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Article type : Original Manuscript

Cereal bars functionalized with tempeh: nutritional composition, isoflavones content and consumers acceptance

Tempeh as a cereal bar ingredient

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ABSTRACT

The aim of this study was to produce cereal bars (CB) added with tempeh flour (TF) and evaluate the nutritional composition, fatty acids profile, isoflavones content, and sensory acceptance. Tempeh was produced from organic soybeans cotyledons fermented by *Rhizophus oligosporus* followed by freeze-drying and milling in order to obtain the TF. The addition of 0% (control), 10%, 15%, and 20% TF to CB resulted in both protein and lipid content increase and a higher polyunsaturated/saturated fatty acids ratio. The content of isoflavone aglycones, the forms considered to have higher biological activity, was higher in TF than in soybean, which was also reflected in the CB chemical composition. An acceptance test showed that CB added with TF (up to 15%) were sensory accepted. Overall results suggest that the addition of TF as an ingredient in cereal bars allows improving the chemical and functional characteristics of this type of ready-to-eat foods.

Keywords: Isoflavone aglycones, fatty acids profile, *Rhizophus oligosporus*, sensory acceptance, soybean.

INTRODUCTION

Isoflavones are a well-known group of flavonoids that are abundantly present in the cotyledons and hypocotyls of soybeans (*Glycine max*) and have been attracting an increasing attention due to its potential health benefits (Chen *et al.,* 2013). Besides the capacity to alleviate menopause symptoms due to their phytoestrogen properties (Ye *et al*., 2012), isoflavone intake has also been associated with cardiovascular disease risk reduction, osteoporosis prevention, cancer cell growth inhibition, antioxidant and anti-inflammatory activity (Chen *et al.,* 2013; Ahmad *et al*., 2014; Xiao *et al.*, 2017; Gagné & Maizes, 2018; Jayachandran & Xu, 2019).

Isoflavones are found in four distinct forms namely aglycones (daidzein, genistein and glycitein), β-glycosides (daidzin, genistin, and glycitin), acetyl glycosides (6”-O-acetyl dadzin, 6”-O-acetyl genistin and 6”-O-acetyl glycitin) and malonyl glycosides (6”-O-malonyl daidzin, 6”-O-malonyl genistin, and 6”-O-malonyl glycitin). However, according to previous studies, aglycones are the ones related to a higher biological activity since the conjugated forms are poorly absorbed in the small intestine (Larkin *et al*., 2008). In soybeans, the predominant forms are the malonyl glycosides (Handa *et al*., 2014). Nevertheless, the amount of the different isoflavone forms can be affected by processing conditions, such as fermentation, heat treatment, enzyme hydrolysis, baking (Wang & Murphy, 1996), and ultrasound treatment (Falcão *et al.,* 2018). Fermented soybean products, in particular, are rich in the easily absorbed aglycones, which are formed as the result of enzymatic activity (Larkin *et al*., 2008), thus being predominant in this type of products and making their consumption highly desirable in terms of health benefits.

For centuries, different fermented soybean products have been consumed in Eastern Asia as staple foods, being several of them increasingly available throughout the world as consumers are becoming more conscious about the health-benefits of the foods they eat (Cao *et al*., 2019). Tempeh is a traditional Indonesian food made by soybeans fermentation by *Rhizophus oligosporus*, being rich in protein and lipid and having notable nutritional/functional properties. Tempeh is manufactured with soaked, dehulled and cooked soybeans, whose surfaces are then covered with mycelium, becoming bound into a compact white cake (Handoyo & Morita, 2006; Nishinari *et al*., 2018). During the fermentation process, *R. oligosporus* enzymatic activity leads to a significant increase in water-soluble nutrients, enhancing vitamins biosynthesis and leading to a remarkable vitamin B_{12} presence in tempeh since no plant-based foods contain such vitamin unless fermented or contaminated (Wolkers-Rooijackers *et al*., 2018). Additionally, other biochemical process are responsible for the lipid content decrease, protein hydrolysis, allergenicity reduction, and isoflavones content changes, mainly due to the conversion of glycosides to aglycone forms (Borges *et al*., 2016; Haron *et al*., 2009; Puteri *et al*., 2018). Therefore, this fermentation process, besides conferring unique flavors, boosts the nutritional and biological properties of the final product (Jayachandran & Xu, 2019). Such tempeh characteristics suggest its use as a bioactive ingredient in food products aiming to improve their functional and nutritional properties. Cereal bars are widely consumed as fast snacks as they are practical, easy to carry and consequently well adapted to the busy day lives of modern societies. Moreover, they are also perceived by several consumers as healthy foods and thus consumed in order to complement the regular diet (Brito *et al*., 2013). The production of cereal bars is relatively simple and because of the versatility of their formulation, they can be easily incorporated with functional ingredients providing consumers with healthier ready-to-eat options.

