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Effect of flaxseed flour incorporation on the physical properties and consumer

acceptability of cereal bars

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ABSTRACT

Extensive research has revealed numerous nutritional and health benefits of flaxseed due primarily to its nutrients content. The objective of this study was to evaluate the effect of flaxseed flour addition on the physical and sensory characteristics of cereal bars. Four formulations of the flaxseed cereal bars were prepared by partially replacing oats with flaxseed flour added at levels of 0 (control), 6, 12, and 18%. There were no significant differences (P > 0.05) in water activity, moisture, and firmness values between the flaxseed bars and control. Flaxseed addition significantly (P < 0.05) decreased lightness and increased redness of the bars. There were no significant differences (P > 0.05)between the 12% flax cereal bars and the control with respect to sensory attributes and overall acceptability. The overall acceptability for both 12% flax bars and the control was in between "like moderately" and "like slightly" on the 9-point hedonic scale. The overall acceptability was most highly correlated with flavor acceptability for both control (r = (0.80) and 12% flax (r = 0.82) cereal bars. Flaxseed bars provided 12% dietary fiber of the daily recommended value. These results indicated that flaxseed flour incorporation up to 12% substantially enhanced the nutritional qualities of the cereal bars without affecting their sensory and quality properties.

Key words: Physical properties, consumer acceptability, nutritional composition, flaxseed, cereal bars

INTRODUCTION

Consumers are increasingly interested in functional foods due to the growing awareness of the link between diet and health. Flaxseed (*Linum usitatissimum*) is an ancient crop that has been gaining recognition as a functional food source (Oomah, 2001; Hall, 2002; Hall, 2006; Katare et al., 2012). The renewed interest in flaxseed as a functional food source is due to its unique nutrient profile and potential to affect the risk and course of cardiovascular disease and certain types of cancers (Adlercreutz et al., 1992; Bougnoux and Chajes, 2003; Saarinen et al., 2003). Flaxseed is a rich source of α linolenic acid (ALA), lignans and dietary fiber (Daun et al., 2003; Hall, 2006; Zhang et al., 2011). These nutrients make flaxseed an appealing functional food ingredient (Fitzapatrick, 2007).

Flaxseed incorporation in food products has been the subject of many studies. Ahmed (1999) evaluated the effect of flaxseed meal on the physicochemical properties of an extruded corn snack and reported that with increasing flaxseed meal content, bulk density and breaking strength increased while the expansion ratio decreased. Spaghetti containing flaxseed meal was weaker both before and after cooking compared with spaghetti made with 100% semolina (Manthey et al., 2000). Shearer and Davis (2005) studied the physicochemical properties of whole-wheat muffins and batter prepared with 0 -5% flaxseed meal and observed changes in batter viscosity, muffin color and texture. The effect of flaxseed (7.3%, 11.6%, and 15.5%) on the sensory properties of muffins was evaluated and majority of consumers rated control muffin higher than the 11.6% flaxseed muffin for all sensory attributes and overall acceptability (Ramcharitar et al., 2005). Khouryieh and Aramouni (2012) evaluated the physical and sensory properties of

flaxseed cookies and indicated that cookies containing flaxseed flour had firmer texture, darker color and lower water activity than the control. Chen et al. (1994) studied the oxidative stability of whole and ground flaxseed, either alone or as an ingredient in a muffin mix. The ALA content decreased from 55.1 to 51.3% in ground flaxseeds and to 51.7% in lipid extracts. Whole or ground flaxseed or lipid extracts showed long term stability at room temperature for 280d. Chen et al. (1994) concluded that under typical baking conditions, there was minimal loss of ALA from flaxseed. Malcolmson et al. (2000) examined the storage stability of milled flaxseed at ambient temperatures for 128d and concluded that no significant increase in peroxide values or conjugated double bonds was found during the 128 d-storage period; a trained sensory panel could not detect any differences in the odor properties of fresh or stored milled samples. In a different study conducted by Przybylski and Daun (2001) it was reported that ALA content in milled flaxseed stored at ambient temperatures for up to 20m was relatively unchanged and demonstrated resistance to oxidative deterioration. These studies suggest that flaxseed nutrients, lignans and ALA, are stable under normal processing and storage conditions, and flaxseed ingredient creates acceptable food products.

