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ORIGINAL RESEARCH PAPER

STUDIES ON THE USE OF CAROB POWDER AND *BACILLUS SUBTILIS* FOR IMPROVING FUNCTIONALITY OF THE CEREAL BARS

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Abstract

The present study aimed to obtain and investigate the physicochemical and sensory characteristics of fiber-rich cereal bars containing probiotics. Different cereal bars formulations were tested, by adding *Bacillus subtilis* (Probisis®) cells, and/or carob powder for improving functional properties and enhancement of the color and flavor. Proximate composition, texture, color, and antioxidant activity of the cereal bars were measured. Water activity was registered below 0.5, which ensures a good microbiological stability of the products. Microbiological analyses showed that bacteria strains were viable in cereal bars after 28 days of storage at room temperature, with 8.2 to $9.5 \cdot 10^8$ CFU/g. Total phenols content and antioxidant activity were higher in carob containing cereal bars. Obtained cereal bars were well appreciated by sensory panelists, *B. subtilis* exerting no effect on sensory attributes. Carob powder addition was appreciated in terms of color and texture, affecting however taste and flavor.

Keywords: *Bacillus subtilis*, carob, antioxidant activity, cereal bars

Introduction

Consumer choices are constantly changing, in search for products that meet their food needs and preferences. In this context, the snack market has grown steadily in recent years, and cereal bars are an increasingly common choice for quick snacks. In terms of cereal bar snacks, consumers are looking for functional products, that provide not only essential nutrients but also different benefits for health, like fiber rich content, ingredients with antioxidant potential, etc. Considering these aspects, cereal bars can be considered a standard, well-accepted and convenient snack, which

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would be an ideal food matrix to provide functional compounds (Sun-Waterhouse *et al.*, 2010).

Cereal bars are products that can be easily reformulated, allowing the inclusion of various ingredients, such as vitamins, minerals, fiber, proteins, and bioactive compounds, that might contribute to nutritional value or might increase acceptability of consumers who associate cereal bars with healthy products (de Melo *et al.*, 2020; Muniz *et al.*, 2020).

The closer a product is to the needs of consumers, the greater the chance of successful acceptance on the market (Rozenfeld and Amaral, 2006). Over time, cereal bars evolved by combining innovation, practicality, and health in a single food (Salazar *et al.*, 2019).

New functional foods are formulated taking into account health benefits and safety, being used to prevent or even treat various biological dysfunctions (Gutkoski *et al.*, 2007).

Probiotics represent one of the largest functional food markets, with most of the products available being represented by dairy derivatives, such as fermented milk products (*e.g*. yoghurt), cheeses, and frozen desserts. Non-dairy probiotic products present a considerable growth potential for the food industry and can be widely explored by developing new ingredients, processes, and products.

Cereal bars enriched with probiotics can positively influence the composition of the intestinal microbiota together with overall health and well-being. However, the use of probiotic microorganisms in food remains limited due to the difficulty of maintaining viability during processing and storage throughout the product's shelf life (Bampi *et al.*, 2016). Different studies investigated the possibility of incorporating probiotics in cereal or fruit based bar snacks. In this respect, Maisto *et al*. (2021) studied the potential of obtaining date fruit bars supplemented with different species of *Lactobacillus* spp. The process involved fermentation in order to reach probiotic concentrations of bacteria. Bampi *et al*. (2016) used microencapsulation of *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *Lactis*, while Henriques (2011) used *Bifidobacterium animalis* Bb12 and *Lactobacillus acidophilus* L10 for obtaining cereal bars with probiotics.

The use of *Bacillus* species as a probiotic has acquired great interest since the sporulated forms have high stability and resistance to the surrounding atmospheric conditions, heating and drying, together with stability in various pH conditions. Thus, many studies showed that they are able to survive while passing through the gastrointestinal tract, and populate the intestine with many viable cells (Cutting, 2011; Lefevre *et al.*, 2011; Lefevre *et al.*, 2017; Villéger *et al.*, 2022). The probiotic potential of *B. subtilis* CU1 (CNCM I-2745) was evaluated by Lefevre *et al.* (2017) in a study on healthy elderly subjects, the strain being well tolerated and having beneficial effects on immune health. According to the study, *B. subtilis* CU1 is safe and can be used as probiotic for human consumption.