The aim of this study was to develop cereal bars added with different amounts of tempeh flour as a bioactive ingredient and evaluate the nutritional composition, fatty acids profile, isoflavones content, and sensory acceptance of the obtained novel products. The addition of tempeh in cereal bars could improve its functional and chemical properties, and may potentially popularize tempeh consumption in western countries, where its consumption is almost negligible.

MATERIAL AND METHODS

Materials

The tempeh was obtained by the fermentation of organic soybeans (*Glycine max*, certificate 53/2016, Rede de Agroecologia, Marechal Cândido Rondon, Paraná, Brazil) by *Rhizopus oligosporus*

(INTSOY - International Soybean Program, University of Illinois, EUA). The other ingredients used for cereal bars production were described in Table 1. All reagents were of analytical grade.

Tempeh and Tempeh Flour Production

The methodology described by Borges *et al*. (2016) was applied for the tempeh production. Organic soybeans cotyledons were selected and hydrated (1:10; w/v) for 18 h at room temperature. After manual peeling, the grains were cooked for 45 min in 1.5 L of water and 12.5 mL of white vinegar (4% acidity). The cotyledons were drained, dried, cooled down to 25 °C, inoculated with *Rhizopus oligosporus* (100:0.7, w/w), placed in perforated polypropylene bags and incubated at 30 °C for 40 h. Then, tempeh was lyophilized (Labconco, FreeZone 6L, Kansas, USA) for 24 h at 40 °C at a pressure < 0.5 mBar. The lyophilized tempeh was milled using an SL31 knife mill (Solab, Piracicaba, Brazil) and the tempeh flour (TF) obtained was added to cereal bars (CB).

Cereal Bar Production

CB formulations added with TF and control (without TF) are shown in Table 1. The TF addition in the proportion of 10%, 15%, and 20% was based on preliminary tests in order to avoid the excessive bitter taste. For CB production, the ingredients were mixed, stored under refrigeration for up to 4 h and the CB were cut using a 20 mm x 20 mm x 10 mm mold. The samples were stored in polyethylene bags and kept under refrigeration (5 ± 1 °C) until the analyses of nutritional composition, fatty acid profile, isoflavones content, and sensory acceptance.

Insert Table 1.

Nutritional Composition

Moisture, protein, lipid, and ash were determined according to AOAC (2005) methodologies and the carbohydrate content was estimated by difference.

Fatty Acid Profile

The lipids were extracted according to the method of Bligh & Dyer (1959) with modifications. Transesterification of fatty acids was achieved according to method 5509 of the International Organization for Standardization (ISO, 2017). Fatty acid methyl esters were analyzed by Gas Chromatography (Perkin Elmer, Clarus 680 GC, Waltham, MA, USA) with flame ionization detection (FID) and a fused silica capillary column (100 m x 0.25 mm) with a 0.25 µm CP 7420 cyanopropyl polysiloxane stationary phase. The carrier gas was helium (1.1 mL min^{-1}), and the flame gases were hydrogen and synthetic air (40 and 400 mL min^{-1}, respectively). The split was 1:150, and the column temperature was set to 80 °C for 1 min;

ramped at 20 °C min^{-1} to 160 °C, at 1 °C min^{-1} to 198 °C, and at 5 °C min^{-1} to 250 °C; and kept at 250 °C for 1.6 min. The injector and detector temperatures were set at 240 °C and 250 °C, respectively. An Integrator-Processor CG-300 (Scientific Instruments CG) was used for peak area determination, and peaks were identified by comparison of retention times with FAME standards (37 Component FAME mix, 18919-1AMP, Supelco, USA).