Cereal bars are commonly referred to as snack bars. Cereal bars are often made of a base of processed cereal grains and may contain different ingredients such as nuts, seeds, fruit, raisins, and chocolate. Cereal bars have been used for multiple purposes such as breakfast, snacks, energy, meal replacement, and weight control. Cereal bars are considered healthy foods because they are generally rich in fiber and low in fat (Carvalho et al., 2011). An enriched cereal bar product containing a significant amount of flaxseed and having acceptable sensory characteristics would provide an excellent source for consumers to increase consumption of fiber and ω -3 fatty acids. There have been no published studies on the effect of flaxseed flour on the quality properties of cereal bars. The optimal level of flaxseed flour to be incorporated into products without affecting the physical and sensory properties of foods is not clear. The objective of this study was to evaluate the effect of flaxseed flour on the physical characteristics and consumer acceptability of the cereal bars.

MATERIALS AND METHODS

Materials

Golden flaxseed flour was purchased from Pizzey's Milling (Gurnee, IL, USA). The nutritional composition of the flaxseed flour is given in Table 1. On arrival, the flaxseed flour was stored in Ziploc plastic bags and kept at 5°C in a refrigerator until used. SUPRO® 313 isolated soy protein (91% protein, and 4.5% moisture) was donated by Solae Company (Saint Louis, MO, USA). KRYSTAR 300 Crystalline fructose was donated by Tate and Lyle (Decatur, IL, USA). Rolled oats, all purpose flour, honey, canola oil, salt, and cinnamon were purchased from local stores.

Cereal bar preparation

The cereal bar formula was made from 31.3% rolled oats, 12.5% all purpose flour, 9.4% soy protein isolate, 18.8% crystalline fructose, 12.5% honey, 2.5% canola oil, 0.6% cinnamon, 0.6% salt and 11.9% water. Preliminary work was conducted to determine an appropriate flaxseed levels in the formulations. The flaxseed cereal bars were prepared by partially replacing oats with flaxseed flour added at levels of 0 (control), 6, 12, and 18% (total weight basis). The cereal bar formula varied only in the amount of rolled oats and flaxseed flour. Dry ingredients (rolled oats, flaxseed flour, all purpose flour, isolated soy protein, salt, and cinnamon) were mixed using a KitchenAid mixer (St. Joseph, MI, USA) for 2 min at speed 2 to give a uniform mixture. Liquid ingredients (honey and canola oil) were added and mixed for 2 min. Water was incorporated slowly and the entire dough was mixed at speed 2 for 2 min. After mixing, the dough was poured into greased aluminum pans. The dough was compressed with a spatula into metal pans to form a uniform mass. The bars were baked in a conventional oven at 148.9°C for 22 min. The pans were cooled to room temperature and the dough was cut into uniform bars (8 X 3 X 2 cm). The bars were stored into Ziploc bags at room temperature and evaluated the next day after baking.

Physical measurements of cereal bars

Moisture content. Moisture content of the cereal bars was determined according to the AACC method 44-19 (AACC, 2000), using isotemp premium oven 700 series (Fisher Scientific Inc, Dubuque, Iowa). Two grams of crushed cereal bars were placed in weighed aluminum dishes and dried in a hot air oven at 130 °C for 2 h.

Water activity. The water activity was measured using an Aqua Lab water activity meter series 3TE (Decagon Devices Inc., Pullman, WA). Bar samples were crushed into small pieces and loosely placed into the plastic sample cups until the cups were approximately half full. The cups with the bar samples were then placed into the measuring chamber one at a time.

Texture. The TA.XT*Plus* Texture Analyzer (Stable Micro System, Scarsdale, NY, USA) with 5-kg load cell was used to measure the hardness of the bars. Bar hardness was measured using a three point bending test. The hardness of the bars was indicated by the maximum peak force required to break the bars. A 5.0-mm stainless steel punch probe

was used. On the basis of preliminary trials, texture parameters were set to measure compression force at return to start cycle, a pretest speed, test speed, and post-test speed of 10 mm/s, 2mm/s and 10mm/s, respectively, and distance of 5mm. The force-time plots were analyzed to determine the maximum force required to break the bars. A minimum of 9 measurements for each bar were analyzed.

