Probiotic dairy dessert supplemented with whey protein concentrate: effect on the viability of *Lactobacillus acidophilus*, on texture, physicochemical and sensory features

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Summary

This study aimed at developing a flan-type dairy dessert supplemented with *Lactobacillus acidophilus* La-5 and whey protein concentrate (WPC). Four flan-type dairy desserts containing *L. acidophilus* La-5 were produced: F1 (0% WPC), F2 (1.5% WPC), F3 (3% WPC) and F4 (4.5% WPC). Instrumental texture, physicochemical and microbiological analyses were done throughout 28 days of refrigerated storage. Sensory evaluation and purchase intention tests were carried out after 7 and 14 days. Initial counts of probiotic bacteria were 9.30 log CFU·g^{-1}, 9.16 log CFU·g^{-1}, 9.19 log CFU·g^{-1} and 9.40 log CFU·g^{-1} (F1, F2, F3 and F4, respectively). During storage, *L. acidophilus* La-5 counts were above the minimum required for probiotic food (6.00 log CFU·g^{-1}), varying from 9.40 log CFU·g^{-1} to 8.53 log CFU·g^{-1} (F4 and F1, respectively). All formulations presented an increase in hardness, adhesiveness, titratable acidity ($p < 0.05$) and a decrease in pH ($p < 0.05$). All formulations exhibited excellent sensory acceptance ($p > 0.05$). The manufacture of a probiotic flan-type dairy dessert enriched with WPC was feasible, with suitable counts of *L. acidophilus* La-5 for a probiotic food and good acceptance in both sampling periods of sensory evaluation.

Keywords

whey protein concentrate; flan; dairy product; functional food

The demand for ready-to-eat dairy desserts has grown significantly due to the use of new technologies and ingredients that provide alternatives to classical desserts, obtaining thus products with different characteristics, new flavours and higher nutritional value [1]. Furthermore, the food industry has developed products with functional properties, aiming at fulfilling consumers' expectations, since consumers are interested in desserts with functional claims [2]. Within this context, food containing probiotic microorganisms, as well as proteins with biological value such as whey proteins, are frequent targets of research [3, 4].

Besides lactose, residual lipids and casein, whey proteins contain soluble milk proteins. Within this group are the β-lactoglobulin (2–4 g·l^{-1}), α-lactalbumin (1–1.5 g·l^{-1}), bovine serum albumin (0.1–0.4 g·l^{-1}), immunoglobulin (0.6–1.0 g·l^{-1}) and lactoferrin (approx. 0.1 g·l^{-1}) [5]. Utilization of bovine whey proteins in food formulations is motivated mainly by their health benefits. Ingestion of food supplemented with these proteins can result in health benefits connected to stimulation of protein synthesis [6], assistance in the production and secretion of hormones [7], and prevention of hepatic steatosis associated with weight gain in elderly women [8]. Moreover, in mice, the ingestion of bovine whey proteins was proven to inhibit ulcerative lesions [9], improve insulin resistance [10] and improve muscle mass gain [11]. Technologically, whey proteins may improve food characteristics, since they can promote gelation, emulsification and foaming [5, 12].

Addition of probiotics to diet has also been

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an alternative to promote health benefits. Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [13]. These microorganisms can support the reduction of lactose intolerance, inhibition of pathogenic microorganisms, prevention of diarrhoea, reduction of blood cholesterol levels, maintenance of remission in ulcerative colitis and increase of immune response [14–18]. Studies on animals showed the anticarcinogenic activity of probiotics [19]. In humans, a positive effect on irritable bowel syndrome and on life quality of patients with colorectal cancer was reported [20]. To obtain the desired effect [14–18], the probiotic should be present in adequate amounts in products, presenting populations of 10^6–10^7 CFU per gram or per millilitre of the product [21]. In addition, probiotic products should maintain their physicochemical and sensory characteristics during storage [22].

