Andean lupin (*Lupinus mutabilis* Sweet): Processing effects on markers of heat damage, chemical composition and *in vitro* protein digestibility.

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Abstract

Background and objective: Andean lupin (*Lupinus mutabilis* Sweet) has health benefits with promising possibilities for food industry. The aim of this research was to determine the effect of various processing (water debittering, extrusion, and spray-drying), on the markers of heat damage and *in vitro* protein digestibility in Andean lupin.

Findings: The proteins and lipids (47.4 and 16.2 g/100 g dry matter) of untreated Andean lupin were modified by processing. The extruded products had a higher protein content (55.7 g/100 g) and digestibility (68.1%) with low heat damage (8.7 mg furosine/100 g protein) than debittering lupins. A limited heat damage was found for spray-dried products with addition of maltodextrin, these values were 54.1 mg furosine/100 g protein; 0.60 mg hydroxymethylfurfural/kg; 0.58 mg glycosylisomaltol/kg, and digestibility (72.8-74.0%).

Conclusions: The chemical composition of Andean lupin was modified by the technological processes (debittering, extrusion and spray-drying) applied. Processing enhanced the digestibility, without inducing relevant heat damage.

Significance and novelty: The most sensitive heat damage marker identified for lupin was furosine.

Key words: debittering; extrusion; heat damage; *Lupinus mutabilis*; spray-drying

1. Introduction

Andean lupin (*Lupinus mutabilis* Sweet), also known as *chocho* or *tarwi*, is a close relative of *Lupinus albus, Lupinus luteus* and *Lupinus angustifolius,* three economically important pulses cropped worldwide (Villarino et al., 2015). The pulses of all four species are rich in proteins, lipids (high in mono-and polyunsaturated fatty acids) and biologically active substances (Bähr, Fechner, Hasenkopf, Mittermaier & Jahreis, 2014; Carvajal-Larenas, Linnemann, Nout, Koziol & van Boekel, 2016).

L. mutabilis is a promising source of nutrients and bioactive components with many benefits for health (Carvajal-Larenas et al., 2016; Hickisch, Beer, Vogel & Toelstede, 2016; Caligari et al., 2000; Gross et al., 1988), and has high contents of protein and lipid (32-53% and 13-25%, respectively) (Carvajal-Larenas et al., 2016). Nutritional studies show that lupins can be compared with soybean (Kaczmarska, Chandra-Hioe, Frank & Arcot, 2018) and, employed in the enrichment of wheat flour, enhance amino acid balance and increase the protein content of many products, as bakery, dietary and functional foods (Villarino et al., 2015; Güemes-Vera, Esperza & Dávila-Ortiz, 2004).

All lupins contain antinutritional factors, mainly bitter alkaloids, on average higher in *L. mutabilis* (28.0 g/kg) and lower in *L. albus* (1.8 g/kg; Carvajal-Larenas et al., 2016), whose content must be reduced by boiling and soaking in running water (Musco et al., 2017). There is not enough information on the chemical characteristic changes caused by different type of processing (debittering, drying, extrusion and spray drying) on *Lupinus mutabilis*.

Processing of pulses, as soaking and extrusion, modifies many chemical, enzymatic and digestibility characteristics (Palanisamy, Franke, Berger, Heinz and Töpfl, 2019; El-Hady & Habiba, 2003). This processing might lead to formation of toxic compounds, like hydroxymethyl-furfural and furosine (Hidalgo & Brandolini, 2011; Islam, Khalil, Islam

71 & Gan, 2014). Extrusion improves the properties of dietary fiber of lupin seed coats,
72 soluble dietary fiber, and inactivates many food enzymes (Zhong, Fang, Wahlqvist,
73 Hodgson & Johnson, 2019). Spray-drying is another processing that improves nutritional
74 value, solubility, stability, flow properties, and reduces bioactive compounds degradation
75 (Sosnik & Seremeta, 2015). Therefore, the effect of processing should be analyzed when
76 developing innovative food products. However, the information about Andean lupin
77 nutritional properties after processing is still limited, hindering the development of new
78 and/or functional products. The aim of this research was to study the effect of different
79 food processes (debittering, extrusion and spray-drying) on the markers of heat damage,
80 *in vitro* protein digestibility, chemical composition and color of Andean lupin.
81
82 **2. Materials and Methods**
83 *2.1 Materials*
84 Three *Lupinus mutabilis* genotypes from different regions of Peru (Altagracia, from
85 Ancash, Andenes, from Cusco, and Yunguyo, from Puno) were kindly supplied by the
86 Legumes Program of the Universidad Nacional Agraria la Molina, Lima, Peru.
87
88 *2.2 Lupin grains processing*
89 *2.2.1 Debittering*
90 The debittering of whole lupin grains, needed for the removal of toxic alkaloids, was
91 carried out by soaking and washing according to Jacobsen and Mujica (2006), Erbas
92 (2010) and Ertaş and Bilgiçli (2012), with modifications. The lupin grains were hydrated
93 for 12 h at room temperature with a 1:6 (w/v) lupin:water ratio. Then, hydrated grains
94 were boiled (hydrated grains:water 1:3 w/v) for 1 h, changing of water each 30 min;
95 afterwards, soaked in water (cooked grains:water 1:3 w/v) at room temperature for 5 days;

the water was changed daily. Finally, the grains were dried at 50 °C in a hot air tray dryer (Xinhang, SW-10S, China) for 18 hours, and stored under dark at room temperature until milling.

