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Enhancing the Nutritional Value of Oat Bars

Azza A. Omran*

Department of Crops Technology Research, Food Technology Research Institute, Agricultural Research Center, Egypt
*Corresponding author: azzaa_omran@yahoo.com

Abstract The present work was designed to prepare four different oat bar formulas (F_1, F_2, F_3 and F_4) from quick oat flakes, sunflower, chickpea and pumpkin seeds. Chemical, physical, textural, microbiological and sensory evaluation were performed. The results indicated that the substitution of oat with sunflower, chickpea and pumpkin seeds significantly enhanced the nutritional value of the resultant bars. Protein and fat contents significantly increased. Total phenols, total flavonoids, tannins and antioxidant activity significantly increased in F_2, F_3 and F_4 compared to F_1. Substitution of oat with sunflower, chickpea and pumpkin seeds has no significant effect on taste, color, flavor and overall acceptability but, it had a significant effect on appearance. Hardness, microbiological counts and peroxide values increased while water activity decreased during storage period for three months. The results clearly demonstrated the value of substituting oat with sunflower, chickpea and pumpkin seeds to produce oat bars with good nutritional value and stability up to two months. Sunflower formula was the most valuable addition with the highest acceptability followed by pumpkin formula.

Keywords: *oat bars, sunflower, chickpea, pumpkin, nutritional quality, storage experiments, sensory attributes*

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1. Introduction

Recently, the consumption of fast food and snacks significantly increased, revealing a trend of change in lifestyle of the population. This is due to the facilities for the purchase of pre-prepared, frozen and ready market foods. Among these foods stand out the snacks, which are defined as small meals, light or substantial and may be related to the attributes of healthy and would be an ideal food format to deliver fruit-derived phenolic antioxidants and fiber. Because of the growing consumer demand for healthy, natural and convenient foods, attempts are being made to improve snack foods nutritional values by modifying their nutritive composition [1]. The greatest difficulty in obtaining a good cereal bar is a combination of several ingredients with specific functionality such as grains, nuts, seeds, dried fruits, raisins, thickening agents, sweeteners and flavorings, and turns them into a product with flavor, texture and decent appearance, to achieve specific nutrient goals. Cereal bars have been used for multiple purposes such as breakfast, snacks, energy and meal replacement. There is a trend towards producing natural snacks with a good fiber, calorie and protein supply [2]. Cereal bars have emerged as an important breakfast cereal mixture product. It is considered a ready-to-eat cereal, produced from the mixture of grain components with other ingredients such as chopped nuts and fruit pieces, through extensive processing. The principal grains used in the manufacture of cereal bars include corn, rice, wheat, oats and barley, which are overall considered healthy ingredients [3].

Oats (*Avena sativa* L.) have a long tradition of use in food and is widely recognized as an inexpensive and healthy food. The world production of oats reached over 22 million tonnes in 2014 and the major producers are Russia, Canada, Poland, Australia, Finland, and USA [4]. Unlike wheat, it is usually consumed in a whole-grain form. Oat is higher in protein, calcium and essential fatty acids than are other grains. Oat is also high in dietary fiber, including soluble fiber and β-glucan, which has been shown to reduce serum cholesterol [5,6]. Oat flakes are made by passing whole groats, which have been tempered or steamed, between a pair of rollers and the grains are subjected to low moisture, high shear and high temperature for a short time. The gap between the rollers determines the thickness of the flakes. The smaller flake size of quick oats require a shorter cooking time [7].

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crop grown in the world. Sunflower seed is a package of healthy unsaturated fats, protein, fiber and other important nutrients like vitamin E, selenium, copper, zinc, folate, iron and phytochemicals. Sunflower polyphenols can be used as effective antioxidants for sunflower [8,9].

Chickpea (*Cicer arietinum* L.) is one of the oldest and most widely consumed legume in the world due to its relatively high in protein content and wide adaptability as a food grain. It is the second most widely grown legumes in the world. The origin of the chickpeas is thought to have been Levant and Ancient Egypt [10]. It is a good source of protein and carbohydrate. Its protein quality is better than other legumes such as pigeon pea, black gram and green gram [11].

Pumpkin *(Cucurbita maxima*), a member of Cucurbitaceae family has been used frequently as a functional food or medicine [12], because its a good source of polysaccharides, protein and phenolic glycosides. Besides,

it is rich in unsaturated fatty acids and tocopherols and with very high oxidative stability [13,14].

Dried fruit is a fruit has been used in baking mixes and breakfast cereals. Its consumption is rapidly increased by people due to the awareness regarding their health benefits [15].

As the formulation and development of nutritious complementary food from locally and readily available raw materials have received a lot of attention, the present work was aimed to prepare oat bars formula, enriched with different seeds and dried fruits and evaluating their physicochemical, textural, microbiological and sensorial properties to assess their nutritional quality.

2. Materials and Methods

2.1. Materials

Whole sunflower (*Helianthus annuus* L., Sakha 53 variety) and flaxseed seeds (*Linum usitatissimum L*., Sakha 2 variety) were obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Quick oat flakes, sesame seeds, raisins, dried apricots and plums, hulled chickpea, whole pumpkin seeds, sucrose, cinnamon and glucose syrup (79.50% total soluble solids) were obtained from local markets, Cairo, Egypt. Pepsin, pancreatin enzymes, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] were obtained from Sigma–Aldrich Chemical Co., St. Louis, USA. All other chemicals were of analytical grade.

