0.22 <sup>bc</sup> |
| pH                                       | 6.13 ± 0.01 <sup>d</sup>   | 6.11 ± 0.02 <sup>d</sup>    | 6.13 ± 0.01 <sup>d</sup>   | 6.14 ± 0.01 <sup>d</sup>   | 6.33 ± 0.01 <sup>a</sup>   | 6.21 ± 0.01 <sup>c</sup>   | 6.29 ± 0.04 <sup>ab</sup>  | 6.33 ± 0.01 <sup>a</sup>    | 6.24 ± 0.01 <sup>bc</sup>  |
| a <sub>w</sub>                           | 0.98 ± 0.00 <sup>b</sup>   | 0.98 ± 0.00 <sup>b</sup>    | 0.98 ± 0.00 <sup>b</sup>   | 0.98 ± 0.00 <sup>b</sup>   | 0.98 ± 0.00 <sup>b</sup>   | 0.98 ± 0.00 <sup>b</sup>   | 0.99 ± 0.00 <sup>a</sup>   | 0.99 ± 0.00 <sup>a</sup>    | 0.99 ± 0.00 <sup>a</sup>   |

Values are means ± standard deviations of three replicates (n = 3). Means with different superscript letters in a row indicate significant (p < 0.05) differences by Tukey's test. Carbohydrate: calculated by difference. F1: control; F2: 2.5% oil, 2.5% flour and 2.5% seed; F3: 2.5% oil and 2.5% seed; F4: 2.5% flour and 2.5% seed; F5: 2.5% oil and 2.5% seed; F6: 5% oil, 5% flour and 5% seed; F7: 5% oil and 5% flour; F8: 5% flour and 5% seed; F9: 5% oil and 5% seed. a<sub>w</sub> - water activity.

raw beef patties reduced the redness ( $a^*$ ). Similar results were obtained previously (Elif Bilek and Turhan, 2009). This is because flavonoids present in flaxseed react to proteins (Arts *et al.*, 2002), forming individual pro-oxidant active isomers. This effect results in oxidised myoglobin or metmyoglobin (Chaijan, 2008). In cooked products there was also  $a^*$  reduction in most formulations, however F2 and F9 results was similar to control ( $p > 0.05$ ). Higher yellowness ( $b^*$ ) was verified for raw and cooked formulations with higher flaxseed contents. The yellowish colour of golden flaxseed-based ingredients might be responsible for this result. The  $L^*$ ,  $a^*$  and  $b^*$  values decreased after the beef patties cooking, due to the fat loss and oxidation that occurred during this process. In general, raw and cooked beef patties could be considered light-coloured ( $L^*$  values greater than 50%), yellow tone ( $b^*$ ) and red sub-tone ( $a^*$ ).

Table 3. Colour parameters  $L^*$ ,  $a^*$  and  $b^*$  of beef patties formulated with different additions of golden flaxseed blends.

