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The functional and organoleptic characterization of a dairy-free dessert containing a novel probiotic food ingredient

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New eating habits and diversification of tastes of consumers have led to the scientific community and the food industry to expand the range of probiotic foods and novel probiotic ingredients. Scant information is available about the viability and functionality of probiotics during shelf life and its effect on the nutritional characteristics of dairy-free products. The aim of the study was to formulate a fermented dairy-free dessert using a novel food ingredient based on a pumpkin by-product and containing *Lactobacillus casei* (ATCC®393™) (NFI). The effect of NFI and the soluble solids (SS) of soy milk on the probiotic viability, physical stability, colour, and firmness of dairy-free dessert was studied using a response surface methodology. The different levels of SS and NFI significantly ($p < 0.05$) affected the response variables. Thereafter, two formulations were selected and the physico-chemical, nutritional and organoleptic characterization were evaluated. The *L. casei* count reached the desired therapeutic level (>10^7 UFC mL^{-1}) after gastrointestinal digestion at 21 days of storage. In general, both the fermentation process and storage reduced ($p < 0.05$) the content of phytic acid, raffinose and stachyose, which implies a nutritional improvement of the final product. Scores above 5.0 on a 9-point scale were obtained for colour, odour, texture and overall acceptability in the consumer acceptance test. Therefore, a dairy-free dessert with good physical properties, suitable nutritional characteristics, and sensorial acceptability could be successfully formulated with the NFI.

Introduction

L. casei is a Gram-positive and facultative anaerobic lactic acid bacteria (LAB) that has remarkable phenotypic and genotypic variability, allowing it to colonize diverse ecosystems, including the human gastrointestinal tract. There is scientific evidence that supports the potential effect of *L. casei* ATCC®393™ against the proliferation of colon carcinoma cells,[1] reduction of intestinal cholesterol absorption,[2,3] and the capacity to modulate the microbial intestinal flora and adhere to the colon epithelium within levels compatible with the physiological effect.[4–6]

Health benefits are only obtained when a probiotic strain reaches the target site in a metabolically active state and in sufficient numbers. For this reason, probiotics must maintain their effectiveness and potentiality during processing conditions and shelf life of food, resist exposure to gastric and intestinal juices, and finally, achieve and colonize the intestinal tract.[7,8] Therefore, probiotic survival during processes and storage must be controlled from a nutritional and regulation point of view.[9,10]

Probiotic microorganisms are generally encapsulated in dairy products, with yogurt being the most popular probiotic food.[11] In the past few years, there has been a growing demand for dairy-free probiotic foods that can be consumed by people with new eating habits, such as vegans or vegetarians, and also by lactose or animal milk protein intolerant consumers.[12] These new trends and the diversification of tastes of consumers have led to the expansion of the range of probiotic foods, by the scientific community and the food industry, seeking to use different vehicular matrices, such as edible coatings or films, confectionary or baked products, fruit drinks and vegetables.[13–15]

Among the vegetable matrices without lactose and animal milk proteins are soybean and its derivatives, such as tofu, soy milk (SM) or fermented SM. Soy-based products are widely consumed all over the world, especially in countries of East and South Asia, being considered the main source of dietary

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proteins. Moreover, the consumption of fermented soy products has been associated with additional health benefits due to their functional properties such as hypolipidemic, anticholesterolemic and antiatherogenic ones.[11]

The acceptability of soy-based products is hampered by the beany flavour, and the presence of undesirable compounds such as phytic acid (PA), raffinose and stachyose.[16] Nevertheless, the fermentation process of SM with LAB and the addition of fruit pulp could be good options in improving the digestibility, palatability, and the acceptability of the final product.[17–19]

Some studies carried out in fermented SM with LAB have revealed that the fermentation process significantly reduces these compounds.[20–22] However, very little information exists about the content of PA and α-galactooligosaccharides (α-GOS) during the storage of fermented dairy-free products.

On the other hand, the physical stability of the fermented products during storage impacts consumer acceptability. In order to prevent the syneresis, the food industry uses strategies such as an increase in the solid content or the addition of stabilizing agents.[23,24] In a previous work, the authors profitably used pumpkin by-products for the formulation of a novel food ingredient containing *Lactobacillus casei* (ATCC®393™) (NFI).[13] The NFI provides not only stabilized probiotic cells but also dietary fibre from the pumpkin matrix. Pumpkin fibre could exert technological functionality in food formulation and the physicochemical properties had not been changed substantially due to the probiotic presence: the values are in the order of data previously reported (ash content 3.78 ± 0.7; protein content 5.7 ± 0.3; total dietary fibre 69.1 ± 0.7; g 100 g^{-1} db).[25,26] Besides, the NFI was previously studied as a dietary supplement in two commercial beverages, SM with apple juice and chocolate milk, presenting, in both cases, good sensorial acceptance as well as an improvement in probiotic survival during simulated gastric stress.[13] These promising results have led the authors to study its application as a food ingredient in complex matrices.

The objective of the present work was to design and optimize the formulation of a dairy-free dessert using the NFI applying the response surface methodology. Thereafter, two formulations were selected for chemical, physical, nutritional and organoleptic characterization.

Materials and methods

Microorganism and culture stock preparation

The strain of *L. casei* (ATCC®393™) was purchased from Microbiologics. The freeze-drying cell culture was activated following the protocol suggested by the supplier.

