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RESEARCH

The addition of Andean lupin (Lupinus mutabilis) protein concentrate enhances the nutritive value and the antioxidant activity of yoghurt

La adición de un concentrado de proteínas de altramuz andino (Lupinus mutabilis) mejora el valor nutritivo y la actividad antioxidante del yogur

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ABSTRACT

Introduction: Andean lupin seeds are under-exploited sources of nutrients and bioactive compounds.

Materials and methods: Seeds were debittered to remove toxic alkaloids, partially defatted with 70% ethanol, dried at 60°C and milled. The defatted flour was suspended in water adjusted at pH 7 with 1N NaOH. The protein extract was filtered and spray-dried. Enriched yoghurts were obtained with the addition of lupin protein concentrate at 0.5, 1 and 1.5%. The chemical and fatty acid composition, the contents of polyphenols and flavonoids as well as the antioxidant activity were determined. The microbial quality and physicochemical properties (pH values, lactic acid content and percentage of syneresis) were evaluated in yoghurts during 28 days of storage.

Results: Andean lupin concentrate showed high protein and carbohydrate contents (69% and 20%), unsaturated fatty acids (77%) mainly oleic, linoleic and α-linolenic acids as well as low atherogenic and thrombogenic indexes (0.07 and 0.44). The addition of the lupin protein concentrate at 1.5% into yoghurts increased the protein content. Enriched yoghurts showed higher unsaturated fatty acids, total polyphenol and flavonoid contents as well as lower atherogenic and thrombogenic indexes compared to the control. The lupin protein concentrate increased the ABTS radical scavenging activity, the ferric reducing power and the total antioxidant activity in yoghurts. Enriched and control yoghurts showed similar lactic acid bacterial counts. The counts for total and thermotolerant coliforms were under three MPN/mL and yeast and molds under 10 CFU/ mL. The pH values in enriched products were higher at days 1 and 7 of storage (4.82 and 4.95) compared to the control sample (4.72 and 4.85). The lactic acid content increased in all formulations at day 21. Enriched yoghurts showed lower syneresis than the control.

Conclusions: Yoghurts enriched with lupin protein concentrate could represent nutritive and functional alternatives to prevent chronic diseases when consuming in a healthy diet.

Keywords: Lupinus; proteins; unsaturated fatty acids; polyphenols; flavonoids

RESUMEN

Introducción: Las semillas de altramuces andinos son fuentes poco explotadas de nutrientes y compuestos bioactivos.

Materiales y Métodos: las semillas de altramuz se desamargaron para eliminar alcaloides tóxicos, se desgrasaron parcialmente con etanol al 70%, se secaron a 60 ° C y se molieron. La harina desgrasada se suspendió en agua ajustada a pH 7 con NaOH 1 N y el extracto de proteína se filtró y se secó por pulverización. Se obtuvieron yogures enriquecidos con la adición de concentrado de proteína de altramuz al 0,5, 1 y 1,5%. Se determinó la composición química y de ácidos grasos, el contenido de polifenoles y flavonoides, así como la actividad antioxidante. Se evaluó la calidad microbiana y las propiedades fisicoquímicas (valores de pH, contenido de ácido láctico y porcentaje de sinéresis) en yogures durante 28 días de almacenamiento.

Resultados: El concentrado de altramuz andino presentó altos contenidos de proteínas y carbohidratos (69% y 20%), ácidos grasos insaturados (77%) principalmente ácidos oleico, linoleico y α-linolénico, así como bajos índices aterogénicos y trombogénicos (0,07 y 0,44). La adición de concentrado de proteína de altramuz al 1,5% en los yogures aumentó el contenido de proteína. Los yogures enriquecidos mostraron mayor contenido de ácidos grasos insaturados, polifenoles totales y flavonoides, así como índices aterogénicos y trombogénicos más bajos en comparación con el control. El concentrado de altramuz aumentó la actividad captadora de radicales ABTS, el poder reductor férrico y la actividad antioxidante total en yogures. Los yogures enriquecidos y control mostraron recuentos de bacterias de ácido láctico similares. Los recuentos de coliformes totales y termotolerantes se mantuvieron por debajo de tres NMP/mL, levaduras y mohos por debajo de 10 UFC/mL. Los valores de pH en los productos enriquecidos fueron mayores en los días 1 y 7 de almacenamiento (4,82 y 4,95) en comparación con la muestra control (4,72 y 4,85). El contenido de ácido láctico aumentó en todas las formulaciones al día 21. Los yogures enriquecidos mostraron menor sinéresis que el control.

Conclusiones: Los yogures enriquecidos con concentrado de proteína de altramuz podrían representar alternativas nutritivas y funcionales para prevenir enfermedades crónicas al consumirse en una dieta saludable.

Palabras clave: Lupinus; proteínas; ácidos grasos insaturados; polifenoles; flavonoides

KEY MESSAGES

- Low atherogenic and thrombogenic indexes were evinced in the protein concentrate.
- The lupin protein concentrate when adding into yoghurts increased the ABTS radical scavenging activity, the ferric reducing power and the total antioxidant activity
- Yoghurts enriched with lupin protein concentrate could represent nutritive and functional alternatives to prevent chronic diseases when consuming in a healthy diet.