| Formulation           | Lightness ( $L^*$ )        | Redness ( $a^*$ )         | Yellowness ( $b^*$ )       |
|-----------------------|----------------------------|---------------------------|----------------------------|
| <b>Raw patties</b>    |                            |                           |                            |
| F1                    | 57.01 ± 0.28 <sup>b</sup>  | 19.22 ± 0.51 <sup>a</sup> | 19.94 ± 0.90 <sup>c</sup>  |
| F2                    | 55.61 ± 0.34 <sup>bc</sup> | 11.93 ± 0.61 <sup>c</sup> | 19.25 ± 0.64 <sup>c</sup>  |
| F3                    | 56.35 ± 1.63 <sup>b</sup>  | 16.10 ± 0.59 <sup>b</sup> | 19.92 ± 0.71 <sup>c</sup>  |
| F4                    | 56.65 ± 1.26 <sup>b</sup>  | 14.93 ± 0.53 <sup>c</sup> | 21.49 ± 0.90 <sup>b</sup>  |
| F5                    | 62.48 ± 1.42 <sup>a</sup>  | 10.72 ± 0.43 <sup>f</sup> | 19.15 ± 0.39 <sup>c</sup>  |
| F6                    | 62.46 ± 1.70 <sup>a</sup>  | 10.29 ± 0.44 <sup>f</sup> | 21.24 ± 0.71 <sup>b</sup>  |
| F7                    | 63.94 ± 1.00 <sup>a</sup>  | 9.88 ± 0.29 <sup>f</sup>  | 21.47 ± 0.40 <sup>b</sup>  |
| F8                    | 55.45 ± 1.51 <sup>bc</sup> | 13.87 ± 0.55 <sup>d</sup> | 23.28 ± 0.41 <sup>a</sup>  |
| F9                    | 54.82 ± 0.62 <sup>c</sup>  | 13.95 ± 0.66 <sup>d</sup> | 23.11 ± 0.76 <sup>a</sup>  |
| <b>Cooked patties</b> |                            |                           |                            |
| F1                    | 56.51 ± 0.46 <sup>cd</sup> | 6.85 ± 0.07 <sup>a</sup>  | 14.65 ± 0.19 <sup>g</sup>  |
| F2                    | 52.45 ± 0.49 <sup>f</sup>  | 6.74 ± 0.10 <sup>a</sup>  | 17.65 ± 0.49 <sup>d</sup>  |
| F3                    | 57.90 ± 0.74 <sup>bc</sup> | 5.23 ± 0.06 <sup>d</sup>  | 15.34 ± 0.40 <sup>f</sup>  |
| F4                    | 61.28 ± 0.40 <sup>a</sup>  | 4.57 ± 0.06 <sup>c</sup>  | 15.60 ± 0.28 <sup>f</sup>  |
| F5                    | 55.16 ± 1.63 <sup>de</sup> | 6.46 ± 0.11 <sup>b</sup>  | 17.90 ± 0.40 <sup>cd</sup> |
| F6                    | 58.07 ± 0.19 <sup>b</sup>  | 6.24 ± 0.11 <sup>c</sup>  | 18.37 ± 0.37 <sup>b</sup>  |
| F7                    | 58.68 ± 0.44 <sup>b</sup>  | 6.38 ± 0.07 <sup>bc</sup> | 18.27 ± 0.19 <sup>bc</sup> |
| F8                    | 55.35 ± 0.64 <sup>de</sup> | 6.48 ± 0.02 <sup>b</sup>  | 19.47 ± 0.11 <sup>a</sup>  |
| F9                    | 54.41 ± 0.14 <sup>c</sup>  | 6.84 ± 0.16 <sup>a</sup>  | 18.53 ± 0.11 <sup>b</sup>  |

Values are means ± standard deviations of three replicates ( $n = 3$ ). Means with different superscript letters in a column indicate significant ( $p < 0.05$ ) differences by Tukey's test. Carbohydrate: calculated by difference. F1: control; F2: 2.5% oil, 2.5% flour and 2.5% seed; F3: 2.5% oil and 2.5% seed; F4: 2.5% flour and 2.5% seed; F5: 2.5% oil and 2.5% flour; F6: 5% oil, 5% flour and 5% seed; F7: 5% oil and 5% flour; F8: 5% flour and 5% seed; F9: 5% oil and 5% seed.

The fatty acid profiles of beef patties are presented in Table 4. The most prevalent fatty acids in raw and cooked beef patties were palmitic and stearic (SFA); oleic (MUFA) and; linoleic and  $\alpha$ -linolenic (PUFA). These results corroborate with Pelser *et al.* (2007) and Elif Bilek and Turhan (2009). The *trans* fatty acids remained in minimum amounts in all formulations (maximum of 0.09 g 100 g<sup>-1</sup> product).

The golden flaxseed blends addition in raw and cooked beef patties increased absolute values of fatty acids SFA, MUFA and PUFA in almost all formulations. There was a large variation in amount (g 100 g<sup>-1</sup> product) of SFA, MUFA and PUFA between raw (3.13 to 3.65, 2.89 to 4.27 and 1.66 to 5.51, respectively) and cooked samples (3.25 to 4.22, 3.20 to 4.85 and 2.17 to 7.09, respectively). The highest levels of SFA, MUFA and PUFA were verified for formulation F6. The control sample had the lowest concentrations ( $p < 0.05$ ). Despite of this, golden flaxseed blends addition in beef patties reduced total percentage of SFA and MUFA and increased PUFA in raw and cooked products. This effect occurred with greater intensity in beef patties added with oil, which contained high linoleic and  $\alpha$ -linolenic fatty acids amounts.

