**Effects of two debittering processes on the alkaloid content and quality characteristics
of lupin (*Lupinus mutabilis* Sweet)**

Elena Villacrés^{1*}, Javier Álvarez¹, and Cristina M. Rosell²

¹Nutrition and Quality Department, Instituto Nacional de Investigaciones Agropecuarias,
INIAP. Ecuador, elena.villacres@iniap.gob.ec

² Institute of Agrochemistry and Food Technology, IATA, CSIC, Valencia, Spain,
crorell@iata.csic.es

***Corresponding author:**

Tel-fax: +593 3007134, Panamericana Sur Km 1, Mejía, Ecuador. e-mail:
elena.villacres@iniap.gob.ec

Abstract

Background: The presence of quinolizidine alkaloids (QAs) in the species *Lupinus mutabilis* Sweet limits the expansion of its consumption and use, despite its high protein content. Therefore, the objective of this research was to determine the effect of two thermal treatments, aqueous (ATT) and saline (STT) on QAs and total protein contents, as well as on the texture (fracturability and hardness), the attributes of the visual perception Hue (H^*), Luminosity (L^*) and chromatism (C^*) and grain size in three lupin varieties (INIAP-450, INIAP-451 and Criollo). In addition, the water consumption of each treatment was measured. **Results:** The debittering process with the ATT helped to concentrate the total nitrogen by 560 g kg⁻¹ and decreased the grain hardness to 2037gf in the Criollo variety, while the chromatic parameters H^* and C^* increased in the three varieties. The STT was more efficient than the ATT in terms of the time required and the volume of water used to reduce the QAs to safe levels for consumption (2.5-3.5 g kg⁻¹). The size of the grain increased to 4 times its original size; the luminosity L^* decreased during cooking to a value of 41.49 in the Criollo variety and then increased to 57.42 during grain washing.

Conclusions: the STT was advisable for lupin debittering, although the extent of the effect was dependent on the variety.

Keywords: Quinolizidine alkaloids, protein, lupin, fracturability, chromaticity

1. Introduction

The species *Lupinus mutabilis* Sweet, which is native in the Andes, is currently cultivated in Ecuador, Peru and Bolivia, with a certain level of agronomic and agro-industrial technological development. This specie is known to be resistant to adverse conditions, such as pests, diseases, drought and frost¹. Lupin has attracted attention not only as feed supplement but also as grain for human food in the current decade, due to its high protein percentage (340-430 g kg⁻¹ of dry matter) and acceptable contents of essential amino acids. In addition, the grain contains vitamins, minerals, unsaturated fat, dietary fiber^{2,3} and phytochemical compounds such as total phenolic compounds and flavonoids⁴. However, the use of the species *L. mutabilis* has been limited by the presence of toxic substances, mostly quinolizidine alkaloids (QAs)⁵. These compounds confer a very bitter taste⁶ and a certain degree of toxicity⁷. In the specie *L. mutabilis*, the alkaloid levels ranges from 30 to 40 g kg⁻¹⁸ and a debittering process must be applied to decrease those levels. The debittered lupin seeds are also referred to as sweet lupin when the alkaloid content has been reduced to less than 0.2 g kg⁻¹, which is the maximum concentration currently permitted and adequate for safe consumption⁹. The FAO recommends that seeds cultivated for human consumption should contain less than 1.0 g kg⁻¹ (wet weight) or 3.3 g kg⁻¹ (dry weight) alkaloids. Higher content is sensory perceptible, unpalatable and potentially toxic¹⁰.

In Andean countries, the grain of *L. mutabilis* is debittered by successive washes with water, which reduces the concentrations of these substances to safe levels for

consumption¹¹. The artisanal process use a large volume of water ($193 \text{ m}^3 \text{ t}^{-1}$) and requires a long process time (5 to 7 days), which turns into a substantial economic cost and significant loss of water soluble nutrients such as some vitamins and mineral, flavonoids, monosaccharides and sucrose¹². Through this process, including hydration, cooking and washing, the alkaloid level of bitter lupins ($0.5\text{-}40.0 \text{ g kg}^{-1}$) is easily decreased to levels that are safe for human consumption¹³. Several authors determined that the cooking and washing time and number of washing stages significantly influence the final alkaloid content of the lupin grain^{13,16,9}. Recently, a mathematical model was generated to improve the artisanal process¹⁷. However, this model has not been validated experimentally. The greatest changes in the physical characteristics and chemical composition of the grain occur during the debittering process. Among the most noticeable are the increase in grain size, difference in color and decrease in its hardness¹³. Texture is an important quality aspect of lupin. It is dictated by the structure of the grain that, in turn, depends on an interaction of chemical components and physical forces^{14,15}. When legumes are processed thermally, first turgor is destroyed, leading to a loss of crisp succulence. Blanching, cooking and sterilization affect the tissues of vegetables, resulting in a decrease in hardness that is mainly due to changes in cell-wall pectins during heating, that led to the formation of soluble pectins by degradation of methylated pectins. The most common debittering method use water at room temperature for washing the grain after cooking, resulting in more acceptable sensorial properties than either debittering with 0.5% NaHCO_3 at room temperature ($\sim 25^\circ\text{C}$) or debittered with hot water (65°C). However, these processes take between 5 to 6 days¹³. In the case of chickpea, some reduction in the hydration time was reported when was soaked in NaCl ¹⁶. Nevertheless, when applying it to lupin, cooking with 40% NaCl was needed to decrease up to 51.22% of the alkaloids, but the grain had a

salty taste and a subsequent treatment of the process effluents was required to remove the high level of NaCl, making the process costly¹⁷. NaCl increases the porous microstructure of the grain, facilitates the penetration of water and the diffusion of the alkaloids, reduces the interactions between the minerals and the pectin and increases the solubility of the protein¹⁸. Aqueous and alkaline thermal treatments have been tested for debittering processes. In the case of *L. campestris* and *G. max*, the application of an aqueous thermal treatment resulted in a 56 % alkaloids decrease after 3 h, whereas in the case of alkaline treatment a greater decrease (76.5 %) has been observed¹⁹. The objective of this study was to improve the debittering process by evaluating the critical factors of the process, including temperature, time and the use of sodium chloride (NaCl), and their effects on QA and total protein contents, as well as on the texture (fracturability and hardness) and grain color. These critical factors were evaluated in three different lupin varieties to check the validity of the conditions.