INTRODUCTION

Andean lupin (family: Fabaceae, genus: *Lupinus*, species: *Lupinus mutabilis*) is an under-exploited legume originated in the Andean region (from Ecuador to the northwest of Argentina (1). Seeds are sources of nutrient such as proteins (35-50%), essential fatty acids (oleic and linoleic acids) as well as insoluble dietary fiber (30-40%) (1,2). Andean lupin also has bioactive compounds (i.e, polyphenols and flavonoids) (2). The flavonoid compounds are located in the cotyledon of seeds and correspond to the isoflavones class (9.8 to 87 mg/100g) (3). It has been reported that lupin isoflavones have antioxidant activity (3).

Seeds have toxic and bitter alkaloids that must be eliminated after consumption (1). The alkaloid level for a safety consumption has been stablished in 0.02-0.07 g/100g (4). The traditional aqueous debittering process to eliminate alkaloids includes a soaking step of seeds at variable temperatures (25-60°C), followed by cooking and washing for 3-5 days, until no bitter test is appreciable (5). After debittering, flours and protein concentrates can be obtained to enrich the protein content of pasta, snacks and bread (6). Moreover, the protein concentrates from other lupin species (L. angustifolius) showed high unsaturated fatty acid content (above 60%) and remaining compounds such as polyphenols (7).

Yoghurt is, among dairy, the highly consumed food in world. It is an excellent vehicle to deliver proteins, minerals and bioactive compounds (8). Legume flours and protein concentrates from i.e., lentil, chickpea and pea have been added into yoghurts to enriched the protein and fiber content, as well as to promote the growth of lactic acid bacteria (9, 10, 11). Enriched yoghurts presented higher viscosity and similar physicochemical properties (pH and lactic acid content) than non-enriched samples (9, 10, 11). The oligosaccharides of lentil flour also promoted antioxidant properties in yoghurts (9). On the other hand, the aqueous extracts of mushroom and olive fruits increased the contents of polyphenols in yoghurts as well as the antioxidant activity (8). Essential oils from peppermint and lemon grass leaves increased the unsaturated fatty acid content and preserved the microbial quality by inhibiting the growth of pathogenic bacteria (8). In this sense, Andean lupin protein concentrate could be added into yoghurt not only to increase the nutritional value but also to promote other functional properties such as antioxidant. We hypothesized that the use of Andean lupin protein concentrate in the formulation of yoghurts would enhance the nutritive value (contents of proteins and unsaturated fatty acids) and increase

the antioxidant activity of yoghurt, without changes in the physicochemical properties and microbial quality. Therefore, the aim of this study was to obtain a protein concentrate from edible Andean lupin seeds to enhance the nutritive value and antioxidant activity of yoghurts. The chemical and fatty acid composition and the contents of polyphenols and flavonoids were evaluated in the protein concentrate and enriched yoghurts. In addition, the antioxidant activity and stability during storage (physicochemical properties and microbial quality) of yoghurts were analyzed.

MATERIALS AND METHODS

Lupin protein concentrate

Andean lupin seeds were debittered by soaking at 25 ºC for 18 h, cooking at 97 °C for 1 h, and washing for five days (5). Seeds were dried at 60°C and milled. The alkaloid content of dried lupin seeds was determined by titration with 1N NaOH after the extraction with chloroform (12). Results were expressed as g of lupanine/100g in a dry basis (d.b.).

Dried edible seeds were partially defatted with an aqueous-ethanol solution (30:70 v/v; 1:4 w/v, 24h), to facilitate the production of the protein concentrate (13). After 24 h of extraction, the solid phase was recovered by filtration and the flour dried at 60 °C (13). The defatted four was suspended at 30 ºC (1:10 w/v); adjusted to pH 7 with 1N NaOH and extracted for 1 h under constant stirring (7). The lupin enriched extract was separated from the insoluble fibers by centrifuging at 3300g (7). To extend the conservation of lupin extract, it was spray-dried at constant temperature of hot air = 170 °C and exhausted air= 80 °C using 2% maltodextrin as wall-material.

Yoghurt production

The lupin protein concentrate was suspended in filter water at 0.5, 1, 1.5, 3, 5, 7, 10%, stirred at 600xg for 3 min (until undissolved particles were not observed) and heated at 90°C for 10 min, then cow´s skimmed milk powder (13.5%), sweetener composed by sucralose and potassium acesulfame (1.5%) and gelatin (0.5%) were added to the suspensions. The products were heated at 85 ºC for 4 s, added with vanilla (0.4 %) and potassium sorbate (0.3%) and cooled at 44°C. Lactic acid bacteria composed by Streptococcus thermophilus and Lactobacillus delbrueckii

bulgaricus (Lyofast Sacco® Y 450B, 5U, C1985970A) were added in quantities recommended by the manufacturer (0.04%). Yoghurts were placed in individual plastic containers of 40 mL (50 x 50 x 75 mm of width x depth x height) and incubated at 43-45 ºC until they reached pH 4.8 (14). A control yoghurt without the addition of lupin concentrate (CY) was also obtained. Products were stored at 5°C.