The combination of these two components in a flan-type dairy dessert is a promising alternative in the market of dairy desserts. However, the addition of whey proteins may modify the characteristics of the product such as hardness, its acceptance, and the survival of the probiotic microorganisms. Thus, the aim of this study was to develop a flan-type dairy dessert containing the probiotic microorganism *Lactobacillus acidophilus* La-5, supplemented with different percentages of whey protein concentrate. The physicochemical, instrumental texture and microbiological characteristics (*L. acidophilus* La-5 and contaminants – coagulase positive *Staphylococcus*, moulds and yeasts, total coliforms and *Escherichia coli*) were evaluated, as well as the sensory acceptance of the products.

MATERIAL AND METHODS

Inoculum preparation containing the probiotic culture

Lyophilized culture of *Lactobacillus acidophilus* La-5 (Christian Hansen, Hoersholm, Denmark) type DVS (Direct Vat Set) was used, which was stored frozen after opening the package, according to the manufacturer's instructions. The *L. acidophilus* La-5 culture was pre-incubated in milk prior to the manufacturing process. For that, 3 g of the culture were dissolved in 60 ml of whole ultra-high temperature-treated milk (UHT milk; Polly, Londrina, Brazil) under aseptic conditions, and the inoculum was incubated at 37 °C for 2 h. The purpose of this step was to obtain minimum initial populations of 6.00 log CFU·g^{-1} in products.

Manufacture of flan-type dairy dessert

Four dairy dessert formulations (F1, F2, F3 and F4) were produced, in triplicate, on different days. For the desserts preparation, the following ingredients were used: 225 ml whole UHT milk (Polly), 50.2 g refined sugar (União, Sertãozinho, Brazil), 0.10 g xanthan gum (Danisco, Cotia, Brazil), 3.7 g unflavoured colourless gelatine (Dr. Oetker, São Paulo, Brazil), 2.0 ml vanilla flavour (Dr. Oetker), 3.0 g probiotic culture *Lactobacillus acidophilus* La-5 (Christian Hansen), and whey protein concentrate (WPC; Arla Food Ingredients, Sønderhøj, Denmark) in different amounts 4.63 g (F2), 9.27 g (F3) and 13.90 g (F4). F1 was not supplemented with WPC (control formulation).

Milk was mixed with sugar and WPC according to the quantities described previously. The mixture was pasteurized (90 °C, 20 min) and cooled at 40 ° C in an ice bath, under constant agitation using a mixer (Arno, São Paulo, Brazil). Then, gelatine, xanthan gum, vanilla flavour and preinoculum containing the probiotics were added. The mixture was kept in an ice bath, and the ingredients were gently mixed with a spatula until the mixture reached a temperature of 15 °C. The desserts were placed in 140 ml plastic containers with lids (Copobrás, Içara, Brazil) previously sanitized with 0.5% sodium hypochlorite, and stored under refrigeration (5 °C ± 1 °C) for up to 28 days.

Sampling period and storage

During the storage period, instrumental texture, microbiological and physicochemical analyses were performed once a week. Sensory evaluation was carried out after 7 days and 14 days of storage. The mean composition was determined on the first day after production (day 1). Viability of *L. acidophilus* La-5 was also determined on day 0 (day of production) and on day 1.

Enumeration of *Lactobacillus acidophilus* La-5

The viability of *L. acidophilus* La-5 was monitored during the storage period. For this purpose, portions of 25 g of flan-type dairy dessert samples were homogenized with 225 ml of 0.1% peptone water (Oxoid, Basingstoke, United Kingdom) using a Bag Mixer (Interscience, St. Nom, France). Subsequent decimal dilutions were prepared using the same diluent. For enumeration of the microorganism, 1 ml aliquots of each dilution were transferred to sterile Petri dishes, and De Man – Rogosa - Sharpe agar (Himedia, Mumbai, India) cooled to 45 °C was added. The plates were incubated at 37 °C for 48 h [23]. The analyses were carried out in duplicate.