Preparation of the NFI supporting *Lactobacillus casei*

The preparation of the NFI, from pumpkin and supporting probiotic microorganisms, was carried out as previously reported.[13] Briefly, ≈10.0 g of pumpkin by-products was vacuum dried (Christ 1–4 LD) and mixed with 100.0 mL of water and sterilised at 121 °C for 15 min. Then, it was cooled and inoculated with a suspension of *L. casei* (≈10^3 CFU mL^{-1}) followed by 24 h of incubation at 37 °C. Subsequently, it was centrifuged (Eppendorf 5804R) and the pellet was vacuum dried at 25 °C and 4.5 Pa for 24 h. Finally, the dried powder was milled (Cannoíserve Coquette CG-0700), sieved through a stainless steel mesh with a particle size of 840 μ (ASTM N° 20, Zonytest), and stored at 25 °C.

Soy milk preparation

Soybeans (*Glycine max*) were purchased from the local market. The SM preparation was carried out according to Li *et al.*[18] with modifications. Approximately 250 g of soybeans were mixed with 250 mL of distilled water (w/v) and left at room temperature overnight. Thereafter, they were ground with a blender until a homogeneous mix was obtained. Immediately, the mix was placed in a stainless steel vessel and 1.250 mL of distilled water (ratio dry soybeans/water = 1 : 6; w/v) were added. The mix was boiled for 30 min with constant stirring. Then, the suspension was ground for 2 min, cooled at room temperature and filtered to separate the aqueous solution (SM) from the solid residue (okara). This SM reached 7° Brix and 88.2 ± 0.4% moisture content.

A rotavapor (BÜCHI Labortechnik R-124) connected to a thermal bath (BÜCHI Labortechnik B-840) was used to obtain an SM with different concentrations of soluble solids (SS) (°Bx). The evaporation conditions were carried out at 70 °C under vacuum with 20 revolutions per minute (rpm). Finally, the SM was stored at refrigeration temperature (8 °C) for 2 to 3 days, until use.

Preparation of the soy milk based dessert

Approximately 35 g of SM with different levels of SS within the range 6–16 °Bx were placed in a 50 mL conical tube. Equal quantities of strawberry pulp (3.5 g 35 g^{-1} SM, Bahia Regional Trade S.A.), artificial strawberry essence (0.35 g 35 g^{-1} SM, Sensaciones, Prindal) and stevia (0.35 g 35 g^{-1} SM, Hileret Stevia) as a non-caloric natural sweetener were added. All systems were sterilised at 121 °C for 15 min and rapidly cooled in a water bath at 0 °C. According to the experimental design, different levels of the NFI within the range 0.035–1.05 g 35 g^{-1} SM were added to each formulation followed by a homogenization process at 9500 rpm for 30 seconds (Ultra-Turrax® IKA T25) under aseptic conditions using a laminar flow cabinet. The systems were incubated in a chamber at 37 °C with orbital shaking at 60 rpm for 20 h, and finally stored at a refrigeration temperature of 8 °C for 21 days.

The pH value, titratable acidity, viability of *L. casei*, water holding capacity, colour and texture were determined for each dessert during storage.

Probiotic microorganism counting

Viable cell numbers of *L. casei* were determined by surface-plating 100 μL of decimal serial dilutions in MRS agar (Biokar Diagnostics). Plates were incubated at 37 °C for 72 h under aerobic conditions. The increase of *L. casei* during the storage

period (ΔCFU) was expressed as the difference between the final and initial counting cells. Each determination was performed at least in duplicate. Mean values and standard deviations (SD) are reported.

Physicochemical characterization

The superficial pH was determined with a surface combined glass electrode Ag°/AgCl connected to a pH meter (Cole-Parmer). Titratable acidity was determined with 0.01 M NaOH and phenolphthalein as an indicator. The results are expressed as gram of lactic acid 100 mL^{-1} of dessert.

The moisture of the sample was determined using infrared heating (Ohaus MB45) until a constant weight was reached.

Instrumental colour measurement was determined on the samples after the fermentation process using a colorimeter (Minolta CM-508D). Measurements were made in the CIE Lab space under illuminant D65 and with a viewing angle of 2°. The lightness (L^*) was used to quantify the darkness/clarity, the coordinate a^*, to determine the redness/greenness, and the b^* coordinate was used to quantify the yellowness/blueness.

From the parameters a^* and b^*, the hue angle (h^*) was calculated using the following equation:

$$h^* = \tan^{-1}\ (b^*/a^*) \quad (1)$$

Prior to measurements, the colorimeter was calibrated using a white reference standard ceramic plate. Samples were placed in a clear glass container and colour measurements were performed at least in duplicate from independent samples.

Texture was evaluated through a cylinder penetration test[27] using a Universal testing machine (Instron 3345) provided with a 100 N-load cell. A stainless steel cylinder puncture (h = 152.5 mm, ∅ = 20 mm) was introduced vertically into ~7 mL of dessert at a constant speed of 5 mm s^{-1}. Force (N)–displacement (mm) curves were recorded up to 70% of sample deformation. The strain is $\varepsilon = D/H$, where D is the displacement and H is the initial height of the sample and the stress is $\sigma = F/A$, MPa, where F is the force and A is the cross-sectional area. The measurements were carried out at 8 °C and at least four replicates were assayed.

Short-term physical stability

After the homogenisation procedure, ~2 mL of dessert were placed into a 2.5 mL Eppendorf tube and immediately incubation was carried out at 37 °C with orbital shaking at 60 rpm for 20 h. Then, the samples were refrigerated at 8 °C for 12 h. The samples were centrifuged at 6 °C at 5000 rpm for 40 min, the expelled water was carefully removed and the pellet was weighed according to the method of Granato *et al.*[28] The water holding capacity (WHC) was calculated using the following expression:

$$\text{WHC}\ (\%) = [1 - ((\text{IW} - \text{PW}))/\text{IW}] \times 100 \quad (2)$$

where IW is the initial weight of the sample and PW is the weight of the pellet after centrifugation. The WHC was determined at least in duplicate from independent samples.