To determine the final concentrations in which the protein concentrate could be added into yoghurts, the flavor of each formulation was tested by a laboratory panel. The final formulations used to determine the nutritional value and antioxidant activity were yoghurts enriched with 0.5, 1 and 1.5% of lupin protein concentrate (hereafter: Y0.5, Y1 and Y1.5).

Chemical composition

The chemical composition of the lupin protein concentrate and yoghurts (Y0.5, Y1 and Y1.5) was analyzed according to the AOAC official methods (15). The moisture content was evaluated after dehydration of samples at 100-105 ºC, ashes after calcination at 550 ºC, and proteins with the Kjeldhal method (N x 6.25). The fat content of the protein concentrate was analyzed by Soxhlet extraction with petroleum ether at 60 ºC for 16 h (15). Yoghurts were analyzed for their fat contents by applying the Monjonnier method (16). The percentage of carbohydrates were calculated as follows: 100% – (moisture+ protein+ ashes+ fat). Results of the protein concentrate and yoghurts were expressed in g/100g dry basis and in g/100 mL of fresh weight (f.w.), respectively.

Fatty acid composition

The fatty acid profile was evaluated in fatty acid methyl esters (FAME) by GC – IT/ MS using a CGC Agilent 6850 chromatograph (17). FAME were separated with a DB-23 Agilent capillary column (60 m length × 0.25 mm internal diameter × 0.25 µm thickness). The operation conditions were: column flow = 1.0 mL/min; linear velocity = 24 cm/s; detector temperature = 280 °C; injector temperature = 250 °C; oven temperature = 110–215 °C to 5 °C/min; 215 °C for 24 min; carrier gas - helium; injection volume = 2 µL; injection split ratio 1:50 (11). The total saturated (SFAs), unsaturated (UFAs), monounsaturated (MUFAs), and polyunsaturated (PUFAs) fatty acids were calculated as the sums of individual fatty acids. The UFA/SFA ratio, the atherogenic (AI) and thrombogenic (TI) indexes were calculated according to the equations AI= C12:00+C14:00+C16:00/ 4 (n-6+n-3) + C18:1 + other MUFAs and TI= C14:00+C16:00+C18:00/ 0.5

C18:1 + 0.5 another MUFAs + 0.5 n-6 + 3 n-3 + (n-3/ n-6) (18). Results of the fatty acid composition were expressed in g/100g of the total fatty acids identified.

Polyphenols and flavonoids

The lupin protein concentrate was reconstituted in 70% aqueous ethanol solutions (1:10 w/v) and left to stand for 24 h, then filtered and the extract collected (19). Yoghurts were freeze-dried and then 0.5g of each sample were extracted with 19.20 mL of 50% ethanol for 2 h under shaking and then centrifuged at 3300g, for 1 h at room temperature (20). The contents of total polyphenols (TPC) were analyzed in ethanolic extracts with the Folin-Ciocalteu reagent at 765 nm (21). Gallic acid (GA) was used as standard and results were expressed in mg of GA equivalents/100 mL of the ethanolic extract. Flavonoids (TFC) were determined with NaNO2 and AlCl3 reagents at 510 nm (19). Catechin was used as standard and results were expressed in mg of catechin equivalents (CE)/100 mL of the extract.

Antioxidant activity

ABTS radical scavenging activity

The ABTS radical scavenging activity was evaluated using the ABTS radical dissolved in distilled water to yield a 7 mM solution. The solution was incubated with a 2.45 mM potassium persulfate solution for 16 h in darkness at room temperature and subsequently diluted with methanol to a final absorbance of 0.7 at 734 nm. To determine the antioxidant activity, 100 µL of the ethanolic extract was placed in a cuvette containing 300 µL of the ABTS solution. The absorbance was measured at 734 nm, and results were expressed as SC50 values (the concentration of the extract in mg GAE necessary to scavenge 50% of the ABTS radical) (22).

Phosphomolybdenum inhibition

The total antioxidant capacity assay was carried out following the phosphomolybdenum method (23). The ethanolic extract (100 µL) was shaken with 1 mL of the reagent solution (0.6N sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in test tubes, covered and incubated in a water bath at 95°C for 90 min. After cooling, the absorbance was measured at 765 nm. The antioxidant capacity was expressed in mg of ascorbic acid equivalents (AAE)/100 mL extract.

Ferric reducing power

The ferric reducing power (FRP) was evaluated in aliquots of ethanolic extracts (500 µL) mixed with phosphate buffer (250 µL 0.2M, pH 6.6) and potassium ferricyanide (250 µL) and kept at 50 °C for 20 minutes. A trichloroacetic acid solution (2 mL, 100 mg/L) (250 µL) and 0.1% (w/v) ferric chloride solution (250 µl) were added and the absorbance was measured after 10 minutes at 700 nm. The increase in the absorbance of the reaction mixture suggests a high reducing power. Results were expressed in mg ascorbic acid equivalents AAE/mL of extract (24).