Considering nutritional question, *trans* fatty acids may contribute to cardiovascular diseases development in humans (de Souza *et al.*, 2015). The addition of higher golden flaxseed levels in beef patties did not increase total *trans* fatty acids content in relation to control sample. Thus, it can be used without nutritional impairment. On the other hand, PUFA increased in beef patties added with golden flaxseed blends, which is favourable for human consumption. Especially, considering that  $\alpha$ -linolenic fatty acid has a cardio-protective effect. In addition, it reduces related complications to obesity and to diabetes mellitus (Rodriguez-Leyva *et al.*, 2010). The cooked beef patties (~ 100 g) added with golden flaxseed blends ranged from 52.3% (F4) to 222.5% (F6) of ideal recommendation for  $\alpha$ -linolenic acid consumption (2.22 g/day) (Simopoulos *et al.*, 1999). These values were well above the  $\alpha$ -linolenic fatty acid adequacy recommendation of control formulation (1.8%), which promotes a significant contribution to healthy diet.

A healthy recommendation for PUFA/SFA ratio is 0.45 or higher, while the proportion of  $n-6/n-3$  ratio should not be greater than 4 (Cardiovascular Review Group, 1994). Researchers have shown that higher PUFA intake and a diet with lower  $n-6/n-3$  ratio may reduce development risk and assisting in disease treatment. Some examples are the obesity and related metabolic disorders, breast cancer and

Table 4. Fatty acid composition of beef patties formulated with different additions of golden flaxseed blends.