2. Materials and methods

2.1 Materials

The following varieties of *Lupinus mutabilis* Sweet were used: INIAP-450, INIAP-451 and Criollo, which were grown in the Santa Catalina Experimental Station with the geographic location of altitude 3050 m.a.s.l., latitude UTM 9959382 m S, longitude 17 M0772618 m W. INIAP-450 was obtained by selection from a germplasm population introduced in Peru in 1992, with the identification of ECU-2659²⁰; INIAP-451 variety was obtained by selection through participatory processes from the ECU-2658-2 line²¹ and Criollo variety is a native material from Chimborazo province. The grain harvested was threshed and

classified in a *Crippen* Mfg. Inc. (Michigan, USA) using sieves with pore sizes of 12, 11 and 10 mm. For this study, grains with an average diameter of 10 to 12 mm were used.

The debittering process was performed in a 46 cm diameter x 17 cm high reactor, which was connected to a digital thermostatic immersion circulator (Cole Parmer Polystat, model 01266-02, USA) to control the temperature and velocity of water flow. The samples were subjected to two different debittering treatments (aqueous and saline thermal). Three batches of each process were performed.

2.2. Aqueous thermal treatment (ATT)

The grains were subjected to an aqueous thermal treatment consisting of three stages: hydration (10 h), cooking (1 h) and washing (73 h), and samples were collected along the treatment (Figure 1). The grain was hydrated with stationary water at an initial temperature of 80°C for 10 h (T1) at a 1:3 ratio (grain:water). Cooking was carried out in water at 91°C for 1 h (T2), a 1:3 ratio (grain:water) was used, and a water change was performed after the first 30 min of cooking. The aqueous washing of the grain was performed with a stirring system (10.6 L/min), maintaining a ratio of 1:15 (grain:water). The first washing step was made at 35°C for 28 h, with water changes occurring at the following time intervals: 3 h (T3), 3 h (T4), 16 h (T5), 3 h (T6) and 3 h (T7). In the second stage of washing, the water temperature was maintained at 18°C for 45 h, and the water was replaced at 18 h (T8), 3 h (T9), 3 h (T10), 18 h (T11) and 3 h (T12). The debittering of the grain through the application of the aqueous thermal treatment took 84 h.

2.3. Saline heat treatment (STT)

The flowchart of lupin debittering through the application of the saline heat treatments is shown in Figure 1. The saline heat treatment was performed by the addition of 0.5% (w/v)

sodium chloride to the water used for hydration, cooking and first wash of the grain at 35°C (Figure 1). Hydration was performed at an initial temperature of 80°C for 8 h (T1 s), and a 1:3 ratio (grain:saline water) was used in a steady state (without agitation). Then, the grain was cooked at 91°C for 1 h (T2s) using a 1:3 ratio (grain:saline water); a water change was performed after the first 30 min of cooking. Grain was washed with saline water using a stirring system (10.6 L/min). In the first 6 h of washing at 35°C, a ratio of 1:15 (grain:saline water) was used, and for the following washes, the proportions 1:5 and 1:7.5 (grain:saline water) were used. Saline water changes and sampling were performed at the following time intervals: 3 h (T3s), 3 h (T4s), 16 h (T5s), 3 h (T6s) and 3 h (T7s). Afterwards, the saline water was replaced with water at 18°C to remove the NaCl retained in the grain; then, two water changes were implemented: 18 h (T8s) and 3 h (T9s).

2.4 Analytical methods

2.4.1 Total nitrogen content

The total nitrogen content was determined according to the standard method 955.04 from AOAC²².

2.4.2 Total alkaloid content (QAs)

The method described by Gross⁸ was used to determine the total alkaloid contents, with some modification of the titration. Five milliliters of 0.01 N sulfuric acid and two drops of methyl red were added to the concentrated chloroform extract, and the acid excess was titrated with 0.01 N NaOH. For the calculation, 1 mL of 0.01 N H₂SO₄ was equivalent to 2.48 mg of lupanin. The total alkaloid content was reported as the lupanin content²³, which is the most abundant alkaloid (55-66 %) in *L. mutabilis* specie²⁴.

2.4.3 Fracturability and hardness

Fracturability and hardness were measured using a previously reported method²⁵. The grains were compressed in a texture analyzer (texturometer TA-XT2i, Micro Systems, Godalming, UK) with a load cell of 5.00 kg and a stainless steel cylindrical probe with a diameter of 5 mm (P5) at a speed of 1 mm/s. The grains were compressed in two cycles up to 50% of their initial height, with an interval of 3 s between each cycle. The strength of the first significant break in the positive area of the first bite defines fracturability. The hardness is the strength of the peak during the first compression cycle²⁶.

2.4.4 Color measurement

Color is a quality parameter, particularly when lupin is used as egg substitute. Color measurements were performed using a Portable Spectrophotometer (Lange Spectro-Color d/8° model LZM 268, Chelmsford, United Kingdom) based on the CIE L^* , a^* , b^* color system. The following attributes of visual sensation were measured: L^* (luminosity), C^* (chromatism) and H^* (hue). The color differences between the raw and processed lupin seeds were calculated using the following equation: $\Delta E = (\Delta L^{*2} + \Delta C^{*2} + \Delta H^{*2})^{0.5}$, where ΔL^* , ΔC^* and ΔH^* are the differences in the attributes of the visual perception between the raw and processed seeds²⁷.

2.4.5 Grain size

The larger and smaller diameters of the grains subjected to different treatments were measured with a *Mitutoyo* digital pachymeter (Suzano, Brazil).

2.4.6 Hydration capacity

One hundred grains of lupin with an average diameter of 10-12 mm were weighed and then soaked in water for 8 to 10 h. The surface liquid was drained, and the grains weighed again.

The initial weight was subtracted from this value and the amount of embedded water was calculated. Hard seeds do not embed water as fast as normal seeds; their weight at the end of soaking does not change significantly and they float in the hydration water. Normal seeds swell and descend to the bottom of the soaking container. Floating grains are counted and the percentage of hydrated grains is established by calculating the difference. This parameter allows to know the ability of lupin seeds to rehydrate so that the cooking is more uniform²⁸.

2.5. Statistical analysis

The statistical analysis was performed using the Infostat program (Córdoba, Argentina). The normal distribution of the data was verified with the Kolmogorov-Smirnov goodness of fit test. A multivariate analysis of variance (ANOVA) was applied, and the Tukey test with a significance level of 95% ($P < 0.05$) was used to establish significant differences between samples. All analyses were performed in triplicate; the data are presented as means $\pm$ standard deviations.