As far as our knowledge is concerned there are no studies investigating the possibility of obtaining cereal bars containing *B. subtilis*. Thus, the aim of the present research was to obtain cereal bars with improved functionality, by the addition of carob powder and/or *B. subtilis* CU1 cells. The viability of the *B. subtilis* CU1 cells in the complex food matrix, represented by the cereal bars, was tested upon 28 days of storage at room temperature.

Materials and methods

Formulations of the cereal bars

All ingredients used for cereal bars manufacturing were bought from a local market. Four different cereal bars formulations were prepared and the ingredients used are presented in Table 1.

Table 1. Formulations of functional cereal bars developed in the present study.

Ingredient	S0 (g/100g)	S0B (g/100g)	SC (g/100g)	SCB (g/100g)
Wheat germ	10	10	10	10
Amaranth, expanded	5	5	5	5
Buckwheat, expanded	15	15	15	15
Cranberry	15	15	12	12
Roasted peanuts without salt	12	12	11	11
Carob powder	-	-	4	4
Glycerol	2	2	2	2
Rice syrup	24	24	24	24
Quinoa syrup	9	9	9	9
Shea Butter	1.6	1.6	1.6	1.6
Ascorbic acid	0.4	0.4	0.4	0.4
Erythritol	5	5	5	5
B. subtilis cells	-	1	-	1

S0 – simple cereal bar; S0B – cereal bar with the addition of *B. subtilis* cells; SC – cereal bar with carob powder; SCB – cereal bar with carob powder and *B. subtilis*

The technological process involved mixing so that the qualities and characteristics of the ingredients were not affected. The solid ingredients were mixed with the fat and syrups forming the "dough", representing the basis for further development of functional bars. The addition of carob powder, in samples coded SC and SCB, was considered for providing functional properties (antioxidant potential) and for sensorial benefits. The *B. subtilis* CU1 (Probisis® B) (Lesaffre, France) cells were added to the S0B and SCB samples, at concentration 10^{11} of spores/100 g cereal bars, as indicated in the technical data sheet. The *Bacillus subtilis* cells is patented by Lesaffre, France, and registered in the CNCM collection (Collection Nationale de Cultures de Microorganisms, Institut Pasteur). A control sample (S0) with no carob powder or starter culture addition was considered in the study.

For each formulation the obtained mixture was put into a mold and then rolled. Lamination was performed by applying light mechanical pressure in order to obtain a uniform product sheet. After cooling the product for 24 hours, the product sheet was cut into 9×3×1 cm bars, each weighing 35g. Each bar was wrapped in plastic foil and kept at room temperature for 28 days, for further analysis.

Proximate composition

The moisture content of obtained cereal bars was determined according to the AACC 44-51 method (AACC International, 2000). Total titratable acidity (TTA), pH (WTW InoLab pH7100, Weilheim, Germany), and the ash content were determined according to SR ISO 2171: 2002, 91:2007 methods (ASRO, 2008). Water activity (a_w) was measured using the Fast lab water activity meter (GBX, Loire, France). Energetic value of the obtained cereal bars was calculated taking into account the provisions of European Council (Regulation (EU) No 1169/2011), taking into account proximal composition of raw materials, as specified by the manufacturers.

Antioxidant activity and total phenolic content determination

The cereal bars were minced into homogeneous blends using a kitchen mixing tool. Bioactive compounds extraction was performed using 80% methanol at room temperature for 2 hours, under magnetic agitation. Then the mixture was centrifuged at 3000 rpm for 10 min, and the obtained supernatant was used for further determinations.

Total Phenolic Content

A volume of 0.2 ml extract was added over 1.5 ml of freshly prepared diluted solution (1:10) of Folin Ciocâlteu reagent. After 5 min, 1.5 ml of Na_2CO_3 solution ($60g \cdot L^{-1}$) was added to the mixture. The absorbance was read at 725 nm, after 90 minutes of maintaining at room temperature (Li *et al.*, 2008). The total content of phenols was expressed in mg of Gallic acid (GA)/100g.

