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EFFECT OF PROCESSING ON THE DIFFUSION OF ALKALOIDS AND QUALITY OF *LUPINUS MUTABILIS* SWEET

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ABSTRACT

Cold water processing by soaking, cooking and washing has been used for hundreds of years to produce debittered lupine in the Andean region. The process of debittering lupine (*Lupinus mutabilis* Sweet) was investigated at semi-industrial village-scale and laboratory scale in Ecuador. The process took 5.7 ± 1.0 days, removed 94.9% of the total alkaloids, used water at almost 62 times the weight of the raw dry and bitter lupine, and caused a 22% loss of total solids, principally fat, minerals and carbohydrates. During the debittering process the microbiological quality deteriorated. Mathematical modeling based on Fickian diffusion suggested that the diffusion coefficient of alkaloids would be expected to be between 10^{-10} and 10^{-11} m²/s because the lupine endosperm is a polymer matrix whose properties change during processing. Of the process operations, cooking was the most efficient at removing alkaloids both in terms of time and water used, followed by soaking and washing.

PRACTICAL APPLICATIONS

The current debittering process of bitter toxic lupine in San Pedro, Ecuador, is effective but consumes much water and time. During the process, not only alkaloids are removed but also 22% of total solids, principally fats, minerals and carbohydrates. The microbiological quality of the product deteriorates during this long processing time. Improving the efficiency of the debittering process would reduce water consumption, save time and improve the nutritional and microbiological quality of the final product. Future work will focus on strategies to optimize the debittering process.

INTRODUCTION

Lupine is a tall-growing grain legume (Pate *et al.* 1985), which is employed as a protein source in human and animal nutrition (Güemes-Vera *et al.* 2008). Some lupine species have functional properties in bakery and pastry products (Güemes-Vera *et al.* 2008), e.g., by increasing water absorption of the dough (Güemes-Vera *et al.* 2008). The consumption of lupine might have beneficial effects on human health (Güemes-Vera *et al.* 2008) as they contain antimutagenic, anticarcinogenic, hypocholesterolemic, and antioxidant phenolic compounds, and prebiotic oligosaccharides, which favor the proliferation of bifidobacteria (Jiménez-Martínez *et al.* 2003b).

The FAO (2009) reported that more than 585,000 metric tons of lupine were produced in 2007, with the legume being cultivated in Germany, Poland, the Russian Federation and Mediterranean countries as well as in Australia, South Africa and South America. Four major crop species are cultivated, namely *Lupinus albus* L., *L. luteus* L., *L. angustifolius* L. and *L. mutabilis* Sweet, of which the latter is cultivated in Ecuador, Peru and Chile, and which shows the highest average content, on a dry weight basis, of protein (440 g/kg) and lipids (180 g/kg) (Pate *et al.* 1985). *L. mutabilis* is commonly known in Chile as “lupino” (Peralta *et al.* 2001), in Peru as “tarhui” (Aguilera and Trier 1978; Torres-Tello *et al.* 1980) or “tarwi” (Aguilera and Trier 1978; Santos *et al.* 1997; Caicedo *et al.* 2001) depending on

the translation from the Quechua, and as “chocho” in Ecuador (Aguilera and Trier 1978; Villacrés *et al.* 2003).

In Ecuador, lupine can be cultivated in the harsh climates of the Andes and in poor soils. It is an important food crop whose production increased from 662 metric tons in 1986 (Moncayo *et al.* 2000) to 789 tons in 2000 (Junovich 2003), with up to 90% of this production destined for human consumption (Aguilera and Trier 1978; Petterson and Crosbie 1990; Gueguen and Cerletti 1994; Zduńczyk *et al.* 1996; Santos *et al.* 1997; Caicedo *et al.* 2001). Its nutritional value is similar to that of soya bean (Aguilera and Trier 1978; Petterson and Crosbie 1990; Gueguen and Cerletti 1994; Zduńczyk *et al.* 1996; Santos *et al.* 1997), and therefore the crop is known as the soya bean of the Andes (Villacrés *et al.* 2003). In spite of its importance, lupine has been studied much less than soya bean (Gueguen and Cerletti 1994). The use of lupine as a protein-rich food crop is hampered by its high alkaloid content (Aguilera and Trier 1978; Aguilera *et al.* 1983; Petterson and Crosbie 1990), which has a useful function in the lupine plant because it gives high resistance to microbial infections and insect attacks (Ciesiolka *et al.* 2005). These bitter tasting and toxic components must be removed before consumption (Aguilera and Trier 1978; Aguilera *et al.* 1983; Beirao da Costa 1989; Petterson and Crosbie 1990; Haq 1993; Gueguen and Cerletti 1994; Zduńczyk *et al.* 1996). Detoxification was attempted by chemical (Von Baer *et al.* 1979; Torres-Tello *et al.* 1980; Ortiz and Mukherjee 1982; Chango *et al.* 1993; Touche *et al.* 1997; Nossak *et al.* 2000; Jiménez-Martínez *et al.* 2003a) and biological methods (Szakács and Stankovics 1983; Agosin *et al.* 1989; Camacho *et al.* 1991; Santana *et al.* 1996; Santana and Empis 2001). As an extraction solvent, water in combination with several chemicals (Gueguen and Cerletti 1994) or water-alcohol mixtures were tested (Kahnt and Kurz 1989). Water as the only solvent was investigated both at a laboratory (Torres-Tello *et al.* 1980; Aguilera *et al.* 1983) and at commercial scale (Rossetto 1989), and was considered (Chajuss 1989) the most economical way to remove alkaloids. The use of water is advantageous because it prevents environmental contamination with chemical products and does not require the recovery of organic solvents (Rossetto 1989).