3. Results and discussion

3.1. Quinolizidine alkaloids

The quinolizidine alkaloids (QAs) contents of the grain gradually decreased as a function of the processing stages and washing time (Figures 2 and 3). In the aqueous thermal treatment (ATT), after hydration (T1) the content of QAs in the varieties INIAP-450, INIAP-451 and Criollo decreased by 39.8, 35.7 and 29.6 g kg⁻¹ in relation to raw grain, which was due to the water solubility of the QAs in the seeds, in which the QAs are present in the form of salts that are solubilized in polar solvents such as water¹⁹. The initial temperature of the

soaking water (80°C) accelerated its penetration in the grain, requiring 10 h to hydrate 98% of the seeds, compared to 14 h that are typically applied in the artisanal debittering process¹². In the first stage (T1s) of STT, 98% of the grains were hydrated after 8 h of soaking and the decreases in the QAs contents of the seeds were 7.33, 6.71 and 9.97% in INIAP-450, INIAP-451 and Criollo, respectively. This decrease is attributed to the NaCl, which increases the porous microstructure of the grain, facilitates the penetration of water and the diffusion of the alkaloids¹⁸. The osmoactive effect of sodium chloride may have favored the countercurrent mass transfer between grain tissues and the saline solution²⁹. Cooking contributed to a greater elimination of the QAs. In the cooking stage (T2) of ATT, the QAs contents of INIAP-450, INIAP-451 and Criollo decreased by 19.3, 20.25 and 11.30%, while in the STT, after cooking (T2s) the QAs contents were reduced to an even greater extent (27.04, 22.79 and 27.02%). During the washing stage, a progressive reduction in the QAs contents was observed in the three varieties evaluated. In ATT, the QAs contents were reduced by 76.66, 75.58 and 72.72% after 28 h of washing the grain at 35°C (T3-T7). However, with the saline heat treatment (T3s-T7s), at the same washing time a greater reduction in the QA contents (80.31, 78.88 and 77.05%) was registered in the three varieties (INIAP-450, INIAP-451 and Criollo, respectively). These results were affected significantly ($P < 0.05$) by the processing stage and variety, likely due to the increase in the ionic strength resulting from the dissociation of NaCl, which favors a greater diffusion of the QAs in the extraction medium³⁰. The alkaloids reduction reached after hydration, cooking and washing stages (T1-T7) of ATT process in INIAP-450, INIAP-451 and Criollo was 81.87, 81.14 and 76.46%, respectively). In the same stages (T1s-T7s) of the STT, the reduction percentages were 86.49, 84.69 and 84.77% in INIAP-450, INIAP-451 and Criollo, respectively. After washing (T7) at 35°C, a decrease in grain hardness was

observed; therefore, the temperature of the washing water was decreased to 18°C for the last washing stage that lasted 45 h in the aqueous thermal treatment (T8-T12) and 21 h in the saline heat treatment (T8 and T9). In the saline heat treatment, the final wash was performed with water alone to facilitate the removal of the sodium chloride retained in the grain. The alkaloid content of the seeds was affected by the treatment stage and the grain variety ($P < 0.05$). In both treatments, after washing at 18°C, the residual QAs content ranged from 0.25-0.35% (d.b.), a level considered safe for human consumption, as established by FAO²³. The debittering process for 1 kg of grain with ATT required 87 h and 96 L of water, with 11 changes, representing a saving of 97 L compared with the artisanal process¹². The STT was performed in 58 h and 66 L of water was used, with seven changes, which represents a decrease of 26 h in the processing time and a saving of 30 L in the volume of water used compared with the ATT and 127 L with respect to the artisanal process that uses 193 L from rivers or watersheds. In STT, the total volume of water used was similar to that obtained through mathematical modeling³¹, however, the time required to reduce the QAs content was reduced considerably compared to that study, which mentions 4.4 days as total process time.

3.2. Total nitrogen content

During the debittering process, the chemical composition of the grain in the native state was modified following the application of the two treatments (ATT and STT) (Table 1). The total nitrogen content increased slightly in the three varieties of grain during hydration, which was attributed to the reduction in the content of water-soluble compounds (some vitamins, mineral, flavonoids, monosaccharides and sucrose¹²), including QAs. The thermal

treatment also improve nutritional value through conversion of native protein into more digestible denatured forms³².

During cooking, a decrease in the total nitrogen was recorded compared with the hydrated grain. Probably in the cooking process at 91°C globular proteins are not completely denatured, leaving a fraction with the capacity to imbibe water, which is linked to a decrease in the vulnerability to enzymatic attack and to lower digestibility³³. Comparing both treatments, the greatest loss was registered with the application of STT in INIAP-450 (6.41%) ($P < 0.001$), due to an increase in the solubility of the protein nitrogen induced by a reduction in the number of the electrostatic protein-protein interactions³³. When the cooked grain was washed, in both treatments a gradual increase in the total nitrogen concentration was recorded in the three varieties. At the debittering end point, the Criollo variety showed the highest total nitrogen content ($P < 0.05$) following the application of ATT (89.69 g kg⁻¹) and STT (87.27 g kg⁻¹). This increase was attributed to the elimination of some carbohydrates, minerals and QAs during water changes¹⁹, while other nutrients, such as protein, remained inside the grain increasing their proportion.

3.3. Fracturability and hardness

Fracturability and hardness are properties that influence the consumer's acceptance of lupin; because of that they were monitored during the debittering process. The greatest effect on the mentioned properties was observed in the cooking stage of ATT and STT treatments (Table 2). Cooking induces the largest alteration in structure and concomitantly in texture. Heating thus allows cells to separate resulting in a softening of the grain. Cell separation has been reported at 76°C for soaked lima beans¹⁵. Heating and soaking also produce changes in the cell inclusions. Protein bodies do not appear to be disrupted,

however, deviations from the normal spherical structures were observed, perhaps due to swelling^{34,35}. The cooking of dried legumes and cereals in a humid environment leads to water migration and simultaneous softening of the grain by degrading the cotyledon tissues in their individual cells, as well as a decrease in the levels of some carbohydrates and an increase in the fiber content^{32,36}. Following the application of the two evaluated treatments (ATT and STT), the fracturability did not exhibit significant changes during the washing of the grain at 35°C and 18°C, while the hardness of the three varieties decreased during the washing stage with respect to the hydrated grain, likely due to the increase in water activity¹³. The hardness of the Criollo variety was reduced to 2037 gf when applying the ATT treatment, resulting in a softer grain at the end of the process. A similar variation was detected in grains subjected to STT, along with a progressive decrease in hardness up to a value of 2182 gf during grain washing at 35°C and 18°C (T8s and T9s). Therefore, the water activity of the seeds increased, whereas the hardness decreased.

