

Snack bars with high soy protein and isoflavone content for use in diets to control dyslipidaemia

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Abstract

The objective was to develop a cereal bar product rich in isoflavones and soy protein to be used in diets for controlling dyslipidaemia. A soy snack bar with 39.88 g/100 g dietary fibre, 34.25 g/100 g protein, 100.39 mg/100 g isoflavones and 245.47 kcal/100 g was produced. The shelf life of the product was tested over a period of 6 months. The hardness, water activity and darkness of the snack bars increased with storage time. Clinical trial was carried out to evaluate the effectiveness of the soy snack bar on the lipid profiles of 22 dyslipidaemic subjects for a period of 45 days. There was a decrease in levels of triglycerides (TGs) (–20%) and an increase in high-density lipoprotein cholesterol (HDL-c) (+8%) comparing with baseline values. Therefore, relatively high intakes of soy protein and isoflavones in moderately hypercholesterolaemic subjects (>200 mg/dl) may have a moderately beneficial effect on plasma lipid profiles, as TG and HDL-c levels, without additional diet restrictions.

Keywords: *functional food, cereal bars, soy, hypercholesterolaemia*

Introduction

Cereal bars are a nutritional food with multiple ingredients, including cereal, fruit, nuts and sugar. Several types of cereal bars exist, including high-protein, high-fibre and high-calorie bars. Moreover, other snack bars are available including fruit bars, crunchy bars, salty bars, low-calorie bars and diet bars. Furthermore, bars with filling, bars with chocolate, bars without chocolate and bars with potentially functional additives such as prebiotics are also available.

Nutrition, convenience, price and sensory attributes are important characteristics in determining the acceptability of a food product (Boustani and Mitchell 1990; Bower and Whitten 2000). According to Roberfroid (1999), one of the major challenges is

to provide busy consumers with healthy ready-to-eat foods.

Cereal bars are practical, easy to manufacture and depending on the ingredients used can be sold at a low price. These products can be conveniently added to a packed lunch or eaten as a snack. Although the process for manufacturing cereal bars is relatively easy, incorporating high amounts of functional components can be difficult due to the individual characteristics of the components and their interactions with corn syrup or other ingredients. In addition, these functional components can be detrimental to sensory characteristics, such as texture and taste, and physical properties, such as water activity.

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Diets for controlling excess body weight, hyperglycaemia and dyslipidaemia typically include low-energy and low-saturated-fat diets, but these diets have limited efficacy due to the required long-term commitment. However, long-term health benefits can be gained from dietary proteins and bioactive non-nutrients (i.e. phytochemicals) that are present in soy (Cederroth and Nef 2009).

Soy products and products with added soy are in demand due to the purported health benefits of soy consumption. Soybeans contain isoflavones and proteins which are compounds that have been shown to improve lipid metabolism by activating receptors for low-density lipoprotein (LDL; Anderson 2003). Moreover, soy proteins have been connected to reductions in menopause symptoms and a reduced risk for several chronic diseases, including cancer, heart disease and osteoporosis (Riaz 1999).

Hypercholesterolaemia is a major risk factor for cardiovascular disease. Some studies have shown that soy protein intake increases high-density lipoprotein cholesterol (HDL-c) and decreases total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-c) and triglycerides (TGs) (Reynolds et al. 2006). The mechanism(s) for the potential hypolipidaemic effect of soy protein appears to be multifactorial. Potential mechanisms include the inhibition of cholesterol absorption, enhanced bile acid excretion, increased receptor-mediated clearance of lipids from the blood, enhanced LDL receptor activity and enhanced 7 α -hydroxylase activity (Wang et al. 2004). The American Food and Drug Administration (FDA 1999) allowed manufacturers of soy-containing foods to indicate the beneficial role of soy protein in reducing the risk of coronary heart disease on their product labels.

The addition of soybean ingredients to food products, such as cereal bars, may be an option to increase the consumption of soybeans, especially in Western countries where the consumption of soy and soy derivatives is low as compared with Asian countries where soybeans and soybean derivatives are frequently consumed. The objective of this study was to develop a snack bar with high isoflavone and soy protein content for use in diets to control dyslipidaemia and to provide an additional option of soy products to consumers.

Materials and methods

Materials

The ingredients used to manufacture the snack bar were soy crisps (SUPRO Plus Nuggets 60, Solae Company, São Paulo, Brazil), soy protein isolate (SUPRO 780, Solae Company), toasted soy without salt (Só Soja do Brasil Ltd, Caldas Novas, Brazil), textured soy protein (TEXPRO, Exin Indústria e Comércio, Massaranduba, Brazil), glucose syrup (Glucogil 40/82, Cargill, Guarujá, Brazil), maltodextrin (Maltogil, Cargill, Uberlândia, Brazil)

and palm oil (Vegetable fat 370B, Agropalma, Belém, Brazil). In addition, lecithin, colouring ingredient, glycerine, demerara sugar and dried bananas from a local market were used.

