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ORIGINAL ARTICLE

Lentinula edodes mushroom as an ingredient to enhance the nutritional and functional properties of cereal bars

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Abstract *Lentinula edodes* (shiitake) is the second most cultivated edible mushroom in the world; it has low lipid contents, high protein and it is source of vitamins and minerals. This study aimed to develop and to evaluate two sweet and two salty food bars containing shiitake. The binder elements were heated and then the dried elements were added. The bars were shaped, and the sensorial test was accomplished with hedonic scale of 9 points for analysis of texture, aroma, taste and appearance, and a 5-point scale for buying intention. The centesimal composition included percentages of moisture content, ashes, lipids, proteins and carbohydrate contents. Chemical elements of shiitake were quantified by Energy Dispersive X-ray Fluorescence. Glucans were determined using a commercial kit. Phenolic compounds were determined with the Folin-Ciocalteu reagent. The shelf life was evaluated by microbiological control, up to 180 days, at temperatures of 25 °C and 37 °C. The sweet bar 1 (SwB1) had better sensory analysis and buying intention. Shiitake showed high concentrations of calcium, iron, phosphorus, potassium, zinc, manganese, phenolic compounds and glucans. SwB1-bar maintained shiitake nutritional characteristics. SwB1-bars did not present microorganisms for up to 180 days of shelf life, neither at 25 °C nor at 37 °C, and they followed the standards determined by National Health Surveillance Agency. Sweet bars are an easy marketing alternative due to their stability, low-cost of production and good acceptance, as well as flexibility to add other functional ingredients beneficial to health, such as shiitake.

Keywords *Lentinula edodes* · Functional bars · Food development · Nutritional composition · Chemical elements

Introduction

There are thousands of species of mushrooms in the world; however, only about 25 varieties are cultivated with culinary characteristics and medicinal properties (Ng and Tan 2017). Among those varieties, is the *Lentinula edodes* (popularly known as shiitake). Shiitake is the second most cultivated edible mushroom in the world, accounting for 25% of the production. Shiitake is a complete nutritional food type because it has low lipid content, high protein content, vitamins, minerals and fibers (Akesowan 2016).

Shiitake presents a variety of bioactive compounds with great therapeutic application, such as polysaccharides, glycoproteins and phenolic compounds, namely protocatechuic, p-hydroxybenzoic, p-coumaric and cinnamic acids (Roncero-Ramos et al. 2017; Rathore et al. 2017). According to Rincão et al. (2012), aqueous and methanolic extracts, and polysaccharide isolated from shiitake, were able to inhibit the replication of herpes virus and polio type 1 in vitro. At an appropriate dose, the mushroom presents immunomodulatory action, anticancer (Pinya et al. 2019), antiviral (Rincão et al. 2012), hipoglicemic and other health promoting properties (Spim et al. 2017).

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According to Dietary Reference Intake (DRI 2005), dietary fiber helps in the prevention and supportive treatment of heart disease, with a recommendation of 20–38 g of daily fiber intake. The impact of shiitake fibers on rat health was assessed by Anwar et al. (2019). Since intestinal microbiome can be modulated by diet, that author evaluated Wistar rats submitted to a control diet, a hypercholester, olemic diet and a hypercholesterolemic diet with 5% shiitake. Triglycerides, total cholesterol and low-density lipoprotein (LDL) concentrations were lower and high-density lipoprotein (HDL) concentrations were higher after the shiitake supplementation. Intestinal microbiome of the group supplemented with shiitake presented greater species richness, characterized by the abundance of *Clostridium* and *Bacteroides* spp.

β-Glucans present in shiitake can also act on lipid metabolism, reducing total cholesterol levels (Spim et al. 2017). The polysaccharides can be considered functional food. They can be used as prebiotics, especially by regulating the intestinal microbiota and the health of the individual (Anwar et al. 2019).

Based on their nutritional complexity, mushrooms can be used for the development of functional food. Functional bars, for example, are an easy alternative to be marketed due to their stability, easy transportation, low-cost of production and good acceptance (Hadi et al. 2018), besides the flexibility of other functional ingredients to be added to the bar. The use of shiitake in biotechnology and in the prevention or treatment of chronic diseases, especially hypercholesterolemia and diabetes, has grown in recent decades. For this reason, the present study developed, characterized and evaluated the acceptance of the functional bars supplemented with shiitake.