2.2.2 Milling

The bitter and debittered lupin grains were ground separately with a Grindomix GM 200 knife mill (Retsch GmbH, Germany) at 6000 RPM for 35 s; each flour was sieved through a 2.0 mm mesh, packed in high-density polyethylene bags with hermetic closure and stored at 4 °C until analysis.

2.2.3 Extrusion

The extrusion was performed on debittered flour with a DSE32 laboratory extruder (Jinan Dingrun Machinery Co., China) at a pressure of 20 Mpa. The humidity of debittered flour was increased until 35% to enter to extruder. The temperatures in the different section of the extruder were 95, 120, 140 and 130 °C, respectively (Lampart-Szczapa et al., 2006). The extrusion pellets were milled with a Grindomix GM 200 knife mill (Retsch GmbH, Germany) at 6000 RPM for 35 s. The extruded flours were packed in high-density polyethylene bags with hermetic closure and stored at 4 °C until further analysis.

2.2.4 Spray-drying

To obtain a lupin drink, debittered lupin whole grains were hydrated for 12 h at room temperature (1:6 w/v lupin:water), peeled, ground for 15 min in a blender (Oster®, BLSTBC4129-053, Mexico) after adding cold boiled water (1:4 w/v ratio), and filtered through a thin-mesh cloth to remove coarse material. The lupin drink was fed to a laboratory SD-Basic spray-dryer (LabPlant, United Kingdom), with the addition (6%

w/w) of a coating agent (gum arabic or maltodextrin; Frutarom SAC, Peru). The working conditions were: inlet temperature 170 °C, outlet temperature 80-90 °C (Boostani, Aminlari, Moosavi-nasab, Niakosari, & Mesbahi, 2017), 400-600 kPa and feeding speed 12.5 mL/min. The spray-dried lupin powder was stored in airtight dark glass jars at 4 ºC until analysis.

2.3. Analyses

Chemical composition was assessed by the official methods 920.87 for proteins (conversion factor 6.25), 923.05 for lipids, 923.03 for ash and 925.10 for moisture (AOAC, 2000). Total carbohydrates were computed by difference. Sugars were assessed by HPLC, following Hidalgo and Brandolini (2011). The contents are expressed as dry matter basis (DM). The heat damage indices furosine (in milligrams of furosine/100 g protein), hydroxymethylfurfural (HMF) and glucosylisomaltol (GLI) (mg/kg DM) were determined by HPLC as performed by Hidalgo and Brandolini (2011). Water activity (a_w) was measured with an AQUALAB (Decagon Devices Inc., USA). Color was assessed in triplicate using the CIE lab scale (L^*, a^*, b^*) with a Chroma meter II Reflectance (Minolta Camera Co. LTD, Japan), and color difference (ΔE) was measured according to the equation: $\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2}$

The *in vitro* digestibility of the proteins was evaluated following Almeida, Monteiro, Costa-Lima, Alvares and Conte-Junior (2015), with minor modifications. Exactly 250 mg of each sample were suspended in 15 mL 0.1 N HCl containing 1.5 mg/mL pepsin and incubated for 3 h at 37 °C in a water bath. The pepsin hydrolysis was stopped with the addition of 7.5 mL 0.5 N NaOH. The pancreatic digestion was started by the addition of 10 mL 0.2 mol/L phosphate buffer (pH 8.0) containing 10 mg pancreatin, and 1 mL sodium azide 0.005 mol/L to prevent microbial growth; the mix was incubated for 18

hours at 37°C. After pancreatic hydrolysis, 1 mL 10g/100 mL trichloroacetic acid was added, followed by centrifugation at 1000 g for 20 min. The supernatant was collected and the total protein content (N x 6.25) was assessed. Casein powder isolate was used as a reference. The digestibility values were computed with the equation: *Digestibility (%) = (Ps/Pt) x 100*, where *Ps* and *Pt* represent supernatant and total protein content, respectively.

All tests were performed in triplicate on two different batches of each product.