Nutritional characterization of the optimised formulations

Chemical composition. Protein, fat, ash, and total dietary fibre content were determined following the AOAC methods 920.87, 920.39, 923.03 and 991.43, respectively.[29] Available carbohydrate was calculated by the difference method and, the metabolizable energy was estimated using the energy conversion factors.[30]

Probiotic survival to "*in vitro*" gastrointestinal digestion. The simulated gastrointestinal digestion (SGID) procedure was conducted as previously reported.[13] Briefly, ~5 mL of sample were mixed with 5 mL of artificial saliva solution for 2 min in a vortex (minishaker IKA® MSI). Then, 30 mL of the gastric solution [0.3%, w/v pepsin (Merck, 0.7 FIP-U mg^{-1}) in 0.01 M HCl] with a pH of ≈2.0 was added followed by incubation at 37 °C for 2 h. Subsequently, the pH value was adjusted to 7.5–8.0 with sterile 2 M NaOH, and finally, 30 mL of the intestinal solution [0.6%, w/v bile salts in 0.05 M KH_2PO_4] were added, followed by incubation at 37 °C for 2 h. The SGID was performed at least in duplicate, and probiotic viability was determined by plate counting at the end of gastric and intestinal digestion.

Determination of phytic acid and non-digestible oligosaccharides. The PA content in the dessert was determined using the method described by Gao *et al.*[31] and Lai *et al.*,[32] with minor modifications. Briefly, ~0.2500 g of freeze-dried dessert was re-suspended in 5 mL of 2.4% (v/v) HCl with constant stirring for 16 h. Then, 0.5 g of NaCl was added and the sample was stored at −20 °C for 20 min followed by centrifugation at 10 °C at 3000 rpm for 20 min. An aliquot of 750 μL of the supernatant dilution (1 : 25) was measured colorimetrically using the Wade reagent (Shimadzu UV-1800).

The content of PA is expressed as mg PA g^{-1} dessert, dry basis (db).

Raffinose and stachyose content was analysed using a YMC-Pack Polyamine II column (YMC 250 × 4.6 mm) according to the methodology described by Kim *et al.*,[33] with brief modifications. Approximately 0.5 g of the freeze-dried sample were mixed with 10.0 mL of a 20% (v/v) ethanol solution and shaken in a thermal bath at 35 °C for 60 min. The sample was then centrifuged at 6 °C at 4830 rpm for 20 min. The supernatant was collected and filtered through a Sep-Pak® Plus NH_2 solid phase extraction cartridge (Waters), and the filtrate was vacuum dried (Martin Christ Alpha 1–4) for 24 h. The residue was dissolved in distilled water and filtered through a 0.25 μm nylon filter. Finally, 20 μL were injected into a high performance liquid chromatography (HPLC) system comprising an on-line degasser (Waters AF) and a solvent delivery pump (Waters 1525) equipped with a refractive index detector (Waters 2414). The operating conditions were as follows: 35 °C column temperature, 39 °C detector temperature, mobile phase acetonitrile : water (70 : 30, v/v; Biopack), and 1.0 mL s^{-1} flow rate. The calibration curves were performed with the raffinose and stachyose grade HPLC standards (Sigma-Aldrich). The results are expressed as mg g^{-1} dessert (dry basis).

All determinations were performed in duplicate, from independent samples, and the mean values ± SD are reported.

Sensory evaluation. Sixty-two consumers frequent or not frequent of soy products participated voluntarily as untrained panellists. Five mL of each sample was offered at refrigeration temperature in a plastic tray coded with a three-digit random number in individually partitioned booths. The panellists were instructed to rinse their mouths with water and eat a cracker between samples to avoid carryover effects. A Consumer Acceptance Test was carried out to determine the attributes of colour, odour, mouth creaminess, and overall acceptability. The panellists were asked to judge the dessert with respect to their degree of liking or disliking using a semi-structured 9-point hedonic scale. The hedonic scale was based on the assumption that consumer preferences exist on a continuum and that preference can be categorised by responses based on liking and disliking.[34] Additionally, an Intensity Response Scale was used in order to determine the direction of consumer preference.[35]

Experimental design and statistical analysis

The effect of the independent variables on the responses was studied through a central design composed of two factors [NFI and SS of SM] with four levels (−2; −1; +1; +2) and central points (0; 0), using the Response Surface Methodology (RSM). The proposed levels for each factor were determined in previous assays, establishing the NFI range between 0.035 and 1.050 g 35 g^{-1} for SM and between 6.0 and 16.0 °Bx for SS. The coded and uncoded values are detailed in Table 1. The central point was performed in triplicate to calculate the reproducibility of the method. The experimental data were fitted to a second degree polynomial function:

$$\Psi = B_0 + B_1x_1 + B_2x_2 + B_{11}\ {x_1}^2 + B_{22}{x_2}^2 + B_{12}x_1x_2 \qquad (3)$$

where Ψ is the dependent variable, x_1 and x_2 are the independent variables, B_0 is the value of the adjusted response at the centre point of the design, B_1 and B_2 are the linear regression coefficients, B_{11} and B_{22} are the quadratic regression coefficients, and B_{12} is the interaction coefficient.[36] The statistical significance ($p \leq 0.05$) of the terms in the regression equations was analysed using analysis of variance (ANOVA) for each response, with a significance level of 95.0%. The adequacy of the model was evaluated through the coefficient of determination (R^2), adjusted R^2 (R^2_{adj}), lack of fit test ($p \geq 0.05$) and the Durbin–Watson statistic (DW). The desirability function was used to simultaneously optimize several responses.[37]

Statistical analysis of the results was performed through ANOVA for a level of significance (α) of 0.05 followed by a LSD Fisher *post hoc* test to identify significant differences between samples. The *post hoc t* Student test was used in the consumer acceptance examination. All statistical and regression analyses were performed using the Statgraphics Centurion XV program (V 2.15.06).

