Brazilian Journal of
Food Technology

Campinas, v. 16, n. 1, p. 42-51, jan./mar. 2013
http://dx.doi.org/10.1590/S1981-67232013005000001

Development of cassava doughnuts enriched with *Spirulina platensis* biomass

Desenvolvimento de 'sonho de mandioca' enriquecido com biomassa de Spirulina platensis

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Recebido | Received: 02/09/2011
Aprovado | Approved: 16/10/2012
Publicado | Published: mar./2013

Summary

The cyanobacteria *Spirulina platensis* has been cultivated in a fed batch process with urea as the nitrogen source, in order to obtain dehydrated biomass for incorporation into food, aiming at nutritional enrichment and the production of a functional character, due to the amount of proteins, vitamins and several bioactive compounds found in this cyanobacterium. In this study, response surface methodology was used to analyze the substitution of wheat flour by cassava in the development of doughnuts with added *Spirulina platensis* biomass and inverted sugar, in order to increase the rate of the *Maillard's* reaction and mask the green colour of the biomass. The formulations were evaluated in relation to their proximate, sensory and technological compositions, which, when compared to the standard formulation, without the addition of *S. platensis* biomass and inverted sugar, showed the feasibility of adding the biomass to bestow nutritional enrichment without significantly affecting the sensory acceptance of the product or its typical characteristics.

Key words: *Gluten free; Maillard's reaction; Inverted sugar; Nutritional enrichment.*

Resumo

Cultivos da cianobactéria *Spirulina platensis* vêm sendo conduzidos utilizando-se ureia como fonte de nitrogênio, em processo descontínuo alimentado, para obtenção de biomassa desidratada para ser incorporada em alimentos visando enriquecimento nutricional e conferir caráter funcional em virtude da composição rica em proteínas, vitaminas e diversos componentes bioativos. Neste trabalho, foi estudada a substituição da farinha de trigo pela mandioca, além da adição de biomassa de *Spirulina platensis* e açúcar invertido, para desenvolver um 'sonho' com elevada taxa da Reação de Maillard para mascarar a coloração verde da biomassa, por meio da metodologia de superfície de resposta. As formulações foram avaliadas em relação aos aspectos de composição centesimal, sensoriais e tecnológicos que, quando comparados com o padrão, sem adição de biomassa de *S. platensis* e açúcar invertido, demonstraram a viabilidade da adição da biomassa para conferir enriquecimento nutricional sem afetar, de forma significativa, a aceitação sensorial e as características típicas do produto obtido.

Palavras-chave: *Sem glúten; Reação de Maillard; Açúcar invertido; Enriquecimento nutricional.*

1 Introduction

Spirulina platensis is a photosynthetic cyanobacteria, besides being one of the richest sources of protein – up to 74% of its dry mass with 47% essential amino acids, it also contains methionine, which is an amino acid absent in most cyanobacteria and algae. These contents are in accordance with those recommended by FAO/WHO, apart from the sulphur amino acids and lysine. *S. platensis* has a mucopolysaccharide cell wall, which confers high digestibility with a low nucleic acid content when compared to other microbial biomasses. This cyanobacterium also has reasonable contents of chlorophyll, carotenoids, phycocyanin, vitamins, minerals and essential fatty acids, amongst other bioactive compounds important to the development of functional foods (SPOLAORE et al., 2006; COHEN, 1997; IYER et al., 2008).

The use of *S. platensis* as food has been reported since the time of the Spanish colonization in Mexico, also being cited in expeditions to African deserts in the 1960's. Many food and food supplies based on *Spirulina* sp. *can be* found on the market, classified by the FDA (Food and Drugs Administration) as GRAS (Generally Recognized As Safe), a status that guarantees its safety for consumption as a source of several important substances without toxicological effects (MORAIS et al., 2006; McKASLE, 2006; CHAMORRO-CEVALLOS et al., 2008; SALAZAR et al., 1998).

Problems such as malnutrition and underfeeding exist in some Brazilian regions. Aiming to overcome these problems, the hypothesis of using cassava in bakery goods has been raised, allowing for the substitution of an imported raw material and creating jobs and income, resulting in social and nutritional improvements (KATO, 1988). The possibility of incorporating *S. platensis* biomass would confer increased nutritional value, particularly with respect to protein.

The use of cassava in bakery products can be justified by several reasons. First, cassava is one of the most important plants cultivated in Brazil; second, it is cheaper than wheat and third, it has no gluten and can therefore be consumed by celiac patients (CEREDA and VILPOUX, 2003; SDEPANIAN et al., 2001; DIETERICH et al., 1997). Celiac disease is caused by a permanent intolerance to gliadin that unleashes an inflammatory reaction, resulting in bad or no nutrient absorption.