| Fatty acid (g 100 g <sup>-1</sup> product) | F1                        | F2                        | F3                        | F4                        | F5                        | F6                       | F7                       | F8                        | F9                       |
|--|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| <b>Raw patties</b>                         |                           |                           |                           |                           |                           |                          |                          |                           |                          |
| <b>Saturated</b>                           |                           |                           |                           |                           |                           |                          |                          |                           |                          |
| Lauric (C12:0)                             | 0.01 ± 0.00 <sup>a</sup>  | 0.01 ± 0.00 <sup>a</sup> | 0.01 ± 0.00 <sup>a</sup> | 0.01 ± 0.00 <sup>a</sup>  | 0.01 ± 0.00 <sup>a</sup> |
| Myristic (C14:0)                           | 0.10 ± 0.00 <sup>a</sup>  | 0.09 ± 0.00 <sup>b</sup>  | 0.10 ± 0.00 <sup>a</sup>  | 0.10 ± 0.00 <sup>a</sup>  | 0.10 ± 0.00 <sup>a</sup>  | 0.09 ± 0.00 <sup>b</sup> | 0.09 ± 0.00 <sup>b</sup> | 0.09 ± 0.00 <sup>b</sup>  | 0.09 ± 0.00 <sup>b</sup> |
| Pentadecanoic (C15:0)                      | 0.01 ± 0.00 <sup>a</sup>  | 0.01 ± 0.00 <sup>a</sup> | 0.01 ± 0.00 <sup>a</sup> | 0.01 ± 0.00 <sup>a</sup>  | 0.01 ± 0.00 <sup>a</sup> |
| Palmitic (C16:0)                           | 2.15 ± 0.06 <sup>d</sup>  | 2.33 ± 0.02 <sup>b</sup>  | 2.27 ± 0.00 <sup>bc</sup> | 2.21 ± 0.06 <sup>cd</sup> | 2.28 ± 0.00 <sup>bc</sup> | 2.50 ± 0.02 <sup>a</sup> | 2.47 ± 0.03 <sup>a</sup> | 2.33 ± 0.06 <sup>b</sup>  | 2.49 ± 0.02 <sup>a</sup> |
| Margaric (C17:0)                           | 0.04 ± 0.00 <sup>a</sup>  | 0.04 ± 0.00 <sup>a</sup> | 0.04 ± 0.00 <sup>a</sup> | 0.04 ± 0.00 <sup>a</sup>  | 0.04 ± 0.00 <sup>a</sup> |
| Stearic (C18:0)                            | 0.67 ± 0.02 <sup>d</sup>  | 0.80 ± 0.00 <sup>b</sup>  | 0.77 ± 0.03 <sup>bc</sup> | 0.72 ± 0.00 <sup>cd</sup> | 0.75 ± 0.01 <sup>bc</sup> | 0.92 ± 0.03 <sup>a</sup> | 0.87 ± 0.02 <sup>a</sup> | 0.76 ± 0.02 <sup>bc</sup> | 0.87 ± 0.00 <sup>a</sup> |
| Arachidic (C20:0)                          | 0.02 ± 0.00 <sup>b</sup>  | 0.03 ± 0.00 <sup>a</sup>  | 0.02 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>b</sup>  | 0.03 ± 0.00 <sup>a</sup> | 0.03 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>b</sup>  | 0.03 ± 0.00 <sup>a</sup> |
| Behenic (C22:0)                            | 0.00 ± 0.00 <sup>c</sup>  | 0.01 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>a</sup> | 0.01 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>a</sup> |
| Lignoceric (C24:0)                         | 0.00 ± 0.00 <sup>d</sup>  | 0.01 ± 0.00 <sup>c</sup>  | 0.03 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>b</sup> | 0.02 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>b</sup> |
| Total                                      | 3.00 ± 0.08 <sup>e</sup>  | 3.33 ± 0.02 <sup>c</sup>  | 3.24 ± 0.04 <sup>d</sup>  | 3.13 ± 0.01 <sup>de</sup> | 3.23 ± 0.01 <sup>d</sup>  | 3.65 ± 0.00 <sup>a</sup> | 3.56 ± 0.04 <sup>b</sup> | 3.29 ± 0.02 <sup>cd</sup> | 3.58 ± 0.01 <sup>b</sup> |
| Percentage total of fatty acids            | 48.39                     | 29.21                     | 34.47                     | 35.21                     | 34.43                     | 23.73                    | 27.47                    | 33.47                     | 27.67                    |
| <b>Monounsaturated</b>                     |                           |                           |                           |                           |                           |                          |                          |                           |                          |
