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Modelling of the aqueous debittering process of *Lupinus mutabilis* Sweet

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ABSTRACT

We investigated the process of lupin debittering by soaking, cooking and washing in water using a newly designed hydroagitator. The effect on alkaloids content, solids in the product, final weight, processing time and water and energy consumption were expressed in a mathematical model for optimization purposes. Design expert 8 software was used to model the processes. Optimum processing conditions comprised 18 h of soaking, 1 h cooking, 3 changes of water/day and 22 h of agitation/day. For estimating the washing time a mathematic function and non-dimensional constant k were inferred from observation and the software used ($\partial c/\partial t = kc$; $k = -0.188 - 4.973^{-3} \text{ *Agitation} - 0.0043 \text{ *Changes} - 1.681^{-3} \text{ *Agitation} \text{ *Changes}$). The new developed technology could be used to optimize processes such as hydration and/or removal of undesired materials of legumes and other seeds.

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1. Introduction

Lupins (*Lupinus* spp) are legumes (Pate, William, & Farrington, 1985) used principally as a protein source in human and animal nutrition (Güemes-Vera, Peña-Bautista, Jiménez-Martínez, Dávila-Ortiz, & Calderón-Domínguez, 2008). The FAO (2012a) reports that 934,426 metric tons of lupin were produced in 2010 in e.g., Germany, Poland, the Russian Federation, Mediterranean countries as well as in Australia, South Africa, and South America. Four major species of lupins are cultivated, namely *Lupinus albus*, *Lupinus luteus*, *Lupinus angustifolius*, and *Lupinus mutabilis*, of which the latter shows the highest average content, on a dry weight basis, of protein (44 g.100 g⁻¹) and lipids (18 g.100 g⁻¹) (Pate et al., 1985), comparable to soybean in quantity and quality (Santos, Ferreira, & Teixeira, 1997). Whole lupin seeds are consumed as a snack or as an ingredient in fresh salads and soups (Villacrés, Peralta, & Alvarez, 2003). Lupin flour can be used as an ingredient in foods such as

biscuits, baby foods, hamburgers, breads, and pasta (Cremer, 1983; Ruales, Polit, & Nair, 1988). However, lupin also naturally contains about 70 different alkaloids (Ruiz, 1978), which are toxic (Australia New Zealand Food Authority, 2001, pp. 1–21), especially those belonging to the sparteine and lupanine types (Jiménez-Martínez, Hernández-Sánchez, & Dávila-Ortiz, 2003), and thus these must be removed prior to consumption. Because of the nutritional value of *L. mutabilis* the debittering of its seeds has been attempted previously. Most published debittering processes include a soaking stage of the seed with durations ranging up till 18 h (Jiménez-Martínez, Hernández-Sánchez, & Dávila-Ortiz, 2007) or 20 h (Villacrés, Caicedo, & Peralta, 2000), followed by cooking for 0.5 h (Villacrés et al., 2000) up to 6 h (Jiménez-Martínez et al., 2003). A soaking stage is important because it increases the water content of the seed and facilitates the extraction of alkaloids in subsequent stages. The cooking stage is essential to inactivate the germination capacity of the seeds, their enzymes (lipase, lipoxygenase), to eliminate occurring microorganisms for food safety, to reduce the loss of proteins through their coagulation, and to facilitate the leaching of the alkaloids by increasing the cell wall permeability (Gross, Godomar-Galindo, & Schoeneberger, 1983; Jiménez-Martínez et al., 2003).

After soaking and cooking, the alkaloid removal can be achieved by biological (Dagnia, Petterson, Bell, & Flanagan, 1992; Jiménez-Martínez et al., 2007; Santana & Empis, 2001), chemical (Aguilera, Gerngross, & Lusas, 1983; Jiménez-Martínez et al., 2003; Nossak,

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Vilegas, Von Baer, & Lanças, 2000; Ortiz & Mukherjee, 1982; Torres-Tello, Nagata, & Dreifuss-Spiegel, 1980) or aqueous processing (Caicedo, Peralta, Villacrés, & Rivera, 2001; Torres-Tello et al., 1980; Villacrés et al., 2000). Biological processes such as germination, biological digestion, and solid- and liquid-state fermentation are restricted to lupin seeds with an alkaloid content up to $1.1 \text{ g} \cdot 100 \text{ g}^{-1}$ (Szakacs & Stankovics, 1984), and consume energy and have a duration up to 5 days (Santana, Pinto, Fialho, Saa-Correia, & Empis, 2002). Chemical treatments were suitable for lupin seeds with alkaloid contents up to $4.2 \text{ g} \cdot 100 \text{ g}^{-1} \text{ d.w.}$ (Ortiz & Mukherjee, 1982), but have disadvantages including material and nutritional losses (Gueguen & Cerletti, 1994), uncertainty regarding their chemical safety and negative impact on the environment.

Aqueous debittering processes are in use at the household and commercial scale, to remove alkaloids from whole seeds for human consumption purposes. The lupin seeds are soaked for 14–20 h, and then boiled for 0.5–2 h, followed by washing in cold water for 4–5 d (Villacrés et al., 2000). After washing, the product still requires a thermal treatment (boiling for 10 min) to render it bacteriologically safe for consumption (Villacrés et al., 2000) as a snack, as an ingredient of cold salads, or in warm dishes. The debittered lupin seeds can also be packed for distribution to supermarkets (Caicedo et al., 2001; Peralta, Mazón, & Villacrés, 2001; Villacrés et al., 2003).

The aqueous treatment is applied to lupin seeds with high alkaloids content (up to $4.2 \text{ g} \cdot 100 \text{ g}^{-1}$) (Torres-Tello et al., 1980; Villacrés et al., 2000), and although this process is still not very efficient (FAO, 2012b) because of its high consumption of water (63 kg water per kg seed) (Caicedo et al., 2001), time (5–6 d) (Villacrés et al., 2000) and its high loss of solids ($0.27 \text{ kg} \cdot \text{kg}^{-1}$ dry seed) (Torres-Tello et al., 1980), it has been stated that the use of only water is advantageous because it avoids chemical waste disposal (Rossetto, 1989) as well as undesirable quality changes that occur in the other debittering processes.

