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Germination, an Effective Process to Increase the Nutritional Value and Reduce Non-Nutritive Factors of Lupine Grain (*Lupinus mutabilis* Sweet)

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Abstract This study was designed to develop and evaluate the processing technology for germinated lupine, in order to increase the nutritional value of the grain and decrease the content of non-nutritive factors. For this study, INIAP-450 lupine variety was used. The water absorption rate was tested with three grain sizes (7, 8, 9 mm in diameter), determining a similar behavior, with a sudden increase in moisture during the first three hours of soaking, it was slow in the next 12 h and was constant from that time. A 90% germination and lower content of non-nutritive factors were reached under the following conditions: Grain moisture (45%), temperature (20°C), germination time (4 days). Germinated lupine was debittered after 40 h in water through stirring. With the use of germination, the non-nutritive factors: raffinose, stachyose and alkaloids, decreased significantly, while the minerals: calcium (0.63%), magnesium (0.078%), copper (112 ppm), iron (121 ppm), manganese (101 ppm) and zinc (184 ppm), were increased. Germination not only improved the nutritional content of the grain, it also increased the availability of nutrients, expressed in digestibility and protein solubility, the profile of fatty acids and amino acids of germinated and debittered grain. The nutritional and organoleptic benefits obtained with the germination of lupine, make it advisable to apply this process in the food industry in order to offer consumers a new product, with functional properties and high nutritional value.

Keywords Germinated lupine, Alkaloids, Stachyose, Raffinose, Availability of nutrients

1. Introduction

Lupine is a legume grown in the highlands of Ecuador. Its importance lies in the content and nutritional value of its grain, it has between 41-52% protein on dry matter; it is the richest grain that contains this nutrient and can replace meat and milk, in regions where these foods are not readily available. Also, the root system of the plant has the ability to fix atmospheric nitrogen, thereby improving soil fertility and can also be used as an alternative rotation with other crops. In addition, production, processing and marketing of this legume, now constitute a source of employment and income for poor farmers in marginal areas [1].

In recent decades, Ecuador has experienced changes in eating habits, demographic, health and socioeconomic conditions that have influenced the pattern of food consumption, with a trend towards Western diets and a detriment in traditional food intake; this is leading to a state of protein-calorie malnutrition, with deteriorating health and increased morbidity and mortality from chronic diseases [2]. The increase in production and consumption of lupine, can help change this situation, through the offering of a new product and differentiation in the common practice of eating lupine with roasted corn, ceviche or chili.

Germination offers this opportunity, through chemical reactions occurring in the grain, such as the synthesis of enzymes that convert proteins into amino acids, the complex carbohydrates into simple sugars and fats into fatty acids [3]. Germination is the most effective technique that can provide the body with concentrated vital energy; when germinated grains are consumed, these may act on human metabolism, leading to regeneration of the bloodstream and digestive processes, it does not generate uric acid and persons suffering from gout can consume it with confidence. The germinated grains can easily be stored and transported, its production does not require sacrificial work or high costs [4].

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2. Materials and Methods

For this study, INIAP-450 lupine variety was used. Sieves 22, 20 and 19 mesh were used for the classification of grain, obtaining lots of 7, 8 and 9 mm in diameter.

Subsequently, impurities (mainly damaged grain) which failed to be mechanically separated were removed. Then the grain was codified and stored in a cool, dry place, according to the diameter of each category, for the development of objectives and corresponding analysis.

To determine the water absorption rate, the grain was classified into three categories according to the following diameter: 7, 8, and 9 mm. Then, 25 grams of sample was weighed and placed in stainless steel baskets, they were soaked in a tank provided with constant stirring at a temperature of 16°C. The grain was steeped for 24 h and sampled every three hours to determine the rate of water absorption at different time.

To determine the necessary conditions for an optimal germination of the grain, three grain diameters: 7, 8, and 9 mm were worked with, these were hydrated at two levels of humidity: 45 and 50%, two temperatures (16, 20°C) and three processing time (2, 3 and 4 days).

For the removal of alkaloids from germinated grain, it was cooked for 60 minutes and distributed under the following conditions: germinated lupine in water with and without agitation in stainless steel baskets under the conditions mentioned, in a soak tank at a constant temperature of 16°C. In this test, the total debittering time of sprouted grain was determined and the residual content of alkaloids was measured.

