Managing technological aspects of *Lupinus mutabilis* from a food sovereignty perspective in Ecuador

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Chapter 1

General introduction and thesis outline

Introduction

Global poverty and nutritional status

In our day and age, the world population consists of almost 7 billion people (Hammond, 2007) and it will be a major challenge to feed them all. Poverty and malnutrition have been present for a long time and easy solutions do not exist. These two related issues, malnutrition and poverty, are first considered in more detail. They play a role in the background for the research described in this thesis, though they are not the direct subject of the thesis.

Malnutrition

"Malnutrition is a broad term that refers to all forms of poor nutrition. Malnutrition is caused by a complex array of factors, including dietary inadequacy (deficiencies, excesses or imbalances in energy, protein and micronutrients), infections, economics and socio-cultural factors. Malnutrition includes under-nutrition as well as overweight and obesity" (United Nations, 2010). Undernutrition is when insufficient food intake and repeated infections result in one or more of the following: underweight for age, short for age (stunted) and thin for height (wasted) (United Nations, 2010). However, the physical appearance is only one of the consequences because the intellectual capacity of children will also be affected, making them adults with limited capabilities, and therefore less competitive and with fewer opportunities. These adults will procreate undernourished children to continue the cycle. Data of global and regional malnutrition are presented in Table 1 and Figure 1. Undernutrition (as wasting) affected 925 million people of all ages in 2010 (United Nations, 2010), which was about 1 out of 7 persons. Note that undernutrition coexists with overweight, pointing at the necessity to correct imbalances.

People are also considered to be undernourished when they suffer from vitamin and/or mineral deficiencies, so-called micronutrient malnutrition (United Nations, 2010). According to Céspedes (2007), regional adviser on micronutrients and nutrition of the World Food Program, more than 2 billion people are affected by micronutrient deficiencies in all developing countries.

Table 1. Malnutrition prevalence for preschool children (data 2010) by geographic region[1]

Geographic region	Prevalence of stunted (short for age) (%)	Prevalence of underweight for age (%)	Prevalence of wasting (thin for height) (%)	Prevalence of overweight (%)
Latin America & the Caribbean	13.4	3.5	1.4	7.1
Africa	35.6	17.9	8.5	7.1
Asia[2]	26.8	20.0	10.2	4.6
Oceania[3]	35.5	14.2	4.3	3.6
Developed countries	7.2	2.3	1.8	14.1
World	25.7	16.1	8.1	6.5

[1] World Health Organization, 2013
[2] Excluding Japan
[3] Excluding Australia and New Zealand

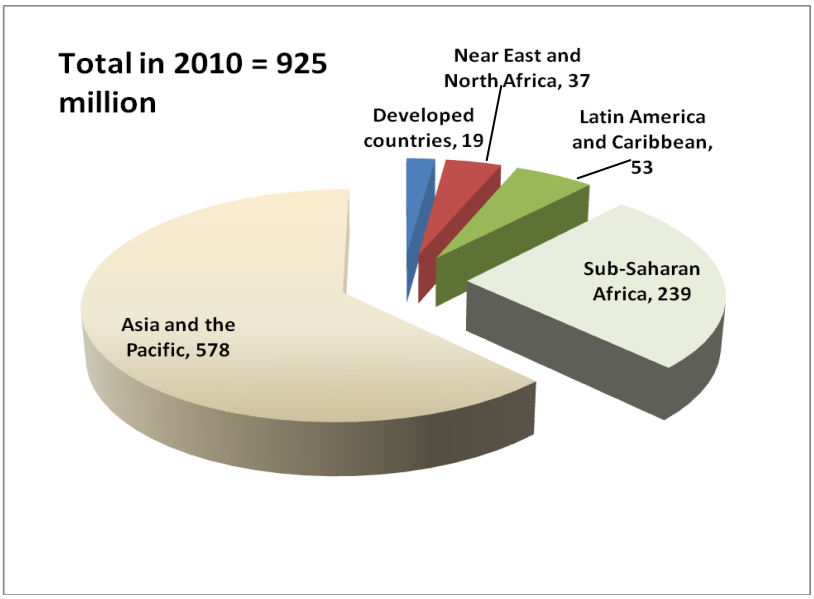

Figure 1. Undernutrition (as wasting) in 2010, by region (millions of people of all ages) [1]

[1] United Nations, 2010

From them, an estimated 163 million children are vitamin A deficient (by low serum retinol), with a prevalence of about 30% in South Central Asia (which includes India) and about 40% in Central and West Africa. South and Central America and the Caribbean have the lowest prevalence of the developing world, near to 10% (United Nations, 2010).

Table 2. Anaemia in selected population groups[1] and prevalence of inadequate zinc intake[2] per region

Region	Anaemia[1]						Zinc: prevalence of inadequate intake[2]
	Pre-SAC[3]		NPW[3]		PW[3]		
	Prevalence (%) (95 % C.I.)[4]	No. affected (millions) (95 % C.I.)[4]	Prevalence (%) (95% C.I.)[4]	No. affected (millions) (95% C.I.)[4]	Prevalence (%) (95% C.I.)[4]	No. Affected (millions) (95 % C.I.)[4]	(Range in %)
Africa	64.6 (61.7, 67.5)	93.2 (89.1, 97.4)	44.4 (40.9, 47.8)	82.9 (76.5, 89.0)	55.8 (51.9, 59.6)	19.3 (18.0, 20.7)	36.5- 61.6
Asia	47.7 (45.2, 50.3)	170.0 (161.0, 178.9)	33.0 (31.3, 34.7)	318.3 (302.0, 334.6)	41.6 (39.0, 44.2)	31.7 (29.7, 33.6)	33.5-72.5
Europe	16.7 (10.5, 23.0)	6.1 (3.8, 8.4)	15.2 (10.5, 19.9)	26.6 (18.4, 34.9)	18.7 (12.3, 25.1) 1	1.4 (0.9, 1.8)	3.9-12.7
LAC[5]	39.5 (36.0, 43.0)	22.3 (20.3, 24.3)	23.5 (15.9, 31.0)	33.0 (22.4, 43.6)	31.1 (21.8, 40.4)	3.6 (2.5, 4.7)	26.0-68.4
NA[5]	3.4 (2.0, 4.9)	0.8 (0.4, 1.1)	7.6 (5.9, 9.4)	6.0 (4.6, 7.3)	6.1 (3.4, 8.8)	0.3 (0.2, 0.4)	6.3 (0-16.1)[4]
Oceania	28.0 (15.8, 40.2)	0.7 (0.4, 1.0)	20.2 (9.5, 30.9)	1.5 (0.7, 2.4)	30.4 (17.0, 43.9)	0.2 (0.1, 0.2)	N/A[6]

[1] McLean et al., 2008

[2] Caufield and Black, 2004

[3] Pre-SAC, preschool-aged children (0.00–4.99 years); NPW, non-pregnant women (15.00–49.99 years); PW, pregnant women.

[4] 95% C.I. (confidence interval).

[5] LAC, Latin America and the Caribbean; NA, Northern America.

[6] Not available.

Concerning anaemia, a prevalence of 24.8 % at global level has been reported, which means that about 1.6 billion people are affected. From them 163.8, 859.7 and 597.7 million people are elderly, adults and children (0.00–14.99 years), respectively (McLean et al., 2008). Regarding zinc deficiencies, there are no global data. However, the International Zinc Nutrition Consultative Group (IZiNCG) has estimated them based on the presence and bioavailability of zinc in each country's food supply. According to that technique, the global prevalence of zinc deficiency was estimated at 31%, which are about 2 billion people. However, the prevalence of zinc deficiency ranges from 4–73% across sub regions (Caufield and Black, 2004). Distribution of anaemia and zinc deficiency prevalence per region is presented in Table 2. Zinc deficiency prevalence affects especially the developing world. The available information indicates that any effort to improve the nutrition status globally or locally should be promoted and supported.

Poverty and the role of agriculture

So far it has not been possible to reach the first objective of the millennium "to eradicate extreme poverty and hunger"; there are still many people in that situation. In fact, in 2008 an estimated 2.47 billion people survived with less than 2 dollars per day (poverty) and 1.29 billion people even had less than 1.25 dollars per day (extreme poverty) (World Bank, 2012b). Poor people are predominantly located in Asia, Africa and Latin America and the Caribbean (FAO, 2012; Infante, 2009; Rojas, 2009). Poverty is also related to Gross Net Income (GNI) (Table 3).

Table 3. Poverty, GNI and importance of agriculture by regions[1]

Regions[2]	% of people with less than $ 2.0 / day (data for 2008)	% of people with less than $ 1.25 / day (data for 2008)	GNI[3] per capita ($). (data for 2011)	Employment in agriculture as % of total employment (data for 2008)	Agriculture. Value assed (% of GDP) 2010
LAC (all income levels)	N/A[4]	N/A	11,823	14.0	6.2
LAC (developing countries only)	5.5	3.3	11,586	14.1	6.4
MENA (all income levels)	N/A	N/A	10,865[5]	22.9	7.4[6]
MENA (developing countries only)	3.2	0.6	8,026[7]	27.2	10.5[6]
SSA (all income levels)	N/A	N/A	2,251	N/A	11.2
SSA (developing countries only)	35.2	20.6	2,232	N/A	11.2
CAR	46.8	31.3	810	N/A	56.5[7]
EAP (all income levels)	N/A	N/A	10,405	36.0	3.4
EAP (developing countries only)	11.1	3.4	7,312	39.6	11.2
ECA (all income levels)	N/A	N/A	25,128	9.1	1.9
ECA (developing countries only)	0.6	0.2	14,178	16.4	7.2
World	36.0[8]	19.0[8]	11,573	N/A	2.8

[1] World Bank, 2010b and World Bank, 2010a
[2] LAC Latin-American & Caribbean, MENA Middle East & North Africa, SSA Sub-Sahara Africa, CAR Central African Republic, EAP East Asia & Pacific, ECA Europe & Central Asia.
[3] GNI (Gross Net Income)
[4] Not available, [5] In 2010, [6] In 2007, [7] In 2009
[8] Calculated from World Bank, 2010b and World Bank, 2010a

In order to fight poverty, the generation of employment is crucial. Agriculture is a good source to generate this, especially in the developing world because there this activity is generally still labour intensive. Besides, agriculture contributes significantly to the Gross Domestic Product (GDP) of those economies. The problems of malnutrition and poverty are related. The solution to both problems can be addressed simultaneously by improving agriculture.

Global economy and nutritional status in developing countries

Global economy refers to the production and commercialization of goods and services (including financial products) that independently from where they are produced or created, affects other goods, services and somehow all economies in the world. A particular effect of the global economy is on the nutritional status of people. For example, the proportion of undernourished people in the world began to rise from 2006 onwards after a period of decline that started in 1969. According to the United Nations (2010), the causes for this rise are the global downturn, increments in food prices, reduced remittance streams, contractions in trade, accelerated reductions in foreign direct investment and decreased official development assistance to developing countries. Other authors also mention speculation with food (Infante, 2009) and the use of food as an energy source (Bogarín, 2009; Infante, 2009). In addition, in the global economy there is open competition between multinational food companies and small and local businesses, affecting the social and economic interests of the latter, especially in the developing world (Kloppenburg, 2008; Linnemann and van Boekel, 2007; Wageningen University, 2007; Windfuhr and Jonsén, 2005). According to Wageningen University (2007), that is because the model of vertically integrated and centrally commanded food chains, dominant in the global economy, is not appropriate for many local production networks in developing countries since they are in fact horizontally integrated and participatively commanded. For instance, retailers and small processors compete with global companies, which sell products to consumers based on big advertisement campaigns and with standardized products usually produced/manufactured abroad, according to the scales of economy concept and foreign realities. As a consequence the local chains and local networks have to give up their knowledge, producing and processing systems, products, habits and preferences (INREF, 2010). The local chains become disconnected and the relationships

between their actors vanish. Then, the actors of the once local chain become part of the global chains as suppliers, labourers, sellers or product consumers (INREF, 2010). They do not produce, process, sell or consume according to local necessities but the production and consumption are rather determined by a global food company agenda. Food production and consumption become part of two disconnected, industrialized areas leading to a social organization of global food chains in which both shortcomings, namely undernutrition and abundance (overweight and obesity) exist simultaneously (Windfuhr and Jonsén, 2005). In the developing world, agriculture and local food chains have an important role in generating employment, improving the economy and fighting malnutrition. Therefore, any global or local effort to improve agriculture will be valuable. Since Ecuador is part of the developing world, it is extremely important to re-connect the local food chains there. The present study is a contribution to this goal and details of how the project did this will be presented further. However, in order to know the local context in which the research was performed, introductory information about Ecuador is first presented.

The Ecuadorian reality

Localization and economy

According to the Ecuadorian Institute of Statistics and Census (INEC, 2012a), Ecuador has 14.5 million people distributed over 256.370 km^2. The GNI expressed as PPP (purchasing power parity) was 8310 dollars per capita in 2011 (World Bank, 2010a). Farming activities (agriculture and livestock) are an important component of the economy, which in 2011 represented 8.1 % of GDP, only exceeded by commerce (14.7%), industry (13.9%) and non-oil mining (11.4%) (INEC, 2012c). In addition, farming supplies about 30% of the total commodities used in the industry (Anda, 2011). Farming is also an important source of employment in Ecuador. According to the World Bank (2010b), this sector contributed for 28.7% of total employment in 2009, which is twice the regional value (Table 3).

Regions and food habits

The continental part of Ecuador is crossed from North to South by the Andean mountain range that divides the country in three regions (namely the Coast, the Andes and the Jungle).

In addition to the continental part, Ecuador has an insular region located 1000 km to the west from the Coast region, named the Galapagos Islands (Nutrinet, 2008). Each region has specific ecological, topographic and climatic characteristics, which influence their industrial, commercial, touristic, agricultural, social and cultural (including food habits) development.
The Galapagos archipelago is well known because of its peculiar flora and fauna. It is mainly dedicated to natural touristic activities with controlled fishing and agriculture. The Jungle is a rain forest of middle altitude, predominantly dedicated to the extraction of petroleum and subsistence agriculture. The Coast is a low region, which produces tropical products such as banana, plantain, coffee, coconut, cocoa, pineapple, papaya, cassava and rice. It is also where sea food is captured, specially tuna fish, and where shrimps are farmed. Most agro-industrial and industrial companies dedicated to export are located in this region. The Coastal dishes are similar to those of the Galapagos region and are based on rice, plantain, cassava, fish and poultry (from the Coastal region) and beans, vegetables, milk, pork and beef from the Andean region. The latter region also produces maize, potatoes and fruits. The Andean diet is mainly based on locally produced foods, although it is possible to find food products and dishes from the Coast too (especially sea food, rice and tropical fruits). A difference with the Coastal region is that most of the farms and food factories in the Andean region are dedicated to producing and processing food for national consumption. In this region traditional indigenous foods such as quinoa, maize, broad beans, lupin, whole flours and "panela" (brown sugar loaf) are still cultivated. However, the fast food industry and processed food (pasta, white sugar, sausages, white bread and fizzy drinks) are displacing the local foods, and therefore local chains (Nutrinet, 2008). An analysis of the Ecuadorian diet showed a deficit of fruits and vegetables, which is a paradox in a country where those are abundantly produced (Nutrinet, 2008).

Poverty and nutritional status

Ecuador is a country with plenty of biodiversity and natural resources. However, Ecuador is also a country with a high prevalence of poverty and malnutrition. In 2010, 10.6 % of the population (1.5 million) and 4.6 % (0.7 million) lived below 2.0 and $1.25 per day, respectively (World Bank, 2012a). Poverty is mainly located in some provinces of the Coastal region (Esmeraldas, Los Ríos and Manabí), in parts of the Andean region (Carchi, Bolívar, Cotopaxi, Chimborazo, Cañar, Loja) and especially in the rural areas of these

locations (CEPAR, 2004; INEC, 2012b). In those rural areas, the situation is the worst because poverty is twice that of urban levels and extreme poverty is three times the urban values (INEC, 2012b).

In Ecuador, the low and heterogeneous distribution of income (INEC, 2012b) affects food access and nutritional status of people (CEPAR, 2004); as a result 16 % of the population does not have enough food. Moreover, according to Nutrinet (2008), Ecuadorian poor people do not eat enough protein. The final result is the presence of chronic (stunted), global and acute (wasted) undernutrition in both the urban and rural areas. In urban areas the values (<2.0 standard deviation) were 17, 7.8 and 1.7 %, and for rural areas 30.7, 11.3 and 1.6 %, respectively (CEPAR, 2004). Ecuadorian children of 0-4 years old also presented prevalence of Vitamin A deficiency (10- 19%) and prevalence of insufficient zinc ingestion (59-73%). In addition, the national prevalence of anaemia (Hb < 11 g/ dL) in children of 0-4.99 years old was 6-28%. However, in rural and poor areas of Ecuador this value was up to 50.2% (Céspedes, 2007). Malnutrition and poverty are severe problems that Ecuador suffers from, which have to be solved urgently.

Combating poverty and malnutrition: the Food Sovereignty concept and the TELFUN project

According to the World Bank (2012b) hunger and poverty should be addressed by creating more and better jobs, delivering better education, health services and basic infrastructure and protecting the vulnerable. In addition, FAO (2012) added that those problems should be faced by making investments in agriculture and providing small farmers with access to seeds, fertilizer, adequate technologies, infrastructure, rural credits and markets.

Whereas there is general agreement with the above statements, in practice it turns out to be a difficult and lengthy process to alleviate poverty and hunger. Food scarcity has been an issue since the 70's. In 1974 the First World Conference of the United Nations on the theme of Feeding the World, resulted in the proclamation and signing of The Universal Declaration about Eradicating Hunger and Malnutrition where, among others, the most important resolution exhorted to all governments was to accept the goal that, within the time lapse of one decade, hunger must have been eradicated, and that nobodies' future or prospects should be restrained because of malnutrition (MCDS and FAO, 2011). Nevertheless, four decades later after many meetings, declarations, definitions, and in spite of the effort of

national and international organizations and local governments, food shortages are still amongst us. In fact, hunger has increased during the last decade (FAO, 2012).

In response to this situation, "Via Campesina" and other social organizations have since the 90's proposed the "Food Sovereignty" concept (MCDS and FAO, 2011). Food sovereignty has been defined in several manners, of which the generally accepted formulation is "*the right of peoples to define their own food and agriculture; to protect and regulate domestic agricultural production and trade in order to achieve sustainable development objectives; to determine the extent to which they want to be self reliant; to restrict the dumping of products in their markets; and to provide local fisheries-based communities the priority in managing the use of and the rights to aquatic resources. Food Sovereignty does not negate trade, but rather it promotes the formulation of trade policies and practices that serve the rights of peoples to food and to safe, healthy and ecologically sustainable production"* (Windfuhr and Jonsén, 2005). Food Sovereignty also emphasizes the importance of local food chains and highlights the necessity to re-connect the actors from producers to consumers (MCDS and FAO, 2011).

Inspired by the Food Sovereignty concept, the TELFUN research project was initiated. The acronym TELFUN stands for: "**T**ailoring Food Sciences to **E**ndogenous Patterns of **L**ocal Food Supply for **Fu**ture **N**utrition". The project investigated how traditional local crops can be used to contribute to the local livelihood and improved nutritional status. The project had a multi-disciplinary research approach, since food chains comprise many actors and factors such as producers, processors, retailers, consumers and an intangible component (the socio-economic relationships among actors). The question is how agriculture, product development and technology can provide affordable and nutritious food, taking into account food habits and preferences of local consumers and their nutritional requirements.

In particular, the research project focused on agronomic, technological (science and engineering), nutritional and social aspects of the local food networks. The TELFUN project used protein-rich leguminous seeds as model crops in 3 continents. In India, mung beans were selected as model crop, whereas cowpea was studied in Benin and Ghana, and lupin in Ecuador. These three crops were chosen because they are indigenous and part of the traditional culture, well-adapted and originating from these respective continents, and offer a high nutritional potential. Traditionally, these crops have been used in local diets, which makes them an important crowbar for empowering and reconnecting local food networks (INREF, 2010). In addition, India, Benin, Ghana and Ecuador all face the challenge of

poverty and malnutrition, so any specific research outcome could be of immediate local interest.

The TELFUN project is part of a bigger program of Wageningen University called INREF, which stands for "**In**terdisciplinary **R**esearch and **E**ducational **F**und". The main objective of this program is to generate scientific knowledge and innovation aimed at fulfilling a societal need. Research follows a cross border approach (INREF, 2010), has participative components and considers community preferences, points of view, local practice and adequate technology concepts. Final results are communicated to the community to facilitate application of research outcomes (Urrutia, 2009; Altieri, 2002).

Lupin *(Lupinus mutabilis)* as a food crop

Importance and drawbacks

Lupin (*Lupinus mutabilis*) is a very important legume for Ecuador and for the entire Andean region (Ecuador, Perú, Bolivia and parts of Colombia and Chile) because it has been cultivated, processed and consumed for at least 1500 years (Haq, 1993). The importance of lupin for the Andean region is illustrated in Figure 2. Figure 3 shows some features of lupin. According to the Ecuadorian National Institute of Agriculture and Livestock Research (INIAP), *L.mutabilis* is also important because it is one of three key legumes together with peas (*Pisum sativum)* and kidney beans *(Phaseolus vulgaris*) to improve both the nutritional status and the economic situation of poor people in Ecuador (Caicedo and Peralta, 1999). The nutritional composition of *L. mutabilis* is similar to that of soya bean (Pate et al., 1985; Santos et al., 1997) and therefore, it has been called the soya bean of the Andes (Villacrés et al., 2003). In addition, in Ecuador debittered *L. mutabilis* costs twice or three times less than beef and chicken breast. Considering the poverty, malnutrition and inadequate protein intake of many Ecuadorians, Nutrinet (2008) stressed the importance of balancing their diet and improve its protein quality at a low prices, by stimulating the consumption of traditional combinations of cereals and legumes, such as maize and lupin.

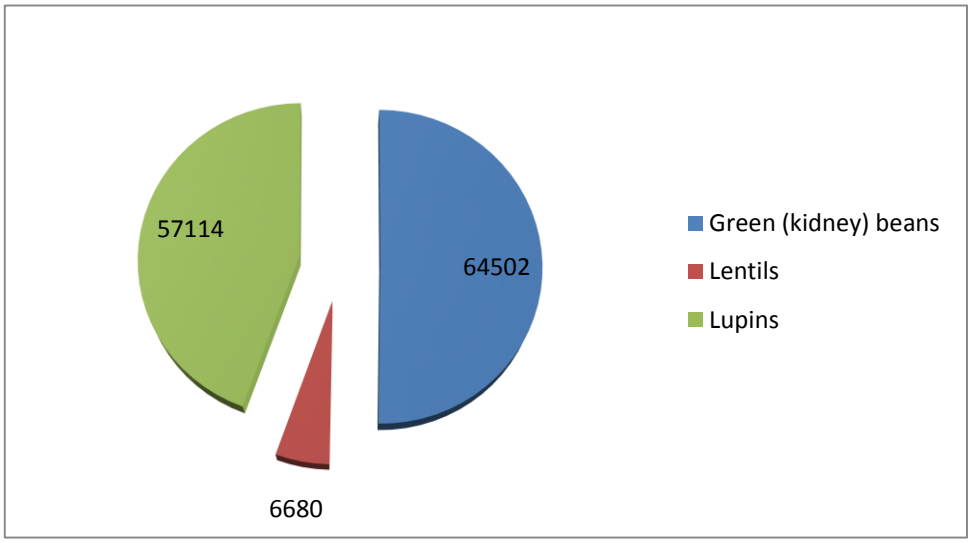

Figure 2. Production (tonnes) of the three main legumes in the Andean Region and Chile in 2011[1]

[1] FAO, 2013

Lupinus mutabilis is important in Ecuador because it can be grown in poor soils and dry climates (Haq, 1993) where other legumes and even specially bred varieties of low-alkaloid *L. mutabilis* (0.018 g/100g dw) cannot grow (Gross et al., 1988). The main reason for this is because only *L. mutabilis* with high alkaloid content, i.e. up to 4.5 g/100g dw (Gueguen and Cerletti, 1994), gives the plant high resistance to microbial infections and insect attacks (Ciesiolka et al., 2005). In addition *L mutabilis* shows a wide genetic variability illustrating adaptation to microhabitats and natural selection (Haq, 1993), which is also a preservation mechanism. Considering that poor and undernourished people live in areas with poor soils, such as in the Andean highlands, the cultivation of *L. mutabilis* for home-consumption and for sale is important for their nutrition and livelihood.

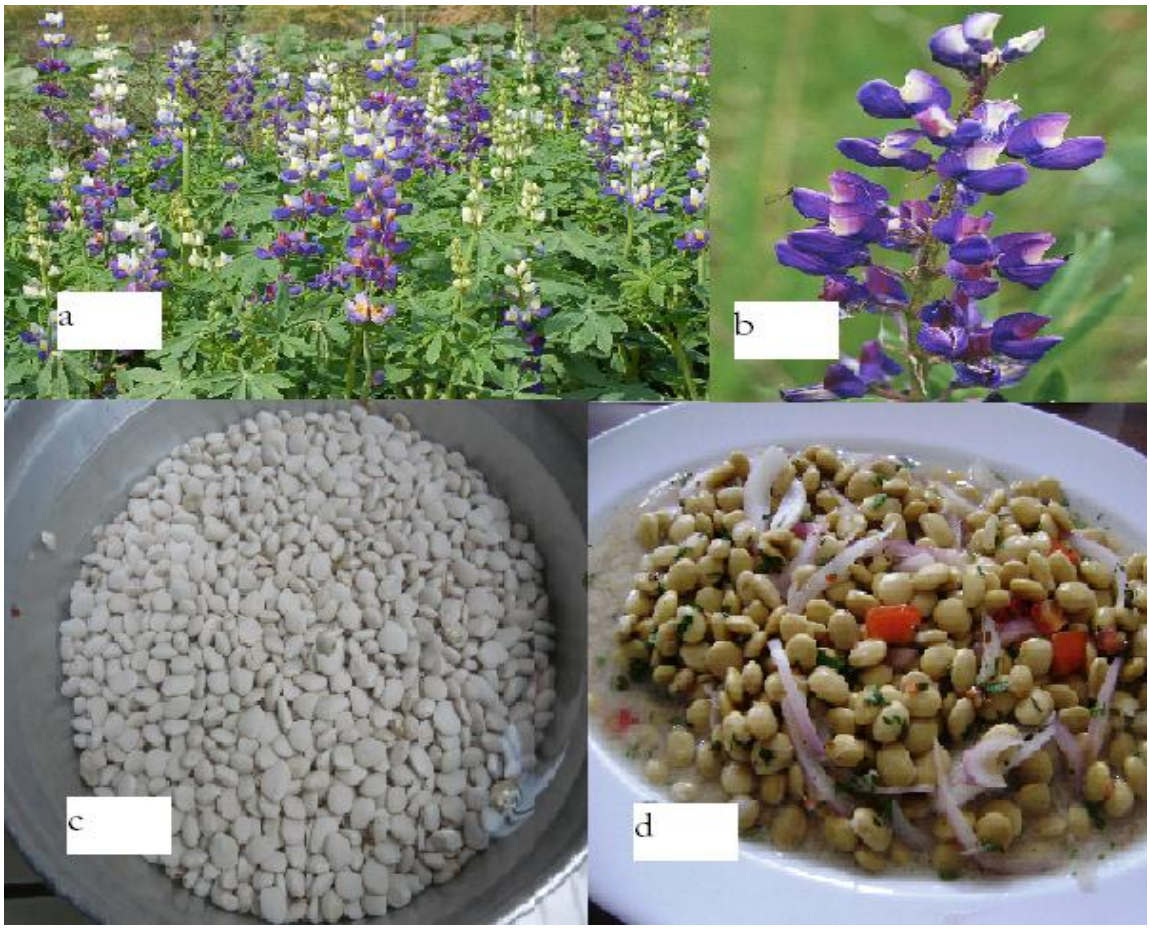

Figure 3. *Lupinus mutabilis* (a) in the field, (b) the stem and florescence, (c) raw seeds, (d) debittered seeds in a fresh salad

A limitation of *L. mutabilis* is its content of toxic quinolizidine alkaloids of which 70 have been distinguished; these alkaloids must be removed before consumption because of their toxicity (Australia New Zealand Food Authority, 2001). The alkaloids toxicity is associated with liver diseases and neuromuscular blockage (Camacho et al., 1991) caused by inhibiting the ganglionic transmission impulse of the sympathetic nervous system (Jiménez-Martínez et al., 2003). Chemically, lupin alkaloids can be distinguished as either free bases (soluble in organic solvents), or salts (Ortiz and Mukherjee, 1982). The major alkaloids in *L. mutabilis* are lupanine ($C_{15}H_{24}N_2O$), sparteine ($C_{15}H_{26}N_2$) and 13-Hydroxylupanine ($C_{15}H_{24}N_2O_2$) (Petterson, 1998) (Jiménez-Martínez et al., 2007), with sparteine being reported as the most toxic when orally ingested (fatal dose: 30 mg kg^{-1} body weight) (Australia New Zealand Food Authority, 2001), see Figure 4.

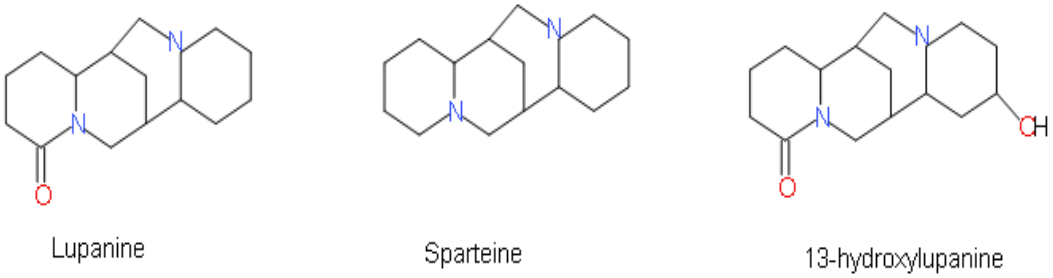

Figure 4. Major alkaloids in *Lupinus mutabilis*

Nevertheless, lupin alkaloids could have some commercial importance because of their pharmacological activity (Ciesiolka et al., 2005; Jiménez-Martínez et al., 2003). In addition, specific protein isolates and concentrates obtained from lupin could also have commercial importance because of their functional properties for chemical and food industries (Doxastakis, 2000; Gueguen and Cerletti, 1994; Moure et al., 2006; Sathe et al., 1982). The development of an efficient technology for debittering lupin and the identification of alternative uses for its components could definitely contribute to reconnect to the actors of local lupin chain. In addition, these benefits are also important contributions to the TELFUN project that encourages the use of traditional and local crops for new purposes (INREF, 2010).

Research area and process for debittering lupin

This research was carried out in the Village of San Pedro de Tanicuchi, located in the rural area of Cotopaxi province at 2981 m between the central and occidental ranges of the Ecuadorian Highlands (Gobierno Autónomo de la Parroquia de Tanicuchi, 2011). The rural area of Cotopaxi province is not only one of the poorer places of Ecuador but also it is a very important centre for lupin processing at both the household and medium scale level (family associations). Although there is no official list of debittering factories, the local people estimate their number to about 40. Since all work is manual, this debittering process is a very important way to generate employment locally.

Regarding the processes for debittering of lupin, the only food-grade approach known is by aqueous extraction (Figure 4) of the legume destined for human consumption in fresh salads,

soups, or as a snack. This process is cited to be advantageous because it is the most economic way to remove alkaloids (Chajuss, 1989) and it only uses water (Rossetto, 1989). However, the process is also reported as inefficient in terms of time and water consumption (Caicedo et al., 2001; Villacrés et al., 2000). In addition, it causes nutrient losses (Torres-Tello et al., 1980), and can potentially be hazardous for consumption because of the poor hygienic quality as evidenced by the presence of coliforms and high counts of Enterobacteriaceae in the debittered seed (Villacrés et al., 2000). The current process needs to be improved in order to expand the market for debittered lupine.

Figure 4. Current debittering process of *Lupinus mutabilis*

Relevance and research questions

Contribution to Food Sovereignty

This thesis describes the options to improve the debittering process of *Lupinus mutabilis* Sweet in terms of reducing water and time consumption and decreasing losses of macronutrients, and iron and zinc. The research will also consider the microbial quality, consumer sensorial preferences and cost of processing options. Moreover, technology

development should take into account the local seeds, processors preferences and ancient and indigenous knowledge.

In short, this research aims to contribute to the re-connection and strengthening of the lupin chain by developing a technology that is in balance with all components of the lupin chain.

Research question

Can the aqueous debittering process of *Lupinus mutabilis* Sweet be optimized, within the framework of the Food Sovereignty concept?

Objectives

The general objective of this research is to optimize the aqueous debittering process of *Lupinus mutabilis* in the framework of the food sovereignty concept, and at the same time to contribute to the knowledge about lupin.

The specific objectives are the following:

- To critically inventorize and review published data on composition, uses, toxicology and debittering of the major lupin species, focusing on *Lupinus mutabilis.*
- To establish the efficiency of the current debittering process as a starting point of optimization.
- To study the effect of processing conditions on efficiency of the aqueous debittering process.
- To estimate mathematic models that reflect the best conditions for debittering lupin from processing and consumers´ points of view.

Outline of the thesis

The present chapter describes the justification and relevance of the PhD research and its objectives. It also outlines and provides general information about lupin. **Chapter 2** presents the nutritional composition, toxicity, anti-nutritional factors, debittering processes and

applications for specific protein concentrates and isolates of four major lupin species namely *Lupinus albus,L. luteus, L. angustifolius* and *L. mutabilis* with emphasis on the last. A critical evaluation of data and suggestions for future research are given. In **chapter 3**, the performance of the current debittering process was investigated, focusing on the weight of final product, remnant solids, chemical composition, water and time consumed and microbial quality. A mathematical model estimated the diffusion coefficient of alkaloids. Based on chapter 2 and 3, it became clear that further research was needed to improve the debittering process in terms of technology used and consumers' preference. Consequently, in **chapter 4** a debittering machine designed and built by the author was used to test different parameters. Optimal conditions, mathematical functions and a non-dimensional constant *k* (to estimate the needed time for debittering) were derived from model simulations. Subsequently, in **chapter 5** the effect of different experimental treatments on consumers'acceptance, expected price and consumers' willingness to pay were modeled and evaluated. Optimal solutions were inferred. Finally, in **chapter 6**, a general discussion is presented on the obtained results and on the degree to which the objectives have been achieved. In addition, recommendations for further research are also given.

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Chapter 2

Lupinus mutabilis: composition, uses, toxicology and debittering

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F.E. Carvajal-Larenas, A.R. Linnemann, M.J.R. Nout, M. Koziol, M.A.J.S. van Boekel. ***Lupinus mutabilis*: composition, uses, toxicology and debittering.** Critical Reviews in Food Science and Nutrition.

Abstract

Lupinus mutabilis has protein (32.0-52.6 g/100g dw) and lipid (13.0-24.6 g/100g dw) contents similar to soya bean (*Glycine max*). The Ω3, Ω6, and Ω9 contents are 1.9-3.0, 26.5-39.6, and 41.2-56.2 g/100g lipid, respectively. Lupins can be used to fortify the protein content of pasta, bread, biscuits, salads, hamburgers, sausages, and can substitute milk and soya bean. Specific lupin protein concentrates or isolates display protein solubility (>90%), water absorption capacity (4.5 g/g dw), oil absorption capacity (3.98 g/g), emulsifying capacity (2000 mL of oil/g), emulsifying stability (100 %, 60 h), foaming capacity (2083 %), foaming stability (78.8 %, 36 h), and least gelation concentration (6 %), which are of industrial interest. Lupins contain bitter alkaloids. Preliminary studies on their toxicity suggest as lethal acute dose for infants and children 10 mg kg^{-1} bw and for adults 25 mg kg^{-1} bw. However, alkaloids can also have medical use for their hypocholesterolemic, anti-arrhythmic and immunosuppressive activity. Bitter lupins can be detoxified by biological, chemical or aqueous processes. The shortest debittering process requires 1 hour. This review presents the nutritional composition of lupins, their uses (as food, medicine and functional protein isolates), toxicology and debittering process scenarios. It critically evaluates the data, infers conclusions and makes suggestions for future research.

Keywords: Nutritional composition, uses, toxicology and debittering

Introduction

Lupins (*Lupinus* spp.) are legumes (Haq, 1993) used principally as a protein source in human and animal nutrition (Güémes-Vera et al., 2008). According to FAO (2012a) more than 934,426 metric tons of lupin were produced in 2010, in Germany, Poland, the Russian Federation and Mediterranean countries as well as in Australia, South Africa, and South America. Four major species of lupins are cultivated, namely *L. albus*, *L. luteus*, *L. angustifolius* and *L. mutabilis*, of which the latter has the highest average content of protein (44 % dw) and lipids (18 % dw) (Pate et al., 1985). Lupins can be used as ingredients for many products such as cakes, snacks, hamburgers, biscuits, babyfoods, soups, salads and substitutes for milk, meat, and soya bean (Cremer, 1983; Güémes-Vera et al., 2008; Ruales et al., 1988; Villacrés et al., 2003). Lupin protein isolates and concentrates display physical and functional properties comparable to those of soya bean (Doxastakis, 2000). Water and oil absorption; emulsifying capacity, activity and stability; foaming capacity and stability; and gelation capacity are properties of lupin protein isolate that are valuable to the food and chemical industry (Doxastakis, 2000; Gueguen and Cerletti, 1994; Moure et al., 2006; Sathe et al., 1982). Alkaloids from lupins, apart from being toxic in human nutrition, could be useful in medical applications for their immunosuppresive, antiarrhytmic and hypocholesterolemic capacity (Ciesiolka et al., 2005; Jiménez-Martínez et al., 2003a). In addition, lupins contain phenolic antioxidant compounds, and prebiotic oligosaccharides, which may favour the proliferation of bifidobacteria (Jiménez-Martínez et al., 2003b). However, despite these facts, little is known about the chemical structure, properties and composition of the four main lupins species (Santos et al., 1997), when compared with soya bean (Gueguen and Cerletti, 1994). The factor limiting the use of lupins is the presence of quinolizidine alkaloids (Jiménez-Martínez et al., 2003a), especially in bitter species or subspecies, which have to be removed before consumption (Australia New Zealand Food Authority, 2001).

To assess the potential of lupins, particularly of *L. mutabilis*, this review critically investigates published data on the composition, uses, toxicity and processing scenarios for the detoxification and debittering of lupin species. Research needs are formulated on the basis of identified knowledge gaps. For each constituent, the published data were converted into the same units, and their average, minimum and maximum values were calculated and reported.

Varieties of *L. mutabilis* cited in this study are, apart from a unspecified variety (Aguilera et al., 1983; Aguilera and Trier, 1978), 'H-1' (Bleitgen et al., 1979), 'Potosi' (Carvalho et al., 2005; Múzquiz et al., 1989; Santos et al., 1997), 'Inti' (Gross et al., 1988; Santos et al., 1997), '2150-Inti' (Gross et al., 1988), 'Multulopa' (Güémes-Vera et al., 2008), 'CTC-177-1', 'Cumbre', 'Garz' (Múzquiz et al., 1989), 'H-6' (Sathe et al., 1982), 'Kayra' (Torres-Tello et al., 1980), and 'Sweet Andino 450' (Villacrés et al., 2000).