3.4. Color of the grain

In addition to the texture, the color of lupin influences the preference of the consumers and their intent to purchase. Tables 3 and 4 show the statistically significant impact ($P < 0.05$) of the variety and treatment stage on grain color. Following the application of ATT, the luminosity (L^*) value ranged from 61.47 to 50.51, while after STT, the variation was higher (61.47-41.50). The darkening of the *Lupinus mutabilis* seeds is attributed to the formation of brown pigments, as a result of Maillard reaction during the cooking, besides to the loss of flavonoid and carotenoids pigments¹³. A decrease in L^* was reported after baking barlotto beans, chickpeas, faba beans, white beans and during processing of soybeans with fluidized superheated-steam^{32,46}. Other authors attribute the reduction in color or darkening of the beans to the coagulation of the proteins³⁷. The chroma or color

level (C^*) was increased during the hydration, cooking and washing of the grain at 35°C and 18°C compared with the raw grain. Similar results were obtained with the application of ATT and STT. The attribute that differentiates the color, Hue (H^*) also increased with cooking and washing. Debittering of the grain with the application of STT caused greater color difference ($\Delta E = 5$ units) in INIAP-451, when the raw and processed seeds were compared. In this case, possibly the browning was partially inhibited by a low concentration of NaCl³⁸. A variation of 88 to 90.4 for L^* and an increase in the values of H^* and C^* , resulting in a greater intensity of the cream color of the grain, was reported for *Lupinus albus* seeds³⁹.

3.5. Grain size

A significant change in the diameters of the three varieties of grain was noted during hydration (T1) and the size increased proportionally to the water absorption capacity of the protein, the components of the fiber, the oligosaccharides and the permeability of the tegument (Table 4). The absorption of water and the increase in grain size continued until the tissues were saturated with water, which was reached in the cooking stage (T2); afterwards, no significant differences were observed in the largest and smallest diameters of grains. In the hydration stage of ATT, the increase in the larger diameter of the grain was 3.35 times the original size for the INIAP-450 variety, 4.19 for INIAP-451 and 4.38 for the Criollo variety. During cooking, the increase was 3.74 times the original size for INIAP-450, 4.04 times for INIAP-451 and 3.65 for the Criollo variety. In the STT, the increase in the larger diameter was 3.53 times the original size for INIAP-450 variety, 3.82 for INIAP-451 and 3.70 for Criollo variety. Similar results were reported for lupin debittering, the grain swelled up to 3 times its original size, depending on the variety²³. During cooking, the smaller diameter of the grain increased 0.79 times for INIAP-450, 1.14 times for INIAP-

451 and 0.98 for the Criollo variety. The increase in the diameter of the seed depended on their final humidity, thus, when the grain was exposed to 40, 50 and 60% humidity, its size increases by 15, 23 and 24.93%, respectively.

4. Conclusions

Two different debittering processes were applied to three different varieties of *L. mutabilis*. Both processes, ATT and STT, resulted in approximately 80% of the alkaloids removed from the grain during soaking, cooking and the first wash of the grain at 35°C. In general, the debittering process with ATT and STT exerted positive effects on reducing the QAs and increasing protein concentrations in the grain in the three varieties. Moreover, the processes improved the grain texture by decreasing hardness and increased grain size. Color was also improved, as evidenced the increase in the tone and chromaticity of the grain, characteristics that affect the consumer's level of acceptance and preference. However, in the saline heat process, the addition of sodium chloride in the first stages of the process helped to optimize the effect of temperature, agitation and water changes on the reduction of the QAs contents. Considering the economy of the process, the debittering of 1 kg of grain by the SST was achieved in 58 h using 66 L of water, which represents a saving of 26 h in the processing time and 30 L in the volume of water compared with the ATT and 127 L compared with the artisanal process, which uses 193 L and take between 5 to 6 days. Hence, considering the current debittering processes, the saline heat process is advisable for lupin debittering. Nevertheless, further studies will be undertaken pursuing an additional reduction of water and the process time to make it more environmentally friendly.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this publication.

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Figure 1. Flowchart of the lupin debittering process with aqueous thermal and saline heat treatments

Figure 2. Variation in the total alkaloid content of lupin grain during the aqueous thermal treatment

T1 = hydration (80°C, 10 h); T2 = cooking (91°C, 1 h); T3 = washed (35°C, 3 h); T4 = washed (35°C, 3 h); T5 = washed (35°C, 16 h); T6 = washed (35°C, 3 h); T7 = washed (35°C, 3 h); T8 = washed (18°C, 18 h); T9 = washed (18°C, 3 h); T10 = washed (18°C, 3 h); T11 = washed (18°C, 18 h); T12 = washed (18°C, 3 h)

Values followed by different letters within a column indicate significant differences ($P < 0.05$). Means $\pm$ standard deviations ($n = 3$).

Figure 3. Variations in the total alkaloid content of lupin grain during the saline heat treatment

T1s = hydration (80°C, 8 h); T2s = cooking (91°C, 1 h); T3s = washed (35°C, 3 h); T4s = washed (35°C, 3 h); T6 = washed (35°C, 16 h); T6s = washed (35°C, 3 h); T7s = washed (35°C, 3 h); T8s = washed (18°C, 18 h); T9s = washed (18°C, 3 h)

Values followed by different letters within a column indicate significant differences ($P < 0.05$). Means $\pm$ standard deviations ($n = 3$)

Table 1. Variations in the total nitrogen content of the lupin grain* subjected to the aqueous thermal and saline heat treatments

