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Sensory evaluation and physicochemical properties of set-type yoghurt fortified with andean lupin (*lupinus mutabilis*) proteins

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AbstractThe aim of this study was to evaluate the sensory characteristics and the physicochemical properties of high-protein yoghurt fortified with Andean lupin (*Lupinus mutabilis*) protein concentrate. Formulations with 0.5, 1 and 1.5% of protein concentrate (containing 69% proteins, 4% fats and 21% carbohydrates) were obtained using a commercial starter culture and cow's milk. The acceptability for colour, flavour, texture and taste was evaluated with a hedonic 9-point scale and 100 adults aged between 18-59 years. Participants selected the attributes more convenient to describe the yoghurts using the CATA (Check-All-That-Apply) test. The chemical composition, pH and acidity as well as the textural properties were evaluated. The yoghurt with 0.5% lupin protein concentrate was acceptable according to the sensory attributes. The protein concentrate, when adding at 1 and 1.5%, increased the bitterness, the residual taste and astringency of formulations. The pH values and lactic acid content in fortified products were similar to the control sample, however the syneresis was lower. The yoghurt with 0.5%. The addition of fruits, cereals or honey could be a strategy to increase the acceptability according to participants' perceptions. The use of yoghurt as dressings in salads could also be a novel form of consumption. The set-type yoghurt fortified with Andean lupin proteins could be alternatives to increase the daily intake of proteins, however some sensory properties should be optimized.

Keywords: yoghurt; legume; proteins; sensory characteristics

Evaluación sensorial y propiedades fisicoquímicas de yogur firme fortificado con proteínas de lupino andino (*Lupinus mutabilis*).

Resumen. El objetivo fue evaluar las características sensoriales y propiedades fisicoquímicas del yogur rico en proteínas fortificado con concentrado proteico de chocho andino (*Lupinus mutabilis*). Se obtuvieron formulaciones con 0,5; 1 y 1,5% de concentrado proteico (69% de proteínas, 4% de grasas y 21% de carbohidratos) utilizando un cultivo iniciador comercial y leche de vaca. Se evaluó aceptabilidad de color, aroma, textura y gusto con escala hedónica de 9 puntos y 100 adultos con edades entre 18-59 años. Los participantes seleccionaron los atributos más convenientes para describir los yogures utilizando la prueba CATA (Check-All-That-Apply). Se evaluó la composición química, el pH y la acidez, así como las propiedades texturales. El yogur con 0,5% de concentrado de proteína de lupino fue aceptable; al adicionarse al 1 y 1,5%, se incrementó el amargor, gusto residual y astringencia de las formulaciones. Los valores de pH y contenido de ácido láctico en los productos fortificados fueron similares a la muestra de control, sin embargo, la sinéresis fue menor. El yogur con 0,5% de concentrado de proteína de chocho mostró mayor firmeza y menor adhesividad. La adición de frutas, cereales o miel podría ser una estrategia para aumentar la aceptabilidad según la percepción de los participantes. El uso de yogur como aderezo en ensaladas también podría ser una forma novedosa de consumo. Los yogures tipo firme fortificados con proteínas de chocho andino podrían ser alternativas para aumentar la ingesta diaria de proteínas, sin embargo, se deben optimizar algunas propiedades sensoriales.

Palabras clave: yogur; legumbre; proteínas; características sensoriales

Introduction

Yoghurt is the product obtained by coagulation and fermentation through the action of proto-symbiotic microorganisms of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* with the addition or not of other lactic acid bacteria which contribute to the determination of the characteristics of the finished product. (1). The mandatory ingredients allowed in the formulation are milk or reconstituted milk standardized in its content of fat and lactic acid bacteria cultures. Optional ingredients can also be used such as concentrated milk, cream, butter, milk powder, fruit, honey, coconut, cereals, vegetables. Non-dairy ingredients, alone or in combination, must be present in a maximum proportion of 30% (m/m) of the final product (3). In recent years, there has been a rise in consumer demand for high-protein yoghurt, namely that containing a minimum of 5.6% protein and less than 15% fat (3). The interest in high-protein yoghurt lies on the concept of weight management and maintenance of a healthy life style (3). High-protein yoghurt could be beneficial in infant, elderly, or sports nutrition due to the ability of proteins to increase plasma amino acids and trigger the synthesis of proteins (4). Furthermore, high-protein yoghurts could be beneficial in calorie-restricted diets, because the energy intake from protein seems to have a greater effect on satiety than intake of fat or carbohydrate (5).