DPPH radical scavenging activity (DPPH-RSA)

A volume of 100 μL extract and 3.9 mL DPPH (2,2-Diphenyl-1-picrylhydrazyl) solution were mixed for the analysis. Similarly, a blank test was obtained, replacing the sample with 80 % methanol solution. The absorbance was read at 515 nm after 30 min of rest in dark conditions. The scavenger capacity of antioxidant substances was expressed as IC50, which represents the concentration of the active compound (mg sample) capable of inactivating 50 % of the total molecular DPPH (López-Amorós *et al.*, 2006; Olugbami *et al.*, 2015).

Trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacity (TEAC) was determined using $ABTS^{\bullet+}$ discoloring assay following the method proposed by Arnao *et al.* (2001) with some modifications. A quantity of 0.15 ml of extract was allowed to react with 2.85 ml ABTS solution for 2 hours in the dark. The absorbance was read at 734 nm using a T80+ spectrophotometer (PG Instruments Ltd) against a blank solution consisting of 80% methanol. Results were expressed in mg Trolox equivalent (TE)/100 g.

Texture

The texture of the cereal bars was analyzed using a CT3-1000 Brookfield texture analyzer, by applying a force of 1.96 N according to the method of Samakradhamrongthai *et al.* (2021). All measurements for texture analysis were made in triplicate, after 24 h of bars obtaining.

Color

The color of the obtained cereal bars was measured with a colorimeter (Chroma Meter CR-301, Minolta Co., Osaka, Japan), equipped with D-65 illuminant and standardized with sets of CR-A47 calibration plates and a white plate. CIELAB color space parameters L*, a*, b* were measured and used to calculate the chroma (C) and hue angle (H°).

The chroma value (C), indicating the intensity or saturation of the color, was calculated according to equation 1 (Duta and Culetu, 2015):

$$C = \sqrt{a^{*2} + b^{*2}} \qquad (1)$$

The hue angle (H°), a parameter that proved to be effective in predicting the visual appearance of the color, was calculated according to equation 2 (Ağagündüz *et al.*, 2021):

$$H^{o} = tan^{-1}\left(\frac{b^{*}}{a^{*}}\right) \qquad (2)$$

Microbiological analysis

The cereal bars were evaluated for microbiological quality after 28 days of storage at room temperature through yeasts and molds (ISO 21527-2, 2008), and Enterobacteriaceae (ISO 21528-2, 2017). The spore-forming bacteria viability in the cereal bars was also assessed according to Ciurescu *et al.* (2020) with modifications. Cereal bar samples were thoroughly minced in sterile conditions and the vegetative cell was thermally inactivated (80°C, 10 min) to assess the viability of spore-forming. Serial dilutions were obtained, then spread on Luria-Bertani (LB) agar (Merck, Darmstadt, Germany) and incubated at 35±2°C for 48 h. The results were expressed as CFU/g.

Sensory evaluation

Sensory analysis was performed by a group of trained tasters (n = 12) to assess the degree of acceptability, using a hedonic method of assessment by scoring from 1 = "I do not like it at all" to 9 - "I like it very much". A value of six ("I like") on the 9-point hedonic scale was considered the minimum level of product acceptability (Aigster *et al.*, 2011).

A second sensory test (n = 12) was performed based on the observations made by the panelists, and the assessment was made using the 5-point test (Bulancea and

Iordachescu, 2006). For a correct classification in a certain quality class, ranges of variation of the actual score were used for each product (5-4.5 = "Very good", 4.5-3.5 = "Good", 3.5-2.5 = "Satisfactory", 2.5-1.5 = "Unsatisfactory", 1.5-0.5 = "Bad", < 0. 5= "Very bad").

Statistical analysis

For statistical analysis, the Minitab ver. 17 program was used. The ANOVA unifactorial test with a 95% confidence level and Tukey post-hoc analysis were applied to determine the statistical differences between samples. The experiments were carried out at least in triplicate. The values were expressed as average ± standard deviation (SD).

Results and discussion

Nutritional value of cereal bars

The nutritional value of food is given by the composition in nutrients (proteins, carbohydrates, lipids, vitamins, and mineral salts), the ratio between these components, their quality, the extent to which they are digested and used and by how the product meets the needs of the body (Segal, 2010).