| Palmitoleic (C16:1 n-7)                    | 0.05 ± 0.00 <sup>b</sup>  | 0.06 ± 0.00 <sup>a</sup>  | 0.06 ± 0.00 <sup>a</sup>  | 0.05 ± 0.00 <sup>b</sup>  | 0.06 ± 0.00 <sup>a</sup>  | 0.06 ± 0.00 <sup>a</sup> | 0.06 ± 0.00 <sup>a</sup> | 0.05 ± 0.00 <sup>b</sup>  | 0.06 ± 0.00 <sup>a</sup> |
| Margaroleic (C17:1 n-7)                    | 0.02 ± 0.00 <sup>a</sup>  | 0.02 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>a</sup>  | 0.02 ± 0.00 <sup>a</sup> |
| Oleic (C18:1 n-9)                          | 2.43 ± 0.07 <sup>f</sup>  | 3.32 ± 0.03 <sup>c</sup>  | 3.10 ± 0.00 <sup>d</sup>  | 2.81 ± 0.05 <sup>e</sup>  | 3.10 ± 0.00 <sup>d</sup>  | 4.17 ± 0.03 <sup>a</sup> | 3.76 ± 0.04 <sup>b</sup> | 3.16 ± 0.01 <sup>d</sup>  | 3.72 ± 0.18 <sup>b</sup> |
| Gadololeic (C20:1 n-9)                     | 0.01 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>a</sup> | 0.01 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>a</sup> |
| Total                                      | 2.51 ± 0.07 <sup>f</sup>  | 3.41 ± 0.02 <sup>c</sup>  | 3.19 ± 0.00 <sup>d</sup>  | 2.89 ± 0.04 <sup>e</sup>  | 3.19 ± 0.00 <sup>d</sup>  | 4.27 ± 0.03 <sup>a</sup> | 3.86 ± 0.04 <sup>b</sup> | 3.24 ± 0.01 <sup>d</sup>  | 3.82 ± 0.08 <sup>b</sup> |
| Percentage total of fatty acids            | 40.48                     | 29.91                     | 33.94                     | 32.51                     | 34.01                     | 27.76                    | 29.78                    | 32.96                     | 29.52                    |
| <b>Polysaturated</b>                       |                           |                           |                           |                           |                           |                          |                          |                           |                          |
| Linoleic (C18:2 n-6)                       | 0.52 ± 0.00 <sup>f</sup>  | 1.22 ± 0.04 <sup>e</sup>  | 1.05 ± 0.05 <sup>d</sup>  | 0.80 ± 0.00 <sup>e</sup>  | 1.05 ± 0.03 <sup>d</sup>  | 1.86 ± 0.06 <sup>a</sup> | 1.56 ± 0.00 <sup>b</sup> | 1.04 ± 0.00 <sup>d</sup>  | 1.57 ± 0.01 <sup>b</sup> |
| α-Linolenic (C18:3 n-3)                    | 0.03 ± 0.00 <sup>g</sup>  | 2.03 ± 0.06 <sup>c</sup>  | 1.45 ± 0.03 <sup>d</sup>  | 0.85 ± 0.00 <sup>f</sup>  | 1.46 ± 0.05 <sup>d</sup>  | 3.63 ± 0.06 <sup>a</sup> | 3.10 ± 0.08 <sup>b</sup> | 1.23 ± 0.02 <sup>c</sup>  | 3.09 ± 0.08 <sup>b</sup> |
| Docosapentaenoic (DPA) (C22:5 n-3)         | 0.01 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>a</sup> | 0.01 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>a</sup> |
| Total                                      | 0.56 ± 0.01 <sup>g</sup>  | 3.26 ± 0.01 <sup>c</sup>  | 2.51 ± 0.03 <sup>d</sup>  | 1.66 ± 0.00 <sup>f</sup>  | 2.52 ± 0.09 <sup>d</sup>  | 5.51 ± 0.01 <sup>a</sup> | 4.68 ± 0.06 <sup>b</sup> | 2.28 ± 0.02 <sup>c</sup>  | 4.68 ± 0.02 <sup>b</sup> |
| Percentage total of fatty acids            | 9.03                      | 28.60                     | 26.70                     | 18.67                     | 26.87                     | 35.83                    | 36.11                    | 23.19                     | 36.17                    |
| <b>Ratios</b>                              |                           |                           |                           |                           |                           |                          |                          |                           |                          |
| n-6/n-3                                    | 13.00 ± 0.07 <sup>a</sup> | 0.60 ± 0.03 <sup>de</sup> | 0.72 ± 0.03 <sup>cd</sup> | 0.93 ± 0.00 <sup>b</sup>  | 0.71 ± 0.01 <sup>c</sup>  | 0.51 ± 0.02 <sup>f</sup> | 0.50 ± 0.01 <sup>f</sup> | 0.84 ± 0.01 <sup>b</sup>  | 0.50 ± 0.00 <sup>f</sup> |
| PUFA/SFA                                   | 0.19 ± 0.00 <sup>g</sup>  | 0.98 ± 0.01 <sup>c</sup>  | 0.77 ± 0.01 <sup>d</sup>  | 0.53 ± 0.01 <sup>f</sup>  | 0.78 ± 0.02 <sup>d</sup>  | 1.51 ± 0.01 <sup>a</sup> | 1.31 ± 0.03 <sup>b</sup> | 0.69 ± 0.01 <sup>e</sup>  | 1.31 ± 0.01 <sup>b</sup> |