Chemical composition of the ingredients and the soy snack bars

The chemical composition of the individual soy ingredients and of the total bar formulation were determined. Moisture (air oven at 105°C), fat (Soxhlet), ash (muffle furnace at 550°C), protein (Kjeldahl) and dietary fibre (total, soluble and insoluble fibres were determined using an enzymatic method) were analysed by official AOAC methods (2000). The nitrogen/protein conversion factor used to calculate total protein content was 6.25. The carbohydrate content was estimated by the mass remaining after protein, fat, ash and water contents were subtracted from the total mass. The caloric value was calculated by applying calorie conversion factors to carbohydrates (4 kcal/g), proteins (4 kcal/g) and lipids (9 kcal/g) and are expressed as kcal/100 g.

The chemical composition of the ingredients was determined to characterize the soy ingredients and to determine the amount of protein in each ingredient for calculating the predicted amounts needed to achieve at least 25 g of vegetable protein in 100 g of final product using the following formula: $\%P_{\text{bar}} = \sum \% \text{Ing} \times \%P_{\text{ing}} / 100$, where $\%P_{\text{bar}}$ is the percentage of protein in a soy bar (predicted value), $\% \text{Ing}$ is the percentage of the ingredient in the mixture and $\%P_{\text{ing}}$ is the protein percentage of the ingredient.

Extraction and determination of isoflavones by HPLC from soy ingredients and the soy snack bar

Isoflavones quantitative analysis was carried out by high-pressure liquid chromatography (HPLC) according to Berhow (2002). The isoflavones were extracted according to Carrão-Panizzi et al. (2002). The soy snack bars and its ingredients were grinded in a 'coffee grinder mill' and the obtained flours were defatted at room temperature with hexane (HPLC grade). Approximately 100 mg of soy snack bars and its ingredients, defatted flours, were placed in test tubes and the isoflavones were extracted with 4.0 ml of 70% ethanol solution containing 0.1% of acetic acid for 15–17 h at room temperature. The extracts were then transferred to Eppendorf tubes and centrifuged at 4°C, 21.000g for 15 min. The supernatants were filtered using 0.43 μm filters (Millipore) and 20 μl was used to separate and quantify the isoflavones in a chromatographer (Waters 2690) equipped with a photodiode array detector (Waters 996) and oven (CTO-10AS VP) with temperature setting at 26°C. The isoflavones were eluted in a reverse-phase column (YMC Pack ODS-AM C18) 4.6 mm \times 250 mm and 5 μm particles by the linear gradient system. The initial gradient

system condition consisted of 20% methanol containing 0.025% trifluoroacetic acid (TFA), pH 3.0 and 80% H₂O containing 0.025% TFA (pH 3.0). The proportion of 90% of methanol and 10% of H₂O was reached in 35 min of elution, and the isoflavones were separated and detected at 260 nm. The gradient proportion of 100% of methanol and 0% of H₂O was reached at 40 min, remaining in this condition for 5 min and then returned to the initial condition of 20% methanol and 80% H₂O in 10 min. The final elution time was 55 min. The solvent flow rate was 1 ml/min.

Isoflavones were identified and quantified by comparing the standard genistin, daidzin and glycitin curves. The concentration of malonyl-glycosides and aglycones was calculated from the standard curves of their corresponding beta-glycosides, using the similarity of the molar extinction coefficients of malonyl-isoflavones and their beta-glycosides (Coward et al. 1998). The isoflavone contents were expressed in mg/100 g of defatted soy snack bars and in mg/100 g of defatted ingredients flours on a dry weight basis.

Soy snack bar formulation and production

Together, the soy ingredients in the soy bar had sufficient amounts of soy protein and isoflavones to provide the health benefits described by previous studies (Cederroth and Nef 2009) when three bars are consumed per day. The predicted amounts of protein and isoflavones present in 100 g of the bars were approximately 30 g and 100 mg, respectively. The ingredients were used in the amounts defined by the previously mentioned laboratory tests.

The bars were manufactured in batches of 3.0 kg. In the manufacturing process, syrup was heated at 100°C and 78°Brix and blended with the other ingredients to form crude dough. The dough was laminated and cut to form individual bars, which were packaged in aluminium foil, sealed in packs and stored at room temperature (21–25°C) prior to analysis. Bars 28 g each, approximately, having dimensions 10.0 × 2.7 × 1.4 cm were obtained.

Shelf-life study

The texture, colour, water activity and microbiological analyses of the cereal bars were performed after bars were manufactured. Measurements were taken every 30 days for 6 months. Samples were randomly collected for analysis during the storage period. Ten soy bars were randomly selected and tested for texture or water activity, and five bars were selected for microbiological and colour analyses.

Instrumental texture. The hardness (peak force during first compression) of the soy bars was determined using a TA.XT2 Texture Analyser (Stable Micro System, UK) with a HDP/BSK blade set, with a knife

probe and XTRAD software. Ten samples with similar sizes (10.0 cm × 2.7 cm × 1.4 cm) were cut with a knife using a cross-head speed of 0.5 mm/s, a distance of 15 mm and a force threshold of 0.05 N.