Other lupins cited are *L. albus* 'Multolupa' (Agosin et al., 1989; Aguilera and Trier, 1978; King et al., 1985; Múzquiz et al., 1989), 'Astra' (Aguilera and Trier, 1978; Bleitgen et al., 1979), 'Tifwhite' (Aguilera et al., 1983), 'Ares' and 'Typ Top' (D´Agostina et al., 2006), 'SP', 'AL' and 'Kali' (Múzquiz et al., 1989); a *L. angustifolius* unspecified variety (Lqari et al., 2002), 'Uniwhite' (Múzquiz et al., 1989), 'Fest', 'Unicorp', 'LCFM' (Múzquiz et al., 1989); a *L. campestris* unspecified variety (Jiménez-Martínez et al., 2003a); *L. luteus* 'Aurea' (Aguilera and Trier, 1978), 'Tremosilla', 'Gyulatanyai', 'SAH' and 'Afus' (Múzquiz et al., 1989); *Lupinus termis* (Rhama and Narasinga, 1984); and *Lupinus tricolor* SODIRO (Castillo, 1965).

Nutrient composition of lupins

Macronutrients

The average moisture content (Table 1) of whole raw lupin *(Lupinus* spp.*)* seeds varies from 8.1 - 9.4 g/100g fresh weight. The metabolic energy content varies slightly from 2032 kJ/100g dw for *L. angustifolius,* to 2078 kJ/100g dw for *L. albus,* and to 2164 kJ/100g dw for *L. luteus*. These values are lower than those reported for *L. mutabilis* (2307 kJ/100g dw) (Villacrés et al., 2000). This could be explained by the higher lipid content reported for *L. mutabilis*. The average crude protein content in lupins varies from 33.9 - 43.3 g/100g dw. The lower value is for *L. angustifolius* and the higher for *L. mutabilis*. However, despite the fact that almost all publications agree that the protein content in *L. mutabilis* is highest amongst the major lupin species, this is based on averages only. When we consider data within the species, we observe, for example for *L. mutabilis*, that crude protein ranges from 32.0 - 52.6 g/100 dw. This wide range in *L. mutabilis* is associated with genetic and agronomic factors. Indeed, (Haq, 1993) mentioned that *L. mutabilis* has a wide genetic variability illustrating adaptation to microhabitats and natural selection. This variability has especially been noted in

plant shape, vegetative growth, susceptibility to frost and diseases, protein, oil, and alkaloid content (Haq, 1993). Carvalho et al. (2004) grew *L. mutabilis* 'Potosi' in pots with a layer of gravel at the bottom and filled with sandy soil, watered every day, added no fertilizers and obtained seeds with just 11.2 % of protein dw, 8.5 % of oil dw, and 28.3 % of crude fibre dw, showing that a limited availability of nutrienst may affect the composition of lupin.

In addition, total protein content is often (but not always) estimated by multiplying the total nitrogen value by the factor 6.25 (Santos et al., 1997). However, according to several authors (Aguilera and Trier, 1978; Gueguen and Cerletti, 1994) this procedure overestimates the protein values because living tissues and legume seeds in particular, contain considerable amounts of non-protein nitrogenous compounds and because of the high degree of amidation of these proteins (Doxastakis, 2000). Santos et al. (1997) mentioned that a factor of 5.7 would be more suitable as a conversion factor for legume proteins, and for lupin seeds even a lower factor (5.4) was proposed (the difference resulting from the fact that in the case of lupin a portion of the nitrogen measured originates from alkaloids. Gueguen and Cerletti (1994) and Aguilera and Trier (1978) suggested 5.5 and 5.7 as conversion factors, respectively.

The reported lipid content in raw lupins (Table 1) ranges from 5.5 g/100g dw in *L. luteus* to 18.9 g/100g dw in *L. mutabilis*. However, among the varieties of *L. mutabilis*, lipid content may range from 13.0 - 24.6 g/100 dw. This range in lipid content can be explained at least partially by genetic and agronomical factors (Carvalho et al., 2004; Haq, 1993). For example, Carvalho et al. (2005) showed that the composition of lupin (and its lipid fraction particularly) can be affected by water stress, *i.e.*, lipid content was reduced by half in conditions of water stress.

Francki et al. (2002) mentioned that Total-Acid-Glycerols (TAGs) are rapidly accumulated during mid-stages of seed development. However, the seeds of late-maturing varieties usually accumulate larger amounts of lipid than those of early-maturing varieties because the plants with a longer growing season have a longer time available to convert carbohydrates into lipids. This is only true, however, if late-maturing varieties get enough time in the field because the last stage of maturation is of critical importance for oil content (Bélteky and Kovács, 1984).

The average fibre content varies from 8.2 g/100g dw in *L. mutabilis* to 16.0 g/100g dw in *L. angustifolius*. We note that *L. mutabilis* has the lowest average fibre content of the lupin species reported in Table 1, and that the fibre content varies widely between lupin species.

The reported average values for ash content vary from 3.0 g/100g dw in *L. angustifolius* to

3.9 g/100g dw in *L. mutabilis.* The variability in content of fibre and ash also can be explained partially by agronomic factors, *i.e*., crude fibre and ash decreased with about 10% as a result of water stress (95)(Carvalho et al., 2005).

The average carbohydrate content in lupin species was reported excluding the fibre content, and varied from 32.9 g/100 dw in *L. mutabilis* to 47.6 g/100g dw in *L. angustifolius*. The differences in carbohydrate content probably can be explained by the same arguments that explain variations in other macronutrients; however, that it is not stated as such in the investigated studies since the carbohydrate content was generally deternined by difference (Güémes-Vera et al., 2008).

Based on average values presented in Table 1, raw *L. mutabilis* has the highest protein and lipid, and the lowest fibre and carbohydrate content among the major lupin species. However, some minor species such as *L. campestris* (Jiménez-Martínez et al., 2003a) were reported to have similar amounts of protein (44.9 ± 2.0), lipid (13.1 ± 2.0), crude fibre (14.7 ± 1.1), ash (3.5 ± 0.1), and carbohydrate (24.7 ± 1.3 g/100 dw) as *L. mutabilis.*

Regarding the composition of whole debittered lupins, there is a paucity of published data, except for debittered *L. mutabilis* and *L. campestris*. The average composition of debittered *L. mutabilis* as presented in Table 1 is higher in crude protein and carbohydrates than in *L. campestris* debittered by a wet process (Jiménez-Martínez et al., 2003a), which contained crude protein 50 ± 0.5 g/100g dw, lipids 21.2 ± 0.5 g/100 dw, fibre 10.2 ± 0.2 g/100 dw, ash 3 ± 0.0 g/100g dw and carbohydrates 15.6 ± 0.2 g/100 dw. All data on the composition of whole debittered lupins were based on wet debittering processes, which obviously cause losses of soluble dry matter into the process water. This then can result in apparent increases of *e.g.* the crude protein content due to preferential leaching-out of dissolved carbohydrates and minerals. Regarding the crude lipid content in debittered lupins, there is a different situation. In *L. mutabilis* the crude lipid content decreases and this might be because of a sort of micelle formation with lecithin present in this lupin (FAO., 2012b; Rozan et al., 1997). However, fat content in debittered *L campestris* increases compared to the raw material. We did not find an explanation in literature for this difference.

Regarding the fatty acid composition, the aqueous debittering process apparently does not significantly affect the profile of fatty acids of *L. mutabilis* (Table 1). This is important because lupin species contain approximately 80 % of unsaturated fatty acids in the lipid fraction.

So far several reasons have been given for the wide variability in the macronutrient composition of raw and debittered lupins. However, that variability may also be influenced by the method of analysis. A wide array of methods for determining macronutrients was reported, such as gravimetry for estimating moisture, fibre, ash (Güémes-Vera et al., 2008); Kjeldahl (Ortiz and Mukherjee, 1982) and micro Kjeldahl (Gross et al., 1988; Güémes-Vera et al., 2008) for crude protein; solvent extractions (Güémes-Vera et al., 2008; Torres-Tello et al., 1980), and Nuclear Magnetic Resonance (NMR) (Gross et al., 1988) for lipids; and estimation of carbohydrates by difference (Güémes-Vera et al., 2008), whereas some authors even omitted to report the methodology used, which precludes an evaluation of exactitude. Moreover, some authors did not specify the variety of *Lupinus* analysed, or did not present the standard deviation of the mean values or the range of variation, making it impossible to determine the precision of the results.

Minerals

The number of authors who investigated the mineral composition in lupins is rather low. Whole raw lupins present a mineral composition that shows a wide variability (Table 1); especially in the reported manganese content for *L. albus* (83.5 mg/100g dw) when compared with other lupins (2.1-8.6 mg/100g dw). These variations could be partly explained by agronomical aspects. Field studies on the accumulation of mineral elements provide evidence of significant differences between species grown at on the same site and, within a species, when grown on different soil types (Gladstones and Drover, 1962; Walton and Francis, 1975). The amount of mineral elements absorbed by a crop will obviously depend on its productivity of dry-matter and on the availability of specific nutrients in the soil in which it is rooted (Pate et al., 1985). For example, a study conducted under greenhouse conditions showed that the lupin plant is sensitive to deficiencies of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, zinc, iron, and manganese (Rivadeneira et al., 2001). These deficiencies determine that the lupin plant can be dwarfed, weak, with necrosis, discoloration, and with lower content of minerals compared with a plant grown without mineral deficiencies (Rivadeneira et al., 2001).

In the case of whole debittered *L. mutabilis* it can be noted that, with the exception of calcium, iron and zinc, the other mineral contents are lower than in whole raw lupin. This reduction could be attributed to leaching. Remarkable is the decrease of potassium and

magnesium in the debittered product. Perhaps these two minerals were present in a highly soluble chemical form. Increases in calcium, iron and zinc contents could be due to their presence in the form of poorly soluble complexes with *e.g.*, phytic acid. Villacrés et al. (2000) also suggests that increases in iron and zinc contents may be caused by contact of the product with debris present in water used for the debittering process.

Variations in mineral content may also be due to the use of different analytical methods. For example, Peñalosa et al. (1991) determined calcium, potassium, magnesium, phosphorus using an Auto Analyzer. Torres-Tello et al. (1980) determined calcium by permanganometry, iron by orthophenanthroleine, magnesium by complexometry, and phosphorus by spectrophotometry. In addition, authors do not always mention analytical methods and variety of lupin analysed, nor does they always present standard deviations or ranges. Therefore, we did not attempt to explain all observed differences based on limited information; rather, we gathered the scarce information to obtain an impression of the mineral contents in lupin, and to evaluate which knowledge is lacking.

Amino acids

The essential amino acid profile of raw lupins shows little variation among species (Table 2). However, *L. luteus* has a higher cystine and leucine content, *L. albus* a higher tyrosine content, and *L. mutabilis* a higher lysine content.

The content of essential amino acids in debittered lupins was reported only once (Torres-Tello et al., 1980) for *L. mutabilis* (Table 2). In some cases the reported data do not differ greatly from those of raw lupin (namely for glutamic acid, glycine, isoleucine, leucine); in others the contents are higher (for lysine, phenylalanine, proline, serine, threonine), lower (for hystidine, tryptophan, tyrosine and valine), or much lower (for cystine, methionine). It is suggested that the cystine and methionine contents diminish both when the seed is defatted and when the alkaloid extraction is done in an alkaline environment, as Torres-Tello et al. (1980) did Gueguen and Cerletti (1994). This is in agreement with other authors Cerletti et al. (1978), Liener (1994), Maga (1984), Sgarbiere and Galeazzi (1978) who have also suggested that alkaline processing can alter protein quality due to the possibility of disruption of the protein structure and degradation of some amino acids.

Table 1. Composition of whole raw seeds of four lupin species and whole debittered *L. mutabilis*

Material	*L. albus*		*L. angustifolius*		*L. angustifolius*		*L. mutabilis*				Whole debittered *L. mutabilis*			
	$\bar{x}$ [1]	References	$\bar{x}$	References	$\bar{x}$	References	$\bar{x}$	min	max	References	$\bar{x}$	min	max	References
Macronutrients (100g^{-1} dw except for moisture)														
Moisture (g)	8.6	(Petterson, 1998)	9.0	(Petterson and Crosbie, 1990), (Petterson, 1998)	9.4	(Chango et al., 1993b), (Petterson, 1998)	8.1	6.2	9.9	(Petterson, 1998), (Güémes-Vera et al., 2008), (Villacrés et al., 2000), (Caicedo et al., 2001)	74.3	73.6	75.0	(Villacrés et al., 2000), (Caicedo et al., 2001)
Metabolic energy (KJ)	2078	(Petterson, 1998)	2032	(Petterson, 1998)	2164	(Petterson, 1998)	2307	n.a.[2]	n.a.	(Villacrés et al., 2000)	2441.0	n.a.	n.a.	(Villacrés et al., 2000), (Caicedo et al., 2001)
Crude protein (g)	38.2	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Petterson, 1998), (Pate et al., 1985)	33.9	(Haq, 1993), (Petterson and Crosbie, 1990), (Petterson, 1998), (Pate et al., 1985)	42.2	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Petterson, 1998), (Pate et al., 1985)	43.3	32.0	52.6	(Carvalho et al., 2005), (Güémes-Vera et al., 2008), (Gross et al., 1988), (Pate et al., 1985), (Aguilera and Trier, 1978), (Gueguen and Cerletti, 1994), (Haq, 1993), (Ortiz and Mukherjee, 1982), (Jiménez-Martínez et al., 2003a), (Caicedo et al., 2001), (Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)	57.5	51.1	72.0	(Villacrés et al., 2000), (Torres-Tello et al., 1980), (Caicedo et al., 2001), (Carvajal-Larenas et al., 2012)

Material	*L. albus*		*L. angustifolius*		*L. angustifolius*		*L. mutabilis*				Whole debittered *L. mutabilis*			
	$\bar{x}$ [1]	References	$\bar{x}$	References	$\bar{x}$	References	$\bar{x}$	min	max	References	$\bar{x}$	min	max	References
Crude lipids (g)	11.2	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Petterson, 1998), (Pate et al., 1985)	6.3	(Haq, 1993), (Petterson and Crosbie, 1990), (Petterson, 1998), (Pate et al., 1985)	5.5	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Petterson, 1998), (Pate et al., 1985)	18.9	13.0	24.6	(Jiménez-Martínez et al., 2003a), (Ortiz and Mukherjee, 1982), (Haq, 1993), (Aguilera and Trier, 1978), (Pate et al., 1985), (Güémes-Vera et al., 2008), (Carvalho et al., 2005), (Caicedo et al., 2001), (Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)	16.6	8.9	20.4	(Villacrés et al., 2000), (Caicedo et al., 2001), (Carvajal-Larenas et al., 2012)
Fibre (g)	8.9	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Petterson, 1998), (Pate et al., 1985)	16.0	(Haq, 1993), (Petterson and Crosbie, 1990), (Petterson, 1998), (Pate et al., 1985)	15.8	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Petterson, 1998), (Pate et al., 1985)	8.2	6.2	11.0	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Aguilera and Trier, 1978), (Pate et al., 1985), (Güémes-Vera et al., 2008), (Carvalho et al., 2005), (Caicedo et al., 2001), (Villacrés et al., 2000)	7.2	6.9	7.5	(Villacrés et al., 2000), (Torres-Tello et al., 1980), (Caicedo et al., 2001)
Ash (g)	3.4	(Jiménez-Martínez et al., 2003a), (Petterson, 1998)	3.0	(Petterson and Crosbie, 1990), (Petterson, 1998)	3.8	(Jiménez-Martínez et al., 2003a), (Petterson, 1998)	3.9	2.4	5.2	(Jiménez-Martínez et al., 2003a), (Aguilera and Trier, 1978), (Güémes-Vera et al., 2008), (Carvalho et al., 2005), (Caicedo et al.,	2.9	1.9	5.0	(Villacrés et al., 2000), (Torres-Tello et al., 1980), (Caicedo et al., 2001), (Carvajal-

Material	*L. albus*		*L. angustifolius*		*L. angustifolius*		*L. mutabilis*				Whole debittered *L. mutabilis*			
	$\bar{x}$ [1]	References	$\bar{x}$	References	$\bar{x}$	References	$\bar{x}$	min	max	References	$\bar{x}$	min	max	References
										2001), (Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)				Larenas et al., 2012)
Carbohydrates (g)	39.3	(Jiménez-Martínez et al., 2003a), (Pate et al., 1985)	41.0	(Petterson and Crosbie, 1990), (Pate et al., 1985)	38.8	(Jiménez-Martínez et al., 2003a), (Pate et al., 1985)	32.9	26.1	43.2	(Jiménez-Martínez et al., 2003a), (Aguilera and Trier, 1978), (Pate et al., 1985), (Güémes-Vera et al., 2008), (Villacrés et al., 2000), (Caicedo et al., 2001), (Carvajal-Larenas et al., 2012)	26.7	22.0	33.2	(Villacrés et al., 2000), (Torres-Tello et al., 1980), (Caicedo et al., 2001), (Carvajal-Larenas et al., 2012)
Fatty acids (g 100g^{-1} dw)														
C16:0 (Palmitic)	7.9	(Petterson, 1998), (Pate et al., 1985)	10.3	(Petterson, 1998), (Aguilera and Trier, 1978)	5.8	(Petterson, 1998), (Aguilera and Trier, 1978)	10.4	0.6	13.9	(Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988)	11.3	n.a.	n.a.	(Caicedo et al., 2001)
C16:1 (Palmitoleic)	n.a.		n.a.				13.9	13.9	13.9	(Petterson, 1998)	0.2	n.a.	n.a.	(Caicedo et al., 2001)
C16:2 (Hexadecadioneic acid)	n.a.		n.a.		n.a.		0.2	0.2	0.2	(Petterson, 1998)	n.a.	n.a.	n.a.	

Material	*L. albus*		*L. angustifolius*		*L. angustifolius*		*L. mutabilis*				Whole debittered *L. mutabilis*			
	$\bar{x}$ [1]	References	$\bar{x}$	References	$\bar{x}$	References	$\bar{x}$	min	max	References	$\bar{x}$	min	max	References
C18:0 (Stearic)	1.6	(Petterson, 1998), (Pate et al., 1985)	5.2	(Petterson, 1998), (Aguilera and Trier, 1978)	2.0	(Petterson, 1998), (Aguilera and Trier, 1978)	4.7	2.0	8.2	(Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988)	7.3	n.a.	n.a.	(Caicedo et al., 2001)
C18:1 (Oleic)	54.0	(Petterson, 1998), (Pate et al., 1985)	33.9	(Petterson, 1998), (Aguilera and Trier, 1978)	28.5	(Petterson, 1998), (Aguilera and Trier, 1978)	46.4	41.2	56.2	(Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988)	52.5	n.a.	n.a.	(Caicedo et al., 2001)
C18:2 (Linoleic)	18.7	(Petterson, 1998), (Pate et al., 1985)	40.3	(Petterson, 1998), (Aguilera and Trier, 1978)	48.2	(Petterson, 1998), (Aguilera and Trier, 1978)	33.1	26.5	39.6	(Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988)	28.4	n.a.	n.a.	(Caicedo et al., 2001)
C18:3 (Lino-lenic)	8.6	(Petterson, 1998), (Pate et al., 1985)	5.6	(Petterson, 1998), (Aguilera and Trier, 1978)	6.3	(Petterson, 1998), (Aguilera and Trier, 1978)	2.5	1.9	3	(Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988)	3.0	n.a.	n.a.	(Caicedo et al., 2001)
C20:0 (Ara-quidic)	1.0	(Petterson, 1998), (Pate et al., 1985)	0.7	(Petterson, 1998), (Aguilera and Trier, 1978)	2.2	(Petterson, 1998), (Aguilera and Trier, 1978)	0.9	0.9	0.9	(Aguilera and Trier, 1978)	n.a.	n.a.	n.a.	

Material	*L. albus*		*L. angustifolius*		*L. angustifolius*		*L. mutabilis*				Whole debittered *L. mutabilis*			
	$\bar{x}$ [1]	References	$\bar{x}$	References	$\bar{x}$	References	$\bar{x}$	min	max	References	$\bar{x}$	min	max	References
C22:0 (Behenic)	3.3	(Petterson, 1998)	1.5	(Petterson, 1998), (Aguilera and Trier, 1978)	6.6	(Petterson, 1998)	0.6	0.6	0.6	(Aguilera and Trier, 1978)	n.a.	n.a.	n.a.	
Minerals (mg 100 g^{-1} dw)														
Ca	200	(Petterson, 1998)	232	(Petterson, 1998), (Petterson and Crosbie, 1990)	210	(Petterson, 1998)	147	120	180	(Peñaloza et al., 1991), (Petterson, 1998), (Villacrés et al., 2000)	320.7	130	420	(Peñaloza et al., 1991), (Torres-Tello et al., 1980), (Villacrés et al., 2000)
Cu	0.5	(Petterson, 1998)	0.5	(Petterson, 1998), (Petterson and Crosbie, 1990)	0.9	(Petterson, 1998)	1.0	0.8	1.1	(Petterson, 1998), (Villacrés et al., 2000)	1.0	n.a.	n.a.	(Villacrés et al., 2000)
Fe	2.6	(Petterson, 1998)	6.1	(Petterson, 1998), (Petterson and Crosbie, 1990)	9.3	(Petterson, 1998)	5.9	5.0	7.3	(Petterson, 1998), (Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)	12.6	5.8	19.9	(Torres-Tello et al., 1980), (Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)
K	n.a.		n.a.		n.a.		1265	1130	1400	(Peñaloza et al., 1991), (Villacrés et al., 2000)	20.5	15	28.6	(Peñaloza et al., 1991), (Torres-Tello et al., 1980), (Villacrés et al., 2000)

Material	*L. albus*		*L. angustifolius*		*L. angustifolius*		*L. mutabilis*				Whole debittered *L. mutabilis*			
	$\bar{x}$ [1]	References	$\bar{x}$	References	$\bar{x}$	References	$\bar{x}$	min	max	References	$\bar{x}$	min	max	References
Mg	n.a.		n.a.		n.a.		285	240	330	(Peñaloza et al., 1991), (Villacrés et al., 2000)	109.7	69.1	170	(Peñaloza et al., 1991), (Torres-Tello et al., 1980), (Villacrés et al., 2000)
Mn	83.5	(Petterson, 1998)	2.1	(Petterson, 1998), (Petterson and Crosbie, 1990)	8.6	(Petterson, 1998)	3.2	2.6	3.7	(Petterson, 1998), (Villacrés et al., 2000)	2.6	n.a.	n.a.	(Villacrés et al., 2000)
Na	n.a.		n.a.		n.a.		n.a.	n.a.	n.a.		47.2	42.0	52.4	(Torres-Tello et al., 1980), (Villacrés et al., 2000)
P	360	(Petterson, 1998)	321	(Petterson, 1998), (Petterson and Crosbie, 1990)	610	(Petterson, 1998)	753	600	880	(Peñaloza et al., 1991), (Castillo, 1965), (Petterson, 1998), (Villacrés et al., 2000)	793.3	430	1450	(Peñaloza et al., 1991), (Torres-Tello et al., 1980), (Villacrés et al., 2000)
Zn	3.0	(Petterson, 1998)	3.6	(Petterson, 1998), (Petterson and Crosbie, 1990)	5.6	(Petterson, 1998)	3.5	3.4	3.6	(Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)	4.3	3.6	5.0	(Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)

[1] $\bar{x}$ = average
[2] n.a. = not available

Table 2. Amino acid composition of whole seeds of five lupin species

Species	**Raw *L. albus***		**Raw *L. angustifolius***		**Raw *L. luteus***		**Raw *L. mutabilis***				**Debittered *L. mutabilis***				FAO/WHO[2]		
	$\bar{x}$ [1]	**References**	$\bar{x}$	**References**	$\bar{x}$	**References**	$\bar{x}$	**min**	**max**	**References**	$\bar{x}$	**min**	**max**	$\bar{x}$	**(A)**	**(B)**	**(C)**
Amino acid (g 100 g^{-1} proteins)																	
Alanine	n.a. [3]		n.a.		n.a.		3.3	3.2	3.4	(Torres-Tello et al., 1980), (Gross et al., 1988)	4.1	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Arginine	12.4	(Petterson, 1998)	12.0	(Petterson, 1998)	9.1	(Petterson, 1998)	10.2	9.1	11.1	(Torres-Tello et al., 1980), (Petterson, 1998), (Gross et al., 1988)	11.6	n.a	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Aspartic acid	n.a.		n.a.		n.a.		9.6	8.7	10.3	(Torres-Tello et al., 1980), (Gross et al., 1988)	9.6	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Cystine	1.5	(Petterson, 1998), (Haq, 1993)	1.6	(Petterson, 1998), (Haq, 1993)	2.4	(Petterson, 1998), (Haq, 1993)	1.6	1.4	1.7	(Haq, 1993), (Petterson, 1998), (Pate et al., 1985) (Gross et al., 1988)	traces	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Glutamic acid	n.a.		n.a.		n.a.		24.3	22.8	26.3	(Torres-Tello et al., 1980), (Gross et al., 1988)	23.6	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Glycine	n.a.		n.a.		n.a.		3.8	3.7	3.9	(Torres-Tello et al., 1980), (Gross et al., 1988)	3.8	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Histidine	2.0	(Petterson, 1998), (Haq,	2.6	(Petterson, 1998), (Haq,	3.1	(Petterson, 1998), (Haq,	3.5	3.0	4.2	(Torres-Tello et al., 1980), (Petterson, 1998),	2.9	n.a.	n.a.	(Torres-Tello et al.,	n.a.	n.a.	n.a.

Species	**Raw *L. albus***		**Raw *L. angustifolius***		**Raw *L. luteus***		**Raw *L. mutabilis***				**Debittered *L. mutabilis***				FAO/WHO[2]		
	$\bar{x}$ [1]	**References**	$\bar{x}$	**References**	$\bar{x}$	**References**	$\bar{x}$	**min**	**max**	**References**	$\bar{x}$	**min**	**max**	$\bar{x}$	**(A)**	**(B)**	**(C)**
		1993)		1993)		1993)				(Gross et al., 1988)				1980)			
Isoleucine	4.1	(Petterson, 1998), (Haq, 1993)	4.0	(Petterson, 1998), (Haq, 1993)	3.6	(Petterson, 1998), (Haq, 1993)	4.2	3.6	4.8	(Torres-Tello et al., 1980), (Haq, 1993), (Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988), (Güémes-Vera et al., 2008)	4.0	n.a.	n.a.	(Torres-Tello et al., 1980)	4.6	2.8	1.3
Leucine	6.8	(Petterson, 1998), (Haq, 1993)	6.9	(Petterson, 1998), (Haq, 1993)	7.8	(Petterson, 1998), (Haq, 1993)	7.0	5.7	7.8	(Torres-Tello et al., 1980), (Haq, 1993), (Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988), (Güémes-Vera et al., 2008)	6.7	n.a.	n.a.	(Torres-Tello et al., 1980)	9.3	6.6	1.9
Lysine	4.5	(Petterson, 1998), (Haq, 1993)	4.6	(Petterson, 1998), (Haq, 1993)	4.5	(Petterson, 1998), (Haq, 1993)	5.8	5.0	7.3	(Torres-Tello et al., 1980), (Haq, 1993), 56(Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988), (Güémes-Vera et al., 2008)	7.8	n.a.	n.a.	(Torres-Tello et al., 1980)	6.6	5.8	1.6
Methionine	0.7	(Petterson, 1998), (Haq,	0.7	(Petterson, 1998), (Haq,	0.6	(Petterson, 1998), (Haq,	0.8	0.4	1.4	(Haq, 1993), (Aguilera and Trier, 1978), (Petterson, 1998), (Pate	traces	n.a.	n.a.	(Torres-Tello et al.,	n.a.	n.a.	n.a.

Species	**Raw *L. albus***		**Raw *L. angustifolius***		**Raw *L. luteus***		**Raw *L. mutabilis***				**Debittered *L. mutabilis***				FAO/WHO[2]		
	$\bar{x}$ [1]	**References**	$\bar{x}$	**References**	$\bar{x}$	**References**	$\bar{x}$	**min**	**max**	**References**	$\bar{x}$	**min**	**max**	$\bar{x}$	**(A)**	**(B)**	**(C)**
		1993)		1993)		1993)				et al., 1985), (Gross et al., 1988)				1980)			
Methionine +Cystine	2.2	(Petterson, 1998), (Haq, 1993)	2.3	(Petterson, 1998), (Haq, 1993)	3.0	(Petterson, 1998), (Haq, 1993)	2.4	1.8	3.1	(Haq, 1993), (Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988)	traces	n.a.	n.a.	(Torres-Tello et al., 1980)	4.2	2.5	1.7
Phenylalanine	3.4	(Petterson, 1998), (Haq, 1993)	3.7	(Petterson, 1998), (Haq, 1993)	3.7	(Petterson, 1998), (Haq, 1993)	3.5	3.0	4.0	(Torres-Tello et al., 1980), (Aguilera and Trier, 1978), (Pate et al., 1985), (Gross et al., 1988), (Güémes-Vera et al., 2008)	3.8	n.a.	n.a.	(Torres-Tello et al., 1980)	7.2	6.3	1.9
Proline	n.a.		n.a.		n.a.		3.8	3.7	4.0	(Torres-Tello et al., 1980), (Gross et al., 1988)	4.2	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Serine	n.a.		n.a.		n.a.		4.9	4.2	5.3	(Torres-Tello et al., 1980), (Gross et al., 1988)	5.5	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Threonine	3.4	(Petterson, 1998), (Haq, 1993)	3.4	(Petterson, 1998), (Haq, 1993)	3.0	(Petterson, 1998), (Haq, 1993)	3.5	3.0	4.0	(Torres-Tello et al., 1980), (Haq, 1993), (Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988), (Güémes-Vera et al., 2008)	4.1	n.a.	n.a.	(Torres-Tello et al., 1980)	4.3	3.4	0.9

Species	**Raw *L. albus***		**Raw *L. angustifolius***		**Raw *L. luteus***		**Raw *L. mutabilis***				**Debittered *L. mutabilis***				FAO/WHO[2]		
	$\bar{x}$ [1]	**References**	$\bar{x}$	**References**	$\bar{x}$	**References**	$\bar{x}$	**min**	**max**	**References**	$\bar{x}$	**min**	**max**	$\bar{x}$	**(A)**	**(B)**	**(C)**
Tryptophan	0.9	(Petterson, 1998), (Haq, 1993)	0.9	(Petterson, 1998), (Haq, 1993)	0.9	(Petterson, 1998), (Haq, 1993)	0.8	0.5	1.0	(Torres-Tello et al., 1980), (Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988)	0.6	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	n.a.	n.a.
Tyrosine	4.8	(Petterson, 1998), (Haq, 1993)	3.4	(Petterson, 1998), (Haq, 1993)	2.9	(Petterson, 1998), (Haq, 1993)	4.0	3.4	5.1	(Torres-Tello et al., 1980), (Aguilera and Trier, 1978), (Petterson, 1998), (Gross et al., 1988), (Güémes-Vera et al., 2008)	3.4	n.a.	n.a.	(Torres-Tello et al., 1980)	n.a.	0.6	n.a.
Valine	3.8	(Petterson, 1998), (Haq, 1993)	3.7	(Petterson, 1998), (Haq, 1993)	3.4	(Petterson, 1998), (Haq, 1993)	3.8	3.2	4.9	(Torres-Tello et al., 1980), (Haq, 1993), (Aguilera and Trier, 1978), (Petterson, 1998), (Pate et al., 1985), (Gross et al., 1988), (Güémes-Vera et al., 2008)	3.4	n.a.	n.a.	(Torres-Tello et al., 1980)	3.5	3.5	1.3

[1] $\bar{x}$ = average
[2] Recommendation from ref. (Múzquiz et al., 1994): (**A**), children <2 years; (**B**), children 2-5 years; (**C**) adults.
[3] n.a. = not available

Vitamins

Little information is available on the vitamin content in lupin species. Only one study (Castillo, 1965) reported on vitamins in a mixture of raw *L. tricolor* 'Sodiro' and *L. mutabilis* 'Sweet' (Table 3). Vitamins in debittered seeds were published by Castillo (1965), Torres-Tello (1980). The carotene content was reported by Castillo (1965) for the debittered mix as 0.6 mg/100g dw. It is worth noting that the amount of carotene in the whole debittered mix was six times higher than the value reported by the same author in the raw material (0.1 mg/100g dw). We did not find an explanation for this difference in the bibliography. Perhaps it is because the author did not analyse the same samples before and after debittering. The author appeared to have taken random samples of raw and debittered lupin from markets. Another explanation is that the extraction of caretene might be easier in boiled than in raw raw lupin. The reported thiamine content varied from 0.01 mg/100g dw to 0.6 mg/100g dw. The riboflavin content varied from 0.02 mg/100g dw to 0.5 mg/100g dw. The niacin content varied greatly from 0.0 to 4.1 mg/100g dw. The lowest values were reported by Castillo (1965), while the highest were reported by Torres-Tello (1980). Variation in vitamin content in whole debittered lupin could be related to the fact that Torres-Tello (1980) analysed *L. mutabili*s 'Sweet' and Castillo (1965) analysed a mix of *L. mutabilis* 'Sweet' and *L. tricolor* 'Sodiro'. In addition, variations might be caused by the debittering process applied. Torres-Tello (1980) boiled *L. mutabilis* for three times 5 min at 100 ºC, soaked the seeds in alkaline water, and then washed them in running water for 8 to 12 h. Castillo (1965) reported that mixed samples were cooked for 12 h and washed in running water for 7 to 8 days. Castillo (1965) analysed mixed samples that stayed roughly 11 h longer in boiling water and 6 days more in contact with running water, which might explain losses by leaching.

Table 3. Vitamins in lupin seeds

Vitamins (mg/100g)	**Mix of raw *L. tricolor* + *L. mutabilis***	**References**	**Mix of debittered *L. tricolor* + *L. mutabilis***	**References**	**Debittered *L. mutabilis***	**References**
Carotene	0.1	(Castillo, 1965)	0.6	(Castillo, 1965)	not reported	
Thiamine	0.6	(Castillo, 1965)	0.01	(Castillo, 1965)	0.6	(Torres-Tello et al., 1980)
Riboflavin	0.5	(Castillo, 1965)	0.02	(Castillo, 1965)	0.5	(Torres-Tello et al., 1980)
Niacin	4.1	(Castillo, 1965)	0.0	(Castillo, 1965)	3.1	(Torres-Tello et al., 1980)

Alkaloids

Diversity and their occurrence

Almost 70 different quinolizidine alkaloids (QA) have been reported to occur in *Lupinus* species (Australia New Zealand Food Authority, 2001), of which about 28 are free bases (soluble in organic solvents), and the remaining alkaloids are salts (Ortiz and Mukherjee, 1982). The most common alkaloids are shown in Table 4. QA are bitter and toxic compounds, present in the seed endosperm but not in the hull, that can be structurally very similar to sweet-tasting molecules. It is agreed that 25 human G protein-coupled receptors mediate bitter taste perception. However, it is also possible to find alternative mechanisms that mediate bitter taste. For example, lipophylic bitter compounds and bitter salts may activate intracellular signals (Rodgers et al., 2005).

In lupins the reported total alkaloid content varies considerably between authors, and species (Table 4). On average the lower total alkaloid content is reported for *L. albus* (0.186 g/100g dw) and the higher for *L. mutabilis* with 2.8 g/100g dw. However, also in *L. mutabilis*, low total alkaloid contents have been reported. For example, Haq (1993) reported 0.007 g/100g dw in *L. mutabilis* (unspecified variety), and Gross (1988) reported 0.08 g/100g dw in *L. mutabilis* 'Inti' and 0.018 g/100g dw in *L. mutabilis* '2150'.

Regarding the diversity of alkaloids in the major lupin species, the principal alkaloid reported is lupanine ($C_{15}H_{24}N_2O$) (Table 4). Next, 13-hydroxylupanine ($C_{15}H_{24}N_2O_2$) is reported in *L. albus*, *L. angustifolius* and *L. mutabilis.* Sparteine ($C_{15}H_{26}N_2$) is an important component of *L. luteus* and *L. mutabilis.* In *L. mutabilis* 4-hydroxylupanine, D-lupanine, sparteine, 3-hydroxylupanine, and minor components such as anagyrine, 11-12 dehydroasparteine, dehydrolupanine, and 17-oxolupanine, were also reported. The chemical structures of the most frequently occurring alkaloids are presented in Figure 1.

The total alkaloid content was determined by titrimetry (INEN Instituto Ecuatoriano de Normalización, 2005), gas chromatography (GC) (Nossack et al., 2000), high performance liquid chromatography (HPLC) (Jiménez-Martínez et al., 2003a) and capillary gas liquid chromatography (Gross et al., 1988), whereas the identity of alkaloids has been elucidated by gas-liquid chromatography (GLC), capillary GLC-mass spectrometry (Hatzold et al., 1983), gas chromatography and mass spectrometry (Jiménez-Martínez et al., 2007), and gas chromatography and thin layer chromatography (Múzquiz et al., 1989).

Table 4. Alkaloids in *Lupinus* spp.