Table 1. Formula of the oat bars*

Ingredients	Formula (g)			
	F_1	F_2	F_3	F_4
Oat flakes	45.50	40.50	40.50	40.50
Sunflower seeds	-	5.0	-	-
Chickpea seeds	-	-	5.0	-
Pumpkin seeds	-	-	-	5.0
Sesame seeds	2.50	2.50	2.50	2.50
Flaxseed seeds	2.50	2.50	2.50	2.50
Raisins	10.0	10.0	10.0	10.0
Apricot (dried)	2.50	2.50	2.50	2.50
Plums (dried)	2.50	2.50	2.50	2.50
Cinnamon	0.50	0.50	0.50	0.50
Sucrose	20.0	20.0	20.0	20.0
Glucose syrup	14.0	14.0	14.0	14.0
Water	12.0	12.0	12.0	12.0

*F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.

2.2. Preparation of Oat Bars

Sucrose was mixed with glucose syrup and water (Table 1) and the final concentration for the syrup was 68% total soluble solids (using a Laboratory Refractometer, Bellingham and Stanley Ltd, England), the temperature was 105±2°C, then cinnamon was added. The oat bars were prepared according to the method described by Silva de Paula *et al.* [16] with some modification as shown in Table 1. Whole sunflower, pumpkin seeds and hulled chickpea were added as a partial replacement of oat flakes (based on preliminary trials). The oat flakes and different seeds were put on a pan and heated for about two to three min at 115±2°C according to Adebiyi *et al.* [17]. Then, dried fruits were added and mixed well with the prepared sugars syrup mixture in order to obtain a homogenous mixture. After that, sheeted in rectangular frames, glazed with a minimum amount of corn oil, manually pressed and left to cool at room temperature to formulate four oat bars (18±2 g weight, 48±2 mm length, 26±2 mm width and 14±1 mm thickness). The obtained bars were packed in polypropylene metalized bags and stored at 25±2°C for three months for subsequent analyses.

2.3. Chemical Analysis

2.3.1. Proximate Analysis of Raw Materials and Oat Bars

Moisture, protein, fat, crude fiber and ash contents of raw materials and oat bars were determined according to the methods of the AOAC [18]. Carbohydrates were calculated by the difference method [19]. Total calories of the oat bars were estimated by multiplying the crude protein, fat and carbohydrates by calculation as its basis of 4, 9 and 4 kcal/g, respectively according to the method of James [20]. Mineral contents (*e.g.* iron, zinc, copper, manganese, calcium, potassium, magnesium and sodium were determined according to the method outlined in the AOAC [18] using the Perkin Elmer (Model 300, USA) Atomic Absorption Spectrophotometer. Total phosphorus was determined by the colorimetric method of Trough and Mayer [21]. Peroxide value (meq/kg fat) was determined according to the method of the AOAC [18].

2.3.2. Determination of Total and Reducing Sugars and Starch

Total and reducing sugars were determined using Somogyi–Nelson Method according to Somogy [22] and Nelson [23]. Non reducing sugars were calculated by difference between total and reducing sugars. Starch was determined according to the method described by Ranganna [24].

2.3.3. Phytochemical Analysis

Total phenolics content were determined using Folin-Ciocalteu method according to Singleton and Rossi [25]. Gallic acid was used as a standard. Total flavonoids were determined according to the method of Zhishen *et al.* [26]. Catechin was served as a standard. Tannins were determined as described by Price *et al.* [27]. Catechin was used as a standard.

2.3.4. Phytate Determination

Phytate content was determined according to the procedure described by Mohamed *et al.* [28].

2.3.5. Determination of *in vitro* Starch Digestibility (IVSD)

In vitro starch digestibility was determined using pancreatic amylase according to the method of Singh *et al.* [29].

2.3.6. Determination of *in vitro* Protein Digestibility (IVPD)

In vitro protein digestibility was determined according to the method of Akeson and Stahmann [30]. After enzymatic digestion of samples with pepsin and pancreatin, the protein in the resultant supernatant was estimated using the Kjeldahl method [18]. The percentage of protein digestibility was calculated by the ratio of protein in the supernatant to protein in the sample as the following equation:

$$\text{In vitro protein digestibility}(\%) = \frac{\text{N in supernatant} - \text{N in Blank}}{\text{N in sample}} \text{x100.}$$

N = Nitrogen.

2.3.7. Antioxidant Activity

The antioxidant activity was determined using the radical scavenging activity in reacting with DPPH free radical according to Brand-Williams *et al.* [31] and the ABTS assay which was carried out according to Re *et al.* [32]. The radical scavenging percentage was calculated using the following equation:

$$\text{Radical scavenging } (\%) = \left[\left(A_0 - A_1 / A_0 \right) \right] \text{x100}$$

A_0= Absorbance of the control reaction (containing all reagents except the test compounds). A_1= Absorbance in the presence of the tested extracts after 30 min.

2.4. Sensory Evaluation

The oat bar samples were organolyptically evaluated for its some sensory characteristics, *i.e.*, appearance, flavor, taste, texture and overall acceptability by ten panelists according to the method of Stone and Sidel [33].

2.5. Color Measurement

External color of the oat bars was measured by a hand-held Tristimulus reflectance colorimeter Minolta Chromameter (model CR-400, Konica Minolta, Japan). Results recorded in lightness with L^* = (100 for lightness, and zero for darkness), a^* [chromaticity on a green (-) to red (+)] and b^* [chromaticity on a blue (-) to yellow (+)]. Values reported are the means of triplicate determinations.