Aqueous thermal treatment stages				Water used (L)	Saline heat treatment stages				Water used (L)
	INIAP-450	INIAP-451	Criollo			INIAP-450	INIAP-451	Criollo	
Raw grain	75.35 ± 0.22 ^{wx}	74.22 ± 0.59 ^{xy}	77.41 ± 0.48 ^{tu}		Raw grain	75.35 ± 0.22 ^q	74.22 ± 0.59 ^f	77.41 ± 0.48 ^{no}	
T1	80.13 ± 0.05 ^{qr}	79.75 ± 0.09 ^r	82.83 ± 0.14 ^{no}	3	T1s	78.63 ± 0.23 ^m	77.93 ± 0.23 ^{mn}	80.27 ± 0.23 ^{kl}	3
T2	74.99 ± 0.14 ^{xy}	73.59 ± 0.09 ^y	76.58 ± 0.09 ^{uw}	3	T2s	72.10 ± 0.23 ^s	72.33 ± 0.23 ^s	77.47 ± 0.23 ^{no}	3
T3	81.90 ± 0.23 ^{op}	82.83 ± 0.47 ^{mn}	87.83 ± 0.14 ^{fg}	15	T3s	73.64 ± 0.09 ^r	81.20 ± 0.23 ^{jk}	82.74 ± 0.09 ⁱ	15
T4	79.57 ± 0.23 ^r	81.67 ± 0.23 ^{op}	86.10 ± 0.47 ⁱ	15	T4s	75.37 ± 0.23 ^q	84.47 ± 0.23 ^{gh}	85.63 ± 0.47 ^{def}	15
T5	81.43 ± 0.23 ^{op}	83.77 ± 0.47 ^{lm}	87.73 ± 0.23 ^{fgh}	7.5	T5s	77.93 ± 0.47 ^{mn}	86.80 ± 0.70 ^{ab}	87.41 ± 0.09 ^a	5
T6	82.13 ± 0.47 ^{no}	84.47 ± 0.23 ^{kl}	88.67 ± 0.23 ^{cde}	7.5	T6s	76.77 ± 0.23 ^{op}	84.93 ± 0.23 ^{fgh}	86.33 ± 0.23 ^{bcd}	5
T7	81.90 ± 0.70 ^{op}	78.40 ± 0.23 st	88.20 ± 0.23 ^{efg}	15	T7s	76.30 ± 0.23 ^{pq}	84.19 ± 0.05 ^h	85.87 ± 0.23 ^{cde}	5
T8	84.70 ± 0.23 ^k	79.10 ± 0.47 ^{rs}	88.71 ± 0.05 ^{cde}	7.5	T8s	80.03 ± 0.23 ^l	85.26 ± 0.09 ^{efg}	86.66 ± 0.09 ^{abc}	7.5
T9	85.63 ± 0.23 ^{ij}	80.97 ± 0.47 ^{pq}	88.95 ± 0.05 ^{bcd}	7.5	T9s	81.43 ± 0.23 ^j	85.59 ± 0.05 ^{def}	87.27 ± 0.23 ^a	7.5
T10	86.10 ± 0.23 ⁱ	84.93 ± 0.23 ^{jk}	89.18 ± 0.09 ^{abc}	5					
T11	87.03 ± 0.23 ^h	87.73 ± 0.23 ^{fgh}	89.41 ± 0.05 ^{ab}	5					
T12	87.50 ± 0.23 ^{gh}	88.39 ± 0.05 ^{def}	89.69 ± 0.05 ^a	5					
Variety (V)				< 0.0001	Variety (V)				< 0.0001
P-value	Treatment stages (TS)			< 0.0001	P-value	Treatment stages (TS)			< 0.0001
	Interaction V*TS			< 0.0001		Interaction V*TS			< 0.0001

* Percentage based on g kg⁻¹ of dry grain. Values followed by different letters within a column indicate significant differences (P < 0.05). Means ± standard deviations (n = 3).

Table 2. Variations in lupin grain texture parameters following aqueous thermal and saline heat treatments

Aqueous thermal treatment stages	Fracturability (gf)			Hardness (gf)			Saline heat treatment stages	Fracturability (gf)			Hardness (gf)		
	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo		INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo
T1	467 ± 3 ^b	470 ± 4 ^b	462 ± 3 ^a	3740 ± 160 ^{i-k}	4265 ± 359 ^k	4090 ± 252 ^{jk}	T1s	470 ± 5 ^c	467 ± 4 ^{bc}	462 ± 1 ^b	4367 ± 376 g	4137 ± 30 g	3421 ± 283 ^f
T2	460 ± 1 ^a	460 ± 2 ^a	459 ± 6 ^a	2794 ± 320 ^{b-h}	2645 ± 301 ^{a-f}	2876 ± 329 ^{b-h}	T2s	462 ± 2 ^a	461 ± 2 ^a	459 ± 1 ^a	2720 ± 456 ^{a-e}	2786 ± 313 ^{a-f}	2182 ± 236 ^a
T3	461 ± 1 ^a	461 ± 3 ^a	460 ± 1 ^a	3111 ± 153 ^{b-h}	2947 ± 98 ^{b-h}	3031 ± 288 ^{b-i}	T3s	462 ± 2 ^a	462 ± 2 ^a	460 ± 2 ^a	2684 ± 514 ^{a-e}	2804 ± 288 ^{a-f}	2489 ± 238 ^a
T4	459 ± 2 ^a	460 ± 2 ^a	459 ± 3 ^a	3092 ± 324 ^{b-i}	2695 ± 252 ^c	2501 ± 348 ^{ab}	T4s	462 ± 2 ^a	462 ± 1 ^a	461 ± 1 ^a	2568 ± 274 ^{a-e}	2859 ± 196 ^{a-f}	2642 ± 163 ^{a-e}
T5	459 ± 2 ^a	460 ± 1 ^a	461 ± 1 ^a	3296 ± 353 ^{d-i}	2764 ± 234 ^{a-g}	2752 ± 402 ^{a-h}	T5s	462 ± 2 ^a	462 ± 1 ^a	461 ± 2 ^a	3037 ± 309 ^{c-f}	2954 ± 258 ^{b-f}	2893 ± 332 ^{b-f}
T6	460 ± 2 ^a	460 ± 1 ^a	460 ± 2 ^a	3342 ± 573 ^{e-i}	2884 ± 258 ^{bc}	2788 ± 353 ^{b-h}	T6s	461 ± 1 ^a	461 ± 3 ^a	461 ± 2 ^a	2431 ± 29 ^{a-d}	2646 ± 262 ^{a-e}	2346 ± 158 ^{ab}
T7	460 ± 2 ^a	458 ± 2 ^a	460 ± 1 ^a	3110 ± 252 ^{b-i}	2786 ± 304 ^{b-h}	2628 ± 228 ^{a-e}	T7s	461 ± 1 ^a	461 ± 2 ^a	460 ± 2 ^a	2404 ± 159 ^{a-c}	2570 ± 290 ^{a-e}	2321 ± 388 ^{ab}
T8	460 ± 2 ^a	458 ± 2 ^a	461 ± 2 ^a	3256 ± 321 ^{c-i}	3415 ± 241 h ^{ij}	2798 ± 194 ^{b-h}	T8s	462 ± 2 ^a	461 ± 3 ^a	461 ± 1 ^a	2755 ± 351 ^{a-f}	2895 ± 128 ^{b-f}	2461 ± 456 ^{a-d}
T9	460 ± 0.2 ^a	460 ± 2 ^b	462 ± 2 ^a	3360 ± 385 ^{f-i}	3433 ± 350 h ^{ij}	2812 ± 133 ^{b-h}	T9s	462 ± 1 ^a	461 ± 2 ^a	463 ± 1 ^a	3085 ± 216 ^{def}	3156 ± 460 ^{ef}	2704 ± 422 ^{a-e}
T10	460 ± 2 ^a	460 ± 2 ^a	462 ± 1 ^a	3365 ± 710 g ⁻ⁱ	3433 ± 216 h ^{ij}	2868 ± 271 ^{b-h}							
T11	461 ± 2 ^a	459 ± 1 ^a	462 ± 1 ^a	3343 ± 351 ^{e-i}	2602 ± 197 ^{a-d}	2560 ± 381 ^{abc}							
T12	460 ± 2 ^a	458 ± 2 ^a	462 ± 1 ^a	2965 ± 352 ^{b-h}	2592 ± 426 ^{a-d}	2038 ± 83 ^a							
Variety (V)	0.723			< 0.0001			Variety (V)	0.000			< 0.0001		
Treatment stages (TS)	< 0.0001			< 0.0001			Treatment stages (TS)	< 0.0001			< 0.0001		
Interaction V*TS	< 0.0001			< 0.0001			Interaction V*TS	0.0044			0.0812		

gf = grams of force. Values followed by different letters within a column indicate significant differences ($P < 0.05$). Means ± standard deviations (n = 3).