A negative control, consisting of the non germinated and debittered grain was used for determining the effect of germination on the nutritional content of the grain. All samples were lyophilized, prior to analysis of the following variables: proximal composition, soluble protein, total and reducing sugars, total starch, vitamin C, protein digestibility, minerals, amino acids, alkaloids, oligosaccharides, fatty acids, A and E vitamins.

3. Results and Discussion

Water absorption rate

The water absorption rate was greater during the first three hours of contact between the water and the grain with the different types of grain tested; the water absorption rate was similar (statistical range a) for different grain sizes. After six hours of soaking, the absorption rate was halved and after twelve hours of hydration, the speed of absorption became constant. This result is due to the low initial grain moisture, whose cells, in contact with water tend to quickly saturate in this element.

As time passes, the grains absorb more water, the speed of absorption decreases, thereby these reach a constant behavior when the lupine tissues are completely soaked and reach the saturation (Fig.1). This event occurs after 12 h of soaking. Overall, it was determined that the absorption rate is inversely proportional to the initial moisture content of the grain, regardless of its size.

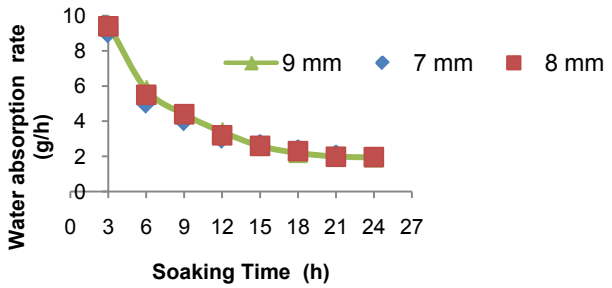

Figure 1. Water absorption speed in relation to grain size

Appropriate conditions for germination of the grain

A higher percentage of germination was achieved when grain moisture reached 45% (Table 2). Higher concentrations may result in grain oxygen deficiency, thereby killing the seed or decreasing the speed of germination. The temperature at which the lupine grain germinated effectively was 20°C; possibly at this level, the enzyme system is efficiently activated, greater water absorption occurs, the nutrients are transported better in the embryo, the enzyme systems needed for hydrolysis are activated, producing soluble and insoluble compounds [5].

Table 1. Content of non-nutritive compounds in the crude lupine

Control	Raffinose (%)	Estachyose (%)	Total alkaloids (%)
Crude lupine	1.54 ± 0,23	3.51 ± 0,17	3.6 ± 0,13

Average of 3 replicates ± S.D.

Table 2. Effect of germination on raffinose content of lupine (%)

Temperature (°C)		16ºC			20ºC		
Time (days)		2	3	4	2	3	4
Humidy (%)	45	0.23± 0,03	ND*	ND*	0.50 ± 0.15	0.21 ± 0.03	ND*
	50	0.38 ± 0,05	0.12 ± 0.03	ND*	0.21± 0.04	0.20 ± 0,02	ND*

*ND: not detectable
Average of 3 replicates ± S.D.

Time is another factor that affects the process of germination. It was observed that the number of sprouted grains increases, depending on the processing time until the 4th day, in which the highest number of sprouted grains (97.18%), were counted. So this period was established as optimal.

Contents of non-nutritive compounds in raw sprouted grain

Crude grain was used as a negative control; Table 1 shows the concentrations of non-nutritive compounds that were determined.

Table 3. Effect of different treatments on the raffinose concentration

Treatments		Average (%)	Statistical ranges
T11	$a_1b_1c_1$	0.24 ± 0.01	d
T7	$a_ob_1c_o$	0.19 ± 0,08	cd
T5	$a_1b_oc_1$	0.16 ± 0.02	bcd
T3	$a_ob_oc_2$	0.16 ± 0.02	bcd
T2	$a_ob_oc_1$	0.15 ± 0.01	bcd
T1	$a_ob_oc_o$	0.15 ± 0.03	bcd
T9	$a_ob_1c_2$	0.14 ± 0.02	bc
T10	$a_1b_1c_o$	0.08 ± 0.01	ab
T6	$a_1b_oc_2$	0.001 ± 0.0005	a
T4	$a_1b_oc_o$	0.001 ± 0.0011	a
T8	$a_ob_1c_1$	0.001 ± 0.0007	a
T12	$a_1b_1c_2$	0.001 ± 0,0011	a

Average of 3 replicates ± S.D.