Enumeration of microbiological contaminants

The following microorganisms were enumerated: coagulase-positive *Staphylococcus*, moulds and yeasts, total coliforms, and *Escherichia coli*. The samples were prepared as described previously. For enumeration of coagulase-positive *Staphylococcus*, 1 ml aliquots of each dilution were transferred to Staph Express Petrifilm plates (3M Microbiology, St. Paul, Minnesota, USA) and incubated at 37 °C for 24 h. For moulds and yeasts counts, 1 ml aliquots of each dilution were transferred to Petrifilm Moulds and Yeasts Count Plates (YM Petrifilm, 3M Microbiology) and incubated at 25 °C for 5 days [24]. Petrifilm EC plates (EC Petrifilm, 3M Microbiology) were used for enumeration of total coliforms and *E. coli*, incubated at 37 °C for 24 h and 48 h for total coliforms and *E. coli*, respectively. All analyses were carried out in duplicate.

Determination of physicochemical parameters

During the storage period, pH and titratable acidity were determined. The pH values were determined with a pH meter model Tec 3MP equipped with a penetration electrode (Tecnal, Piracicaba, Brazil). Titratable acidity was determined using Dornic solution (Merck, Darmstadt, Germany) in the presence of phenolphthalein indicator, and the results were expressed in percentage. The mean composition was determined for the final product (after one day of storage at 5 °C ± 1 °C). Ash was determined gravimetrically by incineration of 5 g sample at 550 °C (FDG Equipamentos, São Paulo, Brazil). Protein was estimated by determining the N content by Kjeldahl method by using a micro-Kjeldahl equipment (Tecnal) and multiplying the result by a conversion factor (6.38; AOAC 991.23) [25]. Fat was determined through lipids extraction by the Mojonnier method (AOAC 989.05) [25]. Moisture content was determined by oven drying method at 105 °C (Nova Ética, Vargem Grande Paulista, Brazil) using 5 g of sample (AOAC 926.08) [25]. Carbohydrate content was calculated by difference to achieve 100% of total contents. All analyses were carried out in triplicate.

Instrumental texture

Hardness (force required to compress food between the molar teeth, or between the tongue and palate) and adhesiveness (force required to remove food from palate, lips and teeth during chewing) [26] were determined using sample compression test, using acrylic cylindrical probe TA3/100, 25.4 mm in diameter. Samples were kept in their original containers under refrigeration at 5 °C ± 1 °C until analysis. The analysis was performed in CT3 Texture Analyzer (Brookfield, Middleboro, Massachusetts, USA) controlled by a computer. Data were collected through the Texture CT V1.4 Build 17 software (Brookfield). All analyses were performed in quadruplicate, using compression speed of 1 $mm \cdot s^{-1}$, and distance of 10 mm as established by Corrêa et al. [27].

Sensory evaluation

After approval by the Ethics Committee on Research in Human Beings (protocol number 579.932), the sensory evaluation of the flan-type dairy dessert was performed after 7 and 14 days of storage at 5 °C ± 1 °C. An acceptance test was carried out with 50 consumers in each test (untrained panellists), recruited among students, teachers and employees at the university, using a 9-point hedonic scale (1 = dislike extremely; 5 = neither like, nor dislike and 9 = like extremely). The purchase intention test was also performed, in which consumers were asked whether they intend to buy the desserts [28]. For that, 10 g sample were presented monadically in white plastic dish coded with three-digit numbers, following a completely randomized block design.

Tab. 1. Mean composition of flan-type dairy desserts.

Formulation	WPC	Protein	Lipids	Ash	Moisture	Carbohydrates
	[%]					
F1	0	4.2 ± 0.3^{a}	3.7 ± 0.4^{a}	2.0 ± 0.0^{a}	76.8 ± 3.4^{a}	13.3 ± 2.7^{a}
F2	1.5	4.7 ± 0.1^{b}	3.5 ± 0.4^{a}	2.0 ± 0.0^{a}	77.6 ± 1.1^{a}	12.2 ± 1.3^{a}
F3	3	6.1 ± 1.0^{c}	3.7 ± 0.1^{a}	2.0 ± 0.0^{a}	73.5 ± 1.2^{a}	14.7 ± 1.8^{a}
F4	4.5	7.7 ± 0.2^{d}	3.6 ± 0.4^{a}	2.1 ± 0.0^{a}	74.0 ± 1.0^{a}	12.6 ± 1.2^{a}

Values are expressed as mean ± standard deviation after 1 day of storage at 5 °C ± 1 °C.
Different lower case letters in superscript in the same column indicate significant differences ($p < 0.05$) between formulations (F1, F2, F3 and F4) for each constituent.
WPC – whey protein concentrate, F1 – control, 0% WPC, F2 – 1.5% WPC, F3 – 3% WPC, F4 – 4.5% WPC.