Materials and methods

Acquisition and processing of shiitake

Raw shiitake mushrooms were provided by the Yuri Cogumelos company located in Sorocaba (São Paulo State, Brazil). Raw mushrooms were shredded in a multiprocessor food (Philco®), weighed, and dried at 50 ± 2 °C for 48 h. After drying, the dried mushrooms were weighed again and showed a moisture content from 87 to 92%, meaning that 100 g of raw mushrooms produced about 10 g of dried mushrooms. Afterwards, they were stored in a dry and airy place, protected from light, until the time of making the functional bars.

Development of functional bars supplemented with Shiitake

The amount of dried shiitake added to the bars was selected based on Spim et al. (2017) study, in which 100 mg dried shiitake/kg/day (corresponding to 7 g of dried shiitake for a 70 kg individual) has demonstrated hypocholesterolemia and hypoglycemic effects in pre-clinical investigation; and no toxicity (Grotto et al. 2015).

Two functional Sweet bars (SwB) and two functional Salty bars (SaB) were designed and developed for comparative purposes in the acceptability test. To develop the functional bars, assuming the individual would have to eat two functional bars, the amount of dried shiitake used in a 25 g bar was 3.5 g.

The ingredients used in SwB were dried shiitake, oats, quinoa, chia, prune, flaxseed, and chestnut, peanut and sugars. The ingredients used in SaB were dried shiitake, oats, quinoa, rice flakes, flaxseed and sesame seeds, dried tomatoes, peanut butter, parmesan cheese and condiments parsley, garlic, onion, oregano, thyme, bay leaf, pepper and salt. The general process of producing the functional bars is shown in Fig. 1.

The binder elements were heated under stirring at 115 ± 3 °C, until reaching Brix Grade 85–89. The other ingredients were added to the hot syrup and homogenized. The blends were molded in a bar mold of 25 ± 1 g. After cooling, the functional bars were packed in a polypropylene plastic vacuum package.

Sensory analysis and purchase intention

After defining the formulations of the functional bars supplemented with shiitake, the bars were evaluated by 82 untrained tasters, of both genders. The study is approved by

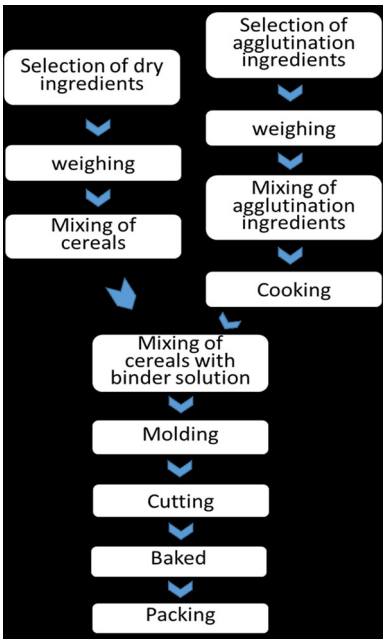

Fig. 1 Flowchart of the production process of functional bars supplemented with Shiitake

the Ethics Committee of the University of Sorocaba (approval number 2.016.736/CAAE 65105416.8.0000). Each consumer received two copies of the Informed Consent Form (Silva et al. 2014) and they participated with complete freedom to express discomfort or dissatisfaction.

The sensory analysis was performed maintaining the anonymity of the participants and the confidentiality of the results. Samples of all four formulations were supplied individually, with water. The test was carried out at the Laboratory of Sensorial Evaluation of Experimental Food Cooking (University of Sorocaba). The bars were analyzed for texture, aroma, taste and appearance by a sensorial acceptance test. For the quantification of the results, **a** hedonic 9-point scale was used: I liked extremely "9", I liked it very much "8", I liked it moderately "7", I liked it slightly "6", indifferent "5", slightly displeased "4", I moderately disliked "3", I disliked "2" and I greatly disliked "1" (Praseptiangga et al. 2019; Samuel and Peerkhan 2020). The acceptance range was from 6 to 9 points.

And a hedonic 5-point scale was used for the purchase intention survey: would certainly buy "5", possibly buy "4", maybe buy "3", possibly would not buy "2" and certainly would not buy "1" (Silva et al. 2014).

