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Characterization of the biochemical and lipid profiles of white lupine (*Lupinus albus*) grain and its derivatives

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Abstract. Lupine is a legume with the high contents of protein and lipids unsaturated, vitamins, minerals and dietary fiber and thus its nutritional quality has properties beneficial to health. The aims of this study were to produce flours from white lupine grain using different thermal treatments, to chemically characterize them and to compare them to those of raw and pickled lupine grains, as well as to determine their chemical compositions, lipid profiles and contents of anti-nutritional factors. To preparation of lupine flours the heat treatment was applied at 100 °C for 60 min and 150 °C per 30 min in an oven with circulating air. The moisture, protein, lipid, mineral and dietary-fiber contents of the four lupine products tested were determined. The trypsin-inhibitory activity levels were determined using casein as the substrate. The fatty acid composition were determined by gas chromatography. The results show us the samples had high levels of protein, unsaturated fatty acids, and dietary fiber. Heat treatment did not affect the nutrient content of the grain. However, pickled lupine grains had lower levels of nutrients and minerals and a higher sodium level than those of the other lupine products examined, compromising the nutritional quality of the grain. The analyses conducted in the present study indicate that *Lupinus albus* grains currently contain alkaloids but due its high nutritional value it is possible to incorporate this legume into the human diet.

Keywords: White lupine; Thermal treatments; Chemical composition.

Introduction

The lupine (*Lupinus* spp.) plant, which has been cultivated for approximately 4000 years. Currently, this legume is used mainly in animal husbandry and agronomy as a green manure due to its great ability to fix nitrogen in the soil (Barradas et al., 2001). However, there is increased interest worldwide in potentially utilizing the lupine as an ingredient in foods for human consumption in the form of shakes, breads, cookies and snacks (Ribeiro, 2006).

The lupine is outstanding among the grain legumes due to the high contents of protein and lipids and predominance of unsaturated rather than saturated fatty acids in the grain, which account for 80% of the total fat available (Neves et al., 2006). Lupine grain is also rich in vitamins, minerals and dietary fiber (Van Barneveld, 1999).

Regarding the benefits of lupine consumption to human health, its antioxidant activity

and ability to reduce the serum cholesterol level have been much discussed (Yoshie-Stark et al., 2004). The intake of food products, such as flours and isolates, containing lupine proteins has been associated with a decreased risk of diseases such as obesity, diabetes and cardiovascular disease (Volek and Marounek, 2011).

Despite the high nutritional quality of this legume, it is difficult to introduce lupine grains into food due to cultural issues and its content of anti-nutritional factors, such as trypsin inhibitors and alkaloids, which negatively affect its bioavailability and digestibility (Botaro, 2010). Therefore, these anti-nutritional compounds must be inactivated prior to the consumption of the grain, which is conducted mainly by heat application because these factors are thermolabile. However, if the heat treatment is inadequate or excessive, the nutritional quality of these legumes will be decreased (Monteiro et al., 2010).

Thus, the aims of this study were to produce heat-treated flours using white lupine grain, to attempt to inactivate their anti-nutritional factors, to chemically characterize these flours, and to compare their chemical compositions, lipid profiles and nutritional qualities with those of raw lupine grain and of pickled lupine grain acquired in the market.

Methods

Materials

Raw white lupine (*Lupinus albus*) grain was provided by the Agronomic Institute of Paraná (IAPAR) and pickled lupine grain was purchased at a supermarket. The reagents used for determining the lipid profiles were of chromatographic grade, including a standard mixture of fatty, saturated and unsaturated acids containing between 8 and 22 carbons (Supelco 37 FAME mix, Sigma, USA). The other reagents were of analytical grade.