The ingredients used to obtain the cereals bars were chosen such as to allow classification as *Clean Label* products, attesting the high quality of the finished product.

Information regarding nutritional quality of the cereal bars are presented in table 2.

Table 2. Information regarding the nutritional value of obtained cereal bars.

Nutritional composition	S0 and S0B	SC and SCB
Energy, kJ/100 g	1511	1509
kcal/100g	361.1	359.3
Protein, g/100g	8.9	8.8
Carbohydrate, g/100g	56.7	57.3
- sugars, g/100g	23.1	22.0
- polyols, g/100g	2	2
Fiber, g/100g	3.3	3.6
Fat, g/100g	9.7	9.2
-saturated, g/100g	2.1	2.0
Salt, g/100g	0.07	0.07

The energy value of the cereal bars with no carob powder addition (S0 and S0B) was 361.1 kcal. On the other hand, since the carob powder was used to substitute part of the cranberries in the cereal bar formulation, the samples SC and SCB exhibited slightly lower energy value of 359.3 kcal (Table 2). Comparable results in terms of energy value were recorded by Muniz *et al.* (2020), which achieved values between 367 kcal/100 g for the bars obtained with honey, coconut oil, oatmeal, and raisins, and values between 299 and 321 kcal for the bars obtained with honey, coconut oil, oatmeal, fermented cashew paste, fermented guava peels, and raisins. The fiber content of the cereal bars investigated in the present study depended on the presence of the carob powder, being 3.3 g/100 g in case of S0 and S0B and 3.6 g/100 g in case of SC and SCB samples. In the study conducted by Sampaio *et al.* (2010) on food bars containing rice flakes, oatmeal, corn flakes, dehydrated apples, chocolate, corn syrup, and brown sugar, the total fiber content of 6.01% was obtained. Taking into account the fiber content, the cereal bars developed in the present study can be labeled as "source of fiber", in agreement with EC Regulation 1924/2006. The carbohydrate content of the samples ranged from 56.7 to 57.3 g/100g. Similar high carbohydrate contents were reported in the literature for the cereal bars obtained with cereals and fruits; nut cream cereal bars (*Lecythis prisons* Camb.) (63.9%), cereal bars with Sterculia seeds (*Sterculia striata*) (70.7%), tonka berry cereal bars (*Dipteryx lacunifera* Ducke) (69.3%) (Carvalho, 2008), and gluten-free cereal bars with pseudo-cereal varieties (68.33–71.57%) (Souza *et al.*, 2014). There are many studies about cereal bars containing expanded rice, cereals, and fruits with a high carbohydrate content (Freitas and Moretti, 2006). In addition, incorporating honey and sugar syrup into cereal bars as binding agents contribute to the high carbohydrate content (Agbaje *et al.*, 2016).

Physical chemical characteristics of the cereal bars

The physical chemical properties of the cereal bars obtained in the present study are presented in Table 3.

It can be noted that the cereal bars water content ranged between 9.59 and 10.12%, ensuring good products stability and long shelf life. Water activity (a_w) was found to be between 0.42 and 0.47. All samples had water activity values below 0.50, which ensures microbiological stability throughout a storage period. As a rule, cereal-based bars water activity can vary from 0.1 to 0.5 (Aramouni and Abu-Ghoush, 2011). Bars with a low water activity value will have a crispy texture. Water activity profoundly influence the speed of many chemical reactions in food and the microbial growth rate. Molds and yeasts will grow at water activities of 0.7 - 0.8, while bacterial growth is recorded when water activity reaches 0.8. Therefore, it was essential to control the activity of the water in the obtained bars to avoid these types of growth.

Table 3. Physical chemical properties of the obtained cereal bars.