	Lupinus albus	*Lupinus angustifolius*	*Lupinus luteus*	*Lupinus mutabilis*			
Alkaloids				**Average**	**Min**	**Max**	**References**
Content (g/100g seed) dw	0.186 (0.005-0.367) [1]	0.7545 (0.095-1.4) [1]	0.985 (0.47-1.5) [1]	2.8	0.007	4.5	(Jiménez-Martínez et al., 2003a), (Haq, 1993), (Beirao da Costa, 1989), (Gueguen and Cerletti, 1994), (Hatzold et al., 1983), (Nossack et al., 2000), (Gross et al., 1988), (Villacrés et al., 2000), (Carvajal-Larenas et al., 2012)
Composition (%)							
Lupanine	70[2]	70[2]	60[2]	64.4	46.0	84.5	(Petterson, 1998), (Hatzold et al., 1983), (Jiménez-Martínez et al., 2007), (Múzquiz et al., 1989)
D-Lupanine	n.a.[3]	n.a.	n.a.	13.0	n.a.	n.a.	(Jiménez-Martínez et al., 2007)
Sparteine	n.a.	n.a.	30[2]	12.6	6.6	19.1	(Petterson, 1998), (Hatzold et al., 1983), (Jiménez-Martínez et al., 2007), (Múzquiz et al., 1989)
Albine	15[2]	n.a.	n.a.	n.a.	n.a.	n.a.	
3-Hydroxylupanine	n.a.	n.a.	n.a.	12	n.a.	n.a.	(Petterson, 1998)
13-Hydroxylupanine	8[2]	12[2]	n.a.	9.5	1.6	14.9	(Petterson, 1998), (Hatzold et al., 1983), (Jiménez-Martínez et al., 2007)
4-Hydroxylupanine	n.a.	n.a.	n.a.	4.9	1.1	8.7	(Hatzold et al., 1983), (Jiménez-Martínez et al., 2007)
Tetrahydrorhombifoline	n.a.	n.a.	n.a.	2.8	2.0	3.5	(Petterson, 1998), (Hatzold et al., 1983)
Angustifoline/ oxoasparteine	n.a.	10[2]	n.a.	2.3	0.6	5.4	(Petterson, 1998), (Hatzold et al., 1983), (Múzquiz et al., 1989)
4,13-dihydroxylupanine	n.a.	n.a.	n.a.	2.1	n.a.	n.a.	(Hatzold et al., 1983)

	Lupinus albus	*Lupinus angustifolius*	*Lupinus luteus*	*Lupinus mutabilis*			
Alkaloids				**Average**	**Min**	**Max**	**References**
13-(angeloyloxi) lupanine	n.a.	n.a.	n.a.	1.8	1.6	2.0	(Petterson, 1998), (Hatzold et al., 1983)
cis-13-(cinnamoyloxy)lupanine	n.a.	n.a.	n.a.	1.2	n.a.	n.a.	(Hatzold et al., 1983)
Ammodendrine	n.a.	n.a.	n.a.	1.1	0.2	2.0	(Petterson, 1998), (Hatzold et al., 1983)
Multiflorine	3[2]	n.a.	n.a.	1.0	0.1	1.8	(Hatzold et al., 1983), (Jiménez-Martínez et al., 2007)
Oxo-esparteine	n.a.	n.a.	n.a.	0.5	n.a.	n.a.	(Jiménez-Martínez et al., 2007)
trans-13-(cinnamoyloxy) lupanine	n.a.	n.a.	n.a.	0.4	n.a.	n.a.	(Hatzold et al., 1983)
13-(tigloyloxy)lupanine	n.a.	n.a.	n.a.	0.3	n.a.	n.a.	(Hatzold et al., 1983)
α-isolupanine	n.a.	n.a.	n.a.	0.3	n.a.	n.a.	(Hatzold et al., 1983)
4-(angeloyloxy)lupanine	n.a.	n.a.	n.a.	0.3	n.a.	n.a.	(Hatzold et al., 1983)
13-(benzoyloxy)lupanine	n.a.	n.a.	n.a.	0.2	n.a.	n.a.	(Hatzold et al., 1983)
13-(angelolyloxy)-4-hydroxylupanine	n.a.	n.a.	n.a.	0.2	n.a.	n.a.	(Hatzold et al., 1983)
17-oxolupanine	n.a.	n.a.	n.a.	0.1	n.a.	n.a.	(Hatzold et al., 1983)
dehydrolupanine	n.a.	n.a.	n.a.	0.1	n.a.	n.a.	(Hatzold et al., 1983)
11-12-dehydroasparteine	n.a.	n.a.	n.a.	0.1	n.a.	n.a.	(Hatzold et al., 1983)
Anagyrine	n.a.	n.a.	n.a.	0.03	n.a.	n.a.	(Hatzold et al., 1983)

[1] (Haq, 1993), [2] (Petterson, 1998), [3] n.a. = not available

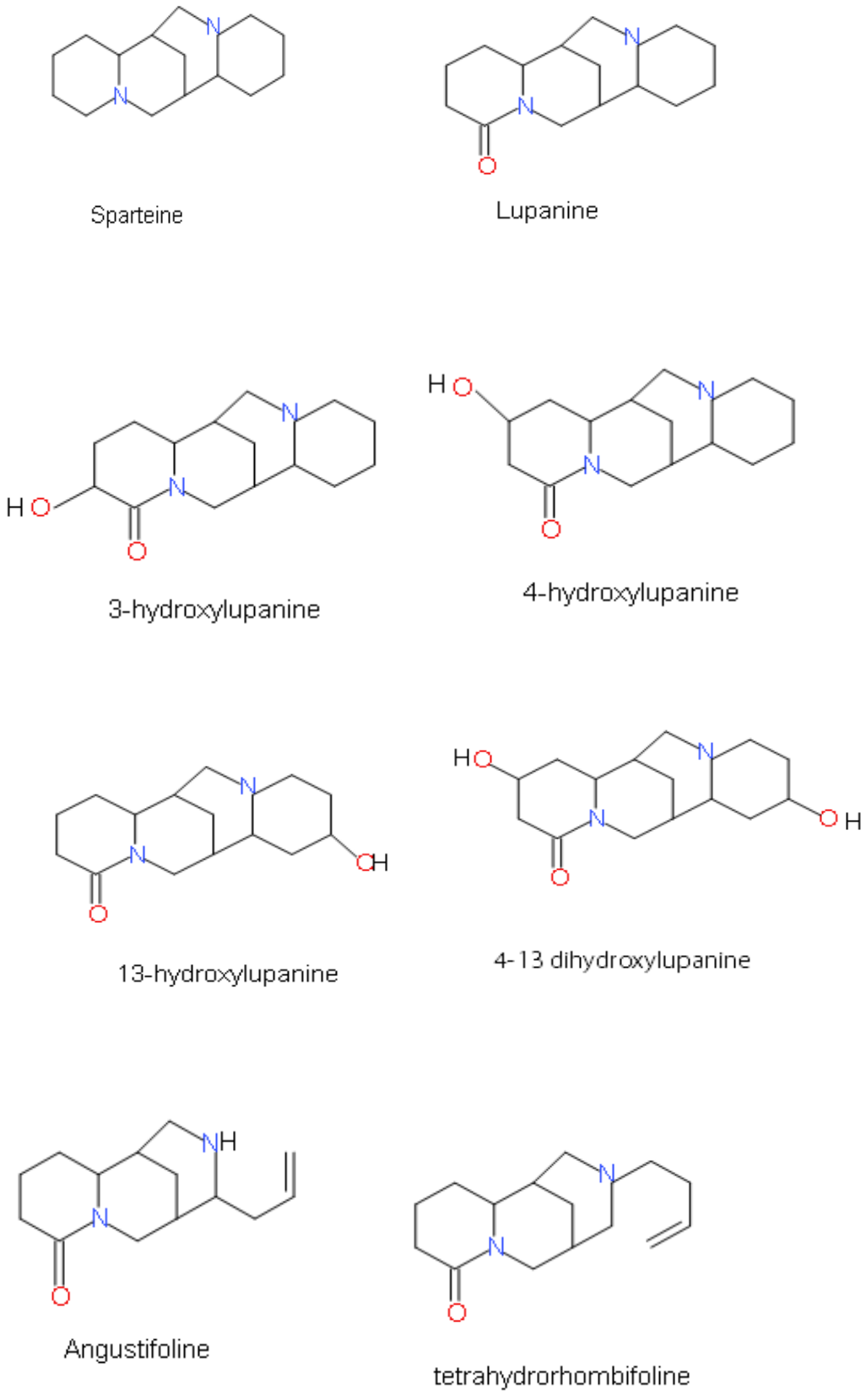

Figure 1. Structure of *Lupinus* spp. alkaloids

Apart from differences due to the analytical techniques used, variation in the reported alkaloid contents and their diversity can be explained, by (i) the fact that the studied species and variety were not always the same; (ii) the presence of a wide genetic variability illustrating adaptation to microhabitats and natural selection such as reported for *L. mutabilis* (Haq, 1993); (iii) environmental and agronomical conditions: favourable moisture conditions would reduce alkaloid content, whereas maritime conditions could be associated with higher levels (Bélteky and Kovács, 1984), the amount of nitrogen as well as the intensity of sunlight and temperature might affect the amount of alkaloids directly (Jambrina-Alonso, 1983; Wink and Witte, 1984) even in maturing seeds (Wink and Witte, 1984), and in the shade, the alkaloid content would increase (Bélteky and Kovács, 1984); (iv) the "turnover" effect can affect alkaloid contents within the same plant, depending on the weather, day and hour of the day (Wink and Witte, 1984). The turnover effect is manifested when alkaloids produced in leaves (from carbon and nitrogen) are then transported in the phloem like amino acids and rapidly degraded in the target tissues, which probably use the nitrogen and carbon for the synthesis of storage protein (Wink and Witte, 1984). Diurnal variation of QA formation, transport, and turnover was studied in fruiting lupins. In phloem sap of seeds of *L. albus*, alkaloid contents changed from about 4 mg/g at 17h00 to about 3 at 11h00 and 21h00 (Wink and Witte, 1984), showing that apparently QA are not waste or end products, but that they are metabolically dynamic compounds.

Toxicity in humans

Table 5 summarizes published data on toxic effects of alkaloids in man. Most of the information comes from reports of accidents. Alkaloids are associated with liver diseases and neuromuscular blockage (Camacho et al., 1991) caused by inhibiting the ganglionic transmission impulse of the sympathic nervous system (Jiménez-Martínez et al., 2003a). Intoxications with alkaloids can be acute or chronic. Regarding acute intoxication with orally ingested sparteine, one report mentioned that it was mortal in a dose >30 mg kg^{-1} body weight (bw), whereas mixed alkaloids ingested by 5 people in a dose between 11 mg kg^{-1} bw and 46 mg kg^{-1} bw were lethal for 3, and caused a serious intoxication to 2 (Australia New Zealand Food Authority, 2001). Cremer (1983) reported that alkaloid doses between 10 and 25, and 25 to 45 mg kg^{-1} bw were toxic for small children, and adults, respectively. Aguilera and Trier (1978) mention similar toxic levels; however, in this report the intake by adults was

reported as non-fatal poisoning, and the intake by children was reported as fatal. In another study, a single dose of 10 mg of lupanine or 13-hydroxylupanine was administered orally to 11 volunteers. In all subjects, more than 90 % of both alkaloids was excreted unchanged via the urine with a half-life of 6-7 h (Australia New Zealand Food Authority, 2001). These results suggest that the minimum lethal acute dose is 10 mg total alkaloids kg^{-1} bw for infants and children, and 25 mg total alkaloids kg^{-1} bw for adults, respectively.

Table 5. Studies of alkaloid toxicity on humans

Material	Type of exposure	Evaluation way	Dose unit	Aver age	Min	Max	References
Sparteine	Oral-acute	Mortal doses	mg kg^{-1} body weight	>30	n.a.[1]	n.a.	(Australia New Zealand Food Authority, 2001)
Mixed alkaloids	Oral-acute	5 people, 3 lethal, 2serious intoxication	mg kg^{-1} bw	28.5	11	46	(Australia New Zealand Food Authority, 2001)
Total alkaloids	Not specified, oral presumably	Toxic for small children	mg kg^{-1} bw	17.5	10	25	(Cremer, 1983)
Total alkaloids	Not specified, oral presumably	Toxic for adult	mg kg^{-1} bw	35	25	45	(Cremer, 1983)
Total alkaloids	Not specified, oral presumably	Non-fatal in adults	mg kg^{-1} bw	36	25	46	(Aguilera and Trier, 1978)
Total alkaloids	Not specified, oral presumably	Fatal cases in infants and children	mg kg^{-1} bw	18	11	25	(Aguilera and Trier, 1978)
Lupanine or 13-hydroxylupanine	Oral-acute (capsule) administered to 11 volunteers	No reported complications	mg per ingestion	10	n.a.	n.a.	(Australia New Zealand Food Authority, 2001)
Total alkaloids	Oral (diet based in *Lupinus albus* flour 0.02 % alkaloids) on 20 military cadets, 4 weeks	Blood analysis, no complications . Good digestibility	mg per day	12	n.a.	n.a.	(Aguilera and Trier, 1978)
Total alkaloids	Not specified, oral presumably	Safe doses	mg per day	500	n.a.	n.a.	(Aguilera and Trier, 1978)

[1] n.a. = not available

On the other hand, when chronic toxicity of lupins is studied in human beings, it should be considered that the use of debittered lupins in Europe and South America over thousands of years would provide indicative evidence of safety (Cremer, 1983; Petterson, 1998). In fact, nowadays *Lupinus* spp. are still consumed in the Andean region (Cremer, 1983) and around the world FAO (2012b). Information about studies of chronic toxicity of lupin alkaloids in human beings is very scarce. Once a tolerance test was carried out with 20 military cadets in Perú. They received an average daily ration of 60 g of *L. albus* flour containing <0.02 % alkaloids (equivalent with a daily dose of 12 mg alkaloids). That dose was served as 49 different dishes, which were administered during 4 weeks. Results showed good digestibility of lupin dishes and no significant changes in main blood indicators (Aguilera and Trier, 1978).

The maximum allowed total alkaloid content in debittered lupin seed was established as 700 mg kg^{-1} seed by the Ecuadorean Institute of Standards (INEN Instituto Ecuatoriano de Normalización, 2005). This is higher than the level of 400 or 500 mg kg^{-1} seed proposed by Muzquiz et al. (1994) for food and feed use. Jiménez-Martínez et al. (2003a) suggested even a lower maximum level of 300 mg kg^{-1} in feed, as higher levels would result ´in a decrease in nutrient ingestion and consequently a decrease in animal growth´. In Europe, a daily dose of 0.35 mg kg body $weight^{-1}$ was reported to be tolerated in adults without adverse effects (Australia New Zealand Food Authority, 2001); however, this value was not considered safe for all individuals in the population. Therefore, a factor of 10 was applied to account for the uncertainties in the data and human variations. As a result the provisional tolerable daily intake for humans was suggested as 0.035 mg kg body $weight^{-1}$ body weight day^{-1} (Australia New Zealand Food Authority, 2001), which is very different from the 500 mg day^{-1} proposed as a safe dose by Aguilera and Trier (1978). This huge difference has important implications for the amount of lupin that somebody would be allowed to eat. For example, considering 0.035 mg kg^{-1} day^{-1} as the maximum tolerated daily intake as proposed by the Australia New Zealand Food Authority (2001) and the maximum alkaloid content in debittered lupin seeds according to the Ecuadorean Institute of Standards INEN (2005) (0.07%), an adult weighing 70 kg would be allowed to eat a maximum of 3.5 g of (debittered) lupin per day. However, in the Andean region the portion size of debittered *L. mutabilis* is often much bigger than this amount, *i.e.* 5-10 times more (personal observation). On the other hand, considering 500 mg day^{-1} as the safe dose as proposed by Aguilera and Trier (1978) and again the maximum alkaloid content according to the Ecuadorean Institute of Standards INEN (2005) for

debittered lupin seeds (700 mg kg^{-1}seed), a 70-kg adult could safely consume 714 g of debittered lupin per day. This shows the uncertainty about a safe daily amount of debittered lupin for human beings, and at the same time points out the necessity to determine this more accurately, especially where lupin is consumed not as just ingredient but also as snack or main dish.

Another toxicity risk associated with lupin consumption is the sometimes lethal effect of phomopsins, mycotoxins that can be formed by the fungus *Diaporthe toxica*, which occasionally infests lupins. The infested seeds are smaller, discoloured, and less dense than non infested seeds. The phomopsins are concentrated initially in the seed coats, and are not found in the cotyledons until there has been a heavy fungal invasion (Petterson, 1998). Clinical effects of phomopsins are functional failure of liver and fatty infiltrations. According to Petterson (1998), the National Food Authority in Australia and the Department of Health in the United Kingdom mention the value of 5 µg phomopsin kg^{-1} seed as the maximum amount allowed for human consumption. Since discoloured seeds are easily recognized and removed, both by manual grading and machine colour-sorting, the only possible risk of phomopsin ingestion would seem to come from the consumption of very lightly discoloured seed coats or from lupin flour made with infested seeds (Petterson, 1998). A moisture content of lupin seed below 10 % (Table 1) does not favour fungal activity (Petterson, 1998); however, the presence of phomopsin is a risk that needs to be considered at all times.

Allergenicity and anti-nutritional factors

A minority of people are lupin sensitive (Petterson, 1998). In a skin-shot test on 200 Chilean children using extracts from lupins and other foods, it was shown that sensitivity for lupin (3 %) was similar to sensitivity for eggs (3 %), wheat (2 %), but much less than for cow's milk (8 %) or soya beans (22 %) (Petterson, 1998). According to the European Food Safety Authority (2005), there were just a few reported cases of allergic reactions to lupin-containing products reported during the period 1994-2005. The main people at risk were those who are allergic to peanuts (0.7-1.5% of the European population). The major allergen identified was a β-conglutin protein of 43-45 kDa. However, other possible allergens of lupin have also been identified, namely the conglutin gamma (2S albumin) and 11S globulin.

Anti-nutritional factors such as phytic acid, saponins and tannins are also present in lupins (Table 6). The amount of phytate in lupins is too low to be of concern (Petterson, 1998). The

amount of phytic acid reported for lupins varies from 1.42 to 2.74 g/100g dw (Múzquiz et al., 1989). The small amounts of sapogenins in seeds of lupins are also considered very low and of little concern (Pate et al., 1985). The saponin content in lupins of up 1.7 g/100g dw was reported by Múzquiz et al. (1989) as similar or lower than in soya bean. Concerning the toxicity of tannins, a possible relationship between the presence of condensed tannins and oesophageal cancer was suggested (Jiménez-Martínez et al., 2003a). Although no-effect levels for tannins on growth have not yet been established, Jiménez Martínez et al. (2003a) reported that a 0.1 % of concentration of tannic acid (a hydrolysable form) in diets given to chickens did not cause any harmful effect. The tannic acid content in *L. mutabilis* was reported as 58 mg/100g by Jiménez Martínez et al. (2007). In lupins small amounts of cyanogenic compounds, hemagglutinins and trypsin inhibitor activity were detected but considered not to be of anti-nutritional significance (Pate et al., 1985). Indeed, "several authors have reported the absence of hemagglutin activity in the test based on red blood cells of sheep; chickens, rabbits and humans type O" (Múzquiz et al., 1989). In *L. mutabilis* the trypsin activity was reported as 1.16 trypsin inhibitor units (T.I.U.), which is considerably lower than in soya bean (30.1 T.I.U.) (Haq, 1993). Absence of vicine and convicine, based on a quantitative ultraviolet spectrophotometry test (the vicine and/or convicine detection limit of the test was 0.3 g kg^{-1}) (Olsen and Andersen, 1978), was reported for the main lupin species (Múzquiz et al., 1989).

Oligosaccharides may be considered anti-nutritional factors when occurring in large quantities, because they cannot be metabolized by monogastric animals and pass through to the colon, where bacterial digestion may produce carbon dioxide, methane, and hydrogen. The final result is discomfort and flatulence (Petterson, 1998), and the enhanced bowel movement may reduce nutrient uptake. The oligosaccharides in lupin species belong to the raffinose family (Petterson, 1998). The oligosaccharides found in lupins are stachyose, and raffinose (Múzquiz et al., 1989) (Table 6). Other reported oligosaccharides in *L. mutabilis* are verbascose, ranging from 0.8 g/100g dw (Gross et al., 1988) to 4.5 g/100g dw (Harpal and Gladstones, 1986), and ajugose 0.2 g/100g dw (Andersen et al., 2005). According to Jiménez Martínez et al. (2007), oligosaccharides in the seed can be reduced by up to 93% of their original content by soaking, cooking and fermentation processes. On the other hand, oligosaccharides are also reported to have health benefits because of their role as osmotic regulators in the gastrointestinal tract (Petterson, 1998). It will be of interest to investigate which oligosaccharides cause such beneficial effect and at what levels and conditions.

Table 6. Allergenicity and anti-nutritional factors

Compound	*L. albus*	References	*L. angusti-folius*	References	*L. luteus*	References	*L. muta-bilis*	References	Soya bean *Glycine max*	References
Phytic acid (g/100g)	1.42 ±0.06	(Múzquiz et al., 1989)	1.45 ±0.4	(Múzquiz et al., 1989)	2.72 ±0.9	(Múzquiz et al., 1989)	2.74 ±0.4	(Múzquiz et al., 1989)	1.23 ±2.3	(Múzquiz et al., 1989)
Saponins (g/100g)	0.9 ±0.02	(Múzquiz et al., 1989)	0.9 ±0.04	(Múzquiz et al., 1989)	1.22 ±0.05	(Múzquiz et al., 1989)	1.7 ±0.08	(Múzquiz et al., 1989)	1.7 ±0.01	(Múzquiz et al., 1989)
Condensed tannins (g/100 g)	0.01	(Petterson and Mackintosh, 1994)	0.01	(Petterson and Mackintosh, 1994)	0.02	(Zdunczyk et al., 1994)	0.06	(Jiménez-Martínez et al., 2007)	n.a.	
Trypsin inhibitors (g/100g)	0.01	(Petterson and Mackintosh, 1994)	0.01	(Petterson and Mackintosh, 1994)	0.03	(Zdunczyk et al., 1994)	n.a.		n.a.	
Stachyose (g/100g)	0.8 ±0.2	(Múzquiz et al., 1989)	0.3 ±0.0	(Múzquiz et al., 1989)	1.0 ±0.2	(Múzquiz et al., 1989)	1.3 ±0.3	(Múzquiz et al., 1989)	3.7 ±0.7	(Múzquiz et al., 1989)
Raffinose (g/100g)	0.8 ±0.2	(Múzquiz et al., 1989)	0.6 ±0.0	(Múzquiz et al., 1989)	1.0 ±0.2	(Múzquiz et al., 1989)	0.9 ±0.2	(Múzquiz et al., 1989)	1.0 ±0.2	(Múzquiz et al., 1989)

n.a. = not available

Debittering processes

Biological processes

Biological methods to debitter lupin are mainly based on fungal or bacterial fermentation as summarized in Table 7. A study on bacterial fermentation performed on *L. albus* 'Multolupa' (Camacho et al., 1991) investigated the effects of *Lactobacillus acidophilus, L. buchneri, L. cellobiosus*, and *L. fermentum*, which revealed that the alkaloid content could be reduced to 41.1 % of the initial value. This reduction was obtained at pH ≤ 4.5 with the strain *Lactobacillus acidophilus* B-1910. An additional reported benefit of *L. acidophilus* B-1910 was the reduction of the oligosaccharide content. Moreover, the riboflavin content was increased. Unfortunately, no control experiment was included in this study to assess the loss

of alkaloids by leaching (diffusion) only. Santana and Empis (2001) reported the reduction of the alkaloid content in *L. albus* flour by bacterial fermentation with two unnamed strains (IST20B and IST40D) (not identified but genetically closely related to *Acidovorax*) (Santana et al., 1996). Those strains were isolated from soil that had recently been used to produce *L. luteus* seed. The maximum QA reduction (50 %) was obtained by using 5 g lupin flour in 20 mL of a suspension (IST20B) at pH 7 with a bacteria concentration of ≈ 0.85 g of dry biomass per litre, incubated during 120 h at 31.2 ºC. *Lactobacillus plantarum* species were also reported to reduce the alkaloid content in *L. albus* (Szakács and Stankovics, 1983). In this study, soaked and dehulled seeds were put in contact with different lactic acid bacteria. The best results were reported at 37 ºC. The alkaloid content was reduced from 1.1 % initially to 0.1 % after soaking, dehulling, fermenting, and washing the seeds. In this study the effect of the separate steps on alkaloid reduction was not presented. These results show that the use of different bacteria can reduce the alkaloid content by about 50 % when suspensions or slurries of lupin are made. Apparently such acid cultures with a high alpha-galactosidase activity cause significant reductions in the amount of alkaloids (Camacho et al., 1991).

Fungal fermentation (Table 7) is mostly carried out as a solid-state fermentation to produce tempeh or other mycelium-penetrated masses by dehulling, soaking, cooking, inoculating with fungal spores, and incubation of the legume seeds. These operations allow proliferation of, *e.g*., *Rhizopus* mycelium on and throughout the seed (Jiménez-Martínez et al., 2007). It is stated that solid-state fermentation can result in small increases in crude protein, riboflavine and niacin contents. In addition, the process would decrease oligosaccharides and the QA content (Jiménez-Martínez et al., 2007). Peñaloza et al. (1991) produced tempeh on *L. mutabilis* Sweet inoculated with *Rhizopus oligosporus* UCW-FF8001 at about 3 x 10^5 c.f.u. g^{-1} of cooked beans. The appearance of the tempeh cake was reported as very good and comparable to that of tempeh from soya bean. Unfortunately, the rate of alkaloid reduction was not stated in the data. Jiménez-Martínez (2007) also made tempeh with *L. mutabilis.* In this study, 50 g of washed lupin seeds were soaked for 10-18 h in a watery solution of lactic acid (1g L^{-1}), washed again, dehulled, and autoclaved at 121 ºC and a pressure of 1 kg cm^{-2} during 5 minutes in a fresh solution of lactic acid of an equal concentration. Next, the seeds were washed and inoculated with *R. oligosporus* NRRL-2710 and incubated at 30 ºC for 48 h. After soaking and cooking the alkaloid content was reduced to 65 % of the initial value, and after fermentation to 9 % of the initial value (7.9 g kg^{-1}). Agosin et al. (1989) reported a study carried out on bitter (8.0 g kg^{-1} dw QA) and sweet (0.3 g kg^{-1} dw QA) *L. albus* 'Multolupa'.

The seeds were dehulled, 5 mm-ground, and inoculated with *Rhizopus oligosporus* NRRL 2710 spores. The complete process took 2 h-energy (100 ºC) for cooking the seeds, and 45 h (30 ºC) for the fermentation. In sweet lupin, even though the alkaloid content was reduced to about 43 % of the initial value, the effect of fermentation (a reduction of 3.6 %) was small compared to that of extraction during soaking and cooking. In addition, 50 % of the lipid fraction was metabolized; linolenic and erucic acids were degraded, whereas no significant improvement of protein digestibility was observed. Sensory evaluation (on a 5-point hedonic scale by 28 untrained panellists) showed promising results for deep-fried lupin tempeh. In bitter lupin, the initial alkaloid content was reduced to 50 % after soaking and cooking. The fermentation process did not cause any detectable reduction of alkaloids, indicating that *R. oligosporus* NRL 2710 could not degrade alkaloids of lupin. Cakes made from bitter lupin were reported less compact than those from sweet lupin. Alkaloids were determined by titration. The latter two studies showed different extents of reduction of alkaloid content in seeds treated by fermentation with *R. oligosporus* NRL 2710. We did not find an explanation for this difference in literature. It may be caused by the use of different lupin species, or by the use of an acid environment during soaking, which –as reported by Jiménez-Martínez et al. (2007)- might facilitate the actions of *R. oligosporus* NRRL-2710 on alkaloid reduction in the fermentation process. Camacho et al. (1991) reported a reduction of alkaloid content at pH 4.5. Another cause could be a deficiency in nutrients during fermentation as Peñaloza et al. (1991) suggested.

Germination is another approach to reduce the alkaloid content. A study by Dagnia et al. (1992) in *L. angustifolius* 'Gungurru' showed that germination reduced the alkaloid content from 0.72 to 0.16 g kg^{-1}, which is equivalent to a 78 % decrease after 6 days (with 7 days total processing time). In this study, the phytate concentration also decreased, namely from 4.7 to 1.6 g kg^{-1}.

The information about biological approaches to debitter lupins is scarce. Nevertheless, based on the studies presented above, some suggestions can be made. First, biological processes do not produce significant chemical residues; however, they require water for washing and sometimes lactic acid solutions. Second, biological processes require preparatory operations such as dehulling, optional grinding, soaking, and cooking. These physical treatments obviously contribute to the reduction of alkaloids in the seed. In order to distinguish the separate effects of biological and physical treatments, it is essential to perform biological studies that include appropriate controls. Third, all reported studies started with seeds that

had initial alkaloid contents up to 11 g kg^{-1}. We did not find studies on the debittering of seeds with higher alkaloid contents such as 30 or 35 g kg^{-1}, which would facilitate an assessment of the applicability of biological methods to debitter lupin seeds such as *L. mutabilis.* Four, in general the biological methods might reduce anti-nutritional components of lupin seeds (Beirao da Costa, 1989; Szakács and Stankovics, 1983), and also in some cases fat, protein content and PER values. Five, all reported treatments were carried out within 48 and 168 h and required an amount of water between 8 to 40 fold the seed weight. Temperatures used varied between 30 ºC and 37 ºC, except for the germination that was at 20-25 ºC. So the evidence to date shows that the debittering process cannot be achieved in less than two days and always uses substantial quantities of water and energy.

Chemical extraction

In plant materials, alkaloids are known to occur partly as free bases and partly as salts that are insoluble in most organic solvents. A common practice to isolate alkaloids from plant sources, prior to their characterization, consists of a treatment with a base that converts such salts into free alkaloids, which, as they are soluble in organic solvents, can be easily recovered by extraction (Ortiz and Mukherjee, 1982). Chemical approaches to extract alkaloids can be distinguished as (i) extraction with hexane and basic solutions, (ii) basic extractions and (iii) mixed alcohol extractions (Table 7).

Extractions with hexane and basic solutions were performed by Ortiz and Mukherjee (1982) and Torres-Tello et al. (1980). In these studies, *L. mutabilis* was crushed, flaked or dehulled, and split. Initially, the seeds were brought in contact with hexane, followed by a basic solution. These procedures extracted between 80 % and 96.9 % of original alkaloids and required between 3 h and 24 h of processing time.

Basic extractions have been tested with *L. campestri* (Jiménez-Martínez et al., 2003a) and *L. mutabilis* (Aguilera et al., 1983; Torres-Tello et al., 1980). These studies showed that this type of extraction can reduce alkaloid contents up to 99.9 %. This required less than one day for whole seeds and less than 1 h for lupin flour (90 % passed 100 mesh screens). This might be because a reduction in particle size of lupin (especially when lupin is processed into flour) increases the contact with water, thus facilitating the diffusion of alkaloids, especially at raised temperatures. These processes also use energy up to 6 h (*i.e.* about 50 MJ kg^{-1}) and cause material losses, mainly of carbohydrates. This could be explained by their solubility in

an aqueous environment. The process carried out by Aguilera et al. (1983) also extracted oil and protein from lupin. These authors did not report material loss and the explanation could be due the fact that they used centrifugation as a separation-extraction procedure. This principle might be an important asset for recovering material in other approaches as well.

Ethanol mixed with hexane or with CO_2 can also be used to extract alkaloids (Nossack et al., 2000; Torres-Tello et al., 1980). In the first case, the seed was dehulled and split, and the alkaloid reduction (97.9 %) was achieved in about 20 h. In the latter case, the seed was powered to 70-100 mesh. The process was carried out in 0.33 h and achieved a reduction of 39.8 mg of alkaloids / g of seed (Table 7).

All treatments discussed so far were carried out at laboratory scale. However, Chajuss (1989) proposed a larger-scale commercial procedure for extracting alkaloids and fat, and for producing protein concentrates and intermediate products from *L. mutabilis* and *L. albus.* This process includes dehulling, flaking and treatment with hexane to extract lipids. The lipid-free fraction is treated with warm aqueous alcohol and then washed to separate protein concentrate and soluble extract (molasses). In this study 2000 kg of raw lupin yielded 1000 kg of protein isolate (720 g protein kg^{-1}, 7 g oil kg^{-1}), 280 kg of food-grade, degummed, refined and bleached oil, 600 kg of lupin alkaloid-sugar extract (molasses 100 g protein kg^{-1}, 14 g oil kg^{-1}, 300 g moisture kg^{-1}, oligosaccharides, minor components), and 240 kg of hulls (80 g protein kg^{-1}, 20 g oil kg^{-1}). Molasses could be used as soil fertilizer, plant growth promoter and insect repellent, whereas hulls could be used possibly as a “green manure” and soil conditioner, or as energy source.

Regarding the chemical alkaloid extraction scenarios, it is important to note that chemical extractions can be performed on lupin seeds with high alkaloid contents (between 19.4 and 42 g kg^{-1}). Basic debittering would decrease the methionine availability in lupin (Gueguen and Cerletti, 1994). This is an important nutritional issue to be considered. All chemical treatments require additional equipment and facilities for safe operation and disposal of waste. Chemical treatments might add residues, which could pose health risks and could affect the taste of the product. They require considerable amounts of water (24 to 60 fold the weight of lupin seed, or even more).

Table 7. Debittering processes of lupin and impact on several variables

Method	General substrate	Specific substrate	Other inputs	Alkaloid reduction (%)	Residue manage-ment	Effect on sensory properties	Effect on nutritional composition	Used time (h)	Relation used water: seed	Used energy	Method used to determine alkaloid content	Reference
Biological methods												
Bacterial fermen-tation	Dehulled, soaked, grinded *L albus* cv. Multolupa	Suspension 12 % lupin floor, inoculated with 1% v/v	*L.acido-phylus, L. buchneri, L. cellobiosus* and *L. fermentum*	≤ 41.1, Best result with *L. acido-philus*	No residues (n.r.)	n.a.	No differences in protein ratio or protein digestibility	> 24	10.3: 1	24 h, 37 ºC	Titrimetric method	(Camacho et al., 1991)
Bacterial fermen-tation	Decorticated *Lupinus albus* flour, 21.5 g alkaloid kg^{-1}	Particle size 0.5-1.0mm	Bacterial strains IST 20B, IST 40D	50	n.r.	n.a.	n.a.	120	n.m	120 h, 31.2 ºC	GC	(Santana and Empis, 2001)
Fungal fermen-tation	*L mutabilis* (7.92 g alkaloid kg^{-1})	Soaked 18 h in 1g L^{-1} lactic acid sol., dehulled, cooked 121 ºC, 5min	*R. oligosporus* 1g kg^{-1}	91	n.a.	n.a.	Red. olygosac-charies	67	>40:1	48 h, 30 ºC	Gas chromato-graphy/ Mass Spectrometry	(Jiménez-Martínez et al., 2007)
Fungal fermen-tation	*L. mutabilis* (8.03 and 0.285 g alkaloid kg^{-1})	Dehulled, 5 mm – grind ed	*R. oligosporus* spores NRRL 2710	50-57	n.r.	Good results in fried product	50 % fat loss, NPR almost constant	60	8:1	45 h, 30 ºC, 2 h, 100 ºC	Titrimetric method	(Agosin et al., 1989)
Germi-nation	*Lupinus angustifolius* 'Gungurru' seed , 0.72 g alkaloid kg^{-1}	24 h-soaked seeds	Water	78	n.r.	n.a.	Increment in protein content PER goes from 1.45 to 0.44	168	n.a.	168 h, 20-25 ºC	n.a.	(Dagnia et al., 1992)

Method	General substrate	Specific substrate	Other inputs	Alkaloid reduction (%)	Residue manage-ment	Effect on sensory properties	Effect on nutritional composition	Used time (h)	Relation used water: seed	Used energy	Method used to determine alkaloid content	Reference
Chemical methods												
Hexane-basic solution extraction	*Lupinus mutabilis* 42 g alkaloid kg^{-1}	Defatted, flaked lupin	Hexane: Sodium carbonate (15%), hydrochloric acid (5%)	80-90	n.a.	n.a.	Reduced fat content	3	n.a.	3 h, 60 ºC	Titrimetric method	(Ortiz and Mukherjee, 1982)
Hexane-basic solution extraction	*Lupinus mutabilis* vs. Keyra 19.4 g alkaloid kg^{-1}	Dehulled split seed	Hexane, ammoniac solution 2N, absolute ethanol	96.9	n.a.	No-bitter taste in product	13 % solids loss	≈ 24	n.a.	n.a.	Gravimetric method	(Torres-Tello et al., 1980)
Basic extraction	*Lupinus campestris* 27.4 g alkaloid kg^{-1}	Whole seed	Solution $NaHCO_3$	99.9	n.a.	n.a.	Reduced carbohydrates content	6	n.a.	6 h boiling	HPLC	(Jiménez-Martínez et al., 2003a)
Basic extraction	*Lupinus mutabilis* 20 g alkaloid kg^{-1}	Dehulled cracked, grounded (100 mesh)	NaOH. Centrifuge	99.9	n.a.	n.a.	Incremented protein content to 78-83 %, 80 % oil recovered	>0.7	60:1	0.7 h, 65 ºC	Titrimetric method	(Aguilera et al., 1983)
Basic extraction	*Lupinus mutabilis* vs. Keyra 19.4 g alkaloid kg^{-}	Whole seed	Alkaline medium	98.6	n.a.	n.a.	13 % solid loss	> 17h	8 h running water	5 h, 70 ºC. 15 min. 100 ºC	Gravimetric method	(Torres-Tello et al., 1980)
Alcoholic extraction	*Lupinus mutabilis* 'Keyra' 19.4 g alkaloid kg^{-1}	Dehulled split seed	Pre-treated with ethy-lene at 60ºC. Hexane-ethanol-water (75:15:1)	97.9	n.a.	No-bitter taste in product	12 % solids loss	>20h	n.a.	60 ºC, 5 h	Gravimetric method	(Torres-Tello et al., 1980)

Method	General substrate	Specific substrate	Other inputs	Alkaloid reduction (%)	Residue manage-ment	Effect on sensory properties	Effect on nutritional composition	Used time (h)	Relation used water: seed	Used energy	Method used to determine alkaloid content	Reference
Alcoholic extraction	*Lupinus mutabilis*	Dried, powered seed 70-100 mesh	Supercritical fluid: CO_2 10 %, ethanol absolute, 80 atm, 60 ºC	39.8 mg /1g of seed	n.a.	n.a.	n.a.	0.33	24:1	0.3 h 60 ºC	High Resolution Gas Chromato-graphy	(Nossack et al., 2000)
Aqueous extractions												
Cold-watery extraction	*Lupinus mutabilis* ' Keyra' 19.4 g alkaloid kg^{-1}	Dehulled split seed	Cold water	95.4	n.r.	n.a.	26.9 % solids loss	72 h	n.a.	0.5 h, 90 ºC	Gravimetric method	(Torres-Tello et al., 1980)
Cold-watery extraction	*Lupinus mutabilis* Sweet	Whole seed	Cold water	97.4	n.a.	n.a.	Increased protein, fat, Zn, Fe Reduced fibre, ash, carbohydrates	120-144	n.a.	0.5-2 h boiling	n.a.	(Villacrés et al., 2000)
Warm-watery extraction	*Lupinus mutabilis*	Whole seed	Warm water	93.3	Solid floccu-lation	70-94 % liking level	Increased protein content	90	63:1	90 h, 40 ºC	n.a.	(Caicedo et al., 2001)

Aqueous processing

Cold and warm aqueous processing of lupin seeds reduces the alkaloid content (Table 7). In a study on *L. mutabilis* 'Kayra' (Torres-Tello et al., 1980), 95.4 % of initial alkaloids were removed. The seeds were dehulled, split, and then cooked at 90 ºC for 0.5 h followed by extraction with cold water for 72 h. Villacrés et al. (2000) reported a traditional process applied at commercial scale to whole seeds of *L. mutabilis*. The seeds were soaked for 14-20 h at room temperature (≈15 ºC), then cooked for 0.5-2 h and washed for 96 h to 120 h at room temperature again. The process took between 120-144 h in total and removed 97.4 % of the initial alkaloid content. In another study, Caicedo et al. (2001) used warm water (40 ºC) to debitter whole seeds of *L. mutabilis*. This process was also carried out at commercial scale. It took 90 h and used water in the ratio 63:1 (w: w) water: seed. The process removed 93.3 % of alkaloids.