Colour. The colour measurement was performed as described by Oliveira et al. (2003), with some adaptations. One light source was used on the sample, and the distance between the camera and the samples was 18 cm. The images were captured using a digital camera (Sony Cyber-shot, 7.2 mega pixels). The digital images were converted to mean red, green and blue (RGB) values by a pixel-to-pixel application. This colour model is the most basic and well known and is based on the perception of colour (Adobe Technical Guides 2000). The data were converted to the CIELAB system by obtaining light (L*), red/green colour (a*) and blue/yellow colour (b*) values. A colour system adopted by the Commission Internationale de l'Éclairage (CIELAB) was used to measure development of 'L', 'a' and 'b' values based on distinctions of the optical nerve and brain (Adobe Technical Guides 2000).

Water activity (*a_w*). Water activity of the soy bars was measured using a water activity meter (AquaLab model CX-2, Decagon Devices, Inc., Pullman, WA) under controlled temperature.

Microbiological analyses. The presence and amount of yeasts, moulds, *Staphylococcus aureus*, *Salmonella* sp. and coliforms were determined at 45°C, and the amount of *Bacillus cereus* was determined according to AOAC official methods (1998; n. 2001.05, 967.25, 991.14 and 980.31).

Sensory analyses. Sensory evaluation of the final product in terms of general appearance, colour, flavour, taste and texture was performed by 50 individuals who were faculty, staff and students at the Universidade Estadual de Londrina. These participants were randomly invited to participate in this study. A verbally anchored 9-point structured hedonic scale was used to evaluate overall acceptability (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike, 5-neither like nor dislike, 6-like, 7-like moderately, 8-like very much and 9-like extremely). The panellists tasted the snack bar samples and chose the point on the scale that best matched their opinion of the product. The panellists were asked about purchase intent (yes or no) and whether that intent would change if they were informed that the product could help reduce the risk of developing diseases such as cardiovascular disease (yes or no). This evaluation was carried out

approximately three months after manufacture of the snack bars.

Clinical trial

Twenty-two dyslipidaemic patients were selected from a cardiology clinic in Londrina (Paraná, Brazil) and were instructed to consume three soy bars per day between the main meals over a period of 45 days. The patients were sedentary and were instructed not to make any changes in their daily lifestyle. To profile the patients, they were asked to answer questionnaires about their lifestyle, socio-economic status, food frequency and daily routine. Body weight, height and waist circumference were measured to calculate body mass index (BMI) and cardiologic risks. The criterion for inclusion was patients greater than or equal to 18 years old having CHOL plasma levels greater than or equal to 200 mg/dl and who had no other diseases. All participants were informed of the procedures they would undergo and they signed an informed consent document. This study and the consent terms were approved by the Human Ethics Research Committee at Londrina University Hospital (No. 099/09). Approximately 20 ml of blood was collected in vacuum tubes after a 12-h fast before the start of the clinical trial (day 0) and after 45 days for the following biochemical analyses: CHOL, HDL-c, LDL-c, TGs and glucose. A biochemical autoanalyser (Dade AR, Newark, NJ, USA) was used in conjunction with Dade Behring kits to quantify those levels in the patient samples. LDL-c was calculated by the Friedewald equation (Friedewald et al. 1972).

Statistical analyses

To assess the significance ($p < 0.05$) of the results, the dependent variable values (L^* , a^* and b^*) for the snack bars were subjected to an analysis of variance, which was then followed by Tukey's test. Linear models were adjusted for the hardness and water activity. Data analyses were carried out using the Statistica software (version 7.1; Statistica 2006). For the clinical trial, data-sets were analysed by Graphpad InStat version 3.10 using the two-tailed paired t test with $p < 0.05$, and Kolmogorov–Smirnov normality test. The data are presented as mean \pm SE.

Results

Composition of ingredients and soy snack bars

The amount of protein (%) and isoflavone (mg/100 g) in each ingredient, respectively, was 61.57 and 148.32 in soy crisps, 84.86 and 110.53 in soy protein isolate, 38.67 and 115.11 in toasted soy and 50.67 and 351.38 in textured soy protein.

The snack bar formula developed is presented in Table I and the proportion of syrup to the mixture of

Table I. Ingredients and proportions used in the soy bar formulation.