The aim of the present study is to measure the effect of the process variables soaking, cooking and washing, on debittering efficiency (consumption of water, time and energy and residual concentration of alkaloids) and product yield (solids, and seed fresh weight). Data will form the basis of a mathematical model that may be used for optimization purposes.

2. Materials and methods

2.1. Raw lupin

A batch of raw bitter *L. mutabilis* Sweet (150 kg, alkaloids content $2.65 \text{ g} \cdot 100 \text{ g}^{-1} \pm 0.02 \text{ g} \cdot 100 \text{ g}^{-1} \text{ d.w.}$) was obtained by pooling 15 kg contributions from 10 lupin village processors selected at random from the village of San Pedro, Cotopaxi Province, Ecuador. All processors used the same variety of raw lupin. The contributed lupin was mixed, put in jute bags and stored in an environmental chamber at 16°C and 80% Relative Humidity.

2.2. Operations to be tested

Raw, whole lupin seeds were debittered under controlled laboratory conditions in two stages. First, nine different conditions of soaking and cooking were analysed. Next, nine different conditions of washing were studied.

2.2.1. Soaking and cooking conditions

Soaking was carried out at room temperature (18°C). The initial weight ratio of water: raw seeds dry weight was 3:1. Later, more (measured) water was added if required to ensure that the seeds always remained under water. Soaking was carried out for 0, 18 and 36 h.

Cooking was carried out at 91.9°C , corresponding to the boiling point of water in Cumbayá, Quito, Ecuador (altitude 2433 m). Petroleum gas was used as fuel. Soaked seeds were added to boiling water, and cooking time was recorded from the moment that lupin came in contact with boiling water. Similar as with soaking, the initial weight ratio of cooking water:soaked seeds was 3:1. Cooking was done for 1, 3 and 6 h.

In the experiment, soaking and cooking treatments were combined in 9 sets as follows. Soaking 0 h with cooking 1 h (S0C1); soaking 0 h with cooking 3 h (S0C3); soaking 0 h with cooking 6 h (S0C6); soaking 18 h with cooking 1 h (S18C1); soaking 18 h with cooking 3 h (S18C3); soaking 18 h with cooking 6 h (S18C6); soaking 36 h with cooking 1 h (S36C1); soaking 36 h with cooking 3 h (S36C3); and soaking 36 h with cooking 6 h (S36C6). Table 1 summarizes the treatments and data obtained.

During the experiment the water and seed weight, as well as the consumed amount of petroleum gas were recorded (weighing scale ES 200L, Ohaus Corporation, NJ, U.S.A.). During the experimental part, seed samples were taken and their moisture content was measured according to AOAC 925.09 (2005), as well as their alkaloids content as described below.

2.2.2. Washing conditions

Nine experiments were conducted to estimate the effect of limited water volumes and agitation conditions on alkaloids removal and other variables mentioned before. The conditions tested included the number of times the water was changed per day (3, 6, and 9 times), and the duration of hydro-agitation per day (0, 11, and 22 h). These were tested in 9 combinations as follows: 3 d^{-1} water changes with 0 h d^{-1} of hydro-agitation (W3H0); 6 d^{-1} water changes with 0 h d^{-1} of hydro-agitation (W6H0); 9 d^{-1} water changes with 0 h d^{-1} of hydro-agitation (W9H0); 3 d^{-1} water changes with 11 h d^{-1} of hydro-agitation (W3H11); 6 d^{-1} water changes with 11 h d^{-1} of hydro-agitation (W6H11); 9 d^{-1} water changes with 11 h d^{-1} of hydro-agitation (W9H11); 3 d^{-1} water changes with 22 h d^{-1} of hydro-agitation (W3H22); 6 d^{-1} water changes with 22 h d^{-1} of hydro-agitation (W6H22); and 9 d^{-1} water changes with 22 h d^{-1} of hydro-agitation (W9H22). Table 2 summarizes the treatments and data obtained. The 9 combinations were chosen based on i) the previous studies made by other authors. For example, treatment (W3H0) was reported by Villacrés et al. (2000) as current debittering process. Caicedo et al. (2001) used agitation 24 h/day but kept constant 3 changes of water per day (W3H24). Torres-Tello et al. (1980) worked with running (unquantified) water all the time (WnH24). ii) Based on theoretical considerations. For example, we noted that by increasing the frequency of changing water we can reduce the average viscosity of solvent (η) and therefore speed up the debittering process (Equation Stokes–Einstein $D = KT/6\eta\pi r$) (Chang, 1977). In addition, the consideration of the factor agitation time is based on Crank (1975) who mentions that solutes (alkaloids) concentration at the interface seed-water is lowered by agitation and thus, the diffusion of alkaloids from the seeds is increased. iii) By our previous essays. We worked with more and less changes of water and the significant effects are present in the studied range. Regarding agitation time we found effects at all times. We choose 0 h of agitation to compare with current debittering process (W3H0). We choose 22 h, to see the maximum effect of agitation (from 24 h/day, 2 h/day are used in changing water and weighing lupin and water), and 11 h/day as an intermediate point.

Each condition was tested as follows. Lupin was soaked and cooked following the previously selected conditions. Then, 20 kg of soaked and cooked lupin were put in two plastic net bags (10 kg per bag). These were put in a stainless steel tank of 0.6 m length, 0.45 m width and 0.40 m depth. Then, 33 kg of water at $14\text{--}16^\circ\text{C}$ were

Table 1

Soaking-cooking stage: Factors, levels and experimental results.