Nutritional composition

The centesimal composition was assessed by the percentages of moisture, ashes, lipids, proteins and carbohydrates, in shiitake *in natura* and in the functional bar supplemented with shiitake selected in sensory analysis.

For the moisture content, 3 g of raw shiitake was dried at 105 °C until constant weight. To determine the ashes, 3 g of the samples were charred and incinerated in Mufla (Quimis Enila) at 550 °C. For the lipid extraction, 100 mL of ethyl ether solvent was used for each sample for 6 h in the Soxhlet extractor (Samuel and Peerkhan 2020).

Proteins were quantified using the micro Kjeldahl method (Zhou et al. 2015), with some modifications. The method consisted of three phases: digestion, distillation and titration. The digestion was based on the oxidation of 70 mg of the sample at 380 °C with 5 mL concentrated sulfuric acid, 100 mg potassium sulfate and 100 mg copper sulfate for six hours at 380 °C. In the distiller, nitrogen from proteins was reduced in ammonium sulfate. Upon contact with 5 mL 5% boric acid, the ammonium sulfate was transformed in ammonium borate, titrated with 37% hydrochloric acid. The centesimal composition of the mushroom was determined by adding the percentages of moisture, ashes, lipids, proteins and subtracting of 100%, the difference was the percentage of carbohydrates.

The fibers were quantified using Mccleary et al. (2010) and filtration manual techniques. For this, 2 g of dry lipid-free samples were digested with 50 mL concentrated glacial acetic acid, 5 ml concentrated nitric acid, 2 g trichloroacetic acid and 5 g diatomaceous sand by 45 min on heating. Subsequently, the samples were manually filtered, washed with boiling water, 20 ml 70% alcohol and 20 ml ethyl ether. Subsequently, the sample was dried, weighed and incinerated at 550 °C to ashes. The difference between the initial weight of the sample and the ash weight represents the total fibers.

Concentration of chemical elements

Chemical elements from shiitake *in natura* were assessed using the technique of Energy Dispersive X-ray Fluorescence (ED-XRF) (Amptek®). ED-XRF is a nuclear analytical technique used for the analysis of solid samples to determine the concentration of several elements, so that a previous chemical treatment will not be necessary (Nascimento Filho, 1999). Essential and non-essential chemical elements were quantified: calcium (Ca), iron (Fe), titanium (Ti), phosphorous (P), sulfur (S), chlorine (Cl), potassium (K), manganese (Mn), zinc (Zn), rubidium (Rb) and cadmium (Cd).

Determination of glucans

The determination of total glucans was carried out in shiitake *in natura* and in the selected functional bar, using the commercial Yeast & Mushroom-Megazyme® kit. For this purpose, 100 mg of samples and 1.5 mL of 37% HCl were incubated for 45 min at 30 °C. The volume was adjusted to 10 mL with deionized water and incubated for 2 h. Later, 10 mL of 2 M KOH was added and the contents completed to 100 mL with 200 mM sodium acetate buffer (pH 5.0) and centrifuged at 1500 rpm for 10 min. The supernatant (0.1 mL) was mixed to 0.1 ml of the exo-β-1,3-glucanase and β-glycosidase solution and incubated at 40 °C for 60 min. Then, 1.5 mL of glucose oxidase/peroxidase was added and incubated for an additional 20 min. at 40 °C. The absorbance was read at 510 nm.

For α-glucans, 2 mL of KOH was added to 100 mg of the samples. After 20 min of stirring, 0.2 mL of amyloglucosidase and invertase were added, incubated at 40 °C for 30 min. The tubes were centrifuged at 1500 rpm for 10 min. To a 0.1 mL of the supernatant, 0.1 mL of sodium acetate buffer and 3.0 mL of enzyme reagent were added, and incubated for 20 min at 40 °C. The absorbance was measured at 510 nm (Lambda 35, PerkinElmer, Waltham, MA, USA). For the quantification of β-glucan, α-glucan was subtracted from total glucan. All analyses were performed in triplicate.