Table 4. (Cont.)

|  |                           |                          |                           |                          |                          |                          |                          |                          |                          |
|--|---------------------------|--------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Total                                  | 0.68 ± 0.00 <sup>b</sup>  | 3.71 ± 0.03 <sup>d</sup> | 2.89 ± 0.01 <sup>f</sup>  | 2.17 ± 0.00 <sup>g</sup> | 2.83 ± 0.00 <sup>f</sup> | 7.09 ± 0.01 <sup>a</sup> | 5.71 ± 0.01 <sup>b</sup> | 3.31 ± 0.02 <sup>e</sup> | 5.45 ± 0.00 <sup>e</sup> |
| Percentage total of fatty acids        | 9.62                      | 29.42                    | 27.74                     | 22.72                    | 27.42                    | 39.81                    | 36.39                    | 29.37                    | 37.87                    |
| <b>Ratios</b>                          |                           |                          |                           |                          |                          |                          |                          |                          |                          |
| <i>n</i> -6/ <i>n</i> -3               | 12.60 ± 0.08 <sup>a</sup> | 0.58 ± 0.00 <sup>c</sup> | 0.62 ± 0.00 <sup>c</sup>  | 0.85 ± 0.00 <sup>b</sup> | 0.65 ± 0.02 <sup>c</sup> | 0.43 ± 0.00 <sup>d</sup> | 0.46 ± 0.00 <sup>d</sup> | 0.60 ± 0.01 <sup>e</sup> | 0.46 ± 0.00 <sup>d</sup> |
| PUFA/SFA                               | 0.23 ± 0.00 <sup>g</sup>  | 0.98 ± 0.01 <sup>d</sup> | 0.89 ± 0.01 <sup>e</sup>  | 0.67 ± 0.02 <sup>f</sup> | 0.87 ± 0.01 <sup>e</sup> | 1.68 ± 0.00 <sup>a</sup> | 1.40 ± 0.00 <sup>e</sup> | 0.99 ± 0.00 <sup>d</sup> | 1.51 ± 0.01 <sup>b</sup> |
| <b>Trans</b>                           |                           |                          |                           |                          |                          |                          |                          |                          |                          |
| Elaidic (C18:1 <i>trans</i> 9)         | 0.01 ± 0.00 <sup>d</sup>  | 0.05 ± 0.00 <sup>a</sup> | 0.04 ± 0.00 <sup>b</sup>  | 0.01 ± 0.00 <sup>d</sup> | 0.04 ± 0.00 <sup>b</sup> | 0.04 ± 0.00 <sup>b</sup> | 0.05 ± 0.00 <sup>a</sup> | 0.03 ± 0.00 <sup>c</sup> | 0.03 ± 0.00 <sup>c</sup> |
| Linolelaic (C18:2 <i>trans</i> 9, t12) | 0.02 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>b</sup> | 0.02 ± 0.00 <sup>b</sup>  | 0.02 ± 0.00 <sup>b</sup> | 0.02 ± 0.00 <sup>b</sup> | 0.03 ± 0.00 <sup>a</sup> | 0.03 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>b</sup> | 0.02 ± 0.00 <sup>b</sup> |
| Linolenic (C18:3 <i>trans</i> )        | 0.01 ± 0.00 <sup>c</sup>  | 0.02 ± 0.00 <sup>b</sup> | 0.01 ± 0.00 <sup>c</sup>  | 0.01 ± 0.00 <sup>c</sup> | 0.01 ± 0.00 <sup>c</sup> | 0.03 ± 0.00 <sup>a</sup> | 0.02 ± 0.00 <sup>b</sup> | 0.01 ± 0.00 <sup>c</sup> | 0.02 ± 0.00 <sup>b</sup> |
| Total                                  | 0.04 ± 0.00 <sup>e</sup>  | 0.09 ± 0.00 <sup>b</sup> | 0.07 ± 0.00 <sup>c</sup>  | 0.04 ± 0.00 <sup>e</sup> | 0.07 ± 0.00 <sup>c</sup> | 0.10 ± 0.00 <sup>a</sup> | 0.10 ± 0.00 <sup>a</sup> | 0.06 ± 0.00 <sup>d</sup> | 0.07 ± 0.00 <sup>c</sup> |
| Percentage total of fatty acids        | 0.57                      | 0.71                     | 0.67                      | 0.42                     | 0.68                     | 0.56                     | 0.64                     | 0.53                     | 0.49                     |
| Others fatty acids                     | 0.69 ± 0.01 <sup>g</sup>  | 1.13 ± 0.00 <sup>d</sup> | 0.95 ± 0.02 <sup>ef</sup> | 0.89 ± 0.00 <sup>f</sup> | 0.90 ± 0.00 <sup>f</sup> | 1.55 ± 0.01 <sup>a</sup> | 1.41 ± 0.02 <sup>b</sup> | 1.01 ± 0.00 <sup>e</sup> | 1.24 ± 0.00 <sup>c</sup> |
| Percentage total of fatty acids        | 9.76                      | 8.96                     | 9.12                      | 9.32                     | 8.72                     | 8.70                     | 8.99                     | 8.96                     | 8.62                     |

Values are means ± standard deviations of three replicates (*n* = 3). Means with different superscript letters in a row indicate significant (*p* < 0.05) differences by Tukey's test. Carbohydrate: calculated by difference. F1: control; F2: 2.5% oil, 2.5% flour and 2.5% seed; F3: 2.5% oil and 2.5% seed; F4: 2.5% flour and 2.5% seed; F5: 2.5% oil and 2.5% flour; F6: 5% oil, 5% flour and 5% seed; F7: 5% oil and 5% flour; F8: 5% flour and 5% seed; F9: 5% oil and 5% seed. PUFA - polyunsaturated fatty acids, SFA - saturated fatty acids.