Run	Soaking time (h)	Cooking time (h)	Processing time (h)	Alkaloid content (g 100 g ⁻¹ d.w.)	Water use (l/kg raw lupin d.w.)	Final weight (kg/kg raw lupin d.w.)	Solids (kg/kg raw lupin d.w.)	Energy (MJ/kg raw lupin d.w.)
1	18	3	21	1.84	8.05	2.6	0.92	24.8
2	36	6	42	1.46	9.36	2.6	0.86	44.6
3	18	0	18	2.47	3.23	2.4	0.93	0.0
4	0	0	0	2.66	0.00	1.0	1.00	0.0
5	18	6	24	1.74	9.29	2.7	0.90	44.6
6	0	6	6	1.83	8.09	2.4	0.91	44.6
7	0	3	3	2.11	6.04	2.3	0.95	34.7
8	36	0	36	1.99	3.44	2.5	0.93	0.0
9	36	6	42	1.50	9.36	2.6	0.86	49.6
10	18	3	21	1.64	8.09	2.6	0.91	34.7
11	36	1	37	1.54	6.67	2.6	0.91	9.9
12	36	1	37	1.51	6.68	2.6	0.90	9.9
13	0	0	0	2.63	0.00	1.0	1.00	0.0
14	36	3	39	1.42	8.08	2.7	0.88	34.7
15	18	1	19	1.60	6.52	2.5	0.88	9.9
16	18	6	24	1.68	9.29	2.6	0.88	49.6
17	18	1	19	1.89	6.52	2.6	0.91	9.9
18	0	3	3	1.98	5.72	2.3	0.95	24.8
19	36	0	36	1.60	3.44	2.5	0.93	0.0
20	18	0	18	2.21	3.23	2.4	0.94	0.0
21	36	3	39	1.42	8.08	2.7	0.89	24.8
22	0	6	6	2.08	8.09	2.3	0.91	49.6
23	0	1	1	1.88	3.67	2.0	0.96	9.9
24	0	1	1	1.63	3.67	1.9	0.94	9.9

added into the tank. This tank containing the water and the seeds was situated in a temperature controlled chamber (14–16 °C). The amount of water and lupin were the minimum necessary for keeping the seed under water all the time, and to allow the water to circulate through the bags with lupin. Next, the hydro-agitation system designed by one of the authors (FECL) (Fig. 1) was started for the washing stage. The hydro-agitation system re-circulates water 60 times h⁻¹ and injects water at 48 kPa. Table 3 presents the combination of experimental treatments.

2.3. Alkaloids content

2.3.1. Sample pretreatment

Raw whole lupin seeds were milled using a 4E mill model (The Strub Company, Hatboro, PA, U.S.A.), sieved with a vibrating sieve (Meinzer II, Series 0447, Fairfax, VA, U.S.A.) equipped with a 60 mesh sieve (Dual Model, MFG Co. Chicago, IL, U.S.A.), and the throughs were collected for analysis. Debittered seeds were chopped for 2 min in a food processor (model HC 3000, Black & Decker Corporation, Towson, Maryland, U.S.A.).

2.3.2. Alkaloid determination

Alkaloid concentrations were determined by titration using the methodology described by von Baer, Reimerdes, and Feldheim (1979) with the modification suggested by the Ecuadorian Institute of Standards (INEN, 2005, pp. 1–7). To 0.2 g of lupin, 0.6 g of basic Al₂O₃ was added and blended to a fine powder. Then 0.2 ml of KOH (150.4 g l⁻¹) 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 (Nerín & 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 sulphuric acid (0.49 g l⁻¹) and 2 drops of methyl red indicator were added to the vial. The excess acid was titrated with NaOH (0.40 g l⁻¹). The concentration of total alkaloids (TA), expressed as lupanine content (g.100 g⁻¹), was calculated as follows:

$$TA = 0.248 \cdot V / \text{Weight of sample (g)} \quad (1)$$

V = volume (ml) of sulphuric acid (0.49 g l⁻¹) that reacted.

All alkaloid determinations were performed in duplicate. All chemical and solvents used were A.R. grade obtained from Merck Ecuador, Quito, Ecuador.

3. Theoretical considerations

3.1. Soaking and cooking processes

The aqueous debittering of lupin occurs through the diffusion of alkaloids. In the ideal system consisting of only the material to diffuse (solute) and solvent, the diffusion constant (*D*) for spherical particles, is given by the Stokes–Einstein Equation (Chang, 1977):

$$D = KT / 6\eta\pi r \quad (2)$$

where *KT* is a measure of the thermal energy of the molecule, η is a measure of the viscosity of solvent and *r* is the radius of the particle. The Stokes–Einstein equation is important in our study because it states the inverse relationship of solutions' viscosity (η) and the diffusion (*D*) of materials (alkaloids) $D = KT / 6\eta\pi r$ (Chang, 1977). Diminishing the average viscosity of solutions by increasing the number of water changes will speed up the alkaloid diffusion. The diffusion of alkaloids from stirred (agitated) solutions in steady conditions can be solved by the equation proposed by Crank (1975). However, although that equation takes in consideration variation of mass transfer, it assumes that the alkaloid's diffusion coefficient is constant. This approach is not suitable for our study because during lupin processing the polymer matrix is in dynamic change continuously. For example, the raw material has a water content of about 7%, but at the end of washing the water content is increased

Table 2

Washing stage: Factors, levels and experimental results.