The protein content in yoghurt can be increased prior to fermentation due to the addition of milk powder, whey powder and micellar casein concentrates or achieved after fermentation by draining, evaporation or membrane filtration (3). A plain yoghurt with a high consumer acceptance should in general have a smooth, uniform and spoonable texture. It should be free from lumps, graininess, and visual whey separation, and it has a clean and typical yoghurt flavour (6,7). The composition of the milk base and the processing parameters and conditions have an influence on the sensory and physical properties of yoghurt. High-protein yoghurt fortified with milk powder showed higher sensory acceptability compared to products added with whey powder and caseinates. However, the perception of bitterness was a limitation to consume these products. Other

sensory defects such as graininess, bitterness, too acidic flavour, and whey separation were also perceived (6).

One strategy to obtain high-protein yoghurt is the use of legume proteins. The ingredients based on legumes are a trend due to the increasing demand of vegetarian people that revolve around the care of the environment (8). The use of legume proteins in the formulation of different fermented foods could increase the intake of protein at lower costs in low-income countries (9). In addition, the research of food innovations that diversify the use of legumes may promote their cultivation in sustainable cropping systems while improving the nutritional quality of products (6).

The limitation to use legume proteins in food formulation is their off-flavour as well as the presence of anti-nutritional factors (10). The off-flavour is produced due to inadequate storage of legumes, overheating of protein extracts, among others, and it limits the use of legume ingredients in product development (10). Major anti-nutritional factors in legumes include saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitor, and goitrogens. Anti-nutritional factors combine with nutrients and reduce the nutrient bioavailability. These compounds can be reduced by applying different methods and technologies, such as fermentation, germination, autoclaving, soaking etc (11). Edible legume ingredients such as flour, protein concentrates are used in the formulation of various foods to increase the nutritional value, the healthy properties and functional characteristics of products (10). In yoghurt, the addition of legume flour and protein concentrate increased the protein and fibre contents, the viscosity of formulations and the antioxidant properties of the product (12,13, 14).

Andean lupin (*Lupinus mutabilis*) is a legume native from South America which is consumed in the traditional cuisine of Peru, Bolivia and Ecuador (15).

The genus *Lupinus* (family Fabaceae) comprises about 267 species of lupines that grow in various regions, from sea level to the Andes (16). There are four species consumed: white lupine (*L. albus*) with wide distribution worldwide, blue (*L. angustifolius*), yellow (*L. luteus*) and Andean (*L. mutabilis*). The latter originates from the region from Ecuador to northwestern Argentina (17, 18). In 2019, 1,006,842 tons of lupines were produced in the world, Australia and New Zealand were the countries where the highest production was recorded (19).

Andean lupin has the highest protein and fat contents (40-50 g/100g and 20-30 g/100g, respectively) compared to other legumes such as pea and chickpea (20). Seeds are sources of bioactive compounds such as polyphenols and carotenoids with antioxidant and antihypertensive properties (21). Andean lupin has monounsaturated and polyunsaturated fatty acids which can promote the cardiovascular health. Proteins are represented by globulins (80%) as in other legumes and there is a small proportion of albumins and prolamins (15). The amino acids cysteine and methionine are limiting; while lysine, leucine, isoleucine, tyrosine, and glutamic acid are abundant (15, 18).

Grains contain a significant amount of dietary fiber but no starch. The shells are composed of cellulose, hemicellulose and pectin, while in the cotyledons there are polysaccharides composed of galactose, arabinose and uronic acid (22). Lupines contain bitter alkaloids from the quinolizidine family. These compounds are toxic so they must be removed (23). Most of the alkaloids are soluble in water, the traditional debittering process includes soaking the seeds for 18-20 h, followed by cooking between 0.5 and 6 h (24).

Several ingredients are obtained from Andean lupin seeds (i.e: protein concentrates, isolates, edible oil, lupin flour) (20).

The lupin protein concentrate, which has ~60% of lupin proteins, has been used in the production of bread and noodles showing good sensory characteristics and high consumer acceptability. The incorporation of lupin products such as flour into fermented foods was limited due to the persistent after-taste perceived in formulations (23). The after-taste could be attributed to the remanent of alkaloids, toxic and bitter compounds for humans and animals (15). Their presence in food represents a concern for safe consumption since their ingestion can cause intoxication. Alkaloids can be removed by soaking, cooking and washing lupin seeds, until a safe level of consumption <0.02 g/100g seeds (15). There are also improved varieties with lower alkaloid content, however these are not available in Latin America (25).