Raffinose concentration in the sprouted grain

The concentration of raffinose decreased as the germination time increases, registering a 85% decrease after two days of processing in all treatments, and reaching undetectable levels in the third and fourth day of germination (Table 2).

The initial concentration of raffinose (1.54%) decreased significantly with $a_1b_1c_2$ treatments (45% humidity, 20°C temperature, 4 days), $a_ob_1c_1$ (50% humidity, 20°C temperature, 3 days), $a_1b_oc_o$ (45% humidity, 16°C temperature, 2 days), $a_1b_oc_2$ (45% humidity, 16°C temperature, 4 days), so these were in the first statistical range "a". The coefficient of variation (2.98%) showed an acceptable degree of dispersion of data obtained from different samples.

Stachyose content

According to the data in Table 4, stachyose suffers a significant decrease from an initial concentration of 3.6% to 0.66% on the fourth day of germination at 20°C. However, this non nutritional compound did not disappear completely during the monitoring period, as in the case of raffinose.

The presence of stachyose in all treatments was possibly due to its high molecular weight structure, which requires a higher enzyme activity to be completely hydrolyzed; this condition was achieved in raffinose, because it has lower molecular weight [6].

Alkaloid content

Unlike oligosaccharides, total alkaloid content decreases slowly, as germination time increases, in each of the treatments tested; a reduced content of these compounds was obtained after 4 days of germination at 20°C (Table 5).

The germination process did not completely remove all the alkaloids contents present in the grains; this is possibly due to its chemical structure, based on a group called quinolizidine.

Determination of wash time for the removal of residual alkaloids in the germinated grain

The data in Table 6 show that the studied factors: grain distribution and water condition, influence debittering time. When the grains were willing to bulk and soak under constant agitation, debittering time decreased to 39 h. Similarly, when the grain was placed in stainless steel baskets and washed with stirred water, there was debittering in an average time of 49 h, concluding that water condition (moving or stationary), is the most influential factor for debittering time. The grain that was debittered in stationary water, used the same time similar to those required in the traditional washing process, due to the static condition of the water.

Table 4. Effect of germination on stachyose content of lupine

Temperature (ºC)		16			20		
Time (days)		2	3	4	2	3	4
Humidity (%)	45	2.12 ± 0.21	1.09 ± 0.10	0.72 ± 0.23	1. 22 ±0.10	1.20 ±0.10	0.66 ± 0.22
	50	1.79 ± 0.12	1.42 ± 0.28	0.85 ± 0.11	2.11 ± 0.27	1.23 ± 0.24	1.17 ± 0.39

Average of 3 replicates ± S.D.

Table 5. Effect of germination on alkaloid content of lupine

Temperature (ºC)		16			20		
Time (days)		2	3	4	2	3	4
Humidity (%)	45	3.21 ± 0.9	3.07 ± 1.10	2.99 ± 1.03	3.08 ± 0.83	3.04 ± 0.69	2.63 ± 0.61
	50	3.56 ± 1.06	2.90 ± 0.96	2.86 ± 1.07	3.48 ± 0.58	2.92 ± 0.86	2.63 ± 0.75

Average of 3 replicates ± S.D.

Table 6. Time required for the removal of alkaloids of sprouted grain

Sprouted grain	Time (h)
In baskets with stationary water	84
Bulk with water stirred	40
In baskets with stirred water	49
Bulk with unstirred water	64
Technically debittered lupine	96

Residuals alkaloids

The difference in the residual concentration of alkaloids, depending on the condition of the grain and the water used was determined. The arrangement of the grain in bulk and stirred water allowed a greater removal of alkaloids. In the sprouted grain, removal of alkaloids is facilitated by the breaking of the husk [7]. The final concentration of alkaloids in sprouted lupine was placed in stainless steel baskets and was washed with unstirred water which was slightly higher than the previous sample. However, the recorded values are within the quality specifications necessary for human consumption (0.0054 to 0.02%).

Effect of germination on the nutritional value of grain

The fiber content was lower in germinated lupine when compared with non-germinated grain, because during the process of germination, the cotyledon husk is easily stripped, when it is soaked and washed. The ash represents the total amount of inorganic compounds or minerals and its content was increased in the sprouted lupine grain, while non-germinated grain that did not experience this process.