	Ingredients	Proportion (%)
Dry ingredients	Soy crisps	24.0
	Soy protein isolate	6.3
	Soy toast without salt	13.0
	Textured soy protein	8.0
	Dried banana	10.0
	Total dry ingredients	61.2
Syrup ingredients	Glucose syrup	27.5
	Raw sugar	2.2
	Maltodextrin	1.8
	Water	4.3
	Palm oil	1.5
	Lecithin	0.5
	Colouring	0.7
	Glycerine	0.3
	Total syrup ingredients	38.8
	Total	100

dry soy ingredients and fruit was 38.8:61.2. Chemical composition of snack bars was 14.30% of carbohydrates no-fibre, 39.88% of total dietary fibre (35.56% insoluble fibre and 4.32 soluble fibre), 8.82% of lipids, 34.25% of protein and 2.75% of ash, indicating high percentages of protein and dietary fibre, indicating that the bars have potential health benefits. However, it is necessary to observe the potential antinutritional effects of fibres on nutrient biodisponibility, which is not assessed in this study. The bar developed in the present study has only 245.47 kcal/100 g due to the high amount of fibre.

Among the 12 isoflavones that were analysed in the soy bars for this study, genistin, acetyl-genistin, daidzin and genistein were detected in high quantities when the bars were first produced and 6 months after storage. The total isoflavone content in the snack bars was 100.39 mg/100 g. In addition, there was no significant loss of phytoestrogens during storage (Table II).

Table II. Isoflavone content of soy bars during storage.

Isoflavones	mg/100 g	
	Day 0	After 6 months
Daidzin	14.10	14.52
Glycitin	0.00	0.00
Genistin	30.97	31.00
6''-O-malonyl-daidzin	5.81	5.31
6''-O-malonyl-glycitin	0.00	0.00
6''-O-malonyl-genistin	11.05	10.85
6''-O-acetyl-daidzin	2.92	2.98
6''-O-acetyl-glycitin	0.00	0.00
6''-O-acetyl-genistin	16.08	15.98
Daidzein	5.92	5.90
Glycitein	0.00	0.00
Genistein	13.53	12.93
Total	100.39	99.47

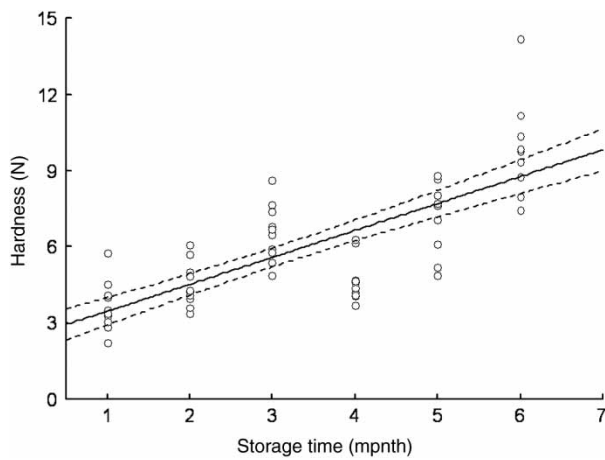


Figure 1. Hardness of soy snack bars during 6 months of storage at room temperature (21–25°C).

Shelf life

The soy snack bars formulated in this study had hardness values ranging from $2.54 \text{ N} \pm 0.29 \text{ N}$ when the bars were manufactured. These values increased to $10.00 \text{ N} \pm 1.92 \text{ N}$ after 6 months of storage (Figure 1). The following linear model for hardness (H) was used with $R = 0.81$: $H = 2.414 + 1.059 \times \text{months of storage}$. Figure 1 shows an increase in the hardness of the soy snack bars over time.

Images of soy snack bars immediately taken after the bars were manufactured and during 6 months of storage are shown in Figure 2. RGB values obtained from the images were converted to CIELAB values to calculate the average colour of the bars. The images demonstrated the various components of the bars in addition to the changes in the colour palate and colour lightness intensity of the samples during storage. The L^* , a^* and b^* values of soy bars evaluated during the six-month storage period are listed in Table III. The L^* and b^* values decreased (from 52.92 to 42.46 and from 53.69 to 47.06, respectively; $p < 0.05$) during storage and the a^* values significantly increased (from 11.96 to 18.32; $p < 0.05$) during storage. These results suggest that the bars became darker, redder and less yellow over time (Figure 2).

The soy snack bars formulated for this study had a_w values ranging from 0.629 ± 0.005 after the bars

were manufactured to 0.651 ± 0.006 after 6 months of storage under room temperature (21–25°C). The following linear model was used to determine the water activity with $R = 0.62$: $a_w = 0.621 + 0.006 \times \text{months of storage}$ (Figure 3). There was no significant effect of storage on the microbiological contamination of the product (data not shown). During 6 months of storage, there were no major changes in the microbiological parameters. There were negative results (were not detected) for coliforms, *S. aureus*, *B. cereus* and *Salmonella* sp., and there were fewer than 10 yeast and mould cells in each sample. The maintenance of water activity may have produced this microbiological stability.

The mean sensory acceptability value of the soy snack bars was 7.1 ± 1.3 , with a 70% approval rating (scores ≥ 7). Among panellists who gave favourable scores for the bars, 54% indicated that flavour was the most influential attribute. Among panellists who gave unfavourable scores for the bars, 36% indicated that texture was the most influential attribute and 25% indicated that appearance was the most influential attribute. When panellists were asked whether they would purchase this product, 83% of the panellists responded that they would buy the product. When the question was repeated after stating that this product could help reduce the risk of developing diseases such as cardiovascular disease, 94% of the panellists said that they would buy the product due to their concerns with maintaining and improving their health.