Results and discussion

Effect of the NFI concentration and SS on probiotic viability, physical stability, texture and colour of the dairy-free dessert

The results corresponding to ΔUFC, WHC and firmness are summarised in Table 1, whereas, the regression coefficients of the correspondingly fitted second degree polynomial function are detailed in Table 2. As can be observed, $R^2 > 94\%$, $R^2_{adj} > 88\%$, and $p > 0.05$ for a lack of fit test and DW > 1 confirmed that the equations predict adequately the variability of the studied responses for the range analysed in the experimental design.

After the fermentation process, all systems presented pH values from 4.52 ± 0.05 to 4.98 ± 0.05, showing a decrease with respect to the initial pH of SM (6.1 ± 0.3). In concordance with pH, the lactic acid concentration ranged between 0.78 ± 0.03 and 1.47 ± 0.03 g of lactic acid 100 mL^{-1} dessert (data not shown). The viable count of *L. casei* presented values from 9.45 to 10.05 log (CFU mL^{-1} dessert), and was in agreement with

Table 1 Matrix of the experimental design with the independent variables and the physicochemical properties, increase in *L. casei* viability, texture and colour for the formulation of the dairy-free dessert

	Independent variables		Dependent variables				
Systems	SS[b]	NFI[c]	ΔCFU[d]	WHC[e]	Firmness[f]	h^*[g]	Lightness
1[a]	11 (0)	0.5425 (0)	0.69 ± 0.03^{cde}	82.3 ± 0.7^{cde}	2.04 ± 0.08^{d}	66 ± 3^{ab}	53 ± 1^{c}
2	8.5 (−1)	0.7969 (1)	0.88 ± 0.07^{de}	80.6 ± 0.9^{cd}	1.4 ± 0.1^{c}	65.21 ± 0.05^{b}	50.3 ± 0.3^{b}
3	8.5 (−1)	0.2889 (−1)	0.73 ± 0.03^{cde}	76.5 ± 0.5^{b}	0.8 ± 0.01^{b}	45.8 ± 0.4^{e}	50.92 ± 0.06^{b}
4	6 (−2)	0.5425 (0)	0.89 ± 0.01^{e}	72.3 ± 2.3^{a}	0.43 ± 0.08^{a}	55.2 ± 0.2^{d}	47.57 ± 0.08^{a}
5	13.5 (1)	0.2880 (−1)	0.41 ± 0.06^{ab}	84.6 ± 0.2^{e}	2.05 ± 0.06^{d}	63 ± 1^{c}	54.71 ± 0.06^{d}
6	11 (0)	0.0357 (−2)	2.07 ± 0.04^{g}	80.36 ± 0.02^{c}	1.2 ± 0.3^{bc}	39.4 ± 0.05^{f}	53.57 ± 0.03^{c}
7	16 (2)	0.5433 (0)	1.3 ± 0.3^{f}	89.1 ± 0.2^{f}	2.6 ± 0.2^{e}	75.2 ± 0.7^{g}	56.4 ± 0.4^{e}
*8	11 (0)	0.5425 (0)	0.65 ± 0.07^{c}	83.4 ± 1.8^{cde}	2.3 ± 0.2^{de}	68 ± 1^{a}	54.4 ± 0.3^{d}
9	13.5 (1)	0.7965 (1)	0.33 ± 0.02^{a}	91 ± 1^{f}	3.8 ± 0.3^{f}	78.1 ± 0.4^{h}	55.0 ± 0.4^{d}
*10	11 (0)	0.5425 (0)	0.7 ± 0.1^{cd}	83 ± 5^{cde}	2.3 ± 0.1^{de}	67.8 ± 0.4^{a}	53.58 ± 0.06^{c}
11	11 (0)	1.0503 (2)	0.58 ± 0.05^{bc}	84 ± 2^{de}	2.6 ± 0.3^{e}	77.4 ± 0.7^{h}	53.0 ± 0.1^{c}

[a] Central systems from the experimental design. [b] SS: soluble solids in soy milk (SS) expressed in degrees Brix (°Bx). [c] NFI: novel food ingredient concentration in grams added to 35 g of soy milk. [b,c]. The codes for each factor are detailed in brackets. [d] ΔCFU: increase in viability of *L. casei*. [e] WHC: water holding capacity expressed in percentage (%). [f] Firmness expressed in kPa. [g] *: hue angle expressed in degree (°).