Germinated lupine showed higher amount of nitrogen-free extract (ELN) compared to non-germinated lupine; also, total and reducing sugars slightly increased in germinated grain.

Soluble protein

According to Grosch [8], the behavior of protein in terms of solubility is diverse and depends on the number of polar and non-polar groups of amino acids that make up the polymer. In the germination process, it seems that the hydrophilic groups predominate in the proteins, which causes an increase in the content of soluble protein.

Table 7. Proximal composition of debitter lupine with and without application of the germination process

Debitter lupine		
Analysis	Non-germinated	Germinated
Protein (%)	51.18 ± 0.58	50.10 ± 0.62
Ether extract (%)	21.89 ± 0.29	20.90 ± 0.38
Fiber (%)	13.52 ± 0.31	11.52 ± 0.57
Ash (%)	1.91 ± 0.06	2.50 ± 0.09
Moisture (%)	1.35 ± 0.06	0.62 ± 0.02
Nitrogen free extract (%)	10.00 ± 0,27	14.44 ± 0.50

Average of 3 replicates ± S.D.

Protein digestibility

The digestibility of the protein is indicative of the availability of amino acids. According to the results shown in Table 8, protein digestibility is greater in sprouted lupine, reaching a value of 87.4%, relative to non germinated lupine (85.89%); this is possibly due to the initial splitting of the high molecular weight molecules to lower weight; by the action of proteases, enzymes that are activated at the germination process.

Table 8. Germination effect on other nutritional components of the debitter lupine grain

Analysis	Non-germinated	Germinated
Soluble protein (%)	12.81 ± 0.52	15.70 ± 0.50
Total sugars (%)	1.28 ± 0,06	1.75 ± 0.08
Reducing sugars (%)	0.40 ± 0.03	0.44 ± 0.06
Starch (%)	1.63 ± 0.04	1.18 ± 0.09
Vitamin C (% ascorbic acid)	0.006 ± 0.0006	0.02 ± 0.01
Digestible protein (%)	85.89 ± 0.63	87.40 ± 0.51
Alkaloids (%)	0.01 ± 0.003	0.004 ± 0.001

Average of 3 replicates ± S.D.

Amino acids

The amino acid profile is important because it gives information about the quality of the protein of germinated and non-germinated lupine and its nutritional value. Table 9 shows that the content of certain essential amino acids such as threonine, valine, methionine, isoleucine, tyrosine, histidine, and lysine, were elevated in the sprouted grain. However, some non-essential amino acids, decreased in concentration, as in the case of proline and aspartic acid. The essential amino acids play important roles in the body, for example histidine helps in the removal of excess metals from the body, it stimulates the synthesis of collagen, which is the main structural protein in the extracellular space in the various connective tissues in animals. Threonine is frequently found in the active centers of enzymes, like isoleucine. Methionine plays a special role in protein biosynthesis. The tyrosine by enzymatic oxidation is converted into melanin, blackish brown. The rest of the essential amino acids are possibly involved in the invigoration-reconstitution of all tissues, whereas the function of amino acids, its concentration in the sprouted grain could constitute a valuable contribution to the diet, helping in the growth, development and maintenance of structures in humans.

Fatty acids

The major essential fatty acid in germinated lupine was linoleic acid (C18:2 n-6) with a concentration of 5.61%, and also linolenic acid (C18: 3 omega 3), which increased to 56.11%. These fatty acids are essential because they aren´t synthesized by the body, they are required to be obtained from food, because they play an important role in the maintenance of health, in the synthesis of many cellular

structures and various biologically important compounds. Germination improved balance of polyunsaturated fatty acids, from 10:1 to 4:1, these ratios are close to the ideal condition of 5: 1, cited by Biolley [8].

Table 9. Amino Acids in germinated and non-germinated and debitter lupine

Amino acids (g/100 g protein)	Non germinated	Germinated
Aspartic acid	8.56 ± 0.44	8.46 ± 0.32
* Threonine	3.08 ± 0.09	3.18 ± 0.10
Serina	4.46 ± 0.45	4.59 ± 0.31
Glutamic acid	22.46 ± 0.53	20.75 ± 0.42
Proline	3.65 ± 0.48	3.15 ± 0.32
Glycine	3.53 ± 0.28	3.23 ± 0.33
Alanine	3.13 ± 0.08	2.54 ± 0.10
Cysteine	2.28 ± 0.10	2.88 ± 0.09
* Valine	3.09 ± 0.09	3.11 ± 0.07
Methionine	0.25 ± 0.03	0.32 ± 0.08
* Isoleucine	3.77 ± 0.38	3.86 ± 0.21
Leucine	6.22 ± 0.06	6.10 ± 0.07
Tyrosine	3.05 ± 0.05	3.47 ±0.09
Phenyalanine	3.93 ± 0.09	3.92 ± 0.11
Histidine*	2.42 ± 0.14	3.07 ± 0.10
* Lysine	4.34 ± 0.11	4.54 ± 0.14
Arginine	9.01 ± 0.10	8.13 ± 0.07