In the Andean Region the process of debittering lupine with water has been in use since pre-Inca times (Torres-Tello *et al.* 1980). In Ecuador, the National Agricultural Research Centre (INIAP) has proposed a commercial wet-warm debittering process, which consists of soaking the lupine for 14–16 h at 40°C, boiling for 40 min, and then washing at 40°C (Caicedo *et al.* 2001). The process uses potable water and applies good manufacturing practices (Caicedo *et al.* 2001). However, limited information is available on the consequences of debittering of lupine with water as regards (1) the amount of water and time used in

each stage of the process and in the process as a whole; (2) the alkaloid content in lupine before and after each stage of the debittering process; (3) the changes in the nutritional composition of debittered lupine regarding macro- and micronutrients; (4) the microbiological quality of the lupine before and after debittering; (5) an estimation of the diffusion time of alkaloids in water; and (6) a model of the diffusion of alkaloids in water.

The current investigation was performed to contribute to the knowledge about the wet processing of lupine in cold water and to offer insights to improve the present system to the benefit of processors and consumers of lupine.

MATERIALS AND METHODS

Field Survey

The debittering process with cold water is used on both household and commercial scale in the village of San Pedro in the Province of Cotopaxi in Ecuador. In this village, the debittering is performed in approximately 40 small factories, of which 10 were selected and surveyed to define the debittering process in use.

Raw Lupine

A composite batch of raw bitter lupine (15 kg) was obtained by pooling equal quantities obtained from five processors (3 kg from each) using random sampling in the village of San Pedro, Cotopaxi Province, Ecuador. All the processors used *Lupinus mutabilis* Sweet. From this batch, a sample of 500 g was reserved for microbiological determinations that were performed. The remaining 14.5 kg were stored dry and were used for the debittering experiments and for the determination of macronutrients, iron, zinc and total alkaloid concentrations. All determinations were performed in duplicate.

Sampling

Samples were taken at 4-h intervals for soaking experiments, at 15-min intervals for cooking effect, for washing effect and materials balance at 12 h during the first 2 days, followed by 6-h intervals the next day; during the final washing period, samples were evaluated each hour.

For composition and microbiological quality, samples were evaluated at the end of the process.

For analysis of efficiency the amount of water was registered every time it was added. Time was registered at the end of the process, and alkaloids were analyzed during and at the end of the process.

Debittered Lupine

For microbiological determinations, debittered lupine was randomly sampled from five processors (800 g from each). The samples were collected in sterile containers and kept refrigerated until analysis.

For the determination of macronutrients, iron, zinc and total alkaloid concentrations, lupine was randomly sampled from the same five processors (500 g from each). All samples were analyzed in duplicate.

Determination of Macronutrients, Iron, Zinc and Microbiological Quality in Raw and Debittered Lupine

In both raw bitter, and debittered lupine, protein was determined by Association of Official Analytical Chemists (AOAC) method 2001.11 using the default N : P conversion factor of 6.25, fat by AOAC 920.39C, ash by AOAC 923.03, moisture content by AOAC 925.09 and carbohydrates, which included fiber, were calculated as the difference (100% – %moisture – %protein – %fat – %ash – %alkaloids). Iron and zinc were determined using AOAC 999.11 method (AOAC 2005).

Total mesophilic aerobic plate count (colony forming units [cfu]) was determined by AOAC method 966.23, fungal plate count by AOAC 997.02 (AOAC 2005) and *E. coli* (most probable number [MPN]) by the method of the Ecuadorean Institute of Standards (Instituto Ecuatoriano de Normalización) (INEN Instituto Ecuatoriano de Normalización 1990).

Determination of Alkaloids

For the determination of alkaloid concentrations in raw bitter lupine, 1 kg of the previously pooled sample of 14.5 kg was milled using a model 4E mill (The Strub Company, Hatboro, PA) and sieved with a shaking 60 mesh sieve (Meinzer II, Series 0447, Fairfax, VA; dual model, MFG Co. Chicago, IL), with the throughs taken for analysis. The lupine was not dehulled before milling and sieving. All alkaloid determinations were performed in duplicate.

All chemicals were analytical grade obtained from Merck Ecuador, Quito, Ecuador: chloroform (catalog number 1024452500), Al_2O_3 (1010671000), Dragendorff's reagent (1020350100) and methyl red indicator (1060760100). A 0.01 M solution of sodium hydroxide was prepared by diluting a standard solution 0.1 M of sodium hydroxide (Merck Ecuador, 109141100) in freshly distilled water. A solution 0.005 M of sulfuric acid was obtained by diluting sulfuric acid 0.05 M (for titration, 1099840001) in freshly distilled water. Potassium hydroxide solution (2.68 M,

catalog number 0421) was obtained from AIC Cia. Ltda., Quito, Ecuador.