Statistical analysis

Statistical analysis was performed using Statistica v.8.0 software (Statsoft, Tulsa, Oklahoma, USA). Normality and variance homogenity were evaluated using Shapiro-Wilks and Brown-Forsythe test, respectively, adopting α of 0.05. When the variance homogenity was not observed, data were analysed using the non-parametric Kruskal-Wallis test and Mann-Whitney U test for identification of contrasts ($p < 0.05$). When the variance homogenity was identified, parametric tests and Tukey's test were performed to identify the significant differences between means ($p < 0.05$) [29–31]. For comparison between the different storage periods for the same formulation, when the variance homogenity was not observed, data were analysed by non-parametric variance analysis, with application of Friedman test and Least Significant Difference (LSD) rank) to determine the contrasts ($p < 0.05$) [31]. When variance homogenity was observed, analysis of variance (ANOVA) and Tukey's test were performed to detect significant differences ($p < 0.05$) between means [30].

RESULTS AND DISCUSSION

Physicochemical parameters

Tab. 1 shows the mean composition of all formulations. Although no significant differences were observed in lipids, ash, moisture and carbohydrates contents for all formulations ($p > 0.05$), a significant difference ($p < 0.05$) was observed for protein, since products were supplemented with WPC. The results of pH and titratable acidity are presented in Tab. 2.

A significant decrease ($p < 0.05$) in pH values was observed in all formulations when results obtained on day 7 and day 28 were compared. No differences were observed between F1, F2, F3 and F4 when formulations were compared weekly, until the 21st day of storage. At day 28, a significant difference was observed, since F3 and F4 showed higher pH values than those observed for the other formulations (F1 and F2; $p < 0.05$). Significant changes were observed in acidity during storage ($p < 0.05$) when values obtained on day 7 and day 28 were compared for each formulation. Probably, this difference was associated with acid production by *L. acidophilus* La-5.

Similar results were obtained by several authors in dairy desserts containing probiotic microorganisms. Irkin and Guldas [32] found no significant differences in pH values of puddings containing *L. acidophilus* LAFTI L10 and *Bifidobacterium animalis* ssp. *lactis* LAFTI B94 during 20 days of storage. Pereira et al. [33] developed a "petit suisse" cheese containing *L. acidophilus* La-5 and *Bifidobacterium* sp. BL04, and observed a decrease in pH (4.84 to 4.45) during the storage period. Patel et al. [34] produced a chocolate mousse with *L. paracasei* NCDC 22, and found pH and acidity values to change from 6.6% and 0.3% (day 1) to 5.9% and 0.7% (day 28). Ribeiro et al. [35] investigated "petit-suisse" cheese containing *L. acidophilus*, and observed a decrease in pH values from 4.71 (day 1) to 4.39 (day 28).

According to Antunes et al. [36], WPC can improve the buffering capacity of dairy products due to its content of proteins and phosphates. Proteins and phosphates form a particle network when mixed with water. This gel is stabilized due to thermal treatment during food manufacture. However, this effect was not observed in the present study. Similarly to the observations in the present study, Buriti et al. [37] found that mousse formulations

Tab. 2. Physicochemical parameters of flan-type dairy desserts.