"Sonho" is a typical Brazilian product, similar to a doughnut. It is a kind of bread according to its official definition and to the technology used to make it. It is usually produced and commercialized in bakeries (MASSON et al., 1999; GALERA, 2006).

The addition of *Spirulina* to cassava doughnut dough could provide functional characteristics and nutritional enrichment. Furthermore, the use of hydrolyzed sugar could mask some of the probable negative effects on its sensory aspects, such as the colour, providing better acceptance (HASLER, 1998).

Thus, the aim of this research was to use response surface methodology to study the substitution of wheat flour by cassava in the development of doughnuts with added *Spirulina platensis* biomass and inverted sugar, in order to increase the rate of the Maillard's reaction and mask the green colour of the biomass.

2 Material and methods

The microorganism was cultivated in modified Paoletti et al. (1975) culture medium, substituting the KNO_3 by urea. Paoletti et al. (1975) medium contains the following nutrients ($g.L^{-1}$): $NaHCO_3$, 15.15; Na_2CO_3, 8.89; NaCl, 0.92; Na_2SO_4, 1.88; KNO_3, 2.57; K_2HPO_4, 0.50; $MgSO_4.7H_2O$, 0.25; $CaCl_2.2H_2O$, 0.05; Fe-EDTA complex ($mg.L^{-1}$): EDTA-Na_2, 29.8; $FeSO_4.7H_2O$, 24.9; microelement solution ($mg.L^{-1}$): H_3BO_4, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $Na_2MoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$,0.079; $Co(NO_3)_2.H_2O$, 0.049. The starting pH of the culture medium was 9.5 ± 0.2 and it was allowed to vary freely during each test. Fluorescent lamps were used as the source of light and were fixed at a certain distance from the cultivation tanks, so that they could provide a light intensity 42 μmol. photons.$m^{-2}.s^{-1}$. Open ponds containing paddle wheels at 18 rpm were used for culture homogenization and heaters were used to maintain the temperature at 30 °C. Urea was added daily by a fed batch process for 14 days using an exponential feeding rate protocol (DANESI et al., 2002). Distilled water was added to the systems throughout cultivation, so that the volume was kept constant at 5L. The biomass obtained was removed after 14 days of cultivation and centrifuged (1685 xg) (Metrotern, MTD III Plus) at room temperature. The sediments were dried at 55 °C for 24 hours, milled in a pounder and stored under refrigeration until used.

To make the doughnuts, the formulation components were weighed. The cassava was cooked and mashed. The mass obtained was weighed and mixed with the following ingredients: water, sucrose, whole egg powder, salt, instant dry baking powder, inverted sugar and *S. platensis*.

Response surface methodology was used to verify the effects of the concentration of *S. platensis* added (2.59 to 5.41% w/w) and the partial substitution of sucrose by inverted sugar (4.59 to 17.05% inverted sugar), considering its sweetness level, on the making of the doughnuts, considering the physicochemical and technological properties conferred by these additions. An experimental design based on the methodology called "star planning", proposed by Barros Neto et al. (2001), was used, consisting of a 2^2 factorial design with axial points ($\alpha = (2^n)^{1/4}$) and central points, aiming to evaluate the influence of adding the inverted sugar and *S. platensis* on the composition and technological properties of the doughnuts (Table 1). The central point was repeated three times to check the reproducibility of the results. The levels of the independent variables used in this research are represented as the percentage of *S. platensis* added and the percentage substitution of sucrose by inverted sugar (ESTELLER et al., 2004) (Table 1).

Regression analyses were carried out using the S-PLUS 2000 program. Significance levels of $(p) \leq 0.05$ or $(p) \leq 0.10$ were considered in both regression analyses and in their analyses of variance (ANOVA). Equation 1 was used to evaluate the effect of the independent variables on the dependent variables.

$$\hat{y} = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_1^2 + \sum_{ij=1}^{4} b_{ij} X_i X_j \quad (1)$$

b_o, b_I, b_{II}, b_{ij} are the regression coefficients in the model and X_I and X_j are the independent variables, in coded values.

After kneading the dough until homogeneous, the doughnuts were moulded into little 10 g balls, placed on baking trays and allowed to ferment in an oven at 35 °C for 30 minutes. The doughnuts were baked in a pre-heated oven at 130 °C for 15 minutes, allowed to cool at room temperature and stored in plastic pots for subsequent technological analyses. They were then mashed (Marconi mill, model CFW08) and conserved in a freezer until the physicochemical analyses. The determinations were carried out according to conventional methods in triplicate (HORWITZ, 1997). The carbohydrates were calculated by difference and the caloric values were calculated from the sum of the protein content × 4, lipids × 9 and carbohydrates × 4.