Run	Agitation time (h)	Changes of water (times/d.)	Processing time (d)	Alkaloids g 100 g ⁻¹ d.w.	Final weight (kg/kg raw lupin d.w.)	Water use (l/kg raw lupin d.w.)	Solids (kg/kg raw lupine d.w.)	Energy (MJ/kg raw lupine d.w.)
1	22	3	2.29	0.25	2.78	28.6	0.79	8.0
2	11	9	0.95	0.70	2.70	35.2	0.73	1.7
3	0	3	1.92	1.58	3.10	21.3	0.88	0.0
4	22	6	2.44	0.25	2.86	63.2	0.79	8.5
5	22	9	1.93	0.26	2.63	71.1	0.70	6.7
6	11	6	1.97	0.82	2.81	48.7	0.75	3.4
7	0	6	3.97	0.26	3.04	84.7	0.81	0.0
8	22	9	0.93	1.27	2.71	32.5	0.70	3.2
9	0	9	1.95	0.46	3.05	63.9	0.79	0.0
10	11	6	1.97	0.82	2.89	50.1	0.78	3.4
11	0	6	3.97	0.26	3.08	84.5	0.82	0.0
12	0	9	3.95	0.24	3.01	124.3	0.80	0.0
13	22	9	2.15	0.25	2.56	83.5	0.71	7.5
14	0	6	1.97	1.29	3.13	42.2	0.82	0.0
15	22	9	0.93	1.12	2.76	33.4	0.72	3.2
16	11	3	0.96	0.58	2.82	12.4	0.78	1.7
17	11	6	3.30	0.26	2.72	81.2	0.74	5.8
18	22	3	3.60	0.25	2.77	45.4	0.77	12.6
19	22	6	1.94	0.27	2.97	46.5	0.77	6.8
20	0	9	2.95	0.27	2.95	95.9	0.78	0.0
21	22	6	0.94	0.53	2.99	21.1	0.79	3.3
22	11	9	2.29	0.25	2.70	81.9	0.75	4.0
23	0	9	2.95	0.27	3.02	95.9	0.80	0.0
24	11	9	2.29	0.25	2.78	85.3	0.78	4.0
25	0	3	6.25	0.25	2.96	67.5	0.84	0.0
26	11	6	3.30	0.26	2.83	83.5	0.77	5.8
27	11	9	0.95	0.74	2.83	36.6	0.77	1.7
28	11	3	3.96	0.25	2.97	50.3	0.83	6.9
29	0	0	0.00	1.60	2.53	0.0	0.88	0.0
30	0	6	4.47	0.24	3.01	95.1	0.83	0.0
31	22	9	1.93	0.26	2.66	69.0	0.70	6.7
32	22	6	1.94	0.27	2.91	46.5	0.76	6.8
33	22	3	2.29	0.25	2.82	28.9	0.80	8.0
34	11	6	0.97	0.73	3.06	25.1	0.88	1.7
35	11	3	0.96	0.52	2.84	12.4	0.79	1.7
36	22	3	0.95	0.80	2.78	12.3	0.80	3.3
37	11	6	0.97	0.87	2.81	24.4	0.81	1.7
38	0	3	6.25	0.25	3.09	67.5	0.87	0.0
39	22	6	0.94	0.45	3.04	21.1	0.81	3.3
40	22	3	0.95	0.94	2.82	12.4	0.81	3.3
41	22	3	3.60	0.25	2.75	44.9	0.77	12.6
42	0	3	3.92	0.59	3.18	42.6	0.87	0.0
43	11	3	2.96	0.62	2.97	37.0	0.82	5.2
44	22	6	2.44	0.25	2.91	63.2	0.81	8.5
45	0	3	1.92	1.86	3.08	21.3	0.88	0.0
46	11	9	1.95	0.25	2.75	73.1	0.76	3.4
47	0	6	4.47	0.24	2.94	95.3	0.82	0.0
48	0	0	0.00	1.89	2.59	0.0	0.91	0.0
49	0	9	3.95	0.24	3.09	124.3	0.78	0.0
50	11	3	2.96	0.55	2.71	37.0	0.74	5.2
51	22	9	2.15	0.25	2.56	81.2	0.71	7.5
52	0	3	3.92	0.51	3.23	42.6	0.88	0.0
53	0	6	1.97	1.26	3.09	42.3	0.81	0.0
54	0	9	1.95	0.54	3.10	63.9	0.80	0.0
55	11	9	1.95	0.25	2.65	70.2	0.73	3.4
56	11	3	3.96	0.25	2.71	50.3	0.76	6.9

to about 70–75%. According to Crank (1975) and Walstra (2003), the diffusion coefficients depend on water content of polymers. In addition the viscosity of the solution (water + alkaloids) and the alkaloid content in the seed are changing continuously because alkaloids are taken from the seed. Those effects cause unsteady conditions. Further, in a non-ideal system (diffusion of particles present in matrix material interacting with solvent) the diffusion is slower than in pure solvent (Van Boekel, 2009; Walstra, 2003) because of the presence of macromolecules and networks that strongly hinder the process lowering the diffusion coefficient orders of magnitude (Van Boekel, 2009). For example, the diffusion of salt in water is about $10^{-9} \text{ m}^2 \text{ s}^{-1}$, whereas in meat it is about half that value, and in hard cheeses about 0.2 times (Walstra, 2003). In

addition, the solutes diffusion is a situation where the concentration (c) of solute in the seed matrix is not homogeneous, but is time (t) and location dependent. This can be expressed by the Fick's second law that in the case of diffusion in three directions (x, y, z) can be derived as (Van Boekel, 2009):

$$\partial c / \partial t = \left(\partial^2 c / \partial x^2 \right) + \left(\partial^2 c / \partial y^2 \right) + \left(\partial^2 c / \partial z^2 \right) \quad (3)$$

In systems where seeds are exposed without agitation and water is used sparingly, the solutes (alkaloids) concentration at the interface seed–water is higher than in the solvent (water) (Crank, 1975) which restricts the concentration gradient and thus, the diffusion of alkaloids from the seeds. On the other hand, if the same

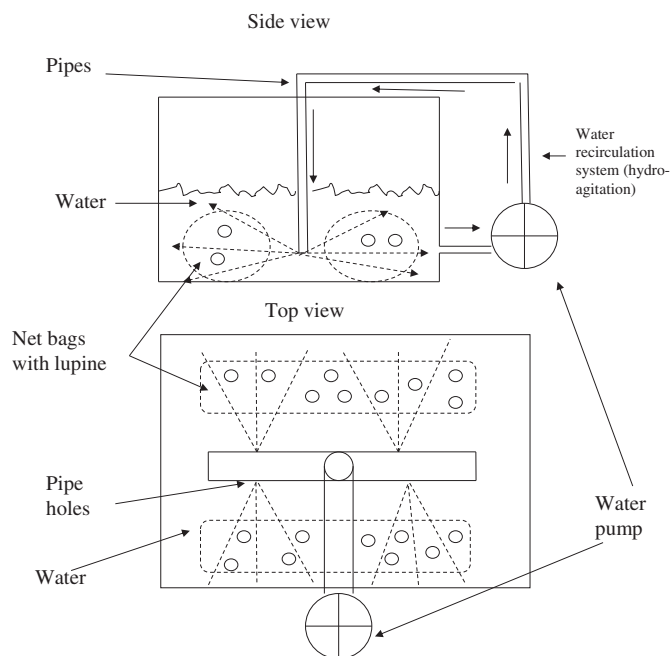


Fig. 1. Hydro-agitation system.

volume of water is well agitated, the concentration of alkaloids at the seed–water interface will be lowered and thus the diffusion would take place more rapidly.