Preparation of lupine flours

To inactivate the anti-nutritional factors present, the heat treatment described by Monteiro et al. (2010) was applied with modifications. To prepare flour 1, raw whole grains were heated in an oven with circulating air (Quimis, Q31M242, Diadema, Brazil) at 100 °C for 60 min. To prepare flour 2, a temperature-time binomial regime of 150 °C per 30 min was employed using the same equipment. The grains were ground in a fine-cutting mill (Tecnal, TE 020, Piracicaba, Brazil), and the particles were subsequently sorted according to size by up to 42-mesh per sieve (Bertel Metallurgical Industries Ltda, Caieiras, Brazil). The raw lupine grains were ground under the same conditions as the treated grains.

Preparation of pickled lupine samples

Pickled lupine grains were ground in a food processor (Arno, WWBC, São Paulo, Brazil). After grinding, the pickled lupine samples were frozen and then were lyophilized (Labconco Free Zone, Missouri, USA) for 20 h.

Determining the chemical composition of raw lupine grain, lupine flours and pickled lupine grain

The moisture, protein, lipid, mineral and dietary-fiber contents of the four lupine products tested were determined in triplicate according to AOAC methods (AOAC, 2007). The conversion factor of nitrogen to protein that was used was 6.25.

The chromium, iron, manganese, sodium, copper and zinc levels were determined according to the methods of the Institute Adolfo Lutz (IAL, 2004). Mineral stock solutions were prepared by dissolving 1 g of each standard mineral (Sigma-Aldrich, St. Louis, MO, USA) in 1 L of acidified aqueous 10% (v/v) hydrochloric acid in a volumetric flask. Working solutions of 1.00, 2.00, 5.00, 7.5 and 10.00 mg/L were also prepared in acidified aqueous 10% (v/v) hydrochloric acid on the same day that the assays

were conducted and were stored at 8 °C until the calibration curve constructed for each of the evaluated minerals were analyzed. Lupine samples were subjected to thermal and chemical treatments to prepare their ashes and promote the release of mineral elements for analysis using an ICP-OES system (Optima 2000DV model, Perkin Elmer).

Determining the lipid profile Extraction

Two types of lipid extraction techniques were utilized. Lipid extraction under cold conditions was performed according to the modified method of Bligh and Dyer (1959). Lipid extraction under hot conditions was performed according to the method of Boschini et al. (2008), using a modified Soxhlet-type apparatus, by extracting the samples for 6 h at 69 °C and using n-hexane as the solvent.

Preparation of fatty acid methyl esters

The lipids were esterified utilizing two procedures. The first procedure was performed as described by Hartman and Lago (1973). Samples of 100 mg of total extracted lipids were weighed in glass tubes with screw caps. To saponify the lipids, 4 mL of a methanolic solution of 0.5 M NaOH was added, and the esterification was performed by adding 5 mL of a mixture of NH₄Cl, chromatographic grade methanol and H₂SO₄, stirring and heating the resulting mixtures in a water bath at 70 °C for 5 min.

The second esterification procedure employed alkaline catalysis and was performed according to the method described by Bannon et al. (1982). A 150 mg aliquot of the extracted lipid fractions were weighed in glass tubes with screw caps, and 5.0 mL of a solution of 0.25 mol/L NaOHCH₃ in methanol-diethyl ether (1:1) was added, and after the mixtures were stirred, 3.0 mL of iso-octane and 15 mL of a saturated NaCl solution were added.

Gas Chromatographic Analysis

The lipid profiles were determined using a Shimadzu GC-2010 automatic-injection gas chromatograph (Shimadzu GC-2010) equipped with a flame-ionization detector (FID) and a split injector. The injector temperature was maintained at 180 °C, and the detector temperature was maintained at 250 °C. The analyses were performed using a Stabilwax capillary column suitable for FAME detection (30 m x 0.53 mm, i.d. x 1 µm film thickness), using the following isothermal program: 180 °C for 5 min, increasing by 5 °C / min to 210 °C and maintaining this temperature for 15 min. The injection volume was 1 µL. The carrier gas was high-purity nitrogen applied with a head pressure. The following isothermal program was applied to control the column oven temperature: 180 °C for 5 min, increasing by 5 °C / min to 210 °C, and maintaining this temperature for 15 min.