2.4 Statistical analysis

The results of the assays underwent the analysis of variance (ANOVA) considering treatments and genotypes as factors and are expressed as mean value ± standard deviation. When significant differences were found ($p \leq 0.05$), Tukey's multiple range test was used to discriminate between mean values at a 95% significance level. The statistical analyses were performed with the software Minitab v. 17 (Minitab Inc, State College, PA, USA).

3. Results and discussion

3.1 Analysis of variance for processing and cultivars

The analysis of variance (Table 1) showed highly significant differences among treatments (types of processing), among cultivars (*altagracia*, *andenes* and *yunguyo*) and their interaction for almost all the traits analyzed; the exception was maltose content, modified only by the treatments. The treatment effect was predominant (Supplementary Table 1), explaining between 72.4% (*a**) and 100% (moisture) of total variation; cultivar differences accounted only for 0.0-6.6%, while the interaction sometimes described a sizeable portion of variation, as for furosine (14.0%), glucose (18.3%), fructose (19.7%) and *a** (21.3%). Therefore, for ease of presentation, the Tables will report the mean

171 results of each treatment, while a detailed view of cultivar performance under the different
172 treatments is presented in the Supplementary Tables.
173
174 **3.2 Effect of process on *in vitro* protein digestibility and chemical composition.**
175 **3.2.1 Debittering of lupin**
176 Using the same methodology of this research, Cortés-Avendaño et al. (2020) found low
177 alkaloid levels (~ 0.001 g/100g DM) after debittering process in ten cultivars of *L.*
178 *mutabilis*. The debittered lupin had higher protein content (54.4±2.61 g/100 g DM) than
179 the bitter seeds (protein content of 47.4±2.80 g/100 g DM) (Table 2); Altagracia was the
180 cultivar with the highest protein value (57.8 g/100 g; Supplementary Table 2). An increase
181 of proteins, after the soaking and washing, is reported in different lupin species; for the
182 case of *L. mutabilis* from 41.4 to 55.9 g/100 g (Carvajal-Larenas, van Boekel, Koziol,
183 Nout, & Linnemann, 2014), while in *L. albus* it rose from 41.3 to 51.6 g/100 g (Erbas,
184 2010). The augmented protein content is consequence of a change in the dry matter
185 composition due to the leaching of hydrosoluble molecules (minerals, alkaloids,
186 flavonoids, sugars, starch and other oligosaccharides) and to some hull loss during the
187 debittering process.
188 The debittering process significantly improved the *in vitro* digestibility of proteins (Table
189 2), which increased from 61.2% (bitter flour) to 63.7% (debittered flour). Soaking and
190 cooking reduce the presence of antinutrients such as phytic acid, tannins, α-amylase and
191 trypsin inhibitors; furthermore, the heating steps denature the proteins, making them more
192 available for digestion (El-Hady & Habiba, 2003).
193 Lipids content of bitter lupins (16.2±1.03 g/100 g) was similar to the *L. mutabilis* results
194 by Schoeneberger, Gross, Cremer and Elmadfa (1982) and Gross et al. (1988), and it is
195 largely superior to the lipid content of *L. albus, L. luteus* and *L. angustifolius* (Bähr et al.,

196 2014; Erbaş, Certel & Uslu, 2005; Erbas, 2010; Musco et al., 2017; Sujak, Kotlarz, &
197 Strobel, 2006); this lipid content increased to 24.8 g/100 g after debittering process and
198 also it is in good agreement with the results of Schoeneberger et al. (1982), who noticed
199 an augmentation from 15 to 26.9% after cooking and watering for three days Andean
200 lupin seeds.
201 In *L. mutabilis* the main soluble minerals are Ca, P, Na and K (Marroú, González &
202 Flores, 2011). Ash content of bitter lupins decreased from 4.8±0.21 g/100 g to 1.83 g/100
203 g after debittering (Table 2). The ash content of bitter lupins agreed with previously
204 reported for *L. mutabilis* from Ecuador (5.0 g/100 g DM) and was within the variation
205 (2.4-5.2 g/100 g DM) early summarized; also, a similar trend of ash decreasing (from 5.0
206 to 1.9 g/100 g) due to debittering was found (Carvajal-Larenas et al. (2014); Carvajal-
207 Larenas et al. (2016)). Meanwhile, a more limited reduction (from 2.57 to 2.55 g/100 g)
208 was observed in *L. albus* by Erbas (2010).
209
210 Total carbohydrates content was 31.7±2.5 g/100 g in bitter lupins, and it was in the range
211 (26.1-43.2 g/100 g) observed by Carvajal-Larenas et al. (2016). This content was
212 modified by debittering process. The debittering process had a negative effect on total
213 carbohydrates content (including fiber), which dropped from 31.6 to 18.9 g/100 g (Table
214 2), a 40% decrease from the raw seed values. Not many data on carbohydrates behavior
215 during debittering are available in literature, but in *L. albus* a decrease from 17.4 to 14.1
216 g/100 g is reported for the sum of crude fiber and starch by Erbas (2010). Interestingly,
217 debittering completely removed all sugars that might be implied in further degradation
218 reaction such as Maillard and others. Table 3 and Supplementary Table 3 reported the
219 concentrations of the sugars found in Andean bitter lupin accessions; the reducing sugars
220 were scarce (0.86 g/100 g), and sucrose was more abundant (4.34±1.33 g/100 g).