Characteristics	S0	S0B	SC	SCB
Water, %	9.89±0.04^{B}	10.07±0.03^{A}	9.59±0.02^{C}	10.12±0.03^{A}
Water activity (a_w)	0.47±0.01^{A}	0.44±0.01^{AB}	0.42±0.01^{B}	0.46±0.01^{AB}
Acidity, °A	5.66±0.04^{B}	5.87±0.03^{B}	7.08±0.03^{A}	7.24±0.02^{A}
pH	5.50±0.01^{A}	5.53±0.01^{A}	5.54±0.01^{A}	5.56±0.01^{A}
Ash, %	1.12±0.09^{A}	1.17±0.03^{A}	1.27±0.04^{A}	1.29±0.03^{A}

The results are expressed as average± SD, n=3. The different letters in a row denote statistical differences at $p < 0.05$

In terms of samples acidity, it can be observed that bars containing carob powder presented significant higher values ($p<0.05$). Carob powder content lead to the increase of samples acidity, with no effect on pH value ($p<0.05$).

Antioxidant activity and total phenolic content

The total phenolics content of the cereal bar samples ranged from 10.49 mg GA/100g to 12.69 mg GA/100g (Table 4). As expected, the cereal bars with carob (SC and SCB) presented a higher content of total phenols ($p<0.05$). Carob was reported to be rich in phenolic compounds like gallic acid, gallotannins, cinnamic acid, myricetin, and flavonoids (Gioxari *et al.*, 2022). Youssef *et al.* (2013) reported 11 phenolic compounds in the carob powder, among which pyrogallol, catechol, chlorogenic and protocatechuic acids are the most abundant.

The IC_{50} value is defined as the concentration of antioxidants that determines a decrease of 50% of the DPPH absorbance (Chen *et al.*, 2013). Thus, with the IC_{50} value being higher, the antioxidant activity is lower. Carob powder containing samples showed a higher amount of total phenolic compounds and lower IC_{50} values, while the presence of *B. subtilis* had no effect on antioxidant activity of obtained bars ($p>0.05$). As regards TEAC, lower values were recorded for samples containing carob ($p<0.05$). The same trends were observed by Maisto *et al.* (2021), recording values up to 77.56 mg GA/date bars of 10 g and 150.13 mg TE/date bars of 10g. In our study, S0 and S0B samples with no carob powder but having a higher amount of cranberries (15 g/100g *vs*. 12g/100g), exhibited higher TEAC values. The different active compounds found in cranberries with regard to carob, might explain the significant differences obtained.

Table 4. Antioxidant activity and total phenolic content of the obtained cereal bars.

Characteristics	S0	S0B	SC	SCB
Total Phenols (mgGA/100g)	10.53 ± 0.14^{B}	10.49 ± 0.11^{B}	12.62 ± 0.14^{A}	12.69 ± 0.17^{A}
DPPH-RSA, IC_{50} (mg)	3.15 ± 0.05^{A}	3.16 ± 0.08^{A}	2.46 ± 0.05^{B}	2.48 ± 0.02^{B}
TEAC, (mgTE/100g)	33.9 ± 0.29^{A}	33.80 ± 0.81^{A}	22.30 ± 0.20^{B}	22.71 ± 0.92^{B}

Results are expressed as average± SD, n=3. Different letters in a row denote statistical differences at $p<0.05$.

Textural characterization of cereal bars

The texture of the cereal bars is a decisive factor in the acceptance of these products, influencing the overall sensory appreciation (Wilkinson *et al.*, 2000). The firmness of the cereal bars can increase during storage, influencing the consumers` acceptability. To prevent this change in texture, it is recommended to add to the recipe between 1-4% glycerol, which is a good humectant preventing the strengthening of the bars (Liu *et al.*, 2009; Pallavi *et al.*, 2015).

The firmness of the obtained cereal bars varied between 22.93 N and 34.06 N (Table 5); no statistical differences within groups of samples with and without carob powder addition ($p>0.05$) was observed. Melati *et al.* (2020) obtained comparable values for some cereal bars based on rice flour and soybean extract (11.42 N - 28.53 N), while Sun-Waterhouse *et al.* (2010) obtained higher values ranging between 44.05 N and 64.40 N for the cereal bars with a high content of dietary fiber.

Table 5. Textural characteristics of the obtained cereal bars.

Characteristics	**S0**	**S0B**	**SC**	**SCB**
Firmness, N	29.86±1.69^{A}	22.93±1.56^{B}	32.25±1.89^{A}	34.06±1.40^{A}
Specific volume, $cm^3/100g$	273±0.72^{B}	279±0.69^{A}	250±0.74^{C}	251±0.48^{C}

Results are expressed as average± SD, n=3. Different letters in a row denote statistical differences at p<0.05.