Determination of total phenolic compounds

Phenolic compounds were determined by weighing 2 g of the shiitake *in natura* or the selected functional bar, in triplicate, in volumetric flasks with methanol 80%. The flasks were shaken every ten minutes for one hour, after being filtered and centrifuged at 3000 rpm for 30 min to obtain the supernatant. The supernatant (2 mL) was transferred to a 25-mL flask; 1 mL of Folin-Ciocalteu reagent was added, and the volume was quenched with 20% sodium carbonate. The reading was carried out in a spectrophotometer at 760 nm (Lapornik et al. 2005). A calibration curve of gallic acid was done at concentrations 12.5; 25.0; 37.5; 50.0; 62.5; 75 and 100 μL/mL. The results were expressed as mg gallic acid equivalent (EAG)/g sample.

Storage and shelf life

Samples of the selected functional bar supplemented with shiitake were ground and diluted in saline (0.9%), followed by serial 1:10 dilutions. The diluted solutions were inoculated in petri dishes, with the culture medium. The culture media used were Plate Count Agar (PCA), indicated for total count of microorganisms, and Potato Dextrose Agar (PDA), used for counting molds and yeasts. The selective culture media used were MacConKey Agar (used to Gram-negative like *Escherichia coli*), Mannitol-Egg Yolk Polymyxin Agar (recommended for the isolation of *Bacillus cereus*), Salt Mannitol Agar (used to identify *Staphylococcus aureus*) and Bismuth Sulfite Agar (for isolation of *Salmonella* sp).

The inoculation of the samples was performed under aseptic conditions. After the incubation time at 37 °C for bacteria and 25 °C for molds and yeasts, the colonies were counted manually and expressed in colony forming units.

The shelf life of the functional bar supplemented with shiitake was accompanied by the growth kinetics of bacteria, molds and yeasts within 1, 7, 15, 30, 60, 90 and 180 days. The results were reported by microbial evaluation, meeting the specifications of the *Codex Alimentarius* (FAO 1980) and Brazilian Food Microbiological Standards (Brasil, 2001).

Statistical analysis

Data were expressed as mean ± standard deviation. Data were submitted to analysis of variance (ANOVA) followed by the Tukey test ($p < 0.05$) and the graphs were produced in GraphPad Prism® 6.

Results and discussion

Development of the functional bars

Pilot tests were performed with different concentrations of binder ingredients. Six sweet functional bars supplemented with shiitake were designed with sugar ranging from 40 to 52%. Two of the tastiest formulations moved forward to the sensory and purchase tests. A sugar concentration of 42% was defined for the sweet bars, presenting satisfactory consistency, appearance, flavor and aroma. All the ingredients used in the functional SwB are described in Table 1. The differences between the bars were the crystallized ginger in SwB2 and the peanut in SwB1, modifying the flavor.

For the functional salty bars, pilot tests were performed with different concentrations of wheat flour, soy lecithin, condiments and xanthan gum. Four salty bars were developed and two of the tastiest bars (presented in Table 1) moved forward to the sensory and purchase tests. The differences between the salty bars were the peanut in the SaB1 and the parmesan cheese in the SaB2.

Sensorial test and purchase intention survey

The sensorial analysis evaluates the flavor and the preferences of the consumers, as well as the acceptance of a new product in the market. Through sensory analysis, it was possible to evaluate the bars supplemented with shiitake for texture, flavor, aroma and appearance (Fig. 2a).

No differences in appearance were observed comparing all functional bars. There were no differences between the SwB1 and 2. There were no differences between the SaB1 and 2. However, a significant decrease in the acceptance rate of SaB was observed compared to SwB, which means the sweet flavor had greater acceptability than the salty one did.

Considering the 9-point hedonic scale as 100%, the acceptability in terms of flavor were 78, 67, 44 and 44% for SwB1, SwB2, SaB1 and SaB2, respectively. Flavor acceptability was 11% higher for SwB1 when compared to SwB2, and 34% higher for SwB1 when compared to both salty bars. Aroma is also an important parameter for pleasantness, and the percentages of acceptability were 78, 67, 56 and 56% for SwB1, SwB2, SaB1 and SaB2, respectively.