2.6. Water Activity (a_w)

The water activity (a_w) of the oat bars was measured using Rotronic Hygrolab3 CH-8303, Switzerland as mentioned by Cadden [34].

2.7. Hardness

The hardness of oat bar samples was measured by Universal Testing Machine (Cometech, B type, Taiwan) provided with software as described by Bourne [35]. Three replicates of each oat bars formula were cut using a flat ended probe (2.50 mm thickness) with a cross-head speed of 1 mm/s at a 20% level of compression. The hardness was recorded by Newton (N).

2.8. Microbiological Examinations

Total plate count (TPC) and yeasts and moulds count of samples were determined according to the APHA [36]. The results of the microbiological analysis were expressed as log cfu/g.

2.9. Statistical Analysis

The obtained data were analyzed using Costat statistical software and were statistically analyzed for means values and standard deviations according to Steel and Torrie [37]. The data were subjected to one-way analysis of variance (ANOVA) at $P<0.05$ followed by Duncan's new multiple range tests to assess differences between samples mean.

3. Results and Discussion

3.1. Chemical Composition of Selected Raw Materials

The chemical composition of selected raw materials (oat flakes, sunflower, chickpea and pumpkin seeds) is presented in Table 2. The results pointed out that sunflower seeds had a high content of ash and crude fiber. While, pumpkin seeds had a high contents of protein and fats (33.48 and 45.02%, respectively). Oat flakes and chickpea had the lowest values of all proximate analysis except for total carbohydrates. The results are close with those found by Brahma *et al.* [38]; Alexandrino *et al.* [39]; Sharma *et al.* [40] and Hassan *et al.* [41] who analyzed oat, sunflower, chickpea and pumpkin seeds, respectively.

3.2. Chemical Composition of Oat Bars

Table 3 displays the chemical composition of four oat bars [oat flakes (F_1), sunflower (F_2), chickpea (F_3) and pumpkin seeds formula (F_4)]. As estimated, substitution with sunflower, chickpea and pumpkin seeds significantly increased protein, ash and fat contents of the oat bars. Data showed that F_4 had the highest contents in protein (10.30%) and fat (6.91%) followed by F_2 (9.92 and 6.31%, respectively). On the other hand, F_4 had the lowest total carbohydrates content (80.89%). Whereas, crude fiber content insignificantly affected by the replacement of oat with different seeds.

The present findings are in accordance with Silva *et al.* [42] who mentioned that the combination between oats and pumpkin increased the contents of crude protein in cereal bars. However, it is possible to notice an increasing trend in the content of fats due to the addition of pumpkin seeds, which has higher content of total fat than that of the oats. Also, it increases the contents of dietary fiber, which are essential to maintain health and reduce the risk of several diseases.

Table 2. Chemical composition of selected raw materials (on dry weight basis)

Constituents	Moisture content	Protein	Fats	Ash	Crude fiber	TC*
Oat flakes	8.45 ^{b} ±0.62	13.62 ^{d} ±0.99	5.26 ^{c} ± 0.75	1.80 ^{b} ± 0.27	2.00 ^{b}± 0.02	79.32 ^{a} ± 1.95
Sunflower seeds	5.00 ^{c} ±0.07	25.49 ^{b} ± 0.17	33.51 ^{b} ± 0.88	4.23 ^{a} ± 1.06	3.65 ^{a}± 0.69	36.77 ^{c} ± 2.05
Chickpea seeds	9.72^{a} ±0.08	19.32 ^{c} ± 0.98	5.35 ^{c} ± 0.25	2.48 ^{b} ± 0.03	1.51 ^{b}± 0.03	72.85 ^{b} ± 1.25
Pumpkin seeds	8.75^{b} ±0.01	33.48 ^{a} ± 0.22	45.02 ^{a} ± 4.42	3.87 ^{a} ± 0.80	3.04 ^{a}± 0.15	17.63 ^{d}± 5.44

*TC: Total carbohydrates were calculated by difference [100 - (protein + fats + ash)].
Values are means of three replicates ±SD, number in the same column followed by the same letter is not significantly different at 0.05.

Table 3. Chemical composition of the oat bars (% on dry weight basis)

Constituents (%) \ Formula	F_1	F_2	F_3	F_4
Moisture content	11.39 ^{a} ± 0.03	11.32 ^{a}± 0.45	11.44 ^{a}± 0.07	11.78 ^{a}± 0.09
Protein	8.42 ^{c} ± 0.14	9.92 ^{ab}± 0.41	9.44 ^{b}± 0.16	10.30 ^{a}± 0.61
Fats	5.53 ^{c} ± 0.10	6.31 ^{b} ± 0.04	5.68 ^{c} ± 0.32	6.91 ^{a}± 0.16
Ash	1.63 ^{b}± 0.07	1.95 ^{a} ± 0.05	1.85 ^{a}± 0.03	1.90 ^{a} ± 0.06
Crude fiber	1.52 ^{a}± 0.06	1.58 ^{a}± 0.09	1.51 ^{a}± 0.11	1.62 ^{a}± 0.05
TC*	84.42 ^{a}± 0.31	81.82 ^{c}± 0.51	83.03 ^{b}± 0.02	80.89 ^{c}± 0.05
Total sugars	21.34 ^{b}± 0.23	21.51 ^{b}± 0.26	22.34 ^{a}± 0.60	22.93 ^{a}± 0.36
Reducing sugars	11.09 ^{ab}±0.12	10.68 ^{b}± 0.31	11.46 ^{a}± 0.16	10.78 ^{b}± 0.20
Non reducing sugars	10.25 ^{c}± 0.12	10.83 ^{b}± 0.06	10.88 ^{b}± 0.43	12.15 ^{a} ± 0.17
Starch	53.78 ^{a}± 1.67	52.81 ^{a}± 1.85	53.50 ^{a}± 1.39	52.48 ^{a}± 0.75
Caloric value (kcal/100g)**	373.18 ^{b}± 0.36	375.80 ^{a}±1.88	372.82 ^{b} ± 1.22	376.65 ^{a}± 0.69

F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.
*TC= Total carbohydrate was calculated by difference [100 - (protein + fats + ash)].
**Caloric value calculated as its basis using factors of 4, 4 and 9 kcal/g for protein, carbohydrates and lipids, respectively.
Values are means of three replicates ±SD, number in the same row followed by the same letter is not significantly different at 0.05 level.