The demand for healthy products is growing every time, particularly those with new, more sustainable and lower cost ingredients (26). There is a potential to produce high-protein yoghurt with Andean lupin protein concentrate to increase the offer of healthy products (24). The use of more refined lupin ingredients such as the protein concentrate could enhance the sensory properties of the final product since it has lower alkaloid contents (24). Thus, the aim of this study was to evaluate the sensory characteristics and physicochemical properties of these products for future industrial applications

Methods

Formulation of set-type yoghurt

The procedure to obtain set-type yoghurt fortified with Andean lupin protein concentrate was previously published (27). Briefly, the lupin seeds were debittered following the traditional aqueous method. Seeds were soaked in water at 25°C for 18 h, then cooked for 1h in boiling water and washed for 5 days at 25°C. The debittered seeds were oven dried at 60°C, milled and sieved to pass through 250 µ. The debittered flour was defatted with an aqueous ethanol solution (70:30 w/v) for 24 hs by maceration, then filtered, the flour was collected and oven dried at 60°C. The defatted lupin flour was extracted at pH 7 with 1M of NaOH solution for 1h at 30°C, then centrifuged at 3300 xg to eliminate the insoluble fibres and the protein solution was spray-dried (28). The chemical composition of lupin protein concentrate corresponded to 69.4% protein, 4% fat, 5.7% ashes, 21% carbohydrates (27).

The ingredients and lactic acid starter culture used in the present study to obtained the yoghurt were different to those previously reported. The set-type yoghurts were formulated with Manfrey® skim milk powder at 13.5%, vanilla essence at 0.4%, Royal® gelatin at 0.5% and freeze-dried thermophilic culture DVS composed by *Streptococcus termophilus* and *Lactobacilus delbruecki bulgaricus* (CHR HANSEN® YF-L812 No 3381849). The Andean lupin protein concentrate was used at 0.5, 1 and 1.5% in filter water. The protein solutions were stirred at 600 xg for 3 min and heated at 90°C for 10 min, then skim milk powder and gelatine were added. The mix was homogenised and heated at 85°C for 4s. The yoghurt samples were added with vanilla essence and cooled at 45°C to inoculate the lactic acid bacteria culture. Bacteria were inoculated at 0.04% as recommended by the manufacturer. Each yoghurt was placed in individual plastic containers and incubated at 43-45°C. A control sample (without the addition of the

Andean lupin protein concentrate) was also obtained following the same procedure. After reaching pH 4.8, the samples were cooled and refrigerated at 5°C for 24 h (27).

Sensory evaluation

The protocol for the sensory analyses was approved by the Ethics Committee of the Faculty of Health Science of the National University of Salta (DC 714/19). Participants (n= 100) aged between 18-59 years were regular consumers of dairy products, with no food allergies or lactose intolerance and they were recruited at the University. The consumers signed an informed agreement to participate in the study. The fortified yoghurts and the control sample in their containers (40g each) were coded with random three-digit numbers. The samples were presented to the consumers in a balanced rotation order (29). Water and biscuit were also served. The participants were instructed to rinse their mouth between tasting the samples.

The yoghurt samples were evaluated according to their acceptability for colour, flavour, taste, texture and the general impression (overall acceptability) using a nine-point hedonic categorical scale rated from 1 (I dislike it extremely) to 9 (I like it extremely). The Check all that apply (CATA) test was used to evaluate the sensory characteristics of yoghurt samples (30). The participants were instructed to select the attributes that they considered convenient to describe the product they were tasting. The CATA questionnaire was composed by sensory and non-sensory terms, randomly presented within the two groups of terms and between products: *bitter, astringent, milk flavour, medicine-like taste, salty, spicy, bitter aftertaste, sweet, acid, firm, thick, vanilla taste, white, whey separation, healthy, nutritive, I would eat it every day, snack/breakfast, dietetic food, light, ugly/nasty; tasty/pleasant*. The CATA terms were selected according to previous studies in which consumers evaluated yoghurt fortified with dairy proteins (31). At the end of the questionnaire, the consumers had to express appreciations about each product as a sentence.