Microbial quality of yoghurts

Microbiological analyses were conducted at 1st, 14th, and 28th days of storage according to the American Public Health Association (APHA) methods (25). Considerable changes in bacterial counts are not expected through storage, so the three stipulated times in which they were evaluated are generally sufficient to determine the microbial quality of the products. The viable counts of Streptococcus thermophilus were enumerated in M17 agar after incubation in aerobiosis at 37 °C for 72 h. The acidified De Man-Rogosa-Sharpe agar (pH 5.4, acidified with 1N HCl) was used to enumerate the Lactobacillus delbrueckii bulgaricus after anaerobic incubation at 37 °C for 72 h in AnaeroGen™ jars (ThermoFisher Scientific, Rodano, Italy). The lactic acid bacteria counts were expressed as logarithm of colony-forming unit CFU/mL. Tryptic Lauryl Sulphate culture medium incubated at 35 °C for 48 h and Brilliant Green Bile medium incubated at 35 ºC for 48 h were used to identified the total coliforms. Thermotolerant coliforms were identified in Escherichia coli medium incubated at 45 ºC for 24 h. Results of total and thermotolerant coliforms were expressed as the Most Probable Number (MPN)/mL. Yeast and molds were incubated in Sabouraud Dextrose Agar at 25 ºC for 5 days and expressed in log CFU/mL.

Physicochemical properties of yoghurts

The pH values, lactic acid content and spontaneous whey expulsion (syneresis) were determined at 1st, 7th, 14th, 21st, and 28th days of storage. The pH was measured with a Tecnopon mPA210 and the lactic acid content was evaluated by titration with 1N NaOH and expressed in g/100 mL (26). The syneresis was determined as the spontaneous whey expulsion after graining yoghurts face down in their individual containers for 2 h (27). Results were expressed as the difference between the initial and the final weights of samples in percentage (27).

Statistical analyses

Results are expressed as mean ± standard deviation. A Shapiro Williams test was conducted to check the normality of the distributions. One-way analysis of variance with multiple comparison Tukey´s test was conducted to test differences among enriched yoghurts and the control sample. All statistical analyses were performed with Infostat software® (student version) (28).

RESULTS

Lupin protein concentrate

The debittered lupin flour showed an alkaloid content of 0.01±0.00g/100g d.b., which was in accordance with the legislation for a safety consumption (0.02-0.07g/100g) (4). The moisture content in the protein concentrate was 6.60±0.23g/100g, protein 69.36±0.00, fat 4.01±0.32 and ashes 5.67±0.33 g/100g. The total carbohydrate content was 20.96±0.40 g/100g. Lower contents of protein (67.95%) and fat (1%) were evaluated in L. angustifolius protein concentrate (7). The differences could be attributed to the initial chemical composition since L. angustifolius seeds have lower contents of protein (33.9%) and fat (6%) than Andean lupin grains (40% and 18%) (1). In addition, L. angustifolius seeds have low alkaloid content (0.7% compared to 2.8% of Andean lupin raw seeds) so they do not need to undergo a debittering process (1). The debittering have been shown to increase the fat in Andean lupin seeds (1), conditioning the subsequent extraction with the defatting solvent. On the other hand, the remaining carbohydrates of the lupin concentrate may correspond to soluble fibers such as oligosaccharides since the major insoluble fibers were separated after centrifugation (29). It would be interesting to evaluate the amount and composition of dietary fiber, however, the focus of this study was to use lupin proteins and remaining compounds (i.e., fatty acids and antioxidants). The chemical composition of the protein concentrate was in accordance with that of commercial fava bean protein concentrate in which it was reported an average protein content of 65%, 3.5-4% fat, 5.5-7% ashes and a carbohydrate content of 17% (30).

Table 1 shows the fatty acid composition of lupin protein concentrate. The palmitic and stearic fatty acids were the major SFAs, whereas the oleic and linoleic fatty acids were the main MUFAs and PUFAs. Total MUFAs and PUFAs accounted for 76% of the fatty acids identified in the concentrated which also increased the UFAs/SFAs ratio (Table 1). The MUFAs and PUFAs content

reported in L. angustifolius concentrate were similar (40% and 27%) to those observed in this study (7). When comparing with the debittered lupin flour, it showed higher total MUFAs (56%) and lower SFAs and PUFAs (15 and 28%) (29). The unsaturated fatty acids could have converted into saturated ones during the extraction of proteins (31). The ethanol used in the defatting process could also have contributed to remove some unsaturated fatty acids (32).

The lupin protein concentrate showed lower AI than that from the debittered lupin flour (0.1) (Table 1). In contrast, the TI was higher compared to other lupin flours (0.1-0.2) (33). The higher TI could be attributed to a higher stearic acid content as well as the presence of trans fatty acids (34).

The lupin protein concentrate showed TPC and TFC of 14.30 mgGAE/100g and 14.10 mgCE/100g. The total phenolic compound content was lower than those of other lupin species (L. albus, L. luteus) varying from 491.51 to 731.14 mg/100 g d.b (35). However, the analyses were conducted in raw seeds instead of protein concentrates. The debittering and defatting processes in seeds could have contributed to the loss of polyphenols or their conversion in other compounds (36). Defatted horse gram flour showed similar total phenolic content (14.3 mg/100g) than lupin concentrate but lower flavonoid content (8.6 g/100g) (37). Some flavonoid compounds detected in the lupin concentrate may corresponded to isoflavones since their presence was reported in seeds (3). In addition, Andean lupin isoflavones presented antioxidant activity (3).

Chemical composition of enriched yoghurts

Table 2 shows the chemical composition of yoghurts enriched with Andean lupin protein concentrate and the control sample.