*Essential amino acids
Average of 3 replicates ± S.D.

Minerals

Table 10 shows an increase in the mineral content of germinated lupine; they are mainly calcium, magnesium, iron, copper, manganese and zinc. This agrees with the results obtained by Muzquiz [9] and Bewley [10]; it is likely that this increase is due to the action of phytase which was activated during germination, helping to free chelated minerals associated with phytic acid.

Table 10. Contents of minerals of the germinated and non-germinated debitter lupine

Mineral	Germinated	Non-germinated
Ca (%)	0.63 ± 0.06	0.37 ± 0.09
Mg (%)	0.078 ± 0.01	0.05 ± 0.01
Na (%)	0.014 ± 0.003	0.01 ± 0.002
K (%)	0.07 ± 0.003	0.07 ± 0.004
P (%)	0.48 ± 0.02	0.37 ± 0.01
Cu (ppm)	112.00 ± 0.14	5.00 ± 0.08
Fe (ppm)	121.00 ± 0.16	61.00 ± 0.14
Mn (ppm)	101.00 ± 0.07	37.00 ± 0.07
Zn (ppm)	184.00 ± 0.10	92.00 ± 0.16

Average of 3 replicates ± S.D.

Oligosaccharides

After the germination process, the grain was boiled and washed to remove residual alkaloids, making it fit for consumption; in the two analyzed samples, the presence of oligosaccharides weren´t detected. The sprouted grain was able to reach this condition 40 h after washing, while the non germinated and debitter grain required additional 48 h to reach this condition. Washing time of the grain and non-nutritional content was decreased with this process, while nutritional profile of the grain was improved.

4. Conclusions

1. Hydration tests showed that the grain diameter doesn´t have any influence on the rate of hydration, but for increased performance and better presentation, the category of 9 mm mesh was selected for germination tests. The water absorption is very important, to assist the unfolding of the reserve substances, into others of lower molecular size, which are easily transported to the point of growth of the embryonic axis.
2. During germination, a yield of 97.18% was achieved with the following operating conditions: Initial grain moisture: 45%, germinating temperature, 20°C, over a period of 96 h (4 days), and a constant relative humidity of 100%.Of these, the most influential factor is the humidity, which determines the success or failure of the germination process.
3. The application of germination substantially reduced the anti-nutritional content of the grain. The raffinose values decreased from 1.54% to undetectable levels, stachyose experienced a decrease of 49%, while the level of alkaloids decreased only by 27%. These results represent an increase in protein digestibility, especially because reducing of both raffinose and stachyose.
4. In sprouted grain, the alkaloids were reduced to levels of 0.004%, this was achieved in an average time of 40 h, with stirred water and periodic changes every 6 h. This represents a saving of 32 h in relation to the time taken for non sprouted and debitter grain. Similarly, the volume of water used for the removal of alkaloids decreased from 63 m^3/t to 50 m^3 /t.
5. The calcium concentration was doubled (0.63%) when lupine was processed mechanically, while the traditional processing of lupine presented a concentration of 0.37%. Magnesium also increased to 0.078% from an initial value of 0.05%. The microelements also increased relative to traditional lupine, which shows the effectiveness of the germination process that improves the grain´s nutritional profile.
6. In the sprouted grain, the linolenic acid content rose from 0.61 to 1.39%, the ratio of Omega 6 /Omega 3 (4:1) was also improved, approaching the ideal ratio (5:1). This shows that fat of germinated lupine is of better nutritional quality than the non-germinated grain.

7. The amino acid profile in the sprouted grain, showed an increase in the essential amino acids, at the expense of a reduction in non-essential amino acids. This is a favorable effect for nutritional, since the essential amino acids can´t be synthesized by the human body.

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