The fatty acid peaks were identified by comparison of their retention times with those of the

reference standards (Supelco 37 FAME Mix, Sigma, USA). The fatty acid contents were expressed in mg/g fat and were determined using equation 1 (below), as proposed by Holland et al. (2002).

$$\text{Equation 1: } CI = \%A \times \%L \times fc/100$$

Where:

CI = fatty acid concentration, expressed in g/100 g

%A = % relative fatty acids

%L = % lipids in the sample

Fc = conversion factor (0.94)

The lipid profile analyses were conducted with a completely randomized design using a mixed design with repeated measures and independent variables with sub-sub divided parts. The sphericity of the data was evaluated using Mauchly's test at 5% probability, and comparisons between the variables were tested using paired t tests coupled with Bonferroni correction at a 0.05 probability (Field, 2005). These tests were performed using SPSS version 11.5 software.

Determining the trypsin-inhibitory activity level

The trypsin-inhibitory activity levels were determined using the method described by Kakade et al. (1969), with some modifications regarding the use of casein (0.005%) as the substrate. To determine the trypsin-inhibitory activity levels of the lupine samples, a calibration curve was constructed using trypsin 0.005%. In determining the levels of trypsin-inhibitory activity, a trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbance

units at 280 nm. Trypsin inhibitory activity is defined as the number of trypsin units inhibited (TUI).

Statistical analysis

All of the experiments were conducted in triplicate. An analysis of variance (unique-factor ANOVA) and a Tukey test at 5% probability were used to compare the values determined in all of analyses. A standard curve of Tyr was created using regression analysis using five different concentrations (Pimentel-Gomes, 1990).

Results and Discussion

Chemical composition of the lupine samples

The results of the analysis of some components of the lupine flours and raw grain, expressed on a dry basis, are shown in Table 1. The moisture contents of the raw grains, flour 1, flour 2 and pickled grains were 8.2, 7.6, 6.6 and 69.3%, respectively, with the first three products having no significant differences. The pickled grains had a significantly higher moisture content than those of the other products, which was expected because this product was prepared by cooking it in damp heat, in which steam moisturized it and softened its fibers. This type of processing can cause the loss of some components through dissolution, significantly changing the nutritional value of the product. The level of retention of these nutrients is directly associated with the amount of water used for cooking as well as the duration of the overall preparation process (Philippi, 2006).

Table 1. Chemical composition of lupine (*Lupinus albus*) samples on a dry basis.

Chemical Composition	Raw lupine grains	Flour 1 (100 °C/60 min)	Flour 2 (150 °C/30 min)	Pickled lupine grains
Total Lipids	10.16 ^{ab} ± 0.42	9.96 ^{ab} ± 0.16	11.59 ^a ± 0.10	8.06 ^b ± 0.41
Proteins	33.81 ^a ± 1.65	35.30 ^a ± 0.41	33.22 ^a ± 0.54	34.26 ^a ± 1.93
Ash	2.71 ^b ± 0.07	2.76 ^b ± 0.06	2.75 ^b ± 0.04	7.86 ^a ± 0.26
Dietary Fiber	34.03 ^b ± 0.86	36.10 ^{ab} ± 0.25	34.78 ^{ab} ± 1.34	39.67 ^a ± 2.11
Carbohydrates ¹	19.28	15.86	17.65	10.13

¹The carbohydrate levels were calculated by difference determinations. Average values ± standard deviation (n = 3) indicated with the same superscripted letter (a, b or c) on the same line do not differ significantly (p ≤ 5; Tukey test).

Regarding the levels of lipids, no significant differences were found between the samples of raw grains, flour 1 and flour 2, which had an average lipid content of 10.6%. It is noteworthy that these values are in accordance with the Taco value, which is 10.3% lipid content for white lupine grain (Taco, 2011).