Table 3. Color variations of lupin grain subjected to aqueous thermal treatment

Aqueous thermal treatment stages	L*			C*			H*			ΔE		
	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo
Raw grain	61.47 $\pm$ 3.74 ^a	60.85 $\pm$ 1.85 ^{ab}	58.96 $\pm$ 1.86 ^{abc}	9.18 $\pm$ 0.75 ^j	10.51 $\pm$ 0.694 ^j	10.88 $\pm$ 0.68 ^j	81.47 $\pm$ 1.69 ^{mm}	80.587 $\pm$ 1.83 ⁿ	80.71 $\pm$ 0.64 ⁿ			
T1	56.76 $\pm$ 3.81 ^{a-d}	53.00 $\pm$ 2.58 ^{d-g}	50.51 $\pm$ 4.47 ^g	16.79 $\pm$ 3.41 ⁱ	21.221 $\pm$ 6.087 ^{a-h}	17.75 $\pm$ 4.49 ^{hi}	84.26 $\pm$ 2.50 ^{k-n}	81.755 $\pm$ 1.77 ^{mm}	82.81 $\pm$ 1.73 ^{lmm}	10.36 $\pm$ 3.28 ^h	13.68 $\pm$ 6.03 ^{c-h}	12.27 $\pm$ 3.55 ^{gh}
T2	56.91 $\pm$ 2.84 ^{a-d}	54.93 $\pm$ 2.96 ^{c-g}	53.303 $\pm$ 3.28 ^{d-g}	21.94 $\pm$ 1.82 ^{a-g}	22.515 $\pm$ 2.429 ^{a-d}	22.94 $\pm$ 2.16 ^{abc}	91.08 $\pm$ 1.35 ^{ab}	90.105 $\pm$ 1.64 ^{abc}	91.31 $\pm$ 2.00 ^a	16.91 $\pm$ 1.38 ^{a-c}	16.81 $\pm$ 1.72 ^{a-d}	17.45 $\pm$ 1.79 ^a
T3	54.22 $\pm$ 1.71 ^{c-g}	54.66 $\pm$ 2.78 ^{c-g}	52.961 $\pm$ 4.60 ^{d-g}	19.06 $\pm$ 2.27 ^{d-i}	24.265 $\pm$ 2.089 ^{ab}	21.79 $\pm$ 1.50 ^{a-g}	89.91 $\pm$ 1.53 ^{abc}	87.935 $\pm$ 2.14 ^{a-j}	89.99 $\pm$ 2.48 ^{abc}	15.15 $\pm$ 1.22 ^{a-g}	17.13 $\pm$ 1.82 ^{ab}	16.31 $\pm$ 1.43 ^{a-e}
T4	53.26 $\pm$ 3.16 ^{d-g}	54.39 $\pm$ 1.71 ^{c-g}	54.48 $\pm$ 2.12 ^{c-g}	19.61 $\pm$ 1.61 ^{c-i}	24.688 $\pm$ 2.861 ^a	22.44 $\pm$ 1.34 ^{a-e}	89.52 $\pm$ 2.07 ^{a-d}	87.951 $\pm$ 1.55 ^{a-j}	89.48 $\pm$ 2.12 ^{a-d}	15.96 $\pm$ 1.19 ^{a-f}	17.42 $\pm$ 2.48 ^a	15.47 $\pm$ 1.08 ^{a-g}
T5	56.20 $\pm$ 3.03 ^{a-e}	55.13 $\pm$ 2.60 ^{c-g}	53.893 $\pm$ 3.18 ^{c-g}	20.07 $\pm$ 1.03 ^{c-i}	21.802 $\pm$ 1.268 ^{a-g}	21.80 $\pm$ 2.33 ^{a-g}	89.30 $\pm$ 1.55 ^{a-f}	86.825 $\pm$ 2.26 ^{c-k}	89.16 $\pm$ 2.44 ^{a-g}	14.78 $\pm$ 0.76 ^{a-g}	14.46 $\pm$ 1.54 ^{a-g}	15.28 $\pm$ 1.52 ^{a-g}
T6	54.51 $\pm$ 4.32 ^{b-e}	55.56 $\pm$ 2.85 ^{b-g}	53.57 $\pm$ 4.06 ^{d-g}	18.62 $\pm$ 1.27 ^{f-i}	20.173 $\pm$ 2.015 ^{c-i}	21.59 $\pm$ 2.33 ^{a-g}	89.33 $\pm$ 1.87 ^{a-f}	89.047 $\pm$ 2.05 ^{a-g}	87.88 $\pm$ 2.50 ^{b-j}	14.75 $\pm$ 1.84 ^{a-g}	14.38 $\pm$ 0.95 ^{a-g}	14.79 $\pm$ 1.37 ^{a-g}
T7	56.07 $\pm$ 3.81 ^{c-fg}	52.75 $\pm$ 2.05 ^{d-g}	54.55 $\pm$ 3.08 ^{c-g}	18.59 $\pm$ 2.09 ^{f-i}	20.280 $\pm$ 1.862 ^{c-i}	20.06 $\pm$ 1.94 ^{c-i}	89.73 $\pm$ 2.40 ^{a-d}	86.458 $\pm$ 1.62 ^{d-k}	89.37 $\pm$ 1.72 ^{a-f}	14.38 $\pm$ 1.21 ^{a-g}	14.27 $\pm$ 1.16 ^{a-g}	13.86 $\pm$ 1.22 ^{b-g}
T8	55.27 $\pm$ 2.76 ^{c-g}	53.87 $\pm$ 1.99 ^{c-g}	56.48 $\pm$ 2.93 ^{a-d}	18.19 $\pm$ 1.96 ^{ghi}	22.255 $\pm$ 1.877 ^{a-f}	22.06 $\pm$ 1.28 ^{a-f}	88.61 $\pm$ 1.18 ^{a-i}	85.800 $\pm$ 1.77 ^{g-i}	88.85 $\pm$ 1.58 ^{a-h}	13.44 $\pm$ 1.35 ^{e-h}	14.88 $\pm$ 1.50 ^{a-g}	14.40 $\pm$ 1.37 ^{a-g}
T9	55.12 $\pm$ 3.59 ^{c-g}	53.54 $\pm$ 2.16 ^{d-g}	50.74 $\pm$ 4.01 ^{fg}	19.83 $\pm$ 1.97 ^{c-i}	21.145 $\pm$ 2.475 ^{a-h}	21.55 $\pm$ 1.73 ^{a-g}	87.98 $\pm$ 2.53 ^{a-j}	85.985 $\pm$ 1.80 ^{f-l}	85.25 $\pm$ 2.49 ^{i-l}	14.63 $\pm$ 1.82 ^{a-g}	14.31 $\pm$ 1.92 ^{a-g}	14.79 $\pm$ 2.59 ^{a-g}
T10	54.51 $\pm$ 3.72 ^{d-g}	53.25 $\pm$ 1.82 ^{c-g}	54.73 $\pm$ 2.72 ^{c-g}	20.02 $\pm$ 2.34 ^{c-i}	23.143 $\pm$ 2.548 ^{abc}	22.44 $\pm$ 1.49 ^{a-e}	88.64 $\pm$ 1.75 ^{a-i}	85.538 $\pm$ 0.99 ^{h-l}	87.37 $\pm$ 2.40 ^{c-k}	15.30 $\pm$ 1.87 ^{a-g}	15.80 $\pm$ 1.49 ^{a-f}	14.46 $\pm$ 1.04 ^{a-g}
T11	56.52 $\pm$ 2.63 ^{a-d}	55.97 $\pm$ 2.97 ^{b-f}	52.29 $\pm$ 1.82 ^{d-g}	18.67 $\pm$ 1.43 ^{e-i}	20.103 $\pm$ 0.734 ^{c-i}	20.23 $\pm$ 1.40 ^{c-i}	89.43 $\pm$ 1.93 ^{a-e}	87.717 $\pm$ 1.72 ^{b-j}	86.07 $\pm$ 1.95 ^{e-l}	13.72 $\pm$ 1.06 ^{c-g}	13.31 $\pm$ 0.76 ^{e-h}	12.95 $\pm$ 1.11 ^{f-h}
T12	54.52 $\pm$ 3.88 ^{c-g}	51.07 $\pm$ 2.74 ^{efg}	54.96 $\pm$ 2.56 ^{c-g}	17.64 $\pm$ 1.68 ^{hi}	20.49 $\pm$ 0.96 ^{b-i}	20.72 $\pm$ 1.45 ^{b-h}	88.40 $\pm$ 1.60 ^{a-i}	84.59 $\pm$ 2.60 ^{j-m}	89.82 $\pm$ 2.70 ^{a-d}	13.55 $\pm$ 1.69 ^{d-h}	14.93 $\pm$ 1.36 ^{a-g}	14.47 $\pm$ 0.97 ^{a-g}
Variety (V)	< 0.0001			< 0.0001			< 0.0001			< 0.0001		
Treatment stages (TS)	< 0.0001			< 0.0001			< 0.0001			< 0.0001		
Interaction V*TS	< 0.0001			< 0.0001			< 0.0001			< 0.0001		