Color analysis. The color of the bars was measured with MiniscanTM MS/S Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA, U.S.A.) at ambient temperature. The colorimeter was calibrated by using white and black standards. The color values L*, a*, and b* were measured with a C illuminant and a 10° standard observer. The dimension L* means lightness, with 100 for white and 0 for black, while the a* and b* values range from 60 (red and yellow respectively) to -60 (green and blue respectively). The color measurement was determined on 3 different locations on the same bar.

Sensory evaluation

Focus group. A focus group session consisting of 6 people was conducted. The main objective of the focus group was to gain an early assessment from panelists on acceptability of cereal bars with 3 levels of ground flaxseed (6.0, 12.0 and 18.0%) prior to performing a consumer acceptance test. The focus group consisted of four females and two males aged 24–32 years. Participants were selected based on their consumption of cereal bars and willingness to participate. The focus group session was held using a roundtable discussion. The session lasted for 2 h with a 10 min break in the middle. Each participant was provided with 3 cereal bars on 3 coded plates. Water was supplied to cleanse palate between samples. The session began with a short introduction, in which

the participants were told that they were going to taste cereal bars formulated with flaxseed flour. The participants were first allowed to discuss the cereal bars freely, and then the moderator asked questions regarding appearance, color, flavor, texture, and overall acceptability of each bar. The participants were asked to list the positive and negative characteristics of the cereal bars (Resurrection, 1998).

Consumer acceptability. The consumer acceptability study was carried out on a laboratory scale. Laboratory acceptance testing is the most frequently used among the various types of sensory acceptance tests (Stone and Sidel, 2004). Prior to tasting, panelists signed informed consent form and were asked a series of questions about demographic information (gender, age, and education), frequency of consumption of cereal bars, and food allergies. Individuals were excluded if they reported allergies to any food product. Based on the focus group assessment, the 12% flax bars were compared to the control to evaluate the product's consumer acceptance. A total of 105 untrained panelists participated in the consumer acceptability study. Each panelist evaluated two samples of the bars in one session. Bars were cut in halves (~30 g/sample) and offered monadically to panelists on odorless plastic plates coded by 3-digit random numbers at room temperature. Water and unsalted crackers were provided to panelists to cleanse their palates between samples. A 9-point hedonic scale (9 = like extremely; 5 = neither like nor dislike; 1= dislike extremely) is used to determine degree of liking for products (Peryam and Girardot 1952).

Nutritional analysis

The nutritional composition of the cereal bars (carbohydrates, fats, protein, fiber, calories, vitamins and minerals) was determined using Genesis R&D software version 7.1

(ESHA Research, Salem, OR, USA). All ingredients for every bar were entered into the Genesis software and the moisture content adjusted to reflect the values obtained by the oven method. The nutritional analysis was used to determine allowable claims for the flax cereal bars.

Statistical Analysis

A completely randomized design with three replications with three subsamples of all experiments was conducted. Statistical analysis was performed using the SAS software version 9.0. (2002-2003). An Analysis of Variance (ANOVA) and Fishers' Least Significant Difference (LSD) comparison was performed on all data to determine the effect of flaxseed flour on the physical and sensory properties of cereal bars. Significant differences among formulations were determined at the significance level of P < 0.05. A correlation analysis between sensory attributes and overall acceptability was performed using SAS Proc Corr.

RESULTS AND DISCUSSION

Physical properties of cereal bars

There were no significant differences (P > 0.05) in water activity (0.69 -0.70) and moisture content (20.7-20.9%) between the cereal bars formulated with flaxseed flour and the control, as shown in Table 2. The addition of flaxseed flour up to 18% did not have any significant effect on the water activity and moisture content of the cereal bars. The water activity concept is widely used to assess the microbial growth, lipid oxidation, non-enzymatic and enzymatic activities, and the texture of finished foods (Rahman and Labuza, 1999). Most food products have a water activity in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. Molds and yeasts usually start to grow at a water activity between 0.7 and 0.8, while the bacterial growth starts to take place at water activity of 0.8.