Nevertheless, the sucrose concentration on Table 3, was below values found by Gross et al. (1988) in two Chilean low-alkaloid strains of *L. mutabilis* (9.0-9.9 g/100 g) obtained by plant breeding but very similar to those described by Erbaş et al. (2005) for *L. albus* (4.1 g/100 g for sucrose).

3.2.2 Extrusion of debittered flour

Extrusion breaks cell wall structures and triggers several chemical and rheological changes; new hydrophobic interactions, hydrogen and disulfide bonds deeply modify proteins aggregation status (Chen, Wei & Zhang, 2011). One of the problems of the extrusion process is the loss of nutrients during the extrusion of the food. In the present investigation, it was possible to adjust the extrusion parameters (low temperature) to minimized lipid losses. Protein concentration slightly increased from 54.5±2.61 to 55.7±2.03 g/100 g DM (Table 2) in comparison with debittered flour, due to a minimum loss of dry matter. Lampart-Szczapa et al. (2006), showed a decrease of soluble proteins from raw to extruded flours in three lupin species (*L. luteus, L. albus and L. angustifolius*); according to them, proteins change to fibrous structure after extrusion due to new bonds formation. In addition, Frías et al. (2011) observed a slight protein increase from 23.6 to 24.1 g/100 g between raw and extruded *Pisum sativum* samples; the extrusion process was performed at 129, 135 and 142 °C.

The technological process of extrusion significantly improved the *in vitro* digestibility of proteins (Table 2), which increased from 63.7% (debittered flour) to 68.1% (extruded flour); El-Hady & Habiba (2003) mentioned that the temperature of extrusion denatures the proteins and reduces the presence of antinutrients, making them more available for digestion. In fact, Palanisamy et al. (2019) found that extrusion significantly improved protein *in vitro* digestibility of lupin extrudates (80.9-85.9%) compared to the raw

material mixture (78.2%) and concluded that the main influencing factors for the improvement of protein digestibility were the protein structure changes. El-Hady and Habiba (2003) noticed that *in vitro* protein digestibility increased from raw to extruded seeds in fava beans (from 75.4% to 80.4%), peas (from 74.5% to 78.1%), chickpeas (from 74.0% to 81.1%) and kidney beans (from 70.6% to 79.3%). Extrusion improved the lupin digestibility, but not to the level of the control casein protein isolate (87.1%).

The lipid content of lupin flour did not change from debittering to extrusion (Table 2); however, Frías et al. (2011) in pea observed moderate increases in lipid concentration after extrusion. Ash content showed marginal changes (1.8 - 1.7±0.34 g/100 g), mainly attributable to Andenes (Supplementary Table 2). Similar minor modifications were observed by El-Hady and Habiba (2003) in pea, chickpea, kidney bean and fava bean, as well as by Frías et al. (2011) in pea. On the other hand, the extrusion did not change very much the carbohydrates content, which hovered around 17.8±1.38 g/100 g (Table 2). Stability of carbohydrate concentration after extrusion was reported by Frías et al. (2011) in pea. The sugars were not detectable (Table 3), because they were already removed by the debittering step.

3.2.3 Spray-dried lupin drink

At the beginning, the lupin drink (8.5% total solids) prepared in this study as input material for the spray-drying process, had the following chemical composition in dry matter: 45.28±4.28 g/100 g (protein), 35.69±3.01 g/100 g (lipid), 18.52±5.94 g/100 g (carbohydrate), and 0.51±0.07 g/100 g (ash). Therefore, lupin drink had a protein content significantly lower than the bitter, debittered and extruded flours, because the sieve cloth strained many solids, which probably retained complex protein aggregates, allowing only the passage of soluble proteins. The lupin drink saw an increase in lipid concentration

(average: 35.7±3.01 g/100 g), because of the removal of solid compounds by sieving, but also a very low ash content (0.5±0.07 g/100 g). Jiménez, Dávila and Hernández (2000) reported in a *L. campestris* drink a protein content of 4.8 g/100 g and a lipid content of 1.4 g/100 in a 11% total solids solution (corresponding to 43.6 g/100 g and 12.7 g/100 g DM), indicating a low density of nutrients in comparison with *L. mutabilis*. Additionally, the solids removed during the lupin drink preparation could be utilized for the preparation of new nutritional-value products, as proposed for other leguminous crops (e.g. soybean) (McClements, Newman & McClements, 2019). Carbohydrates concentration was around 18.5±5.94 g/100 g; however, Altagracia showed a carbohydrate concentration higher than the other two lupins (Supplementary Table 2). Sugar concentration was not measured in the lupin drink, because they are already below the detection limits in the debittered lupin grains.