Then, the speed (duration) of the debittering process depends on the size of alkaloid molecules, temperature of the seeds and solvent, viscosity of solvent, size and water content of the seed, volume of solvent, and level of agitation of the system. It is not possible to change the size of alkaloid molecules, nor of the lupins that will be consumed as intact cotyledons, but the other variables could be changed.

The soaking stage increases the water content in the seed; the cooking stage affects the alkaloids diffusion caused by the effect of temperature and turbulence; the number of changes of water affects the volume and viscosity of solvent; and agitation diminishes the gradient concentration at the seed–solvent interface.

Most studies on improving diffusion explore the use of temperature and/or stirring. However, the approach of reducing viscosity (by increasing changes of water) combined with reduction of alkaloids concentration at the interface seed–water (by hydro-agitation) is a different asset to improve mass transfer. Moreover, the use of the gradient approach with unsteady state considerations led us to model and to obtain an optimum solution that reflects the permanent change of compositions of lupin matrix and solution (water + alkaloids). Finally, the unsteady state approach

allowed us to estimate the processing time required to obtain, within the frame of reference studied, any reduction in alkaloids concentration in the seed and for any processing condition.

4. Results and discussion

4.1. Modelling and determination of best conditions for soaking and cooking

The effects of soaking and cooking are presented in Fig. 2. The relations between processing time (soaking and cooking), and solids d.w. and alkaloids content are inverse. This is in line with the loss of alkaloids by diffusion which also reduces solids content. On the other hand, the product weight f.w., the consumption of water and energy are in positive relation with process duration. The fresh weight increase is a result of the swelling of the seeds. It can be seen from Fig. 2 that soaking and cooking have additional effect on solids content and synergistic effect on d.w., product weight f.w., water use and alkaloids content. The cooking stage uses almost twice the amount of water than soaking. Energy is consumed only during the cooking stage. Fig. 2 shows the optimum combination of soaking and cooking conditions aiming at a minimum use of time, energy, water, and the lowest residual concentration of alkaloids, with maximum final solids d.w. and seed weight f.w.). This optimum was derived using the software expert design 8. The highest desirability (nearly 0.5) was achieved in a range of soaking times from 18 h to 27 h combined with a 1 h cooking period; this approximates some of the local conditions reported elsewhere (Villacr  s et al., 2000). In contrast, the lowest desirability (0.1) results from cooking periods extended to 4 h and longer. The high consumption of energy, water and time diminishes the desirability. Most of the effects of soaking and cooking combinations could be explained through second order mathematic equations (Table 4). For practical reasons, the combination of 18 h soaking and 1 h cooking was selected as the standard pre-treatment for the next stage, i.e. washing.

4.2. Modelling and determination of best condition for washing

The effect of the frequency of daily water changes and of agitation duration is presented in Fig. 3. Most relations between agitation duration and water change frequency could be expressed by second order mathematic equations as shown in Table 5. We observed losses of solids and alkaloids with increased frequency of water changes and longer agitation. The effect of agitation on solids reduction and alkaloids content was stronger than of water changes. The water consumption was directly related to the frequency of water changes, whereas the consumption of energy was related to agitation duration only. A more complex relation can be observed between agitation duration and frequency of water changes, on seed final weight f.w. Increasing water changes up to 5–6 times d^{-1} increased the seed final weight, but at higher frequencies the fresh weight decreased. This phenomenon was independent of agitation. Possibly at low water change frequency, a swelling effect dominates until a water saturation occurs, and at higher frequencies the additional water could not be absorbed by the lupin matrix, but may be involved in associations that induce leaching of substances from lupin; for example, water and fat were reported to form emulsions (Chajuss, 1989) based on the lecithin present in lupin FAO (2012b). Fig. 3 shows the optimum conditions for the washing stage (minimization of time, energy, water, alkaloids, and maximization of solids d.w. and seed weight f.w.). The highest desirability (about 0.78) was obtained at a combination of 3 d^{-1} changes of water and 22 h agitation duration. The time needed to achieve a required alkaloid content equal or less than 0.25 g.100 g $^{-1}$ d.w. under that condition was 3.6 days.

Table 3
Stages, factors and levels of experimentation.

Stage	Factor	Units	Levels		
Soaking–cooking	Soaking (S)	h	0	18	36
	Cooking (C)	h	1	3	6
Washing	Changes of water (W)	Times/day	3	6	9
	Hydro-agitation (H)	h/day	0	11	22
	Time of washing	Days	1/3	2/3	3/3

The levels 1/3, 2/3 and 3/3 mean that the responses (variables) were measured at 1/3, 2/3 and at the end of washing stage (3/3). We use this generic form because each treatment in the washing stage needed different times to reduce alkaloid content to safe limits (0.25 g kg $^{-1}$ dry seed).