The effect of flaxseed flour on the hardness of the snack bars is presented in Table 2. The maximum force required to break the bars was slightly increased as the level of flaxseed flour increased in cereal bars. However, the amount of flaxseed flour used to replace the oats in the cereal bars did not significantly (P > 0.05) affect the hardness of the bars. Since the cereal bar formula varied only in the amount of rolled oats and flaxseed flour, the increase in the bar hardness can be attributed to the differences in the composition of the flaxseed flour and oats. Khouryieh and Aramouni (2012) reported that cookie hardness increased as the level of flaxseed flour increased in cookies, and the increase in the cookie hardness was attributed to the high protein content of flaxseed flour. Shearer and Davis (2005) observed a firmer texture for muffins containing up to 5% flaxseed than the control muffins as shown by the maximum force required to compress the muffins.

Color is one of the most important quality factors affecting consumers' perception and acceptance of food products (Schwartz et al, 2007). Significant differences (P < 0.05) were found between the L*, a* and b* values of the four cereal bars (Table 2). The cereal bars with flaxseed flour were significantly (P < 0.05) darker than the control bars as indicated by lower L* values. The 6% and 12% flax bars had significantly (P < 0.05) higher a* (redness) values than the control, while the 18% flax bars were not higher (P > 0.05). The 18% flax bars had a significantly lower b* (yellowness) values than the other treatments. The changes in color values observed for flaxseed bars can be related to the Maillard browning reactions possibility between flaxseed protein and other ingredients such as honey. These results are consistent with Khouryieh and Aramouni (2012), who concluded that cookies with increased flaxseed flour levels were significantly darker and redder than the control as indicated by lower L* and higher a* values. The results are also in agreement with Koca and Anil (2007), who reported that crumb "L" values decreased and "a" values increased with increasing flaxseed flour levels in bread formulations. Ahmed (1999) reported that flaxseed significantly (P < 0.05) reduced brightness and increased redness in corn-flax extruded snack. Similar trends were also shown in flaxseed containing baked goods, as reported by Shearer and Davis (2005) and Alpaslan and Hayta (2006).

Sensory evaluation

The early assessment for the cereal bar formulations conducted by the focus group revealed that the 12% flaxseed bars had the highest ratings for all sensory attributes. The majority of the participants agreed that the content of flaxseed in 12% flax bars was just about right. The focus group participants described the 12% flax bars as "having a pleasant taste," "nice nutty flavor," "good texture," "not dark in color," and "good appearance." The 6% flaxseed bars were similar to the 12% flaxseed bars. The 18% flaxseed bars had the lowest ratings. The 18% flax bars were described as "having an unpleasant taste," "too dry," "dark in color," and "unattractive appearance." Therefore, the 12% flaxseed bars were selected to conduct the consumer acceptability study.

A total of 105 panelists participated in the consumer acceptability study including 69 females and 36 males, accounting for 65.7 and 34.3% respectively, between 18 and 65

years of age (74% ages 18-30, 17% ages 31-55, 9% ages 56-65). With regards to education 12% finished high school, 38% had some college or technical school, 29% were college grad and 21% were post grads. Mean acceptance scores of sensory attributes (appearance, color, flavor, texture, and overall acceptability) are shown in Figure 1. The 12% flaxseed cereal bars were compared with the control (0% flaxseed flour). There were no significant differences (P > 0.05) between the 12% flaxseed bars and the control with respect to appearance, color, flavor, texture, and overall acceptability. The overall acceptability for both flaxseed cereal bars was in between "like moderately" and "like slightly" on 9-point hedonic scale. The mean value of overall acceptability for the 12% flaxseed bars was 6.2, comparing to 6.3 for the control. These results may indicate that subjects were unable to distinguish the 12% flaxseed bars from the control bars. These results are consistent with Khouryieh and Aramouni (2012), who reported that 6 and 12% flaxseed cookies were the most preferred among consumers, while the flaxseed flour substituted at 18% was shown to have a negative effect on all the sensory attributes. Consumer acceptability of muffins with flaxseed was investigated by Ramcharitar et al. (2005) who reported that the majority of consumers rated (P < 0.001) the control muffin (0% flaxseed) higher than the 11.6% flaxseed muffin for all sensory attributes and overall acceptability. The high acceptance of our 12% flaxseed formulation by consumers may be related to the desirable flavor of cinnamon. Many consumers who participated in the acceptance test commented that the cinnamon aroma of the cereal bars was very strong and appealing. Yeu et al. (2008) reported that addition of cinnamon flavor improved overall, aroma, and taste acceptance scores in an extruded soy-based high-protein breakfast cereal by masking the undesirable soy aromas of the samples.