Alkaloid concentrations were determined by titration using the methodology described by von Baer *et al.* (1979) with the modification suggested by the Ecuadorean Institute of Standards (INEN Instituto Ecuatoriano de Normalización 2005): this is the current official method in Ecuador. To 0.2 g lupine ground to pass a 60 mesh screen, 0.6 g of basic Al_2O_3 was added and blended to a fine powder. Then 0.2 mL of 2.68 M KOH was added and blended again to a homogeneous paste. This paste was then transferred to centrifuge tubes, and 6 mL of chloroform was added, followed by mixing with a glass stirring rod and centrifuging for 2 min at 900 g. The supernatant was poured through a cotton filter into a glass vial. The process of adding chloroform, mixing, centrifuging and filtering was repeated at least 10 times, until absence of alkaloids in the final extract could be demonstrated. This was done by adding four to five drops of 0.005 M sulfuric acid and three to four drops of Dragendorff's reagent to 1 mL of the final extract: a negative reaction (absence of orange colored spots) should result (Nerín and Garnica 1986). Finally, the funnel used for the filtration was rinsed with 15 mL of chloroform. All extracts, including the last 15 mL wash, were collected in the glass vial and were evaporated at 30°C until 1 mL remained; this was further evaporated when cooling the sample in a 15°C water bath. For the determination of alkaloid concentrations, 5 mL of 0.005 M sulfuric acid and two drops of methyl red indicator were added to the vial. The excess acid was titrated with 0.01 M NaOH. The concentration of total alkaloids, expressed as lupanine content (g/100g), was calculated as follows (INEN Instituto Ecuatoriano de Normalización 2005):

Total alkaloids expressed as lupanine (g/100 g)

$$= \frac{0.248 \times V}{\text{weight of sample (g)}} \quad (1)$$

$V = V_0 - V_1$

$V_0 = 5 \text{ mL}$

$V_1 = \text{ml used in titration}$

0.248 represents the weight of lupanine (g) in a solution 0.01 M (2.48 g) $\times 100$ (to express the result directly in percentage)/1,000 (transformation of ml of acid to liters).

Water used in the Debittering Process

Raw, dehulled lupine was debittered in the laboratory by the same procedure as used by the local processors (Fig. 1 and Table 1, using the maximum time for soaking and boiling and the minimum time for washing), which are conditions similar to those of the procedure proposed by INIAP

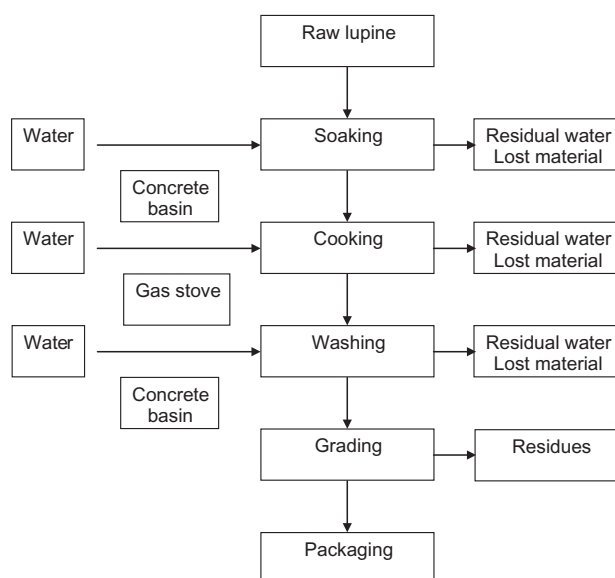


FIG. 1. PROCESS FOR DEBITTERING LUPINE

Performed in the village of San Pedro, Cotopaxi Province, Ecuador.

(Caicedo *et al.* 2001). Each batch started with 1,000 g of raw, dehulled lupine.

The minimum water : lupine ratio used was 2.5:1 (w/w) for soaking, boiling, and washing of the raw lupine. This amount of water was the minimum needed to keep the lupine covered by water at all times. During the washing process the water was changed three times per day, namely at 9:00 a.m., 12:00 p.m. and 4:00 p.m. The total amount of water used was established as the difference between the water added before each operation and that remaining after the operation.

Efficiency of Each Operation in the Debittering Process

Total alkaloid concentrations were determined before and after each operation with the methodology described above. This information was combined with that for the water used for debittering and the time required for each operation to estimate the efficiency of each step of the debittering process. The equations used were

$$\text{Water efficiency (g/kg)} = \frac{\text{alkaloids extracted (g/kg raw dry lupine)}}{\text{water used (kg/kg raw dry lupine)}} \quad (2)$$

Where water efficiency is expressed in g of alkaloids extracted per kg of water used.

$$\text{Time efficiency (g/kg x h)} = \frac{\text{alkaloids extracted (g/kg raw dry lupine)}}{\text{time used (h)}} \quad (3)$$

Where time efficiency is expressed in g of alkaloids extracted per kg of raw lupine and hour of extraction.

Estimation of the Diffusion Time of Alkaloids in Water

To assess possible improvements in efficiency, a theoretical diffusion time for alkaloids in lupine was calculated and compared with that used in the current debittering process. The estimated D -value was obtained without stirring or agitation.

The theoretical diffusion time, t , was calculated according to Walstra (2003) as follows:

$$t = x^2/D \quad (4)$$

Where:

t = time (s) required to travel a distance x

x = Distance of diffusion (m)

D = Diffusion coefficient (m^2/s)

The maximum distance (x) that an alkaloid molecule would travel in a hydrated seed of lupine was assumed to be the distance between the geometric center of the seed and the integument, approximately 6.4×10^{-3} m. To estimate the diffusion time of alkaloids in lupine the diffusion coefficient of sucrose in most fruits (D) was used, namely $1 \times 10^{-10} \text{ m}^2/\text{s}$ (Walstra 2003).