Regarding the ash contents, the highest ash content was found in the sample of pickled lupine grains (7.9%), whereas the average ash content of the other samples was 2.7%, and their contents did not significantly differ. This result was expected because during the pickling process, the grains are immersed in a brine consisting of a saturated saline solution and both the salt and water of the brine are

absorbed by the grains during storage (Evangelista, 2001).

Quantitative analysis of the protein contents of the samples showed no significant differences between them. According to Brazilian standards, the raw lupine grains and the two flours obtained due to the thermal treatment of these grains would be considered foods with high protein contents (Brasil, 1998).

Table 2 shows the levels of total dietary fiber and those of the soluble and insoluble fiber fractions in the four lupine samples.

The lupine samples contained high amounts of dietary fiber, with their levels ranging from 34.9% to 39.7% for the raw lupine grains, two flours and pickled grains. In all of the samples, there was a

predominance of insoluble dietary fiber (29.6%), and the values for these samples did not significantly differ. The average content of soluble fiber in the four lupine samples was 6.5%, and their values did not significantly differ. According to the Taco tables, the dietary fiber content of raw lupine grains is 32.2%, consistent with the content observed in this study (Taco, 2011). Due to the high content of dietary fiber in the raw lupine grains and the two flours, they would be considered foods with high

fiber contents according to Brazilian standard (Brasil, 1998).

Table 3 shows the levels of trace elements (in mg/kg) found in the four lupine samples. The pickled lupine grains had a significantly higher level of sodium compared with that of the other samples (8744.0 mg/kg). This higher sodium content can be attributed to the technological process applied to this sample, in which the product is sold immersed in brine.

Table 2. Fiber levels (soluble, insoluble, and total dietary) of lupine (*Lupinus albus*) samples on a dry basis.

Fiber	Raw lupine grains	Flour 1 (100°C/60 min)	Flour 2 (150°C/30 min)	Pickled lupine grains
Dietary Fiber	34.03 ^b ± 0.86	36.10 ^{ab} ± 0.25	34.78 ^{ab} ± 1.34	39.67 ^a ± 2.11
Insoluble Fiber	29.72 ^a ± 2.4	29.50 ^a ± 0.32	28.27 ^a ± 0.93	30.91 ^a ± 1.80
Soluble Fiber	4.24 ^a ± 1.77	6.60 ^a ± 0.46	6.50 ^a ± 0.79	8.7 ^a ± 0.73

Average values ± standard deviation (n = 3) indicated with the same superscripted letter (a, b or c) on the same line do not differ significantly (p ≤ 5; Tukey test).

Table 3. Trace minerals levels (mg/kg) of lupine (*Lupinus albus*) samples on a dry basis.

Trace minerals	Raw lupine grains	Flour 1 (100 °C/60 min)	Flour 2 (150 °C/30 min)	Pickled lupine grains
Chromium	0.53 ^a ± 0.08	0.52 ^a ± 0.07	0.58 ^a ± 0.14	0.31 ^b ± 0.05
Copper	13.89 ^a ± 0.56	12.99 ^a ± 1.40	12.89 ^a ± 0.15	4.05 ^b ± 0.52
Iron	42.61 ^a ± 5.14	30.71 ^{ab} ± 4.51	27.08 ^{ab} ± 0.66	16.71 ^b ± 1.04
Manganese	1679.98 ^a ± 14.14	1893.31 ^{ab} ± 64.48	1759.98 ^b ± 77.62	70.78 ^c ± 10.09
Nickel	0.66 ^a ± 0.07	0.62 ^a ± 0.79	0.80 ^a ± 0.13	0.03 ^b ± 0.01

Average values ± standard deviation (n = 3) indicated with the same superscripted letter (a, b or c) on the same line do not differ significantly (p ≤ 5; Tukey test).