A correlation between sensory attributes and overall acceptability was conducted to better understand the relationships between sensory attributes of cereal bars and assess the impact of sensory attributes on the overall acceptability (Table 3). The overall acceptability was most highly correlated with flavor acceptability for both control (r =(0.80) and 12% flax (r = 0.82) cereal bars. For the control cereal bars, color scores followed next and positively correlated (r = 0.74) while, for the 12% flax cereal bars, the sensory texture scores were next to the flavor and positively correlated (r = 0.67) with overall acceptability. Overall acceptability was also moderately correlated with sensory scores of appearance for both control (r = 0.58) and 12% flax (r = 0.51) cereal bars. These results suggest that the flavor attribute was the most critical factor in consumer preferences for overall acceptability. These results are in agreement with Ramcharitar et al. (2005) who reported that flaxseed muffin flavor was most strongly and positively correlated (p < 0.01, r = 0.82) with overall acceptability, which highlighted the importance of developing strategies to minimize undesirable flavors arising from the use of flaxseed flour. Khouryieh and Aramouni (2012) reported that the flaxseed cookie flavor was the most highly correlated with the overall acceptability (r = 0.90).

Nutritional analysis

The nutrient contents for the cereal bars formulated with flaxseed flour are shown in Table 4. The official serving size by Food and Drug Administration for cereal bars is 1 bar (40g). Nutritional labels of various commercial bars in the United States have a range of net weights per 1 bar serving size from 40 to 60g, depending on the type of bar. The total fat content in the cereal bar formulations increased as the percent of flaxseed flour increased. While the amount of monounsaturated fatty acids remained constant, the amount of polyunsaturated fatty acids increased substantially in the flaxseed formulations comparing to the control. The amount of the polyunsaturated fatty acids increased by 50% in the 6% flaxseed formulation and by 75% in both the 12 and 18% flaxseed formulations comparing to the control. This increase is attributed to the high fat content of flaxseed flour. Mentes et al. (2008) reported that oil content and proportion of linolenic acid in fatty acid composition increased as the ratio of flaxseed flour in bread formulation was increased. According to Daun et al. (2003) flaxseed oil content has a range of 38-45% fat depending on location, cultivar, and environmental conditions, of which 73% is polyunsaturated fatty acids and 18% monounsaturated fatty acids. Although the amount of total carbohydrates decreased as the amount of flaxseed flour increased in the cereal bar formulations, the amount of dietary fiber increased by 33% for both the 12 and 18% flaxseed formulations. The high increase in fiber content can be related to the differences in composition of flaxseed flour. Flaxseed is a rich source of dietary fiber and accounts for 28% of its composition (Hall et al., 2006). Dietary fiber has been widely viewed as a component essential in lowering the risk of colon cancer (Hall et al., 2006). The nutrient content indicated that the 12% and 18% flax bar formulations would be considered a "good source of fiber," providing 12% of the daily reference value. A "good source of fiber" claim requires fiber content of 10-19% of the daily reference value (21CFR 101.54).

CONCLUSIONS

The cereal bars quality was strongly affected by the chemical composition of the flaxseed flour. Flaxseed flour incorporation up to 12% substantially enhanced the nutritional qualities of the cereal bars without affecting their sensory and quality

properties. Consumer acceptability results indicated that subjects were unable to distinguish between the 12% flax cereal bars and the control. The correlation results suggested that the flavor attribute was the most critical factor in consumer preferences for overall acceptability of flaxseed cereal bars. The amounts of the polyunsaturated fat and dietary fiber increased substantially in the flaxseed cereal bars. The nutrient content indicated that the 12% and 18% flax bar formulations would be considered a "good source of fiber," providing 12% of the daily reference value.