Diffusion Modeling

To estimate the kinetics of the removal of alkaloids by boiling, soaking and washing, we modeled based on Fickian diffusion (Van Boekel 2009). However, it must be stressed that the process of extraction of alkaloids is very complex. The primarily solid matrix of lupine seed consists mainly of cell walls, protein and starch, forming a polymer network through which the alkaloids must diffuse to reach the surrounding water. A correction to the diffusion constant is needed due to tortuosity (Walstra 2003), meaning that the diffusing molecules need to travel around obstacles. According to Walstra (2003) this could lead to a correction factor

TABLE 1. TIME USED FOR THE DEBITTERING PROCESS OF LUPINE IN THE VILLAGE OF SAN PEDRO, COTOPAXI PROVINCE, ECUADOR

| | Soaking (h) | Cooking (h) | Washing (d) | Total time (d) |
|---------------------|----------------|----------------|----------------|-------------------|
| Average \pm S.D.* | 12 \pm 4.6 | 0.4 \pm 0.4 | 5.2 \pm 0.8 | 5.7 \pm 1.0 |
| Minimum time | 8.0 | 0.2 | 4.5 | |
| Maximum time | 18.0 | 1.0 | 6.0 | |

* Values represent means \pm standard deviation ($n = 10$).

The end point for the last step is determined by tasting. The time needed for grading and packing depends on the quantity of lupine.

of up to 0.4 for the diffusion coefficient. Furthermore, during soaking the seeds will imbibe water, and this absorbed water will cause some swelling. While this may enhance the diffusion process because the molecules can now move in a more aqueous environment, it implies that the diffusion constant will change as the process advances. Finally, there could be a considerable constraint for the molecules to cross the barrier between the seed integument and the water, slowing down the extraction process. We did not attempt to model all these processes together. Rather, we wanted to investigate the degree to which Fickian diffusion can describe the observed phenomena.

The starting point for modeling is the second diffusion law of Fick (Crank 1975):

$$\frac{\partial c_s}{\partial t} = D_s \frac{\partial^2 c_s}{\partial x^2} \quad (5)$$

where c_s is the concentration of alkaloids in the seed (g/kg), D_s the diffusion constant in m^2/s , x the distance over which the molecules diffuse, t the time in s. The boundary conditions needed to solve this equation are (1) only one-dimensional radial diffusion is considered over distance x , (2) the alkaloids are homogeneously distributed within the seed at time zero, (3) the surrounding water is free of alkaloids at the start, (4) there is no concentration gradient of alkaloids in the water, and (5) the mass balance at the contact side is described as

$$K_{s/w} \frac{V_w}{A} \frac{\partial c_s}{\partial x} = -D_s \frac{\partial c_s}{\partial x} \text{ at } x = L_s \quad (6)$$

where $K_{s/w}$ is the partitioning coefficient describing the partitioning between the seed and the water, V_w is the volume of the water in m^3 , L_s the distance in m and A is the contact area in m^2 . We considered the case of one lupine seed, with average diameter of 0.0128 m, a contact area for radial diffusion of $25 \times 10^{-6} \text{ m}^2$, being present in a volume of 2 mL of water.

These equations were numerically solved using the software program Athena Visual Studio (<http://www.athenavision.com>). Since there is no information about the magnitude of the partitioning coefficient we assumed $K_{s/w} = 1$ and we simulated various values for D_s .

Alternatives of Washing and Optimization of this Process

In accordance with Eqs. (5) and (6) variations in the alkaloid concentration in the seed depend on several factors, most of which cannot be varied. However, the volume of used water can be changed and the impact of this variation on alkaloids content, solids in the product and amount of water used can be measured as a function of time.

In order to do this, a factorial design $(3A \times 3B) + 1 = 9$ treatments + 1 reference point was used.

Factor A was “changes of water per day” (Ch) with levels 3, 6 and 9. The changes of water were spaced evenly during the day. For example, for level 3 changes of water per day water was changed at 8:00 a.m., 4:00 p.m. and 10:00 p.m.

Factor B was “processing time” (t) with levels t1, t2 and t3 days. We express the levels in a generic form because each treatment needed different processing times to achieve a safe level of alkaloids (maximum 0.25% d.w.). Levels t3 were taken at the end of the process. Levels t1 and t2 were taken at equal intervals.

The nine treatments were as follows: three changes of water, time 1 (Ch3, t1); three changes of water, time 2 (Ch3, t2); three changes of water, time 3 (Ch3, t3); six changes of water, time 1 (Ch6, t1); six changes of water, time 2 (Ch6, t2); six changes of water, time 3 (Ch6, t3); nine changes of water, time 1 (Ch9, t1); nine changes of water, time 2 (Ch9, t2); and nine changes of water, time 3 (Ch9, t3). The reference point is the lupine before washing (Ch0, t0). Variables measured were alkaloids content (% d.w.), water used (l/kg d.w. raw lupine) and solids in the product (kg/kg d.w. raw lupine).

The results were analyzed by using the software program Design-Expert 8 (Stat-Ease, Inc., Minneapolis, MN; response surface approach) aiming at an optimum solution (minimization of processing time, water used and maximization of solids in the product) and keeping the alkaloids content at safe level (maximum 0.25% d.w.).