The samples were evaluated by means of the physical analyses of weight, raw and baked volumes and texture. Ten doughnuts were made with each formulation, and of these, three were chosen at random for weighing. The volume was determined by first measuring the volume of a certain amount of colza seeds in a cylinder, removing the seeds, placing the product to be analyzed in the cylinder, re-adding the same amount of seeds and determining the volume of the product from the increase in volume (GALERA, 2006). The specific volumes were calculated from the ratio "volume/weight" ($mL.g^{-1}$) (HOSENEY, 1990).

The expansion coefficient or expansion rate was calculated according to Equation 2:

$$Ce = \frac{V_2 - V_1}{V_1} \times 100\% \quad (2)$$

where: Ce: expansion rate; V_2: raw dough volume; V_1: baked dough volume.

The maximum shearing force to cut the cassava doughnuts was determined using a TA-XTIIi texturometer (Stable MicroSystems, Surrey, England) applying the force perpendicular to the sample contact area, with compression of up to 80% deformation considering the initial sample height. The Warner Bratzler rectangular steel blade probe (HDP/BSW, Stable MicroSystems, Surrey, England) was used. The parameters used to determine the compression force were: pre-test speed, 3 $mm.s^{-1}$; test speed, 1 $mm.s^{-1}$; post-test speed, 10 $mm.s^{-1}$. The trigger force limit used was 0.1 N (to allow the sample to accommodate itself under the blade at the beginning of the test). The rate of data acquisition was 200 pps (points per second) (ROSENTHAL, 1999).

The doughnut formulations with the highest and lowest percentages of added *S. platensis* were filled with guava jam and submitted to a sensory evaluation (standard and formulations 5 and 6). The degree of acceptance was estimated with respect to the attributes of appearance, smell, flavour, texture, overall impression and purchase intention, applying the acceptance test with 34 doughnut consumers, including UEM students and employees – Campus Umuarama. Most were female (68%), aged between 18 and 35 years old (70%), had concluded high school (17%) and had a family income ranging between 5 and 10 minimal wages (56%).

The panellists were first asked "how much they liked or disliked" the doughnuts, using a 9-point structured hedonic scale (1 = disliked extremely; 9 = liked extremely). For the purchase intention analysis, a 5-point structured scale (1 = I would never buy it; 5 = I would certainly buy

Table 1. Treatments of the central composite rotational design.

Treatments	Independent variables			
	Coded		Real values (% w/w)	
	Inverted sugar (X_1)	*S. platensis* (X_2)	Inverted sugar*	*S. platensis*
1	−1	−1	5	3
2	+1	−1	15	3
3	−1	+1	5	5
4	+1	+1	15	5
5	0	−1.414	10	2.59
6	0	+1.414	10	5.41
7	−1.414	0	4.59	4
8	+1.414	0	17.05	4
9	0	0	10	4
10	0	0	10	4
11	0	0	10	4

*Percent substitution of sucrose by inverted sugar, respecting the sweetness power of sucrose, so as not to alter the formulation/treatment sweetness.

it) was used. The three doughnut samples were evaluated in the Laboratory of Sensory Analyses, in individual booths under white light, in a single sensory session. Sample presentation was monadic and the consumers were asked to clean their palate with water after each test (MEILGAARD et al., 1991). The results were submitted to an analysis of variance (ANOVA) and to a means comparison test (Tukey's test).

The project was approved by the Ethics Research Committee of the State University of Maringá (CAAE, n. 0477.0.093.000-10) and was carried out in the same Institution.

3 Results and discussion

The *S. platensis* was cultivated under conditions defined in previous works (DANESI et al., 2002, 2004, 2011; RANGEL-YAGUI et al., 2004) in order to avoid ammonia toxicity, and showed typical cellular growth. The biomass obtained presented a proximate composition comparable to reports in the literature (SHIMAMATSU, 2004; MORAIS et al., 2006; SPOLAORE et al., 2006). The addition of *S. platensis* to the doughnuts promoted an increase in protein content. Formulation 6, with an addition of 10% w/w of *S. platensis*, showed an increase of 66.7% in protein content in relation to the standard formulation. Table 2 shows the results obtained for the proximate composition.