After spray-drying, the dried drink obtained showed a protein content (31.7-31.9 g/100 g DM) lower the flours obtained by the other process studied. This content was similar when using different wall materials such gum arabic or maltodextrin during spray-drying (Table 2). The presence of the wall materials (6%), which have a very poor/null protein content, had a diluting effect on the protein content.

The *in vitro* digestibility of proteins in spray-dried powder was 74.0% with gum arabic and 72.8% with maltodextrin, and the different genotypes showed similar behaviors. Therefore, spray-drying increased significantly the *in vitro* digestibility of proteins of debittered flours (63.7%). Spray-drying improved the digestibility, but not to the level of the control casein protein isolate (87.1%). Similarly, Almeida et al. (2015) reported an *in vitro* protein digestibility of soybean powder (55.2%) much lower than casein powder (83.7%). The nutritive value of legume proteins is lower than animal proteins because of

poor digestibility, deficiency of sulphur-rich amino acids and presence of antinutritional factors.

The lipid concentration was also low (15.7-17.7 g/100 g DM), i.e. about half that of the lupin drink, and lower than those of the other three treatments; the gum arabic addition conserved a marginally high lipid content in comparison to the maltodextrin.

The addition of wall materials during the spray-drying contributed to a slight increase in the ash content in comparison with the filtered suspension (to 0.75 g/100 g DM) for the maltodextrin-added and a four times higher (from 0.51±0.07 to 1.90 g/100 g DM) for the gum arabic-added samples (Table 2); this is due to the higher ash content of gum arabic (~2%) compared to maltodextrin (~0.45%). The gum arabic spray-drying Altagracia had an ash concentration that was one-half of the other two accessions (Supplementary Table 2). Similarly, wall materials contributed to the increase in carbohydrates content, 48.48 g/100 g (samples with gum arabic) and 51.90 g/100 g (samples with maltodextrin). The presence of sugars in spray dried powder (absent in the lupin drink) was totally due to the wall materials: in particular, the gum arabic supplied fructose and sucrose, while the maltodextrin contributed glucose, maltose and sucrose (Table 3).

3.3 Effect of process on markers of heat damage

3.3.1 Color and water activity

The *L**, *a** and *b** of the bitter lupin (Table 4 and Supplementary Table 4) were 84.6±0.90, -2.10±0.44 and 20.3±1.55, respectively, indicating a pale yellow-greenish tinge. Not much information is available about lupin color, but Mohamed and Rayas-Duarte (1995) recorded 82.8 (*L**), -1.98 (*a**) and +21.3 (*b**), while Yorgancilar and Bilgiçli (2014) reported range values for *L. albus*, for *L**, *a** and *b**, of 65.2-67.1, 3.4-7.0

and 16.5-20.3, respectively. In *L. angustifolius* Rumiyati, James and Jayasena (2015) observed *L**, *a** and *b** values of 90.6, -1.3 and 28.5, respectively.

Debittering led to a slight variation in color parameters in comparison with bitter lupin, ΔE = 5.03±1.32 (Table 4), possibly as the result of the hydration and cooking operations applied to remove the alkaloids that ended in the removing of some pigments. The extrusion (ΔE =10.50±3.25) reduced the luminosity (75.1±1.72) and increased *a** (-0.94±0.48) and *b** (23.8±1.90). Rumiyati et al. (2015) mentioned that the high extrusion temperature influences the color of the lupin. Spray-drying powder using maltodextrin was the most different in color (ΔE = 15.24±1.03) followed by the powder using gum arabic (ΔE = 14.56±1.05). The differences are mainly due to the luminosity of the spray-drying flours which was higher (92.0-92.6) than that of the other processed lupin while *b** was lower (7.7-7.8), suggesting that the wall materials improved the luminosity; maltodextrin increased *L** more than gum arabic.

The water activity of the bitter and debittered samples was very similar (0.57±0.01 and 0.58±0.01) and within the range of soybean flour (0.55-0.66) reported by Paucar-Menacho et al. (2010). The extrusion increased a_w (0.71±0.03); the lowest water activity was scored in the two spray-dried powders (gum arabic: 0.40±0.02 and maltodextrin: 0.41±0.02). An increase in water activity favors brown color development (bitter and debittered lupin) but after a_w reaches around 0.7 (extruded lupin) browning rates diminishes.