Isoflavones Forms Content

Isoflavones forms content was carried out after the lyophilized samples were defatted with hexane in a 1:10 g mL^{-1} ratio by continuous agitation (Marconi, MA 140/CFT, Piracicaba, Brazil) for 1 h at 25 °C, followed by vacuum filtration. The prepared samples (200 mg) were added with 8 mL of extraction solution containing ultrapure water, acetone, and ethanol (1:1:1, v:v:v), as described by Yoshiara *et al*. (2012). The separation and quantification of isoflavones were carried out according to the method described by Handa *et al*. (2014) modified by Falcão et al. (2018). An ultra-high performance liquid chromatography (Acquity UPLC® System, Waters, USA) equipped with a diode array detector (Waters) and a reversed-phase BEH C18 column (2.1 mm x 50 mm, 1.7 μm, Waters) were employed. The samples were filtered (Millex filter-H, 0.22 μm) and aliquots of 1.4 μL were automatic injected into UPLC. The binary mobile phase containing 0.4% formic acid (A) and acetonitrile (B), and the following gradient elution was used: 0 min, 95% A; 8.5 min, 20% A; the flow rate was 0.3 mL min^{-1} and the column temperature was kept at 27 °C. For UPLC analysis, the identification of the compounds was based on the retention times and UV spectra at a 260 nm. Quantification was performed by external standardization using 6-point analytical curves (0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005 mg mL^{-1}) with triplicate measurements ($R^2 \geq 0.999$ and $P < 0.001$).

Sensory Acceptance

This study was authorized by the Ethics Committee of the Faculdade Integrado - Campo Mourão, Paraná, Brazil (Certificate 64104117.4.0000.0092) and carried out after microbiological evaluation attesting the samples suitability (data not shown). The panel was composed by 135 untrained assessors (consumers) recruited at Universidade Tecnológica Federal do Paraná, Campus Medianeira, Paraná, Brazil. The assessors, comprised mostly of CB consumers (93%), were students, professors, and other university employees, mostly aged between 18 and 25 years (88%), of both genders (53.3% female, 46.7% male), and from medium to high educational level (91% attending undergraduate school and 9% post graduated). Sensory tests were conducted in four individual booths under white light, in two sections (one in the morning and one in the afternoon) in the same day on the Sensory Analysis Laboratory. The samples CB-A, CB-B, CB-C and CB-D (about 15 g of each) were served in white plate coded with three randomized digit in a monadic way considering a complete balanced blocks in a randomized order. The assessors were instructed to clean their palate with water before and between every sample evaluation. A 10 cm hybrid hedonic scale anchored with verbal terms (0 = disliked extremely, 5 = neither liked, nor disliked, 10 = like extremely) (Villanueva, Petenate, and Silva, 2005) was used to evaluate acceptance (color, appearance, texture, flavor, and overall impression attributes) of the samples.

Statistical Analysis

The data were evaluated by a one-way ANOVA, considering samples as the source of variation, and Tukey's means test ($P < 0.05$). Sensory analysis was evaluated by a two-way ANOVA, considering formulations and assessors as the sources of variation, and Tukey's means test ($P < 0.05$). The analyses were carried out by Statistica 8.0 (Statsoft, Tulsa, USA). The internal preference map was generated by multidimensional scaling and cluster analysis using the Senstools software version 2.3.28 (OP & P Product Research, Utrecht, Netherlands).

RESULTS AND DISCUSSION

Impact of Tempeh Flour Content on Nutritional Value

The results for the proximate composition obtained for soybean, tempeh, tempeh flour (TF) and the four different cereal bars (CB) are shown in Table 2. Moisture content for tempeh was higher ($P < 0.05$) than for soybean and TF due to the soybean hydration before fermentation. Soaking of soybean is considered an important pre-treatment step in the manufacturing of soybean products such as tempeh, as it facilitates the processing by improving the softness of the material (Falcão et al., 2018). Thus, the tempeh with high moisture content (64.84 g 100 g^{-1}) was lyophilized and subsequently processed into TF in order to achieve a low moisture content (2.93 g 100 g^{-1}) and allowing for further addition as an ingredient in the CB formulation. The protein content of soybean was similar to the values reported in the literature, while being lower than that observed for tempeh and TF. The fermentation by *Rhizopus oligosporus* is known to produce a variety of enzyme-like proteases causing significant increase in protein content for tempeh (Tahir et al., 2018). The loss of soluble substances, such as carbohydrates and minerals (Table 2), may be caused by the pre-treatment steps of hydration and cooking during tempeh processing. Lipid content was significantly reduced in tempeh compared to soybean, probably because the lipids are the main source of energy for *R. oligosporus* during the tempeh fermentation process (Prinyawiwatkul et al., 2013).

Regarding the produced CB, for all cases the moisture content was lower than 15% (Table 2), which is in accordance with the current Brazilian legislation (Brazil, 2005). The addition of TF in increasing amounts (from 10 to 20%) to CB significantly affected their protein content, causing an increase of CB protein content and consequently a relative decrease in the carbohydrates content. The lipid content also increased with the addition of TF to CB formulations; however, values for CB-C were near, whereas CB-A and CB-B values were lower than those reported by Gutkoski et al. (2007) who studied CB with high dietary fiber content.