Values followed by different letters within a column indicate significant differences ($P < 0.05$). Means $\pm$ standard deviations ($n = 10$).

Table 4. Color variations of lupin grain subjected to saline thermal treatment

Saline heat treatment stages	L*			C*			H*			ΔE		
	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo
Raw grain	61.47 $\pm$ 3.74 ^a	60.85 $\pm$ 1.85 ^a	58.97 $\pm$ 1.86 ^{abc}	9.18 $\pm$ 0.75 ^j	10.51 $\pm$ 0.65 ^j	10.88 $\pm$ 0.68 ^j	81.47 $\pm$ 1.69 ^d	80.59 $\pm$ 1.83 ^{de}	80.71 $\pm$ 0.64 ^{de}			
T1s	47.24 $\pm$ 1.91 ^j	50.72 $\pm$ 2.59 ^{fj}	41.50 $\pm$ 4.77 ^k	16.87 $\pm$ 2.30 ⁱ	15.67 $\pm$ 3.58 ⁱ	20.18 $\pm$ 2.20 ^h	81.31 $\pm$ 0.96 ^d	77.92 $\pm$ 1.36 ^e	81.52 $\pm$ 2.00 ^d	16.27 $\pm$ 2.45 ^{d-f}	12.05 $\pm$ 3.39 ^g	20.15 $\pm$ 4.01 ^{ab}
T2s	51.56 $\pm$ 2.64 ^{e-j}	50.95 $\pm$ 2.22 ^{e-j}	49.52 $\pm$ 5.80 ^{bij}	21.71 $\pm$ 1.10 ^{e-h}	23.45 $\pm$ 2.13 ^{e-g}	25.69 $\pm$ 1.65 ^{ab}	88.95 $\pm$ 1.00 ^{abc}	87.28 $\pm$ 0.99 ^c	88.96 $\pm$ 3.23 ^{abc}	17.81 $\pm$ 1.50 ^{a-f}	17.79 $\pm$ 1.86 ^{a-f}	20.42 $\pm$ 1.35 ^a
T3s	49.85 $\pm$ 2.92 ^{g-j}	49.45 $\pm$ 3.33 ^{ij}	50.68 $\pm$ 3.89 ^{fj}	20.38 $\pm$ 0.57 ^{gh}	24.89 $\pm$ 1.81 ^{a-d}	25.02 $\pm$ 1.68 ^{abc}	89.46 $\pm$ 1.23 ^{abc}	87.28 $\pm$ 1.09 ^c	88.63 $\pm$ 1.80 ^{abc}	18.19 $\pm$ 1.68 ^{a-e}	19.82 $\pm$ 1.81 ^{a-c}	18.64 $\pm$ 1.73 ^{a-d}
T4s	55.31 $\pm$ 3.48 ^{b-g}	55.81 $\pm$ 1.28 ^{b-e}	53.00 $\pm$ 3.63 ^{d-i}	22.77 $\pm$ 3.24 ^{b-h}	27.41 $\pm$ 3.62 ^a	23.60 $\pm$ 1.43 ^{b-f}	91.12 $\pm$ 2.19 ^a	89.50 $\pm$ 1.04 ^{abc}	89.74 $\pm$ 2.90 ^{abc}	18.37 $\pm$ 1.80 ^{a-e}	19.92 $\pm$ 2.90 ^{ab}	17.30 $\pm$ 1.03 ^{b-f}
T5s	59.22 $\pm$ 1.08 ^{ab}	57.42 $\pm$ 2.45 ^{a-d}	55.60 $\pm$ 3.21 ^{b-f}	21.06 $\pm$ 1.26 ^{fgh}	24.72 $\pm$ 1.65 ^{a-e}	25.17 $\pm$ 0.91 ^{ab}	90.40 $\pm$ 1.19 ^{ab}	89.94 $\pm$ 2.27 ^{abc}	88.96 $\pm$ 3.08 ^{abc}	15.14 $\pm$ 0.69 ^f	17.68 $\pm$ 1.05 ^{a-f}	17.37 $\pm$ 0.72 ^{b-f}
T6s	55.21 $\pm$ 1.67 ^{b-g}	55.56 $\pm$ 3.19 ^{b-f}	55.73 $\pm$ 3.28 ^{b-e}	21.14 $\pm$ 1.81 ^{fgh}	23.89 $\pm$ 2.16 ^{b-f}	25.25 $\pm$ 2.28 ^{ab}	90.47 $\pm$ 1.18 ^{ab}	89.47 $\pm$ 2.56 ^{abc}	88.79 $\pm$ 1.70 ^{abc}	16.36 $\pm$ 1.52 ^{d-f}	17.40 $\pm$ 1.63 ^{b-f}	17.21 $\pm$ 1.89 ^{b-f}