The moisture content varied between 30.74 and 39.90% and the standard sample showed a value of 35.18%, i.e., almost the mean of the formulations tested, although there were no significant differences amongst the samples. Esteller and Lannes (2005) analyzed different types of bread (sandwich loaf, hot dog rolls, hamburger rolls, French bread, Italian bread, Ciabatta, cheese rolls – a baked cassava starch and cheese delicacy) and toasts in relation to the parameters of dough porosity, texture, colour, specific volume, density and moisture content. The moisture contents of the products varied between 4.88% for the toast and 33.28% for the hamburger and hot dog rolls. Galera (2006) evaluated the partial substitution of wheat flour by rice flour to produce doughnuts. The moisture content found in the different formulations varied from 22.7 to 30.6%. Thus the moisture content found in the samples was coherent with values previously found in other bakery products. The slightly higher moisture content found, as compared to the other products, was due to the presence of cassava in the formulation. Cassava has a high water holding capacity due to the presence of starch and fibre, in addition to the presence of *S. platensis*, which may also contribute to the increase in water holding capacity of the dough.

The moisture content of doughnuts is directly related to the smoothness of the final product. However, during storage undesirable effects may occur, such as syneresis and water migration to the product surface, causing sensory alterations and leading to dissolution of the sugar sprinkled on the doughnut surface, as well as alterations in texture leading to a loss of smoothness (GALERA, 2006).

These alterations are similar to bread staling and are not related to a loss in moisture content of the dough. A piece of bread analyzed after five days (stored under "proper conditions") would have the same moisture content as a "fresh" piece of bread, though the sensation to the palate is drier. Bread hardening may be linked to starch retrogradation and to the behaviour of some proteins and pentosans (BATTOCHIO et al., 2006).

The protein content of the doughnuts varied from 7.26 in the standard formulation to 12.19% in the formulation with the greatest addition of *S. platensis*. In general, an increase in the *S. platensis* content in the

Table 2. Proximate composition (% wet basis) of the standard cassava doughnuts and of those produced with the addition of *S. platensis* biomass and inverted sugar.

Formulation	Mean (%) ± Standard deviation					Carbohydrate	Calories
	Moisture	Ash	Protein	Lipid	Fibre	(%)	(Kcal)
Standard	35.81 ± 0.12	1.20 ± 0.15	7.34 ± 0.30	5.82 ± 0.68	1.98 ± 0.00	47.85	273.14
1	31.84 ± 0.93	2.31 ± 0.12	8.95 ± 0.21	11.51 ± 0.41	3.01 ± 0.06	42.38	308.91
2	27.37 ± 0.69	2.73 ± 0.13	8.41 ± 0.65	11.03 ± 0.71	2.75 ± 0.22	47.71	323.75
3	38.68 ± 0.48	2.38 ± 0.01	10.58 ± 0.64	12.40 ± 0.44	4.91 ± 0.23	31.05	278.12
4	32.41 ± 0.37	2.49 ± 0.05	10.03 ± 0.29	11.85 ± 0.27	5.00 ± 0.15	38.22	299.65
5	39.90 ± 0.73	0.60 ± 0.09	7.26 ± 0.48	7.07 ± 0.11	1.83 ± 0.02	43.34	266.03
6	31.60 ± 0.40	0.80 ± 0.05	12.19 ± 0.29	12.11 ± 0.08	5.09 ± 0.21	38.21	310.59
7	32.40 ± 0.24	2.69 ± 0.12	10.16 ± 0.34	8.41 ± 0.40	3.94 ± 0.04	42.40	285.93
8	36.11 ± 0.05	2.91 ± 0.08	9.92 ± 0.38	8.13 ± 0.57	4.27 ± 0.31	38.66	267.49
9	33.94 ± 0.32	2.25 ± 0.09	9.76 ± 0.15	9.26 ± 0.44	3.94 ± 0.04	40.85	285.78
10	30.74 ± 0.16	2.16 ± 0.03	9.70 ± 0.14	10.24 ± 0.46	3.89 ± 0.01	43.27	304.04
11	34.47 ± 0.10	2.41 ± 0.05	9.94 ± 0.09	10.68 ± 0.66	3.96 ± 0.01	38.54	290.04

formulations promoted an increase in the average protein content of the doughnuts.

Table 3 shows the multiple regressions for the concentration of protein, ash and fibre in relation to the addition of inverted sugar and *S. platensis* biomass.