Formulation	Storage time [d]	pH	Titratable acidity [%]
F1	7	4.50 ± 0.19^{Aa}	0.2 ± 0.0^{Aa}
	14	4.23 ± 0.05^{Ab}	0.3 ± 0.0^{Ab}
	21	4.26 ± 0.05^{Ab}	0.3 ± 0.0^{Ac}
	28	4.05 ± 0.03^{Ac}	0.3 ± 0.0^{Ac}
F2	7	4.30 ± 0.06^{Aa}	0.2 ± 0.0^{Ba}
	14	4.22 ± 0.04^{Ab}	0.2 ± 0.0^{Bb}
	21	4.13 ± 0.03^{Ac}	0.3 ± 0.0^{Bc}
	28	4.05 ± 0.04^{Ad}	0.3 ± 0.0^{Bc}
F3	7	4.42 ± 0.04^{Aa}	0.2 ± 0.0^{ACa}
	14	4.28 ± 0.02^{Ab}	0.3 ± 0.0^{Ab}
	21	4.18 ± 0.03^{Ac}	0.3 ± 0.0^{Ac}
	28	4.13 ± 0.03^{Bc}	0.3 ± 0.0^{ABc}
F4	7	4.56 ± 0.34^{Aa}	0.3 ± 0.0^{Ca}
	14	4.26 ± 0.02^{Ab}	0.3 ± 0.0^{Ab}
	21	4.22 ± 0.04^{Ab}	0.3 ± 0.0^{Ac}
	28	4.18 ± 0.05^{Bb}	0.4 ± 0.0^{Ac}

Values are expressed as mean ± standard deviation after storage at 5 °C ± 1 °C. Titratable acidity is expressed as percentage of lactic acid.
Different uppercase letters in superscript within the same column indicate significant differences ($p < 0.05$) between formulations (F1, F2, F3 and F4) at the same storage time.
Different lower case letters in superscript within the same column indicate significant differences ($p < 0.05$) between the storage time for each formulation.
F1 – control, 0% WPC, F2 – 1.5% WPC, F3 – 3% WPC, F4 – 4.5% WPC.

Tab. 3. Populations of *Lactobacillus acidophilus* La-5 in flan-type dairy desserts.

Storage time [d]	*Lactobacillus acidophilus* La-5 populations [log CFU·g^{-1}]			
	F1	F2	F3	F4
1	9.30 ± 0.02^{Aa}	9.16 ± 0.04^{Ba}	9.19 ± 0.02^{Ba}	9.40 ± 0.05^{Aa}
7	9.08 ± 0.12^{Aab}	8.96 ± 0.09^{Ab}	9.07 ± 0.02^{Aa}	9.26 ± 0.08^{Ba}
14	8.95 ± 0.02^{Abc}	8.94 ± 0.04^{Ab}	8.96 ± 0.01^{Aab}	9.12 ± 0.13^{Bab}
21	8.84 ± 0.22^{Ac}	8.92 ± 0.04^{Ab}	8.71 ± 0.32^{Ab}	8.89 ± 0.41^{Aab}
28	8.53 ± 0.38^{Ad}	8.88 ± 0.05^{Ab}	8.61 ± 0.34^{Ab}	8.61 ± 0.38^{Ab}

Values are expressed as mean ± standard deviation after storage at 5 °C ± 1 °C.
Different uppercase letters in superscript within the same row indicate significant differences ($p < 0.05$) between formulations (F1, F2, F3 and F4) at the same storage time. Different lower case letters in superscript within the same column indicate significant differences ($p < 0.05$) between the storage time for each formulation.
F1 – control, 0% WPC, F2 – 1.5% WPC, F3 – 3% WPC, F4 – 4.5% WPC.

with higher WPC contents showed a significant change in pH values.

Microbiological analysis

The counts of *Lactobacillus acidophilus* La-5 from the flan-type dairy desserts are presented in Tab. 3. A significant reduction in *L. acidophilus* La-5 populations was observed during the storage period for all formulations ($p < 0.05$), when values observed on day 1 and day 28 were compared. However, the counts remained above the minimum required for desserts to be considered a probiotic food, once these should contain viable populations of 10^6 CFU·g^{-1} to exert beneficial effects after ingestion [38]. Even at the last storage week, the probiotic counts remained above the minimum required by law, with populations of 8.53 log CFU·g^{-1}, 8.88 log CFU·g^{-1}, 8.61 log CFU·g^{-1} and 8.61 log CFU·g^{-1} in formulations F1, F2, F3, and F4, respectively.