Insert Table 2.

Impact of Tempeh Flour Content on Fatty Acid Profile

The soybean fermentation by *R. oligosporus* resulted in lipid content decrease, and only a few authors have reported the effects of this change on the fatty acid profile (Prinyawiwatkul et al., 1996). Table 3 shows the obtained fatty acid profile for the different samples under study. It can be observed that the sum of saturated fatty acids (SFA) for tempeh and TF was lower than for soybean, mainly due to a decrease in the relative percentage of palmitic (16:0) and stearic (18:0) acids. Additionally, tempeh and TF presented a higher polyunsaturated fatty acids (PUFA) content compared to that of soybean, corroborating with the higher relative percentages of linoleic (18:2n-6) and γ-linolenic (18:3n-6) acids, with this last being found only in tempeh and products thereof. γ-Linolenic acid is considered as being relatively unusual in plants and has been associated with interesting nutritional and medicinal properties, such as its pronounced anti-inflammatory capacity (Horrobin, 1992; Chang et al., 2009).

These results may be linked to the strong *R. oligosporus*, which consequently increased the polyunsaturated/saturated fatty acids (PUFA/SFA) ratio in the present study.

The fatty acid profile of CB was significantly influenced by the addition of TF, with the increase of TF amount added as an ingredient resulting on the simultaneous reduction of SFA and PUFA increase ($P < 0.05$) (Table 3). Despite the increase of lipid content as a consequence of TF addition (Table 2), the cereal bars added with TF presented a higher PUFA/SFA ratio (Table 3). In the last decades, the health effects of different groups of dietary fatty acids have been extensively studied. Despite recent studies have shown inconsistent results regarding the effects of dietary SFA (eg. while the PREDIMED study showed that SFA intake was not associated with total mortality, in a prospective analysis from the China Health and Nutrition Survey (1989–2011) total SFA and even-chain SFA intake was associated with higher total mortality in women (Zhuang et al., 2019), in general, SFA have been considered has having adverse effects on the cardiovascular system due to their negative impact on cholesterol metabolism (Baum et al., 2012). Therefore, to decrease the risk of cardiovascular diseases, several health authorities have been recommending a limited intake of SFA and its replace with PUFA (Baum et al., 2012; Santos et al., 2013). In this view, the increase of PUFA/SFA ratio imparted by TF addition to the cereal bars can be perceived as beneficial comparatively to the control.

Insert Table 3.

Impact of Tempeh Flour Content on Isoflavones Forms Content

The different identified forms and total isoflavone content of soybean, TF and formulated CB are presented in Table 4. In total, seven different forms were detected with soybeans showing a clear predominance of the poorly absorbed conjugated forms. The total isoflavones content for TF was lower ($P < 0.05$) than for soybean (Table 4), which may be due to processing conditions used during tempeh preparation including soybean maceration, cooking, and fermentation (Wang & Murphy, 1996). However, despite the low concentration of total isoflavones in TF, a higher concentrations of the aglycones forms (daidzein, glycitein, and genestein) were found (74.50%), while it represented 4.07% of the total isoflavones in soybean (Table 4). The present study is also in good agreement with Haron et al. (2009) that reported daidzein and genistein increase as being the main changes observed in the processing method caused by the conversion of isoflavone glycoside forms into their corresponding aglycones. The content increase of isoflavones in aglycone forms is considered advantageous from a health perspective since these aglycones have higher bioavailability, metabolic activity, and a faster absorption when compared to their glycosylated forms (Chen et al., 2013).

Insert Table 4.

The consumption of isoflavones, mainly the aglycone forms, may offer potential benefits for the human organism, as shown by both in vitro and in vivo studies (Xiao et al., 2017). Falcão et al. (2019) in a biological activity in vitro study demonstrated that especially genistein aglycone, can serve as a source of anti-inflammatory molecule in soy foods. Among the human health benefits, the consuming soy isoflavones are associated with lower incidence of some diseases, like type two diabetes, breast cancer in pre-and postmenopausal, cardiovascular disease, and on the prevention and treatment of osteoporosis (Pabich & Materska, 2019).