3.3.2 Furosine, HMF and GLI

Furosine was the most sensitive marker of heat damage for Andean lupin. In general, the heat damage of the samples was limited (Table 5). Furosine content was low, in bitter, debittered and extruded lupins (8.7-10.5 mg/100 g protein), increased slightly after gum

arabic spray-drying (13.4±2.0 mg/100 g protein) and showed a marked raise (54.1±20.7 mg/100 g protein) after maltodextrin spray-drying. The spike was particularly strong for the Andenes accession, which reached 80.5 mg/100 g protein (Supplementary Table 5); the reducing sugars content was similar among cultivars (Supplementary Table 3), thus the higher furosine formation in this cultivar may be related to a different ε-amino acid content. These values are comparable to those reported by Arnoldi et al. (2007) (25.7-53.6 mg/100 g protein) for lupin protein spray-dryed isolates and far lower than the 200-1000 mg/100 g protein reported for ten infant formulas. Furosine is an early indicator of quality changes associated to Maillard (Ruffian-Henares & García-Villanova, 2008). Additionally, HMF and GLI, which monitor heat damage during the intermediate-advanced steps of the Maillard reaction, were below the detection limit in the bitter, debittered and extruded flours. Spray-drying led to a low but detectable HMF content in the samples with gum arabic (0.11±0.09 mg/kg); gum arabic has no maltose, therefore GLI was not observed. A higher concentration of HMF, along with the presence of GLI (0.58±0.00 mg/kg), was detected in spray-dried flours with maltodextrin (0.60±0.09 mg/kg), thus indicating that this wall material (a complex oligosaccharide rich in glucose monomers) was involved in stronger (but still limited) heat damage.

4. Conclusions

The chemical composition of Andean lupin flours was modified by the technological processes (debittering, extrusion and spray-drying) applied. The five days water washing used in debittering reduced soluble sugars (fructose, glucose, maltose and sucrose). The dry matter chemical composition changed in debittered grains; proteins and lipids increased and ashes and carbohydrates decreased. Debittering and spray-drying diluted the intense yellow color of the flour. The protein *in vitro* digestibility was highest in the

spray-dried samples. Processing enhanced the protein *in vitro* digestibility of Andean lupin, without inducing relevant heat damage. Bitter, debittered and extruded lupin had very low furosine content and below detection HMF and GLI; however, all the heat damage indices were found, at low levels, in the spray-dried samples. The most sensitive heat damage marker identified for processed lupin was furosine. Processed Andean lupins are an alternative for human nutrition due to its protein and lipid high content.

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References

Almeida, C. C., Monteiro, M. L. G., Costa-Lima, B. R. C. da., Alvares, T. S., & Conte-Junior, C. A. (2015). *In vitro* digestibility of commercial whey protein supplements. *LWT - Food Science and Technology, 61*, 7–11. https://doi.org/10.1016/j.lwt.2014.11.038

AOAC (2000). *Official methods of analysis* (17th ed.). Gaithersburg: Association of Official Analytical Chemists International.

Arnoldi, A., Resta, D., Brambilla, F., Boschin, G., D'Agostina, A., Sirtori, E., & O'Kane, F. (2007). Parameters for the evaluation of the thermal damage and nutraceutical potential of lupin-based ingredients and food products. *Molecular Nutrition and Food Research, 51*, 431–436. https://doi.org/10.1002/mnfr.200600246

Bähr, M., Fechner, A., Hasenkopf, K., Mittermaier, S., & Jahreis, G. (2014). Chemical composition of dehulled seeds of selected lupin cultivars in comparison to pea and soya bean. *LWT - Food Science and Technology*, *59*, 587–590. https://doi.org/10.1016/j.lwt.2014.05.026

Boostani, S., Aminlari, M., Moosavi-nasab, M., Niakosari, M., & Mesbahi, G. (2017). Fabrication and characterisation of soy protein isolate-grafted dextran biopolymer: A novel ingredient in spray-dried soy beverage formulation. International Journal of Biological Macromolecules, 102, 297–307. https://doi.org/10.1016/j.ijbiomac.2017.04.019

Caligari, P. D. S., Römer, P., Rahim, M. A., Huyghe, C., Neves-Martins, J., & Sawicka-Sienkiewicz, E. J. (2000). The potential of *Lupinus mutabilis* as a crop. In *Linking research and marketing opportunities for pulses in the 21st century* (pp. 569-573). Springer, Dordrecht.