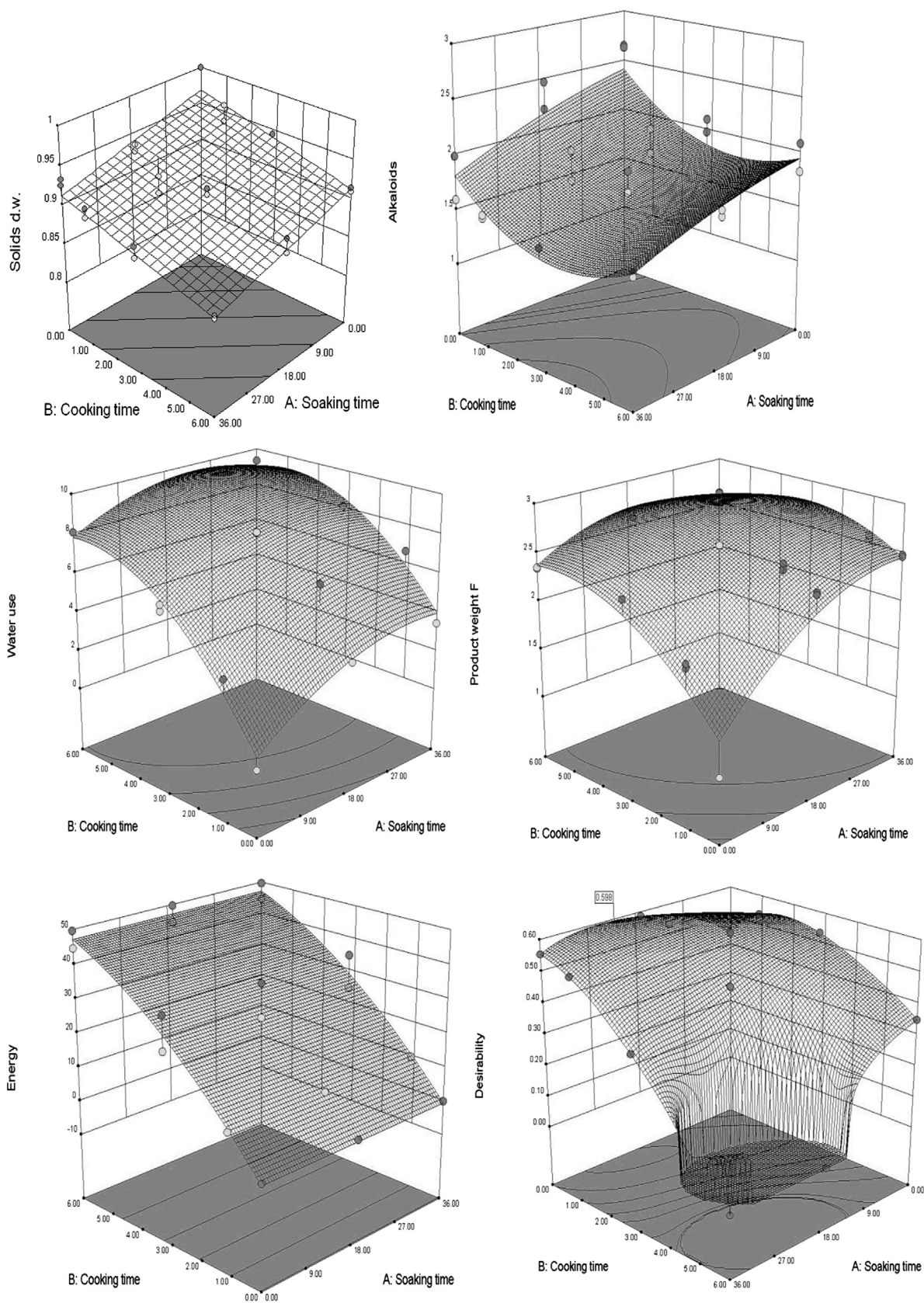


Fig. 2. Effect of soaking and cooking times. This figure shows the effect of soaking and cooking conditions on solids of lupin d.w. (dry base), alkaloids content, water consumption, product weight (fresh weight), energy used, and the desirability (the best conditions for carrying the soaking and cooking processes).

Table 4

Mathematical equations describing the effect of soaking and cooking times on the amount of water and energy used, as well as, on the amount of solids, weight, and residual alkaloid concentrations in the processed lupin. All values are expressed per 1 kg of raw lupin in dry weight. Soaking and cooking times are expressed in hours.

Response	Unit	Equation ^a	r^2	Prob. >F
Water use	l	Water use = + 0.59819 + (0.21924 * Soaking time) + (2.56740 * Cooking time) - (0.010017 * Soaking time * Cooking time) - (3.48278 * 10 ⁻³ * Soaking time ²) - (0.22677 * Cooking time ²)	0.96	0.0001
Energy use	kJ	Energy use = -0.46944 - (3.86340 * 10 ⁻¹⁶ * Soaking time) + (11.88615 * Cooking time) + (1.41324 * 10 ⁻¹⁷ * Soaking time * Cooking time) + (5.63832 * 10 ⁻¹⁸ * Soaking time ²) - (0.65721 * Cooking time ²)	0.98	0.0001
Solids in product	kg d.w.	Product weight D = + 0.97031 - (1.58177 * 10 ⁻³ * Soaking time) - (9.35757 * 10 ⁻³ * Cooking time)	0.79	0.0001
Product weight	kg f.w.	Product weight F = + 1.35480 + (0.063536 * Soaking time) + (0.38716 * Cooking time) - (4.66917 * 10 ⁻³ * Soaking time * Cooking time) - (9.14795 * 10 ⁻⁴ * Soaking time ²) - (0.036117 * Cooking time ²)	0.86	0.0001
Alkaloids	%	Alkaloids = + 2.42216 - (0.010481 * Soaking time) - (0.29499 * Cooking time) + (6.76939 * 10 ⁻⁴ * Soaking time * Cooking time) - (1.75126 * 10 ⁻⁴ * Soaking time ²) + (0.036103 * Cooking time ²)	0.70	0.0003

f.w. fresh weight, d.w. dry weight.

^a Data in duplicate.