Physicochemical properties

Since the yoghurts were formulated with different ingredients, the control of physicochemical properties were necessary. The pH value, lactic acid content and percentage of syneresis were determined after 24 h of refrigerated storage. The pH values were evaluated with a HANNA® digital pHmeter and the lactic acid content was evaluated using the acid-basic titration method and phenolphthalein as indicator (32). The syneresis was determined as the percentage of whey expulsion (33). Briefly, yoghurts in their containers were drained face down for 2h, the whey was collected in a tared glass vessel and yoghurts were weighed. The percentage of whey expelled was calculated as the difference between the initial weight of the container and the weight after draining the yoghurt whey.

The chemical composition of fortified yoghurt and the control sample was determined according to the AOAC methods: the moisture content was evaluated by dehydration of samples in vacuum oven at 105°C, the ashes by muffle incineration of samples at 550°C, proteins were determined by the Kjeldhal method and the factor used to convert nitrogen into proteins was 6.25. The fat content was evaluated by applying the Gerber method, in which the separated fat was measured directly in a calibrated butyrometer (32). The total carbohydrate content was calculated by applying the equation= 100 - (moisture + proteins + fats + ashes).

The textural properties were analysed by uniaxial penetration of yoghurt samples using a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK). Each

product in the individual container was conditioned at 8°C. The penetration was done with a 25x35 mm cylindrical flat probe to 45 mm depth at 3 mm s⁻¹ rate and a compression force of 15g. The probe was placed at 20 mm initial distance from each sample. The following parameters were evaluated: the firmness (N) defined as the maximum force to achieve a given deformation which was represented by the peak force of the penetration cycle; the firmness work done (mJ) defined as the energy required to drive the probe during the downward penetration step which was represented by the area under the positive peak. The adhesive force (N) defines as the maximum force generated during the probe upstroke, was represented by the negative peak force. These parameters were calculated by the Texture Expert Exceed software® (34).

Statistical analysis

The results of the acceptability evaluation and physicochemical properties were expressed in means \pm standard deviations. The ANOVA and Tukey's tests were applied to evaluate the differences among yoghurt samples, using a significance level $p < 0.05$. The frequencies of mention of each CATA term selected by the consumers were calculated for each product, and the Cochran's Q test was applied to test differences between the sensory characteristics. Statistical analyses were performed with the student version of the Infostat® software (35).

Results

Sensory evaluation

The consumers who evaluated the yoghurt samples corresponded to 33% of men, 67% women aged between 18-28 (12%), 28-38 (20%), 38-48 (25%) and 48-58 years (43%). The yoghurt with 0.5% lupin protein concentrate and the control sample were acceptable for consumers according to all the attributes evaluated (Table 1). The colour, flavour and texture attributes in yoghurts with 1 and 1.5% of the concentrate were considered indifferent to consumers. The control samples achieved the highest scores for all the attributes evaluated by participants.

Table 1.

Scores for the sensory evaluation of yoghurts fortified with 0.5, 1 and 1.5% Andean lupin protein concentrate and the control sample

Attributes	Y0.5	Y1	Y1.5	Control
Overall acceptability	6.8 \pm 0.7c	3.9 \pm 2.5a	4.7 \pm 2.6b	7.3 \pm 1.7c
Colour	6.9 \pm 0.9c	5.7 \pm 1.3b	5.1 \pm 2.3a	7.1 \pm 1.6c
Flavour	6.9 \pm 0.9c	5.9 \pm 1.5b	4.9 \pm 2.6a	7.2 \pm 1.6c
Texture	7.1 \pm 1.0c	5.9 \pm 1.4b	4.5 \pm 2.3a	7.4 \pm 1.7c
Taste	7.0 \pm 0.5c	3.3 \pm 1.4b	2.7 \pm 2.1a	7.5 \pm 1.7d

Values followed by different letters between columns indicate significant differences ($p < 0.05$).

The CATA test revealed that yoghurt samples achieved significant differences in almost all the attributes, except for the term *firm* (Table 2). The participants indicated that products with greater proportions of lupin concentrate (1 and 1.5%) were less thick and had less superficial whey/liquid (Table 2). The fortified products were described as astringent, pungent, with medicine-like flavour and bitter (Table 2). The residual taste was also perceived in those products. The yoghurt with 1% of protein concentrate was perceived as salty. The fortified yoghurts were also perceived as spicy and less sweet than the control sample. Yoghurts with 1 and 1.5% of protein concentrate were perceived by consumers as products with less milky and vanilla flavour (Table 2). The yoghurts fortified with Andean lupin protein concentrate were perceived as more acidic and with less white colour than the control sample (Table 2).