The volume of the product can be a key factor in the consumers` decision when purchasing the product. As can be seen from table 5, cereal bars containing carob powder presented a smaller volume in comparison to S0 and S0B (p<0.05). This difference can be explained by the difference in the recipe. As can be seen from table 1, carob powder addition was done by substitution, reducing cranberries and roasted peanuts quantities, which contributed to the volume decrease of the sample.

Color

The values of the color parameters L* (brightness), a* (red), b* (yellow), chromatic value (C), and hue angle (H^{o}) for the obtained cereal bars are shown in Table 6. All color values showed statistically significant differences ($p < 0.05$) between samples with and without carob powder. Adding carob powder to the cereal bars has led to color changes.

Table 6. Color parameters of the obtained cereal bars.

Sample	**L***	**a***	**b***	**C**	**H°**
S0	48.75±0.03^{A}	5.66±0.04^{B}	8.61±0.01^{A}	10.85±0.04^{A}	52.55±0.16^{A}
S0B	48.65±0.03^{A}	5.65±0.02^{B}	8.47±0.06^{A}	10.65±0.08^{A}	52.61±0.03^{A}
SC	43.87±0.05^{B}	6.60±0.05^{A}	5.80±0.04^{B}	8.10±0.05^{B}	45.70±0.00^{B}
SCB	43.78±0.04^{B}	6.47±0.06^{A}	5.74±0.03^{B}	8.05±0.04^{B}	45.48±0.03^{B}

Results are expressed as average± SD, n=3. The different letters between columns denote the statistical difference at p<0.05.

The L* value ranged between 43.78±0.04 (SCB) and 48.65±0.03 (S0B). The brightness of the samples decreased considerably with the addition of carob powder, while the yellowness value decreased (p<0.05). Carob powder addition leads to a significant increase in redness values and decrease in yellowness (p<0.05). Chroma (C) and hue angle (H^{o}) were also influenced by carob powder addition (p<0.05). The higher the C value, the more pure or intense the color is. The values recorded for C suggest expressive features in terms of purity and color (Melati *et al.*, 2020). The H^{o} values, that display the color tone, ranged from 45.48 to 52.61 being significantly decreased (p<0.05) by carob powder addition, as can be seen from table 6. The H^{o} value is associated with the saturation index and results in a vector that directs product color determination and intensity (Melati *et al.*, 2020).

Microbiological analysis

Based on the a_w results presented in Table 3, it was expected that the packed cereal bars would show microbiological stability because the low moisture content doesn`t allow the growth of bacteria, yeasts, and molds. The microbial load of the cereal bares was determined after 28 days of storage. The microorganisms analyzed were aerobic plate count, yeasts, molds, and Enterobacteriaceae (Table 7). The results indicated that the samples subjected to microbiological analysis comply with the limitations imposed by the Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, section *Dehydrated infant formulas and dietary foods*. The chosen section of this regulation was used because it is the closest to the characteristics of the new product obtained and presents the most restrictive limitations. No significant differences were recorded in terms of CFU/g between the two samples containing *B. subtilis* cells regarding the microbial load, as expected.

Table 7. Microbiological analyses of the obtained cereal bars after 28 days of storage at room temperature.

Method/Sample	**Cereal bars**			
	S0	S0B	SC	SCB
Yeasts and molds (CFU/g)	< LOQ	< LOQ	< LOQ	< LOQ
Enterobacteria (CFU/g)	< LOQ	< LOQ	< LOQ	< LOQ
Bacillus subtilis CU1 CFU/g	-	$9.5 \cdot 10^8$	-	$8.2 \cdot 10^8$

LOQ-limit of quantification (<10 CFU/g)