When different formulations were compared, significant differences were only detected on days 1, 7 and 14. On days 7 and 14, higher *L. acidophilus* La-5 populations were observed in the formulation containing the highest WPC percentage (F4, 4.5%) when compared to the other formulations ($p < 0.05$). On days 21 and 28, stabilization of *L. acidophilus* La-5 populations was observed for all formulations, with no statistical differences ($p > 0.05$). Probiotic bacteria are mainly incorporated into dairy products. However, the intrinsic characteristic of food such as type, presence of oxygen, manufacture, constituents and temperature of storage may affect probiotic viability. Therefore, the study of flan as a food matrix for probiotic and WPC delivery might contribute to the development of a product with suitable probiotic populations and protein, which presents an interesting alternative for the consumer.

The results on *L. acidophilus* counts are in agreement with other studies on probiotic dairy desserts. Helland et al. [39] obtained *L. acidophilus* La-5 populations ranging between 7 log CFU·g^{-1} and 8 log CFU·g^{-1} in puddings stored under refrigeration (4–6 °C) for 21 days. Silva et al. [40] produced a probiotic chocolate flan and found *L. casei* populations of 10^9 CFU·g^{-1} after 15 days of storage at 4 °C. However, a few studies have associated probiotics and WPC in dairy desserts. Antunes et al. [36] found that *L. acidophilus* populations increased by 1.8 log in yogurt supplemented with WPC.

Janer et al. [41] found an increase in *Bifidobacterium lactis* population in milk supplemented with 2% WPC. According to these authors, the effect of WPC on the multiplication of bifidobacteria was due to the enzymatic cleavage process, producing bifidogenic compounds. This phenomenon was not observed for *L. acidophilus* La-5 in this study.

Regarding contaminant microorganisms, coliforms, *E. coli*, coagulase-positive *Staphylococcus*, yeasts and moulds were not detected in the samples of the present study, indicating that these formulations were microbiologically safe for human consumption.

Hardness and adhesiveness analysis

The results of the instrumental texture parameters (hardness and adhesiveness) are presented in Tab. 4. Although a significant increase in hardness was observed in the formulations F1, F2, F3 and F4 during storage ($p < 0.05$), no differences were observed for the formulations F2 and F3 when compared to F1 (control; $p > 0.05$). On the other hand, the formulation F4 showed significantly higher hardness values when compared to formulations F1, F2 and F3 ($p < 0.05$). This result may be due to the gelation process resulting from WPC supplementation, once β-lactoglobulin con-

tains free sulfhydryl groups, which contributes to the formation of sulfhydryl-disulfide bonds, resulting in formation of a gel changing the food structure [5, 42].

Likewise, ANTUNES et al. [43] reported that the increase in protein content in food leds to an increased hardness, so the higher the WPC content, the greater the force required to break the gel is. VIDIGAL et al. [44] evaluated the texture of low-fat dairy desserts produced with addition of WPC, and obtained results similar to those found in this study. The authors observed an increase in hardness due to the higher percentage of WPC added to the formulations.

For dairy desserts, adhesiveness is an important parameter to be determined. All formulations showed significant increase in adhesiveness during storage ($p < 0.05$). The formulation F4 presented higher adhesiveness when compared to the other formulations ($p < 0.05$), which was also observed for the hardness parameter. However, lower WPC percentage also affected adhesiveness, since F2, F3 and F4 were statistically different from F1 (control), which showed a lower adhesiveness during storage ($p < 0.05$). Similar behaviour has been reported by BURITI et al. [37] in probiotic mousse containing WPC.

For all formulations studied, the changes detected in the instrumental texture profile did not affect the product's sensory acceptance.

Sensory analysis

After 7 days of storage, the mean scores assigned by the panellists for the dairy desserts were 7.62, 7.66, 7.26 and 6.88 (corresponding to "like slightly" and "like moderately" in the hedonic scale), for F1, F2, F3 and F4, respectively. On day 14, mean scores obtained were 7.73, 7.50, 7.46, and 7.76, for F1, F2, F3 and F4, respectively. No significant difference was observed among the product's acceptance for the same storage period ($p > 0.05$). Likewise, no significant differences were detected ($p > 0.05$) when the formulations were assessed over the storage period (after 7 and 14 days).