The purchase intention survey is presented in Fig. 2b. Both salty bars did not have good acceptability among the volunteers, and the intent-to-buy scales were predominantly “certainly not to buy” and “possibly would not buy”. On the other hand, SwB1 and 2 presented the preference of the volunteers. SwB1 presented 70% of the preference with “possibly would buy” and “certainly

Table 1 Ingredients used in the 25 g functional Sweet Bars (SwB) and Salty Bars (SaB)

Ingredients SwB	SwB1		SwB2		Ingredients SaB	SaB1		SaB2	
	%	g	%	g		%	g	%	g
Dried Shiitake	14.0	3.50	14.0	3.50	Dried Shiitake	14.0	3.50	14.0	3.50
Flakes oat	12.0	3,00	12.0	3.00	Flakes oat	12.0	3.00	12.0	3.00
Brown sugar	8.3	2.10	8.3	2,10	Brazil nut	12.0	3.00	12.0	3.00
White sugar	30.0	7.50	30.0	7.50	Quinoa grains	10.0	2.50	10.0	2.50
Dry plum	9.0	2.25	9.0	2.25	Sesame seed	10.0	2.50	10.0	2.50
Brazilian nut	7.0	1.75	7.0	1.75	Flaxseed	9.0	2.25	8.0	2.00
Flaxseed	3.2	0.80	3.2	0.80	Dried tomato	8.0	2.00	8.0	2.00
Glucose	1.6	0.40	1.6	0.40	Peanut	14.0	3.50	–	–
Soy lecithin	0.4	0.10	0.4	0.10	Peanut butter	3.0	0.75	3.0	0.75
Quinoa grains	3.5	0.86	3.5	0.86	Parmesan cheese	–	–	15.0	3.75
Crystallized ginger	–	–	8.0	2.00	White flour	3.0	0.75	3.0	0.75
Peanut	8.0	2.00	–	–	Whole wheat flour	3.0	0.75	3.0	0.75
Chia seed	2.0	0.50	2.0	0.50	Xanthan gum	1.0	0.25	1.0	0.25
Food glycerin	0.5	0.12	0.5	0.12	Soy lecithin	0.6	0.15	0.6	0.15
Coconut oil	0.5	0.12	0.5	0.12	Spice	0.4	0.10	0.4	0.10
Total	100	25.0	100	25.0	Total	100	25.0	100	25.0

Fig. 2 Sensory analysis and purchase intention of functional Sweet bars 1 (SwB1) and 2 (SwB2), and Salty bars 1 (SaB1) and 2 (SaB2) in texture, flavor, aroma and appearance. Results are presented as mean ± standard deviation of hedonic 9-points scale. *Different from SaB1 ($p < 0.05$); #Different from SaB2 ($p < 0.05$)

would buy" whereas SwB2 presented 56% of the preference.

Therefore, the functional SwB1, with peanut flavor, was chosen as the tastiest bar, and the other analyses (centesimal composition, microbiological, glucans) were done in that bar.

In another study, cookies containing shiitake were produced and analyzed sensorially (Toan and Thu 2018). Wheat flour was replaced with shiitake flour, at concentrations of 5%, 10% and 15%. The authors achieved a significant increase in the concentration of fibers and proteins in the cookie with shiitake flour compared to wheat flour. The cookie with 5% shiitake was the one that pleased the participants the most.

Centesimal composition, glucans and total phenolic compounds

The results of the centesimal composition are shown in Table 2.

SwB1 had the percentage of moisture within the limit established by the national commission on food standards—cereals and products derived from cereals cannot have more than 15% moisture (Brasil 1978). SwB1 also had lipids possibly due to the Brazilian nut and peanut. Comparatively, gluten-free cereal bars of amaranth and flaxseed were developed, and the authors found 9.12% moisture, 7.12% proteins and 7.87% lipids, differences presented according to the ingredients (Pagamunici et al. 2014).

Table 2 Centesimal composition of shiitake *in natura* and functional SwB1 supplemented with shiitake

	Shiitake	SwB1
Moisture (%)	90.3 ± 0.3	14.50 ± 0.23
Ashes (%)	0.62 ± 0.01	1.45 ± 0.12
Lipids (%)	0.40 ± 0.16	14.12 ± 4.04
Proteins (%)	2.27 ± 0.23	11.29 ± 0.22
Carbohydrates (%)	5.76 ± 0.41	61.99 ± 4.08
Fibers (%)	9.99 ± 2.00	5.54 ± 1.50
α-glucans (%)	0.30 ± 0.01	16.46 ± 0.01
β-glucans (%)	26.5 ± 0.01	15.96 ± 0.01
Total Phenolic Compounds *	176 ± 29	82 ± 5

*Data expressed as gallic acid equivalents (mg GAE/g) dry sample

Carbohydrate concentration in SwB1 increased substantially compared to shiitake *in natura* due to its composition in sugars, fibers and glucans. In the study of Silva et al. (2014), cereal bars were developed with 12.5% of pumpkin seed flour. The bars presented 11.45% humidity, 63.34% carbohydrates, 12% protein, 5.51% lipids and 6.17% insoluble fibers, differing from this study only in the lipid concentration.