Table 2 Estimated regression coefficients of the fitted second-degree polynomial for the response variables for the dairy-free dessert

	Regression coefficients				
	ΔCFU	WHC	Firmness	Hue angle	Lightness
Constant	−0.23	53.45	−0.0027	−14.48	37.73
A: NFI	2.40*	0.91*	−0.0019*	96.86*	−2.48
B: SS	0.09	3.39*	0.00064*	6.03*	2.15*
AA	2.53*	−4.24	−0.0013	−38.44	−1.45
AB	−0.59*	0.84	0.00046	−1.71	0.32
BB	0.012	−0.097	−0.000028*	−0.13	−0.066
R^2	98.7	95.7	94.5	97.0	97.6
R^2_{adj}	96.6	91.4	88.9	93.4	95.2
Lack of fit	0.21	0.10	0.15	0.47	0.80
DW	2.63	2.34	1.82	1.69	1.94

*Significant difference ($p \leq 0.05$) with a significance level of 95%. A: NFI, novel food ingredient expressed in grams 35 g^{-1} SM. B: SS, soluble solids of soy milk expressed in ° Bx. A, B: linear terms. AA, BB: quadratic terms. AB: interaction term. R^2: coefficient of determination (%). R^2_{adj}: adjusted coefficient of determination (%). Lack of fit: *p*-value. DW: Durbin–Watson statistic. ΔCFU: increase in *L. casei* viable cell count. WHC: water holding capacity.

the current local[38] and the international (FDA; EFSA) regulations to consider the product as a probiotic at the time of consumption.[39]

The value of ΔCFU was positively affected ($p < 0.05$) by the NFI as can be observed from the linear and quadratic coefficients. The latter implies that the function has a minimum value. The coefficient of the interaction terms between the independent variables was significant ($p < 0.05$) and antagonistic (Table 2). Fig. 1A illustrates the effect of NFI and SS on the response variable ΔCFU. Higher ΔCFU is observed when SS increases in the first half of NFI concentration, and this effect is reversed at higher NFI level. A dessert with a higher concentration of NFI represents a greater concentration of probiotic cells initially and consequently lower ΔCFU, explaining in part, the antagonistic interaction between NFI and SS.

Syneresis represents the expulsion of water from the gel network and is perceived visually as water in the surface, affecting the acceptability of the probiotic product in consumers. WHC is a useful tool to describe the ability of a food matrix to retain free water when an external force is applied.[28] The WHC of the systems ranged from 72.3 ± 2.3 to 91 ± 1% (Table 1). In Table 2, it is observed that the WHC was positively affected ($p < 0.05$) by NFI and SS, the SS being the independent variable with the greatest effect in the Pareto diagram (data not shown). Fig. 1B shows the effect of the independent variables on the physical stability of the dessert, where higher WHC values are obtained with the highest level of NFI and SS. The increase of SS in the dessert formulation would be associated, in part, to an increase in the protein content, which in turn would directly favour the formation of the gel network.[40]

In fermented dairy foods such as yogurt, the firmness is directly related to the total of solids and the protein content. An increase in the protein content increases the degree of cross-linking between proteins, resulting in a gel with a dense and rigid structure.[11] The coefficient of the NFI linear term was significant ($p < 0.05$) and negative for the firmness response of the dairy-free dessert, whereas the coefficient of the SS linear term was significant ($p < 0.05$) and positive (Table 2). The highest firmness values are obtained with the highest levels for NFI and SS (Fig. 1C). However, the coefficient of the quadratic term of SS was negative and significant ($p < 0.05$) in this response, which means that in the SS range studied, there is a maximum value for firmness.

Although the increase in SS is directly associated with an increase in the protein content, the highest values of firmness were observed in the central levels of the SS, possibly because there is a limit of solids and proteins that can interact in the gel network (Table 1). The same tendency was observed for the NFI; however, in this case, the coefficient of the quadratic term was not significant ($p > 0.05$). Carbohydrate characteristics of dietary fibre from NFI could also contribute to the rheological behaviour.[41]

h^* is considered a colour attribute related to differences in absorbance at different wavelengths, where 0° represents a red hue while values of 90° represent a yellow hue.[42] In the present work, it was possible to observe that the coefficient of the linear term for the NFI and SS were significant ($p < 0.05$) and positive (Table 2). As is shown in Fig. 1D, the highest values for h^* were observed with the maximum level of NFI and were associated with the more yellow desserts, whereas lower h^* values were obtained with the minimum level of NFI and were represented by the more red desserts. Consequently, a negative correlation was observed between the a^* coordinate and the h^* (Pearson correlation coefficient: −0.84, $p = 0.0023$), presenting a^* values from 4.5 ± 0.2 to 11.29 ± 0.04, which confirms the above premise.

In relation to L^*, this parameter was positively affected ($p < 0.05$) by the SS (Table 2). As shown in the response surface (Fig. 1E), the highest values in L^* were observed with the highest concentration of SS. As a reference, an image of the desserts obtained in the experimental design is shown in Fig. 2.

Physicochemical and nutritional characterization of the dairy-free dessert selected formulations

Optimization of the dairy-free dessert was carried out by maximizing the dependent variables: firmness, WHC and a^* coordinate, obtaining a desirability value of 0.70. The suitable combination obtained for the optimized product, Dessert A, was 12.5 °Bx of SS and 0.0357 g NFI 35 g^{-1} SM.

In order to eliminate the concentration process of the SM and to increase the NFI content, a second formulation named Dessert B was proposed using freshly extracted SM (SS: 7 °Bx) and 0.4375 g NFI 35 g^{-1} SM.