The isoflavones intake expressed as aglycone equivalent to the general population range from 0.1 to 3.3 mg day^{-1} in Western countries, from 0.49–1 mg day^{-1} in European countries, and from 8 to 50 mg day^{-1} in Asian countries (Mortensen et al., 2009; Pabich & Materska, 2019). However, the Asian countries has been recommend soy products intake between 20 and 50 g to meting nutrients need. In contrast, evidences suggests that the at least 10% of Asian population consumes as much as 100 mg of isoflavones day^{-1} (Messina, Nagata & Wu, 2006). The differences in isoflavone intake are related to the fact that in Western countries less legumes (including soy) and more processed products as well as carbohydrates and meat are consumed (Pabich & Materska, 2019).

In the present study, the cereal bars added with TF (CB-B, CB-C and CB-D) resulted in substantial isoflavone aglycones content, improving CB functional properties. Therefore, considering that Western population do not usually consume soybean neither its derivatives, the developed products could contribute to an increase on aglycone isoflavones consumption by that population.

Impact of Tempeh Flour Content on Cereal Bars Sensory Acceptance

Table 5 shows the mean scores for each attribute assigned by consumers to each formulation. Regarding sensory acceptance, CB-A (control) was better accepted ($P < 0.05$) compared with CB-B, CB-C and CB-D for attributes of color, flavor, appearance, and overall impression. For the attribute texture, CB with 10% TF was similar to the control CB, which differed only from CB-C and CB-D ($P < 0.05$). In general, the acceptance of the evaluated attributes decreased with the increase of TF in CB, with a significant reduction being observed for CB-D (20% of TF) ($P < 0.05$). However, suitable acceptance levels were obtained for CB added of TF up to 15%, with average scores ranging from 5.8 to 7.0. Nevertheless, among the evaluated attributes, flavor was the one with the lower score for CB-B, CB-C and CB-D (Table 5). Although the manufacturing process of cereal bars can be considered as relatively easy, it has been referred that incorporating functional ingredients can be detrimental to sensory characteristics, such as texture and flavor (Lobato et al., 2012; Ramírez-Jiménez et al., 2018). Previously, Freitas & Moretti (2006) reported acceptance levels, with scores ranging from 4.03 to 6.58 (9-point hedonic scale), when evaluating high protein and vitamin content cereal bars added with approximately 15% of texturized soy protein.

Insert Table 5.

Isoflavones are recognized by the bitter taste that they confer on foods (Roland et al., 2011). Therefore, the lower scores attributed to flavor attribute for the CB added with tempeh (ranging from 5.1 to 5.8) maybe related to the bitter taste imparted by the addition of this ingredient. In fact, 36.7% of assessors indicated flavor as the least appreciated attribute for CB-B (10% of TF), and a further 15.1% specifically indicated the perception of a bitter taste, while for CB-C (15% of TF) the indexes were 36.9% and 17.4%, respectively. For CB-D (20% of TF), 40.8% of the assessors did not appreciated the flavor. In addition of being a bitter food with astringent taste, strangeness and low acceptance levels might occur due to a lack of familiarity with soybean products consumption. Bedani and Rossi (2005) observed that assessors did not appreciate soybean and the lack of consumption habits were among the main reasons for the non-consumption of soybeans and its derivatives. In the present study, a percentage of 44% of assessors stated that they had never consumed soy products, 38% said they consume it occasionally and only 19% consume it once a week or more, with no significant difference between gender and soybean-based food consumption. Soybean milk (41%), soybean oil (29%), textured protein (14%), *shoyu* (7%), and others (10%) were amongst the most consumed soybean products. Regarding tempeh, 83% of assessors declared they did not know the product, 13% heard about it, and only 4% have consumed it.

According to Degaspari, Mottin & Blinder (2009), flavor is one of the most influential attributes for CB consumers when choosing a product at the purchase time. Nevertheless, nutritional value is generally considered the second most important factor. Moreover, in the last years, consumers are increasingly demanding and preferring foods with perceived health properties (Ramírez-Jiménez et al., 2018), which can influence their choice towards more nutritious foods over the tasty ones. In fact, in a previous study comprising the development of high soy protein snack bars to control dyslipidaemia, the percentage of panelists that responded that they would buy the product increased from 83% to 94% after knowing that the product could help reduce the risk of cardiovascular diseases (Lobato et al., 2012).

In the individual evaluation of acceptance results, using the internal preference map, it was possible to verify a segmentation of consumers. A two-dimensional solution was responsible for 81% of the variance explained for the overall impression, according to Figure 1. Dimension 1 discriminated CB-A from the others added with TF, with CB-A allocated separately on the left-hand side of the map, while the others were allocated on the right-hand side (Figure 1). Regardless of the grades obtained for the overall impression of CB added with TF (Table 5), it can be observed that a smaller and specific group of consumers prefers it (corresponding to 23% consumers: group 2: 14 consumers, group 3: 9 consumers, and group 4: 8 consumers) (Figure 1), which corresponds to a significant number of assessors who could become potential consumers.