The combined effect of the best condition for soaking and cooking S18C1 and the best condition for washing W3H22 generated the following outcomes: 4.4 days of total processing time (0.8 d for soaking and cooking + 3.6 d for washing), which is shorter than the 5–6 days reported by Villacrés et al. (2000), but longer than the 3.7 days mentioned by Caicedo et al. (2001). However, all of the processing carried out by the latter was at 40 °C with the exception of cooking that was carried out at boiling point. The processes S18C1 + W3H22 consumed about 51 kg of water.kg⁻¹ dry seed, which is lower than the 63 kg of water.kg⁻¹ seed reported by Caicedo et al. (2001). The solids in the product were about 0.77 kg.kg⁻¹ dry seed, indicating a loss of solid material (including alkaloids) of 0.23 kg.kg⁻¹ dry seed. This is less than the 0.27 kg.kg⁻¹ dry seed reported by Torres-Tello et al. (1980). As for seed f.w. the value obtained for the conditions S18C1 + W3H22 was about 2.8 kg.kg⁻¹ dry seed. We did not find literature data to compare, except for Caicedo et al. (2001) mentioning a swelling index of debittered lupin of 2.3 times the raw material. Regarding the energy consumption under the best conditions, our process consumed 22.5 MJ.kg⁻¹ dry seed. In the absence of published data, we estimate that this energy consumption under optimum conditions would be similar to the process described by Villacrés et al. (2000), but only half the energy used in the process described by Caicedo et al. (2001) (42 MJ.kg⁻¹ dry seed) and less than half the energy used in the process of Jiménez-Martínez et al. (2003) (50 MJ.kg⁻¹ dry seed). The optimal solution was developed based on the behaviour (Response Surface) of all treatments applied. If the starting conditions for washing change, that situation will affect or benefit all treatments. To verify, we ran the model using soaked and cooked lupin obtained with different desirability (combination of factors). In each case, the optimum solution for washing stage was the same found in the study, namely W3H22.

Nonetheless, some other solutions, mainly based on one variable, were found that could be of practical interest. For example, the shortest process time (about 3 d) was obtained with S18C1 + 2 d of washing (W9H11 or W6H22); the highest seed weight f.w. (about 3.0 kg.kg⁻¹ dry seed) and the highest solids in product (about 0.88 kg.kg⁻¹ dry seed) could be obtained with W6H0. This process however, required about 5.5 days and consumed more than 100 kg of water.kg⁻¹ dry seed.

The soaking-cooking stage as well as the washing stage, each involve two factors, namely agitation time and changes of water.

The resulting 4 factors could be evaluated in one experiment. However, for practical considerations we did not do this. The most important consideration refers to the number of runs needed to achieve a valid solution. For example, a model 3⁴ requires 81 + *n* treatments. Each one requires 5 days in average to complete the experiments that means about 1 year and 2 months. In addition because we wanted to know, in unsteady conditions, the best treatment but also the models of mass transfer as function of alkaloid concentration and processing conditions, we needed to estimate the constant *k* for each treatment. Because we did not know the trend of those relations, we needed to measure the responses in at least 3 points during each treatment (adding a 5th factor, time). Then, the number of analysis, time and cost involved would have been much higher compared with the study as presented. In addition the interactions of 4 or 5 factors are more difficult to analyse than those of 2 or 3 factors. Finally, the complexity of obtained equations would be higher which would reduce their practical usage.

4.3. Estimation of time needed to reduce the alkaloid content

In order to estimate the time needed to reach a specific residual alkaloid content in the debittered seed after washing (agitation time and number of water changes) a mathematic function was inferred from the data, using the expert design 8 software. The washing process responds to function

$$\partial c / \partial t = kc \quad (4)$$

Where,

c = alkaloid content (g.100 g⁻¹ d.w.)

t = time required for reaching the desired alkaloids content (h)

k = non-dimensional constant expressed in function of agitation time and changes of water. This equation was inferred by us from experimental data by using the expert design 8 software. However, similar functions were also described by Crank (1975).

Fig. 3 shows the graphical representation of *k* vs. agitation duration and change frequency, and in Table 5 the equation is presented.