The proximate composition of desserts A and B resulted in respectively 6.22 ± 0.08% and 3.25 ± 0.04% for protein, 4.5 ± 0.5% and 2.0 ± 0.2 for lipids, 1.37 ± 0.01% and 0.75 ± 0.01% for ash, and 0.51 ± 0.03% and 0.81 ± 0.04 for the total dietary fibre content. Available carbohydrate content could be calculated by difference, resulting in 11 ± 1% and 8.9 ± 0.7% for

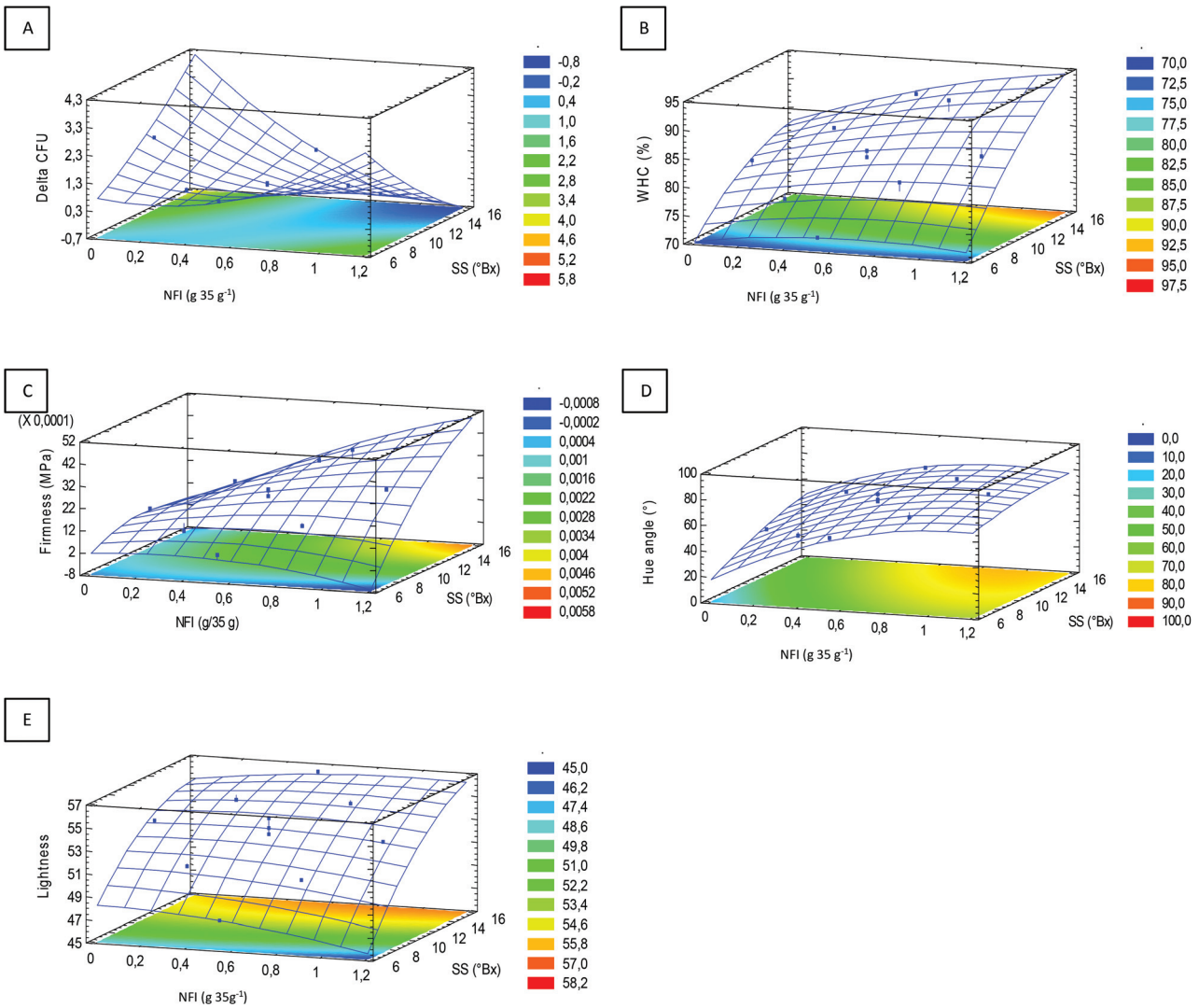

Fig. 1 Response surface graphs of the response variables (A) delta UFC, (B) WHC, (C) firmness, (D) hue angle, and (E) lightness as a function of the NFI and SS concentration. NFI: novel food ingredient (g 35 g^{-1} SM). SS: soluble solids of soy milk (°Bx). ΔCFU: increase in *L. casei* viable cell count. WHC: water holding capacity.

desserts A and B, respectively. From this characterization, the metabolized energy could be estimated as 450 ± 40 kJ g^{-1} (109 ± 9 kcal g^{-1}) for Dessert A and 290 ± 20 kJ g^{-1} (69 ± 5 kcal g^{-1}) for Dessert B. These values are in the order of those previously reported by other authors for similar soy-based products.[43]

The viability and functionality of *L. casei* and PA, raffinose and stachyose content were studied in both the proposed dessert formulations.

After the fermentation process, pH and titratable acidity were 4.73 ± 0.04 and 0.71 ± 0.05 g of lactic acid 100 mL^{-1} in Dessert A. Meanwhile, Dessert B presented a pH and titratable acidity of 4.36 ± 0.01 and 0.63 ± 0.08 g of lactic acid 100 mL^{-1} of dessert. The initial pH in SM was pH 6.1 ± 0.3; therefore, the production of lactic acid due to the fermentation of carbohydrates by probiotic microorganisms probably caused the protein gelation giving place to the formation of a gel network.[44] The three-dimensional gel network retained 72 ± 2% and 56 ± 3% of the aqueous content in the desserts A and B, respectively.

The difference between the initial viable *L. casei* concentration added to the formulation through the NFI and the viable probiotic count after the fermentation process was 4.30 ± 0.06 in Dessert A and 4.6 ± 0.3 in Dessert B. The viable *L. casei* concentration was stable ($p > 0.05$) during 21 days of storage at 8 °C, showing a probiotic count of 9.54 ± 0.09 log (CFU mL^{-1}) and 11.0 ± 0.5 log (CFU mL^{-1}) in desserts A and B, respectively (ΔCFU_A = 0.35 and ΔCFU_B = 0.28). In line with these results, authors such as Içier *et al.*[17] have reported a viable count of *L. acidophilus* of 8.9–9.1 log (CFU g^{-1}) in SM with apple juice (15–25%) after 21 days of storage at 4 °C.