Table 1. Fatty acid composition (g/100g of the total fatty acids identified) of Andean lupin protein concentrate

Fatty acid composition	Enriched extract
Saturated fatty acids (SFAs)	
C10:0 (capric acid)	0.00±0.00
C12:0 (lauric acid)	0.31±0.10
C14:0 (myristic acid)	0.31±0.08
C15:0 (pentadecylic acid)	0.05±0.01
C16:0 (palmitic acid)	12.90±0.04
C16:1 (palmitoleic acid)	0.32±0.02
C17:0 (heptadecanoic acid)	0.03±0.03
C17:1 (heptadecenoic acid)	0.04±0.01
C18:0 (stearic acid)	6.80±0.31
C20:0 (arachidic acid)	0.65±0.05
C22:0 (behenic acid)	0.80±0.01
C24:0 (lignoceric acid)	0.27±0.04
Total SFAs	22.45±0.16
Monounsaturated fatty acids (MUFAs)	
C18:1 trans (elaidic acid)	0.57±0.07
C18:1 (oleic acid)	44.4±0.20
C20:1 (eicosenoic acid)	0.13±0.01
Total MUFAs	45.10±0.13
Polyunsaturated fatty acids (PUFAs)	
C18:2 trans (linolelaidic acid)	0.10±0.00
C18:2 (linoleic acid)	29.86±0.04
C18:3 (α-linolenic acid)	2.32±0.06
Total PUFAs	32.27±0.10
UFAs/SFAs ratio	3.40±0.02
Atherogenic index (AI)	0.07±0.02
Thrombogenic index (TI)	0.44±0.02

Table 2. Chemical composition (g/100 mL fresh weight) of yoghurts enriched with Andean lupin protein concentrate at 0.5, 1 and 1.5% (Y0.5, Y1, Y1.5) and control yoghurt (CY).

Fatty acid composition	**Y0.5**	**Y1**	**Y2**	**Y3**
Chemical composition				
Moisture	84.48±0.24^{bc}	84.94±0.06^{ab}	84.14±0.01^{c}	85.13±0.05^{a}
Protein	5.93±0.21^{b}	5.80±0.01^{b}	7.45±0.06^{a}	6.00±0.12^{b}
Fat	6.97±0.04^{b}	7.75±0.10^{a}	6.08±0.08^{c}	7.48±0.19^{ab}
Ash	1.52±0.07^{a}	1.49±0.11^{a}	1.74±0.08^{a}	1.40±0.06^{a}
Carbohydrates	1.10± 0.30^{b}	0.02±0.04^{a}	0.50±0.06^{ab}	0.01±0.00^{c}

Means ± standard errors (n=3) followed by a different letter between columns are significantly different ($p<0.05$).

The moisture contents were similar among enriched yoghurts, but lower compared to the control sample. The addition of the lupin protein concentrate significantly increased the protein content in Y1.5 (Table 2). Yoghurts supplemented with lentil flour and protein concentrates from pinto and kidney beans also showed higher protein contents compared to non-supplemented yoghurts (37,38). The reported protein content varied from 5.6 to 8.6 at levels up to 4%, similar to those observed in this study (37). Y1.5 showed lower fat content compared to Y0.5, Y1 and CY (Table 2). Results are in contrast with those of yoghurts supplemented with lentil flour and kidney bean protein concentrates, in which the fat content increased with the increase in the fortification levels (37,38). Different concentrations of the supplements used (up to 10%) as well as their lower protein contents (below 30%) could explain the increases (38). The addition of the lupin concentrate increased the carbohydrate content in enriched yoghurts; however, the highest content was observed in Y0.5 (Table 2). (37) reported an increase in the fiber content when lentil flour was added into yoghurts. The evaluation of the total and soluble dietary fiber could be useful to discriminate polysaccharides from sugars in yoghurts enriched with the lupin concentrate. Finally, no significant differences were observed in the ash content in enriched yoghurts and the control sample. An increase in the ash content was reported when protein concentrates from pinto and kidney beans were added into yoghurts; mainly attributed to the higher fortification level (from 2.5 to 10%) (38).

Fatty acid composition, TPC and TFC of enriched yoghurts

Table 3 shows the fatty acid composition, TPC and TFC of yoghurts enriched with lupin concentrate and the control sample. Enriched yoghurts showed lower contents of SFAs compared to the control sample. Results could be explained by the lower concentration of myristic and capric fatty acids in enriched yoghurts (Table 3). The MUFAs increased in enriched yoghurts, in particular the oleic acid content. In addition, the lupin concentrate increased the PUFAs in yoghurts, attributed to the increase in the linoleic and α- linolenic acid contents (Table 3). Similar results were reported in yoghurts made from peanut and groundnut milks, which showed higher oleic and linoleic acids and lower saturated fatty acids compared to the cow´s yoghurt (39). Higher α-linolenic acid content was evinced in Y1 and Y1.5, whereas Y1.5 and CY showed higher cervonic acid content (Table 3).