Pickled lupine grain samples had lower levels of trace minerals than did the other samples. This phenomenon was most likely due to these components migrating into the water used in the cooking process of preparing pickled lupine grains (Ciabotti et al., 2006). Comparing these results and those of the pickled lupine grain with the data in the Taco tables confirmed that the pickled lupine grain contained significantly lower levels of trace minerals. In the relevant Taco table, raw lupine grains were shown to contain 0.79 mg/kg of copper and 2.8 mg/kg of iron, whereas the levels of these two minerals in pickled lupine grains were 0.27 mg/kg and 0.3 mg/kg, respectively (Taco, 2011). According to Brazilian standards, based on these levels and those of the other minerals analyzed, raw lupine grains and the two heat-treated flours can be classified as foods with high iron contents and low sodium contents (Brasil, 1998).

Lipid profiles of the lupine samples

Among the fatty acids analyzed to construct fatty acid profiles of the lupine samples, five were saturated fatty acids (lauric, myristic, palmitic, stearic and arachidic acids) and four were unsaturated fatty acids (palmitoleic, oleic, linoleic and linolenic acids), which contained 12 to 20 carbon atoms and thus are

considered long-chain fatty acids (LCT). Lauric, myristic, and palmitoleic acids were found in only trace amounts in the lupine samples and therefore were not quantified.

The average fatty acid contents of the samples determined using different extraction and esterification methods, shown in Table 4, were expressed as percentages (%). It is noteworthy that these data reveal only the differences between the samples without considering the types of extraction and esterification methods used.

Among the fatty acids evaluated in this study, the content of oleic acid was greatest (63%), followed by that of linoleic acid (14%), with a predominance of unsaturated fatty acids present in all of the samples analyzed. The lipid profile of raw lupine grains was found to contain 7.6% palmitic acid, 2.2% stearic acid, 63% oleic acid, 16.6% linoleic acid, 6.9% linolenic acid and 4.6% arachidic acid.

Knowledge of the lipid profiles of foods is crucial to achieving nutritional safety. Based on the types and proportions of the unsaturated fatty acids present, a food may tend to be atherogenic, high in saturated fats or hypocholesterolemic, with greater percentage of unsaturated fatty acids (Moraes, 2006). Thus, it is important to determine the fatty

acid contents of the samples, separating them into the categories of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), as shown in Table 4.

Table 4. Saturated, monounsaturated and polyunsaturated fatty acid levels (g/100 g of sample) of lupine (*Lupinus albus*) samples on a dry basis.

Fatty acids	Raw lupine grains	Flour 1 (100°C/60 min)	Flour 2 (150°C/30 min)	Pickled lupine grains
Palmitic C16:0	0.72 ^b ± 0.04	0.71 ^b ± 0.04	0.85 ^a ± 0.03	0.61 ^c ± 0.04
Stearic C18:0	0.21 ^b ± 0.01	0.20 ^b ± 0.00	0.23 ^a ± 0.00	0.15 ^c ± 0.01
Arachidic C20:0	0.43 ^b ± 0.01	0.41 ^c ± 0.01	0.46 ^a ± 0.03	0.37 ^d ± 0.03
Oleic C18:1	6.07 ^b ± 0.02	5.98 ^c ± 0.05	6.96 ^a ± 0.04	4.70 ^d ± 0.02
Linoleic C18:2	1.39 ^c ± 0.02	1.35 ^b ± 0.02	1.58 ^a ± 0.02	1.11 ^d ± 0.01
Linolenic C18:3	0.66 ^b ± 0.03	0.64 ^b ± 0.03	0.74 ^a ± 0.03	0.58 ^c ± 0.03
SFA	1.37 ^b ± 0.01	1.33 ^c ± 0.00	1.55 ^a ± 0.01	1.14 ^d ± 0.00
MUFA	6.07 ^b ± 0.01	5.98 ^c ± 0.01	6.96 ^a ± 0.02	4.70 ^d ± 0.00
PUFA	2.06 ^b ± 0.01	2.00 ^c ± 0.01	2.33 ^a ± 0.01	1.69 ^d ± 0.00
Total	9.51 ^b ± 0.00	9.32 ^c ± 0.00	10.84 ^a ± 0.00	7.54 ^d ± 0.00

Average values ± standard deviation (n = 3) indicated with the same superscripted letter (a, b or c) on the same line do not differ significantly (p ≤ 5; Tukey test). Saturated Fatty Acids = SFA; Monounsaturated Fatty Acids = MUFA; Polyunsaturated Fatty Acids = PUFA.