Regarding the purchase intention of the desserts on day 7, the percentage of consumers who said they would buy the desserts were 84%, 90%, 76% and 74% for F1, F2, F3 and F4, respectively, while on day 14, these percentages were 85%, 90%, 80% and 88%, respectively.

Many consumers classified the formulation F4 as "firmer", "firm", or "consistent". This result was due to the higher WPC percentage (4.5%), confirming the results of instrumental texture analysis (Tab. 4). However, the results showed that the addition of the probiotic culture and WPC at different amounts did not affect the product's acceptance.

VIDIGAL et al. [45] investigated the sensory acceptance of dairy desserts containing whey protein concentrate and found that the supplementation with 1.5% and 3.0% of WPC contributed to a better acceptance of the products. In the present study, no significant differences were observed in the formulations containing WPC when compared to the control, demonstrating that even the highest WPC percentage (3% and 4.5%) did not affect the acceptance of the flan-type dairy desserts. Also, the addition of a probiotic culture did not affect the acceptance of the products, as also reported by IRKIN and GULDAS [32], who observed that the addition of *L. acidophilus* and *Bifidobacterium animalis* ssp. *lactis* did not affect the sensory acceptance of puddings.

The flan-type dairy desserts developed had a good sensory quality in both storage periods evaluated, which is an important feature for

Tab. 4. Hardness and adhesiveness of flan-type dairy desserts.

Formulation	Storage time [d]	Hardness [N]	Adhesiveness [mJ]
F1	7	1.70 ± 0.06^{Aa}	0.05 ± 0.06^{Aa}
	14	2.09 ± 0.44^{Aab}	0.16 ± 0.14^{Ab}
	21	2.67 ± 0.26^{Ab}	0.25 ± 0.17^{Ab}
	28	2.51 ± 0.14^{Ab}	0.10 ± 0.08^{Ac}
F2	7	1.86 ± 0.18^{Aa}	0.18 ± 0.05^{Ba}
	14	1.31 ± 0.08^{Ab}	0.25 ± 0.26^{Ba}
	21	2.65 ± 0.17^{Aa}	0.35 ± 0.13^{Ba}
	28	2.37 ± 0.13^{Ac}	0.55 ± 0.54^{Bb}
F3	7	1.77 ± 0.10^{Aa}	0.70 ± 0.22^{Ca}
	14	1.82 ± 0.09^{Aa}	0.80 ± 0.52^{Ca}
	21	2.01 ± 0.18^{Aab}	1.80 ± 0.54^{Cb}
	28	2.12 ± 0.14^{Ab}	1.80 ± 0.23^{Ca}
F4	7	4.69 ± 0.33^{Ba}	1.98 ± 0.83^{Da}
	14	5.49 ± 0.29^{Bb}	1.80 ± 0.26^{Da}
	21	5.10 ± 0.28^{Bc}	1.58 ± 0.28^{Cb}
	28	5.03 ± 0.41^{Bc}	2.58 ± 1.83^{Dc}

Values are expressed as mean ± standard deviation after storage at 5 °C ± 1 °C.
Different uppercase letters in superscript within the same column indicate significant differences ($p < 0.05$) between formulations (F1, F2, F3 and F4) at the same storage time.
Different lower case letters in superscript within the same column indicate significant differences ($p < 0.05$) between the storage time for each formulation.
F1 – control, 0% WPC, F2 – 1.5% WPC, F3 – 3% WPC, F4 – 4.5% WPC.

a newly developed food product. Also, the supplementation with probiotic *L. acidophilus* La-5 and protein assured the health and nutritional claims of the products.

CONCLUSION

The results showed that the probiotic *Lactobacillus acidophilus* La-5 exhibited an excellent behaviour in the flan matrix, since probiotic populations were above the minimum required for probiotic food during storage for all formulations studied. Despite that some formulations had a firmer texture, the production of a probiotic dessert enriched with WPC is feasible since it did not affect negatively the *L. acidophilus* La-5 population and the sensory acceptance of the products.

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