Lupin concentrate is rich in α-linolenic acid compared to groundnut (0.54 g/100g) and cow´s milk (0.5) (39), so its addition effectively increased the content of this fatty acid in enriched yoghurts. Yoghurts enriched with lupin concentrate also showed higher UFA/SFA ratios than the control sample, because of their higher contents of MUFAs and PUFAs (Table 3). Consequently, lower AI and TI were evinced in enriched yoghurts. The AI and TI decreased with the increase in the fortification level (Table 3). The AI indicates the relationship between the sum of saturated and unsaturated fatty acids. The main classes of SFAs, which include C12:0, C14:0, and C16:0, with the exception of C18:0, are considered pro-atherogenic (they favor the adhesion of lipids to cells of the circulatory and immunological systems) (34). UFAs are considered to be anti-atherogenic as they inhibit the accumulation of plaque and reduce the levels of phospholipids, cholesterol, and esterified fatty acids. The TI characterizes the thrombogenic potential of fatty acids, indicating the tendency to form clots in blood vessels (34). Therefore, the consumption of enriched yoghurts with the lupin concentrate showing low AI and TI, could have beneficial effects on cardiovascular health.

A higher content of the unidentified fatty acid was detected in the control sample compared to enriched yoghurts (Table 3). This fatty acid could be typical of cow's milk such as 16:1 trans-3 fatty acid. The isomer had been reported to decrease in milk when cows were fed with diets enriched in plant oils (40).

Table 3. Chemical composition (g/100 mL fresh weight) of yoghurts enriched with Andean lupin protein concentrate at 0.5, 1 and 1.5% (Y0.5, Y1, Y1.5) and control yoghurt (CY).

Fatty acid composition	Y0.5	Y1	Y2	Y3
Saturated fatty acids (SFAs)				
C6:0 (caproic acid)	2.55±0.25^{a}	1.70±0.00^{a}	1.55±0.25^{a}	1.55±0.05^{a}
C8:0 (caprylic acid)	4.20±0.70^{a}	3.65±0.35^{a}	5.85±1.65^{a}	9.30±0.10^{a}
C10:0 (capric acid)	1.50±0.10^{a}	1.75±0.05^{a}	1.87±0.17^{a}	3.30±0.10^{b}
C12:0 (lauric acid)	2.40±0.30^{a}	2.30±0.10^{a}	2.40±0.10^{a}	2.90±0.00^{a}
C14:0 (myristic acid)	6.55±0.45^{b}	7.30±0.40^{ab}	7.49±0.42^{ab}	8.90±0.00^{a}
C15:0 (pentadecylic acid)	0.65±0.05^{a}	0.75±0.05^{a}	0.75±0.05^{a}	0.85±0.05^{a}
C16:0 (palmitic acid)	25.75±0.55^{a}	26.95±0.35^{a}	26.15±0.95^{a}	28.10±0.20^{a}
C16:1 (palmitoleic acid)	1.00±0.20^{a}	0.95±0.15^{a}	1.10±0.00^{a}	1.40±0.10^{a}
C17:0 (heptadecanoic acid)	0.40±0.00^{a}	0.50±0.00^{a}	0.45±0.05^{a}	0.50±0.00^{a}
C17:1 (heptadecenoic acid)	0.15±0.05^{a}	0.10±0.00^{a}	0.10±0.00^{a}	0.10±0.00^{a}
C18:0 (stearic acid)	14.25±2.55^{a}	13.65±0.55^{a}	11.45±0.35^{a}	12.50±0.20^{a}
C20:0 (arachidic acid)	0.40±0.10^{a}	0.40±0.00^{a}	0.40±0.00^{a}	0.35±0.05^{a}
C22:0 (behenic acid)	0.40±0.00^{a}	0.45±0.05^{a}	0.50±0.00^{a}	0.45±0.05^{a}
C24: 0 (lignoceric acid)	0.40±0.00^{a}	0.40±0.00^{a}	0.45±0.05^{a}	0.40±0.00^{a}
Total SFAs	60.60±1.10^{a}	60.85±0.65^{a}	60.50±0.03^{a}	70.60±0.60^{b}
Monounsaturated fatty acids (MUFAs)				
C18:1 trans (vaccenic acid)	1.35±0.15^{a}	1.75±0.15^{a}	1.65±0.25^{a}	1.70±0.20^{a}
C18:1 (oleic acid)	23.75±0.25^{a}	24.15±0.35^{a}	24.50±0.10^{a}	21.30±0.20^{b}
C20:1 (eicosenoic acid)	0.25±0.05^{a}	0.30±0.00^{a}	0.30±0.00^{a}	0.30±0.00^{a}
Total MUFAs	25.35±0.15^{a}	26.20±0.50^{a}	26.45±0.15^{a}	23.30±0.00^{b}
Polyunsaturated fatty acids (PUFAs)				
C18:2 trans (linolelaidic acid)	0.20±0.10^{a}	0.08±0.01^{a}	0.05±0.05^{a}	0.00±0.00^{a}
C18:2 (linoleic acid)	5.60±2.00^{ab}	6.35±0.05^{ab}	9.30±0.20^{a}	3.09±0.02^{b}
C18:3 (α-linolenic acid)	0.45±0.15^{bc}	2.00±0.00^{a}	0.85±0.05^{b}	0.35±0.05^{c}
C18:4 (cervonic acid)	0.10±0.00^{c}	0.22±0.02^{b}	0.30±0.00^{a}	0.30±0.00^{a}
C20:4 (arachidonic acid)	0.15±0.05^{a}	0.20±0.00^{a}	0.20±0.00^{a}	0.20±0.00^{a}
Total PUFAs	6.50±2.30^{ab}	8.85±0.04^{ab}	10.70±0.20^{a}	3.94±0.06^{b}