The product with 0.5% of the protein concentrate and the control sample were considered pleasant according to consumers. The fortified yoghurts with 1 and 1.5% were perceived as less nutritious, healthy, and light than the rest formulations evaluated (Table 2). They were not perceived as dietetic foods, for daily consumption or as a breakfast (Table 2). Some appreciations evoked by participants about these yoghurts were: "*I liked it at the beginning but in the end, I had a bitter and astringent taste*", "*It left in my mouth a medicinal-like taste*". The forms of consumption evoked were: "*If I put fruits, cereal and honey I would eat it as breakfast*", "*If it was accompanied with some fruit, it would be better*". The occasions of consumption these products were in salads or as a dressing as it was expressed by participants: "*in salads would be a good alternative*" or "*as a salad dressing*".

Table 2.

Frequency of mention of sensory characteristics evaluated thorough the CATA test in fortified yoghurts with 0.5, 1 and 1.5% Andean lupin protein concentrate and the control sample

CATA terms	Y0.5	Y1	Y1.5	Control
Bitter	4a	74b	94b	0a
Astringent	15b	22b	18b	6a
Milk flavour	29b	16b	0a	24b
Medicine-like flavour	21b	40b	51b	0a
Salty	0a	6b	0a	0a
Spicy	10b	12b	8b	1a
Bitter aftertaste	38b	32b	61b	3a
Sweet	23b	9a	10a	55c
Firm	61a	64a	64a	53a
Acid	12ab	22ab	37b	0a
White colour	45ab	30ab	23a	61b
Tasty/pleasant	13a	9a	2a	49b
Ugly/nasty	0a	26b	24b	0a
Vanilla taste	24a	33ab	20a	46b
Nutritive	14b	4a	2a	16b
Eat it every day	6a	4a	2a	22b
Snack	2a	0a	8b	35c
Breakfast	6a	2a	0a	19b

Residual taste	39b	32b	39b	10a
Healthy	14a	8a	6a	33b
Light	8a	16a	6a	41b
Dietetic food	20b	28b	10a	31b
Thick	26b	12a	15a	17a
Whey separation	17a	8a	22ab	44b

Values followed by different letters between columns indicate significant differences ($p < 0.05$).

Physicochemical properties

The pH values after 24 h of refrigerate storage corresponded to 4.8 ± 0.0^a in Y0.5; Y1 and Y1.5, while in the control sample it was 4.7 ± 0.0^b . The pH was higher in fortified yoghurts as it was previously observed in yoghurts fortified with legume proteins (25). At day 1, the lactic acid content was 1.2; 1.1 and 1.1 g/100 g in yoghurts fortified with 0.5, 1 and 1.5% protein concentrate and 1.1 g/100g in the control sample. No significant differences were observed in the lactic acid content between samples. The syneresis values corresponded to 0.2 ± 0.0^c ; 0.4 ± 0.1^c and $0.5 \pm 0.2(b)$ in fortified yoghurts, whereas in the control sample it was 2.1 ± 0.0^a .

Table 3 shows the chemical composition of yoghurts. The fortified yoghurts showed lower moisture content than the control sample. The fortified yoghurts also showed higher protein and fat contents, but lower carbohydrate content compared to the control. The content of ashes were similar among fortified and non-fortified yoghurts.

Table 3. Chemical composition (g/100g) of fortified yoghurts with 0.5, 1 and 1.5% Andean lupin protein concentrate and the control sample