Additionally, the *L. casei* functionality was investigated through an *in vitro* SGID. At initial storage, the probiotic survival to the SGID was 78 ± 6% in Dessert A and 85 ± 7% in Dessert B, denoting the absence of significant differences ($p >$

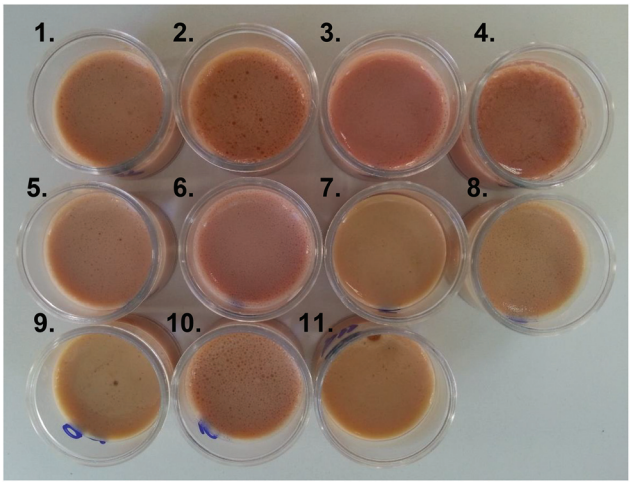

Fig. 2 Picture of dairy-free desserts obtained from the different systems of the experimental design formulated with the novel ingredient supporting *L. casei*.

0.05) between systems. After 21 days of storage, a significant ($p < 0.05$) decrease was observed in probiotic survival in dessert B, the resistance to SGID being 65 ± 6% in Dessert B and 86 ± 6% in Dessert A. In formulation B, a greater concentration of probiotic microorganisms (≈10^6 CFU mL^{-1} of dessert) was added, which under equal fermentation conditions could be in a stationary stage of growth with respect to formulation A (≈10^5 CFU mL^{-1} of dessert) which would probably be found in a stage of exponential growth. This is seen, in part, by the higher ($p < 0.05$) pH values observed in dessert A under the same conditions of time and temperature during fermentation, which could possibly explain the increased resistance of probiotics to SGID.[13] Despite the differences observed, it can be inferred that both proposed formulations had a significant survival of *L. casei* cells (>1.7 × 10^7 CFU mL^{-1} of dessert) after SGID at the end of 21 days of storage. It must be highlighted that the probiotic survival showed through NFI addition and at 21 days of storage was greater than that reported by Wang *et al.*[45] in fermented SM with free cells of *L. casei* Zhang and without storage. These authors reported a surviving to the gastric conditions between 50% and 60%.

In Table 3 the contents of PA, raffinose and stachyose in raw SM (without heat treatment) and in the desserts fermented with NFI at the baseline and at the end of the storage are summarised.

Myo-inositol hexaphosphate or PA is present in soybean seeds and in derived soy products such as SM. Its quantification in the final product is of interest from a nutritional point of view since PA forms complexes with proteins and metal ions, especially iron, zinc, magnesium and calcium, reducing their absorption through the intestinal epithelium.[45]

Considering the proportion of SM in the dessert and the moisture content of 76.8 ± 0.3% for Dessert A and 84.3 ± 0.4% for Dessert B, a reduction in PA content of ≈39.42 and 49.1%, respectively, can be calculated during the fermentation of both desserts. After 21 days of storage, the reduction of PA was even greater with respect to the initial content in raw SM, presenting a total reduction of ≈73.75% in Dessert A and 75.5% in Dessert B (Table 3).

There are some LABs that can synthesize phytases and hydrolyze PA by dephosphorylation and hydrolysis, leading to the formation of myo-inositol and organic phosphorus, and therefore, improve the bioavailability of minerals and proteins.[46] Therefore, the reductions observed in the PA content in dairy-free desserts could probably be explained due to the presence of phytases in *L. casei*. Tang *et al.*[45] have confirmed the presence of phytases in *L. casei* ASCC290 with an acceleration of enzymatic activity in acid medium (pH 5.0). In this study, both formulations presented pH values lower than 4.8.

The content of PA in terms of absolute values reported in the literature is very varied, and several factors such as the preparation of SM, boiling time, autoclaving process, fermentation process and the extraction method could affect the final content of PA and, as a consequence, make the comparison between different literature references[21,47] more difficult.

Human beings lack pancreatic α-galactosidase, which is necessary for the hydrolysis of α-GOS, such as raffinose and stachyose. The presence of α-GOS is associated with abdominal discomfort in consumers of soy-based foods because these compounds can be fermented by microorganisms producing gas in the colon and inducing gastrointestinal disorders in sensitive people.[48] Nevertheless, the presence of α-galactosidase in LAB could hydrolyse these undesirable compounds and avoid the effect of abdominal distension without eliminating the probiotic effect.[49,50]

In the present study, the content of α-GOS was significantly ($p < 0.05$) reduced after the fermentation of SM. In Dessert A,

Table 3 Phytic acid, raffinose and stachyose content in the dairy-free desserts and in raw soy milk