Aqueous alkaloid extraction has the following characteristics: (i) the alkaloid content is reduced, but it takes about 3 days when the seed is dehulled and split or 4-5 days for whole seeds; (ii) the debittering process uses large volumes of water that can be treated and reused; (iii) at present, the cold aqueous extraction is the only food-grade method known and applied at a commercial scale. When destined as flour in formulated foods, lupin flour could be extracted more rapidly than whole seeds used for direct consumption as a snack or salad ingredient; (iv) it does not pose the risk of chemical residues nor requires the recovery of chemical reagents.

Uses

Food uses

Lupin seeds are utilized both as food for human beings, and as feed for pigs, sheep, poultry and ruminants (Cremer, 1983; Villacrés et al., 2000). For human consumption, debittered *Lupinus* can be eaten directly as a snack (Villacrés et al., 2003), and can be used as ingredient in many different products such as fresh salads, soups, cakes, snacks, hamburgers, biscuits, bread, foods for babies, substitutes of milk and in main dishes (Cremer, 1983; Güémes-Vera et al., 2008; Ruales et al., 1988; Villacrés et al., 2003).

Nutritional value of lupin and its products

The protein efficiency ratio (PER) of *L. mutabilis* was reported to be between 0.83 and 1 (Chango et al., 1993a; Ortiz et al., 1975; Petterson, 1998) and could be increased by adding methionine (Haq, 1993; Ortiz et al., 1975). Studies on rats show that whole seed supplemented with about 0.2 % DL-methionine increased the PER value to about that of casein, *i.e.* 2.5 (Petterson, 1998).

In vitro protein digestibility of *L. mutabilis* flour and its protein concentrate were reported as 71.1 and 77.6 %, respectively. Those values increased to 75.1 and 80.1 %, respectively, when the samples were cooked for 30 min in moist heat (Sathe et al., 1982), while the apparent digestibility of *L. mutabilis* was reported as 81.8 % compared with 87.1 % for casein when fed to children (Petterson, 1998). These values are similar to those reported by Gueguen and Cerletti (1994), who found an apparent digestibility of 84 % for both raw and processed seeds, and oil cake. These authors also reported the true digestibility of *L. mutabilis* protein isolate as 92 %, which is comparable with that of casein. Protein digestibility –corrected amino acid scores (PD-CAAS) of lupins were around 0.7, compared with 1.0 for casein and 0.7 for field peas (*Pisum sativum*) (Petterson, 1998).

L. mutabilis seeds, debittered with alcohol and/or water, and enriched with DL-methionine (20 g kg^{-1} of the protein) or fortified with complementary protein carriers rich in sulphur-containing amino acids, *e.g*. cereal proteins, fish products and hen eggs (whole) have been proposed as promising sources of nutrition for humans and animals (Gueguen and Cerletti, 1994; FAO, 2013).

Lupin seed and its derivates (flour, protein concentrates and isolates) have also been used to improve the nutritional properties, specially the protein level, of lupin-enriched foods (Güémes-Vera et al., 2008). For example, the PER of bread with 10 % of *L. mutabilis* flour rose from 28 % (in bread without lupin) to 56 % (control = casein 100 %) (Gueguen and Cerletti, 1994), or according to Gross et al. (1983) from 28 % to 76 %. In another study Jiménez-Martínez et al. (2003b) prepared milk from wild *Lupinus campestri*. In order to compare it with cow's milk and soya bean milk, these products were chemically analyzed. Results showed that the protein and fat content were the highest in *L. campestri* milk (protein 58.0 % d.w, fat 29.4 %) compared with commercial soya bean milk (protein 39.1 % d.w, fat 7.0 %) and cow's (semi skimmed) milk (protein 26.2 % d.w, fat 13.4 %). In a similar study rice, a blend with *Lupinus mutabilis* (rice: lupin 80:20 w/w) and a blend with soya bean

(*Glycine max* 'Iniap-Jupiter') (rice: soya bean 80:20 w/w) were used by Ruales et al. (1988) to make extruded products. The chemical composition showed that the addition of dehulled soya bean grits and *L. mutabilis* flour increased the nutritional value of the product. However, the nutritional value of the product containing lupin was the highest (15.3 % protein, 6.0 % fat, 3.5 % fibre, 1.3 % ashes) compared with the products made with soya bean grits (12.6 % protein, 5.5 % fat, 1.4 % fibre, 1.0 % ashes) and with rice grits only (6.7 % protein, 0.4 % fat, 1.4 % fibre, 0.5 % ashes). The mineral analysis showed that the rice-lupin product had the following composition (mg kg^{-1} d.w.): Zn 42.1, Fe 56.8, Ca 129, Mg 948 and Cu 9.2. The rice-soya bean product had (mg kg^{-1} d.w.): Zn 27.4, Fe 17.2, Ca 275, Mg 719 and Cu 10.9. Finally, the rice product had (mg kg^{-1} d.w.): Zn 14.8, Fe 13.7, Ca 114, Mg 399 and Cu 3.0. Note that the product made with the rice-lupin blend had the highest mineral content compared with two other products. Only the calcium content in the rice-lupin product was significantly lower than in the rice-soya bean product. These results suggest that *L. mutabilis* can be used to improve the nutritional composition of different products because lupins increase the nutrient content (Jiménez-Martínez et al., 2003b). Lupins can also improve the biological quality of proteins when they are used in combination with cereals (Jiménez-Martínez and Dávila-Ortiz, 2006; Ruales et al., 1988).

Sensory acceptance

In general, lupin products present a good sensory acceptance (Cremer, 1983; Gross et al., 1976; Jiménez-Martínez et al., 2003b), which can be higher than for soya bean products (Jiménez-Martínez et al., 2003b). Gross et al. (1983) found that bread made with 90 % wheat flour and 10 % *L. mutabilis* flour had an acceptability (72.7 / 100) similar to bread made with 100% wheat flour (74.8 / 100). Bread made with 90 % wheat flour + 10 % *L. albus* flour scored slightly lower (71.6 / 100) than *L. mutabilis* bread, and bread made with 90 % wheat flour + 10 % soya bean flour had the lowest score (61.0 / 100). On the other hand, another study showed that the acceptance of lupin products can also be lower than that of traditional products (Alamanou et al., 1996; Güémes-Vera et al., 2008). For example, *Lupinus albus* 'Graecus' protein isolate added at 1, 2 and 3 % to frankfurter sausages had a lower acceptance than the control (0% addition) (Alamanou et al., 1996). For the sensory evaluation, panellists were instructed to evaluate the appearance, the texture, the flavour and the juiciness of the products and express their overall acceptability on a 6-point hedonic scale (6 = extremely like, 1 extremely dislike). Results showed that sausages made with 1 or 2 %

protein isolate were liked (4.0 / 6). However, the scores were nearly 1 point lower than for sausages made without lupin isolate (4.8 / 6). Sausages made with 3 % protein isolate had a very bad score of only 2 out of 6. This suggests that there is room for improvement of the sensory attributes of lupin-based products (Linsberger-Martin et al., 2010). This improvement could be achieved by developing and adding flavours, colours and additives but also by studying the effect of processing conditions on sensory attributes. For example, according to Gross et al. (1983) roasting before milling considerably enhanced the organoleptic characteristics of the grain. By doing this, the lupin flour takes on a neutral flavour or a slightly nutty taste in accordance with the degree of roasting. Fermentation would also improve the taste and texture of some lupin products (Villacrés et al., 2006). We encourage researchers to study the sensory improvement of lupin-based products in order to increase their consumption.

Pharmaceutical uses

Quinolizidine alkaloids are known to have a high pharmacological activity (Jiménez-Martínez et al., 2003a). Many pharmaceutic and cosmetic uses for lupin seeds have been described since the 16th century (Aguilera and Trier, 1978). Ciesiolka et al. (2005) suggested, based on *in vitro* studies, that the hypocholesterolemic activity was associated with stimulation of low-density lipoprotein (LDL) receptors by a well-defined protein component of the lupin seeds. Extract from *L. angustifolius* (alkaloid content about 110 g kg^{-1} dw) showed pharmacological properties, such as a decrease of arterial blood pressure of rats (Ciesiolka et al., 2005). Sparteine is also used in cardiac medicine due to its anti-arrhythmic capacity (Ciesiolka et al., 2005; Hatzold et al., 1983), and it is frequently used in obstetrics as it induces the contraction of the uterus and hastens partition (Hatzold et al., 1983).

Composition, structure, physical and functional properties of lupin proteins

The functional properties of proteins that are relevant to food production are related to their physicochemical and structural properties, such as size, shape, composition, hydrophobicity/hydrophilicity ratio, net charge, structural arrangements, and adaptability of domain structures of the whole molecule to changes in environmental conditions (Hettiarachchy and Ziegler, 1994; Kinsella, 1976). Lupin protein composition and structure

are therefore presented and discussed to provide understanding of the functional properties of lupin flour and its derived products.

Composition and structure of lupin proteins

The major protein classes in legume seeds are globulins and albumins (Table 8); prolamin and glutelin fractions are also present but in very low quantities (Doxastakis, 2000). Globulins are proteins extracted at high ionic strength, and represent 90 % of the protein in soya bean and about 80 % in *L. albus*) (Gueguen and Cerletti, 1994). *L. mutabilis* 'Potosi' and 'Inti' were reported to contain about 11 % and 13 % more globulin, respectively, than *L. albus* (Santos et al., 1997). Denaturing PAGE (Polyacrylamine Gel Electrophoresis) analysis showed that the globulins of *L. mutabilis* are composed of polypeptides with higher molecular masses than those of *L. albus*. Some of these polypeptides are linked by disulfide bonds (Santos et al., 1997). The complexity of the globulin fraction is due to the presence of different families of proteins (legumin-like and vicilin-like proteins, and lupin conglutins γ and δ), and the presence of oligomeric components (12S and m7S). These proteins and components have different types of associations, protomer sizes and compositions (Doxastakis, 2000).

Legumin-like proteins correspond to the fraction of globulin polypeptides with sedimentation coefficients of about 11-12S. In *L. angustifolius* this fraction shows molecular masses between 185-315 kDa (Doxastakis, 2000), similar to that of *L. albus*. However, legumin-like protein α-glutinin from *L. mutabilis* was reported to differ considerably in structure and composition from that of *L. albus* (Santos et al., 1997). In *L. albus*, the α-conglutin fraction represents about 33 % of total protein (Duranti et al., 1981) and is composed of four main types of subunits, with molecular masses between 50 and 60 kDa (Santos et al., 1997). Upon reduction, each of the main subunits is split into an acid (heavier) polypeptide chain (38-50 kDa) and a basic (lighter) polypeptide chain (19 kDa) (Santos et al., 1997).

In *L. mutabilis* 'Potosi' α-conglutin is formed by four main types of subunits (50-65 kDa) and two minor types (40-42 kDa), which upon reduction produce a number of undetermined heavier polypeptide chains and two lighter ones (18 and 19 kDa). *L. mutabilis* 'Inti' differs from 'Potosi' as the first consists of five main types of subunits (namely of 32, 40, 45, 49 and 53 kDa), which upon reduction produce four main types of polypeptide chains (18, 19, 31 and 37 kDa) (Santos et al., 1997). In the case of soya bean the 11S fraction is glycinin, and

represents 20-35 % of total protein. In soya bean 11S proteins are hexamers (αβ)6 of relative molecular weights of about 350-400 kDa. Each subunit of the hexamer consists of two components; the acid (α) of 40 kDa and the basic (β) of 20 kDa bound by disulphide bonds (Gueguen and Cerletti, 1994). For all these 11S like proteins, the acid polypeptides have significantly lower hydrophobicity compared with the basic units and are mainly located on the exterior of the molecule (Doxastakis, 2000). Moreover, studies on *L. albus* have shown that assembly of the subunits in the oligomer is likely to be dictated by the distribution of polarity in the polypeptide (Duranti et al., 1988; Guerrieri and Cerletti, 1990). Consequently, the polarity of the 11S oligomer would be the result of its polypeptide composition (*i.e.* amount and type of polypeptides) and structure (spatial distribution of polypeptides in the oligomer). Because variations in the composition and structure are reported not only between species but also between different genotypes of soya bean (Gueguen and Cerletti, 1994) and *L. mutabilis* (Santos et al., 1997), we may expect different functional behaviour of 11S fractions between and within species of lupins and soya bean.

Vicilin-like proteins are polypeptides with a sedimentation time of 7S. However, this group includes polypeptides 4S, 5S, 6S and 7S for *L. albus* (Duranti et al., 1981) and polypeptides with 7.4S (Joubert, 1956) and *β*-conglutin (Aguilera and Garcia, 1989) for *L. angustifolius* and *L. luteus* (Doxastakis, 2000). For *L. albus*, the vicilin fraction represents about 44 % of total protein (Duranti et al., 1981). Beta-conglutin from *L. albus* is composed of more than 20 polypeptide chains without disulfide bonds, with molecular masses ranging from 15 to 65 kDa (Santos et al., 1997). For *L. mutabilis* 'Potosi' and 'Inti', *β*-conglutin is composed of seven major polypeptide chains (with molecular masses ranging from 50 to 67 kDa), two polypeptide chains with molecular masses in the range of 33-38 kDa, and a number of minor polypeptides. The presence of disulfide bonds was not detected (Santos et al., 1997). Blagrove and Gillespie (1975)mention the presence of a 30 kDa subunit, a major component of *β*-conglutin, in *L. angustifolius* and other Old and New World lupin species and observed that it is absent in the American *L. elegans* and *L. mutabilis*. In soya bean, the 7S fraction (*β* and *γ* conglycinin) constitutes 30-35 % of total protein (Peng et al., 1984). Beta-conglycinin, the major 7S fraction, has 6 components, from which three are principals (α, α' and β) with relative molecular weights of 42-57 kDa. The *γ* conglycinin (*i.e.* the 7S minor fraction) has been less studied. The difference in the composition of the *β*-conglutin of *L. mutabilis* and *L. albus* (Santos et al., 1997) and the difference with the 7S fraction of soya bean (Gueguen and Cerletti, 1994) is evident.

Table 8. Protein fractions in the different lupin species

Protein fractions and families		**Lupin species**								**Soya bean**	**Reference**
Fraction	**Family**	***L. albus***	**Reference**	***L. angusti-folius***	**Reference**	***L. luteus***	**Reference**	***L. mutabilis***	**Reference**		
Globu-lins (%)	All	80	(Gueguen and Cerletti, 1994)	n.a.		n.a.		≈ 91-94	(Santos et al., 1997)	90	(Gueguen and Cerletti, 1994)
	Legumin-like proteins	33 % (α-conglutin). 4 main subunits 50-60 kDa	(Duranti et al., 1981), (Santos et al., 1997)	185-315 kDa	(Doxastakis, 2000)	n.a.		**(**α-conglutin in'Potosi') 4 main subunits 50-65kDa 2 minor subunits 40-42 kDa	(Santos et al., 1997)	**20-35 % (**glycinin) Hexamers: 3 subunits 40 kDa, 3 subunits 20 kDa	(Gueguen and Cerletti, 1994)
	Vicilin-like proteins	44 % of total protein *β*-conglutin > 20 polypeptides (15-65 kDa)	(Duranti et al., 1981), (Santos et al., 1997)	7.4S and *β*-conglutin (30 kDa principally)	(Joubert, 1956), (Aguilera and Garcia, 1989), (Blagrove and Gillespie, 1975)	7.4S and *β*-conglutin	(Joubert, 1956), (Aguilera and Garcia, 1989)	*β*-conglutin 7 polypeptides (50-67 kDa), (33-38 kDa)	(Santos et al., 1997)	30-35 % of total protein mainly. *β* conglycinin, 6 polypeptides (42-57 kDa)	(Peng et al., 1984)
	Lupin conglutin γ	6 % of total protein. A single subunit of 42-43 kDa	(Duranti et al., 1981) (Santos et al., 1997),	Monomer of 43-45 kDa	(Blagrove et al., 1980)	n.a.		6 % of total protein. A single subunit of 42-43 kDa	(Duranti et al., 1981), (Santos et al., 1997)	n.a.	
	Lupin conglutin δ	10-12 % of total protein	(Duranti et al., 1981)	10-12 % of total protein (80 % as δ_2 of 14 kDa	(Lilley, 1986a), (Lilley and Iuglis, 1986)	n.a.		n.a.		n.a.	

Protein fractions and families		Lupin species								Soya bean	Reference
Fraction	**Family**	***L. albus***	**Reference**	***L. angusti-folius***	**Reference**	***L. luteus***	**Reference**	***L. mutabilis***	**Reference**		
Albu-mins		12.8 % of total protein 20 poly-peptides of 6-117 kDa	(Hudson, 1994), (Cerletti et al., 1978), (Doxastakis, 2000)	5-10 % of total protein 20 polypeptide s of 6-117 kDa	(Hudson, 1994), (Blagrove and Guillespie, 1978), (Doxastakis, 2000)	15.4 % of total protein 13 polypeptide s of 6-117 kDa	(Hudson, 1994), (Konopoka-Waliszkiewi cz, 1988), (Doxastakis, 2000)	≈ 6.4 % of total protein Abundant 34 kDa	(Santos et al., 1997)	10 % of total protein	(Hudson, 1994)

The vicilin protein shows surface hydrophobicity, which permits the self-association of proteins into micelle arrangements. At pH 6-6.8 this hydrophobicity is high and precipitated micelles show visco-elastic properties similar to wheat gluten (Gueguen and Cerletti, 1994). The variation in the composition of vicilin-like proteins from lupin species and soya bean are expected to influence the hydrophobic behaviour of their proteins.

Oligomers 12S, 7S, m7S and system 12S↔7S

12S and 7S type proteins aggregate, forming a structure called 12S oligomer or 7S oligomer, respectively. The 12S oligomer is more compact and resistant to endogenous proteases, than the 7S oligomer (Duranti et al., 1988). This gives the 12S structure rigidity, not only due to the disulphide bonds but also because of hydrophobic interactions resulting from the high hydrophobicity of the basic subunits (Gueguen and Cerletti, 1994), which are present in the interior of the structure.

The 12S oligomer can dissociate to a smaller 7S species until an equilibrium is reached (system 12S↔7S). However, this equilibrium is dynamic. In *L. albus*, for instance, this equilibrium is reversibly shifted towards the high M_r form by increased ionic strength and protein concentration (Gueguen and Cerletti, 1994). On the other hand, when the equilibrium 12S↔7S is shifted toward the 7S form, the secondary structure and the net charge of the protein become more like those of m7S molecules (Duranti et al., 1988). This m7S molecule (158 kDa) is a modification of the 7S oligomer but without the capacity to produce 12S oligomers (Duranti et al., 1988). The system 12S↔7S has a structure that consists for 15 % of α helix, 37 % β strand and 48 % coil, whereas this is 20 %, 34 % and 46 %, respectively, in the m7S oligomer (Duranti et al., 1988). Thus the functional behaviour of lupin protein will depend on the association state (*i.e.* the 12S↔7S equilibrium). This behaviour depends especially on the composition of the subunits in the α-chains (Guerrieri and Cerletti, 1990).

Lupin conglutin γ is a globulin protein that in the case of *L. albus* and *L. mutabilis* 'Potosi' and 'Inti' is reported to consist of a single subunit (monomer) of 42-43 kDa composed of two polypeptide chains linked by disulfide bonds (18-30 kDa) (Santos et al., 1997). These monomers would be associated in various states of 92, 150 and 300 kDa representing 6 % of total seed protein (Duranti et al., 1981). The small subunits precipitate at pH 5.6-5.9 and the large ones at pH 6.2-6.8 (Restani et al., 1981). In *L. angustifolius* the association has been reported to vary between 280 kDa (by sedimentation equilibrium) and 320 kDa (gel

permeation). Dissociation generates monomers of 43-45 kDa and subunits of 28-30 kDa and 16.5 kDa (Blagrove et al., 1980). The small subunits precipitate at pH 6.9 and the larger ones at pH 7.8-8.0 (Blagrove et al., 1980). Of the globulins, conglutin γ from *L. albus* has the highest amount of bound sugar (Duranti et al., 1981). Pentoses and hexoses are present, with galactose as the major component (Duranti et al., 1981). In *L. angustifolius* also a high (bound) carbohydrate content was reported (Gueguen and Cerletti, 1994).

Lupin conglutin δ is a sulphur-rich 2S globulin present in *L. albus* (Cerletti, 1983; Duranti et al., 1981), *L. luteus* (Gerritsen, 1956; Joubert, 1956) and *L. angustifolius* (Lilley, 1986a, b). It was not reported in *L. mutabilis*. Lupin conglutin δ represents between 10-12 % of total protein of *L. albus* (Duranti et al., 1981) and *L. angustifolius* (Lilley, 1986a). In *L. angustifolius* 80 % of conglutin δ is conglutin δ_2 of 14 kDa, composed of two subunits of 9.401 and 4.597 kDa with two intra-chain and two inter-chain disulphide bonds and one free SH (Lilley and Iuglis, 1986). Conglutin δ_2 can produce a dimer, conglutin δ_1 (28 kDa, 2.8S), which at low ionic strength and neutral pH associates reversibly to an oligomer of 56 kDa and 4.1S (Gueguen and Cerletti, 1994). The presence of a disulphide crosslink (in the part of conglutin known as α helix) gives stability to the conglutins δ_1 and δ_2. However, addition of 1M guanidine hydrochloride causes denaturation of the helix structure (Youle and Huang, 1981). Lupin conglutin δ is the most acidic globulin in lupin seed because of the high amounts of glutamic acid (Duranti et al., 1981). This acidic nature influences the behaviour of the total protein (Gueguen and Cerletti, 1994) by increasing the hydrophilicity.

Albumins are defined as the water-soluble fraction of the protein from legume seeds, and represent 12.8, 15.4, between 5-10 and 10 % of the total seed protein of *L. albus, L. luteus, L. angustifolius* and soya bean, respectively (Hudson, 1994). *L. albus* is reported to have an albumin content that is about twice that of *L. mutabilis* 'Potosi' and 'Inti' (Santos et al., 1997). The albumin fraction includes molecules that belong to the functional proteins of the seed. Many are enzymes such as glycosidases and proteases. Others play an important role in plant defence, such as trypsin inhibitors and lectins. Albumin is characterized by a high lysine and sulphur amino acid content, especially methionine (Cerletti, 1983; Gueguen, 1991; Smith and Circle, 1978). However, Santos et al. (1997) report that the presence of disulfide bonds is not apparent in *L. albus* and *L. mutabilis* 'Inti' and 'Potosi' after electrophoresis performed under non reducing conditions, and that SDS electrophoresis on polyacrylamine gel (SDS-PAGE) showed about 20 polypeptides in *L. albus* (Cerletti et al., 1978) and *L. angustifolius* (Blagrove and Guillespie, 1978) and 13 in *L. luteus* (Konopoka-Waliszkiewicz, 1988) with

apparent molecular masses from 117 kDa to 6 kDa (Doxastakis, 2000). The polypeptide patterns of the two *L. mutabilis* analyzed by R (reducing)-SDS- PAGE are virtually identical but differ considerably from that of *L. albus*. Particularly evident is the presence of abundant 34 kDa albumin in *L. mutabilis* cotyledons (Santos et al., 1997), which are apparently not present in *L. albus*.

Physical and functional properties of lupin proteins (lupin flour, protein isolates and concentrates)

Table 9 presents the physical and functional properties of the most important *Lupinus* spp. flours, their protein concentrates and isolates. In the following text, the term concentrate is used when the protein content is between 72.8 and 83.8 %, and the term isolate when the protein content is 83.9-87.4 %.

Isoelectric point

The isoelectric point of the protein of *L. mutabilis* was reported to vary from pH 4.0 to 6.0 (Aguilera et al., 1983; Aguilera and Trier, 1978; Bleitgen et al., 1979). In *L. albus* 'Multolupa' it was between pH 4.2 to 6.4 (King et al., 1985) and in *L. angustifolius* between pH 4.3 (Lqari et al., 2002) and pH 4.5 (Sathe et al., 1982). These values confirm that lupin proteins consist of different subunits or groups, each with different properties. For example, the protein fraction from *L. albus* 'Multolupa' that precipitates at pH 5.4, is reported to have a higher amino acid score than protein fractions obtained at a pH 4.2 or 6.4 (King et al., 1985).

Protein solubility

Protein solubility is the percentage of soluble nitrogen/ total nitrogen. A higher solubility is attributed to an elevated charge and the electrostatic repulsion and ionic hydration occurring at a pH above and below the isoelectric pH (Doxastakis, 2000; Moure et al., 2006). For example, *L. angustifolius* slurry (20 mg flour 1-h homogenized with 20 ml solution 0.1 M NaCl at pH 7) showed a protein solubility of 13.1 % (Lqari et al., 2002). However, when –in the slurry- flour was replaced by *L. angustifolius* protein isolate, which first was solubilised at

pH 10.5 or 12, and then precipitated at pH 4.3, the protein solubility increased to 19.2 and 33.8 %, respectively (Lqari et al., 2002).

The ionic strength can also affect the solubility of lupin proteins. In a 1 % slurry made from *L. albus* 'Ares' and 'Typ Top' isolates, the protein solubility was about 10 % when the slurry was treated at pH 8.6 followed by precipitation of the protein at pH between 4.2 and 5.1 and then freeze dried (D´Agostina et al., 2006). In this case the protein solubility was measured at pH 5 and at an ionic strength $u = 0$. This protein solubility, however, was increased to about 90 % when all parameters remained the same, except for the ionic strength u, which was increased to 1.0 with sodium chloride. Other studies show the same behaviour (Manrique et al., 1974; Sathe et al., 1982). The direct effect of ionic strength on protein solubility is clear. At pH 7 however, the effect of u on protein solubility is lower than at a pH closer to the isoelectric point. Temperature also affects protein solubility. On the one hand, heat treatments (80-100 °C) have been reported to have greater adverse effects on solubility when the precipitate is kept at its isoelectric point (King et al., 1985). Apparently the intermolecular attraction due to the pH at the isoelectric point is added to the effect of aggregation (coagulation) caused by the high temperatures. On the other hand, temperatures below 60 °C are reported to be beneficial for protein solubility. King et al. (1985) suggest that nitrogen solubility is increased up to 100 % if isolates are taken to pH 6 before drying and subsequently heated at 60 °C for 20 min. In this case the heating could increase the protein dispersibility. All these results indicate that protein solubility depends on pH, temperature and ionic strength. Isolates obtained by precipitation at the isoelectric pH, kept at that pH in an environment having an ionic strength of $u = 0$ and heated above 80 °C will probably show very low solubility. On the other hand, isolates obtained by solubilisation of protein at pH 8-10 in an environment with an ionic strength of $u = 1$, will show a higher solubility when they are precipitated at their isoelectric point and taken to pH 6-8 before drying and finally heated at temperatures below 60 °C. In the latter case, the intermolecular repulsion would be greater, avoiding the formation of aggregates and thereby facilitating the solubility (King et al., 1985).

Water absorption

The amount of water absorbed by flour, protein concentrate or isolate is closely related to its amino acid profile, conformation, hydrophobicity, pH, thermal treatment, ionic strength,

amount of protein and presence of fat. The amount of water absorbed is also influenced by the technological process used to obtain the flour, concentrate or isolate, for example soaking, fermentation or germination (Moure et al., 2006; Sathe et al., 1982). Moreover, even lupin protein derivates with the same protein content may show different functional properties because, for instance, the ratio of the different globulin fractions differs among lupin varieties (Cerletti et al., 1978).

The water absorption capacity of lupin flour reportedly varies from 2.4 g water/g flour dw in *L. angustifolius* (Lqari et al., 2002) and 2.3 g water/g flour dw in *L. albus* 'Multolupa' (Agosin et al., 1989) to 1.2 g water/g flour dw in *L. mutabilis* (Sathe et al., 1982) (Table 8). This implies that the water absorption capacities of *L. angustifolius* and *L. albus* are similar to that of soya bean (2.0- 2.4 water/g flour dw) (Sathe et al., 1982). The same authors hypothesized that the water absorption capacity of *L. mutabilis* flour is lower due to the presence of fat (17.9 %). In addition, the low water absorption capacity can be related to a low availability of polar amino acids, which are the primary sites for water interaction of proteins (Sathe et al., 1982). This is corroborated by the probable absence of lupin conglutin δ and the lower amount of albumin in protein from *L. mutabilis*.

Water absorption for soya bean protein concentrate is reported to vary between 3.0 and 4.0 g water/ g of concentrate dw (Sathe et al., 1982) and soya bean protein isolates absorb up to 8 g water /g isolated dw. In protein concentrates and isolates of lupin the values of water absorption vary more widely, namely between 0.5-6.0 g water/g of protein dw (King et al., 1985; Lqari et al., 2002; Sathe et al., 1982). This wide variation between and within species can at least be partially understood by the conditions in which those concentrates or isolates were obtained. For instance, Lqari et al. (2002) found for *L. angustifolius* that isolates (83.9-87.4 % protein) extracted at pH 12 or pH 10.5 with 0.25 % Na_2SO_4 followed by precipitation at pH 4.3 showed a water absorption of 4.5 and 3.8 g/ g protein, respectively. These data agree with King et al. (1985), who found that a sample of *L. albus* 'Multolupa' protein isolate could absorb water about 6 times its weight when that isolate was extracted at pH 8.6 followed by a precipitation at pH 4.2-5.1, then freeze-dried and later heated at 100 °C for 20 min. However, the same lupin protein absorbed just 0.5 times its weight when the sample was neutralized before drying and the sample was not heated. Higher water absorption seems also to be related to the electric charge. Exposure to basic pH, especially 10-12, followed by acid precipitation could cause denaturation (unfolding) of proteins (Lqari et al., 2002), leading to an increase of the hydrophilicity thereby enhancing the water absorption. Isolates that in

addition to exposure to a basic pH (8.6) and precipitation at an acidic pH (4.9) were heated (100 °C x 20 minutes) before drying showed higher water absorption than those treated at temperatures of 80 °C or lower. Temperatures of 100 °C might cause the unfolding of proteins too (King et al., 1985).

The protein content also would affect the water absorption capacity. Protein isolates of *L. albus* (95.7 % protein) absorbed 6 g water g^{-1} protein dw (King et al., 1985). Water absorption apparently increased when the protein content in the isolate had more hydrophilic structures (acid polypeptides) in the periphery (11S-like proteins) (Gueguen and Cerletti, 1994; Moure et al., 2006) available to bind water molecules. Soaking also affects the water absorption capacity. *L. mutabilis* seeds increased their size by 3 times when soaked for 18 hours (Gross et al., 1983). Other factors such as germination, fermentation, toasting and autoclaving reportedly increase the water absorption capacity of meals (Moure et al., 2006). This could be related to denaturation (unfolding) of proteins leading to increased hydrophilicity, which enhances higher water absorption. In addition certain processing steps, for instance, soaking combined with alkaline extraction of proteins, also improves the capacity of a protein isolate to absorb water because this process removes compounds such as lipids and polyphenols (Lqari et al., 2002).

Oil absorption

Oil absorption amounts to 1.7 g oil g^{-1} seed dw for *L. mutabilis*, and 1.5 g oil g^{-1} dw flour for *L. angustifolius* (Lqari et al., 2002) (Table 9). Those values are higher than the 0.8 g oil g^{-1} flour reported for soya bean (Moure et al., 2006). Protein concentrates and isolates show higher oil absorption than lupin and soya bean flour. For *L. mutabilis* concentrate a value of 2.9 g oil g^{-1} full-fat concentrate was reported and 3.9 g oil g^{-1} defatted concentrate (Sathe et al., 1982), showing a inverse relation between fat content and oil absorption capacity, suggesting a lipophilic nature of lupin proteins (Sathe et al., 1982). Processing conditions also influence oil absorption capacity. *L. angustifolius* solubilised at pH 12 and then precipitated at pH 4.3 absorbed 2.0 g oil g^{-1} isolate. However, when the dissolution was done at pH 10.5 with 0.25 % Na_2SO_3, followed by precipitation at pH 4.3, the absorption rose to 3.1 g oil g^{-1} isolate. On the other hand, oil absorption in *L. albus* is reported to be between 1.0-1.8 g g^{-1} isolate. In this case the protein precipitation was at a pH between 7 and 4.5. This difference in the amount of oil absorbed would result from the effect of pH during processing. Alkaline

extraction allows removal of undesirable compounds in the protein isolate (fibre, sugars, polyphenols, lipids, and alkaloids) (Lqari et al., 2002), thus increasing the functionality of protein isolates. However, alkaline processing can also alter protein quality due to the possibility of disruption of the protein structure and degradation of some amino acids (Cerletti et al., 1978; Liener, 1994; Maga, 1984; Sgarbiere and Galeazzi, 1978). To avoid degradation of amino acids, Lqari et al. (2002) recommend alkaline processing at pH 10.5 but not at pH 12. In general, soya bean concentrates and isolates show oil absorption values between 0.9 to 2.9 g g^{-1} concentrate or isolate (Moure et al., 2006). These values are lower than reported for *L. mutabilis*, suggesting that the latter can be used to (partly) replace soya bean protein in foods to improve oil absorption capacity.

Emulsifying capacity

Emulsifying capacity is defined as the quantity (in g) of emulsified oil per g of flour, concentrate or isolate (Sathe et al., 1982). Some others report emulsifying capacity as ml of emulsified oil per g of protein. In the case of *L. mutabilis* flour the emulsifying capacity is 55.1 g g^{-1} lupin flour and 89.9 g g^{-1} concentrate (Sathe et al., 1982). In both cases, the emulsifying capacity was measured on slurries with 2 % flour or concentrate and 98 % water. Apparently, the emulsifying capacity decreases with increasing amounts of concentrate in the slurry (Table 9). In the case of *L. albus* protein isolate the reported emulsifying capacity varies between 370-570 ml g^{-1} isolate (D´Agostina et al., 2006), or 326-502 g g^{-1} isolate when the oil density is 0.88 kg l^{-1} (Sathe et al., 1982), in a slurry with a concentration of 1% (D´Agostina et al., 2006). In *L. albus* the emulsifying capacity is reported to be 1000-2000 ml g^{-1} isolate in a slurry with a concentration of just 0.04 % (King et al., 1985). According to King et al. (1985), this concentration of 0.04 % protein is the minimum value, which emulsified the maximum amount of oil. High protein concentrations did not emulsify more oil, possibly because it becomes more difficult to expose hydrophobic areas that can interact with the lipid phase at increasing concentrations (King et al., 1985). Emulsifying capacity is also pH dependent (Moure et al., 2006). In *L. mutabilis*, slurries with 2 % protein concentrate had different emulsifying capacity depending on the pH. For example, the emulsifying capacity was reported as 315.5, 222.2, 80.0, 155.5, and 137.8 g g^{-1} concentrate at pH 2, 4, 6, 8, 10, respectively (Sathe et al., 1982). In *L. albus* the emulsifying capacity is reported as 1000 ml at pH 5 and 2000 ml at pH 8 (King et al., 1985). Note that the emulsifying capacity

at pH 2 (in the case of *L. mutabili*s) or pH 8 (in the case of *L. albus*) is higher than at their isoelectric pH (4-5), probably due to an increased oil solubility in those conditions (King et al., 1985) by unfolding of proteins (12S oligomer), thereby facilitating the exposure of hydrophobic groups. Emulsifying properties show a good correlation with the presence of hydrophobic residues on the protein surface (Kato and Nakai, 1980). The emulsifying capacity of protein isolate also has an apparent inverse relation with its solubility in water. For example, King et al. (1985) reported that soya bean concentrates and isolates with a high solubility in water showed an emulsifying capacity of 15 ml g^{-1}, but soya bean isolates and concentrates with a low solubility in water showed an emulsifying capacity of 66 ml g^{-1}. Apparently, highly water soluble proteins are poor emulsifiers because they can cause coalescence. The emulsifying capacity is also reported to depend on ionic strength (Kinsella, 1984). For *L. albus* and soya bean the highest emulsifying capacity was recorded at an ionic strength of 0.5 (using sodium chloride) of the slurry (King et al., 1985). The values for the emulsifying capacity of lupin species are similar or higher than those reported for soya bean (between 15 and 191 ml g^{-1}) (King et al., 1985), which suggests that lupin isolates could well be used as emulsifiers.

Emulsifying activity

The emulsifying activity is expressed as the volume of an emulsified layer at time 0 h/ total volume of all phases and multiplied by 100 (Lqari et al., 2002) to express the result as a percentage. The emulsifying activity of *L. angustifolius* in a slurry of 3.5 % flour w/v (50 water: 50 oil) after homogenisation for 2.5 min. and centrifugation at 1100 x***g*** for 5 min was 74 % (Lqari et al., 2002). When the flour was replaced by *L. angustifolius* isolate, the emulsifying activity ranged between 69.7-74.5 % (Lqari et al., 2002). In both cases the pH was 7. The composition of *L. angustifolius* flour (33.8 % protein, 13.6 % lipids and 7.9 % water) and its protein isolates (83.9-87.4 % protein, ≈ 3.2 % lipids, and ≈ 9.4 % water) apparently does not affect the emulsifying activity. Slurries with 2% *L. mutabilis* concentrate showed an emulsifying activity of 100 % at 21 °C. These samples, however, were not homogenized nor centrifuged (Sathe et al., 1982).