Based on the regression analysis and on the adjusted coefficient of determination (adjusted $R^2 = 0.82$) the coherence of the fit of the model shown in Equation 3 is apparent. As expected, the increase in protein content of the doughnuts was only due to the amount of *S. platensis* added. Figure 1 provides evidence of the importance and influence of adding *S. platensis* biomass as a low cost alternative for the protein enrichment of products based on cassava, which is notably deficient in this compound. The 40% increase in protein content in relation to the standard is significant. Moreover, its digestibility must be pointed out (80%), which is due to its mucopolysaccharide cell wall and the high biological value of this non-conventional protein source (COHEN, 1997; SPOLAORE et al., 2006).

$$\text{Protein (\%)} = 9.718 + 1.278\, X_2 \quad (3)$$

$$\text{Ash (\%)} = 2.273 + 0.450\, X_1^2 - 0.605\, X_2^2 \quad (4)$$

$$\text{Fibre (\%)} = 4.044 + 1.095\, X_2 - 0.237\, X_2^2 \quad (5)$$

The ash content of the doughnuts oscillated between 0.60 and 2.91%. The difficulty in homogenization during the kneading process of bakery products interferes in the results, causing the variations. Such variations may have led to the higher variation in the model adjusted for this variable (adjusted $R^2 = 0.73$) when compared to that obtained for protein content. However, the model indicated that the addition of inverted sugar and *S. platensis* biomass influenced the nutritional enrichment of the formulations analyzed, increasing, the mineral content in a general way when compared with the standard formulation (Table 2), this behaviour being shown in Equation 4 and Figure 2.

It can be seen that the lipid content increased with the addition of *S. platensis* biomass, when compared to the standard sample. The amount of fat added to the formulations was the same. However *Spirulina* contains lipids, including essential fatty acids and other fat-soluble substances (CHAMORRO-CEVALLOS et al., 2008) which contributed to the increase in final lipid content. The non-occurrence of a significant difference amongst the formulations proves this affirmation. The same behaviour

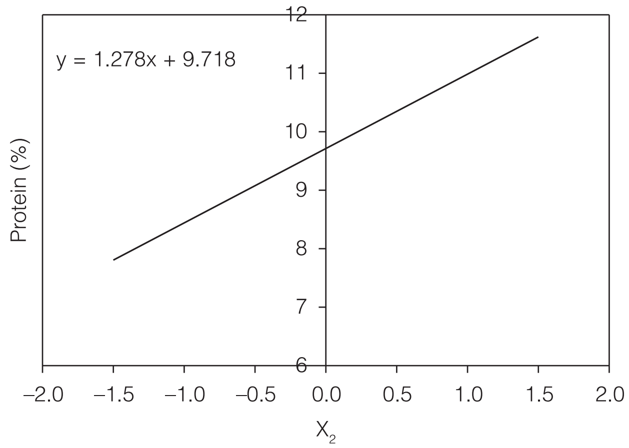

Figure 1. Protein content of the doughnuts as a function of the addition of *S. platensis*.

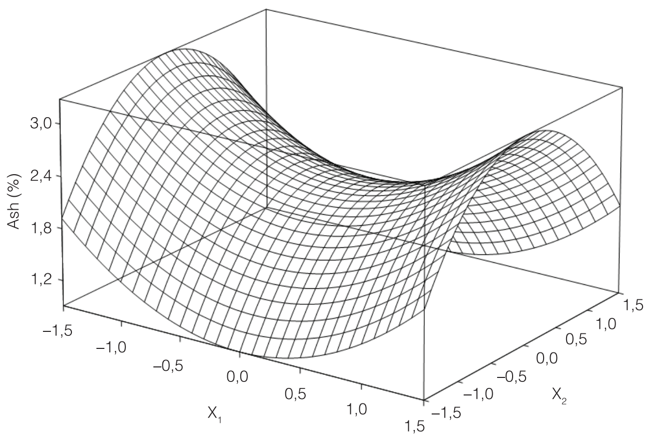

Figure 2. Response surface for ash as a function of the addition of *S. platensis* biomass and inverted sugar.

Table 3. Multivariable Regression Analysis of the total protein[a], ash[b] and fibre[c] contents as a function of the coded values of both the inverted sugar (X_1) and the *S. platensis* biomass (X_2).

Factor	Regression coefficient			Standard error			t-value		
	Protein	Ash	Fibre	Protein	Ash	Fibre	Protein	Ash	Fibre
Intercept	9.718	2.273	4.044	0.159	0.2270	0.0729	60.9055	10.0153	55.4632
X_1	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]
X_1^2	-[d]	0.450	-[d]	-[d]	0.1655	-[d]	-[d]	2.6921	-[d]
X_2	1.278	-[d]	1.095	0.1871	-[d]	0.0614	6.8290	-[d]	17.8484
X_2^2	-[d]	–0.605	–0.237	-[d]	0.1655	0.0698	-[d]	–3.6555	–3.3935
$X_1.X_2$	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]	-[d]

[a]Adjusted R^2: 0.82; [d]$p > 0.05$. ANOVA of regression: $p < 0.0001$; [b]Adjusted R^2: 0.73; [d]$p > 0.05$. ANOVA of regression: $p = 0.0022$; [c]Adjusted R^2: 0.97; [d]$p > 0.05$. ANOVA of regression: $p < 0.0001$.

was observed by Galera (2006) in studies with doughnuts, where the lipid content varied according to the wheat substitution used, without any increase in the addition of fat to the preparation. This may indicate a mechanism of interaction amongst the compounds of the formulation.