Statistical Analysis

Of each sampling point, 3, 5, 6 or even 10 samples were taken. Each sample was analyzed in duplicate.

The Mann–Whitney U -test, a nonparametric test, was used to assess differences between two distributions representing raw and debittered lupine. For this purpose, the software package GRAPH PAD INSTAT T.M. V2.01. was used (copyright 1990–1993, Steve Whetzel, Parke-Davis 930762 A; GraphPad Software Inc., San Diego, CA).

RESULTS AND DISCUSSION

In the village of San Pedro in the Province of Cotopaxi in Ecuador, lupine was debittered with cold water (16°C) according to the procedure shown in Fig. 1, which required on average 5.7 ± 1.0 days (Table 1). The end point of the debittering process was determined by tasting. Local processors complain that this process uses too much water, namely 63 tons of water per ton of raw lupine (Caicedo *et al.* 2001), and too much time.

TABLE 2. COMPOSITION OF RAW AND DEBITTERED LUPINE COLLECTED FROM THE VILLAGE OF SAN PEDRO, COTOPAXI PROVINCE, ECUADOR

| Component | Raw lupine | Debittered lupine | P value |
|----------------|--------------|-------------------|------------|
| Protein* | 41.4 ± 0.08† | 55.9 ± 2.56‡ | P < 0.05§ |
| Fat* | 23.4 ± 0.42 | 8.9 ± 2.57 | P < 0.05 |
| Carbohydrates* | 26.6 ± 0.21 | 33.1 ± 2.50 | P < 0.05 |
| Ash* | 5.0 ± 0.07 | 1.9 ± 0.11 | P < 0.05 |
| Alkaloids* | 3.6 ± 0.07 | 0.2 ± 0.01 | P < 0.0005 |
| Iron¶ | 5.0 ± 0.43 | 5.8 ± 0.61 | NS |
| Zinc¶ | 3.6 ± 0.04 | 3.6 ± 0.45 | NS |

* g/100g dry matter.

† Values represent means ± standard deviation (n = 6).

‡ Values represent means ± standard deviation (n = 5).

§ Mann-Whitney –U-test; NS = not significant.

¶ mg/100g dry matter.

TABLE 3. MICROBIOLOGICAL QUALITY OF RAW AND DEBITTERED LUPINE COLLECTED FROM THE VILLAGE OF SAN PEDRO, COTOPAXI PROVINCE, ECUADOR

| Parameter | Raw lupine* | Debittered Lupine† | P value‡ |
|--|-------------|--------------------|----------|
| Total mesophilic aerobic bacteria (Log cfu§/g) | 2.67 | 6.71 | P < 0.05 |
| Fungi (yeasts and molds) (Log cfu/g) | 2.59 | 1.99 | P < 0.05 |
| <i>E. coli</i> (presence/g) | Present | Present | |

* Values represent means (n = 5).

† Values represent means (n = 5).

‡ Mann-Whitney –U-test.

§ cfu/g = colony-forming units per gram fresh weight.

Processing the lupine for 5.7 days leads to desirable as well as undesirable changes in composition and microbiological quality as shown in Tables 2–4. A comparison of the proximate analyses of raw and debittered lupine based on dry weight as presented in Tables 2 and 4 would seem contradictory. For example, carbohydrates increase in Table 2 but decrease in Table 4. The reason is the important loss of solids taking place during the debittering process. Due to losses of dry matter as specified in Table 4, apparent increases of certain components in the dry matter are revealed. In fact, if the amount of carbohydrate in Table 4 (257.9 g dm: dry matter) is expressed on the basis of the total dry matter (780 g), we obtain the value of 33.1 g/100g dm as in Table 2.

We presented data on the formats of Tables 2 and 4 to point out errors that can be committed when reporting and discussing results of a process where losses of materials take place, without taking these into account. From here onward, our analysis regarding to composition was referred to values reported in Table 4.

Ash (mineral) and alkaloid concentrations were decreased significantly as would be expected from leaching, but interestingly fat concentrations also decreased significantly. Aguilera *et al.* (1983) reported reductions in fat content in debittered lupine ranging from 50.2% to 81.6%, similar to our observation (Table 4). Aqueous extraction of fat is most likely due to some sort of micelle formation with the lecithin present in lupine (Rozan *et al.* 1997).

In debittered lupine, numbers of total mesophilic aerobes were greatly increased (Table 3) to levels exceeding the Ecuadorean Sanitary Norm (INEN Instituto Ecuatoriano de Normalización 2005), which stipulates a maximum total plate count of log 3 cfu/g and the absence of *E. coli* in one gram or ml of sample. As an extreme case, Villacrés *et al.* (2000) reported total plate counts of the order of log 8 cfu/g and the occurrence of *E. coli* at levels ranging between <log 1.48 and >log 3.38 cfu/g, estimated by MPN method. As most of this product is consumed without any further processing, it must represent a potential hazard for human consumption.

A general mass balance for the process showed that the amount of water used is about 62 times the weight of raw dry bitter lupine (Fig. 2), confirming observations by Caicedo *et al.* (2001); the quantity of debittered lupine obtained before grading is 2.8 times that of raw dry lupine, a value similar to the factor reported by Caicedo *et al.* (2001), namely 2.7 when reported on a dry weight basis. Even though the final weight of the debittered lupine is 2.8 times that of the raw dry material, the process is losing 22% of initial solids (Fig. 2), a value similar to the 26.9% loss reported by Torres-Tello *et al.* (1980). This represents a considerable loss. Based on percentages, the principal components lost during debittering are alkaloids (94.9%), ash (minerals) and fat (70.3% each), and carbohydrates (2.9%) (Table 4). Of the micronutrients, 20.8% of the zinc and 9.8% of the iron concentration are lost (Table 4).