Regarding the PUFA levels, all of the samples were found to have a predominance of linoleic acid (14.6%), followed by linolenic acid (7.1%) and arachidic acid (4.5%). Data analysis has been revealed that the isocaloric replacement of saturated fatty acids for polyunsaturated fatty acids reduces the serum total cholesterol and LDL-C fraction, while acting to prevent and treat cardiovascular disease, hypertension, general inflammation, asthma, arthritis and various types of cancer (Suárez-Mahecha et al., 2002).

Fatty acids of the n-6 family (which include linoleic acid and arachidonic acid 20:4) and those of the n-3 family (linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) have different physiological functions and act together to regulate cell membrane-associated biological processes, brain functions and nerve-impulse transmission (Suárez-Mahecha et al., 2002). Thus, the ratio of the daily intake of food sources containing n-6 and n-3 fatty acids is of great importance for human nutrition, as recommended by various authors and the health agencies of various countries.

According to the WHO (1995), the ideal n-6/n-3 fatty-acid ratio would be 5:1 to 10:1. However, other authors recommended n-6/n-3 fatty-acid ratios of 2:1 to 3:1 because these ratios allow a greater level of conversion of alpha-linolenic acid (Master, 1996). The n-6/n-3 fatty acid ratio of the white lupine

samples determined in this study was 2:1. This ratio is consistent with that found by Master (1996), making lupine a food with a proper ratio of n-6 and n-3 fatty acids.

The effects of the extraction and esterification methods employed on the content of fatty acids, independent of the sample analyzed, were conducted. The extraction and esterification methods that were proven more effective were those that provided higher amounts of fatty acids. The most appropriate extraction and esterification methods for each fatty acid observed in this study are presented in Table 5. The extraction methods used significantly affected the amount of most of the fatty acids analyzed, with the exception of oleic and linoleic acids, the levels of which were not affected by these two types of extraction methods. The cold extraction method was more effective in extracting palmitic and stearic acids, whereas the hot hexane extraction method resulted in higher levels of linolenic and arachidic acids in the lipid fraction.

Because lipids have a relatively large range of hydrophobicity, it is almost impossible to extract all of them using a single solvent (Pimentel and Zenebon, 2009). The solvents used to extract lipids should ideally solubilize all of the lipid components and be sufficiently polar to dissociate cellular membrane components and lipoproteins without causing chemical reactions (Tonial et al., 2009).

Table 5. Evaluation of different extraction and esterification methods and the interactive effects of the studied variables on all of the quantified fatty acids.

Fatty Acid	Best extraction	Best esterification
Palmitic C16:0	Cold	HL
Stearic C18:0	Cold	HL
Arachidonic C20:4	Hot	Not significant
Oleic C18:1	Not significant	HL
Linoleic C18:2	Not significant	BA
Linolenic C18:3	Hot	BA

HL esterification: as described by Hartman and Lago (1973). BA esterification: as described by Bannon et al. (1982).

According to Brum et al. (2009), classical lipid-extraction procedures in which samples are subjected to hot solvents in a Soxhlet device should be avoided because they favor peroxidation and hydrolytic reactions and may compromise the analytical results, such as the determined quantities of components of the lipid fraction. These authors also reported that one of the most versatile and effective extraction procedures is that of Bligh and Dyer (Pimentel and Zenebon, 2009), in which a cold mixture of chloroform and methanol is used. Another advantage of methods based on the use of a binary mixture of chloroform and methanol is the efficient extraction of both neutral and being analyzed, the appropriate choice of an extraction method significantly affects the final results.