The fibre content varied from 1.83 to 5.09% and formulation 6 showed the highest value. According to Decree n° 27 of January 13th, 1998, ANVISA (BRASIL, 1998), solid food must contain at least 3 g of fibre/100 g to be considered as a source of fibres, and at least 6 g of fibre/100 g to be considered as a high fibre source. Thus samples 1, 3, 4, 6, 7, 8, 9, 10 and 11 can be considered as sources of dietary fibre. The ingestion of fibre has been decreasing, causing considerable dietary disturbances. The development of products with great sensory acceptance and high fibre contents could minimize these problems and stimulate a healthy lifestyle. The increase in life expectancy has generated a great interest in foods that prevent diseases such as diabetes *mellitus*, cardiovascular diseases, obesity and colon cancer (diseases that can be prevented by fibre), but the foods must be practical, such as bakery products (THARANATHAN and MAHADEVAMMA, 2003). The Adjusted R^2 for fibre obtained from the regression analysis was 0.97, showing the good fit of the model and the contribution to the increase in fibre content made by the addition of biomass to the formulations. This effect is highly evident from Equation 5 and Figure 3. The increases in both protein and fibre contents in the formulations caused by the addition of *S. platensis* biomass, is evidence of the beneficial nutritional effect conferred by this microorganism on the formulations. As expected, the substitution of sucrose by inverted sugar did not influence the fibre content of the formulations.

The raw and baked volumes of the samples were also measured. The expansion coefficients were calculated from these values and the results can be seen

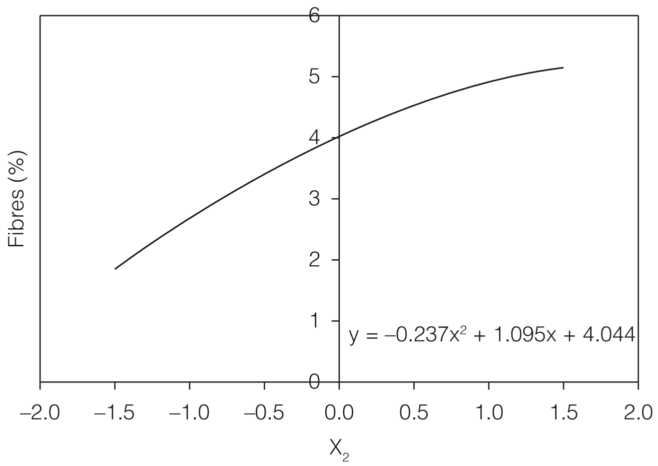

Figure 3. Fibres as a function of the addition of *S. platensis*.

in Table 4. The analysis of variance of the expansion coefficients showed no significant differences amongst the formulations. Thus the addition of inverted sugar and *S. platensis* to the cassava doughnut formulation did not affect the main characteristics of the cassava made dough.

Since the products showed similar behaviour regarding the growth and presence of small cavities on the inside of the products, bestowing softness on the product, it can be inferred that the typical appearance of the bakery product will be accepted by the potential consumers. There is a limited offer of bakery products on the market for those suffering from celiac disease (DIETERICH et al., 1997). Thus cassava based bakery products represent an interesting suggestion, and in such cases, the protein requirements could be supplied by the addition of *S. platensis*.

The addition of inverted sugar and *S. platensis* biomass caused no significant effects ($p>0.05$) on the different formulations in relation to the expansion coefficient and the moisture, lipid and carbohydrate contents. Therefore it was not possible to obtain mathematical models representing the responses.

The analysis of the mechanical properties of the doughnuts made of cassava dough by applying a shearing force to cut the product in the middle, allows one to evaluate the effect of adding different concentrations of inverted sugar and *S. platensis* biomass on the elastic properties of the dough. From the data for maximum compression force with deformation up to 80%, the hardness or resistance to shearing can be evaluated in relation to the composition and sustaining of the product structure.