The debittering process using cold water removed 94.9% of the total alkaloids (Table 4), compared with 79 to 99.9%

TABLE 4. DRY MATTER BALANCE OF NUTRIENTS AND ALKALOIDS DURING THE DEBITTERING OF RAW LUPINE

| Material | Raw | Debittered | Difference g | Difference % |
|----------------------------|--------------|--------------|---------------|--------------|
| Dry Matter (g) | 1,000 | 780.0 | –220.0 | –22.0 |
| Nutrients: | | | | |
| Protein (g) | 414.1 | 435.8 | 21.7 | 5.2 |
| Carbohydrates (g) | 265.6 | 257.9 | –7.7 | –2.9 |
| Fat (g) | 234.2 | 69.5 | –164.7 | –70.3 |
| Ash (g) | 50.5 | 15.0 | –35.5 | –70.3 |
| Iron (mg) | 50.2 | 45.3 | –4.9 | –9.8 |
| Zinc (mg) | 36.0 | 28.5 | –7.5 | –20.8 |
| Total alkaloids (g) | 35.6 | 1.8 | –33.8 | –94.9 |

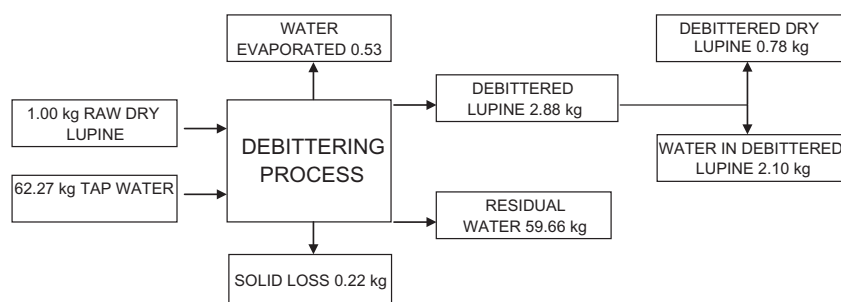


FIG. 2. GENERAL MASS BALANCE EXPRESSED FOR 1 KG OF RAW DRY LUPINE

removal achieved by chemical methods (Torres-Tello *et al.* 1980; Ortiz and Mukherjee 1982; Chango *et al.* 1993; Jiménez-Martínez *et al.* 2003a), 14.9 to 99% by biological methods (Szakács and Stankovics 1983; Agosin *et al.* 1989; Camacho *et al.* 1991; Santana *et al.* 1996; Santana and Empis 2001; Santana *et al.* 2002), 99.9% by water-chemical extraction (Aguilera *et al.* 1983) and 97.7% by warm water extraction (Caicedo *et al.* 2001). This indicates that the use of cold water for debittering lupine can achieve a similar detoxification performance as chemical, biological, warm water and combined forms of alkaloids extraction. This traditional process therefore merits an environmentally friendly upgrading in terms of saving water and time.

Debittering Efficiency of Individual Operations of the Debittering Process

The individual operations in the debittering process show varying degrees of efficiencies regarding the extraction of alkaloids (Table 5). Soaking removed only a small fraction of the total alkaloids and had a low efficiency both in terms of time and the quantity of water used. Despite the fact that the washing operation removed most of the alkaloids, it had a very low efficiency in terms of time and the quantity of water used. The cooking operation had the highest

efficiency when compared with soaking and washing, as well as with the complete process.

Soaking and cooking are important in the hydration of the integument, endosperm and embryo, and thus have a direct impact on the rate of diffusion of alkaloids from the seed. Thus, 1 h of cooking removed 30.7% of the alkaloids, whereas the washing step, conducted at an ambient water temperature of 15°C, required 118.3 h to remove 58.6% of the alkaloids. This implies that the efficiency of the extraction of alkaloids can be improved by changing the conditions of the debittering process. Although the current process takes 5.7 days, the calculation of a theoretical diffusion for alkaloids based on the diffusion of sucrose in fruits (Walstra 2003) suggests that this could be reduced to 4.8 days as explained below.

Figure 3 shows the strong effect of the magnitude of the apparent diffusion coefficient on extraction. The diffusion coefficient of an alkaloid molecule in water is expected to be around 10^{-10} m²/s (Walstra 2003), which is represented in Fig. 3A. However, a lupine seed is not an aqueous environment and consequently the diffusion coefficient would be expected to be lower than 10^{-10} m²/s. On the other hand, the practical situation resulting in adequate detoxification in 5.7 days, strongly suggests a higher value than 10^{-11} m²/s as illustrated in Fig. 3B. Thus, the mathematic model suggests an apparent diffusion coefficient between 1×10^{-10} and 1×10^{-11} m²/s. Indeed, when we substitute in equation (4) t with 492,480 s (experimental debittering time of 5.7 days) maintaining the distance of diffusion (6.4×10^{-3} m), we obtain an estimated alkaloids diffusion coefficient of approximately 8.32×10^{-11} m²/s. Obviously, this value is based on the outcome of the total process. During the debittering of the seed, it is hydrated, then cooked and finally washed under different conditions and consequently the diffusion coefficient is expected to vary between and within process operations.