Clinical trial

Mean ages of subjects who participated in the study were 48.9 ± 7.9 (12 men and 10 women). Table IV shows that there were no significant differences in glucose ($p < 0.750$), CHOL ($p < 0.917$) and LDL-c ($p = 0.262$) levels after soy snack bars were consumed over a 45-day period. Patients had decreased levels of serum TGs (-20% , $p < 0.004$) (Figure 4A). An increase in HDL-c ($+8\%$, $p < 0.023$) was also observed (Figure 4B). To monitor the adherence of the subjects to the treatment, meetings with dieticians was conducted three times during the study in both experimental groups.

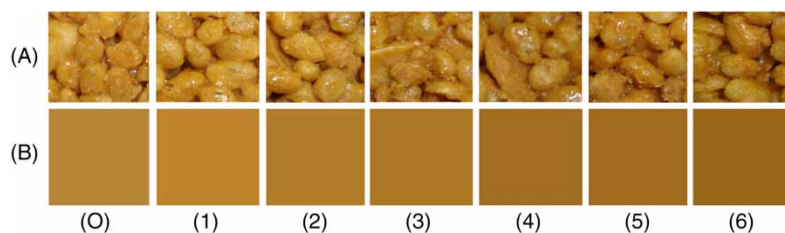


Figure 2. Pictures of soy bars (A) and their average colour (B) of images captured with a digital camera was converted to a mean CIELAB system to obtain L^* , a^* and b^* values immediately after the bars were manufactured (Day 0) and during storage over 6 months (1–6). The numbers below the images refer to months of storage.

Table III. L*, a* and b* values for soy snack bars evaluated immediately after manufacturing and during a storage period of 6 months.

	Storage time* (months)						
	0	1	2	3	4	5	6
L*	52.92 ± 3.45 ^{a,c}	54.48 ± 0.80 ^a	51.85 ± 5.05 ^{a,c}	49.47 ± 5.54 ^{a,b}	47.30 ± 4.41 ^{a,b}	45.31 ± 2.48 ^{b,c}	42.46 ± 6.35 ^b
a*	11.96 ± 1.86 ^a	11.56 ± 0.96 ^a	10.91 ± 1.67 ^a	12.38 ± 1.70 ^a	12.82 ± 1.45 ^a	11.93 ± 1.97 ^a	18.32 ± 2.25 ^b
b*	53.69 ± 2.40 ^a	54.58 ± 1.39 ^a	54.63 ± 2.54 ^a	50.81 ± 1.52 ^{a,b}	51.00 ± 2.19 ^{a,b}	50.65 ± 1.10 ^{a,b}	47.06 ± 5.37 ^b

Note: Different letters in the same line indicate a significant difference ($p < 0.05$); * Mean ± SD.

Body weight and BMI of the subjects who consumed soy snack bars remained relatively constant ($p = 0.2264$), and no significant changes were observed during the course of the study. There were no observed changes for waist circumference ($p = 0.7507$).

Discussion

According to Loveday et al. (2009), high-protein commercial snack bars contain 15–35 g/100 g protein and consist almost exclusively of dairy or soy proteins. These ingredients of the snack bars have health benefits and a low cost. Freitas and Moretti (2006) reported that bars not classified as high-protein bars have an average protein content of 4.0 to a 4.4 g/100 g. Freitas and Moretti (2006) developed a high-protein bar with added soy with the following composition: 60.97% carbohydrate, 15.31% protein, 5.64% lipid and 5.17% dietary fibre.

The bars developed in the present study had only 245.47 kcal/100 g due to the high amount of fibre. Owing to the reduced caloric value, the bars were classified as a 'light food'. The term 'light' is used for foods that present a minimum reduction of 25% of a specific nutrient or total caloric value when compared with the conventional food (Fennema 2000).

Isoflavones include aglycones (daidzein, genistein and glycitein), certain β -glucosides, conjugated malonyl-glucosides and acetyl-glucosides (Góes-Favoni et al. 2004). According to Barnes et al. (1994), the

bioavailability and metabolism of different isoflavones depend on the chemical form of the isoflavone. Aglycones are the most active isoflavones in disease prevention.

Genistin, which was present in high quantities (30.97–31.00 mg/100 g), is a β -glucoside that typically undergoes enzymatic hydrolysis in the small intestine releasing genistein, which is absorbed and prompts biological effects (Dixon 2004). Studies have demonstrated that genistein exhibits a high affinity for oestrogenic receptors (Wober et al. 2002) and has a high amount of activity against several hormone-dependent cancers (Anderson et al. 1999).