The viability of *Bacillus subtilis* cells was evaluated after 28 days of storage at room temperature, and it was found to be $3.3 \cdot 10^{10}$/bar and $2.8 \cdot 10^{10}$/bar in case of S0B and SCB, respectively. As indicated by the producer of *B. subtilis* CU1 (Probisis® B) (Lesaffre, France), the recommended daily dose to be efficient is $2 \cdot 10^9$ spores/day. The probiotic dosage has remained above the amount recommended by the manufacturer to benefit from their probiotic properties. The stability of the shelf life of this probiotic cells was demonstrated in other complex cereal-based food matrices, such as bakery products. For example, the *Bacillus subtilis* Rosell-179, marketed by the Canadian company Lallemand, is successfully used in various bakery products (bread, buns, etc.), added to the dough, or sprayed after cooling. Studies for the *Bacillus subtilis* Rosell-179 showed excellent survival results, opening the door to the idea of healthy bakery products with probiotics, prebiotics, fiber, vitamins, and minerals (Lallemand, 2022). In another study conducted by Permpoonpattana *et al.* (2012), it was found that *Bacillus subtilis* HU58 and PXN21 strains introduced into the composition of whole meal flour biscuits survived baking at 235°C for 8 minutes, with only a one log reduction in viability. The cells of *Bacillus subtilis* used in the present study was chosen due to its ability to survive under restrictive conditions and return to its active state in the human digestive tract, which will exert its beneficial effects throughout the gastrointestinal system. It is a temperature-stable bacterium able to survive at higher temperatures, supplying health benefits if added to food or beverages. A small dose of *B. subtilis* is well tolerated and effective for consumers, increases immunity, and reduces the risk of developing colds, flu, etc. Urdaci *et al.* (2018) indicated that the prophylactic treatments showed strong antidiarrheal activity; *B. subtilis* CU1 was active against distinct experimental diarrhea and could increase the colon's ability to absorb water under diarrheal conditions. Moreover, *B. subtilis* CU1 may decrease intestinal hypersecretion.

Sensory analysis

The sensory analysis was performed based on the observations made by twelve trained panelists. Each taster received an analysis bulletin and a document with the sensory attributes. The results obtained for the hedonic test are presented in figure 1.

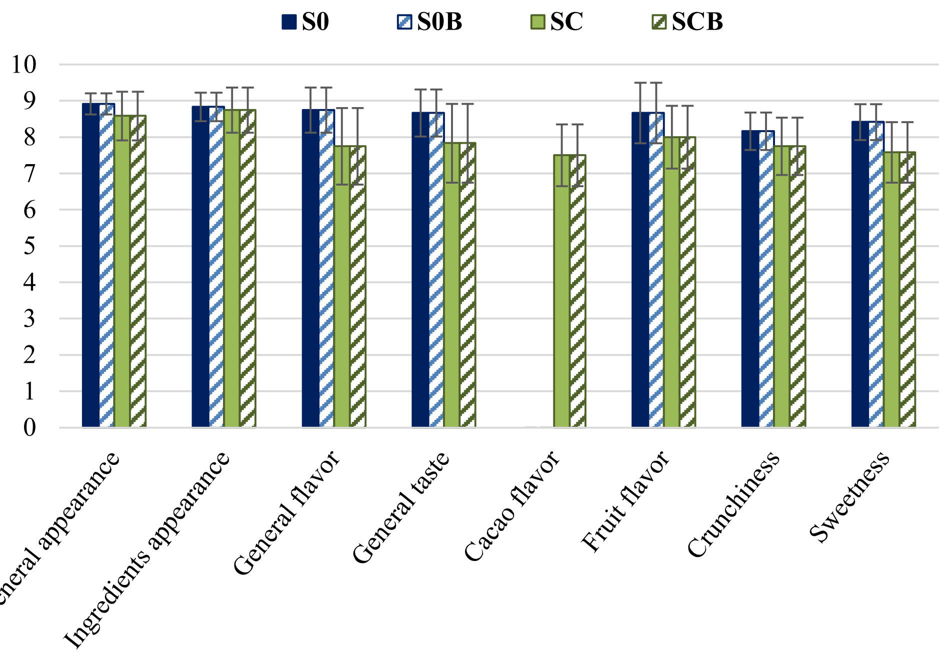

Figure 1. Hedonic appreciation of the obtained cereal bars

The results obtained from the tasting to assess the degree of acceptability by using a hedonic (preferential) method of appreciation by scoring (1 = "I do not like it at all"; 9 - "I like it extremely much") highlighted the fact that the samples were appreciated very well. The scores obtained on all types of bars were above the value of 6 ("I like it"), imposed as the minor limit of acceptability. No changes in the sensory characteristics were noticed in the bars containing *B. subtilis* *(p>0.05)*. The samples without carob powder in the composition were appreciated with significantly higher scores than those with carob powder for most analyzed attributes, with exception of general appearance and ingredients visual appearance where no significant differences were reported (p>0.05). The twelve panelists appreciated better the simple cereal bars (S0 and S0B); the obtained average being situated between 8 and 9 points.