	Phytic acid[1]		Raffinose[2]		Stachyose[3]	
System	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21
Raw SM	0.92 ± 0.03^{b}	ND	7.80 ± 0.02^{g}	ND	29.7 ± 0.2^{l}	ND
Dessert A	0.45 ± 0.05^{c}	0.195 ± 0.005^{e}	6.0 ± 0.2^{h}	6.2 ± 0.2^{h}	24 ± 3^{m}	14.08 ± 0.04^{n}
Dessert B	0.31 ± 0.02^{d}	0.149 ± 0.007^{e}	4.92 ± 0.3^{i}	3.90 ± 0.01^{j}	14.2 ± 0.7^{n}	8.1 ± 0.2^{o}

Different letters denote significant differences ($p \leq 0.05$). [1; 2; 3]: mean values and standard deviation ($n = 4$) are expressed as mg g^{-1} of sample (db). ND: not determined.

the raffinose content dropped by ≈4.74% but remained constant during storage. Meanwhile, in Dessert B, the main reduction was observed during storage (24.6%), Table 3.

Regarding stachyose content, Dessert A did not present changes after fermentation; meanwhile, a reduction of ≈27.9% was observed for Dessert B. At the end of storage, stachyose was hydrolysed by ≈41.3% in Dessert A and ≈58.9% in Dessert B with respect to raw SM (Table 3).

In line with the results in the present work, authors like Battistini *et al.*,[20] Wang *et al.*[22] and Yoon and Hwang[51] had reported that raffinose and stachyose were significantly reduced by LAB and Bifidobacterias in SM during the fermentation stage. It is interesting to highlight that improvement of nutritional quality due to the metabolism and reduction of raffinose and stachyose has only been reported during SM fermentation and, to our knowledge, no reports have been found in the literature that refer to the reduction of these compounds during storage.

Sensory evaluation of the dairy-free dessert

Sixty-two untrained panellists participated during the sensory evaluation sessions, 75.8% of whom were women and 24.2% were men. The age of the participants was between 20 and 55 years.

As regards the colour attribute, desserts A and B received a punctuation of 6 ± 2 and 5 ± 2 in the 9-point scale, being rated as "like slightly" and "neither like nor dislike", respectively. The intensity scale (light [1]–dark [9]) showed a consumer preference towards a light colour than a dark one, receiving a score of 4 ± 2 for Dessert A and 5 ± 2 for Dessert B. It could be inferred that formulation A with a lighter colour was appreciated by consumers, while formulation B had a darker colour, demonstrating a significant ($p < 0.05$) consumer preference to the lighter colour in the dessert.

The odour attribute received a score of 6 ± 2 in the 9-point scale for desserts A and B, both formulations being categorised as "slightly liked". Nevertheless, the intensity scale (none [1]–intense [9]) showed that although the panellists did not perceive an intense odour in the formulations studied, Dessert A was scored "slight but significantly" ($p < 0.05$) with "a more intense odour" with respect to Dessert B (5 ± 1 and 4 ± 2 on the intensity scale, respectively). This fact could be explained by the process of evaporation and concentration of soluble solids in SM during the preparation of Dessert A. In addition, Blagden and Gilliland[52] have reported that the fermentation of soybean milk with *Lactobacillus casei* significantly reduced the presence of compounds such as methanol, acetaldehyde and hexanal which are responsible for the aroma in SM and therefore, the increase in the acceptability of soy foods.

The attribute creaminess in the mouth did not show significant differences ($p > 0.05$) between desserts A and B, receiving a score of 6 ± 2 on the 9-point scale and was rated as "lightly liked". The intensity scale (soft [1]–gritty [9]) did not show significant differences ($p > 0.05$) between the formulations, obtaining an average score of 5 ± 2 on the 9-point scale. These results allow us to infer that the concentrations studied for NFI (0.0357 g NFI 35 g^{-1} and 0.4375 g NFI 35 g^{-1}) seem not to affect the perception of the texture of the dairy-free dessert in the mouth.

The overall acceptability of desserts A and B obtained a score of 6 ± 2 and 5 ± 2 on the 9-point scale, being graded as "like slightly" and "neither like nor dislike", respectively, showing a non-significant trend ($p > 0.05$) towards a greater acceptability of Dessert A compared with Dessert B.

The novel products that obtain high scores in the Affective Test are more likely to be successful in the market.[28] However, it is interesting to highlight that the highest percentage (39%) of panellists reported "almost never" consumed soy-based products, which means that the consumption of soy foods was less than once per month. Therefore, despite not being frequent consumers, they liked the SM-based dessert herein developed.

Conclusions

RSM allowed us to identify the best combination of SS (12.5 °Bx) and the concentration for NFI (0.0357 g NFI 35 g^{-1}) to formulate a dairy-free dessert, optimizing nutritional, textural and colour parameters.

The formulations obtained presented a viable count of *L. casei* >2.7 × 10^9 CFU mL^{-1} dessert for 21 days of storage at refrigeration temperature, and these values were in agreement with the local and international organizations to consider the product as a probiotic at the time of consumption. Besides, a percentage >66% of probiotic cells (>1.7 × 10^7 CFU mL^{-1} dessert) reach the metabolically active lumen of the intestine after *in vitro* gastrointestinal digestion, providing real information about the potential probiotic effect.

In general, the fermentation process of SM and the storage significantly reduced the levels of PA, raffinose and stachyose, improving the nutritional quality of the final product.

The consumer acceptance test performed on both formulations received scores above 5.0–6.0 on a 9-point scale for colour, odour, texture and overall acceptability. Therefore, it can be concluded that the proposed NFI supporting probiotic microorganism allowed formulating an innovative and dairy-free dessert with suitable nutritional properties and sensory acceptability.

Conflicts of interest

There are no conflicts to declare.

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