The values obtained for the shearing force necessary to cut samples of each formulation of cassava doughnut in half were analyzed using a complete factorial experimental design, and the significant negative and positive effects ($p < 0.10$) of adding inverted sugar (–1.9 ± 0.3) and *S. platensis* (1.22 ± 0. 3) were observed. The negative effect of the addition of inverted sugar led to a decrease in the force necessary to cut the samples. On the other hand, the positive effect implied that an increase in the concentration of *Spirulina* in the dough led to an increase in the shearing force necessary to cut the doughnut in half, i.e., it made the dough more resistant to disruption. Furthermore, it can be observed that the effect of adding inverted sugar had a greater impact on shear force than the addition of *S. platensis*.

Probably, the positive effect of adding *S. platensis* and the negative effect of adding inverted sugar were related to the mechanism of action the inverted sugar used to favour protein-protein interactions, such that the network created fixed the water molecules by way of weak bonds (TAKEUCHI, 2001). Thus the proteins in the

Table 4. Means and deviations for shearing force, density, specific volume and expansion coefficient of the cassava doughnut formulations with added *Spirulina platensis* and inverted sugar.

Formulation	Mean ± Standard deviation			
	Force (N)	Density ($g.cm^{-3}$)	Specific volume ($cm^3.g^{-1}$)	Expansion coefficient (%)
Standard	5.64 ± 0.18	2.11 ± 0.15	1.66 ± 0.19	178.95 ± 0.18
1	10.47 ± 0.18	2.13 ± 0.21	1.89 ± 0.18	202.77 ± 0.18
2	7.45 ± 0.51	1.77 ± 0.81	2.19 ± 0.82	189.86 ± 0.88
3	9.03 ± 0.56	1.56 ± 0.15	2.51 ± 0.16	192.66 ± 0.15
4	8.14 ± 0.22	1.70 ± 0.11	2.24 ± 0.12	190.28 ± 0.12
5	8.11 ± 0.42	1.89 ± 0.14	2.07 ± 0.14	193.40 ± 0.14
6	12.11 ± 0.59	1.60 ± 0.16	2.51 ± 0.16	201.33 ± 0.16
7	14.94 ± 0.46	1.34 ± 0.38	3.04 ± 0.31	204.43 ± 0.34
8	12.32 ± 0.72	1.19 ± 0.33	3.50 ± 0.33	212.87 ± 0.31
9	8.06 ± 0.39	1.06 ± 0.55	3.89 ± 0.58	208.66 ± 0.55
10	7.23 ± 0.29	0.95 ± 0.48	4.38 ± 0.44	212.12 ± 0.48
11	7.45 ± 0.91	2.05 ± 0.51	2.11 ± 0.52	223.82 ± 0.52

S. platensis biomass promoted the creation of a protein-protein network during mixing and these interactions were strengthened after baking. Hence it was observed that *S. platensis* promoted an increase in the shearing force, i.e. it made the doughnut stronger. On the other hand, the addition of inverted sugar, which promoted a decrease in the shearing force, was probably related to the fact that it favoured interactions between the proteins and water, weakened the net that surrounded the other components of the cassava doughnut dough.

As well as a greater shearing force, the resistance to deformation and cutting will also be greater, making the doughnut harder to chew and less sensitive to kneading. The desired magnitude of cutting resistance must be evaluated and compared with the sensory acceptance of the target consumers. Thus the evaluation of the texture properties of the doughnuts using a texturometer can be used as an objective parameter to control the attribute of "quality of texture", previously evaluated by the target consumers.

Data on the doughnut market are not available in Brazil, because they are mostly not industrialized. In 2007, research carried out for 52 weeks in the United States showed that more than 204 million doughnuts were commercialized, considering only the 10 main brands on the market (AIB, 2008). These data indicate a promising consumption for this kind of bakery product.

The consumers evaluated the samples in relation to the sensory attributes of acceptance (Table 5) and purchase intention (Figure 4). In the acceptance analysis, if there are two distinct groups of approval-rejection, they are annulled when the mean for each sample is calculated. Thus the presentation of the percent distribution of approval (scores 6 to 9), indifference (score 5) and rejection (scores 1 to 4) allows for a better visualization of the responses given by the panellists using the hedonic scale. There was no significant difference amongst the samples in relation to appearance, smell, flavour and texture. Therefore, none of these attributes contributed more than the others to sample acceptance.