Regarding to Dietary Reference Intakes (DRI) [43] of protein, each 100g oat bar provide 18.30, 21.56, 20.52 and 22.39% from daily intake of protein for female and male aged 19-50y with 55kg body weight (based on 46 g protein/day, respectively) from F_1, F_2, F_3 and F_4, respectively. And 15.04, 17.71, 16.86 and 18.39% from daily intake of protein for male aged 19-50y with 65kg body weight (based on 56g protein/day) from F_1, F_2, F_3 and F_4, respectively.

Each 100g oat bar provide 18.43, 21.03, 18.93 and 23.03% from daily intake of fat (based on 30g fat/ day) from F_1, F_2, F_3 and F_4, respectively according to DRI [43] of daily total fat.

Total sugars significantly increased in different formula. F_2 and F_4 had the lowest content of reducing sugars (10.68 and 10.78%) and starch content (52.81 and 52.48%) compared to other formulas.

Accordingly, the use of pumpkin and sunflower seeds in the formulation of oat bars enhance the nutritional quality which could be used as breakfast, snacks, energy foods.

3.3. Mineral Contents of Oat Bars

Table 4 exhibit the minerals content of the oat bars. Data indicated that substitution of oat with sunflower, chickpea and pumpkin seeds significantly affected the mineral contents in all oat bars. F_1 had the highest content of magnesium, manganese, copper and zinc. While, F_2 had the highest content of calcium and phosphorus. F_3 recorded the highest values of iron and potassium. The differences in minerals are probably due to the type of seeds and ingredients used in the formulation of the oat bars. Similar results were found by Paiva *et al.* [44], who found that cereal bars contain rice, soy and pineapple had higher levels of phosphorus, potassium, magnesium, copper, manganese, zinc and iron. Roy *et al.* [45] mentioned that chickpea is an excellent source of minerals, especially calcium, phosphorous, iron, and magnesium.

Table 4. Minerals content of the oat bars (on dry weight basis)

Minerals (mg/100g) \ **Formula**	**F_1**	**F_2**	**F_3**	**F_4**
Calcium	83.33^{b} ±0.99	85.89^{a} ±0.68	82.17^{b} ±0.57	81.95^{b} ±0.90
Potassium	232.62^{a} ±0.78	229.28^{b} ±0.78	233.37^{a} ±0.57	228.34^{b} ±0.94
Magnesium	106.68^{a} ±1.09	106.59^{a} ±1.28	104.25^{a} ±1.49	105.96^{a} ±1.17
Sodium	92.20^{a} ±0.88	90.81^{a} ±0.35	92.84^{a} ±1.46	87.26^{b} ±1.85
Phosphorus	360.00^{c} ±1.51	372.86^{a} ±2.99	364.29^{b} ±1.98	365.72^{b} ±1.22
Iron	4.23^{a} ±0.18	4.15^{ab} ±0.07	4.24^{ab} ±0.12	4.00^{b} ±0.06
Zinc	2.83^{a} ±0.09	2.62^{b} ±0.05	2.58^{b} ±0.07	2.54^{b}±0.05
Copper	0.68^{a} ±0.05	0.60^{a} ±0.04	0.66^{a} ±0.06	0.62^{a} ±0.03
Manganese	1.93^{a} ±0.03	1.82^{a} ±0.07	1.90^{a} ±0.06	1.82^{a} ±0.05
Na/K ratio	0.396	0.396	0.398	0.382

F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.
Values are means of three replicates ±SD, number in the same row followed by the same letter is not significantly different at 0.05 level.

The reference daily intakes for iron (18 mg or female and 8 mg for male aged from 19-50y) and zinc (8 mg for female and 11 mg for male male aged from 19-50y) according to DRI [46]. Therefore, each 100 g of oat bars provides 23.50 and 52.87% for F_1, 23.06 and 51.87% for F_2, 23.55 and 53.00% for F_3 and 22.22 and 50.00% for F_4 from daily intake of iron for female and male, respectively. Each 100 g of oat bars provides 35.37 and 25.73% for F_1, 32.75 and 23.82% for F_2, 32.25 and 23.45% for F_3 and 31.75 and 23.09% for F_4 from daily intake of zinc for female and male, respectively.

From the above mentioned data about the nutritional characteristics, it could be demonstrated that the oat bars had reasonable amounts of Fe and Zn.

3.4. Phytochemicals and Antioxidant Activity

Table 5 represents the phytochemicals (*e.g.*, total phenol, flavonoids, tannins and phytic acid) and antioxidant activity. Data showed that total phenols, flavonoids and tannins significantly increased in all oat bars. F_2 had the higest total phenols content (123.36 mg/100g) compared to all formula. While, F_4 followed by F_3 recorded the highest contents in total flavonoids and tannins (70.26 and 40.58 mg/100g), respectively. Sharma *et al.* [40] reported that whole chickpea seeds contain from 70.0 to 220.0 mg/100g tannins.