Table 5. Sensory scores of beef patties formulated with different additions of golden flaxseed blends.

| Parameters   | F1                        | F2                        | F3                        | F4                       | F5                         | F6                       | F7                        | F8                       | F9                        |
|--|---------------------------|---------------------------|---------------------------|--------------------------|----------------------------|--------------------------|---------------------------|--------------------------|---------------------------|
| Appearance   | 6.71 ± 1.56 <sup>a</sup>  | 6.72 ± 1.60 <sup>a</sup>  | 6.50 ± 1.95 <sup>a</sup>  | 6.60 ± 1.53 <sup>a</sup> | 7.04 ± 1.49 <sup>a</sup>   | 6.60 ± 1.12 <sup>a</sup> | 6.80 ± 1.31 <sup>a</sup>  | 6.38 ± 1.30 <sup>a</sup> | 6.41 ± 1.33 <sup>a</sup>  |
| Aroma  | 6.84 ± 1.48 <sup>a</sup>  | 6.72 ± 1.37 <sup>a</sup>  | 7.00 ± 1.41 <sup>a</sup>  | 6.88 ± 1.38 <sup>a</sup> | 6.90 ± 1.31 <sup>a</sup>   | 6.31 ± 1.16 <sup>a</sup> | 6.47 ± 1.36 <sup>a</sup>  | 6.54 ± 1.32 <sup>a</sup> | 6.73 ± 1.38 <sup>a</sup>  |
| Flavour  | 6.68 ± 1.55 <sup>a</sup>  | 6.21 ± 1.75 <sup>ab</sup> | 6.51 ± 1.70 <sup>ab</sup> | 6.70 ± 1.23 <sup>a</sup> | 5.64 ± 2.04 <sup>c</sup>   | 5.12 ± 1.88 <sup>c</sup> | 5.78 ± 1.75 <sup>bc</sup> | 5.52 ± 1.55 <sup>c</sup> | 5.74 ± 1.86 <sup>bc</sup> |
| Texture  | 6.20 ± 2.08 <sup>ab</sup> | 6.44 ± 1.86 <sup>ab</sup> | 6.47 ± 1.86 <sup>ab</sup> | 6.76 ± 1.53 <sup>a</sup> | 6.14 ± 1.81 <sup>abc</sup> | 5.47 ± 1.68 <sup>c</sup> | 5.82 ± 1.89 <sup>bc</sup> | 5.29 ± 1.73 <sup>c</sup> | 5.33 ± 1.94 <sup>c</sup>  |
| Positive (yes) overall acceptance (%) <sup>*</sup> | 72                        | 60                        | 64                        | 74                       | 58                         | 34                       | 56                        | 46                       | 44                        |
| Positive (yes) purchase intent (%) <sup>*</sup>    | 68                        | 60                        | 62                        | 70                       | 52                         | 28                       | 48                        | 38                       | 40                        |

Values are means ± standard deviations. Means with different superscript letters in a row indicate significant (*p* < 0.05) differences by Tukey's test. Carbohydrate: calculated by difference. F1: control; F2: 2.5% oil, 2.5% flour and 2.5% seed; F3: 2.5% oil and 2.5% seed; F4: 2.5% flour and 2.5% seed; F5: 2.5% oil and 2.5% flour; F6: 5% oil, 5% flour and 5% seed; F7: 5% oil and 5% flour; F8: 5% flour and 5% seed; F9: 5% oil and 5% seed. <sup>\*</sup>Based on the binomial (yes/no) scale from 50 responses.