The total isoflavone content in the snack bars was 100.39 mg/100 g (Table II) which is similar to values previously reported for soy-based foods (Song et al. 1998). The consumption of a single soy bar (30 g) per day would contribute approximately 10 g of soy protein and 30 mg of total isoflavones. According to Setchell (1998), the daily intake of 25 g of soy protein associated with approximately 30–50 mg of isoflavones daily can reduce serum cholesterol levels. Studies have shown that the consumption of purified isoflavones alone is not as effective for reducing disease risk when compared with the consumption of isoflavones with soy protein (Badger et al. 2002). Thus, it is important to consume isoflavones and soy protein together.

Shelf life can be defined as the length of time that a product can be stored before the appearance of the first characteristic that consumers find unappealing (Loveday et al. 2009). Chemical and physical interactions among the ingredients in snack bars can occur over time and begin to affect the taste and texture of the product. Factors that limit shelf life include chemical reactions or physical modifications. The time from manufacture to consumption of the snack bars may be influenced by the lack of a thermodynamic equilibrium, which is common in heterogeneous multicomponent systems in processed foods (Mezzenga 2007). The thermodynamic incompatibility of certain biopolymers (Tolstoguzov 2003) and existence of chemically heterogeneous micro-environments within foods (Kou et al. 2002) can potentially drive physicochemical reactions during storage (Loveday et al. 2010).

An increase in hardness can be very damaging to the sensorial aspects of bars by reducing the chewiness of the bars. Bower and Whitten (2000) reported that the

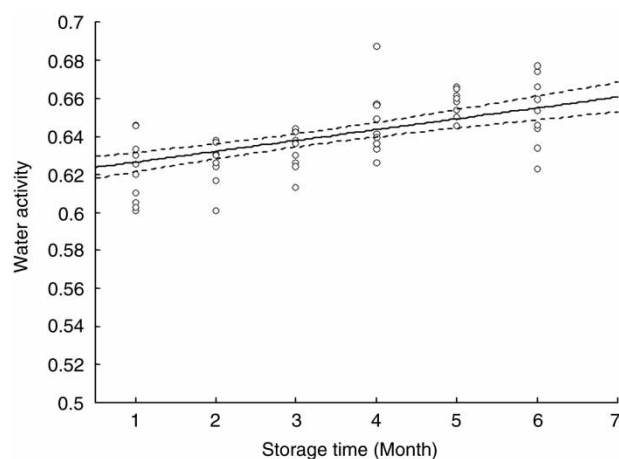


Figure 3. Water activity of soy snack bars during 6 months of storage at room temperature (21–25°C).

Table IV. Baseline and after 45 days of anthropometric characteristics, fasting blood lipids and glucose concentrations ($n = 22$).

Variables*	Baseline	After 45 days	p
CHOL (mg/dl)	246.91 \pm 5.70	246.36 \pm 4.42	0.9169
LDL-c (mg/dl)	150.91 \pm 6.16	157.50 \pm 4.97	0.2621
HDL-c (mg/dl)	56.77 \pm 2.55	61.27 \pm 1.65	0.0228 [†]
TGs (mg/dl)	195.86 \pm 16.98	155.82 \pm 16.02	0.0035 [†]
Glucose (mg/dl)	94.73 \pm 2.36	94.18 \pm 2.05	0.7542
BMI (kg/m ²)	29.12 \pm 1.15	29.25 \pm 1.09	0.2264
Waist circ. (cm) [‡]	96.33 \pm 2.79	96.00 \pm 2.35	0.7507

Note: The following abbreviations are used: BMI, body mass index; CHOL, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol and TGs, triglycerides; $p < 0.05$; *Values are expressed as mean \pm SE; [†]Significant difference between baseline and after 45 days; [‡]Waist circumference.

majority of cereal bar consumers consider chewiness a desirable attribute.

According to Loveday et al. (2009), the shelf life of high-protein bars is often limited by the development of a hard or tough texture, which consumers find unpalatable. Mechanisms producing this texture have not been conclusively identified, but it is possible that protein cross-linking, aggregation or network formation occur during storage (Zhou et al. 2008).