Regarding phytate content, data revealed that phytate content ranged from 483.23-570.18 mg/100g. Phytate content significantly increased compared to F_1, due to its high content in sunflower, chickpea and pumpkin seeds.

Antioxidant activity as DPPH and ABTS significantly increased in all formulas compared to F_1. F_2 had the highest antioxidant percentages as DPPH and ABTS (23.02% and 29.18%). Cereal bar products contain healthy and natural ingredients (cereals, nuts and fruits) that can contribute to a balanced, rich and healthy diet [3].

Generally, substitution of oat with sunflower, chickpea and pumpkin seeds significantly increased phytochemicals and antioxidant activity compared to the F_1.

3.5. *In vitro* Protein (IVPD) and Starch Digestibilities (IVSD)

Table 6 represents the *in vitro* protein digestibility (IVPD) and starch digestibility (IVSD) of different oat bars formula. Substitution of oat with sunflower, chickpea and pumpkin seeds insignificantly affected the IVPD percentages compared to the F_1. The IVPD values ranged from 68.00 to 70.17%, and F_1 had the highest value. The IVPD ranged from 63.14-77.09% and 67.11-80.23% for raw and dehulled chickpea, respectively [47].

The *in vitro* starch digestibility of the oat bars are also shown in the same Table. It could be noticed that F_3 exhibited the highest *in vitro* starch digestibility (69.88%) followed by F_2 (69.15%). However, F_4 had the lowest value (67.49%) compared to all formulas.

Legumes are an important source of dietary protein and starch for human, but their acceptability and utilization has been limited due to some antinutritional substances such as trypsin inhibitors, phytate and tannins *etc*. [48]. However, digestibility of starch can be improved through heat treatments *e.g.* cooking, roasting and autoclaving [49].

3.6. Color of the Oat Bars

Color measurements of the oat bars are demonstrated in Table 7. Data indicated that substitution of oat with sunflower, chickpea and pumpkin seeds insignificantly decreased the lightness (L^*) values of the oat bars comparing with F_1. Lightness of F_3 significantly increased comparing with F_2 and this may be due to the color of the different seeds. Regarding redness (a^*) and yellowness (b^*) values, there were non significant differences amoung all formulas. Where, F_3 recorded the highest b^* value (26.77) and F_1 recorded the lowest ones (25.03) compared with other formula.

Silva *et al.* [42] mentiond that the b^* values characterize the predominance of yellow, and there was no difference between all cereal bars.

Table 5. Phytochemicals and antioxidant activity of the oat bars

Formula	Total Phenoles (mg/100g)	Total Flavonoids (mg/100g)	Tannins (mg/100g)	Phytate (mg/100g)	Antioxidant activity (%)	
					as DPPH	as ABTS
F_1	80.30^{d}± 0.72	54.77^{d} ± 1.18	27.44^{c} ± 0.50	483.23^{d}±2.76	17.33^{d} ± 0.31	21.87^{b} ± 0.85
F_2	123.36^{a}±1.65	66.44^{b} ± 0.53	36.23^{b}±0.64	563.08^{b}±2.67	23.02^{a} ± 0.18	29.18^{a} ± 0.98
F_3	105.52^{c} ±2.35	61.65^{c} ± 1.06	40.58^{a} ±1.07	498.97^{c}±1.07	18.29^{c} ± 0.33	22.62^{b} ± 1.62
F_4	116.71^{b}±1.88	70.26^{a} ± 0.40	40.14^{a} ±1.06	570.18^{a}±3.21	20.48^{b} ±0.50	23.83^{ab}±1.53

F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula. DPPH= 2, 2-diphenyl-1-picrylhydrazyl, ABTS= [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)].
Values are means of three replicates ±SD, number in the same column followed by the same letter is not significantly different at 0.05 level.

Table 6. *In vitro* protein (IVPD) and starch digestibilities (IVSD) of oat bars (%)

Samples	IVPD	IVSD
F_1	70.17^{a} ± 2.79	68.61^{a} ± 2.01
F_2	69.12^{a} ± 0.34	69.15^{a} ± 0.25
F_3	68.00^{a} ± 0.29	69.88^{a} ± 1.49
F_4	69.97^{a} ± 2.43	67.49^{a} ± 1.52

F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.
Values are means of three replicates ±SD, number in the same column followed by the same letter is not significantly different at 0.05 level.

Table 7. Color values of the oat bars *

Color parameters / Formula	*L**	*a**	*b**
F_1	59.42 ^{ab} ±0.50	6.22 ^{a} ±0.55	25.03 ^{a} ±1.70
F_2	56.12 ^{b} ±2.32	5.31 ^{a} ±0.23	25.74 ^{a} ±0.22
F_3	60.22 ^{a} ±2.75	5.91 ^{a} ±0.50	26.77 ^{a} ±1.89
F_4	57.33 ^{ab} ±1.23	5.24 ^{a} ±0.84	25.86 ^{a} ±0.94

F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.
**L* (lightness with L = 100 for lightness, and zero for darkness), a [(chromaticity on a green (-) to red (+)], b [(chromaticity on a blue (-) to yellow (+)], c (color saturation), h [(hue angle where 0°= red to purple, 90°= yellow, 180°= bluish to green and 270°= blue scale.
Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at 0.05 level.