Sensorial appreciation of the obtained cereal bars enhanced with *B. subtilis* with the 5-point scale test is presented in figure 2.

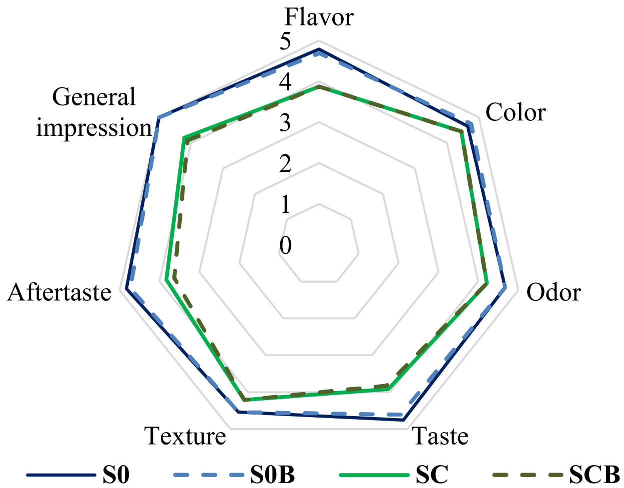

Figure 2. Sensorial appreciation of the obtained cereal bars with the 5-point scale test.

As with the hedonic method of appreciation by scoring from 1 to 9 points, cereal bars with no carob powder in composition were better appreciated, with the overall impression receiving the highest point level value, significantly different ($p<0.05$) in comparison to samples containing carob powder (4.15). Better appreciated, with no significant differences lead by carob addition were color, odor and texture attributes ($p>0.05$).

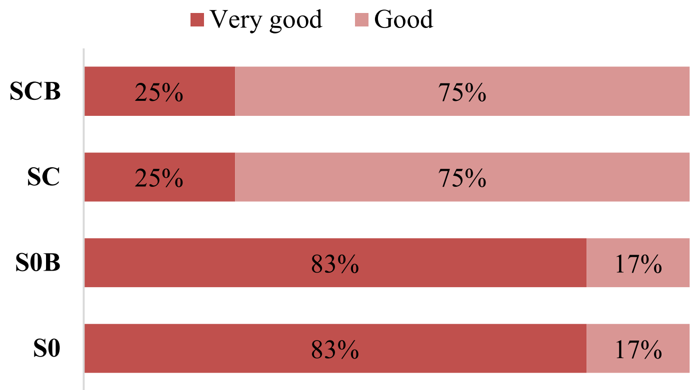

Figure 3. Percentage appreciation of the obtained cereal bars.

At the end of the tasting, panelists were asked to indicate the type of bar they better appreciated. As can be seen from figure 3, with no influence given by *B. subtilis* addition, the majority selected the bars without carob.

Conclusions

In the present study, various type of cereal bars enhanced with *B. subtilis* and/or carob powder were prepared, having adequate moisture content, similar to the products available on the market. Water activity values were below 0.50 for all samples, thus ensuring microbiological stability throughout the shelf life. The *B. subtilis* CU1 cells was successfully incorporated into the formula of the cereal bars, showing viability after 28 days of storage, and having the ability to survive under complex conditions (complex food matrix, low water content). The study found that cereal bars containing *B. subtilis*, presented well-appreciated textural and sensory qualities. The addition of *B. subtilis* did not influence the obtained bars physical chemical, and textural characteristics or antioxidant activity.

Based on the present research, a prototype of a functional product with probiotics can be developed. In order to take advantage of the health benefits attributed to *B. subtilis,* the cells was incorporated into the cereal bars formulation such as to ensure the recommended daily dose of probiotic when consuming a cereal bar daily.

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