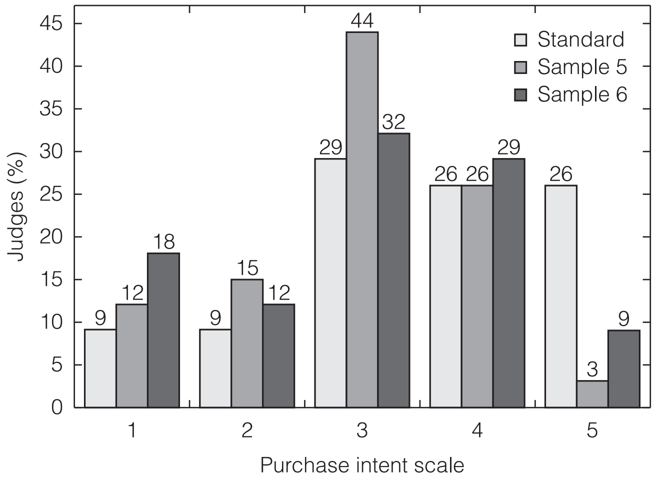

Figure 4. Distribution of the scores in the purchase intent test.

Although the standard formulation (without *S. platensis* biomass) showed a higher portent approval as compared to the formulation with the highest biomass concentration (formulation 6), in relation to the attributes evaluated, there were no significant differences, indicating the feasibility of incorporating the highest concentration of *S. platensis* biomass, resulting in a product with high nutritional value, especially with respect to its protein, lipid and fibre contents.

There was only a significant difference in the means for overall impression, due to a segregation of the samples

Table 5. Average acceptance of the doughnut samples.

Attribute	Sample	Mean	(%) Rejection	(%) Indifference	(%) Approval
Appearance	Standard	6.9^{a}	18	12	70
	Formulation 5	6.4^{a}	9	9	82
	Formulation 6	5.9^{a}	23	6	71
Smell	Standard	7.3^{a}	3	9	88
	Formulation 5	6.8^{a}	9	9	82
	Formulation 6	6.6^{a}	12	21	67
Flavour	Standard	6.8^{a}	9	6	85
	Formulation 5	6.2^{a}	18	6	76
	Formulation 6	6.2^{a}	21	3	76
Texture	Standard	7.1^{a}	9	6	85
	Formulation 5	6.4^{a}	18	3	79
	Formulation 6	6.4^{a}	21	3	76
Overall impression	Standard	6.7^{a}	6	9	85
	Formulation 5	$6.0^{a,b}$	24	12	64
	Formulation 6	5.9^{b}	21	6	73

Means followed by the same letter in the same column, considering each attribute, do not differ by Tukey's test ($p > 0.05$), (% approval - percentage of scores 6 to 9); % indifference – percentage of scores 5; % rejection – percentage of scores 1 to 4).

into two different groups. Formulation 5 was put into both groups.

In a study carried out with 22 Indian dishes enriched with *Spirulina*, good acceptance was shown regarding appearance/colour, texture, smell/flavour and overall acceptance for the samples containing concentrations of 1 g and 2.5 g, when compared to the standard (IYER et al., 2008).

The doughnut acceptance means ranged between 5.9 and 7.3, being ranked as indifference (score 5) and acceptance (scores 6 to 9) and showing that this product has potential for consumption (Table 5).

In the purchase intent test the standard formulation, formulation 5 and formulation 6 showed the highest percentage of scores 3 (29%, 44% and 32%, respectively), which means "Maybe I would buy it/Maybe I would not buy it" on the 5-point structure scale (Figure 4). Despite having a higher percentage of responses in this category, it can be seen there was a greater proportion of scores 4 and 5 than scores 1 and 2 amongst the responses, which means there is a possibility for purchasing the product.

Examples of studies aiming to explore the possibilities of incorporating different raw materials into foods, in order to encourage healthy habits and disease prevention as well as obtaining economic, social and environmental advantages, include the incorporation of single cell protein and cassava into bakery products. Since the scores awarded for sensory acceptance were similar for the standard formulation and for those with the highest and lowest percentages of *S. platensis*, the feasibility of developing these doughnuts was evident, providing a notable increase in nutritional enrichment without changing the doughnut characteristics.

4 Conclusions

The addition of different percentages of *S. platensis* allowed for a better nutritional quality of the cassava doughnut in terms of the protein, mineral, fibre and lipid composition.

The high protein, lipid and fibre concentrations, 12.1, 12.1 and 5.09%, respectively, found in formulation 6, together with the fact that the sensory tests showed that the addition of *S. platensis* to the doughnuts was well accepted by the consumers obtaining similar scores to those of the standard formulation, allows one to indicate the incorporation of 5.41% of *S. platensis* plus 10.0% of inverted sugar into the cassava doughnut formulation. Thus this product has a potential for consumption and commercialization as a low cost product with high nutritional value.

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