Non identified	1.80±0.30^{ab}	1.10±0.10^{b}	2.20±0.40^{ab}	2.70±0.10^{a}
UFA/SFA ratio	0.53±0.05^{a}	0.58±0.02^{a}	0.62±0.01^{a}	0.39±0.01^{b}
Atherogenic index (AI)	0.70±0.01^{b}	0.68±0.01^{b}	0.54±0.02^{a}	1.08±0.01^{c}
Thrombogenic index (TI)	1.62±0.05^{a}	1.39±0.02^{b}	1.52±0.01^{a}	1.98±0.01^{c}
Total polyphenol content (TPC)	4.10±0.16^{a}	4.30±0.18^{ab}	4.29±0.18^{ab}	2.84±0.12^{c}
Total flavonoid content (TFC)	0.42±1.54^{a}	0.59±0.37^{b}	0.63±0.68^{b}	0.36±0.08^{c}

Means ± standard errors (n=3) followed by a different letter between columns are significantly different ($p<0.05$)

The addition of the lupin protein concentrate significantly increased the TPC and TFC in yoghurts (Table 3); as it was previously observed in products enriched with olive fruit, Oyster mushroom extract and mung bean milk (8). The fermentation process could have increased the flavonoid content, as it was reported in yoghurt alternative based on a mixture of cow's milk and chickpea extract (41). Both the lactic acid bacteria mediated and the natural fermentations increased the total phenolic content in all legume varieties (41).

Antioxidant activity of enriched yoghurts

The antioxidant activity of enriched yoghurts was evaluated with three methods and compared to the control sample. The ABTS radical scavenging activity showed a tendency to increase with the addition of the lupin concentrate. The SC50 values were 10.56±0.15, 18.85±0.25 and 40.16±0.25 mgGAE/mL in Y0.5, Y1 and Y1.5, compared to 2.77±0.17 mgGAE/mL in the CY. The FRP corresponded to 15.64±0.10, 28.47±0.09 and 50.27±0.13 mgAAE/mL in enriched yoghurts. Lower FRP was observed in the control sample (7.45±0.13 mgAAE/mL). The total antioxidant activity measured with the phosphomolybdenun method was higher in Y1.5 (64.29±0.15 mgAAE/m) compared to Y1 (48.62±0.14) Y0.5 (28.68±0.12) and CY (10.35±0.15 mgAAE/mL). The association between polyphenols and remaining oligosaccharides of the lupin concentrate could be responsible for the antioxidant activity of yoghurts; as it was previously reported in yoghurts supplemented with lentil flour (9). It would be interesting to evaluate the presence of specific peptides with functional activity such as angiotensin-converting enzyme inhibitory peptides; since they have been shown to increase the antioxidant activity in fermented legume milks and flours (41).

Microbial quality

The counts for S. thermophilus in Y0.5, Y1, Y1.5 and CY were 8.54±0.08, 8.54±0.02, 8.48±0.1, 8.85±0.02 log CFU/mL at day 1 of storage. No significant differences were observed among yoghurts. At day 14, Y0.5 showed the lowest counts for S. thermophilus (8.13±0.03) compared to those of Y1, Y1.5 and the CY (8.91±0.06, 8.79±0.05, 8.78±0.00 log CFU/mL). Enriched yoghurts and the control sample showed similar counts for S. thermophilus at day 28 (8.01±0.06, 8.57±0.14, 8.68±0.05 and 8.34±0.02 log CFU/mL).

At days 1 and 14, the L. bulgaricus counts were similar in Y0.5 and CY (6.12±0.00 and 6.07±0.01 log CFU/mL and 5.75±0.03 and 6.04±0.02 log CFU/mL) but higher compared to those of Y1 and Y1.5 (5.31±0.07, 5.61±0.02 and 5.29±0.03, 5.61±0.03 log CFU/mL). No significant differences were observed in the L. bulgaricus counts at day 28 (Y0.5=6.14±0.08, Y1:5.74±0.06, Y1.5=5.56±0.06 and CY=5.80±0.08 log CFU/mL). Results are in accordance with those reported in yoghurts supplemented protein concentrates from kidney and pinto beans as well as lentil flour; in which no variation in the total bacteria counts was observed (38).

The total and thermotolerant coliforms counts were under three MPN/mL in all samples during storage. Yeast and molds were under 10 CFU/mL in enriched yoghurts and the control sample. The inhibitory effects against pathogenic bacteria could be associated to the presence of potassium sorbate as well as essential oils from the lupin protein concentrate. In previous studies, the addition of zataria, basil, or peppermint

essential oil into probiotic yoghurt formulations provided an inhibitory effect against

Listeria monocytogenes and Escherichia coli (8). Polyphenols and oils play a role in the decontamination from mycotoxigenic fungi and mycotoxins formation in yoghurt (8).