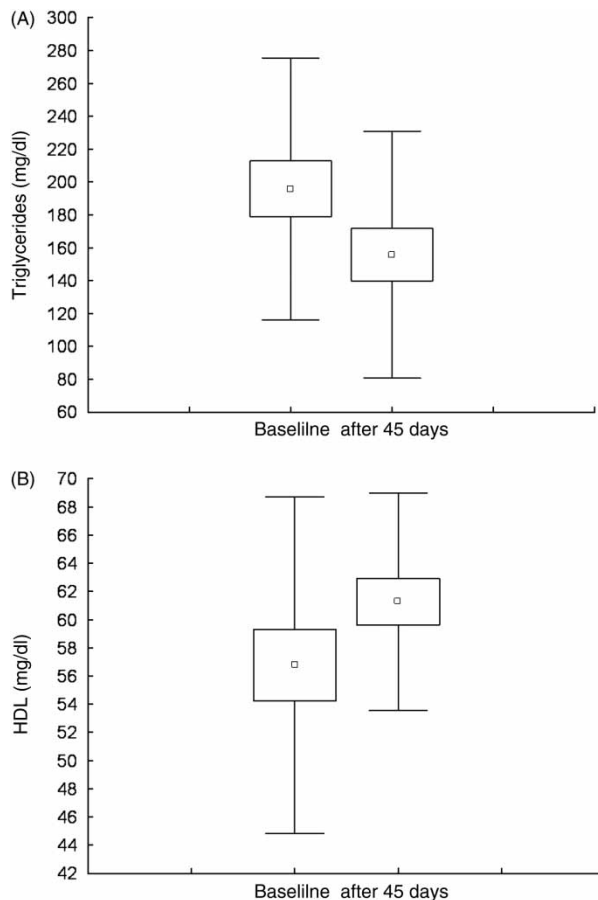


Figure 4. Representation (Box and Whisker plots) of TGs (A) and the HDL (B) profiles at baseline and after 45 days of soy snack bar consumption.

Baier et al. (2007) reported that bar hardening during storage occurs due to the ordered secondary structures in proteins and the low surface hydrophobicity of protein. These authors attributed moisture migration as a driving force for the hardening of protein bars, but they did not specify the source and destination of the migration. Maillard reactions between reducing sugars and reactive lysine residues may have a role in the hardening of protein bars (Loveday et al. 2009).

The colour of the soy bars darkened slightly during storage indicating the onset of final stage Maillard reactions. In the initial and intermediate stages of Maillard reactions, there is little or no colour change.

Theoretically, product darkening (developing a brown colour) occurs due to the reducing sugars (e.g. liquid honey or glucose syrup) or an increase in the amount of reducing sugars available to participate in the Maillard reaction (Yilmaz and Toledo 2005). The shelf life of intermediate moisture foods is often limited by Maillard reactions between the carbonyl groups of reducing carbohydrates and the exterior amine groups of proteins. Maillard reactions can lead to an unappealing texture, flavour, nutritional value and colour of food products (Loveday et al. 2010). Moreover, Maillard reactions occur rapidly at high temperatures and at a_w values between 0.65 and 0.75, which are similar to those at a_w values measured for the bars in this study.

Water activity (a_w) measurements help to predict the mechanical properties, stability and shelf life of foods. The a_w value represents the availability of water in a material to develop chemistry reactions and microbiological modifications, and this physical property influences microbial spoilage, chemical reactivity and enzymatic activity (Labuza 2000). Cereal bars are generally formulated to have moisture between 10 and 15% (w/w) and a a_w value less than 0.65 (Loveday et al. 2009). More specifically, the bars are designed to maintain intermediate a_w values between 0.4 and 0.6 and be stored at room temperature without significant microbial growth. At these a_w values, products are susceptible to physical transformations such as lipid oxidation and non-enzymatic browning (Maillard reactions).

The water activity can be decreased by removing water from the formulation and/or by adding low molecular weight solutes (Loveday et al. 2009). Moreover, to obtain an intermediate a_w value, it is necessary to consider the °Brix of the syrup mix. Higher °Brix values result in harder bars. In general, Brix values in cereal bars are controlled to be between 81 and 84 °Brix by heating the syrup from 100 to 105°C. However, the soy snack bars in this study were manufactured by heating syrup to 100°C and 78°Brix. The addition of soy protein isolates provided the bars with a dry appearance, so the heating temperature and °Brix were decreased to minimize this effect in this study.

There was a small increase or a small oscillation in a_w value during storage. This variation can be attributed to changes in humidity, which was not controlled, or the lack of homogeneity in a heterogeneous, multicomponent product. The changes in a_w values that are likely responsible for affecting the mechanical properties of snack bars may be associated with differences in the microstructure and chemical composition of the product (Lewicki et al. 2004). Freitas and Moretti (2006) manufactured and characterized cereal bars with textured soy protein, wheat germ and oats, and they reported a similar final a_w value (0.637 ± 0.017) for the cereal bars.

The mean sensory acceptability value of the soy snack bar was 7.1 ± 1.3 and was selected the worst and the best attributes. Flavour was one of the best attributes of the soy snack bars, which was promising because the characteristic off-flavour of soy products is often undesirable. The appearance and texture of the bars can be attributed to the reduced amount of syrup used to formulate the bars and to the addition of soy protein isolate, which diminished the humidity of the product.

The panellists showed interest in consuming the soy bar because of potential 'healthiness'. According to Bower and Whitten (2000), however, the healthiness of the bars may not be as important to consumer purchasing habits. Features such as texture, appearance and price have been shown to be more relevant in consumer choices. Bars that are chewy, nutty or contain chocolate are generally more popular than bars that do not have these characteristics. The other desirable characteristics of cereal bars include a light colour and a moist appearance (Dutcoski et al. 2006).