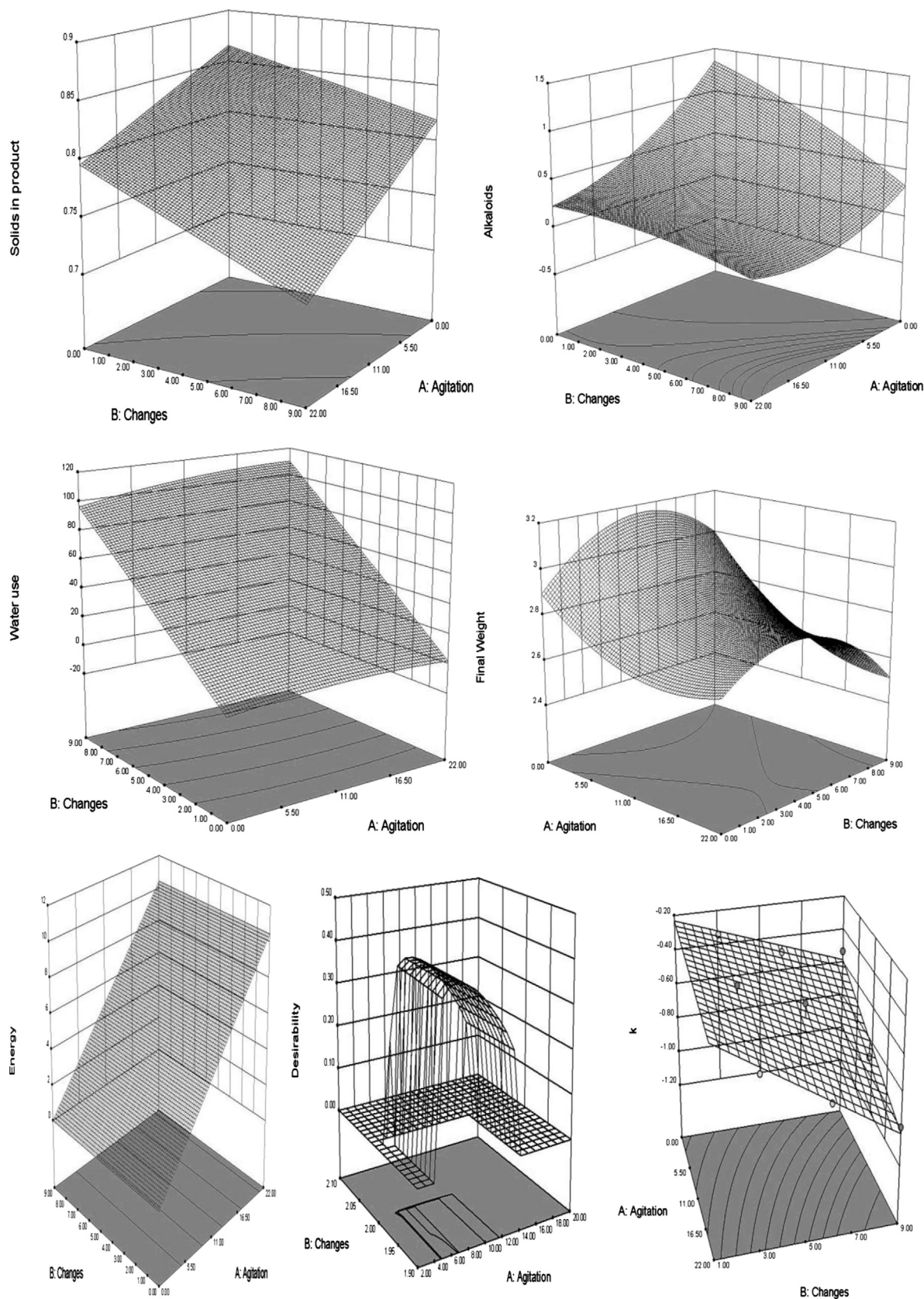


Fig. 3. Effect of agitation duration, and frequency of water changes. This figure shows the effect of washing conditions (agitation duration and frequency of daily water changes) on solids in product, alkaloids, water consumption, product weight (fresh weight), energy used, the desirability (the best conditions for carrying the washing process) and the non-dimensional constant k to estimate the washing time.

Table 5

Mathematical equations describing the effect of washing conditions on the amount of water and energy used, as well as, on the amount of solids, weight, and residual alkaloid concentrations in the processed lupin. All values are expressed per 1 kg of raw lupin in dry weight. Time is expressed in days. Agitation duration is expressed in h per day and frequency of water changes as times per day.

Response	Unit	Equation ^a	r ²	Prob. > F
Water use	l	Water use = + 0.15 003 + (0.23189 * Agitation) + (0.056715 * Changes) + (0.052268 * Time) + (0.057352 * Agitation * Changes) + (0.17243 * Agitation * Time) + (3.63762 * Changes * Time) – (0.028715 * Agitation ²) – (0.024396 * Changes ²) – (0.065985 * Time ²)	1.00	0.0001
Energy use	kJ	Energy = + 1.56854 * 10 ⁻¹⁵ + (1.88219 * 10 ⁻¹⁶ * Agitation) + (1.49132 * 10 ⁻¹⁶ * Changes) + (6.72952 * 10 ⁻¹⁶ * Time) – (1.80946 * 10 ⁻¹⁷ * Agitation * Changes) + (0.15871 * Agitation * Time) – (9.72655 * 10 ⁻¹⁷ * Changes * Time)	1.00	0.0001
Solids in product	kg d.w.	Solids in product = + 0.89303 + (5.68181 * 10 ⁻⁴ * Agitation) – (0.016458 * Changes) – (8.97554 * 10 ⁻³ * Time) – (1.63177 * 10 ⁻⁴ * Agitation * Changes) – (1.25065 * 10 ⁻³ * Agitation * Time) + (3.76413 * 10 ⁻³ * Changes * Time)	0.71	0.0001
Product weight	kg f.w.	Final Weight = + 2.55685 – (0.020706 * Agitation) + (0.14881 * Changes) + (0.16101 * Time) – (1.38852 * 10 ⁻³ * Agitation * Changes) – (3.94451 * 10 ⁻³ * Agitation * Time) – (0.014434 * Changes * Time) + (1.04390 * 10 ⁻³ * Agitation ²) – (0.010413 * Changes ²) – (0.016076 * Time ²)	0.80	0.0001
Alkaloids	%	Alkaloids = + 1.79005 – (0.094894 * Agitation) + (3.40778 * 10 ⁻³ * Changes) – (0.12792 * Time) + (4.11219 * 10 ⁻³ * Agitation * Changes) + (2.06168 * 10 ⁻³ * Agitation * Time) – (0.026879 * Changes * Time) + (1.71437 * 10 ⁻³ * Agitation ²) – (3.97577 * 10 ⁻³ * Changes ²) – (5.85064 * 10 ⁻³ * Time ²)	0.78	0.0001
k		k = –0.18771 – (4.97273 * 10 ⁻³ * Agitation) – (0.043036 * Changes) – (1.68106 * 10 ⁻³ * Agitation * Changes)	0.94	0.0018

f.w. fresh weight.

d.w. dry weight.

^a Data in duplicate.

5. Conclusions

We observed a good agreement between experimental data and the model within the experimental limits. Soaking and cooking have additional effects on solids content, and a synergistic effect on seed fresh weight, water consumption and alkaloids content. In the soaking and cooking process stage, energy consumption depends on cooking time only. During the washing stage, the effect of duration of agitation was stronger than that of the water change frequency. Energy consumption was related to duration of agitation only, and water use to the frequency of water changes. The washing conditions with the highest desirability were: three changes of water, with 22 h of agitation d⁻¹. That combination took a total processing time of 4.4 d and a water consumption of about 51 kg kg⁻¹ raw lupin. However, there were other scenarios that lead to debittered lupin in about three days (processes W9H11 and W6H22). These may be more attractive to processors. The mathematical function (4) and constant *k* are valuable tools to estimate the time required to reduce the alkaloids content during the washing process. The newly developed hydro-agitation technology could be used for optimizing processes such as hydrating and/or removing undesired material for other bitter lupin species, soybean, cowpea, mungbean, or other seeds.

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