3.7. Sensory Evaluation of Oat Bars

Sensory evaluation of oat bars formula are presented in Table 8. Data reveal that the substitution of oat with sunflower, chickpea and pumpkin seeds had partially significantly affects appearance attributes of all oat bars. But, it had no significant effects on flavor, taste, texture and overall acceptability. Sunflower formula (F_2) recorded the highest scores of all tested attributes followed by F_4 which recorded the highest scores for overall acceptability comparing with other formula.

In the sensory acceptance test (a 9-point hedonic scale), all cereal bar samples presented acceptable scores (>5) which were considered a good score and the high acceptance rate obtained by the cereal bars reflects in a large potential purchase of such product [50]. Using of pumpkin in the preparation of the cereal bars led to improve the sensory attributes (*e.g.* texture, flavor and overall appearance), and consumer preference. These products can be classified as light products and an alternative to consumers as healthy and functional food [42].

3.8. Water Activity (a_w) of the Oat Bars

Water activity gives information about the safety and quality of food. It characterizes the different states in which water can be found, which includes how much water is "bound" in the food, how much water is available to participate in chemical or biochemical reactions, and how much water is available to help the growth of microorganisms [51]. Figure 1 represents the a_w values of the oat bars during storage. All the given values of the a_w were measured at 22±1°C and the data demonstrated that using of sunflower, chickpea and pumpkin seeds had insignificantly affected the a_w values of the oat bars at zero time. F_1 recorded the lowest value of a_w (0.683). The a_w significantly decreased during storage for three months as found by Estévez *et al.* [52]. The water activity values of cereal bars containing pineapple peel ranged from 0.66 to 0.72 [53].

Table 8. Sensory evaluation of the oat bar formula

Formula	Appearance (9)	Flavor (9)	Taste (9)	Texture (9)	Overall acceptability (9)
F_1	7.65 ^{b} ± 0.58	7.80 ^{a} ± 0.42	7.60 ^{a} ± 0.70	8.00 ^{a} ± 0.53	7.55 ^{a} ± 0.69
F_2	8.50 ^{a} ± 0.57	8.15 ^{a} ± 0.67	8.10 ^{a} ± 0.70	8.25 ^{a} ± 0.68	8.05 ^{a} ± 0.64
F_3	7.95 ^{ab} ± 0.72	7.80 ^{a} ± 0.71	7.80 ^{a} ± 0.54	7.85 ^{a} ± 0.66	7.90 ^{a} ± 0.53
F_4	8.00 ^{ab} ± 0.75	8.15 ^{a} ± 0.70	7.95 ^{a} ± 0.60	8.00 ^{a} ± 1.00	8.00 ^{a} ± 0.77

F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.
Values are mean of ten replicates ±SD, number in the same column followed by the same letter are not significantly different at 0.05 level.

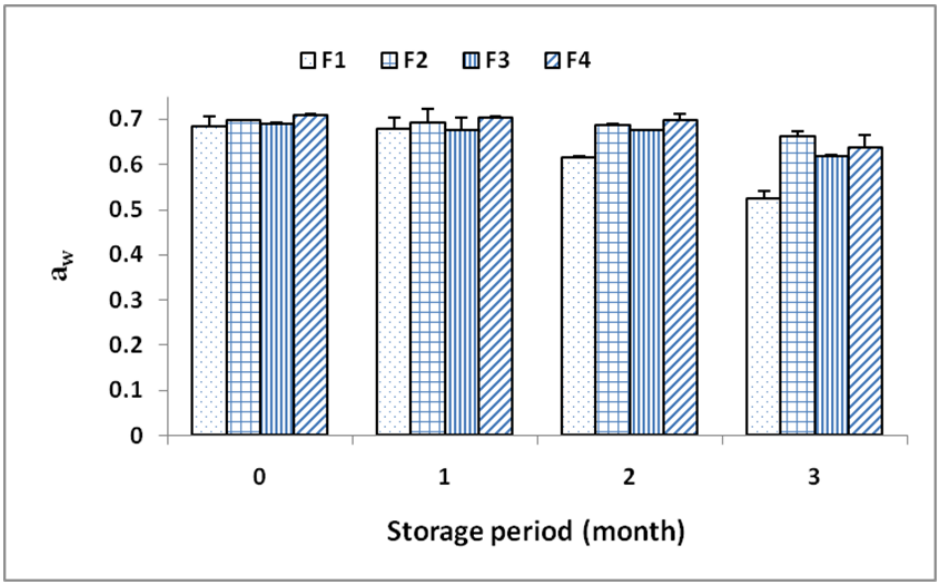

Figure 1. Water activity (a_w) of the oat bars (F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula. Values are mean of three replicates ±SD.)

3.9. Hardness

Figure 2 represents the hardness of the oat bars during storage period for three months. Data show that substitution of oat with sunflower, chickpea and pumpkin seeds (F_2, F_3 and F_4) had significantly decreased of hardness being 6.57, 5.37 and 4.11 N, respectively compared to F_1 (7.01 N) at zero time. From the above mentioned data, it could be stated that F_1 had the highest hardness and F_4 had the lowest ones. This may be due to the higher fat content of F_4 than F_1. After storage for three months, hardness values significantly increased gradually in all formula compared to zero time.

The values of hardness influence in the acceptance of the cereal bars [52]. The addition of pumpkin made cereal bar more compact, which increased its strength to cut and hardness. In the case of the total replacement of oats with pumpkin in the preparation of cereal bar, it is possible to observe a decrease in the strength to cut and hardness [42].

3.10. Peroxide Value

Peroxide value is an indication for lipid oxidation with subsequent formation of peroxides and aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones. These compounds negatively affect physical, chemical and sensory properties of food [54,55].