Carvajal-Larenas, F. E., van Boekel, M. J. A. S., Koziol, M., Nout, M. J. R., & Linnemann, A. R. (2014). Effect of processing on the diffusion of alkaloids and quality of *Lupinus mutabilis* Sweet. *Journal of Food Processing and Preservation, 38*, 1461-1471. https://doi.org/10.1111/jfpp.12105

Carvajal-Larenas, F. E., Linnemann, A. R., Nout, M. J. R., Koziol, M., & van Boekel, M. A. J. S. (2016). *Lupinus mutabilis*: composition, uses, toxicology, and debittering. *Critical Reviews in Food Science and Nutrition*, *56*, 1454-1487. https://doi.org/10.1080/10408398.2013.772089

Chen, F. L., Wei, Y. M., & Zhang, B. (2011). Chemical cross-linking and molecular aggregation of soybean protein during extrusion cooking at low and high moisture content. *LWT - Food Science and Technology*, *44*, 957-962. https://doi.org/10.1016/j.lwt.2010.12.008

El-Hady, E. A. A., & Habiba, R. A. (2003). Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. *LWT - Food Science and Technology*, *36*, 285-293. https://doi.org/10.1016/S0023-6438(02)00217-7

Erbas, M. (2010). The effects of different debittering methods on the production of lupin bean snack from bitter *Lupinus albus* L. seeds. *Journal of Food Quality*, *33*, 742–757. https://doi.org/10.1111/j.1745-4557.2010.00347.x

Erbaş, M., Certel, M., & Uslu, M. K. (2005). Some chemical properties of white lupin seeds (*Lupinus albus* L.). *Food Chemistry*, *89*, 341–345. https://doi.org/10.1016/j.foodchem.2004.02.040

Ertaş, N., & Bilgiçli, N. (2014). Effect of different debittering processes on mineral and phytic acid content of lupin (*Lupinus albus* L.) seeds. *Journal of Food Science and Technology*, *51*, 3348–3354. https://doi.org/10.1007/s13197-012-0837-2

Frías, J., Giacomino, S., Peñas, E., Pellegrino, N., Ferreyra, V., Apro, N., Carrión, M. O., & Vidal-Valverde, C. (2011). Assessment of the nutritional quality of raw and extruded *Pisum sativum* L. var. *laguna* seeds. *LWT - Food Science and Technology*, *44*, 1303-1308. https://doi.org/10.1016/j.lwt.2010.12.025

Gross, R., von Baer, E., Koch, F., Marquard, R., Trugo, L., & Wink, M. (1988). Chemical composition of a new variety of the Andean lupin (*Lupinus mutabilis* cv. Inti) with low-alkaloid content. *Journal of Food Composition and Analysis, 1*, 353–361. https://doi.org/10.1016/0889-1575(88)90035-X

Güemes-Vera, N., Esperza, O. A. R., & Dávila-Ortiz, G. (2004). Structural analysis of the *Lupinus mutabilis* seed, its flour, concentrate, and isolate as well as their behavior when mixed with wheat flour. *LWT - Food Science and Technology, 37*, 283-290. https://doi.org/10.1016/j.lwt.2003.07.001

Hickisch, A., Beer, R., Vogel, R. F., & Toelstede, S. (2016). Influence of lupin-based milk alternative heat treatment and exopolysaccharide-producing lactic acid bacteria on the physical characteristics of lupin-based yogurt alternatives. *Food Research International, 84*, 180–188. https://doi.org/10.1016/j.foodres.2016.03.037

Hidalgo, A., Brandolini, A. (2011). Heat damage of water biscuits from einkorn, durum and bread wheat flours. *Food Chemistry, 128*, 471-478. https://doi.org/10.1016/j.foodchem.2011.03.056 472

Islam, N., Khalil, I., Islam A., & Gan S. H. (2014). Toxic compounds in honey. *Journal of Applied Toxicology. 34, 733–742.*

Jacobsen, S., & Mujica, A. (2006). El tarwi (*Lupinus mutabilis* Sweet.) y sus parientes silvestres. *Revista botánica económica de los andes centrales*. 458-482.

Jiménez, M. C., Dávila, O. G., & Hernández, S. H. (2000). Producing milk-and yoghut-like products from *Lupinus campestris* seed. In: Santen, E. van; Wink, M.;