depressive disorder (Liu *et al.*, 2013; Yang *et al.*, 2014; Husted and Bouzinova, 2016). In the present work, the addition of based ingredients golden flaxseed increased PUFA/SFA ratio and decreased  $n-6/n-3$  ratio in all formulations. This improved the beef patties nutritional profile. Similar results were described by Pelser *et al.* (2007) and Elif Bilek and Turhan (2009). PUFA/SFA and  $n-6/n-3$  ratios of raw and cooked control samples were the least favourable ( $p < 0.05$ ). Flaxseed oil had high PUFA/SFA ratio (6.38) and low  $n-6/n-3$  ratio (0.38), which favours healthier food intake. Raw and cooked beef patties samples F6, F7 and F9 presented the best PUFA/SFA and  $n-6/n-3$  ratios. These formulations contained the highest flaxseed oil content. Valencia *et al.* (2008) found similar effects on pork sausages with flaxseed oil (15%) addition. The authors observed increased PUFA/SFA (0.71) ratio and decreased  $n-6/n-3$  (1.64) ratio.

#### Consumer study

The sensory scores of consumer's studies for beef patties added with golden flaxseed blends and by-products are reported in Table 5. The addition of ingredients based on golden flaxseed did not modify appearance and aroma attributes ( $p > 0.05$ ). However, there was a reduction in flavour and texture for samples with high level addition of golden flaxseed and by-products. Higher flavour scores ( $p < 0.05$ ) were found in F1 and F4 formulations as compared to F5, F6, F7, F6 and F9. Pelser *et al.* (2007) observed that flavour suffered negative influence after flaxseed oil addition in fermented sausages. In addition, they also found that consumers reported a "fish taste" in these products. This effect was related to PUFA lipid oxidation. In this regard, Cameron and Enser (1991) reported that linoleic,  $\alpha$ -linolenic, arachidonic, docosapentaenoic and docosahexaenoic fatty acids had strong correlation for meat quality reduction. This also explains the reduction in beef patties acceptability, since flaxseed and derivatives had high linoleic and  $\alpha$ -linolenic acids levels.

Higher amounts of flour and seed of golden flaxseed in beef patties (F6, F8 and F9) decreased the texture score ( $p < 0.05$ ). These results agree with Elif Bilek and Turhan (2009), who evaluated flaxseed flour addition in beef patties; Turhan *et al.* (2005) studied hazelnut pellicle in low-fat beef burgers, and García *et al.* (2002) incorporated wheat and oat in low fat dry fermented sausages. This effect might be explained by the fact that meat products are not usually having cereal grains and flour in their formulation. Thus, when these ingredients were

added into meat products there may be a reduction in acceptability, since the food would have different sensory characteristics than commonly consumed foods. According to Mehta *et al.* (2015), flavour and texture are the most important sensory attributes in meat products among the consumers. In the present work, this was confirmed, since these attributes were mostly affected by the addition of golden flaxseed blends.

Overall acceptance and purchase intent of less than 60% were verified from F5 formulation, which demonstrated a rejection by consumers. Knowing this, the addition of 2.5% golden flaxseed blends and by-products in beef patties could be considered as having sensory acceptability similar to normal product and is well accepted by consumers. Exception was verified to beef patties with addition of 2.5% oil and 2.5% flour (F5), which obtained low acceptance.

#### Conclusion

The addition of golden flaxseed blends and by-products could be considered an effective strategy to improve the beef patties nutritional profile. There was an increase in content of macronutrients, mineral residue and energy in raw and cooked products following the addition of golden flaxseed. Further, it was possible to reduce cholesterol content in beef patties, thus making them healthier for consumption. Fatty acids profile also improved following the addition of golden flaxseed blends (oil and/or flour and/or seed), mainly with oil use. PUFA/SFA ratio also increased and the  $n-6/n-3$  ratio decreased, thus obtaining values within recommended guideline for a healthy diet. Higher levels of golden flaxseed blends and by-products increased the values of  $L^*$  and  $b^*$  and decreased  $a^*$  values in beef patties. Sensory acceptability decreased for products with higher levels of golden flaxseed. Therefore, the golden flaxseed blends could be used in manufacturing process beef patties, aiming to improve nutritional profile and contribute to healthier food consumption. However, it was demonstrated that level addition close to 2.5% of each by-product were better accepted by consumers.

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