Table 9. Physical and functional properties of lupin flour, concentrates and isolates

Property	Unit	Lupin species					
		***L.* albus**	**Reference**	***L.* angustifolius**	**References**	***L.* mutabilis**	**References**
Seed flour							
Protein iso-electric point	pH	4.5	(D´Agostina et al., 2006)	4.5	(Sathe et al., 1982)	4	(Bleitgen et al., 1979), (Aguilera and Trier, 1978)
		4.2; 5.4; 6.4	(King et al., 1985)	4.3	(Lqari et al., 2002)	4.5	(Bleitgen et al., 1979), (Aguilera et al., 1983)
						6.0	(Aguilera and Trier, 1978)
Protein solubility	Soluble nitrogen (%)	n.a.[1]		13.1 % / pH 7/ solution 1.25 % /+ 0.1M NaCl	(Lqari et al., 2002)	n.a.	
Water absorption	g /g dw	2.3	(Agosin et al., 1989)	2.4	(Lqari et al., 2002)	1.2	(Sathe et al., 1982)
Oil absorption	g /g dw	n.a.		1.5	(Lqari et al., 2002)	1.7	(Sathe et al., 1982)
Emulsifying capacity	g of oil / g of lupin flour	n.a.		n.a.		55.1, (2% flour, 98% water)	(Sathe et al., 1982)
Emulsifying activity	% = (vol (emulsified layer) / total volume) x100	n.a.		74,/pH7/ sol 3.5 % w/v (50water:50oil)	(Lqari et al., 2002)	n.a.	
Emulsifying stability	% = (vol. (emulsified layer after x time) / total volume) x100	n.a.		69.4/pH 7/0.25 h/ 85 °C/0.08 h 1100 x***g*** /sol 3.5%/		70.8/10 h/conc.2 %/21 °C 69/20 h-120 h / conc. 2 % / 21 °C	(Sathe et al., 1982)
Foaming capacity (time 0h)	%= (foam vol. after whipping/ initial vol. of protein sol.) x 100	n.a.		214/ conc. 3 %	(Lqari et al., 2002)	132 / conc. 2 %. 180 / conc. 6 %. 186 / conc. 10%	(Sathe et al., 1982)
Foam stability	%= (foam vol. after some time/ foam volume after whipping) x 100	n.a.		Solution 3 % 82/after 1 h 79.2/ after 2 h	(Lqari et al., 2002)	Conc. 2 %: 93.9/ after 1 h 92.4/ after 2 h 78.8/after 36 h	(Sathe et al., 1982)

Property	Unit	Lupin species					
		***L.* albus**	**Reference**	***L.* angustifolius**	**References**	***L.* mutabilis**	**References**
Least gelation concen-tration	% w / V	n.a.		6.0/ pH 7	(Lqari et al., 2002)	14	(Sathe et al., 1982)
Protein isolate / concentrate							
Protein solubilisation	Soluble nitrogen (%)	10/pH5/*u*=0/1 % sol. 90/pH 5/*u*=1/1 % sol.	(King et al., 1985)	19.2/pH 10.5 33.8/pH 12	(Lqari et al., 2002)	>90% /solution 0.1% in NaOH 0.1N	(Sathe et al., 1982)
Water absorption	g / g dw	0.5/pH 8.6->5.1-> 7 (not heated) 6/pH 8.6->4.9->6 (100 °C)	(King et al., 1985)	3.8/pH 10.5 4.5/pH 12	(Lqari et al., 2002)	1.37 (full-fat) 1.55 (defatted)	(Sathe et al., 1982)
Oil absorption	g /g	1.0-1.8	(D´Agostina et al., 2006)	2.0-3.1	(Lqari et al., 2002)	2.9 (full-fat) 3.9 (defatted)	(Sathe et al., 1982)
Emulsifying capacity	ml or g of oil / g of lupin concentrate	n.a.		n.a.		88.9 (2 % conc. flour, 98 % water.) 82.2 (4 % conc.) 56.9(/10 % conc.) At 2 % conc.: 315.5/pH 2. 222.2/pH 4. 80.0 /pH 6. 155.5/ pH 8. 137.8/pH10.	(Sathe et al., 1982)
	ml of oil / g of protein	370-570 ml /pH 7/conc. 1 %	(D´Agostina et al., 2006)	n.a.		n.a.	
		1000ml/pH 5/conc. 0.04 %	(King et al., 1985)	n.a.		n.a.	
		2000ml/pH 8/conc. 0.04%	(King et al., 1985)	n.a.		n.a.	

Property	Unit	Lupin species					
		***L.* albus**	**Reference**	***L.* angustifolius**	**References**	***L.* mutabilis**	**References**
Emulsifying activity	% = (vol. (emulsified layer) / total volume) x100	n.a.		69.1-74.5/pH 7/ solution 3.5 % (50Water:50oil)	(Lqari et al., 2002)	100/21 °C/conc. 2 %	(Sathe et al., 1982)
Emulsifying stability	% = (vol. (emulsified layer after x time) / total volume) x100	61-63/solution pH 7, prec. pH 4.5. 74-93/ ultra-diafiltration pH 4.5	(D´Agostina et al., 2006)	66.7-71/ pH 7/0.25 h 85°C/0.08 h 1100 x***g*** /conc. 3.5 %	(Lqari et al., 2002)	100/60 h/21 °C/conc. 2 % 91.4/120 h/21 °C/conc. 2 %	(Sathe et al., 1982)
Foaming capacity	%= (foam vol. after whipping/ initial vol. of protein sol) x 100	1102-2083/conc. 5 %	(D´Agostina et al., 2006)	216-219/ sol. 3 %	(Lqari et al., 2002)	150/ conc. 2 % 186/conc. 6 % 192/conc. 10 %	(Sathe et al., 1982)
Foam stability	%= (foam vol. after some time/ foam volume after whipping) x 100	Solution 5 % 68-95/after 1 h	(D´Agostina et al., 2006)	Solution 3 % 81.3-80.7/after 1 h 80.4-79.9/after 2 h	(Lqari et al., 2002)	Solution ,2 % 94.5/after 1 h 88/after 2 h 76/after 36 h	(Sathe et al., 1982)
Least gelation concen-tration	% w / V	14/pH 4.9 16/pH 4.9->7	(King et al., 1985)	10-12/100 °C	(Lqari et al., 2002)	8	(Sathe et al., 1982)

[1] n.a. = not available

Emulsifying stability

The emulsifying stability of protein is based on the ability to absorb the oil-water interfaces, unfold and stabilize oil droplets by forming cohesive and mechanically strong interfacial films which exhibit viscoelasticity (Chou and Morr, 1979; Graham and Phillips, 1980; Kiosseoglou et al., 1989; Velev et al., 1993). The emulsifying stability is expressed as the emulsifying activity after a specific period of time (D´Agostina et al., 2006; Lqari et al., 2002). Sathe et al. (1982) reported that the emulsifying stability of a 2 %- slurry made from *L. mutabilis* flour was 70.8 % after 10 h at 21°C. This value slightly decreased to 69 % after 20 h, and then remained the same up to 120 h. The emulsifying stability of a 2 %-slurry from *L. mutabilis* concentrate was 100 % after 60 h at 21°C and decreased to 91.4 % after 120 h (Sathe et al., 1982). This result indicates that the emulsifying stability of protein concentrate is better, in terms of time and amount of material stabilized, than of lupin flour. The ability of proteins to act as emulsifiers varies with their molecular properties (Kinsella, 1984). The emulsifying stability of a 3.5 %-slurry from *L. angustifolius* flour measured after 0.25 h at 85 °C and 0.08 h at 1100 x***g*** was 69.4 %, whereas a 3.5 %- slurry from *L. angustifolius* isolate treated similarly as its flour had an emulsifying stability of 66.7-71.0 % (Lqari et al., 2002). In this case we do not see much difference between the emulsifying stability of the flour and its isolate, possibly because the measurements were taken after a very short period of time. It would be interesting to know the behaviour of those samples after a couple of hours. The emulsifying stability of protein isolates is also influenced by the procedure used to obtain them. In *L. albus*, protein was isolated by two different approaches. In the first one the protein was obtained by solubilisation at pH 7 followed by precipitation at pH 4.5. In the second approach protein was obtained by ultra-diafiltration at pH 4.5, with a cut-off =10 kDa. Next both protein isolates were emulsified (1 protein:10 oil:10 water) (w:v:v), homogenized at 11,000 rpm for 5 min., heated for 30 min at 80 °C, stored for 12 h at 5 °C, and finally centrifuged for 10 min at 4500 x***g*** and 20 °C. The isolate obtained by the first treatment showed an emulsifying stability of 61-63 %, against 74-93 % for the second isolate (D´Agostina et al., 2006). The isolate obtained by ultra-/diafiltration had a higher emulsifying stability as it contains globulin, albumin and protein-polysaccharide complexes (Alamanou and Doxastakis, 1995), which enhance the emulsifying stability due to steric repulsion effects (Dickinson and Walstra, 1993). Isolate obtained by isoelectric precipitation does not contain albumin, and the amount of protein-polysaccharide complexes is lower. These finding agree

with Kinsella (1984), who reported that the emulsifying stability is influenced by conformation stability and charge.

Foaming capacity

Like emulsions, foams are two-phase systems, with one phase dispersed in an aqueous continuous one. Foam formation is significantly affected by protein surface activity (Moure et al., 2006), processing procedure and protein composition (Tolstogouzov, 1991). Different definitions exist for foaming capacity (D´Agostina et al., 2006; Lqari et al., 2002; Sathe et al., 1982); we use the definition that determines foaming capability as the relation, in percentage, between foam volume after whipping / initial volume of the protein solution x 100. In some cases we recalculated the original data to express the foaming capacity similarly for all studies.

Lupin proteins have the lowest foaming capacity among the proteins from legumes and oilseed crops (Gueguen and Cerletti, 1994). A slurry with 2 % of *L. mutabilis* flour had a foaming capacity of 132 % after whipping for 5 min at 21 ºC. However, in the same conditions, this value increased to 180 and 186 % when the flour content in the slurry was increased to 6 % and 10 %, respectively (Sathe et al., 1982). The foaming capacity for concentrates of *L. mutabilis* is slightly better than for the flour (Sathe et al., 1982) (Table 9). The addition of extra flour, concentrates or isolates to the slurry increases the amount of proteins in the system. Since proteins are surfactant materials (Moure et al., 2006), the interfacial tension in the slurry is reduced (Hettiarachchy and Ziegler, 1994) and the foam volume is increased. Defatting *L. mutabilis* concentrate also increased its foaming capacity, namely from 150 to 158 %, probably because defatting reduces the possible competitive effect of lipids in the interface (Moure et al., 2006). However, defatting also could reduce the foaming capacity when the solvent used, for instance hexane, removes no polar lipids such as triglycerides and excludes polar lipids such as fatty acids and phospholipids (Doxastakis, 2000). This may cause a degree of denaturation in the protein molecule that affects foam formation (Alamanou and Doxastakis, 1997; Kiosseoglou and Perdikis, 1994). Other lupins have a higher foaming capacity than *L. mutabilis*. For instance, in the case of a 3 %-slurry of *L. angustifolius* flour that was homogenized at 10,000 rpm and pH 7, the foaming capacity was 214 % (Lqari et al., 2002). For a 5 %-slurry of *L. albus* protein isolate the foaming capacity was between 1102-2083 % (D´Agostina et al., 2006), which is about five to ten

times the foaming capacity of *L. mutabilis.* Such a difference can be understood, at least partially, by the fact that foaming capacity is not only related to the protein content of the isolate but also to its structure. Special attention should be given to the ratio vicilin / legumin proteins. In pea, the vicilin fraction has been shown to be more active at the air/water interface than legumin (Dagorn-Scaviner et al., 1987). In addition, vicilin had a higher diffusion coefficient and showed higher flexibility than legumin-like protein (Dagorn-Scaviner et al., 1987). Consequently, the equilibrium surface pressure in the interfaces is reached more quickly for the vicilin fraction (Dagorn-Scaviner et al., 1987). *L. albus* and *L. mutabilis* have a very different vicilin and legumin protein structure and composition, as stated earlier, and this difference is apparently in favour of the foaming capacity of protein from *L. albus*.

The addition of NaCl and carbohydrates may improve foaming capacity of lupin protein. Sathe et al. (1982) reported an increase in the foaming capacity from 150 to 174 % when 0.6 % of salt (NaCl) was added to a slurry of 2 % *L. mutabilis* concentrate, which may have been due to increased protein solubility (Sathe et al., 1982). The same authors reported that carbohydrates, such as potato starch, amylopectin, sucrose and amylose, at a concentration of 0.25g/ g concentrate increased the foaming capacity. However, galactose, gum arabic and pectin had the opposite effect. The increase in the foaming capacity is in some cases attributed to the formation of protein- polysaccharide complexes that generate stability due to steric repulsion effects (Dickinson and Walstra, 1993), which are absent with others carbohydrates.

Foam stability

According to Doxastakis (2000), proteins play an important role in accumulating at the bubble surface to produce a viscoelastic adsorbed layer that protects the fill against ruptures and prevents or retards Oswald ripening. Foaming stability is defined as the relation between the foam volume and time (D´Agostina et al., 2006). The foam stability of a 2 % slurry of *L. mutabilis* flour at 21°C is reported as 93.9, 92.4, and 78.8 % after 1, 2 and 36 h, respectively (Sathe et al., 1982). For a 3 % slurry of *L. angustifolius* flour the reported value is 82 % after 1 h and 79.2 % after 2 h (Lqari et al., 2002), and for a 2% slurry of *L. mutabilis* concentrate 94.5, 88 and 76 % after 1 h, 2 h, and 36 h, respectively (Sathe et al., 1982). For a 3 % slurry of *L. angustifolius* isolate the foam stability was about 80 % after 1, or 2 h (Lqari et al.,

2002). In the case of a 5 %-slurry of *L. albus* isolate, the foam stability varied from 68 to 95 % after 1 h (D´Agostina et al., 2006). Variations in foam stability are attributed to protein surface activity, which is related to conformation and ability to unfold at interfaces, as determined by molecular factors (*i.e.*, flexibility, conformational stability, distribution of hydrophobic and hydrophilic residues in the primary structure) (Damodaran, 1997; van Vliet et al., 2002). pH and carbohydrates can also affect foaming stability. Sathe et al. (1982) reported higher foaming stability for a 2 % slurry of *L. mutabilis* concentrate at pH 2 after 2 h (128 %) as compared to foaming stability at pH 4 (114 %), 6 (116 %) or 8 (108 %). The higher foaming stability at the acidic pH range may be due to the formation of stable molecular layers in the air-water interface, which impart texture, stability and elasticity to the foam (Sathe et al., 1982). The addition of 0.25 g carbohydrates (galactose, sucrose, amylose, amylopectin, potato starch, gum arabic and pectin) g^{-1} protein decreased foaming stability after 36 h to 106, 110, 108, 105, 102, 100, and 100 %, respectively, compared with the slurry without carbohydrates (114 %). The adverse effects of carbohydrates on foaming stability of lupin proteins may result from thinning of the films due to a random distribution of carbohydrates and also by an increased coalescence of gas bubbles dispersed in the liquid (Sathe et al., 1982).

Despite the lower efficiency of proteins as compared to low molecular weight surfactants in reducing surface tension, the foams formed with proteins are more stable because proteins give more flexibility and stability to the air-water system by decreasing the interfacial tension (Moure et al., 2006). In other words, lupin proteins have lower foaming capacity than low molecular weight surfactants, but higher foaming stability.

Least gelation concentration

Least gelation concentration is understood as the minimum amount of material that has the capacity of producing a stable gel. A gel is stable when a boiled and cooled sample does not fall down or slip from an inverted test tube (Sathe et al., 1982). This relation is expressed as a percentage of the weight of lupin flour, concentrate or isolate per volume. Heating soya bean protein slurries above their denaturation temperature results in the formation of a high-viscosity progel (Gueguen and Cerletti, 1994). Upon cooling, the proteins -in their unfolded conformation- form the gel through disulfide, hydrogen and hydrophobic interactions (Gueguen and Cerletti, 1994). For *L. mutabilis*, the least gelation concentration has been

reported as 14 % for its flour and as 8 % for its isolate (Sathe et al., 1982). For *L. angustifolius* this value was 6 % for its flour and 10-12 % for its isolate. Both determinations were at pH 7 (Lqari et al., 2002). The high variability in the least gelation concentration of proteins may be related to their composition and degree of unfolding. For instance, in soya bean the 7S protein formed a gel at a lower concentration than the 11S protein (Gueguen and Cerletti, 1994), and so differences in the degree of the denaturalisation might explain why commercial isolates may have different gelling properties that depend on the preparation process (Gueguen and Cerletti, 1994). For instance, the pH used during the preparation of isolates and concentrates affects the last gelation concentration of proteins. For *L. albus* isolate the reported values are 14 % for isolate obtained at pH 4.9 and 16 % after additional neutralisation at pH 7 (King et al., 1985). The acid side of the isoelectric pH helps to form stable gels of globulins because in this environment carboxylic groups are less dissociated, and the interactions between protein molecules and the solvent increase (Gueguen and Cerletti, 1994). In addition, the variability in the least gelation concentration of proteins may be ascribed to the relative ratios of components other than proteins (Thompson and Casey, 1983), such us carbohydrates and lipids, suggesting interactions between those components (Sathe et al., 1982). In general, the least gelation properties of protein from lupin species are similar or in some cases better than those of soya bean, which was reported as 13% for protein isolate (King et al., 1985).

Improving the functional properties of lupin proteins

According to Feeney and Whitaker (1985), the functional properties of lupin proteins can be improved by modifying protein structures and conformation at different levels, and by optimizing characteristics such as size, the hydrophobicity / hydrophilicity ratio (specially at the surface) and the molecular flexibility of proteins. Modification can be achieved by denaturation of proteins using various treatments, like physicochemical (pH variations), physical (heat), chemical (acylation, succinylation) and enzymatic treatments (Gueguen and Cerletti, 1994). The effect of pH and heat was discussed before under the heading Physical and functional properties of lupin proteins (lupin flour, protein isolates and concentrates).
Acylation with succinic anhydride (acting on Lys and Tyr) improves the solubility, as well as the emulsifying, foaming and gelling properties (Beuchat, 1977; Kinsella and Shetty, 1979) by affecting the charge distribution and net charge of protein molecules (Moure et al., 2006).

These functional properties depend, however, on the degree of acylation (Gueguen and Cerletti, 1994). For instance, for faba bean (*Vicia faba*) proteins, 50-70 % of acylation produced a good gel, whereas the emulsion stability and viscosity were significantly enhanced for 97 % of modification (Muschiolik et al., 1987).

Succinylation also increases some functional properties of proteins (Moure et al., 2006). However, the degree of succinylation affects the physical properties of different materials in different ways. For instance, in faba bean the maximum foaming capacity was reached at >80 % succinylation (Gueguen and Cerletti, 1994) and the maximum foaming stability for soya bean glycinin at 25 % succinylation. Other chemical treatments that improve functional properties are acid hydrolysis, alkylation, oxidation, esterification, amidation, deamidation and phosphorylation (Moure et al., 2006).

Enzymatic hydrolysis can increase protein solubility (Arias and Felacio, 1986; Were et al., 1997) by breaking up peptide bonds to produce peptides with desired size, charge and surface properties (Moure et al., 2006) to achieve an elevated charge and electrostatic repulsion. Trypsin treatment of protein products results in higher solubility and water hydration capacity than in the corresponding untreated product (Jones and Tung, 1983). Protein hydrolysis increases the foaming capacity and stability and gelation capacity of flour (Hrčkova et al., 2002; Taha and Ibrahim, 2002).

Physical treatments other than the use of heat, such us high-pressure, improve the functional properties by unfolding and exposing hydrophobic sites (Molina et al., 2002). Co-precipitation of proteins from different vegetable sources with whey proteins yield protein isolates with better functional properties than those of their individual isolates (Lawhon et al., 1980).

Finally, as each protein source may react in a different way to physical, physicochemical, chemical and enzymatic modification treatments, it is necessary to determine and standardize the appropriate treatment for each specific protein application.

Conclusions

Variation in the composition of Lupinus mutabilis

This review shows that most of the reported values on the nutrient contents of raw and debittered lupins vary greatly. The causes of these variations are not precisely known, but

several assumptions can be made. The variations may be due to the quality of the sample (obtained from a few or several plants, from markets or research institutes, storage conditions and age of the sample), the analytical methods used (accuracy, precision), the variety of lupin analyzed, variations within each sample due to agronomical conditions (physical and chemical soil quality, availability of water and sunlight), genetic aspects (which influence plant form, susceptibility to frost and diseases, growth cycle, protein, oil and alkaloid contents) (Haq, 1993), and different factors used for converting free nitrogen into protein (namely 5.4, 5.5, 5.7, and 6.25). In the case of debittered lupin, the methods used for debittering could also influence its nutrient content. However, in spite of the observed variations, *L. mutabilis* showed the highest protein and fat contents among the main lupin species. Most essential amino acids and an substantial amount of unsaturated fatty acids are present in raw lupin.

Alkaloid content

The alkaloid content in *L. mutabilis* reportedly varies between 0.07 and 4.5 g/100g dw. In general the alkaloid content in *L.* m*utabilis* is higher than that of the other main lupin species. Observed variations in alkaloid content in lupins depend on different factors such as analytical procedures, sub-species or ecotype studied, genetic variability, agronomical factors and environmental influences. The main alkaloids reported in *L. mutabilis* are lupanine, sparteine, 3-hydroxylupanine, 13-hydroxylupanine, and 4-hydroxylupanine. From those lupanine is also the main alkaloid reported in other lupins.

Toxicity

Most of the information on toxicity of alkaloids in humans comes from reports of accidental events and from a few studies. Therefore, the toxicity in humans (specially the chronic toxicity) is not well known, but several assumptions can be made. Infants and children are more susceptible to alkaloids than adults. The provisional minimum lethal acute dose of total alkaloids for infants and children is considered to be 10 mg kg^{-1} body weight and for adults this is 25 mg kg^{-1} body weight. As to chronic toxicity, there are no established safe daily doses. Values reported as safe vary from 0.035 mg kg bw^{-1} day^{-1} up to 500 mg per day. Based on the Andean region, the amount of alkaloids in the diet is clearly higher than 0.035 mg kg^{-1}

day^{-1}, suggesting that this value is underestimated. This would imply one of the following options (i) the Andean population developed a certain resistance to alkaloids as compared to the European population, (ii) lupin consumers in the Andean population are suffering from chronic disease, or (iii) the value 0.035 mg kg bw^{-1} day^{-1} is wrong. On the other hand, the value of 500 mg per day seems to contradict even acute doses of 10 mg kg^{-1} bw in children, suggesting that this value is also wrong or at least overestimated. In addition, Jiménez-Mantínez et al. (2003a) suggested that alkaloid contents in the seed that are above 0.03 % could result in a decrease in animal growth. This value is lower than the 0.07 % that is the maximum value accepted by the Ecuadorean Institute of Standards (INEN Instituto Ecuatoriano de Normalización, 2005). In short, there is no established safe daily amount (dose) of alkaloids for human beings, pointing out the necessity to do research in this field.

Allergenicity and anti-nutritional factors

Taking into account the test of allergenicity and the anti-nutritional content of lupins we might consider them as safe for human consumption. However, because there is just one reported study of allergenicity, it is necessary to do more research in this field to supplement the initial findings.

Debittering processes

Biological, chemical, and aqueous debittering processes can reduce the alkaloid content in lupin seeds with different outcomes depending on the conditions. First, bacterial or fungal fermentation reduced alkaloid contents, but from seeds with low alkaloid contents (lower than 1 %). The applicability of the fermentation process as a means to reduce alkaloids in lupin seeds with higher alkaloid contents remains to be investigated. In addition, part of the alkaloid reduction by the fermentation approach is due to the initial processing steps, namely soaking and cooking. For tempeh fabrication, the simultaneous debittering-fermentation process can be accelerated by using lupin seeds that are first dehulled, crashed or flaked, soaked, and cooked, since these operations facilitate the contact between alkaloids and strains. In addition, hydration increases the water content in the seed and facilitates alkaloid extraction during the following steps. Cooking is essential to destroy the germinative capacity of seeds, inhibit enzymatic activity (by lipase, lipoxygenase), eliminate microorganisms

adhered to the seed (which could produce toxins), reduce the loss of proteins through their coagulation and to facilitate the physical washing away of the alkaloids, oligosaccharides, or other anti-nutritional factors (because of increased cell wall permeability) (Gross et al., 1983; Jiménez-Martínez et al., 2003a). During fermentation the K^+ content (and perhaps that of other nutrients) might have to be monitored because the efficiency of the process was reported as K^+ dependent. Fermentation changes the taste and texture of lupin, which can be an advantage or disadvantage depending on the food type that consumers prefer. In addition, most fermentation processes need to use energy for several days, which is a disadvantage for economic and sustainability reasons.

Chemical treatments can reduce the alkaloid content in lupins, even in seeds with high amounts of alkaloids (up to 4.2 %), and in some cases in a short time (*i.e.* less than one hour). Basic treatments diminish the methionine content and therefore reduce lupin PER. Chemical treatments also cause about 13 % material losses. In addition, there is still uncertainty regarding the safety of these chemically treated products, customer acceptance, disposal of chemical compounds, and possibilities of water reutilization.

Aqueous treatments can also reduce the alkaloid content in lupin seeds, even in those with a high alkaloid content. These processes do not require the disposal of chemicals, nor a complicated infrastructure. Moreover, they do not change the availability of methionine. For debittering whole seeds to be used as food for humans, the aqueous treatment is the only process known to be applied on a commercial scale. The aqueous treatment reduces the alkaloid content in the whole seed without changing its natural flavour. This is especially important when the whole seed is eaten as a snack. This process uses significant amounts of water and time, and causes material loss. However, material lost can be recovered by centrifugation, decantation or flocculation. The water used can be treated and reused several times and the speed of the process can possibly be improved by enhancing the diffusion of alkaloids during processing.

When the above methods are compared, we see that particle reduction is applied in biological and chemical processes to speed up the removal of alkaloids, but it is not used in aqueous debittering. Incorporating this pre-treatment in the aqueous debittering process could also speed up the washing out of alkaloids and perhaps of some antinutritional factors. Particle size reduction could also diminish the use of water, energy and labour and can be applied when debittered lupin will be used as flour, or a food ingredient.

When lupin protein is used as functional food ingredient, it is important to consider that most of its functional properties will be modified if protein denaturation occurs during the isolation, for instance, due to physicochemical (pH variations), physical (heat), chemical (acylation) or enzymatic treatments. For example, faba bean protein precipitated at pH 2 has a decreased solubility in both alkaline and acid conditions, but its water adsorption capacity is increased about three-fold (Gueguen and Cerletti, 1994).
Debittering of lupin seeds with high alkaloid contents requires further research, especially with respect to the efficiency, sensorial quality and economic feasibility.

Uses of lupins

After debittering, lupins can be used as a food: eaten directly as snack, or as an ingredient of many products and meals because of the nutritional value of the seed, especially for *L. mutabilis*, which is comparable to that of soya bean. The nutritional value of the seed can be affected by the debittering process applied. However, on the other hand, the nutritional value can be increased by fortifying with DL-methionine or by eating lupin in combination with a product rich in sulphur-containing amino acids, such as cereals. Doing so increases the PER value.
Alkaloids from lupins can be used in the medical field. Some studies suggest that certain QA have pharmacological activity. However, more research is needed to validate preliminary results, and to establish action mechanisms, doses, protocols and contra-indications.
The similarities and differences between lupin and soya proteins in terms of physical characteristics point at opportunities for increasing the use of lupin as an ingredient in the food industry, or even the replacement of soya as a food ingredient in countries where lupin is abundant and when lupin protein shows a better or similar physical behaviour as soya. For example, the structural changes during protein gelation appear to be similar for *L. albus* and soya bean proteins (Kiosseoglou et al., 1999). Lupin applications can benefit from the extensive research in the area of soya bean based food (Doxastakis, 2000). The available research shows that lupins could be used as a functional ingredient for the following properties:
- *L. mutabilis* concentrate for its oil absorption capacity, emulsifying activity, emulsifying stability, protein solubility, foaming stability, and least gelation concentration.

- *L. angustifolius* isolate for its water absorption capacity and flour for its water absorption capacity and least gelation concentration.
- *L. albus* isolate for its emulsifying capacity, protein solubility and foaming capacity.

However, there are so many variables and interactions, which are not yet fully understood, that affect the behaviour of lupin isolates and concentrates, that the suggested applications should be regarded as preliminary and only valid for the conditions and varieties studied.

Recommendations

With respect to future research we recommend:

1. To pay more attention to experimental procedures, such as sampling, storage conditions, specification of genetic make-up, and agronomical conditions of samples to obtain more precise information about the cause of biological variation. In addition more attention should be given to the reporting of the accuracy and precision of the experimental methods and obtained results.
2. To investigate lupin seeds as protein and fat sources (especially for *L. mutabilis*), their flours, protein isolates, and sub-products (alkaloids, oligosaccharides and molasses).
3. To determine the maximum tolerable alkaloid content in the human diet and in the debittered seeds, as the safe doses for humans are still unclear.
4. To further investigate the debittering processes with a focus on the nutritional quality of the debittered seed, the effectiveness of the process expressed as extracted alkaloids, energy and time used, residues generated, solids lost, consumer acceptance, and the possibility to reutilise (or to reduce) processing water (and, if applicable, chemicals), and economic feasibility of the applied technique.

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Chapter 3

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} m^2s^{-1} because the lupine endosperm is a polymer matrix which 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 microbial quality of 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.

Keywords:

Lupine, *Lupinus mutabilis*, alkaloids, debittering, efficiency, diffusion modeling

Introduction

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

The FAO (2009) reported that more than 585,000 metric tonnes 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, Perú, and Chile and which shows the highest average content, on a dry weight basis, of protein (440 g kg^{-1}) and lipids (180 g kg^{-1}) (Pate et al., 1985). *L. mutabilis* is commonly known in Chile as "lupino" (Peralta et al., 2001), in Perú as "tarhui" (Aguilera and Trier, 1978; Torres-Tello et al., 1980) or "tarwi" (Aguilera and Trier, 1978; Caicedo et al., 2001; Santos et al., 1997) 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 which production increased from 662 metric tonnes in 1986 (Moncayo et al., 2000) to 789 t in 2000 (Junovich, 2003), with up to ninety percent of this production destined for human consumption (Aguilera and Trier, 1978; Caicedo et al., 2001; Gueguen and Cerletti, 1994; Petterson and Crosbie, 1990; Santos et al., 1997; Zduńczyk et al., 1996). Its nutritional value is similar to that of soya bean (Aguilera and Trier, 1978; Gueguen and Cerletti, 1994; Petterson and Crosbie, 1990; Santos et al., 1997; Zduńczyk et al., 1996) 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 et al., 1983; Aguilera and Trier, 1978; 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 et al., 1983; Aguilera and Trier, 1978; Beirao da Costa, 1989; Gueguen and Cerletti, 1994; Haq, 1993; Petterson and Crosbie, 1990; Zduńczyk et al., 1996). Detoxification was attempted by chemical (Chango et al., 1993; Jiménez-Martínez et al., 2003a; Nossak et al., 2000; Ortiz and Mukherjee, 1982; Torres-Tello et al., 1980; Touchė et al., 1997; von Baer et al., 1979) and biological methods (Agosin et al., 1989; Camacho et al., 1991; Santana and Empis, 2001; Santana et al., 1996; Szakács and Stankovics, 1983). 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 (Aguilera et al., 1983; Torres-Tello et al., 1980) 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 ºC, boiling for 40 min., and then washing at 40 ºC (Caicedo et al., 2001). The process uses potable water and applies good manufacturing practices (GMP) (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 at 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 5 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 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 two days, followed by 6 h intervals the next day; during the final washing period samples were evaluated each hour.
For composition, 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 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 (cfu, colony forming units) was determined by AOAC method 966.23, fungal plate count by AOAC 997.02 (AOAC, 2005) and *E. coli* (MPN, most probable number) by the method of the Ecuadorean Institute of Standards (Instituto Ecuatoriano de Normalización) (INEN, 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, U.S.A) and sieved with a shaking 60 mesh sieve (Meinzer II, Series 0447, Fairfax, VA, U.S.A, dual model, MFG Co. Chicago, Il, U.S.A.), 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 0.01M solution of sodium hydroxide was prepared by diluting a standard solution 0.1M of sodium hydroxide (Merck Ecuador, 109141100) in freshly distilled water. A solution 0.005M of sulfuric acid was obtained by diluting sulfuric acid 0.05M (for titration, 1099840001) in freshly distilled water. Potassium hydroxide solution (2.68M, 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 4 - 5 drops of 0.005M sulfuric acid and 3 - 4 drops of Dragendorff's reagent to 1 ml of the final extract: a negative reaction (absence of orange coloured 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 ºC until 1 ml remained; this was further evaporated when cooling the sample in a 15 ºC water bath. For the determination of alkaloid concentrations, 5 ml of 0.005M sulfuric acid and 2 drops of methyl red indicator were added to the vial. The excess acid was titrated with 0.01M NaOH. The concentration of total alkaloids, expressed as lupanine content (g $100g^{-1}$), was calculated as follows (INEN Instituto Ecuatoriano de Normalización, 2005):

$$\text{Total alkaloids expressed as lupanine (g/100g)} = \frac{0.248 \times V}{\text{weight of sample (g)}} \qquad (1)$$

$V = V_0 - V_1$

V_0 = 5 ml

V_1 = ml used in titration

0.248 represents the weight of lupanine (g) in a solution 0.01M (2.48g) x 100 (to express the result directly in percentage) /1000 (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 (Figure 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 (Caicedo et al., 2001). Each batch started with 1000 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 am, 12 pm, and 4 pm. 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:

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

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

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

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 ($m^2 s^{-1}$)

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 1×10^{-10} $m^2 s^{-1}$ (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 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^{-1}), D_s the diffusion constant in m^2 s^{-1}, x the distance over which the molecules diffuse, t the time in s. The boundary conditions needed to solve this equation are (i) only one-dimensional radial diffusion is considered over distance x, (ii) the alkaloids are homogeneously distributed within the seed at time zero, (iii) the surrounding water is free of alkaloids at the start, (iv) there is no concentration gradient of alkaloids in the water, and (v)
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 25×10^{-6} m^2, being present in a volume of 2 ml of water.
These equations were numerically solved using the software program Athena Visual Studio (www.athenavisual.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 equations (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 x 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 08h00, 16h00 and 22h00. 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 9 treatments were as follows: 3 changes of water, time 1 (Ch3, t1); 3 changes of water, time 2 (Ch3, t2); 3 changes of water, time 3 (Ch3, t3); 6 changes of water, time 1 (Ch6, t1); 6 changes of water, time 2 (Ch6, t2); 6 changes of water, time 3 (Ch6, t3); 9 changes of water, time 1 (Ch9, t1); 9 changes of water, time 2 (Ch9, t2); and 9 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^{-1} d.w. raw lupine) and solids in the product (kg kg^{-1} d.w. raw lupine).The results were analyzed by using the software program Expert Design 8 (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, three, five, six or even ten samples were taken. Each sample was analyzed in duplicate.

The Mann-Whitney–U-test, a non parametric 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., GraphPad Software Inc., San Diego, California, U.S.A. was used.

Results and discussion

In the village of San Pedro in the Province of Cotopaxi in Ecuador, lupine was debittered with cold water (16 ºC) according to the procedure shown in Figure 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 tonnes of water per tonne of raw lupine (Caicedo et al., 2001), and too much time.

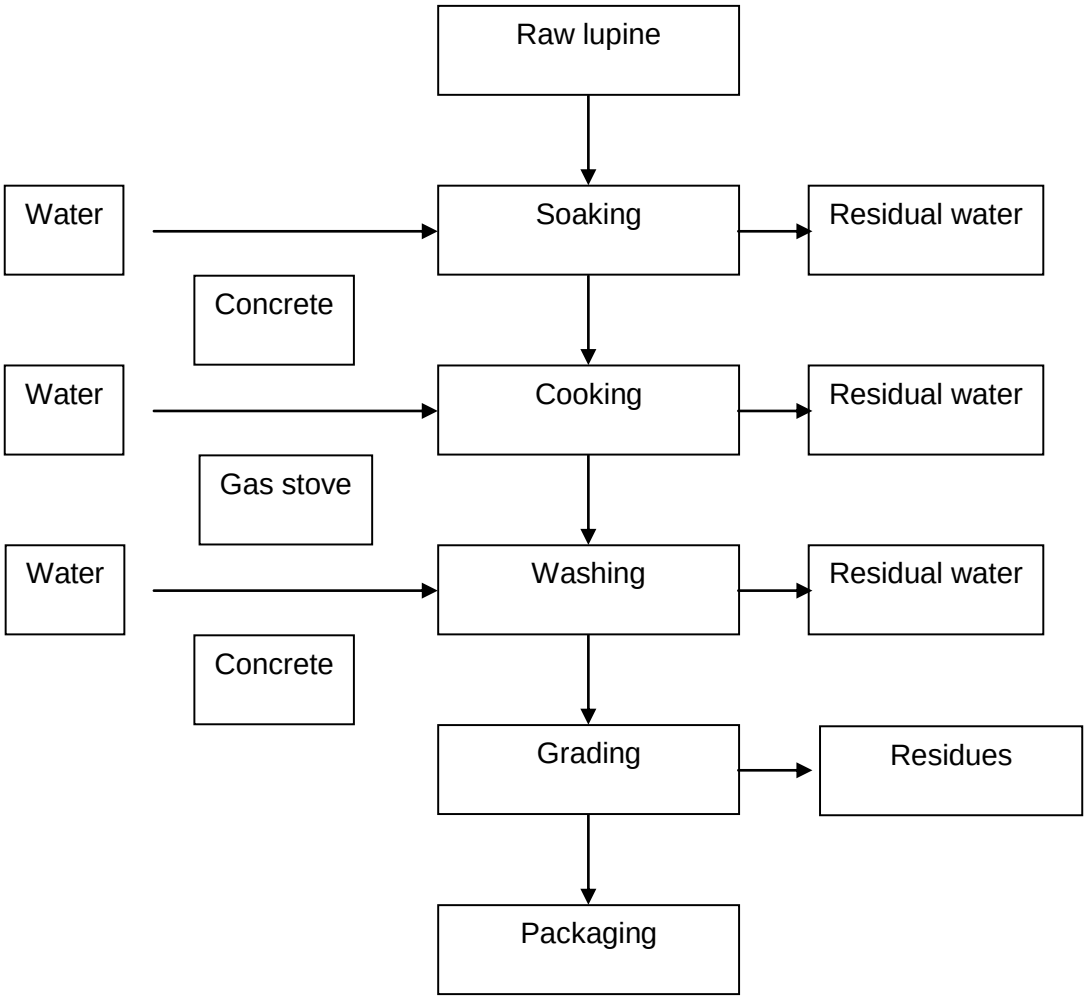


Figure 1. Process for debittering lupine as performed in the village of San Pedro, Cotopaxi Province, Ecuador

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 ± S.D.[1]	12 ± 4.6	0.4 ±0.4	5.2 ± 0.8	5.7 ± 1.0
Minimum time	8.0	0.2	4.5	
Maximum time	18.0	1.0	6.0	

[1] Values represent means ± 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.

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, 3 and 4. A comparison of the proximate analyses of raw and debittered lupine based on dry weight as presented in Table 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 100 g^{-1} dm as in Table 2.

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[1]	41.4 ± 0.08[2]	55.9 ± 2.56[3]	P < 0.05[4]
Fat[1]	23.4 ± 0.42	8.9 ± 2.57	P < 0.05
Carbohydrates[1]	26.6 ± 0.21	33.1 ± 2.50	P < 0.05
Ash[1]	5.0 ± 0.07	1.9 ± 0.11	P < 0.05
Alkaloids[1]	3.6 ± 0.07	0.2 ± 0.01	P < 0.0005
Iron[5]	5.0 ± 0.43	5.8 ± 0.61	NS
Zinc[5]	3.6 ± 0.04	3.6 ± 0.45	NS

[1] g 100g^{-1} dry matter
[2] Values represent means ± standard deviation (n= 6)
[3] Values represent means ± standard deviation (n=5)
[4] Mann- Whitney –U- test; NS=not significant
[5] mg 100g^{-1} dry matter

We presented data on the formats of Tables 2 and 4 to point out errors than can be committed when reporting and discussing results of a process where losses of materials take place, without taking these into account. From here to on 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 Most Probable Number method. As most of this product is consumed without any further processing, it must represent a potential hazard for human consumption.