Regarding the effect of fatty-acid esterification on the fatty acid contents determined, use of the Hartman and Lago method led to the larger detected percentage of three of the six identified fatty acids, namely palmitic, stearic and oleic acid, which were found to have average levels of 0.785 g/100 g, 0.215 g/100 g and 6.32 g/100 g, respectively (Hartman and Lago, 1973).

In contrast, using the BA esterification method led to greater levels of linoleic and linolenic acids being detected, with average levels of 1.45 g/100 g and 0.723 g/100 g, respectively. Only the levels of arachidic acid were not significantly affected by using the different esterification methods.

The levels of trypsin-inhibitory activity in the four samples that were analyzed in this study were

presented as TUI values (trypsin units inhibited/mg of sample) and TUI/mg of the soluble protein. Table 6 shows the soluble protein content and TUI values of the four white lupine (*Lupinus albus*) samples.

The TUI/mg soluble protein values of the first three samples (raw lupine grains, flour 1 and flour 2) were notably significantly different and contained an average of 9.39 TUI/mg of soluble protein. However, pickled lupine grains had a significantly lower TUI/mg level compared with those of the other samples. The same trend was observed for the TUI/mg contents of the samples, in which no differences were found between those of the raw lupine grain, flour 1 or flour 2 samples. The pickled lupine sample had a significantly lower TUI/mg level than those of the other samples.

The thermal stability level of trypsin inhibitors varies greatly, depending, among other factors, on the temperature and duration of the heating process to which they are subjected, the size of the particles including them and their moisture content and the structural conformation of the inhibitor (Carvalho et al., 2002). While the pickled lupine samples were expected to have a significantly lower level of trypsin inhibitors compared with those of the other samples, all of the samples were found to have a very low level of trypsin inhibitors.

In conclusion, despite the fact that the *Lupinus albus* variety (also known as white lupine or sweet lupine) has been modified over time to reduce its content of anti-nutritional factors and alkaloids, the analyses conducted in the present study indicate that *Lupinus albus* grains currently contain alkaloids.

Table 6. Soluble protein levels and trypsin units inhibited in lupine samples and per mg of these samples

Sample	$\mu\text{m PTN sol./}$ mg PTN	UTI/mg PTN	UTI/mg sample
Raw lupine grains	430.69 ^{ab} \pm 2.1	8.7 ^a \pm 0.07	3.73 ^a \pm 0.03
Flour 1 (100 °C/60 min)	501.68 ^a \pm 11.0	9.1 ^a \pm 0.8	4.55 ^a \pm 0.4
Flour 2 (150 °C/30 min)	404.63 ^{ab} \pm 21.6	10.4 ^a \pm 0.7	4.21 ^a \pm 0.3
Pickled lupine grains	307.58 ^b \pm 5.0	5.0 ^b \pm 0.4	1.54 ^b \pm 0.1

Average values \pm standard deviation (n = 3) indicated with the same superscripted letter (a, b or c) on the same line do not differ significantly ($p \leq 5$; Tukey test). Milligram of soluble protein = mg PTN; microgram of soluble protein = $\mu\text{m PTN}$

Conclusion

The dry heat treatment of the lupine grain sample analyzed in this study did not affect or reduce its nutritional quality and did not eliminate all of its anti-nutritional factors. The flours produced using this grain met the Brazilian requirements for foods high in protein, fiber and iron and low in sodium. The samples had high levels of monounsaturated fatty acids, low levels of saturated fatty acids and an appropriate ratio of linoleic and linolenic acids. In the case of the pickled lupine grain, the heat treatment for its production led to the loss of nutrients and minerals and increased its sodium content. Therefore, we suggest that lupine flours should be introduced into the human diet.

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