Data of peroxide value (PV) of oils extracted from oat bars formula during storage at room temperature (25±2°C) for 3 months were illustrated in Table 9. At zero time the PV was 0.87, 1.03, 0.85 and 0.99 meq/kg for oil extracted from F_1, F_2, F_3 and F_4, respectively. The PV values increased with increasing time during storage for one and two months and ranged from 1.46-1.97 and 3.41-4.27 meq/kg, respectively but it was within the acceptable range. The PV gradually increased up to the end of the storage periods in all samples. After storage for 3 months, the PV values in all bar samples increased and ranged from 4.06 to 5.76 meq/kg oil compared to all formulas; however oat bars substituted with pumpkin seeds (F_4) had the highest values (5.76 meq/kg oil). Oat bars could be stored up to two months with safety peroxide values.

Rezig *et al.* [56] indicated that phenolic compounds play a determinant role due to their attributes such as flavor, shelf life and resistance against oil oxidation.

O'Brien [57] stated that a product with peroxide value between 1 and 5 meq/kg is classified at low oxidation state; that between 5 and 10 meq/kg at moderate oxidation and above 10 meq/kg is classified as a high oxidation state. The maximum level of peroxide in virgin oils and cold pressed fats and oils up to 15 milliequivalents of active oxygen/kg oil and other fats and oils up to 10 milliequivalents of active oxygen/kg oil [58].

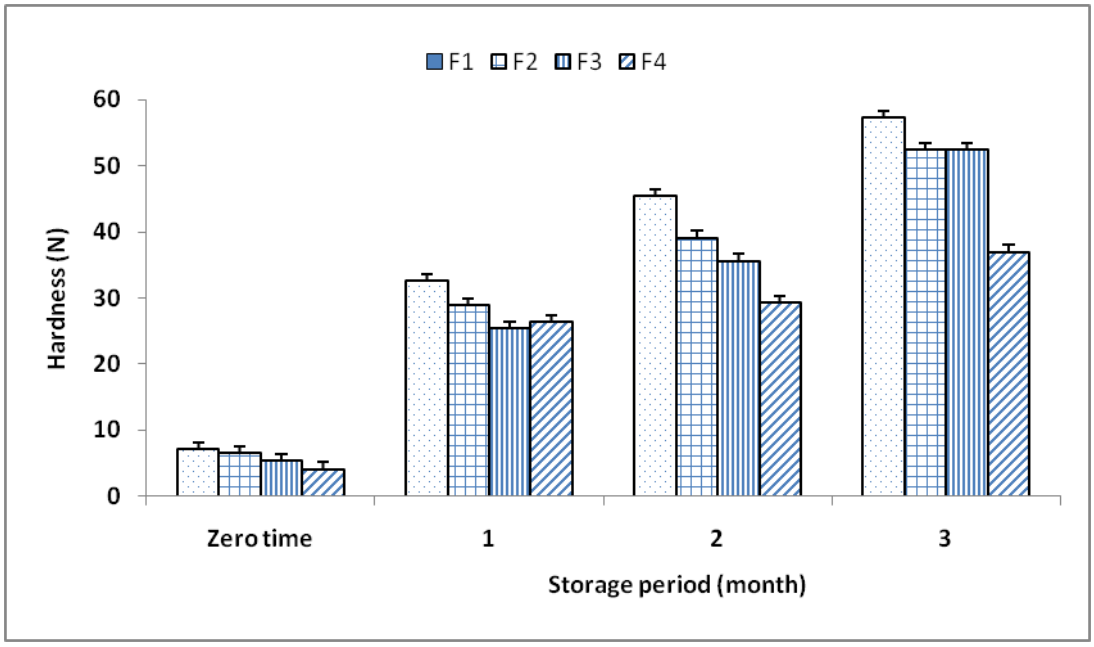

Figure 2. Hardness (N) of the oat bars (F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula. Values are mean of three replicates ±SD.)

Table 9. Peroxide value (PV) of oils of the oat bars formula

Formula	Peroxide value (meq/kg oil)			
	Storage period (month)			
	Zero	1	2	3
F_1	0.87 ^{b} ± 0.03	1.46 ^{c} ± 0.04	3.41 ^{b} ± 0.03	4.06 ^{b} ± 0.04
F_2	1.03 ^{a} ± 0.02	1.76 ^{b} ± 0.14	3.94 ^{ab} ± 0.50	5.68 ^{a} ± 0.06
F_3	0.85 ^{b} ± 0.02	1.59 ^{c} ± 0.06	3.52 ^{b} ± 0.08	4.17 ^{b} ± 0.04
F_4	0.99 ^{a} ± 0.05	1.97 ^{a} ±0.07	4.27 ^{a} ± 0.20	5.76 ^{a} ±0.67

F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.
Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at 0.05 level.

3.11. Microbiological Examinations

Figure 3 and Figure 4 present total plate count (TPC) and yeasts and moulds of oat bars supplemented with sunflower, chickpea pumpkin seeds during storage periods. Data show that the total bacterial count of oat bars affected by replacing of oat with sunflower, chickpea and pumpkin seeds. The results indicated that the addition of sunflower, chickpea and pumpkin seeds showed a decrease in total bacterial count in oat bars at zero time (0.80-0.91 log cfu/g in F_2 to F_4) compared to F_1 (0.92 log cfu/g) which had the highest bactreial count. Total bacterial count in all formula slightly increased after storage for three months (1.95-2.16 Log cfu/g).