Protein concentration in shiitake was low in comparison to the study of Khaskheli et al. (2018), which found 12.1% of proteins in shiitake. The differences can be influenced by the substrate where the mushroom is cultivated, climate, temperature and other care in the mushroom's cultivation (Siwulski et al. 2019). Bach et al. (2018) analyzed the chemical composition of the shiitake produced by two different cultivation methods, in axenic substrates (prepared with eucalyptus sawdust, wheat bran, maize germ and limestone) and (wood logs sawtooth oak) and showed that the physical–chemical composition influences the moisture, protein, lipids, minerals and fibers.

The percentage of α-glucan in shiitake *in natura* was lower than in SwB1. The percentage was possibly higher in the bar due to the presence of oats. The β-glucan presented in shiitake *in natura* was not maintained in the SwB1 even though 16% is a high concentration. In relation to shiitake *in natura*, the β—glucan concentration was similar to that found in a previous study from our group (34.5%) (Spim et al., 2017) and from Kolundžić et al. (2018) (33,98%). Bak et al. (2014) evaluated the stems and mycelia of ten shiitake mushroom cultivars for β-glucan and found a variation ranging from 20.06 to 44.21% in the mycelia and from 29.74 to 56.47% in stems.

Thondre and Henry (2011) investigated the effect of high-purity β-glucans in vitro digestion and in glycemic response in individuals that consumed chapattis, a typical bread in India. The low-purity β-glucans proved to be more effective than the high-purity β-glucans in lowering glycemic response. Therefore, the results depend on the molecular weight, level of purity and preparation methods of β-glucans.

High total phenolic compounds were found (176 mg GAE/g dry basis) in shiitake and (82 mg GAE/g dry basis) in the functional sweet bar, bioactive substances important for cell health. Secondary plant metabolites such as phenolic acids, simple phenols, flavonoids, tannins, lignins, tocopherols, are synthesized during their development and many of them have beneficial effects, such as the antioxidant effect (Ng and Tan 2017). Comparatively, total phenols were evaluated in 17 species of Mexican mushrooms, mostly edible. The authors found from 30.31 to 307.01 mg GAE/100 g in mushroom samples on dry basis (Yahia et al. 2017).

Concentration of chemical elements

The concentrations of the chemical elements—essential and non-essential—are presented in Fig. 3. Shiitake presented high concentrations of K and P (24.5 and 7.9 mg/g, respectively), considered macro-elements. The other elements were found in the order of μg/g, that is, microelements.

Kolundžić et al. (2018) analyzed macro and microelements from three species of mushrooms, including shiitake. The most abundant elements were Ca, K and P, followed by lithium (Li), selenium (Se) and Zn, similar to our findings. On the other hand, some toxic elements such as lead (Pb), arsenic (As) and Cd were detected at higher levels than those allowed.

The variability in the chemical composition of the substrates may interfere both in the nutritional yield and in the levels of toxic elements. The findings of Siwulski et al. (2019) corroborated these results, highlighting the care for the substrates used in the mushrooms cultivation, ensuring consumers' safety.

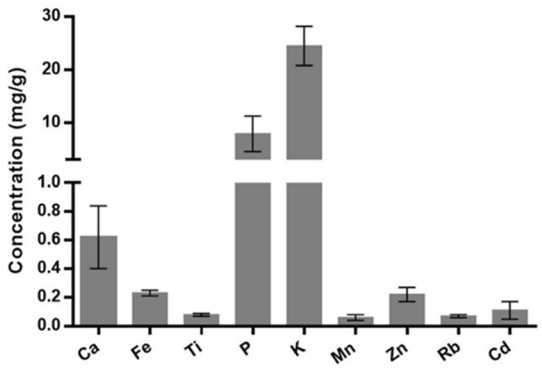

Fig. 3 Essential and non-essential chemical elements of shiitake *in natura* by energy dispersive X-ray fluorescence

In a shiitake from China, K and P concentrations were similar to this study (27.28 and 8.91 mg/g respectively) whereas Ca (0.174 μg/g), iron (Fe) (36.29 μg/g), Zn (7.21 μg/g) and manganese (Mn) (1.74 μg/g) presented significantly lower values (Li et al. 2018).