Insert Figure 1.

CONCLUSIONS

The addition of soybean derivatives as ingredients to frequently consumed foods, such as ready-to-eat cereal bars, can be an interesting approach to increase soybean consumption in western countries. In this work, compared to soybean, tempeh production resulted in a protein increase, a higher PUFA content, a decrease of SFA and a higher content of isoflavone aglycones, which are considered as being more bioaccessible than the conjugated forms. The aforementioned characteristics positively affected the chemical composition of CB added with TF (10, 15 and 20%), resulting on a higher protein, lipid (especially PUFA) and isoflavone aglycones content, thus improving both the nutritional and functional characteristics of the developed cereal bars. The sensory evaluation demonstrated that TF addition negatively influenced different attributes, namely color, flavor, texture, appearance, and overall impression, when compared to CB without TF. Flavor attribute was the one that scored lower comparatively to the control in all the formulations, which can be related to the bitter taste imparted by isoflavones and the unfamiliarity with the tempeh taste. However, it was noted that the CB with TF up to 15% had a positive acceptance from the assessors. Considering the health benefits associated with soybean consumption, the incorporation of tempeh in popular snacks such as CB is proposed as an interesting strategy towards the development of novel food products that are simultaneously convenient and healthy options. Regarding the sensory attributes, the CB formulation may be optimized by maintaining the same basis with the addition of different flavors/flavoring ingredients, in order to improve the sensory acceptance of CB added with TF.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, CNPq, and Fundação Araucária (Process 120/2010, Fundação Araucária-Pronex). The authors acknowledge the Post-graduate Program in Food Science of the Universidade Estadual de Londrina for grant the use of the Senstools.

Research data are not shared

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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FIGURE CAPTION

Figure 1 Internal preference map of cereal bars

Consumers: △ Group 1 (*n* = 104); × Group 2 (*n* = 14); ✳ Group 3 (*n* = 9); ○ Group 4 (*n* = 8). Samples (■): CB-A: control cereal bar (without tempeh flour); CB-B: 10% tempeh flour; CB-C: 15% tempeh flour; CB-D: 20% tempeh flour.

Table 1 Cereal bars formulations

Ingredients (%)	**CB-A**	**CB-B**	**CB-C**	**CB-D**
Chestnut (chopped; Uniagro, Porto Alegre, Brazil)	7.32	7.32	7.32	7.32
Damascus (chopped; Uniagro)	9.15	9.15	9.15	9.15
Tempeh flour	0.00	10.00	15.00	20.00
Maltodextrin (Newnutrition, São Paulo, Brazil)	12.82	12.89	12.83	12.87
Granola (Nestlé, São Paulo, Brazil)	13.73	17.39	12.45	7.41
Rice flakes (Harald, Toledo, Brazil)	14.65	14.65	14.65	14.65
Glucose syrup (Arcolor, São Paulo, Brazil)	28.38	28.38	28.38	28.38
Orange essence (Arcolor)	0.22	0.22	0.22	0.22
Rolled oats (Nestlé)	13.73	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00

CB-A: control cereal bar (without tempeh flour); CB-B: 10% tempeh flour; CB-C: 15% tempeh flour; CB-D: 20% tempeh flour.

Table 2 Soybean, tempeh, tempeh flour, and formulated cereal bars (CB) nutritional composition

Chemical composition	**Soybean**	**Tempeh**	**Tempeh flour**	**CB-A**	**CB-B**	**CB-C**	**CB-D**
Moisture[†]	10.04[d]±0.10	64.84[e]±0.20	2.93[c]±0.06	11.70[c]±0.35	12.61[b]±0.48	13.27[b]±0.39	12.38[b]±0.50
Protein[†‡]	41.00[c]±0.62	55.26[a]±0.37	51.28[b]±0.82	6.24[f]±0.58	9.22[e]±0.11	11.02[d]±0.29	12.31[d]±0.34
Lipids[†‡]	23.20[b]±0.26	11.19[c]±1.23	25.00[a]±0.26	1.81[g]±0.16	4.52[f]±0.52	6.39[e]±0.40	8.09[d]±0.12
Ash[†‡]	5.59[a]±0.00	3.32[b]±0.00	3.01[c]±0.01	1.35[d]±0.04	0.88[f]±0.03	1.12[e]±0.08	1.30[d]±0.08
Carbohydrates[†‡]	30.06[e]±0.020	29.93[e]±0.75	20.70[f]±1.08	78.86[a]±0.46	72.88[b]±0.78	68.35[c]±0.76	65.02[d]±0.68

CB-A: control cereal bar (without tempeh flour); CB-B: 10% tempeh flour; CB-C: 15% tempeh flour; CB-D: 20% tempeh flour. [†] Content expressed in g 100 g^{-1}. [‡] Values expressed in dry basis. Mean ± standard deviation (n = 3 repetitions). Different letters in the same row indicate significant differences by Tukey test ($P < 0.05$).