A dry appearance, which was considered to be one of the worst attributes of the bars developed in this study, can be improved by modifications of the product formulation. Other ingredients may be used to improve the appearance of the bars. According to Ninness (1999), oligofructose can be used as a humectant in a binder system to make the bars softer and more pliable. Brandt (2000) reported that inulin can act as a texture modifier by holding in moisture, which may help the bars to maintain freshness for a longer period of time. Moreover, the addition of these soluble fibres or blends may even reduce the sugar and caloric content of the bars (Dutcoski et al. 2006). These ingredients were not included in the present bar formulation because they may have interacted with the soy ingredients or act as hypocholesterolaemic component. Thus, the effect of these ingredients on patient lipid profiles may have been reduced, hindering the objective of this study, which was to evaluate only the hypocholesterolaemic effect of the soy product.

The bars developed in this study were used in a clinical trial with dyslipidaemic subjects to evaluate the effect of soy consumption on the lipid profiles of the patients. In this study, the patients who consumed soy snack bars had a diet with no limits on saturated fat or

cholesterol ingestion. Moreover, the bar was a multi-component system. Wong et al. (1998) showed that the type of food or food matrix can influence the absorption and physiological effects of a food. The majority of previous clinical studies of soy products used products containing soy flour or soy protein isolate. However, these products are difficult to incorporate into daily meals.

Snack bars have been used as a food matrix to incorporate functional health ingredients, and they have been evaluated in clinical trials (Kaufman et al. 1997; O'Neill et al. 2001; Hallund et al. 2006; Polagruto et al. 2006). The use of bars as a food matrix to present functional ingredients has produced positive results because the patients adhere to the protocol because the bars are convenient.

Several clinical studies have suggested that soy protein or isoflavones can result in significant reductions in plasma concentrations of CHOL and LDL-c in humans exposed to these compounds. However, a significant number of studies have reported no beneficial effects of soy consumption on body weight, serum lipid profiles, fat mass, blood glucose and insulin profiles (Cederroth and Nef 2009).

The consumption of three soy snack bars per day (approximately 30 g of protein and 100 mg of isoflavone/100 g) decreased TGs by 20% ($p < 0.004$) without a low-fat diet (Figure 4A). Clinically, this reduction was considered significant for reducing heart disease. Comparatively, statins, which are used as a hypocholesterolaemic drug, reduce TGs levels by approximately 13% (LaRosa et al. 1999), but these values may be higher depending on the statin administered and baseline TGs levels of the subjects.

According to Wang et al. (2004), the decrease in TGs is associated with soy proteins and not with isoflavones, and they suggested that soy proteins reduce plasma TGs levels through multiple mechanisms, including the suppression of *de novo* lipogenesis (newly synthesized fatty acids derived from carbohydrate metabolism, which are often used for TGs synthesis).

A meta-analysis (Anderson et al. 1995) demonstrated that soy protein, but not casein, reduces TGs (-11%) and LDL-c (-13%) levels. Moreover, the meta-analysis suggested that soy protein supplementation results in greater reductions in TGs and LDL-c levels when compared with casein supplementation in subjects with higher initial CHO levels. Wang et al. (2004) also observed a reduction (12.4%) in TGs levels with a soy protein diet when compared with an animal protein diet. Overall, a majority of studies have reported no significant effects of soy protein on TGs levels.

In the present study, there was an increase in HDL-c ($+8\%$, $p < 0.023$) after the consumption of soy snack bars for 45 days (Figure 4B). In a meta-analysis, Reynolds et al. (2006) showed that the consumption of soy increased HDL-c levels in 25 of the 38 trials

analysed. However, the increase was statistically significant in only two of those trials. In addition, Reynolds et al. (2006) found a positive correlation between the amount of soy protein and isoflavones with net changes in HDL-c. Other components of soy, or their interactions, may also be able to have influenced in lipid metabolism. This would be the case of fibres, which are present in high level in the bars.

According to Anderson and Konz (2001), a 1% decrease in HDL-c levels increases cardiological risk by 3%. Studies have indicated that HDL has antiatherogenic properties and the capacity to promote cholesterol efflux from peripheral tissues by ATP-binding cassette transporter A1 (ABCA1) (Gotto and Brinton 2004).

Conclusions

Several strategies have been investigated for controlling dyslipidaemia without medications. The development of functional foods may expand the available options for controlling dyslipidaemia and for providing health benefits. The soy snack bars manufactured in this study were well accepted in the sensory analysis. During storage, however, the bars developed a dry appearance and their hardness increased. Additional modifications to the bar formulation may prevent these problems. In the clinical trial, consumption of three soy snack bars a day (approximately 30 g of protein and 100 mg/100 g of isoflavones) decreased TGs levels (−20%) and prompted a small increase in HDL-c levels (+8%). We conclude that relatively high intakes of soy protein and isoflavones in moderately hypercholesterolaemic subjects (>200 mg/dl) may have a moderately beneficial effect on plasma lipid profiles (TGs and HDL) without additional diet restrictions. Moreover, snack bars showed a great food matrix to the inclusion of functional ingredients by practicality and possibility to consume between main meals.

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