Shelf life by microbiological control

SwB1 showed an initial total count of microorganisms of 9×10 CFU/g. After 180 days, the total count of microorganisms was 7×10 CFU/g. These results were lower than the maximum limit allowed by National Health Surveillance Agency (ANVISA), evidencing the quality of raw materials and the sanitary control in the preparation of the bars. Microbial food safety is an essential component of food quality to the consumer (Nerín et al. 2016).

When samples of SwB1 were inoculated in a selective culture medium, few units ($< 10^2$ CFU/g) of *S. aureus* and *B. cereus* were found (Table 3). There was no growth for *E. coli and Salmonella sp* (Table 3). Our results were within the limits described in European Union Commission Recommendation (CE N° 1441/2007) and ANVISA.

In a comparative study, breakfast cereals with flakes of barley, oats, rye and wheat were evaluated for bacterial count, packed in three different materials. Total bacterial count and shelf life were evaluated for up to 6 months. The initial count was 5.5×10 CFU/g for all samples, and after 1 month of storage, the samples remained stable (Kince et al. 2017).

Similarly, a bar rich in isoflavones and soy protein was developed to control dyslipidemia. The shelf life of the product was assessed for 6 months, and there were no significant changes in the microbiological parameters in coliforms, *S. aureus*, *B. cereus* and *Salmonella* sp., presenting less than 10 colonies of microorganisms in each sample (Lobato et al. 2011). Cookies and cereal bars were produced with fruit and vegetable residues, and microbiological stability was tested, as well as shelf life for 0, 30 and 90 days. The authors observed that the samples met the standard established by the Brazilian rules, except for the two bars that, after 30 days, showed growth of molds and yeasts. The presence of molds and yeasts is attributed to the high humidity content found in the samples (Ferreira et al. 2015).

According to Nerín et al. (2016), food contamination can occur along the production process. Some contaminants may already be present in the raw materials, but others may be incorporated during handling, packaging, transportation, storage, preheating, disinfection, cleaning and sterilization. These facts highlight the importance of conducting chemical and microbiological analyses during food processing, ensuring safety for consumers (Sharma et al. 2013).

Microbiological criteria and standards for food should be observed, applying, whenever possible, the Hazard Analysis System and Critical Control Points (HACCP) and the microbiological quality of food products (Brasil 2001). Some microorganisms are highly adherent to the grain and cannot be removed by simply washing in liquid medium. However, they can be reduced by removing the surface of the grain using an abrasive grinder. This microbial load reduction is even more important in the case of fungi, as they can potentially produce mycotoxins (Laca et al. 2006).

Conclusion

Both functional sweet bars pleased the volunteers more than the salty ones did, with approval in the sensory analysis and purchase intention. Sweet bar 1 obtained the highest score in the preference of volunteers, with peanut flavor.

SwB1 maintained the shiitake concentrations of proteins, fibers, glucans and phenolic compounds. The development of functional food has brought health benefits and the SwB1 is an easy-to-market alternative due to their stability, easy transportation, low-cost of production and good acceptance, as well as the flexibility to add nutraceuticals such as shiitake.

Table 3 Analysis of the shelf life of the functional SwB1 supplemented with shiitake, in selective culture medias

Counting of microorganisms in Sweet bar 1 supplemented with shiitake								
Samples	*E. coli* (CFU/g)		*S. aureus* (CFU/g)		*B. cereus* (CFU/g)		*Salmonella* sp (CFU/g)	
	24 °C	37 °C	24 °C	37 °C	24 °C	37 °C	24 °C	37 °C
7 days	0	0	2×10	0	0	0	0	0
180 days	0	0	2×10	0	1×10	1×10	0	0
ANVISA	5×10		10^3		5×10^2		0	

ANVISA (Brazilian National Health Surveillance Agency)

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

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