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Component	Amount per 100g
Energy (Kcal)	470
Total Fat (g)	37.0
Saturated (g)	3.4
Polyunsaturated fatty acids (g)	27.0
(Omega 3) α-Linolenic Acid (g)	20.0
(Omega 6) Linoleic Acid (g)	6.0
Monounsaturated fatty acids (g)	6.6
Trans Fatty Acids (g)	< 0.1
Cholesterol (mg)	0
Total Carbohydrates (g)	29.0
Sugars (g)	2.0
Dietary Fiber (g)	27.0
Soluble (g)	9.0
Insoluble (g)	18.0
Protein (g)	20.0
Moisture (g)	8.0
Ash (g)	3.6

Table 1. Proximate composition of flaxseed flour

Flaxseed Level	Water activity	Moisture	Hardness (g)	Color ²		
(%)		content (%)	-	L^*	a*	b*
0	$0.70^{a} \pm 0.01$	$20.7^{a} \pm 1.0$	1895 ^a ± 331	$51.60^{\circ} \pm 1.73$	$14.08^{a} \pm 0.75$	$30.18^{a} \pm 0.69$
6	$0.69^{a} \pm 0.01$	$20.7^{a}\pm1.4$	$1916^{a}\pm254$	$48.63^{ab}\pm2.46$	$15.29^{\circ} \pm 1.04$	$30.27^{a} \pm 1.58$
12	$0.69^{a} \pm 0.01$	$20.9^{a}\pm1.0$	$1912^{a} \pm 291$	$49.68^{b} \pm 1.71$	$14.92^{b} \pm 0.89$	$30.79^{a} \pm 0.71$
18	$0.70^{a} \pm 0.01$	$20.7\ ^{a}\pm2.8$	1940 ^a ± 255	47.55 ^a ± 1.14	$14.47^{ab}\pm0.84$	$28.63^{\mathrm{b}} \pm 1.47$
LSD ³	0.01	1.2	201.3	1.29	0.63	0.84

Table 2. Effects of flaxseed flour on the water activity, moisture content, texture and color of the flaxseed cereal bars¹

^{abc} Means with different superscripts in columns indicate significant difference (P < 0.05). ¹ Values are expressed as means ± standard deviations (n=3) ² L* designates the lightness of the sample, 100 = white, 0 = black; a* (ranges from 60 to -60) indicates redness when positive, greenness when negative; b* (ranges from 60 to -60) indicates yellowness when positive, blueness when negative.

LSD represents least significant difference.

	Overall acceptability			
Sensory attributes	Control	12% Flaxseed		
Appearance	$0.58 (r^2 = 0.34)$	$0.51 (r^2 = 0.26)$		
Color	$0.74 (r^2 = 0.55)$	$0.56 (r^2 = 0.31)$		
Flavor	$0.80 \ (r^2 = 0.64)$	$0.82 (r^2 = 0.67)$		
Texture	$0.71 (r^2 = 0.50)$	$0.67 (r^2 = 0.45)$		

Table 3. Correlation coefficients¹ (r-values) between overall acceptability and sensory attributes of control and flaxseed supplemented flour cereal bars

¹ Significant at the 0.0001 level

		Control		6% Flaxseed		12% Flaxseed	
	Reference Value ¹	Amount	% Daily value	Amount	% Daily value	Amount	% Da value
Total Calories	2,000	130	-	140	-	140	-
Calories from fat	-	20	-	20	-	30	-
Total fat (g)	65	2	3	3	5	3.5	5
Saturated fat (g)	20	0	0	0	0	0.5	2
Trans fat (g)	-	0	-	0	-	0	-
Polyunsaturated fat (g)	-	0.5	-	1	-	2	-
Monounsaturated fat (g)	-	1	-	1	-	1	-
Cholesterol (mg)	300	0	0	0	0	0	0
Sodium (mg)	2,400	130	5	130	5	130	5
Total carbohydrate (g)	300	24	8	22	7	22	7
Dietary fiber (g)	25	2	8	2	8	3	12
Sugars (g)	-	11	-	11	-	11	-
Protein (g)	50	5	-	6	-	6	-
Vitamin A (IU)	-	-	0	-	0	-	0
Vitamin C (mg)	-	-	0	-	0	-	0
Calcium (mg)	-	-	2	-	2	-	2
Iron (mg)	-	-	6	-	6	-	6

 Table 4. Nutritional analysis¹ for control cereal bars and cereal bars formulated with various levels of flaxseed flour

¹ Percent daily values are based on a 2000-calorie diet.



Figure 1. Mean sensory attributes of control and 12% flaxseed cereal bars.

¹ Sensory attributes as determined by consumer evaluation (N=105) on a 9-point hedonic Scale: 1- dislike extremely, 5-neither like nor dislike, 9- like extremely. Sensory values are expressed as means of 3 replicates. Error bars represent standard errors. No significant differences (P > 0.05).