Table 3. Microbiological quality of raw and debittered lupine collected from the village of San Pedro, Cotopaxi Province, Ecuador

Parameter	Raw lupine[1]	Debittered Lupine[2]	P value[3]
Total mesophilic aerobic bacteria (Log cfu[4] g^{-1})	2.67	6.71	P < 0.05
Fungi (yeasts and moulds) (Log cfu g^{-1})	2.59	1.99	P < 0.05
E. coli (presence g^{-1})	Present	Present	

[1] Values represent means (n= 5)
[2] Values represent means (n=5)
[3] Mann-Whitney –U- test
[4] cfu g^{-1} = colony-forming units per gram fresh weight

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 (Figure 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 (Figure 2), a value similar to the 26.9 % loss reported by Torres-Tello *et all*. (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%). During the debittering process other compounds such as oligossacharides were not measured. Of the micronutrients, we only analyzed zinc and iron because those are the most important from a nutritional point of view. 20.8 % of the zinc and 9.8% of the iron concentration are lost (Table 4).

Table 4. Dry matter balance of nutrients and alkaloids during the debittering of raw lupine

Material	Raw	Debittered	Difference (g)	Difference (%)
Dry Matter (g)	**1000**	**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

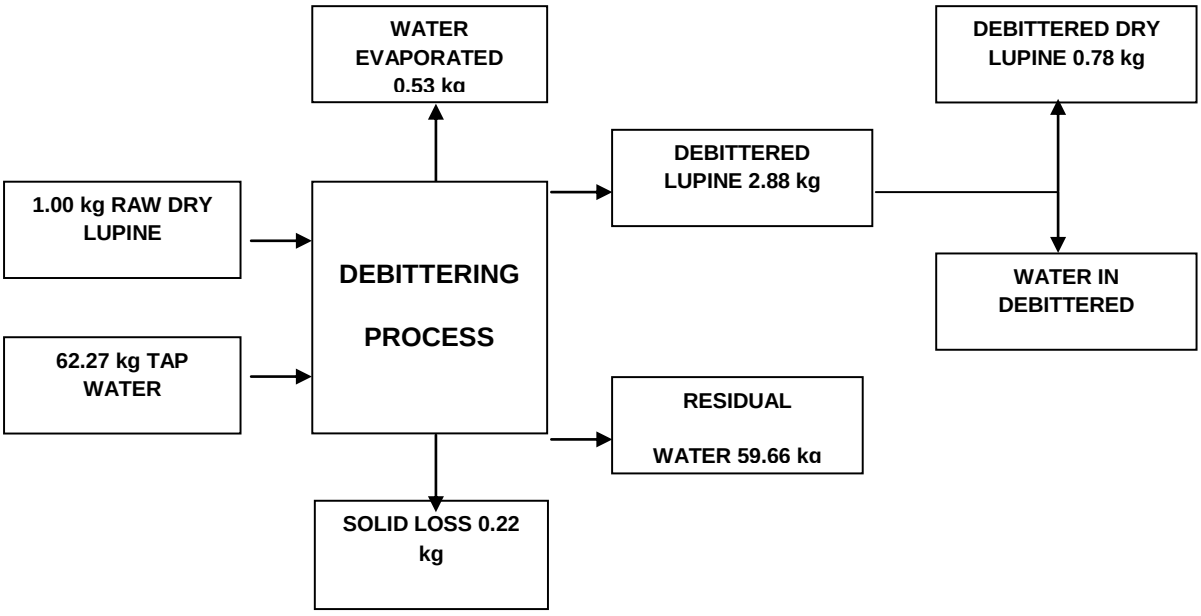


Figure 2. General mass balance for 1 kg of raw dried lupine

The debittering process using cold water removed 94.9 % of the total alkaloids (Table 4), compared with 79 to 99.9 % removal achieved by chemical methods (Chango et al., 1993; Jiménez-Martínez et al., 2003a; Ortiz and Mukherjee, 1982; Torres-Tello et al., 1980), 14.9 to 99 % by biological methods (Agosin et al., 1989; Camacho et al., 1991; Santana and Empis, 2001; Santana et al., 1996; Santana et al., 2002; Szakács and Stankovics, 1983), 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.

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 100 g^{-1} 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^{-1} raw dry lupine)	2.9	2.9	56.5	62.3
Time used (h)	18.0	1.0	118.3	137.3
Water Efficiency[1]	0.69	3.79	0.37	0.55
Time Efficiency[2]	0.11	11.00	0.18	0.25

[1] Alkaloids extracted (g kg^{-1} water)
[2] Alkaloids extracted (g kg^{-1} raw-dry lupine h^{-1})

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 hour of cooking removed 30.7 % of the alkaloids, whereas the washing step, conducted at an ambient water temperature of 15 °C, required 118.3 hours 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} m^2 s^{-1} (Walstra, 2003), which is represented in Figure 3A. However, a lupine seed is not an aqueous environment and consequently the diffusion coefficient would be expected to be lower than 10^{-10} m^2 s^{-1}. On the other hand, the practical situation resulting in adequate detoxification in 5.7 days, strongly suggests a higher value than 10^{-11} m^2 s^{-1} as illustrated in Fig. 3B.

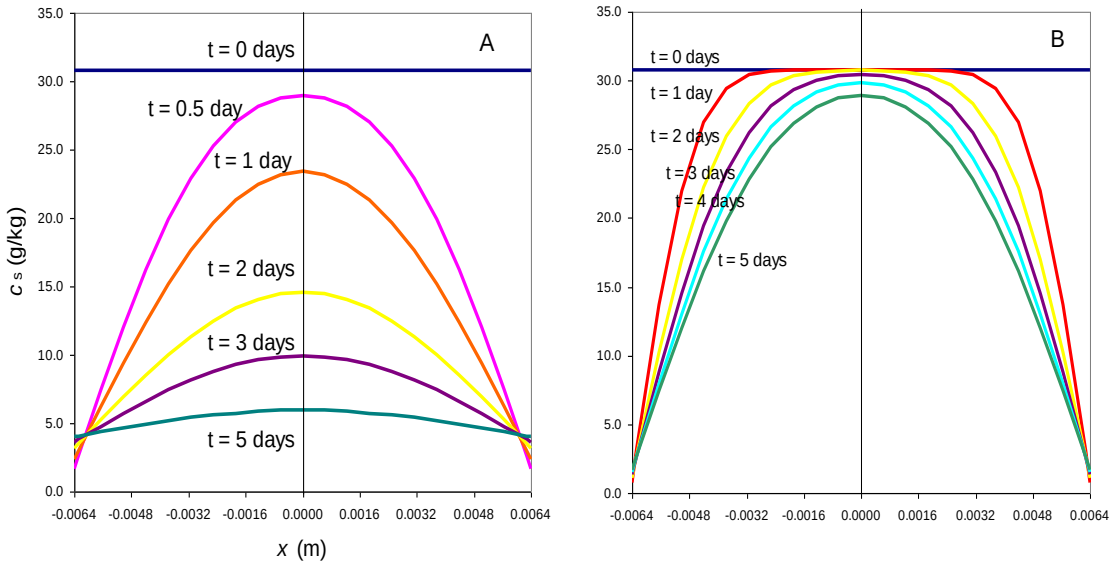


Figure 3. Simulation of extraction according to Fickian diffusion of alkaloids in a lupine seed. Distance x = 0.0128 m, contact area A = 2.5 x10^{-5} m^2, V_w = 2 ml, initial concentration c_s = 30.8 g alkaloid/kg lupine, D_s = 1x10^{-10} (A) and D_s = 1x10^{-11} m^2 s^{-1} (B)

Thus, the mathematic model suggests an apparent diffusion coefficient between 1x10^{-10} and 1x10^{-11} m^2 s^{-1}. Indeed, when we substitute in equation (4) t with 492480 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 $x10^{-11}$ m^2 s^{-1}. 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

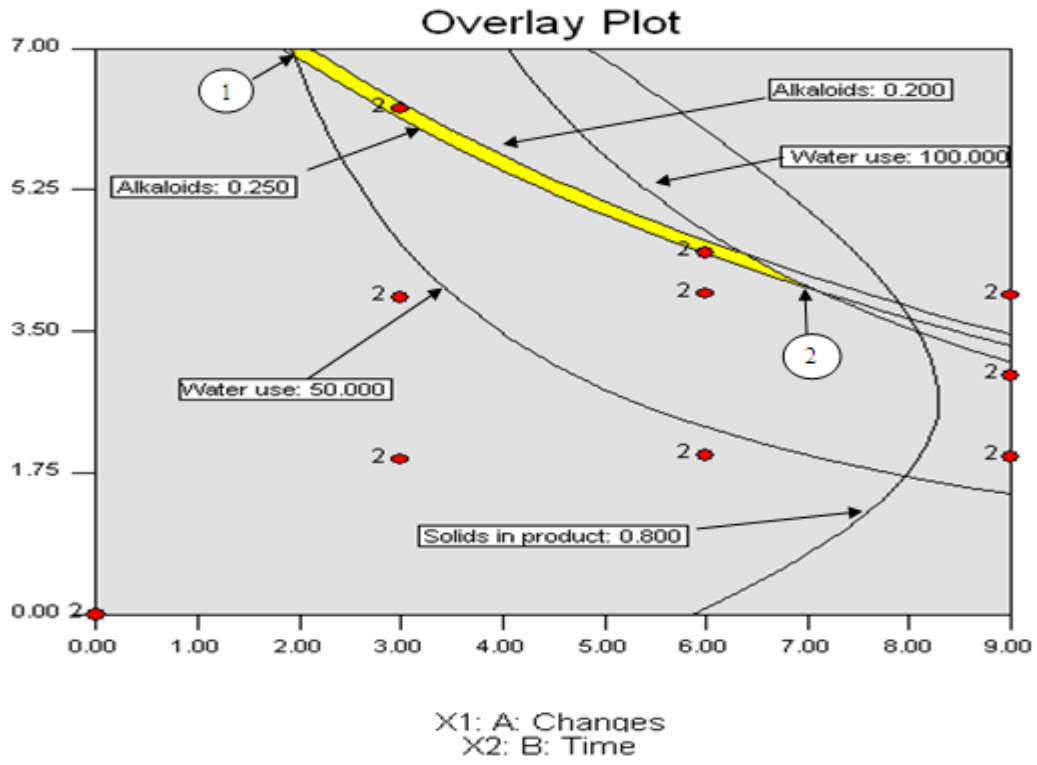
The impact of changes of water on alkaloids content, water used and solids in the product throughout time can be observed in Table 6. Increasing the number of changes of water day $^{-1}$ diminished the time needed to debitter lupine and the solids in the product. However, as it was expected that augmented the amount of water used. The reduction of processing time by increasing the changes of water agrees with Fickian diffusion equations (5) and (6).

Table 6. Treatment tested to optimize the debittering process of lupine (n = 2)

	Factors		Variables		
Treatment code*	Changes of water Times day^{-1}	Time of processing Days	Alkaloids % d.w.	Water used l kg^{-1} d.w. raw lupine	Solids in product Kg kg^{-1} 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)

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.



Point	Optimization Process				
	Changes of water Times day $^{-1}$	Time used Days	Water used l kg^{-1} d.w. raw lupine	Solids kg kg^{-1} 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**

Figure 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^{-1} d.w. raw lupine, solids in product > 0.8 kg kg^{-1} 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).

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 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 (Sievwright and Shipe, 1986). Finally, a continuous replenishment of water in the washing operation might accelerate the diffusion of alkaloids.

Conclusions

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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Chapter 4

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 18h of soaking, 1h cooking, 3 changes of water/day and 22h 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} *****Agitation – 0.0043 * Changes - 1.681^{-3} Agitation* Changes)**.** The new developed technology could be used to optimize processes such as hydratation and/or removal of undesired materials of legumes and other seeds.

Keywords

Extraction, debittering, lupin, alkaloid, modeling, optimization

Introduction

Lupins (*Lupinus spp*) are legumes (Pate et al., 1985) used principally as a protein source in human and animal nutrition (Güémes-Vera et al., 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 *L. albus*, *L. luteus*, *L. angustifolius*. and *L. mutabilis*, of which the latter shows the highest average content, on a dry weight basis, of protein (44 $g.100g^{-1}$) and lipids (18 $g.100g^{-1}$) (Pate et al., 1985), comparable to soybean in quantity and quality (Santos et al., 1997). Whole lupin seeds are consumed as a snack or as an ingredient in fresh salads and soups (Villacrés et al., 2003). Lupin flour can be used as an ingredient in foods such as biscuits, baby foods, hamburgers, breads, and pasta (Cremer, 1983; Ruales et al., 1988). However, lupin also naturally contains about 70 different alkaloids (Ruiz, 1978), which are toxic (Australia New Zealand Food Authority, 2001), especially those belonging to the sparteine and lupanine types (Jiménez-Martínez et al., 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 et al., 2007) or 20 h (Villacrés et al., 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 et al., 1983; Jiménez-Martínez et al., 2003).

After soaking and cooking, the alkaloid removal can be achieved by biological (Dagnia et al., 1992; Jiménez-Martínez et al., 2007; Santana and Empis, 2001), chemical (Aguilera et al., 1983; Jiménez-Martínez et al., 2003; Nossak et al., 2000; Ortiz and Mukherjee, 1982; Torres-Tello et al., 1980) or aqueous processing (Caicedo et al., 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 $g.100g^{-1}$ (Szakács and Stankovics, 1983), and consume energy and have a duration up to

5 days (Santana et al., 2002). Chemical treatments were suitable for lupin seeds with alkaloid contents up to 4.2 g.100g^{-1} dw (Ortiz and Mukherjee, 1982), but have disadvantages including material and nutritional losses (Gueguen and 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 et al., 2001; Villacrés et al., 2003).

The aqueous treatment is applied to lupin seeds with high alkaloids content (up to 4.2 g.100g^{-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 kg.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.

Materials and methods

Raw lupin

A batch of raw bitter *Lupinus mutabilis* Sweet (150 kg, alkaloids content 2.65 g.100g^{-1} ± 0.02 g.100g^{-1} 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 for a maximum of four months in an environmental chamber at 16 °C and 80 %

Relative Humidity. These conditions were used to mimic the prevailing weather conditions during the experiment.

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 analyzed. Next, nine different conditions of washing were studied (Table 1).

Table 1. 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 of processing	1/3	2/3	3/3

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

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 ºC, corresponding to the boiling point of water in Cumbayá, Quito, Ecuador (altitude 2433 m). Petroleum gas (46.2 MJ / kg) was used as fuel (Bosh, 1997). 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 0h with cooking1h (S0C1); soaking 0h with cooking 3h (S0C3); soaking 0h with cooking 6h (S0C6); soaking 18h with cooking 1h (S18C1); soaking 18h with cooking 3h

(S18C3); soaking 18h with cooking 6h (S18C6); soaking 36h with cooking 1h (S36C1); soaking 36h with cooking 3h (S36C3); and soaking 36h with cooking 6h (S36C6). 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.

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 22h). These were tested in 9 combinations as follows: 3 d^{-1} water changes with 0h d^{-1} of hydro-agitation (W3H0); 6 d^{-1} water changes with 0h d^{-1} of hydro-agitation (W6H0); 9 d^{-1} water changes with 0h 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 11h d^{-1} of hydro-agitation (W6H11); 9 d^{-1} water changes with 11h 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 22h d^{-1} of hydro-agitation (W6H22); and 9 d^{-1} water changes with 22h d^{-1} of hydro-agitation (W9H22).

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 (Caicedo et al., 2001) used agitation 24h/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 0h of agitation to compare with current debittering process (W3H0). We choose 22h, to see the maximum effect of agitation (from 24h/day, 2hours/day are used in changing water and weighing lupin and water), and 11h/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 14-16 °C were 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) (Figure 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.

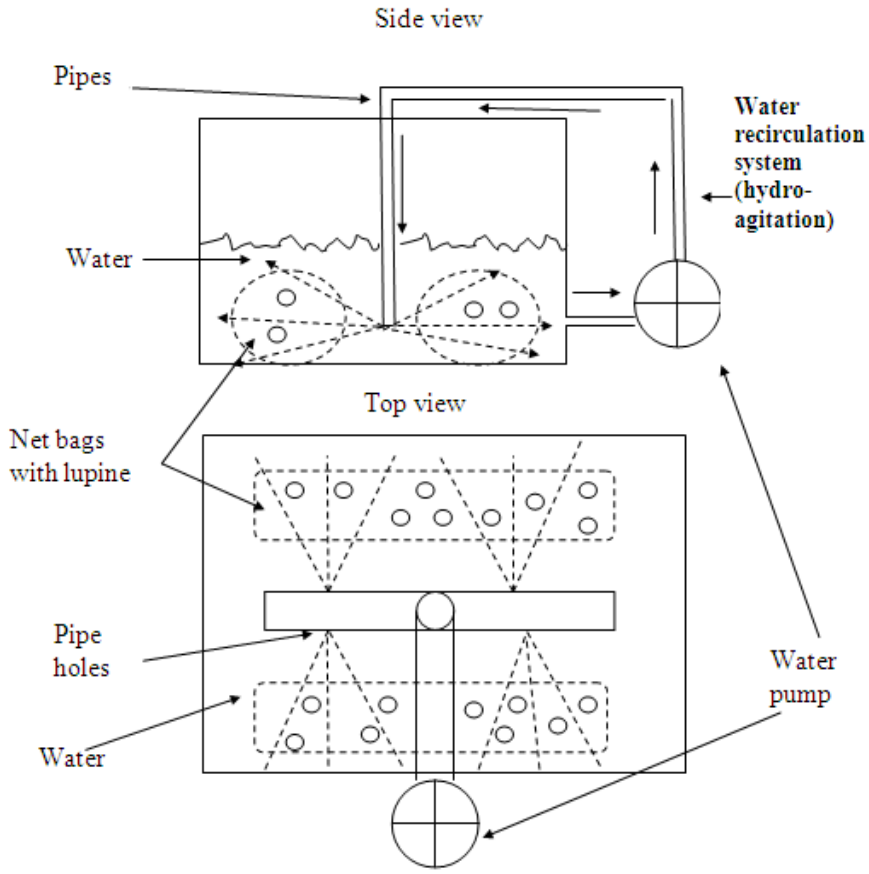

Figure 1. Hydro-agitation system

Alkaloid content

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 minutes in a food processor (model HC 3000, Black & Decker Corporation, Towson, Maryland, U.S.A) to facilitate the extraction of alkaloids.

Alkaloid determination:

Alkaloid concentrations were determined by titration using the methodology described by von Baer et al. (1979) with the modification suggested by the Ecuadorian Institute of Standards (INEN, 2005). To 0.2 g of lupin, 0.6 g of basic Al_2O_3 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 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 ºC until 1 ml remained; this was further evaporated when cooling the sample in a 15 ºC water bath. For the determination of alkaloid concentrations, 5 ml of sulfuric 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.100g^{-1}), was calculated as follows:

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

In which V = volume (ml) of sulfuric 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.

Theoretical considerations

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):

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

Where K*T* 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 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} m^2 $s^{-1,}$ 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):

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

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 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.

Results and discussion

Modeling and determination of best conditions for soaking and cooking

The effects of soaking and cooking are presented in Table 2, Table 3 and Figure 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.

Table 2. Soaking-cooking stage: Factors, levels and experimental results

	Soaking time	Cooking time	Processing time	Alkaloid content	Water use	Final weight	Solids	Energy
Run	(h)	(h)	(h)	(g $100g^{-1}$ d.w.)	(l/kg raw lupine d.w.)	(kg/kg raw lupine d.w.)	(kg/kg raw lupine d.w.)	(MJ/kg raw lupine 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

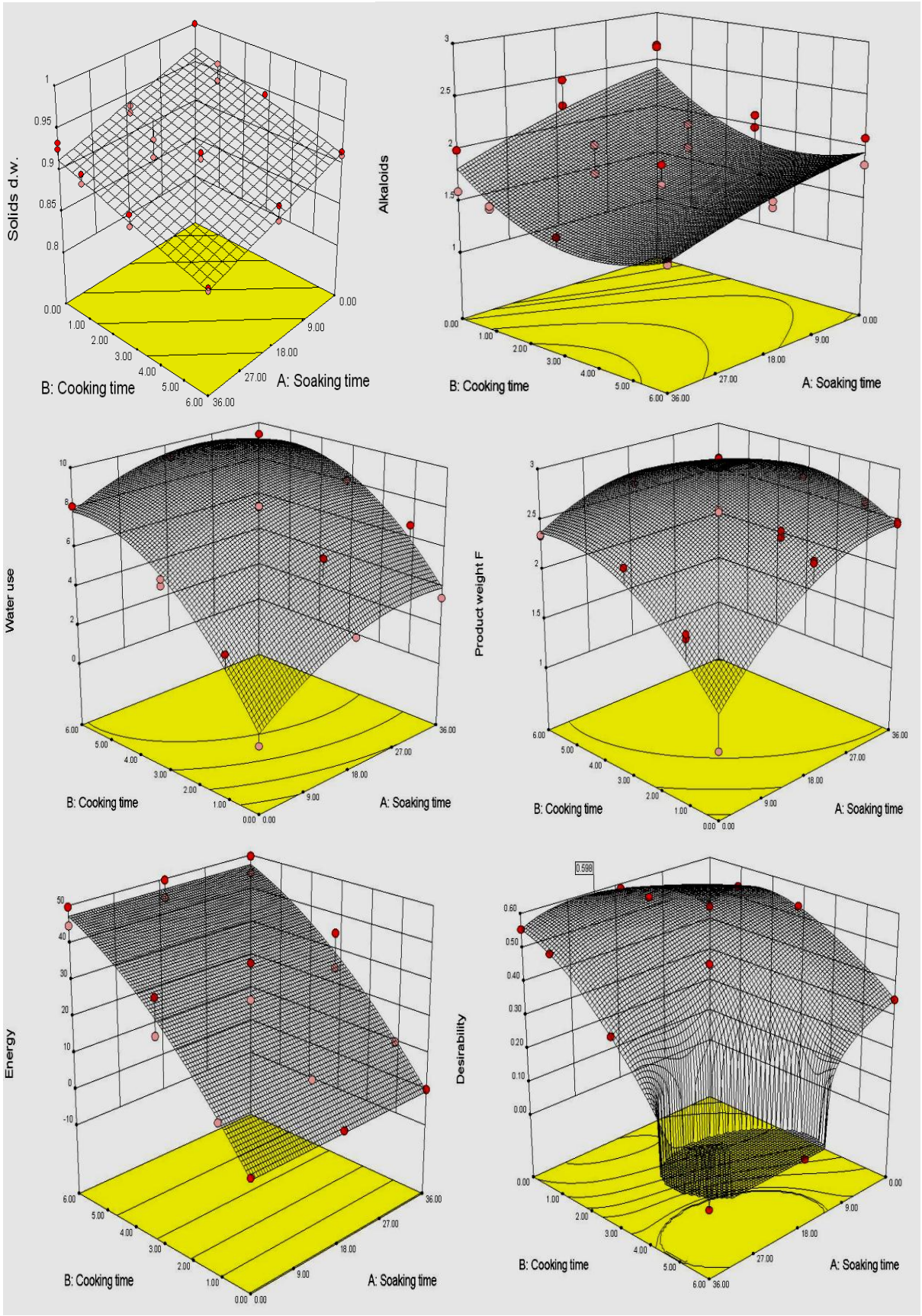

Figure 2. Effect of soaking and cooking times

It can be seen from Figure 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.

Table 3. 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 (*)	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 -3.48278E-003 * Soaking time^2 -0.22677 * Cooking time^2	0.96	0.0001
Energy use	**kJ**	**Energy = -0.46944 -3.86340E-016 * Soaking time +11.88615 * Cooking time +1.41324E-017 * Soaking time * Cooking time +5.63832E-018 * Soaking time^2 -0.65721 * Cooking time^2**	0.98	0.0001
Solids in product	**kg d.w.**	Product weight D = +0.97031 -1.58177E-003 * Soaking time -9.35757E-003 * Cooking time	0.79	0.0001
Product weight	**kg f.w.**	Product weight F = +1.35480 +0.063536 * Soaking time +0.38716 * Cooking time -4.66917E-003 * Soaking time * Cooking time -9.14795E-004 * Soaking time^2 -0.036117 * Cooking time^2	0.86	0.0001
Alkaloids	**%**	Alkaloids = +2.42216 -0.010481 * Soaking time -0.29499 * Cooking time +6.76939E-004 * Soaking time * Cooking time -1.75126E-004 * Soaking time^2 +0.036103 * Cooking time^2	0.70	0.0003

(*) Data in duplicate
f.w. fresh weight, d.w. dry weight

Figure 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 18h to 27h combined with a 1 h cooking period; this approximates some of the local conditions reported elsewhere (Villacrés 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 3). For practical reasons, the combination of 18h soaking and 1h cooking was selected as the standard pre-treatment for the next stage, i.e. washing.

Modeling and determination of best condition for washing

The effect of the frequency of daily water changes and of agitation duration is presented in Table 4, Table 5 and Figure 3.

Table 4. Washing stage: Factors, levels and experimental results

Run	Agitation time (h)	Changes of water (times/d)	Processing time (d)	Alkaloids G $100g^{-1}$ d.w.	Final weight (kg/kg raw lupine d.w.)	Water use (l/kg raw lupine 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

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). Figure 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 $g.100g^{-1}$ d.w. under that condition was 3.6 days. 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 lupine of 2.3 times the raw material.

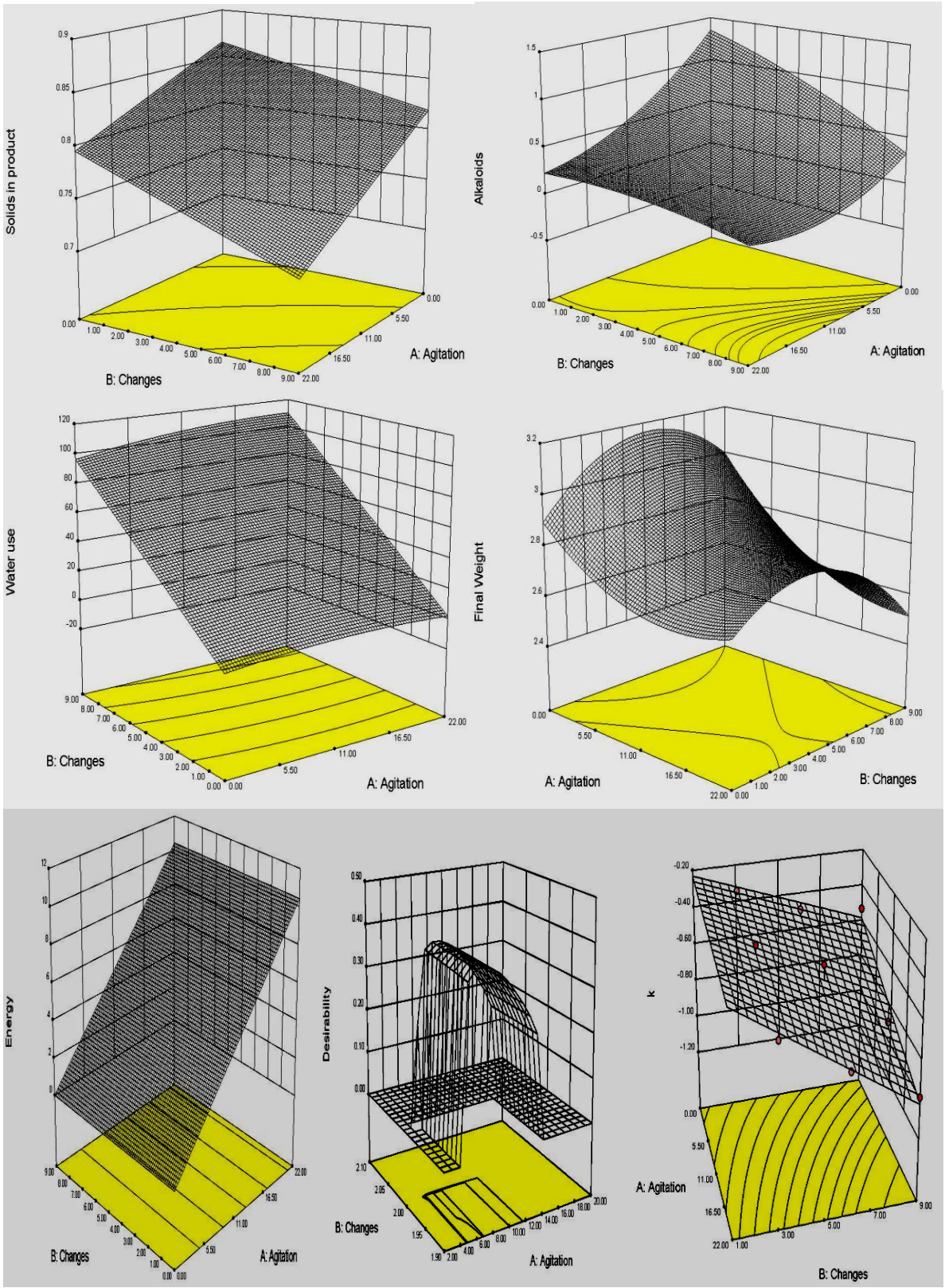

Figure 3. Effect of agitation duration, and frequency of water changes

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 (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 behavior (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 lupine 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 3d) 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 3^4 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 analyze than those of 2 or 3 factors. Finally, the complexity of obtained equations would be higher which would reduce their practical usage.

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 (*)	r^2	Prob. > F
Water use	**l**	Water use = +0.15003 +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 = +1.56854E-015 +1.88219E-016 * Agitation +1.49132E-016 * Changes +6.72952E-016 * Time -1.80946E-017 * Agitation * Changes +0.15871 * Agitation * Time -9.72655E-017 * Changes * Time	1.00	0.0001
Solids in product	**kg d.w.**	Solids in product = +0.89303 +5.68181E-004 * Agitation -0.016458 * Changes -8.97554E-003 * Time -1.63177E-004 * Agitation * Changes -1.25065E-003 * Agitation * Time +3.76413E-003 * Changes * Time	0.71	0.0001
Product weight	**kg f.w**	Final Weight = +2.55685 -0.020706 * Agitation +0.14881 * Changes +0.16101 * Time -1.38852E-003 * Agitation * Changes -3.94451E-003 * Agitation * Time -0.014434 * Changes * Time +1.04390E-003 * Agitation^2 -0.010413 * Changes^2 -0.016076 * Time^2	0.80	0.0001
Alkaloids	**%**	Alkaloids = +1.79005 -0.094894 * Agitation +3.40778E-003 * Changes -0.12792 * Time +4.11219E-003 * Agitation * Changes +2.06168E-003 * Agitation * Time -0.026879 * Changes * Time +1.71437E-003 * Agitation^2 -3.97577E-003 * Changes^2 -5.85064E-003 * Time^2	0.78	0.0001
k		**k = -0.18771 -4.97273E-003 * Agitation -0.043036 * Changes -1.68106E-003 * Agitation * Changes**	0.94	0.0018

(*) Data in duplicate.
f.w. fresh weight.
d.w. dry weight

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 ($g.100g^{-1}$ 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).
Figure 3 shows the graphical representation of k vs. agitation duration and change frequency, and in Table 5 the equation is presented.

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 lupine. 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* might be valuable tools to estimate the time required to reduce the alkaloid content during the washing process. Therefore, an independent validation to confirm these findings is required. 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, soya bean, cowpea, mugbean, or other seeds.

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Chapter 5

Consumer liking and willingness to pay for *Lupinus mutabilis* Sweet in relation to debittering conditions

This chapter has been submitted for publication by

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Abstract

Lupin was debittered by 12 different aqueous treatments and evaluated by 99 consumers. First they scored the products on the basis of liking. Next, they were informed about the price of the products and asked to rank their willingness to pay in relation to their liking scores and product price. Treatments with more processing (i.e. longer agitation times and/ or more frequent changes of water) increased the product price but diminished liking. Consumers did not choose between liking and price; the willingness to pay was the combined effect of both variables. For example, they would accept an increase in price of 0.3 $/kg if liking increased from “like slightly” to “like moderately”. In the studied range, the effect of processing on liking and expected price, as well as their effect on willingness to pay could be described as first order regression equations.

Keywords: Lupin, consumer preferences, process optimization, price

Introduction

Lupins (*Lupinus* spp.) are legumes used principally as a protein source in human and animal nutrition (Güémes-Vera et al., 2008). The FAO (2012) reports that in 2010 934,426 metric tons of lupin were produced in Europe (primarily in Germany, Poland, the Russian Federation and Mediterranean countries), Australia, South Africa, and South America. Four major species of lupins are cultivated, namely *L. albus, L. luteus , L. angustifolius and L. mutabilis*, of which the latter shows the highest average protein and fat contents, namely 44 % and 18 % on a dry weight basis, respectively (Pate et al., 1985), comparable in quantity and quality to what is present in soya bean (Gueguen and Cerletti, 1994; Santos et al., 1997). Whole lupin seeds are consumed as a snack or as an ingredient in fresh salads and soups (Villacrés et al., 2003).

Lupin contains about 70 different alkaloids, which are toxic (Aguilera and Trier, 1978; Australia New Zealand Food Authority, 2001; Camacho et al., 1991; Jiménez-Martínez et al., 2003), especially the sparteine and lupanine types (Jiménez-Martínez et al., 2003), and thus must be removed prior to consumption. Several researchers have attempted to improve the debittering of the seed because the current processes use much water and time (Carvajal-Larenas et al., 2013c), or produce chemical waste, or extract only a limited amount of alkaloids (Carvajal-Larenas et al., 2013b). Most published studies on debittering include a soaking stage of the seed in water, up to 18 h (Jiménez-Martínez et al., 2007) or 20 h (Villacrés et al., 2000), followed by cooking for 0.5 h (Villacrés et al., 2000) to 6 h (Jiménez-Martínez et al., 2003). After soaking and cooking, the alkaloids can be removed by biological (Agosin et al., 1989; Dagnia et al., 1992; Jiménez-Martínez et al., 2007; Santana and Empis, 2001), chemical (Aguilera et al., 1983; Jiménez-Martínez et al., 2003; Nossak et al., 2000; Ortiz and Mukherjee, 1982; Torres-Tello et al., 1980) or an aqueous extraction process (Caicedo et al., 2001; Torres-Tello et al., 1980; Villacrés et al., 2000). An aqueous extraction process is advantageous because it avoids the production of chemical waste (Rossetto, 1989) and can be applied to lupin seeds with high alkaloid contents (up to 4.2 %) (Torres-Tello et al., 1980; Villacrés et al., 2000). Moreover, the aqueous extraction process is the only one practised on a household and commercial scale. In the current aqueous extraction process for debittering lupin, the water is refreshed three times per day and is not agitated (Torres-Tello et al., 1980; Villacrés et al., 2000). After washing, the product may contain *E. coli* and high counts of aerobic bacteria (10^8 cfu/g). Therefore, it still requires a thermal treatment (namely

boiling for 10 minutes) to render it safe for consumption (Torres-Tello et al., 1980; Villacrés et al., 2000). Finally, the debittered seeds are packed for retailing (Caicedo et al., 2001; Villacrés et al., 2003).

The processing conditions of lupin can affect its sensory properties, and therefore change the liking for that product (Frewer et al., 1997). The processing conditions also influence productivity, i.e. the yield of product obtained, and the energy, materials, labour and time used. This in turn affects the processing cost and therefore the price of the final product (Ghasemlou et al., 2013). On the other hand, consumers' willingness to pay is not only influenced by sensory characteristics (liking) but also by other characteristics (Holmquist et al., 2011) such as origin (Stefani et al., 2006), reputation, agronomical characteristics, variety and price of the product (Zhang et al., 2010). Therefore it is important to understand the relationship between these factors. The generated information can help all actors in the lupin processing chain (from product developers, production managers, financial executives to marketers) to design and choose the right product, for a specific market, in the early stages (Frewer et al., 1997).

In this study, we worked with one variety (*Lupinus mutabilis* Sweet) and one origin of the seed (Ecuador). Therefore, the other characteristics addressing the consumers' willingness to pay were narrowed down to product price only. In short, the aims of the study were: i) to measure the impact of processing conditions (number of changes of water/ day and hours of agitation/day) on sensory characteristics (liking), ii) to assess the impact of processing conditions on product price, iii) to estimate the effect of liking and price on willingness to pay, iv) to analyse the findings with regression equations to be used for optimization purposes.

Materials and methods

Raw lupin

A batch of raw bitter *Lupinus mutabilis* Sweet (150 kg, alkaloid content 2.65 % ± 0.02 % d.w.) was obtained by pooling samples of 15 kg from 10 processors selected at random from the village of San Pedro, Cotopaxi Province, Ecuador. All processors were using the same variety of raw lupin. After mixing, the lupin was put in jute bags and stored at 16 °C and 80 % Relative Humidity.

Soaking and cooking conditions

Soaking and cooking processes were carried out as optimized previously (Carvajal-Larenas et al., 2013a). Soaking in tap water was for 18 h at room temperature (18 °C). The initial weight ratio of water: raw seeds (d.w.) was 3:1. More water was added if required to ensure that the seeds always remained under water; this additional water was recorded.

Cooking was carried out for 1 h at 91.9 ^{o}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 the boiling water, and cooking time was recorded from the moment that the lupin came in contact with the boiling water. The initial weight ratio of cooking water: soaked seeds was 3:1.

During the experiment the labour (h), water (kg) and seed weight (kg), as well as the consumed amount of petroleum gas (kg) were recorded (weighing scale ES 200L, Ohaus Corporation, NJ, U.S.A.).

Washing conditions

Twelve experiments were conducted to determine the effect of the amount of washing water and agitation conditions on alkaloid removal, liking, price and willingness to pay. The tested conditions included the number of times the water was changed per day (2, 3, 6, and 9 times), and the duration of hydro-agitation per day (0, 11, and 22 h). These were tested in 12 combinations as shown in Table 1. After soaking and cooking, 20 kg of lupin were put in two plastic net bags (10 kg per each). The bags 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 14-16 °C were added to the tank, which was situated in an environmental chamber (14-16 °C). Next, a hydro-agitation system (Carvajal-Larenas et al., 2013a) was activated and the washing process started. The system re-circulated water 60 times h^{-1} and injected water at 50 kPa into the bags containing lupin. In all experimental treatments, the lupin seeds always remained submerged.

During the experiment the labour (h), electrical energy (kWh), as well as water (kg) and seed weight (kg) were monitored. A ES 200L scale, Ohaus Corporation, NJ, U.S.A. was used for all weight measurements. During the experimental part, seed samples were taken and their alkaloid content was measured by titration as described earlier (Carvajal-Larenas et al.,

2013a). All experiments were terminated when the alkaloid content was reduced to the safe level (maximum 0.26 % d.w.).

Price

The price for the debittered lupin was determined as follows. First we calculated the monthly profits for all treatments if they would be sold at the current price of debittered lupin in the market, minus the production costs, using the following equation:

$$MP = (PP*(1-f) - (Ma+E+L+D))* W*(30/t) \quad (1)$$

Where,
MP= Monthly profit ($/month)
PP=Product Price ($/kg product)
f= fraction of product price that is retained by supermarket (supermarket profits)
Ma= Materials cost ($/kg product)
E= Energy cost ($/kg product)
L= Labour cost ($/kg product)
D= Depreciation ($/kg product)
W= Product obtained (kg/batch)
30= average month (days)
t= time to complete a production batch (days).