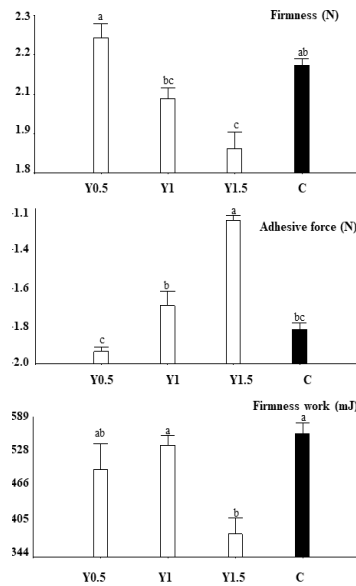
	Y0.5	Y1	Y1.5	Control
Moisture	$80,8 \pm 0,3bc$	$80,4 \pm 0,2b$	$80,0 \pm 0,1c$	$82,6 \pm 0,2a$
Proteins	$6,9 \pm 0,3b$	$6,8 \pm 0,1b$	$7,9 \pm 0,2a$	$5,8 \pm 0,1c$
Fats	$8,1 \pm 0,2b$	$8,5 \pm 0,1b$	$9,0 \pm 0,2c$	$4,2 \pm 0,2a$
Ashes	$1,2 \pm 0,1a$	$1,2 \pm 0,2a$	$1,3 \pm 0,2a$	$1,4 \pm 0,2a$
Carbohydrates	$3,0 \pm 0,2b$	$3,1 \pm 0,2b$	$1,8 \pm 0,2c$	$6,0 \pm 0,0a$

Values followed by different letters between columns indicate significant differences ($p < 0.05$).

Fig. 1 shows the textural properties of yoghurts. The product with 0.5% lupin protein concentrate showed higher firmness but lower adhesiveness compared to the rest of the formulations. Yoghurt with 1.5% showed the highest value for adhesive force. The values for firmness work were similar in fortified yoghurt with 0.5% and 1% protein concentrate and the control sample.

Figure 1

Textural properties (firmness value, adhesiveness and adhesive force value) evaluated in fortified yoghurts with 0.5, 1 and 1.5% Andean lupin protein concentrate and the control sample. Values followed by different letters between bars indicate significant differences ($p < 0.05$).



Discussion and conclusion

Set-type yoghurts fortified with Andean lupin proteins could represent alternatives for consumption in the daily diet. The chemical composition of these products revealed a high protein content ($>5.6\%$), so they could be classified as high-protein foods (3). The fat content was less than 15%, which is an advantage for the protection of cardiovascular health. Food with less content of fats can reduce the blood pressure and cholesterol levels (9). In addition, fortified yoghurt containing live cultures can provide beneficial bacteria which when eaten regularly may support gut health. This is because regular inclusion of fermented foods in diet, including yogurt, increase the microbial diversity of the gut (9). The fermentation also has beneficial effects in the legume ingredient added in the formulation. The process improves the nutritional value of legumes (10,36), such as the protein digestibility and mineral availability. It also reduced the content of antinutritional factors as well as it increases the biological availability of the remanent of total fibre and phenols. The fermentation also improves the viscosity of the products (36) as it was observed in yoghurt with 0.5% protein concentrate (Fig. 1).

To promote the consumption of these promising food, some sensory characteristics should be optimized. The participants perceived the attributes of aroma, colour and texture as indifferent, whereas the flavour and overall acceptability were the properties that they defined as more outstanding and that could limit the consumption (Table 1). An study in which consumers had to express their opinions and perceptions about yoghurts fortified with dairy proteins also found that the addition of whey protein concentrate and caseinates decreased the acceptability and the scores for taste compared to the products supplemented with milk powder (31). The decrease in the acceptability of flavour was explained by the greater perception of the bitter and astringent taste in those products (31). The similar impression could have caused the yoghurt with lupin proteins. In addition, the perception of the bitter taste also influenced the overall acceptability of yoghurt (Table 1 and 2).

The perception of the residual taste and medicine-like flavour in yoghurts could be explained by the interaction between the sweetener and lupin proteins, as well as their binding with the flavour receptors in mouth (37). One strategy raised in a previous study to increase the sensory acceptability was the inclusion of a higher refined product to reduce the perception of bitterness (37). Results of this study showed that the utilization of spray-dried protein extracts of lupins, as a refined product, did not positively impact on the perception of flavour. The bitterness in fortified yoghurts could also be explained due to the presence of plasmin. Plasmin can cause hydrolysis of caseins in yoghurt, leading to the formation of bitter peptides. The bitter taste in yoghurt was positively correlate with astringent mouthfeel. Astringency in milk products can be caused by different compounds, including γ -casein from plasmin-induced degradation of β -CN (3). The combination of lupin proteins with dairy protein powders which have bland flavour as well as the concentration of milk previous to fermentation could provide a high-protein, non-fat yoghurt with good sensory properties (3).