Alternatives of Washing and Optimization of This Process

The impact of changes of water on alkaloids content, water used and solids in the product throughout time can be

TABLE 5. DEBITTERING EFFICIENCY OF INDIVIDUAL OPERATIONS IN EXTRACTING ALKALOIDS FROM LUPINE RELATED TO WATER AND TIME USE (N = 3; LABORATORY SCALE EXPERIMENTS)

| | Operation | | | |
|---|-----------|---------|---------|-------|
| | Soaking | Cooking | Washing | Total |
| Total alkaloids extracted (g/100g dry matter) | 0.2 | 1.1 | 2.1 | 3.4 |
| % of total alkaloids extracted | 5.6 | 30.7 | 58.6 | 94.9 |
| Water used (kg/kg raw dry lupine) | 2.9 | 2.9 | 56.5 | 62.3 |
| Time used (h) | 18.0 | 1.0 | 118.3 | 137.3 |
| Water efficiency* | 0.69 | 3.79 | 0.37 | 0.55 |
| Time efficiency† | 0.11 | 11.00 | 0.18 | 0.25 |

* Alkaloids extracted (g/kg-water).

† Alkaloids extracted (g/kg-raw-dry lupine/h).

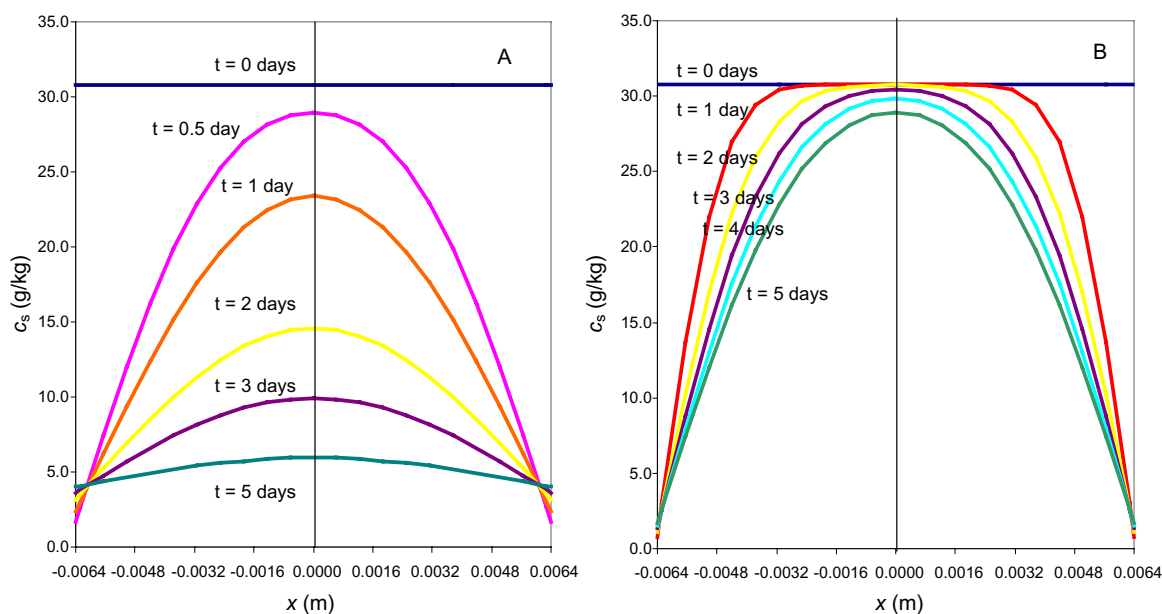


FIG. 3. SIMULATION OF EXTRACTION ACCORDING TO FICKIAN DIFFUSION OF ALKALOIDS IN A LUPINE SEED

Distance $x = 0.0128$ m, contact area $A = 2.5 \times 10^{-5}$ m², $V_w = 2$ mL, initial concentration $c_s = 30.8$ g alkaloid/kg lupine, $D_s = 1 \times 10^{-10}$ m²/s (A) and $D_s = 1 \times 10^{-11}$ m²/s (B).

observed in Table 6. Increasing the number of changes of water/day diminished the time needed to debitter lupine and the solids in the product. However, as it was expected that augmented the amount of water used. The reduction of processing time by increasing the changes of water agrees with Fickian diffusion Eqs. (5) and (6).

Figure 4 shows that lupine can be debittered under different conditions. As an example, we registered two extreme solutions. Unfortunately, the optimization study showed

that it is not possible to reduce the water used and the processing duration at the same time with this approach (Fig. 4).