Then, the process with the highest monthly profits was selected and used as reference for all other processes to calculate the price for that particular treatment at the same profit margin, where equation (1) was reworked into:

$$P_i = \frac{\left(\left(\frac{MP_h * t_i}{W_i * 30}\right) + Ma_i + E_i + L_i + D_i\right)}{(1-f)} \quad (2)$$

Where,

MP_h = Highest monthly profit ($/month)

P = Price that consumer has to pay ($/kg product)

$_i$ = investigated process ($_i$ = 1,…,12)

Liking

Lupin seeds that were debittered according to the twelve conditions mentioned before, were boiled for 10 minutes to ensure bacteriological safety for consumption (Villacrés et al., 2000), packed in polyethylene bags, cooled in a water bath at 16 °C and kept under freezing conditions (-16 °C) until all batches had been produced. Then, all samples were defrosted in a water bath (60 °C) until the samples reached 4 °C. Finally all samples were kept one day under refrigeration conditions (4 °C) to equilibrate the temperature.

Liking was assessed using a convenience sample of the population. Requirements for respondents were that they should eat lupin at least once per month, be healthy and between 18 and 65 years old. Consumers were instructed how to complete the questionnaire and asked not to eat anything for at least 1 h prior to the test.

The number of consumers, the number of samples that each consumer tried, the order of presentation of samples and the code assigned to each sample were obtained throughout a Design Generator Form, Incomplete Block design that the program Qi statistic- Design Express version 1.6 offers (http://www.qistatistics.co.uk/). The obtained design did not have any carry over effect, presented a statistical efficiency (> 80%) and was balanced.

Ninety-nine volunteer consumers (50 men, 49 women, 18 to 68-year old, students, administrators and faculty of the Universidad San Francisco de Quito, Ecuador) tried 4 samples each from left to right. The consumers were physically separated and could not communicate with each other. After tasting each sample consumers had to register the degree of liking of the sample, take two sips of water, and wait for at least 10 s before continuing with the next sample. The form used was a 9-point hedonic scale presented vertically. The highest point corresponded to “like extremely” and scored 9 points; “like very much”, 8 points; “like moderately”, 7 points; “like slightly”, 6 points; “neither like nor dislike”, 5 points; “dislike slightly, 4 points; “dislike moderately”, 3 points; “dislike very much”, 2 points and “dislike extremely”, 1 point.

Willingness to pay

Immediately after finishing the evaluation of the liking, each respondent received information about the price that the 4-previously tried samples would have in the market. Then, each consumer was asked to rank the willingness to pay (W.T.P.) based on their liking evaluation and price. The most preferred sample scored 1 and the least preferred 4.

Data analysis

A parametric ANOVA test was used to assess differences in degree of liking, price and W.T.P. between the twelve debittering treatments. In addition, the two-factor ANOVA test was used to measure the effect of each factor on the response. For these purposes, the software packages GRAPH PAD INSTAT T.M. V2.01., GraphPad Software Inc., San Diego, California, U.S.A and Design-Expert ®, version 8.0, Stat-Ease, Inc., Minneapolis, Minnesota, U.S.A were used. The latter was also used to model the effect of processing conditions on degree of liking, price and W.T.P.

Finally, to select the most desirable combination (*i.e.* maximization of linking, minimization of price and with the best W.T.P), each treatment was scored according to those objectives by using the computer program Design-Expert ®, version 8.0. Treatments distant from the most desirable combination got a low score, and those closer got a higher score. Therefore, the highest score was for the combination (treatment) closest to the most desirable combination.

Results and discussion

Effect of processing conditions on liking of debittered lupin

The effect of number of changes of water (code: W) and hours of agitation (code: H) on liking of debittered lupin is presented on Table 1 and Figures 1A and 1B. The average liking of all samples was between 6.4 (W6H0) and 4.7 (W6H22). The aqueous process applied currently on a commercial scale (W3H0) scored 6.1.

Table 1. Average values ± standard deviation for Liking (scale 1-9), price and willingness to pay (scale 1-4) of debittered lupin obtained from 12 treatments.

Treatment	Code	Liking	Price ($/kg)	Willingness to pay
2 water changes/day, 0h of hydro-agitation/day	W2H0	6.0 ± 1.5	1.62	2.1 ± 1.0
3 water changes/day, 0h of hydro-agitation/day	W3H0	6.1 ± 2.0	1.52	2.1 ± 1.1
6 water changes/day, 0h of hydro-agitation/day	W6H0	6.4 ± 1.9	1.64	2.0 ± 1.0
9 water changes/day, 0h of hydro-agitation/day	W9H0	5.6 ± 1.6	1.72	2.5 ± 1.1
2 water changes/day, 11h of hydro-agitation/day	W2H11	5.6 ± 2.2	1.97	2.5 ± 1.2
3 water changes/day, 11h of hydro-agitation/day	W3H11	5.6 ± 1.8	1.82	2.7± 1.0
6 water changes/day, 11h of hydro-agitation/day	W6H11	5.4 ± 1.8	1.92	2.6 ± 1.1
9 water changes/day, 11h of hydro-agitation/day	W9H11	5.6 ± 1.8	1.83	2.6 ± 1.1
2 water changes/day, 22h of hydro-agitation/day	W2H22	5.9 ± 1.9	2.17	2.8 ± 1.1
3 water changes/day, 22h of hydro-agitation/day	W3H22	5.3 ± 2.0	2.13	3.0 ± 1.1
6 water changes/day, 22h of hydro-agitation/day	W6H22	4.7 ± 1.9	1.92	2.7 ± 1.0
9 water changes/day, 22h of hydro-agitation/day	W9H22	5.6 ± 1.8	2.16	2.6 ± 1.2
ANOVA GLOBAL (0.95) [1]		NS (*)	P < 0.0001	P < 0.00025
ANOVA (factor water changes/day) [2]		P = 0.3748	P = 0.9130	-
ANOVA (factor h of hydroagitation/day)[2]		P = 0.0207	P < 0.0001	-
ANOVA (factor Liking)[2]		-	-	P = 0.0384
ANOVA (factor Price)[2]		-	-	P = 0.0099

[1] Obtained with the software package GRAPH PAD INSTAT T.M. V2.01
[2] Obtained with the software package Design-Expert ®, version 8.0.
(*) NS= Not significant

Although the global ANOVA test did not indicate significant differences between treatments (Table 1), Figure 1A shows an inverse and additive effect on liking of either agitation time or number of water changes. However, the effect of agitation on liking is higher than the effect

of changes of water. This is confirmed by the two-factor ANOVA test. The effect of agitation on liking is significant and the effect of water changes is not. The higher liking (6.1) was estimated when the number of water changes per day was lower than 3 and there was practically no agitation (Figure 1B).

No information is available about the effect of processing conditions on liking and price of debittered lupin nor on the effect of liking and price on willingness to pay. Therefore we compare and discuss our findings with available literature on other products. Thus, Ghasemlou et al. (2013) found for common bean (*Phaseolus vulgaris*) that by changing processing conditions (cooking time, cooking temperature, concentration of added NaCl and $CaCl_2$ the consumers' overall acceptability, on a 9 points hedonic scale, varied from about 2.5/9 to about 8/9. Consumers liked most samples with an average firmness between 12. 5 and 22.5 N, and they disliked most samples with high value of firmness (i.e. 52.9 N). Zhang et al. (2010) reported that winter Anjou pears under different conditions of ripening (0, 2, 4 and 6 days of ethylene treatments) were evaluated by between 100 - 120 consumers on a 9 points hedonic scale. Results showed that the overall desirability varied between 4.3/9 to 7.5/9. In this study consumers liked most products obtained with 6-day ethylene treatment, and disliked most products obtained without ethylene treatment. Holmquist et al. (2011) evaluated the overall preference of three chardonnay wines obtained from three different treatments by 66 consumers on a 9-point hedonic scale. The winning treatment (not aged in oak barrels) obtained 6.5/9. The other treatments scored 5.6/9 and 6.0/9 for 100 % oak-aged and 70 % oak-aged, respectively.

The effect of changes of water and agitation time on liking in this study, through a response surface methodology (RSM), was expressed by a first order regression equation:

$$\text{Liking} = +6.15 - 0.033 * \text{Changes of water} - 0.03 * \text{Agitation}, \; r^2 = 0.49, \; p < 0.047 \qquad (3)$$

Figure 1A

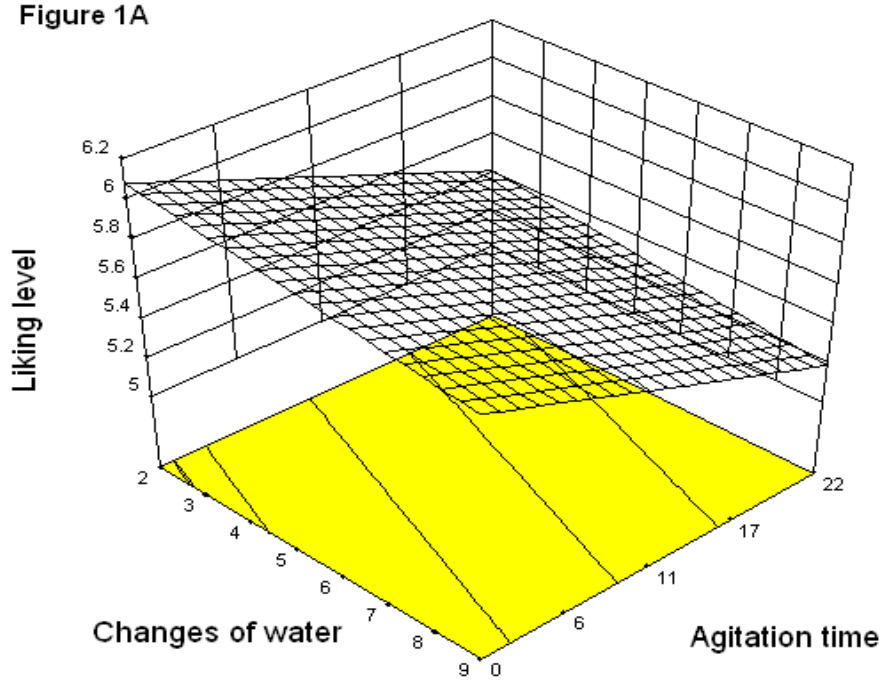

Figure 1B

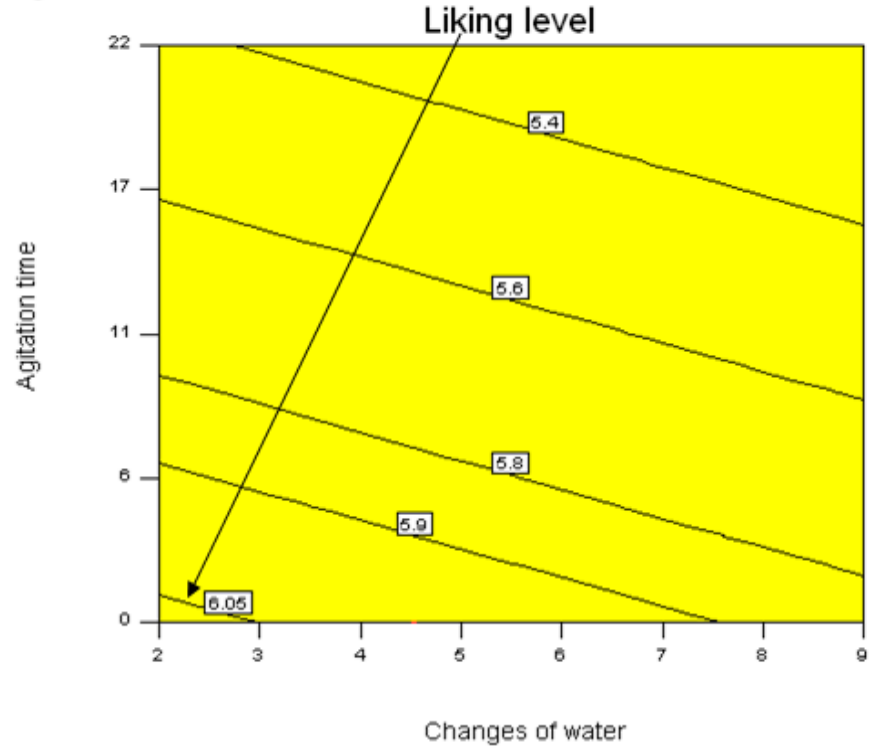

Figure 1. Effect of changes of water and agitation time on liking *

*Representation of this relationship on 3D (Figure 1A) and 2D (Figure 1B)

Although the correlation was not high, it does reflect the liking trend (Figures 1 A and 1B). Response surface methodology for modelling the influence of processing conditions on different sensory attributes was also used by Ghasemlou et al. (2013). The authors measured the combined effect of thirty different processing conditions on two rheological parameters of cooked *Phaseolus vulgaris* (force and deformation) indicating that the RSM methodology is suitable for reflecting differences between a number of processing conditions. The authors did not model that relationship although the sensorial evaluation of all samples was carried out.

Effect of processing conditions on expected price of debittered lupine

The combined effect of processing conditions on price is presented in Table 1, Figures 2 A and 2B. The price varied between 1.52 $/kg (W3H0) and 2.17 $/kg (W2H22) (Table 1). The global ANOVA test presents a significant difference between treatments (Table 1). Figure 2A shows the direct effect of hours of agitation and changes of water on the price of the product. However, the effect of agitation was much larger than the effect of changes of water. This is confirmed by the two-factor ANOVA test. The effect of agitation on price is significant and the effect of water changes is not. Lower prices were obtained when there was no agitation (Table 1 and Figure 2B). The effect of processing conditions on product cost (and price) found in this study is confirmed by Ghasemlou et al. (2013), Zhang et al. (2010) and Holmquist et al. (2011). For example, Zhang et al. (2010) estimated that the costs for pear ripening were associated to the size of operations, increased use of facilities (machinery), energy and ethylene. These authors found an increase of about $ 0.002 per kilogram of pears for every two days of ethylene treatment. Unfortunately, in this study the labour cost was not taken into account. No published mathematic models were found that link processing conditions to product cost or price to compare our findings with. However, as Zhang et al. (2010) pointed out, costs are highly variable and depend on the size of the operations. Therefore, different mathematic models describing process-cost relations can be expected. In this research, a first order regression equation describing the effect of agitation and changes of water on price proved to be adequate:

$$\text{Price (\$/kg)} = 1.63 + 1.11*10^{-3}*\text{Changes of water} + 0.021*\text{Agitation},\ r^2 = 0.85,\ p < 0.0002 \quad (4)$$

Figure 2A

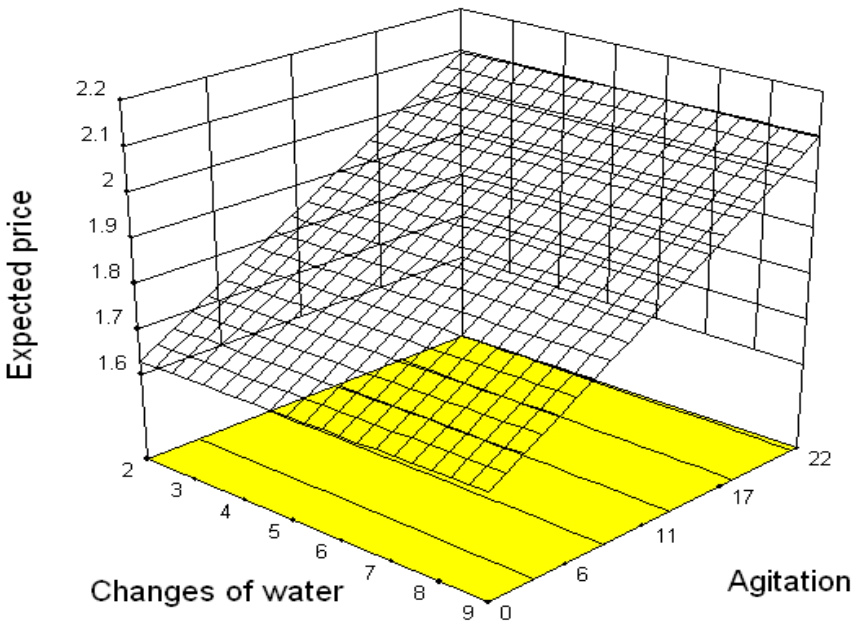

Figure 2B

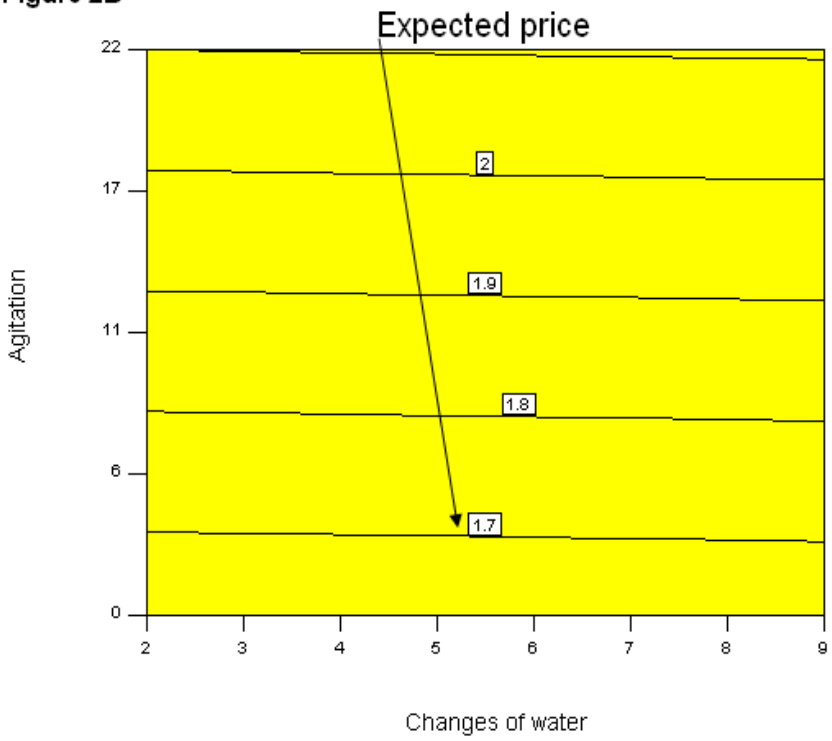

Figure 2. Effect of changes of water and agitation time on price *

* Representation of this relationship on 3D (Figure 2A) and 2D (Figure 2B)

Effect of liking and expected price on W.T.P. and the optimum solution

The effect of liking and price on W.T.P. is presented in Table 1 and Figures 3A and 3B. The W.T.P. can be described by the function:

$$W.T.P = 2.76 - 0.32 * Liking + 0.84 * price,\ r^2 = 0.78,\ p < 0.001 \quad (5)$$

and varies between 2.0 (W6H0) and 3.0 (W3H22) (Table 1), with a significant difference between treatments. In addition, the two-factor ANOVA test also indicated a significant effect of both factors, namely liking and price on W.T.P. (Table 1). Note that the product that was obtained by W6H0 had the highest score for liking, namely 6.4, and the third lowest price of 1.64 $/kg, after 1.52 $/kg (W3H0) and 1.62 $/kg (W2H0) (Table 1). Figure 3A presents the effect of liking and price on W.T.P. Respondents scored a W.T.P. of 2.0, either when the product scored a liking of about 6.2 and would be sold at 1.50 $/kg, or when it would be sold at about 1.8 $/kg when the liking score was 7.0 (Figure 3B). This indicates that when the price increases, the liking should be increased too in order to keep the same willingness to pay (Figure 3B). In other words, respondents would pay 0.3 $/kg more for a product they like better. These findings are in line with Stefani et al. (2006), Zhang et al. (2010), Homburg et al. (2005) and Rosas-Nexticapa et al. (2005), who also reported a positive correlation between products that were liked better and a higher price for a specific product. For example, Zhang et al. (2010) found that compared to the average market price of 0.68 $/kg, consumers were willing to pay a premium of 0.11 $/kg for pears processed under specific conditions of ripening (six-day ethylene treatment). Moreover, in the study performed by Holmquist et al. (2011), consumers were willing to pay $0.67 more for a full bottle of un-oaked chardonnay wine compared with a full oaked bottle of chardonnay because they like the first more.

The relation between liking and price on W.T.P. can be expressed by a first order regression (equation 5) as reported by other studies (Homburg et al., 2005). On the other hand, the same authors (Homburg et al., 2005) found an inverse S-shape function between customer satisfaction (C.S.) and W.T.P. In that study, consumer satisfaction was measured as function of three key attributes, *i.e* quality of food, ambience, and service.

Figure 3A

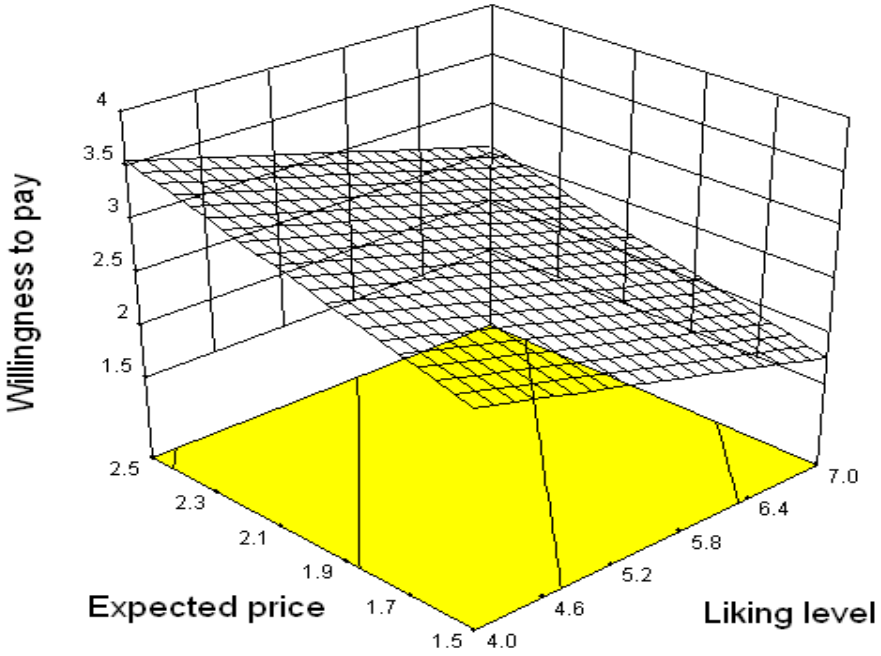

Figure 3B

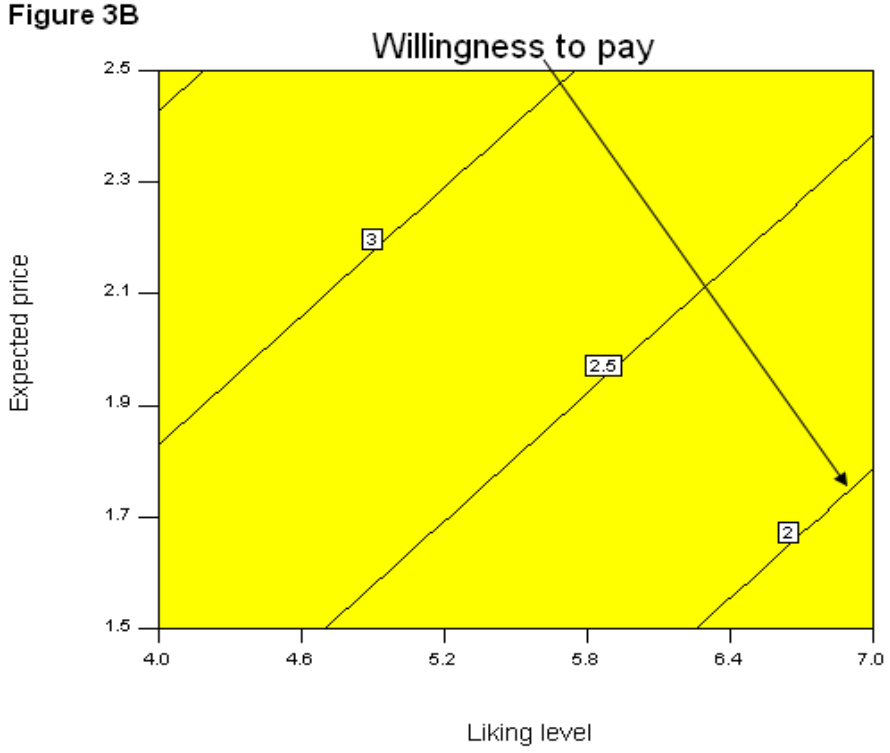

Figure 3. Effect of price and liking on willingness to pay *

* Representation of this relationship on 3D (Figure 3A) and 2D (Figure 3B)

The S-inverse shape function found, described as third order, was concave for low satisfaction levels, convex for high satisfaction levels, and relatively flat (first order) for medium satisfaction levels. This apparent difference between the data of Homburg et al. (2005) and the present study could be explained as follows. First, in the present study we related liking and price to W.T.P. while Homburg et al. (2005) linked C.S to W.T.P without considering the price. Second, in the present study a significant difference between samples was not found. The liking scores for our samples were between 6.4/9 close to "like slightly" and 4.7/9, close to "neither like nor dislike". Therefore, they could be considered of "medium satisfaction level" corresponding to the model found by Homburg et al. (2005) for the first order segment. None of our lupin samples could be considered of high or low satisfaction. Therefore, in our model the concave and convex part would be absent. Third, not all products generate a similar response from consumers. Homburg et al. (2005) did not report for which product they found this relationship, making it impossible to find insights for further analysis. Figure 4A presents the optimization or highest desirability (maximization of liking, minimization of price and with the best possible W.T.P.). The optimal solution (desirability ≈0.77) would be reached with a liking of about 6.9/9 and a price of 1.50 $/kg (Figure 4B).

Figure 4A

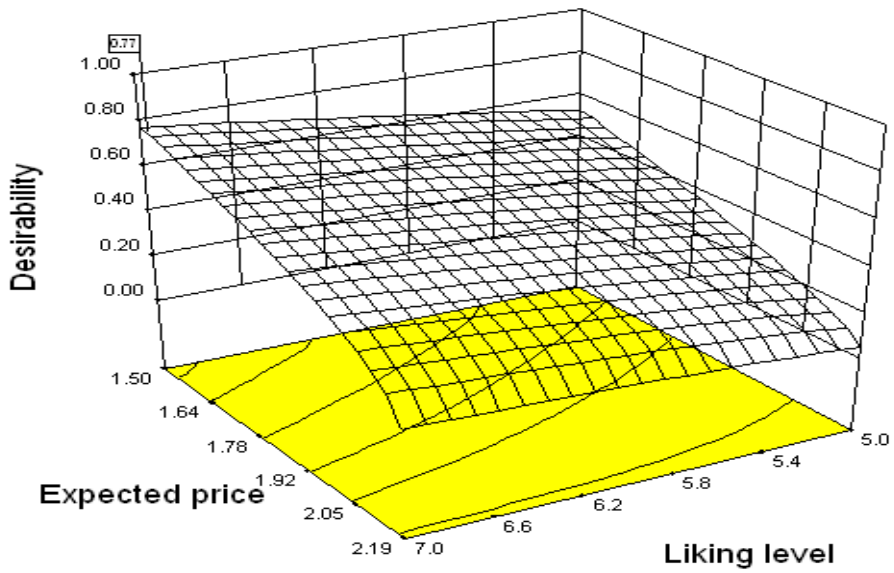

Figure 4B

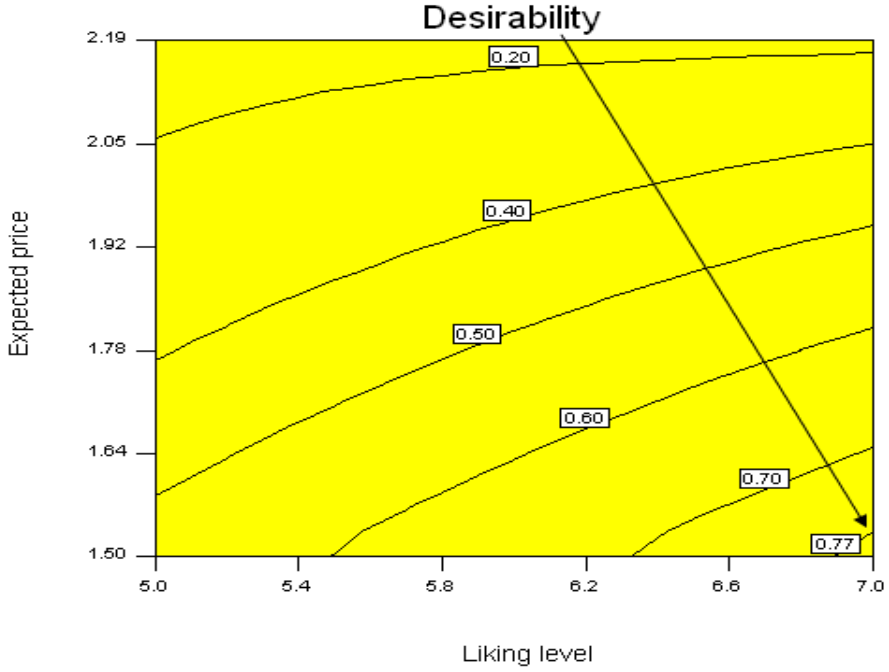

Figure 4. Willingness to pay (W.T.P.) as related to liking level and expected price to determine the most desirable combination ("desirability")*

* Representation of this relationship in 3D (Figure 4A) and 2D (Figure 4B). Treatments distant from the most desirable combination (i.e. maximization of liking, minimization of price and with the best possible W.T.P.) received a low score (0.20 or 0.40), and those closer got a higher score (0.60 or 0.70). Therefore, the highest score (0.77) was for the combination (treatment) closest to the most desirable combination.

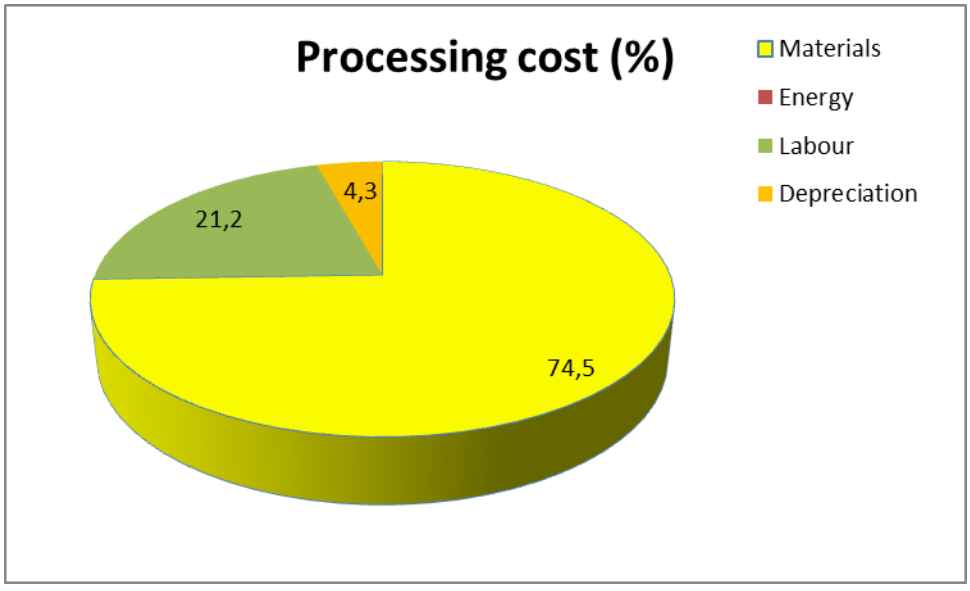

Figure 5. Processing costs (%) for treatment W3H0[1]

[1] Energy is 0%

Treatment W3H0, the current way in which processors in Ecuador debitter lupin, is closest to the optimal combination of liking, price and willingness to pay (Table 1 and Figure 5). Perhaps the reason for this is because consumers preferred what they are acquainted to, for many years, combined with the lowest price (and processing costs) of the product obtained with this treatment since the W.T.P. is the resultant consequence of both variables (liking and price).

Conclusions

Many studies suggest the importance of processing cost and/or price of product in consumer decisions (Bi et al., 2011; Frewer et al., 1997; Homburg et al., 2005; Liu et al., 2006; Rosas-Nexticapa et al., 2005; Zhang et al., 2010). However, most of the published studies did not actually consider that aspect. In the present research it was found that despite the fact that hydro-agitation reduced the processing time and the use of labour, the net effect of increasing processing (more agitation and water changes) increased the final cost and decreased the liking by consumers.

The respondents in this study did not choose between price or liking; they went for both. However, people were willing to pay more if they perceived a benefit from the process used and/or the obtained product. For example, consumers were willing to spend about 0.3 $/kg more for a product for which the liking would increase from 6.2 to 7.

The best product selected by respondents corresponded to that obtained with treatment W3H0 because this had the lowest price and was one of the most liked. However, the obtained optimum was valid within the study conditions only and could change in other scenarios. For example, the price of products could change as a consequence of varying the batch size (economy of scales). On the other hand, the liking of products could also vary. For example, by adding common salt or calcium chloride the firmness and taste of the lupin could change. Moreover, the addition of common salt or calcium chloride could affect the processing time and therefore the processing costs. Therefore, the optimal solution should be considered as a dynamic output that changes in relation to variations between and within factors.

The response surface methodology proved to be a useful tool to compare and model the effect of several processing conditions on liking, cost and W.T.P. It is recommended to compare the response surface methodology with other approaches such as the logit model, double-

bounded model, and linear and non-linear programming to get insights for optimization purposes.

The approach used in this study can probably also be applied to other products to estimate relationships between processing conditions, liking, price and willingness to pay.

The generated information can help developers of lupin-based products, factory managers, financial executives and marketers to choose the best process (product), for a specific market, in the early stages.

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Chapter 6

General discussion

Background

This thesis focused on the question whether the aqueous debittering process of *Lupinus mutabilis* Sweet could be optimized, using a food sovereignty perspective. In this context, the thesis describes and analyzes the potential of local *Lupinus mutabilis* – based food chains to enhance livelihood in the Andean region of Ecuador. The specific aims of the research were:

i. To assemble and critically review published data on the composition, uses, toxicology and debittering methods of lupin species, focusing on *Lupinus mutabilis*;
ii. To determine the efficiency of the locally applied debittering process;
iii. To study the effect of processing parameters on the efficacy of the aqueous debittering process;
iv. To search for optimum conditions for debittering lupin from processors' and consumers´ points of view, using mathematic modelling.

This discussion addresses the extent to which the objectives of this thesis were reached. In addition, the contributions of this research to science and to the objectives of the TELFUN program are discussed, and finally, recommendations for further research are presented.

The potential of lupin for food and non-food uses

The first requirement for this study was to establish the state of the art about *Lupinus* spp. by critically reviewing published data. The scarce data about *Lupinus mutabilis* were compared with those of other lupin species, namely *L. albus, L. luteus* and *L. angustifolius*, as well as with soya bean, when appropriate. It was observed that methods of sampling and identification of lupin varieties were only rarely mentioned in the scientific publications studied. Moreover, authors did not always report the number of repetitions, and the range or standard deviation of data, which hampered the analysis of the accuracy and precision of reported data. Nevertheless, it was clear that the alkaloid content varied among lupin species, as was expected. Our review also indicated that the alkaloid content in lupins varied within the same species, and depended on agronomical conditions of lupin cultivation, such as the amount of water, nutrients, the quantity of light or shadow received, and the type of soil. In addition, we learned that alkaloids are apparently intermediate compounds in the biosynthesis and transport of proteins. This would explain that alkaloid concentrations in the seed could

vary even during the hours of the day. Such agronomic aspects were hardly known and our review pointed out the necessity to investigate them further.

The nutrient composition of lupins, including the contents of oil and minerals, also varied with species and agronomical growing conditions. This research helped to understand the considerable variations in reported data for alkaloids and nutritional content among lupin species and varieties. Moreover, the critical review confirmed that, on average, *Lupinus mutabilis* has the highest nutrient content compared with the other lupin species studied, and that the nutrient content is comparable with that of soya bean. On the negative side, *Lupinus mutabilis* has the highest alkaloid content.

The toxicity of lupins was distinguished into acute and chronic toxicity. The acute toxicity was inferred from animal studies and the registered accidental ingestion by humans. The fatal acute dose of lupin alkaloids was estimated as 10 mg kg^{-1} body weight (bw) for infants and children, and 25 mg kg^{-1} bw for adults. Levels of chronic toxicity are not clear. On the one hand, lupins have been used as food for thousands of years, which gives some confidence about its safety (Cremer, 1983; Petterson, 1998). However, on the other hand, the ingestion of lupin was associated to growth reduction in animals (Jiménez-Martínez et al., 2003). The reported "safe" doses of alkaloids avoiding chronic intoxication, vary widely. According to Aguilera and Trier (1978) the safe dose would be 500 mg day^{-1}. However, the Australian and New Zealand Food Authority (2001) proposed 0.035 mg kg body $weight^{-1}$ day^{-1} as the safe dose (this is equivalent to 2.45 mg day^{-1} for a 70-kg person). This variation suggests that either the chronic dose is a matter of adaptation and thus would not be the same for all people, or that regional variations of toxicity are confounding factors. In any case, presently we are not certain about the safe dose of alkaloids to avoid chronic toxicity. Regarding allergenicity, one report mentions that some people are allergic to lupin protein; their number is comparable to the number of people who are allergic to egg protein (3%) (Petterson, 1998). For the sake of completeness, we want to mention that alkaloids are not only harmful. Isolated lupin alkaloids can apparently be applied as medication (Ciesiolka et al., 2005; Jiménez-Martínez et al., 2003).

We evaluated the published methods for reducing the alkaloid content in lupin seeds to be consumed as whole seeds, as well as a processed ingredient for formulated foods. In the first case, the debittering process by aqueous extraction is the only option to reduce alkaloids in the seed without generating chemical residues in the food or environmental waste. Other (biological) options were reported, but not investigated by us, to be rather ineffective. In the

second case of food use, there are options to shorten the debittering process by crushing and/or powdering the raw lupin enabling a more effective diffusion of alkaloids, as inferred from the Fickian law of diffusion and confirmed by studies performed by Aguilera et al. (1983) and Ortiz and Mukherjee (1982).

Protein concentrates and isolates of lupins could be used in the chemical and food industry, but there is a gap in the understanding of the relationship between protein composition in lupins, its processes of concentration and isolation and its rheological behaviour. Such knowledge is essential for improving the use of lupin proteins as a food additive, as well as for soya bean protein isolates since the physical properties of soya bean isolates vary widely too. Perhaps the factors influencing behaviour of lupin protein are also influencing the protein behaviour of soya bean isolates.

Optimization of the aqueous debittering process

The optimization of the aqueous debittering process was performed in the frame of contributing to food sovereignty, which meant that the research was participative, using the concept of appropriate technology and taking into consideration indigenous knowledge and community preferences. Based on this information, the first step of the optimization process was to select a community interested in improving the process and willing to share its knowledge. This was found in the community of San Pedro de Tanicuchi, Cotopaxi province, Ecuador, relatively close to laboratories and pilot plant (about 2 hours). Moreover, the community is located in a place with a high prevalence of undernutrition, and is one of the poorest places of Ecuador (CEPAR, 2004; INEC, 2012); the debittered product is commercialized in the same province but also in some other cities, such as Quito.