Despite the fortified yoghurts showed similar pH and acidity values measured instrumentally, consumers perceived them as more acidic, which could be explained by two facts. One was that the perception of bitterness could have influenced the perception of acidity or two, the Maillard reaction that may have occurred during the heating in the production of yogurt could have turn lupin proteins more acidic to taste (38). The Maillard reaction is a non-enzymatic reaction that occurs when the carbonyl group of reducing sugars reacts with the amino group of amino acids, polypeptides, or proteins, resulting in the natural production of Maillard reaction products (MRPs), a class of compounds with a wide range of sensory properties (38). Overreactions often turn the food bitter and accumulate burnt flavour. The control of temperature and time of heating of lupin proteins could be an alternative to reduce the Maillard reaction and thus the perception of the acidity in yoghurts.

The rejection of the bitter taste played an important role against the perception of healthy properties. In a previous study, the authors expressed that some consumers are more likely to choose a product perceived as healthier, even if it presents some sensory defect (39). The results of the present research showed that the perception of the healthy attributes was influenced by the taste of yoghurts. According to participants, fortified yoghurts were less dietetic and light than the control (Table 2). Moreover, the fortified yoghurts were perceived as less white than the control sample (Table 2), which could be attributed to the colour of the protein concentrate. Andean lupin concentrate showed a yellow colour tendency, thus, the colour parameters of fortified yoghurts should be measured instrumentally to determine specific differences among products.

The CATA test revealed that yoghurt samples achieved significant differences in almost all the attributes, except for the term *firm* (Table 2). Yoghurts were perceived as similar according to this characteristic, however there were differences in the textural properties of yoghurts (Fig. 1). Previous studies reported an increase in the viscosity of yoghurts due to the addition of lentil and chickpea flours (14, 40) which was explained by the increase in the total solids, as well as the protein and fibre contents of these ingredients. The inclusion of lupin flour in the production of yoghurt turned them less fluid and firmer (25). The lower firmness values and the higher adhesiveness showed in yogurts supplemented with 1.5% Andean lupin protein concentrate could be indicative of a weaker gel network, probably due to a poor interaction between lupin proteins and those of cow's milk (6). The weaker gel could also be attributed to the higher pH values at the end of the fermentation process (4.8). It has been reported that a higher final fermentation pH (\sim 4.8) produced lower apparent viscosity in yoghurts (3). On the other hand, the addition of the lupin protein concentrate was beneficial to reduce the syneresis in

yoghurts, which could be attributed to the ability of legume proteins to absorb the whey expelled by the casein network (6).

The participants evoked new forms of consumption of fortified yoghurts. Although, they would not eat these products in breakfast (Table 2), the combination with fruits, cereal or honey could turn them more acceptable for consumption. The suggestions should be explored in future studies, in terms of sensory and physicochemical properties since it is necessary to determine the shelf life of products with more ingredients. The addition of fruits, cereal or honey in yoghurt can modify the microbiological and physicochemical characteristics (such as pH, acidity and syneresis), which play an important role in consumer acceptability (3). Another tool to increase the consumer acceptability could be the use of other flavouring such as strawberry or peach to mask the bitter taste or the combination of sweeteners of different nature. The bitterness and medicine-like flavour of products (Table 2) may also be perceived due to the interaction of the sweetener with lupin proteins (37), thus, the use of different sweetener could reduce this reaction. The utilization of fortified yoghurts as dressing in salads could also be novel for gastronomy, especially for the elaboration of vegetarian recipes. Work will be done on the development of other products that are more acceptable to the population and that are frequently consumed, such as legume-based snacks or cookies along with other crops of regional interest.

High-protein yoghurts fortified with Andean lupin protein concentrate at 0.5, 1 and 1.5% were obtained. The sensory evaluation with 100 regular yoghurt consumers revealed that only the product with 0.5% protein concentrate was acceptable according to all the attributes evaluated. Fortified yoghurt with Andean lupin proteins were perceived as more acidic, with bitter and residual taste. As the percentage of Andean lupin protein concentrate increased in products, the higher was the bitterness, after-taste, and astringency. In addition, the perception of the healthy properties decreased due to the perception of those sensory defects. The pH values and lactic acid contents were similar in fortified yoghurts compared to the control sample after 1 day of storage. The syneresis was lower in fortified yoghurts than in the control sample. The addition of Andean lupin proteins at 0.5% increase the firmness and decreased the adhesiveness of the product. The addition of fruits, cereals or honey could increase the acceptability of the products as it was evoked by participants. The use of fortified yoghurt as dressings in salads should also be explored.

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