457 Weissmann, S.; Römer, P. *Lupin, an ancient crop for the new millennium:*
458 *Proceedings of the 9th International Lupin Conference, Klink/Muritz, Germany, 20-24*
459 *June, 1999.* (pp. 442-445). International Lupin Association.
460 Kaczmarska, K. T., Chandra-Hioe, M. V., Frank, D., & Arcot, J. (2018). Aroma
461 characteristics of lupin and soybean after germination and effect of fermentation on
462 lupin aroma. *LWT - Food Science and Technology, 87,* 225–233.
463 https://doi.org/10.1016/j.lwt.2017.08.080
464 Lampart-Szczapa, E., Konieczny, P., Nogala-Kałucka, M., Walczak, S., Kossowska, I.,
465 & Malinowska, M. (2006). Some functional properties of lupin proteins modified by
466 lactic fermentation and extrusion. *Food Chemistry, 96,* 290–296.
467 https://doi.org/10.1016/j.foodchem.2005.02.031
468 Marroú, M. E. L., González, M. Y. V., & Flores, S. E. P. (2011). Chemical composition
469 of "oca" (*Oxalis tuberosa*), "arracacha" (*Arracaccia xanthorriza*) and "tarwi" (*Lupinus*
470 *mutabilis*). Formulation of a base mixture for food. *Revista Venezolana de Ciencia y*
471 *Tecnología de Alimentos, 2*: 239-252.
472 McClement, D. J., Newman, E., & McClement, I. F. (2019). Plant-based milks: A review
473 of the science underpinning their design, fabrication, and performance. *Comprehensive*
474 *Reviews in Food Science and Food Safety, 18,* 2047-2067.
475 Mohamed, A. A., & Rayas-Duarte, P. (1995). Composition of *Lupinus albus*. *Cereal*
476 *Chemistry*, *72*, 643-647.
477 Musco, N., Cutrignelli, M. I., Calabrò, S., Tudisco, R., Infascelli, F., Grazioli, R., Lo
478 Presti, V., Gresta, F., & Chiofalo, B. (2017). Comparison of nutritional and
479 antinutritional traits among different species (*Lupinus albus* L., *Lupinus luteus* L.,
480 *Lupinus angustifolius* L.) and varieties of lupin seeds. *Journal of Animal Physiology and*
481 *Animal Nutrition, 101,* 1227–1241. https://doi.org/10.1111/jpn.12643

Palanisamy, M., Franke, K., Berger, R. G., Heinz, V., & Töpfl, S. (2019). High moisture extrusion of lupin protein: Influence of extrusion parameters on extruder responses and product properties. *Journal of the Science of Food and Agriculture*, 99, 2175-2185. https://doi.org/10.1002/jsfa.9410

Cortés-Avendaño, P., Tarvainen, M., Suomela, J. P., Glorio-Paulet, P., Yang, B., & Repo-Carrasco-Valencia, R. (2020). Profile and content of residual alkaloids in ten ecotypes of *Lupinus mutabilis* Sweet after aqueous debittering process. *Plant Foods for Human Nutrition*. Published online: https://doi.org/10.1007/s11130-020-00799-y

Paucar-Menacho, L. M., Amaya-Farfán, J., Berhow, M. A., Mandarino, J. M. G., de Mejia, E. G., & Chang, Y. K. (2010). A high-protein soybean cultivar contains lower isoflavones and saponins but higher minerals and bioactive peptides than a low-protein cultivar. *Food Chemistry, 120*, 15–21. https://doi.org/10.1016/j.foodchem.2009.09.062

Ruffian-Henares, J. A., & García-Villanova, B. (2008). Occurrence of furosine and hydroxymethylfurfural as markers of thermal damage in dehydrated vegetables. *European Food Research and Technology*, *228*, 249-256.

Rumiyati, R., James, A. P., & Jayasena, V. (2015). Effects of lupin incorporation on the physical properties and stability of bioactive constituents in muffins. *International Journal of Food Science and Technology, 50*, 103–110. https://doi.org/10.1111/ijfs.12688

Schoeneberger, H., Gross, R., Cremer, H. D., & Elmadfa, I. (1982). Composition and protein quality of *Lupinus mutabilis*. *The Journal of Nutrition, 112*, 70-76. https://doi.org/10.1093/jn/112.1.70

Sosnik, A., & Seremeta, K. P. (2015). Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric

carriers. *Advances in Colloid and Interface Science, 223,* 40–54. https://doi.org/10.1016/j.cis.2015.05.003

Sujak, A., Kotlarz, A., & Strobel, W. (2006). Compositional and nutritional evaluation of several lupin seeds. *Food Chemistry, 98,* 711–719. https://doi.org/10.1016/j.foodchem.2005.06.036

Villarino, C. B. J., Jayasena, V., Coorey, R., Chakrabarti-Bell, S., Foley, R., Fanning, K., & Johnson, S. K. (2015). The effects of lupin (*Lupinus angustifolius*) addition to wheat bread on its nutritional, phytochemical and bioactive composition and protein quality. *Food Research International, 76,* 58–65. https://doi.org/10.1016/j.foodres.2014.11.046

Yorgancilar, M., & Bilgiçli, N. (2014). Chemical and nutritional changes in bitter and sweet lupin seeds (*Lupinus albus* L.) during bulgur production. *Journal of Food Science and Technology, 51,* 1384-1389. https://doi.org/10.1007/s13197-012-0640-0

Zhong, L., Fang, Z., Wahlqvist, M. L., Hodgson, J. M., & Johnson, S. K. (2019). Extrusion cooking increases soluble dietary fibre of lupin seed coat. *LWT - Food Science and Technology, 99,* 547-554. https://doi.org/10.1016/j.lwt.2018.10.018