Table 3 Soybean, tempeh, tempeh flour, soybean, and formulated cereal bars total fatty acid profiles†

Fatty acid	Soybean	Tempeh	Tempeh flour	CB-A	CB-B	CB-C	CB-D
14:0	N.d.	N.d.	N.d.	0.09^{a}±0.01	0.07^{b}±0.01	0.07^{b}±0.01	0.07^{b}±0.01
16:0	10.52^{b}±0.05	7.28^{c}±0.50	6.84^{c}±0.14	13.83^{a}±0.13	11.08^{b}±0.13	10.34^{b}±0.25	10.21^{b}±0.08
16:1	0.07^{c}±0.01	0.07^{c}±0.01	0.06^{c}±0.01	0.20^{a}±0.01	0.16^{b}±0.01	0.15^{b}±0.01	0.14^{b}±0.01
18:0	4.06^{d}±0.01	2.35^{e}±0.22	2.16^{e}±0.04	8.81^{a}±0.11	7.42^{b}±0.09	6.30^{c}±0.26	5.74^{c}±0.18
18:1n-9t	N.d.	N.d.	N.d.	0.19^{a}±0.01	0.16^{a}±0.01	0.08^{c}±0.03	0.13^{b}±0.02
18:1n-9c	23.05^{e}±0.01	23.67^{e}±0.39	25.36^{d}±0.51	36.00^{a}±0.10	33.38^{b}±0.03	32.81^{bc}±0.10	32.14^{c}±0.13
18:2n-6t	0.05^{e}±0.01	0.07^{de}±0.07	1.40^{a}±0.03	0.10^{cde}±0.02	0.26^{b}±0.01	0.20^{bcd}±0.03	0.22^{bc}±0.02
18:2n-6c	55.99^{c}±0.01	60.62^{a}±0.37	58.41^{b}±0.91	39.97^{f}±0.24	44.85^{e}±0.23	46.93^{d}±0.36	47.87^{d}±0.28
18:3n-6	N.d.	0.76^{a}±0.05	0.70^{a}±0.06	N.d.	0.14^{b}±0.01	0.19^{b}±0.01	0.22^{b}±0.01
20:1n-9	5.47^{a}±0.01	4.43^{b}±0.08	4.33^{b}±0.25	N.d.	1.87^{d}±0.05	2.25^{cd}±0.06	2.56^{c}±0.11
18:3n-3	0.24^{a}±0.01	0.19^{b}±0.01	0.24^{a}±0.01	0.23^{a}±0.01	0.15^{c}±0.01	0.16^{c}±0.01	0.18^{b}±0.01
20:4n-6	0.37^{a}±0.01	0.24^{b}±0.01	0.26^{b}±0.01	0.07^{d}±0.01	0.14^{c}±0.01	0.14^{c}±0.01	0.17^{c}±0.01
22:2	N.d.	N.d.	N.d.	0.20^{a}±0.01	0.21^{a}±0.01	0.21^{a}±0.01	0.20^{a}±0.01
24:0	0.17^{c}±0.01	0.26^{a}±0.01	0.23^{b}±0.01	0.06^{f}±0.01	0.09^{e}±0.01	0.10^{de}±0.01	0.12^{d}±0.01
Σ SFA	14.76^{d}±0.04	9.90^{e}±0.73	9.23^{e}±0.17	22.79^{a}±0.25	18.67^{b}±0.20	16.81^{c}±0.52	16.14^{cd}±0.26
Σ MUFA	28.59^{cd}±0.02	28.17^{d}±0.31	29.75^{c}±0.77	36.67^{a}±0.08	35.61^{ab}±0.06	35.33^{b}±0.13	35.02^{b}±0.02
Σ PUFA	56.65^{b}±0.01	61.93^{a}±0.42	61.02^{a}±0.94	40.59^{e}±0.27	45.74^{d}±0.20	47.85^{c}±0.40	48.88^{c}±0.28
Σ n-6	56.41^{b}±0.01	60.97^{a}±0.37	60.08^{a}±0.88	40.15^{e}±0.25	45.24^{d}±0.20	47.27^{c}±0.39	48.27^{c}±0.27
Σ n-3	0.24^{a}±0.01	0.19^{b}±0.01	0.24^{a}±0.01	0.23^{a}±0.01	0.15^{c}±0.00	0.16^{c}±0.01	0.18^{b}±0.01
PUFA/SFA	1.98^{b}±0.01	2.21^{a}±0.26	2.05^{b}±0.02	1.10^{d}±0.01	1.28^{c}±0.01	1.35^{c}±0.01	1.39^{c}±0.01