After deciding on the research locale, the next step was to document the current process as a starting point for optimization. Based on analyses of macro- and micronutrients, and microbiological composition of lupin from 10 out of 40 factories, it became clear that, from a technological point of view, the process needed improvement in efficiency (time and water used and solids loss) and microbiological quality. The community was informed accordingly; the processors were aware that the process was using too much time and water and they also suspected that the microbial quality required improvement. However, they did not realize that the process was causing losses of considerable amounts of materials. One of the goals of the TELFUN project was achieved; the two way communication was created. The local

community shared its knowledge and the researcher started feeding back to the community. The third step was to create a laboratory-based simulation of the process used in the community in order to study factors and variables in a controlled manner and without interfering with the commercial production. One of the outcomes of this stage was the estimation of the diffusion coefficient of alkaloids in aqueous extraction (Crank, 1975). In this sense this research made a contribution to science as it was the first time that the diffusion coefficient of alkaloids was estimated.

The next step in the participatory research was to meet again with the community and explain them the available options to optimize the debittering process (i.e. by biological, chemical or aqueous processing). The community was informed that the biological methods to debitter lupin are mainly based on fungal (*Rhizopus oligosporus*), or bacterial fermentation (*Lactobacillus acidophilus, L. büchneri, L. cellobiosus, L. fermentum, L. plantarum*) and germination (Camacho et al., 1991; Dagnia et al., 1992; Jiménez-Martínez et al., 2007; Szakács and Stankovics, 1983). These approaches had, on the one hand, the advantage that they did not produce significant chemical residues. However, on the other hand, these treatments required preparatory operations such as dehulling, or grinding. In addition, all reported studies about biological methods to debitter lupin were conducted on a laboratory scale only and always started with seeds that had an initial alkaloid content up to 11 g kg^{-1}, which is about three times lower than the alkaloid content of a bitter lupin species such as *L. mutabilis* (30-35 g kg^{-1}). Moreover, biological treatments would need a substantial energy input because they are carried out at temperatures between 30 °C and 37 °C, except for the germination that was at 20 °C - 25 °C. Considering that this approach needed additional operations and energy, and would only achieve a limited extraction of alkaloids, processors decided that the biological methods were unsuitable for them.

Next, processors of lupin were informed about chemical treatments to debitter lupin. Chemical approaches to extract alkaloids were distinguished as (i) extraction with hexane and basic solutions (sodium carbonate, ammonia solution), (ii) basic extractions (sodium bicarbonate, sodium hydroxide) and (iii) mixed alcohol extraction (ethanol-hexane) (Jiménez-Martínez et al., 2003; Nossack et al., 2000; Ortiz and Mukherjee, 1982; Torres-Tello et al., 1980). Chemical extractions have the advantage that they can be used for lupin seeds with high alkaloid contents (between 19.4 and 42 g kg^{-1}). However, on the other hand, these treatments also need the seeds to be dehulled, crushed and/or split. In addition, chemical treatments might add residues, which could pose health risks and could affect the taste of the

product. All chemical treatments required additional equipment and facilities for safe operation and disposal of waste. Moreover, basic debittering would decrease the methionine availability in lupin (Gueguen and Cerletti, 1994).

Finally, after considering aspects such as the management of chemical waste, the chemical safety of the product and the facilities needed to implement an improved process, the community opted for a trial based on the optimization of the aqueous debittering process. This step also contributed to the objectives of the TELFUN project because the community showed its preference (participative research). In addition, the technology to be used was selected according to the concept of appropriate technology for the community. This optimization was carried out in two stages, first the soaking and cooking operations, followed by the washing operation. This approach allowed a minimization of the number of runs and therefore it reduced the experimental time needed and money used.

Although the soaking operation only resulted in a very small removal of alkaloids, it was essential because it allowed the seed to be hydrated, facilitating the following operations, namely cooking and washing. In case that the seed would not be sufficiently hydrated during the soaking process, the hydration would have to be completed by the following operation (cooking). If the hydration time is extended, the time of the process will be increased. The cooking stage was the most efficient in removing alkaloids, but also the most expensive in terms of energy. However, cooking could not be avoided because during this stage enzymes are inactivated and the lupin protein is coagulated, reducing its loss. This stage also facilitates the leaching of the alkaloids by increasing the cell wall permeability (Gross et al., 1983; Jiménez-Martínez et al., 2003). This shows the necessity to find the ideal combination of soaking and cooking times. The best combination was soaking for 18h and cooking for 1 h (code: S18C1).

The optimization of the washing process, based on the idea of hydro-agitation, was deduced from studying Fick's laws of diffusion, Einstein's and Stokes' equations of diffusion for spherical particles, the concepts of the diffusion mechanics from a stirred solution of limited volume and the unsteady state (Crank, 1975; Chang, 1977; van Boekel, 2009; Walstra, 2003). Debittering of lupin by hydro-agitation had been used earlier by Caicedo et al. (2001). However, they used a process at elevated temperature (40 ^{0}C), did not model the process, did not include different conditions of washing, neither did they state details of hydro-agitation (pressure, number of re-circulations of water per minute).

In our research, it was concluded that two factors, namely agitation time and amount of water used, needed to be investigated. The experimental design considered the application of a broad range of conditions. Since no suitable equipment was commercially available, a hydro-agitator was developed and constructed. The machine had to be simple to operate by the community to comply to the concept of appropriate technology. With this in mind the author designed, constructed and tested a machine, which successfully accomplished the objectives for which it was created, namely to be suitable to study the agitation effect, and be easy to construct, maintain and operate.

Another requirement was a suitable mathematic model to describe the diffusion in non-steady conditions for a product with a shape like lupin (convex). Available models refer to planes, spheres or cylindrical shapes; moreover alkaloid diffusion had not been modelled before. The lupin alkaloids, non-steady state diffusion model was developed based on the surface response methodology from the Design expert 8 computer program (Stat-Ease, Inc., Minneapolis, Minnesota, U.S.A).

The optimum process conditions found were the combination of soaking for 18 h, cooking for 1 h, changing the water 3 times per day and agitating it for 22 h during washing (code: S18C1W3H22). This resulted in a 30 % reduction of processing time, a reduction of about 20 % in water use and a 15 % reduction in the loss of solids as compared with the current process used in San Pedro de Tanicuchi. Other processes such as S18C1W9H11 and S18C1W6H22 could even reduce the time needed by about 50% compared with the current process, but these were less favourable with respect to water use and loss of solids.

Even though this approach of optimizing was successful, it is not the most frequently used procedure. Most optimization approaches compare the final results of several processes without paying attention to the intermediate steps of the process. Therefore, it would be of interest to compare the efficacy of the optimization approach used in this study with others to determine whether this approach could also benefit other products and processes.

The best product from a consumers´ point of view

To determine the best product according to consumers, 99 panellists were asked to compare twelve different products obtained with twelve different conditions for washing, after the optimum soaking and cooking (S18C01) combination. Consumers were not asked to select

the best conditions for soaking and cooking because at this level the amount of alkaloids is still high, making the product unsuitable for human consumption.

The approach for this research differed from other investigations in the sense that usually the researcher first selects the best treatments and confronts consumers only with two or three samples. That approach is efficient because it simplifies the sensorial evaluation and reduces costs. However, such an approach could also be biased. In addition, a valuable opportunity to understand and/or model consumer preferences would be missed. In this study we did the opposite, giving all samples the same chance to be assessed by consumers, avoiding researcher subjectivity. However, since a large number of samples can be inconvenient (with respect to cost, carry over effects, unbalance and reduction of test power), it was decided to control these variables by using Design Generator Form, Incomplete Block design that the program Qi statistic- Design Express version 1.6 offers. The obtained design was a valuable approach because it did not have carry over effects, presented a good statistical efficiency (>80%), was balanced and the number of judges (99) was not excessively high. Thus we were able to model and quantify the relations between liking level, processing conditions and expected price. In addition, we elucidated the relation of elasticity of willingness to pay versus liking level and expected price. In this sense this research is more advanced than what is usual, i.e. the consideration of only two variables and qualitative presentation of findings. To our knowledge, no other study has considered three variables and presented the findings in a quantitative way.

Using this approach and based on the willingness to pay, consumers chose products that had undergone treatments W6H0, W2H0 and W3H0 as the best, indicating that there is a gap between consumer preferences and optimum conditions resulting from technological considerations (yield, efficiency). Finally, once processors were informed of these results, they decided to keep to the "traditional" process W3H0 because of consumers' preferences, instead of adopting a "technologically" more efficient approach.

Consequently, one might say that the effort to optimize the process was unsuccessful as the processing conditions were unchanged. However, that is not the only outcome.

- It has been established that the current process results in a product that is one of the most accepted by consumers and that this is also the cheapest one ($1.52 kg^{-1}). Second, today, a mathematic model of the aqueous process is available. This means that the process can be adapted to different alkaloid contents in the seed, amount of

available water and time needed to complete a process in different scenarios. This is a very important tool to manage the processing and selling operations.

- In addition, based on the microbial results of the product obtained from the processors of San Pedro, they were motivated to improve the microbial quality of lupin in many ways. They invested in constructing a new processing plant with much better conditions than before (the plant has walls, a ceiling, floor tiles, and tiles on the walls). Besides, the layout of the plant was improved; nowadays the raw material is kept on one side of the plant and the debittered product on the other side. Moreover, the workers use protection for the product and themselves. Also a laboratory for line control was included in the plant (Figure 1).
- Because the relationship between liking, price and willingness to pay was substantiated and even quantified, processors know that consumers are inclined to pay more if they, the consumers, note a significant increase in liking. According to Homburg et al. (2005), consumers will also pay more when they perceive increments in quality of food, ambience and /or service. The information for our research has generated four different actions from the lupin processors. One is the improvement in the processing plant as mentioned above. The second action is the acceptance by the processors of the need to include a pasteurization step in the process (suggested 5-10 min at $\approx 90\ ^{0}C$) before packing. The third action concerns a change in the packing process from manual to automatic. The preliminary test of the packing machine is shown in Figure 2, and in February 2013 this machine was bought by the processors. The last action was to motivate them to obtain sanitary certificates from the Ecuadorian Sanitary Institute, which will allow them to sell certified, packaged products with better quality and thus, at a higher price compared with the bulk product. At present (June 2013) they are conducting the required tests and paperwork to obtain that certification.

Figure 1. The new plant for debittering *Lupinus mutabilis*. a) Soaking. b) Cooking. c) Washing

Furthermore processors know now that, for using lupin as a food ingredient, they can crush raw lupin before washing it in order to speed up the debittering process and to reduce the amount of water and time used. Moreover, processors are aware that lupin alkaloids could have different uses and they are planning to study the extraction process. In this sense this research identified the best process from technological and consumer points of view and presented those results to the processors. Therefore, this research accomplished another objective of this thesis and one goal of food sovereignty and the TELFUN project, which was

to actively involve the actors of the food chain, in this case consumers and processors of lupin products.

Figure 2. Optimization of the aqueous debittering process. a) Processors of the community of San Pedro de Tanicuchi receiving information about hydro-agitation. b) Automatic packaging test. c) Debittered lupin in bulk ready to be packaged. d) Packaged lupin.

Contribution of this thesis to the TELFUN project and the food sovereignty concept

Food sovereignty is based on the right of people to decide which food they eat and how to process this food (INREF, 2010). Related to food sovereignty are other concepts such as the importance of selecting the appropriate technology, and the importance of generating research that can be participative, including all actors of the society (producers, processors, and consumers). Food sovereignty points out that in this way the local food chains could become connected, strengthened and consequently contribute to the alleviation of malnutrition and poverty (MCDS and FAO, 2011). In this context the TELFUN program was

designed to investigate how technological improvements and promotion of local crops and indigenous foods could enhance food sovereignty. To achieve this, three interdisciplinary teams consisting of a plant breeder, food technologist, human nutritionist and social scientist were formed to improve the lupin chain in Ecuador, the mung bean chain in India and the cowpea chain in West Africa (Benin and Ghana). In addition, the TELFUN program encouraged researchers to generate research that connects the different realities in India, Ecuador, Benin and Ghana as a way to broaden the view on the processes to be improved. Moreover, the TELFUN program also encouraged researchers to generate knowledge that can be used in actual practice. In the case of Ecuador and after several meetings, discussions and analyses by the lupin team it was clear that in order to strengthen and connect the lupin chain, the food technologist should develop a technology for debittering lupin from a broad viewpoint, satisfying all actors of the lupin chain (from soil to stomach) simultaneously while improving the lupin value. In this regard this research has indeed applied the food sovereignty concept and thereby contributed to the objectives of the TELFUN program, as follows:

- By critically reviewing the potential of lupin components (protein, alkaloids) as industrial materials, this research brought to light other uses for lupin in addition to its traditional use as nutritional food. This is important because sub products such as alkaloids could be used for pharmaceutical purposes. The protein of lupin was found to be a useful food ingredient for the chemical and food industries. Such applications could increase the sovereignty of the community and increase the value of lupin as a cash crop.
- Since safe food products contribute to the objectives of the food sovereignty concept, this research pointed out the necessity to improve not only the efficiency of the current process but also the microbial quality of the product.
- Consumers as part of the community also played an important role when they chose the product that fitted best their taste and wallet. Therefore, this research included another actor of the chain, the consumers, thereby applying the concept of “science within society”.
- The decision about what was the best processing condition was made by consumers and processors, not by the researcher. In this sense, this research accomplished one of the most important goals of food sovereignty, “the right of people to choose the best

food for them" (Windfuhr and Jonsén, 2005). Science created options but the society decided.

- It was not considered opportune that the plant breeder would develop lupin seeds with low alkaloid contents because alkaloids are a defence mechanism of the plant against insects and bacteria. This and the fact that the plant breeder could not totally control the alkaloid content in the lupin because this also depends on genetic and agronomical conditions, necessitated the food technologist to develop a debittering technology that could be easily adapted to seeds with different alkaloid contents. Moreover, since lupin is a food with a high nutritional value, increased consumption would benefit the population. The technologist contributed to this by studying and modelling the effect of processing conditions on liking. Now we know which treatments (and products) are preferred by consumers. In addition, the developed technology for debittering took the processors' points of view into consideration and therefore was participative and developed by an interdisciplinary approach. During the design of this technology it was taken into account that it should be manageable by the local community, thus it was developed considering the concept of appropriate technology. Therefore, another important goal of the TELFUN project was achieved in this research, namely "science within society".
- The findings of this research have been used by the lupin processors to improve the processing plant and the debittering process as mentioned above (Figures 1 and 2). In that sense this research reached another important objective of the TELFUN project, namely that scientifically generated knowledge should be applicable in daily practice.
- Another important contribution of this research refers to patenting. The designed machine might have generated a patent. However, patenting could be seen as conflicting with the concept of food sovereignty. Therefore, this research did not try to get a patent. In contrast, the way that the machine operates has been communicated to the community (Figure 2).

Limitations of this research

This research critically analysed information available on the behaviour of lupin proteins, the acute toxicity, nutritional composition and uses of lupins. However, no experimental work

was done to verify the reported data, or to solve questions or deductions inferred from that data.

This research studied the impact that different aqueous treatments have on several variables, including the content of solids, but did not study the impact of those treatments on the amounts and composition of macro- and micronutrients. This research also did not include laboratory studies to investigate several other aspects of the aqueous technology for debittering lupin, such as the possibility of re-utilising the water, or the impact of crushing or powdering lupin on speeding up the diffusion of alkaloids.

Moreover, this research was conducted within the framework of food sovereignty and the contributions to this concept were stated only qualitatively because they were not quantified. In addition, actions to ensure the sustainability in time of the achievements made by this research were not undertaken.

Recommendations for future research from a technological viewpoint

1. The behaviour of lupin proteins must be linked to the chemical composition of lupin, its agronomical history and the processes of extraction of lupin flour, protein isolates and concentrates.
2. The acute toxicity of lupin needs to be quantified following the protocol of the pharmaceutical industry.
3. The rheological behaviour of lupin mixtures such as doughs, slurries and emulsions and the quality of lupin-based products (such as pasta, bread, sausages and so on) made with those mixtures, is not sufficiently understood. Special attention should be given to developing lupin-products with higher consumer acceptation, especially for taste and texture.
4. When debittered lupin is used as an ingredient, the hydro-agitation system applied to crush and powder raw lupin should be studied in detail because this technology has the potential to reduce the use of water, time and energy.
5. The re-use of water for debittering whole lupin should be studied because the alkaloid concentration in the water during the first half of the washing process is much higher than that of the second half. Therefore, the water used during the second half of the washing stage of one batch could be used for the first half of the washing stage of the next batch, and so on. The amount of water saved would be about 40%.

6. The extraction and study of alkaloid applications should be given attention because this could be a way to generate alternative uses for lupin, improving sovereignty and supporting the economy of local communities.
7. The nutritional value of lupin products needs to be studied in relation to processing. Special attention should be given to variations in macronutrients, minerals and vitamins as affected by different technological approaches of debittering.

Recommendations for studies on food sovereignty

In order to contribute to research based on interdisciplinarity, such as food sovereignty studies, two recommendations are given:

1. Interdisciplinary studies need more time compared with monodisciplinary research. Therefore, this has to be considered when timing multidisciplinary studies.
2. In order to ensure the sustainability in time of the achievements made by this kind of research, food sovereignty studies should include an analysis of the extent in which international trade and patents affect food sovereignty aims. In addition, actions to support achievements with respect to food sovereignty should be presented.

Final conclusion

The research presented in this thesis is only the beginning, there is still much further research needed on lupins. *Lupinus mutabilis* offers a large potential as a food and as an industrial ingredient. This research showed a possible nice future for *L. mutabilis*, but this can only be achieved by sustained efforts.

Food sovereignty is a valuable way to improve the nutritional status and wellbeing of, especially, poor communities. Food sovereignty requires an interdisciplinary approach involving science and technology and social sciences (economics, ethics and politics).

Finally, the concept of food sovereignty might be an inspiration for other fields such as energy and production of technology to contribute to the improvement of the wellbeing of resource-poor people.

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Summary

The present thesis studied the technological aspects of the debittering process of lupin in a food sovereignty framework by addressing four specific objectives: (i) to critically review published data on composition, uses, toxicology and debittering of the major lupin species, focusing on *Lupinus mutabilis*; (ii) to establish the efficiency of the current debittering process as a starting point of optimization; (iii) to study the effect of processing conditions on efficiency of the aqueous debittering process, and (iv) to assess the best conditions for debittering lupin from processing and consumers´ points of view using mathematic modelling.

In chapter 2, the nutritional composition, uses, toxicology and debittering of four main lupin species, namely *Lupinus albus, L. luteus, L. angustifolius and L. mutabilis,* with emphasis on the latter, are summarised. Critical evaluation of data showed that *L. mutabilis* has the highest nutrient content compared with the other lupin species studied, and this nutrient content is comparable with that of soya bean. Variations in lupin composition and alkaloid content depend on genetic and agronomic factors but also on the method of analysis used by researchers. The fatal acute dose of lupin alkaloids was reported as 10 mg kg^{-1} body weight (bw) for infants and children, and 25 mg kg^{-1} bw for adults. As to chronic toxicity, the exact safe dose of alkaloids still needs to be established. Fresh, pasteurized and debittered *L. mutabilis* can be eaten as a snack, or used for making many foods and meals. However, the taste and texture of most of them need improvement. Specific protein isolates from lupin could be used in the food and chemical industry as a gelling agent, foaming agent as well as emulsifier. Isolated alkaloids from lupin could have pharmaceutical applications because of their possible anti-arrhythmic and hypocholesterolemic activity. Besides, alkaloids might decrease arterial blood pressure. Regarding options to debitter lupin to be consumed as whole grain for human beings, aqueous extraction is the only option that reduces alkaloids without generating chemical residues in the food or affecting the environment. Biological options were reported to be rather ineffective.

In chapter 3, the process for debittering lupin (*L. mutabilis*) with cold water was investigated on a laboratory and semi-industrial scale. The investigation estimated the efficiency of each stage of the process (soaking, cooking and washing) in terms of time, amount of removed alkaloids and water used. The outcome showed that cooking was the most efficient. The study also estimated the performance of the process in terms of the weight of the final product, remnant solids and chemical composition as well as microbial quality. The output showed that in the process 22% of total solids was lost, principally fats, minerals and

carbohydrates, and revealed the presence of *E. coli* and a high count of total mesophilic aerobic bacteria (log 6.71 cfu g^{-1}). Mathematical modelling based on Fickian diffusion led to the estimation that the diffusion coefficient of alkaloids through the polymer matrix of lupin endosperm varies between $1x10^{-10}$ and $1x10^{-11}$ m^2 s^{-1} depending on the stage of the process. All these results justified optimization of the aqueous process and some recommendations were given. Thus, increasing the temperature of the process, reducing the tortuosity in the lupin matrix by soaking the seeds in salt solutions and continuous replenishment of water in the washing operation might help to optimize the process.

In chapter 4, the effect of nine conditions of soaking and cooking, and nine conditions of washing (time of agitation and number of water changes) were evaluated on alkaloid content, solids in the product, final weight, processing time and water and energy used in the debittering process. A debittering machine was designed and built by the author to run the washing experiments. Computer software Design-Expert 8 was used to model, for the first time, the soaking and cooking and washing processes. The goal of this study was, besides understanding and modelling the process, to find the optimal processing conditions from a technological point of view, namely minimization of time, energy, water, alkaloids, and maximization of solids d.w. and seed weight f.w. A treatment of soaking for 18 h, cooking for 1 h, 6 changes of water day^{-1} and 11 h of agitation day^{-1} was the optimal processing condition. Overall, the results could be summarized in regression equations, describing the time needed to debitter lupin for any alkaloid concentration and by using any combination of agitation time and changes of water. Since processing time is associated to labour input and logistics, this allows to manage the process. The newly developed technology could also be used for optimizing processes such as hydrating and/or removing undesired material from other bitter lupin species, soya bean, cowpea, mungbean, or other seeds.

In chapter 5, the effect of twelve processing conditions (time of agitation and number of water changes) on liking, expected price and buying preference of consumers was evaluated by using three software programmes. Qi statistic- Design Express version 1.6 was used to calculate the number of panellists needed. GRAPH PAD INSTAT (version 2.01) was used for statistical analysis of the data and Design-Expert 8 was used to model the impact of processing conditions on liking and price. The impact of liking and price on willingness to pay of consumers was also estimated and modelled with this computer program. The main outcome was that, despite the fact that the new technology reduced the processing time and the use of labour, the net effect of increasing processing (agitation and water changes)

augmented the final cost and decreased the liking of obtained products. Another outcome was that people were willing to pay more if they perceived a benefit from the process used and/or the obtained product. For example, consumers would spend about 0.3 $ kg^{-1} more for lupin for which the liking increased from 6.2 to 7 (on a scale from 1 to 9). A treatment of three changes of water day^{-1} and 0 h of agitation day^{-1} was closest to the optimal combination of liking, price and willingness to pay. The approach used in this study also seems suitable to estimate relationships between processing conditions, liking, price and willingness to pay in other products. The generated information can be helpful in decision making, such as selection of consumers´ preferred process and liking in relation to pricing.

In the general discussion (chapter 6), it was concluded that this particular research contributed to the optimization of the process for debittering lupin from consumer and technological points of view. In addition, it was established that *L. mutabilis* offers opportunities as a food ingredient because lupin improves the nutritional content of products when present. Moreover, specific lupin materials can be used in the food, chemical and pharmaceutical industries. In addition, this chapter showed that by optimizing the debittering process and bringing to light new uses for lupin, this research contributed to the TELFUN programme within the food sovereignty concept. Finally, some suggestions were made for future research. The most import are: i) to continue with studies on lupin (as food) and of its protein isolates and alkaloids (as ingredient raw material for the chemical and pharmaceutical industries); and ii) to continue with studies on food sovereignty because it is a valuable way to improve the wellbeing of, especially, poor communities, taking into account that these studies are interdisciplinary and need more time.

Samenvatting

Vanuit het perspectief van voedselsoevereiniteit zijn in dit proefschrift de technologische aspecten van lupine-ontbittering bestudeerd, met de volgende vier doelstellingen: (i) een kritische beschouwing van eerder gepubliceerde wetenschappelijke gegevens over samenstelling, gebruik, toxiciteit en ontbittering van de belangrijkste lupinesoorten, in het bijzonder *Lupinus mutabilis*; (ii) meten van de ontbitterings-effectiviteit van reeds bestaande processen, als uitgangspunt voor optimalisatie; (iii) invloed van procesomstandigheden op de mate van ontbittering in water, en (iv) gebruikmakend van wiskundige modellen, het vinden van de beste procesomstandigheden voor lupine-ontbittering, vanuit het oogpunt van zowel producenten als consumenten.

In hoofdstuk 2 wordt een samenvatting gegeven van de nutriëntensamenstelling, het gebruik, toxiciteit en ontbittering van vier belangrijke lupinesoorten namelijk *Lupinus albus*, *L. luteus*, *L. angustifolius* en *L. mutabilis* met nadruk op de laatste soort. Een kritische beoordeling van de gegevens gaf aan dat *L. mutabilis* het hoogste nutriëntengehalte heeft van de lupinesoorten, en dat de voedingswaarde vergelijkbaar is met die van sojabonen. Verschillen in lupinesamenstelling en hun alkaloïdengehalte zijn afhankelijk van genetische en teeltfactoren maar ook van de analysemethoden die door onderzoekers zijn gebruikt. De acuut lethale dosis van lupine alkaloïden werd gerapporteerd als 10 mg kg^{-1} lichaamsgewicht (lg) voor zuigelingen en kinderen, en 25 mg kg^{-1} lg voor volwassenen. Er is nog geen goede schatting voor chronische toxiciteit. Verse, gepasteuriseerde en ontbitterde *L. mutabilis* worden gegeten als snack, of als maaltijdingrediënt. De smaak en textuur van de meeste lupineproducten verdienen verbetering. Bepaalde eiwit-isolaten uit lupine zouden gebruikt kunnen worden in de voedingsmiddelen- en chemische industrie als verdikkingsmiddel, schuim of emulgator. Alkaloïden uit lupine kunnen farmaceutische toepassingen hebben wegens hun vermeende anti-arrhythmische en cholesterolverlagende werking. Verder zouden ze bloeddrukverlagend werken. Van de mogelijkheden om intacte lupinezaden te ontbitteren voor menselijke consumptie, is extractie in water de enige aanpak die leidt tot alkaloïdenverwijdering zonder chemische residuen in het voedsel of het milieu achter te laten. Biologische ontbitteringsmethoden werden als tamelijk ineffectief beschouwd.

Hoofdstuk 3 beschrijft het ontbitteringsproces van lupine (*L. mutabilis*) met koud water, onderzocht op laboratorium- en op semi-industriële schaal. Dit onderzoek richtte zich op de effectiviteit van elk processtadium (weken, koken en spoelen) met het oog op procesduur, alkaloïdenverwijdering, en waterverbruik. Koken was het meest effectief. Er werd ook gekeken naar de opbrengst aan eindproduct, droge stof-opbrengst, chemische samenstelling

en microbiologische gesteldheid. Tijdens het proces bleek 22% van de droge stof verloren te gaan, vooral in de vorm van vetten, mineralen en koolhydraten. Verder bleken *Escherichia coli*, en een aanzienlijk aantal (log 6,71 kve g^{-1}) mesofiele aerobe bacteriën in het eindproduct aanwezig te zijn. Met behulp van wiskundige modellen gebaseerd op de diffusiewet van Fick kon een schatting worden gemaakt van de diffusiecoëfficient van alkaloïden door de polymere matrix van het lupine endosperm, die varieerde tussen $1x10^{-10}$ en $1x10^{-11}$ m^2s^{-1} afhankelijk van het processtadium. Deze resultaten rechtvaardigen een optimalisatie van het extractieproces in water waarvoor een aantal aanbevelingen werd gegeven. Zo kunnen een hogere procestemperatuur, een vermindering van de tortuositeit van de lupinematrix door de zaden in zoutoplossing te weken, en een continue stroming van het water tijdens het wassen tot een effectiever ontbitteringsproces leiden.

In hoofdstuk 4 werd de invloed van negen week-kook combinaties, en negen was-omstandigheden (doorstromingstijd en aantal waterverversingen) op het gehalte aan alkaloïden, droge stof, het opbrengstgewicht van lupine, en de procesduur, water- en energieverbruik van het ontbitteringsproces bepaald. Hiervoor werd door de auteur een ontbitteringsapparaat ontworpen en gebouwd. Het softwarepakket Design-Expert 8 werd voor de eerste keer gebruikt om het week-, kook-, en wasproces te modelleren. Het doel van dit onderzoek was het proces te begrijpen en te modelleren, en een technologisch optimum te vinden van omstandigheden resulterend in een minimale procesduur, minimaal energie- en waterverbruik, en alkaloïdengehalte, naast een maximale opbrengst zowel als droge stof als eindproduct. Een procescombinatie van 18 uur weken, 1 uur koken, 6 waterverversingen per dag en 11 uur doorstroming per dag tijdens het wassen was het technologisch optimum. De resultaten werden samengevat in regressievergelijkingen waarmee de procesduur kan worden afgeleid voor elke alkaloïdenconcentratie, gebruikmakend van willekeurige combinaties van doorstroomtijd en waterverversing. Aangezien de procesduur consequenties heeft voor arbeidstijden en logistiek kan hiermee het proces gepland worden. De nieuw-ontwikkelde technologie kan ook worden toegepast voor de optimalisatie van andere processen zoals bevochtigingen en/of het verwijderen van ongewenste inhoudsstoffen uit andere bittere lupinesoorten, alsook diverse zaden zoals sojabonen, cowpeas, mungbonen, enzovoort.

In hoofdstuk 5 werd de acceptatie, prijs en koopbereidheid door de consument onderzocht van lupine die op 12 verschillende manieren (combinaties van doorstroomtijd en waterverversingen) was ontbitterd. Hiervoor werden 3 softwarepakketen gebruikt, namelijk Qi statistic – Design express versie 1.6 voor het berekenen van het aantal benodigde

proefpersonen, GRAPH PAD INSTAT versie 2.01 voor statistische analyse van de gegevens, en Design-Expert 8 voor het modelleren van de invloed van procesomstandigheden op acceptatie en prijs. Het belangrijkste resultaat was dat de nieuwe technologie weliswaar de procesduur en benodigde arbeid verminderde, maar dat de extra behandelingen (doorstroming en waterverversing) de kostprijs verhoogden en de acceptatie verminderden. Het bleek ook dat consumenten bereid zijn meer te betalen wanneer zij denken dat het proces of het product voor hun een toegevoegde waarde heeft. Consumenten waren bijvoorbeeld bereid ongeveer 0,3 $ per kg meer te betalen voor lupine waarvan de acceptatiescore van 6,2 naar 7 was gestegen (schaal van 1 – 9). Een procescombinatie met 3 waterverversingen en 0 uur doorstroming per dag benaderde het optimum voor acceptatie, prijs en koopbereidheid. De aanpak van dit onderzoek lijkt ook bruikbaar om de verbanden tussen procesomstandigheden, acceptatie, prijs en koopbereidheid voor andere producten te onderzoeken. De verkregen informatie kan besluitvorming faciliteren, zoals de selectie van processen die tot hoge acceptatie leiden tegen een gunstige prijs.

In hoofdstuk 6 wordt bediscussieerd dat dit onderzoek bijdragen levert aan de ontbitteringstechnologie voor lupine vanuit technologische en consumenten standpunten. Het werd ook duidelijk dat *L. mutabilis* door zijn voedingswaarde mogelijkheden biedt als een voedselingrediënt. In bredere zin zijn bepaalde componenten van lupine interessant voor de voedsel-, chemische- en farmaceutische industrie. Verder wordt er op gewezen dat dit onderzoek ter optimalisering van de lupine-ontbittering en identificatie van mogelijke nieuwe lupinetoepassingen, een bijdrage leverde aan het TELFUN programma gericht op voedselsoevereiniteit.

Tenslotte worden enkele suggesties gedaan voor verder onderzoek. Ten eerste dient het onderzoek voortgezet te worden aan lupine als voedsel, en aan eiwit-isolaten en alkaloïden als ingrediënten voor chemische en farmaceutische industrie. Ten tweede, gezien het interdisciplinaire en tijdrovende karakter van studies m.b.t. voedselsoevereiniteit, dienen deze voortgezet te worden omdat ze kunnen leiden tot een verbetering van het welzijn van kansarme bevolkingen.

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I feel thankful to many people whom I met and who inspired me and/or helped me in one way or another. My apologies in advance if I miss a name. However, rest assured that my thankfulness is not less than for those listed below.

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Bibliography and curriculum vitae of the author

List of publications

Published paper

F.E. Carvajal-Larenas, M.J.R. Nout, M.A.J.S. van Boekel, M. Koziol, A.R. Linnemann, 2013. **Modelling of the aqueous debittering process of *Lupinus mutabilis* Sweet**. LWT - Food Science and Technology. 53:507-516.

Accepted papers

F.E. Carvajal-Larenas, A.R. Linnemann, M.J.R. Nout, M. Koziol, M.A.J.S. van Boekel. ***Lupinus mutabilis*: composition, uses, toxicology and debittering.** Critical Reviews in Food Science and Nutrition.

F.E. Carvajal-Larenas, M.A.J.S. van Boekel, M. Koziol, M.J.R. Nout, A.R. Linnemann. **Effect of processing on the diffusion of alkaloids and quality *of Lupinus mutabilis* Sweet**. Journal of Food Processing and Preservation.

Submitted paper

F.E. Carvajal-Larenas, M. Koziol, A.R. Linnemann, M.J.R. Nout, M.A.J.S. van Boekel. **Liking and willingness to pay for *Lupinus mutabilis* Sweet in relation to debittering conditions**

Curriculum vitae

Francisco Ernesto CARVAJAL LARENAS was born on July 3rd 1967 in Quito, Ecuador. He attended primary and secondary school in Ambato. Then, he followed an 8-year full-time programme and graduated as a Food Engineer at Universidad Técnica de Ambato, Ecuador. In 1993 he was hired by Nabisco Ecuador (Kraft Food) to work as Process Analyst. Next, he was promoted to Quality Analyst, Assistant of Quality, Plant Statistician, Processing Supervisor and finally Manager of the Department of Quality Insurance and New Products Development. In 1997 he moved to Parmalat-Ecuador to work as a Process Manager. Two years later, he was promoted to Plant Manager until 2001.

From 2001 until 2005 he combined three activities, namely i) advisor in manufacturing optimization and profits maximization of food and non-food industries, ii) part time lecturer at Universidad San Francisco de Quito (Food Processing, Process Management and Management of Feasibility Projects) directed to undergraduate students, and iii) Master student in Management (2001-2004) at Universidad Politécnica Javeriana followed by a Masters in Food and Nutrition at Universidad San Francisco de Quito (2003-2005).

In 2004 he became a guest lecturer in Masters of Business Administration programmes at several universities in Ecuador teaching courses in Operations Management, Process Management, Management of Feasibility Projects and Ethics and Global Economy.

Since 2005 he has been a full time lecturer at Universidad San Francisco de Quito in addition to his other two activities (guest lecturer in MBA programs and independent advisor).

In February 2007 he started a PhD research programme at Wageningen University. Since then, his activities as advisor and guest lecturer decreased substantially to give full attention to the PhD programme.

Since December 2011 he has also acted as Plant Director of the Pilot Plant of Universidad San Francisco de Quito.

After graduation he plans to combine lecturing, researching and advising activities. He plans to give emphasis to modelling and process optimization, studies about operations and process management, ethics and global economy and feasibility of projects in food and non-food institutions.

Overview of completed training activities

Discipline specific activities	year
First TELFUN workshop, Wageningen, the Netherlands (oral presentation)	2007
Symposium on food sovereignty promoting or undermining food security, VLAG & ICCO, the Netherlands	2007
Improving the position of smallholders through knowledge exchange between tropical food chains and research, VLAG & other graduate schools, the Netherlands	2007
II Foro de la Realidad nacional agropecuaria (Agrarian reality in Ecuador), Universidad Central del Ecuador, Quito, Ecuador	2007
Congreso Latinoamericano y Caribeño de ciencias sociales (Latin-American and Caribbean congress of social sciences), FLACSO, Quito, Ecuador	2007
Second TELFUN workshop, Quito, Ecuador (oral presentation)	2008
Food sovereignty: origins, meaning and relation with other discourses, Wageningen, the Netherlands	2008
Technologies for sustainable foods networks. Does locality matter?, Wageningen, the Netherlands	2008
Third TELFUN workshop, Hisar, India (oral presentation)	2009
Quesería y productos lácteos (Cheese and dairy products), Taller de Tradiciones, Madrid, Spain	2009
La lucha contra el hambre y la pobreza (Struggling with hunger and poverty), Universidad Complutense de Madrid, Spain	2009
Conservas artesanales (Preserved foods), Taller de Tradiciones, Madrid, Spain	2009
Reaction kinetics in food science, VLAG, Wageningen, the Netherlands	2009
Fourth TELFUN workshop, Tamale, Ghana (oral presentation)	2010
Symposium: Sensory perception and food intake regulation, Wageningen, the Netherlands	2010
Seminario de certificación orgánica (Seminar on organic certification), USFQ, Quito, Ecuador	2010
Simposio en biotecnología agrícola y de alimentos (Symposium on agricultural and food biotechnology), USFQ, Quito, Ecuador	2010
I Simposio de nutrición y salud. Creciendo activo y saludable (Symposium on nutrition and health: Growing active and healthy), USFQ, Quito, Ecuador	2011
III Simposio nacional de agro negocios (III National symposium on agro-business), USFQ, Quito, Ecuador	2011
Simposio control de plagas y enfermedades en cultivos ornamentales (Symposium on management of pests and diseases in ornamental crops), USFQ, Quito, Ecuador	2011
Fifth TELFUN workshop, Cotonou, Benin (oral presentation)	2011
I Simposio de Fisiología Vegetal (I Symposium on Vegetable Physiology), USFQ, Quito, Ecuador	2012

Discipline specific activities, continued	year
I Simposio de alimentos funcionales (I Symposium on functional foods), USFQ, Quito, Ecuador	2012
Desarrollo tecnológico y nuevos mercados para la agricultura (Technological development and new markets for agriculture, oral presentation), USFQ, Quito, Ecuador	2012

General courses	
	2007
Information literacy, Wageningen University, Wageningen, the Netherlands	
Philosophy and ethics of food science & technology, VLAG, Wageningen, the Netherlands	2007
Presentation skills, Wageningen University, Wageningen, the Netherlands	2008
English (grammar), USFQ, Quito, Ecuador	2008
English (speaking), USFQ, Quito, Ecuador	2008
English (composition), USFQ, Quito, Ecuador	2008

Other activities	
	2007
Preparation VLAG research proposal, Wageningen University, Wageningen, the Netherlands	
VLAG PhD week, Wageningen, the Netherlands	2007
PhD trip to Canada (oral presentation)	2008
Group colloquia, Product Design and Quality Management group, Wageningen University, the Netherlands	2007-2010
PhD trip to Australia (oral presentations)	2010