It would be of interest to look for ways to accelerate diffusion, for which several options could be considered either singly or in combination. First, an increase in temperature and its convection effect would seem to be one of the options, but this effect has to be balanced against energy costs and possible other effects on lupine quality, such as an

TABLE 6. TREATMENTS TESTED TO OPTIMIZE THE DEBITTERING PROCESS OF LUPINE (N = 2)

| Treatment code* | Factors | | Variables | | |
|-----------------|------------------|--------------------|-------------|----------------------|-----------------------|
| | Changes of water | Time of processing | Alkaloids | Water used | Solids in product |
| | Times/day | Days | % d.w. | l/kg d.w. raw lupine | kg/kg d.w. raw lupine |
| Ch0t0† | 0 | 0 | 1.74 ± 0.20 | 0.00 ± 0.00 | 0.90 ± 0.02 |
| Ch3t1 | 3 | 1.92 | 1.72 ± 0.20 | 21.31 ± 0.01 | 0.88 ± 0.00 |
| Ch3t2 | 3 | 3.92 | 0.55 ± 0.05 | 42.62 ± 0.02 | 0.87 ± 0.01 |
| Ch3t3 | 3 | 6.25 | 0.25 ± 0.00 | 67.48 ± 0.04 | 0.86 ± 0.03 |
| Ch6t1 | 6 | 1.97 | 1.27 ± 0.03 | 42.28 ± 0.07 | 0.82 ± 0.01 |
| Ch6t2 | 6 | 3.97 | 0.26 ± 0.00 | 84.57 ± 0.14 | 0.81 ± 0.01 |
| Ch6t3 | 6 | 4.47 | 0.24 ± 0.00 | 95.16 ± 0.14 | 0.82 ± 0.02 |
| Ch9t1 | 9 | 1.95 | 0.50 ± 0.05 | 63.92 ± 0.00 | 0.80 ± 0.01 |
| Ch9t2 | 9 | 2.95 | 0.27 ± 0.00 | 95.91 ± 0.00 | 0.79 ± 0.01 |
| Ch9t3 | 9 | 3.95 | 0.24 ± 0.00 | 124.3 ± 0.00 | 0.79 ± 0.01 |

* Treatments were ran at random.

† Starting point (reference).

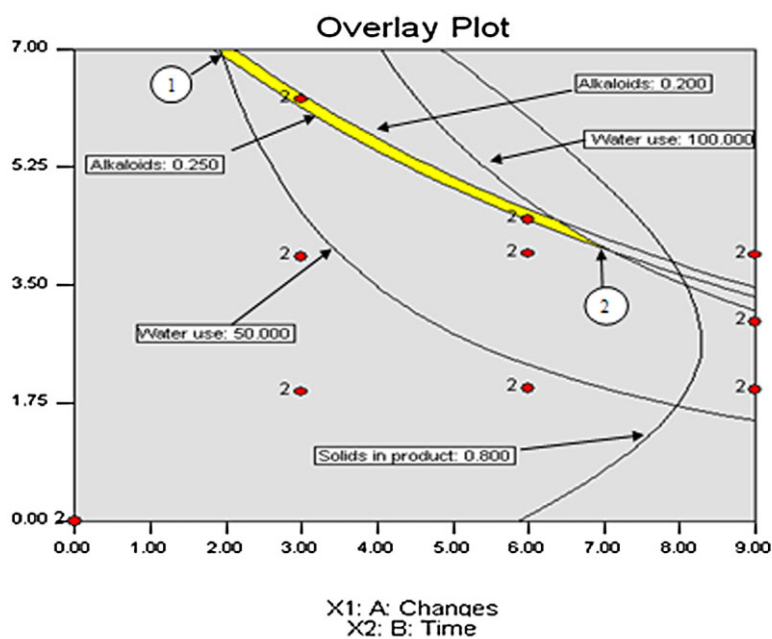


FIG. 4. GRAPHICAL OPTIMIZATION* OF THE WASHING PROCESS WITH THE FOLLOWING RESTRICTIONS: ALKALOIDS $0.20 < X < 0.25\%$ D.W., WATER USED $50 < X < 100$ L/KG D.W. RAW LUPINE, SOLIDS IN PRODUCT > 0.8 KG/KG D.W. RAW LUPINE

*Design-Expert 8-Response surface: Quadratic model; no aliases found; average leverage 0.3; fraction of design space (FDS) statistic = 1 or 100%.

ANOVA response for alkaloids (2FI model, $P < 0.0001$, $r^2 = 0.85$). ANOVA response for water use (quadratic model, $P < 0.0001$, $r^2 = 1.00$). ANOVA response for solids in product (quadratic model, $P < 0.0001$, $r^2 = 0.93$).

| Point | Optimization Process | | | | |
|-------|-------------------------------|-------------------|------------------------------------|---------------------------------|--------------------------------|
| | Changes of water Times/day | Time used Days | Water used L/kg d.w. raw lupine | Solids kg/kg d.w. raw lupine | Alkaloids % d.w. raw lupine |
| 1 | 2.0 | 6.92 | 51.0 | 0.86 | 0.242 |
| 2 | 7.0 | 4.06 | 99.5 | 0.81 | 0.249 |

increased leaching of nutrients or microbial growth. Another option would be to attempt to decrease the tortuosity, for example by modifying the soaking process. Reduced tortuosity might be accomplished by soaking lupine in salt solutions. In black beans, soaking in salt solutions has been postulated to increase protein solubility, reduce interactions between minerals and pectin, and to result in a more porous microstructure, thus facilitating water penetration (Siewright and Shipe 1986). Finally, a continuous replenishment of water in the washing operation might accelerate the diffusion of alkaloids.

CONCLUSION

The current debittering process of lupine in San Pedro, Ecuador, is effective but consumes much water and time. During the process, not only alkaloids are removed but also 22% of total solids, principally fats, minerals and carbohydrates. The microbiological quality of the product deteriorates during this long processing time. Improving the efficiency of the debittering process would reduce water consumption, save time and improve the nutritional and microbiological quality of the final product. Future work will focus on strategies to optimize the debittering process.

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