† The main fatty acids are expressed as a % of the total fatty acids. CB-A: control cereal bar (without tempeh flour); CB-B: 10% tempeh flour; CB-C: 15% tempeh flour; CB-D: 20% tempeh flour. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids. N. d.: not detected. Mean ± standard deviation (n = 3 repetitions). Different letters in the same row indicate significant differences by the Tukey test ($P < 0.05$).

Table 4 Soybean, tempeh flour, and formulated cereal bars isoflavones forms content

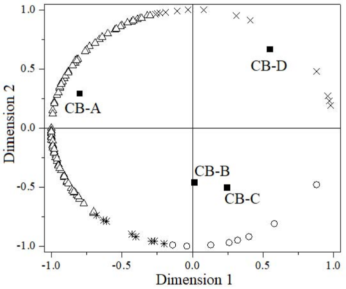
Isoflavones[†] mg 100 g^{-1}	Soybean	Tempeh flour	CB-A	CB-B	CB-C	CB-D
Malonyl daidzin	26.15±0.32	N.d.	N.d.	N.d.	N.d.	N.d.
Malonyl glycitin	13.52±0.53	N.d.	N.d.	N.d.	N.d.	N.d.
Malonyl genistin	33.47^{a}±1.29	8.71^{b}±0.00	N.d.	1.48^{e}±0.04	2.13^{d}±0.05	3.59^{c}±0.05
Acetyl genistin	N.d.	0.69±0.03	N.d.	N.d.	N.d.	N.d.
Daidzein	1.44^{e}±0.20	10.03^{a}±0.27	N.d.	2.23^{d}±0.06	3.10^{c}±0.04	4.89^{b}±0.05
Glycitein	N.d.	1.98±0.02	N.d.	N.d.	N.d.	N.d.
Genistein	1.66^{e}±0.04	15.46^{a}±1.38	N.d.	4.27^{d}±0.15	5.78^{c}±0.11	8.76^{b}±0.22
Total isoflavones	76.24	36.87	N.d.	7.98	11.01	17.24
Total aglycones	3.07±0.18	27.46±1.09	N.d.	6.50±0.19	8.88±0.15	13.65±0.13

[†] Isoflavones content in dry basis. CB-A: control cereal bar (without tempeh flour); CB-B: 10% tempeh flour; CB-C: 15% tempeh flour; CB-D: 20% tempeh flour. N. d.: not detected. Mean ± standard deviation (n = 3 repetitions). Different letters in the same row indicate significant differences by Tukey test ($P < 0.05$). The isoflavones forms daidzin, genistin, glycitin, acetyl daidzin, and acetyl glycitin were not detected.

Table 5 Cereal bars sensory acceptance

Attributes	CB-A	CB-B	CB-C	CB-D
Color	7.6^{a}±1.9	6.9^{b}±2.1	6.5^{bc}±2.1	6.1^{c}±2.2
Appearance	7.6^{a}±2.0	6.7^{b}±2.2	6.4^{bc}±2.2	5.9^{c}±2.2
Texture	7.3^{a}±2.0	7.0^{ab}±2.1	6.8^{b}±2.0	6.2^{c}±2.1
Flavor	8.1^{a}±1.8	5.8^{b}±2.6	5.5^{bc}±2.5	5.1^{c}±2.6
Overall impression	7.6^{a}±1.6	6.3^{b}±2.1	5.9^{bc}±2.1	5.6^{c}±2.1

CB-A: control cereal bar (without tempeh flour); CB-B: 10% tempeh flour; CB-C: 15% tempeh flour; CB-D: 20% tempeh flour. Mean ± standard deviation (n = 135 assessors). Different letters in the same row indicate significant differences by Tukey test ($P < 0.05$), in reference to a 10-cm hedonic scale (0 = disliked extremely, 10 = like extremely).



CB-A
CB-B
CB-C
CB-D
Dimension 1
Dimension 2
1.0
0.5
0.0
-0.5
-1.0