Physicochemical analyses

Figure 1 shows the physicochemical properties of enriched yoghurts and the control sample during storage. At day 1 the pH values of enriched yoghurts were higher than the control sample. The pH increased at day 7 of storage in all samples, however it was significant higher in Y1. From day 14 to 28 all the formulations had similar pH values. Yoghurts fortified with protein concentrates from pinto bean showed higher initial pH values due to the low acidity of legume seeds (38).

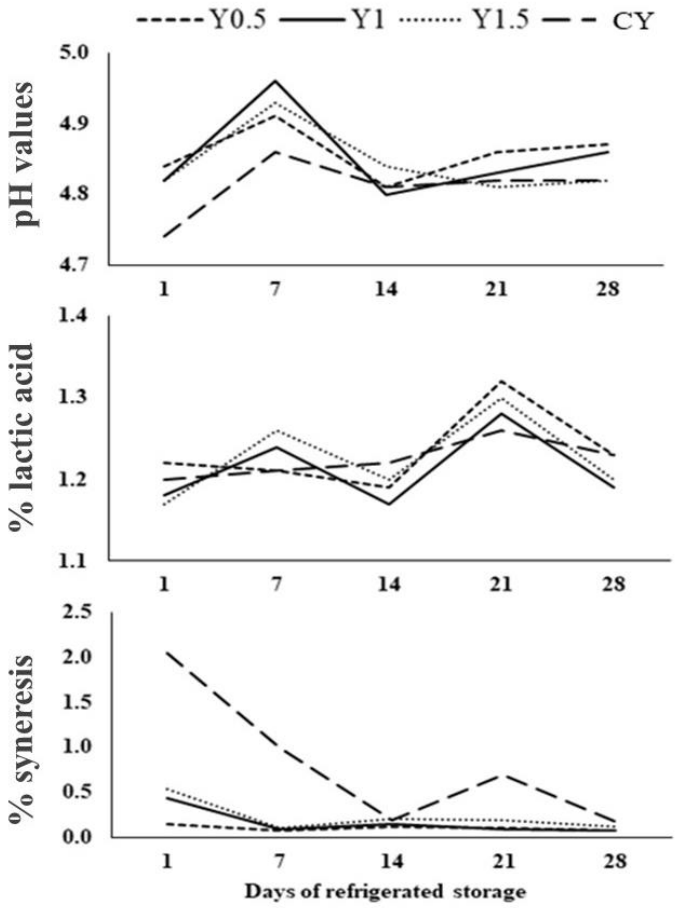

Figure 1. Physicochemical of yoghurts enriched with lupin extract at 0.5, 1 and 1.5% (Y0.5, Y1 and Y0.5) and control sample (CY).

No significant differences were observed in the lactic acid content among yoghurts at 1st, 7th and 14th days of storage (Fig. 1). The lactic acid content increased in the control sample and enriched yoghurts at day 21; which could be attributed to the possible arrangement between lupin and cow proteins or an increase in the activity of lactic acid bacteria (8). The syneresis was higher in CY compared to enriched yoghurts at days 1,7 and 21 (Fig. 1). The lower syneresis in enriched yoghurts could be explained due to the ability of lupin proteins to absorb the whey expulsed by the casein network (42). Previous studies also showed that the addition of chickpea and lentil flours reduced the syneresis of yoghurts due to their interaction between proteins form legumes and those from cow´s milk (9,10).

CONCLUSIONS

Andean lupin concentrate showed high protein content (69% %) and unsaturated fatty acids mainly oleic, linoleic and α-linolenic acids (70%). Low atherogenic and thrombogenic indexes were evinced in the protein concentrate. The addition of the lupin protein concentrates at 0.5, 1 and 1.5% into yoghurts increased the protein content, unsaturated fatty acids, total polyphenol and flavonoid contents. Enriched yoghurts also showed lower saturated fatty acids, atherogenic and thrombogenic indexes compared to the control sample. The lupin protein concentrate when adding into yoghurts increased the ABTS radical scavenging activity, the ferric reducing power and the total antioxidant activity. In addition, enriched yoghurts showed similar lactic acid bacteria counts compared to control sample. The counts for total and thermotolerant coliforms were under three MPN/mL and yeast and mold under 10 CFU/mL during 28 days of storage. The pH values in enriched yoghurts were higher at days 1 and 7 of storage compared to the control sample. The lactic acid content increased in all formulations at 21 days of storage, however, enriched yoghurts showed lower syneresis. Yoghurts enriched with lupin protein concentrate could represent nutritive and functional alternatives to prevent chronic diseases when consuming in a healthy diet.

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AUTHOR´S CONTRIBUTION

CC: bibliographic review, optimization of lupin extract and yoghurt formulations, physicochemical, microbial and textural analyses, manuscript drafting, results and discussion, LCF: optimization of yoghurt formulations, microbial quality during storage, APOL: polyphenols, flavonoids and antioxidant activities, APBR: fatty acid and mineral compositions, DTC: statistical analyses; GV: optimization of yoghurt formulation, results and discussion, AECA: supervisor, funding, reagents and physical space to carry out the experiments, review and correction of the article, AR: director, results and discussion, funding , review and correction of the article.

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COMPETING INTEREST

The authors state that there are no conflicts of interest in preparing the manuscript

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