The changes in yeasts and moulds counts at zero time and during storage period are illustrated in Figure 4. The yeasts and moulds counts were not detected at zero time and increased with progressing of the studied storage periods (1.92-2.07 Log cfu/g). Also, it could be seen that the highest counts of yeasts and moulds were recorded for oat bars (F_1). Decreasing in total bacterial count and yeasts and moulds in oat bars containing sunflower, chickpea and pumpkin seeds could be due to the presence of phytochemicals in those seeds. Phenolic compounds acting as an antioxidant and antimicrobial agents [59].

Luh and Woodroof [60] found that when the moisture content of food is below 8%**,** microorganisms do not grow while when its content is above 18% some microorganisms may be gradually reproduced. Cooksey [61] stated that food products with water activities between 0.30 and 0.85 have some water available in the product that could allow some microorganisms to grow. According to Egyptian Standards [62], total plate count must not be more than 1000 cfu/g and yeasts and moulds must not exceed 10 cells/g in cereal-based foods.

Generally, the microbial load was acceptable for all formula till two months of storage.

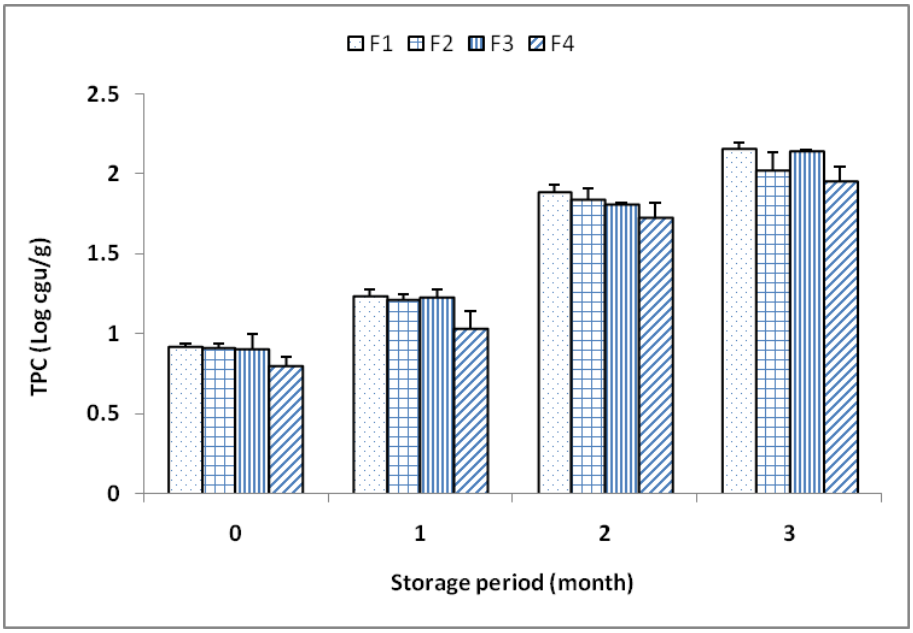

Figure 3. Total plate count (TPC) (Log cfu/g) of the oat bars (Values are mean of three replicates ±SD. F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.)

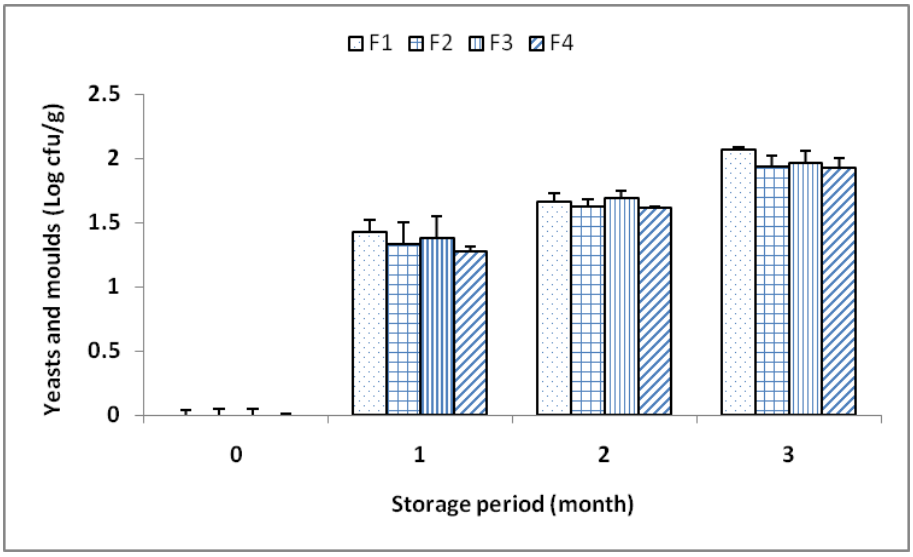

Figure 4. Yeasts and moulds (Log cfu/g) of the oat bars (Values are mean of three replicates ±SD. F_1= Oat flakes formula, F_2= Sunflower seeds formula, F_3= Chickpea seeds formula and F_4= Pumpkin seeds formula.)

4. Conclusion

Finally, it could be clearly concluded that substituting oat bars with sunflower, chickpea and pumpkin seeds enhances the nutritional characteristics and quality of the oat bars. Where, they are a reasonable source of protein, bioactive components, Fe and Zn with good protein digestibility and stability. Besides, it could be stored up to 2 months at room temperature. Along overall sensory quality of the oat bar samples, it had a high acceptable sensory characteristics. The highest acceptability formula was the sunflower followed by pumpkin formula.

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