T7s	56.64 $\pm$ 2.07 ^{a-d}	56.63 $\pm$ 3.22 ^{a-d}	50.47 $\pm$ 2.32 ^{f-j}	21.93 $\pm$ 2.50 ^{c-h}	23.94 $\pm$ 1.17 ^{b-f}	23.60 $\pm$ 1.31 ^{b-f}	89.88 $\pm$ 1.71 ^{abc}	88.49 $\pm$ 1.68 ^{abc}	87.36 $\pm$ 1.03 ^c	16.31 $\pm$ 1.75 ^{d-f}	16.53 $\pm$ 0.76 ^{d-f}	16.86 $\pm$ 1.06 ^{c-f}
T8s	54.81 $\pm$ 2.15 ^{b-h}	53.99 $\pm$ 3.13 ^{c-i}	54.53 $\pm$ 3.15 ^{b-h}	21.79 $\pm$ 1.81 ^{d-h}	23.94 $\pm$ 1.79 ^{b-f}	23.20 $\pm$ 1.33 ^{b-h}	90.74 $\pm$ 1.20 ^a	88.96 $\pm$ 1.98 ^{abc}	89.84 $\pm$ 2.29 ^{abc}	17.21 $\pm$ 1.31 ^{b-f}	17.65 $\pm$ 1.21 ^{a-f}	16.46 $\pm$ 1.11 ^{d-f}
T9s	57.37 $\pm$ 1.60 ^{a-d}	54.98 $\pm$ 3.62 ^{b-fg}	54.89 $\pm$ 1.55 ^{b-g}	21.24 $\pm$ 0.55 ^{fgh}	24.81 $\pm$ 2.08 ^{a-e}	23.26 $\pm$ 1.06 ^{b-h}	90.24 $\pm$ 0.99 ^{abc}	87.69 $\pm$ 1.64 ^{bc}	89.73 $\pm$ 1.31 ^{abc}	15.55 $\pm$ 0.84 ^{ef}	17.50 $\pm$ 1.12 ^{a-f}	15.97 $\pm$ 0.96 ^{d-f}
Variety (V)	< 0.0001			< 0.0001			< 0.0001			< 0.0001		
Treatment stages (TS)	< 0.0001			< 0.0001			< 0.0001			< 0.0001		
Interaction V*TS	< 0.0001			< 0.0001			< 0.0001			< 0.0001		

Values followed by different letters within a column indicate significant differences ($P < 0.05$). Means $\pm$ standard deviations ($n = 10$).

Table 5. Variations in lupin grain size following aqueous thermal and saline heat treatments

Aqueous thermal treatment stages	Large diameter (mm)			Small diameter (mm)			Saline heat treatment stages	Large diameter (mm)			Small diameter (mm)		
	INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo		INIAP-450	INIAP-451	Criollo	INIAP-450	INIAP-451	Criollo
Raw grain	11.4 ± 0.7 ^e	10.8 ± 0.4 ^e	10.9 ± 0.5 ^e	5.5 ± 0.1 ^{de}	5.0 ± 0.1 ^e	5.9 ± 0.1 ^{cd}	Raw grain	11.4 ± 0.7 ^f	10.8 ± 0.4 ^f	10.9 ± 0.5 ^f	5.5 ± 0.5 ^{fg}	5.0 ± 0.3 ^g	5.9 ± 0.4 ^{ef}
T1	14.8 ± 0.8 ^{a-d}	15.0 ± 0.7 ^{abc}	15.3 ± 1.0 ^a	6.2 ± 0.1 ^{a-d}	5.9 ± 0.1 ^{cd}	6.5 ± 0.1 ^{abc}	T1s	15.0 ± 1.0 ^{a-e}	14.6 ± 0.7 ^{a-e}	14.6 ± 0.8 ^{a-e}	6.0 ± 0.7 ^{c-f}	6.1 ± 0.3 ^{b-f}	6.4 ± 0.3 ^{a-e}
T2	15.2 ± 0.6 ^{ab}	14.8 ± 0.5 ^{a-d}	14.5 ± 0.8 ^{a-d}	6.2 ± 0.1 ^{a-d}	6.0 ± 0.1 ^{bcd}	6.5 ± 0.1 ^{abc}	T2s	14.7 ± 0.7 ^{a-e}	14.0 ± 1.1 ^{de}	14.8 ± 1.0 ^{a-e}	6.3 ± 0.6 ^{a-e}	6.2 ± 0.6 ^{b-f}	6.9 ± 0.5 ^{ab}
T3	14.2 ± 0.6 ^{a-d}	14.6 ± 0.5 ^{a-d}	14.7 ± 0.7 ^{a-d}	6.5 ± 0.1 ^{abc}	6.7 ± 0.1 ^{abc}	6.8 ± 0.1 ^a	T3s	14.0 ± 0.8 ^{de}	14.2 ± 0.9 ^{cde}	14.0 ± 0.7 ^e	6.2 ± 0.5 ^{a-f}	6.1 ± 0.5 ^{b-f}	6.5 ± 0.6 ^{a-e}
T4	14.0 ± 0.4 ^{cd}	13.8 ± 0.5 ^{a-d}	13.9 ± 0.4 ^{cd}	6.6 ± 0.1 ^{abc}	6.4 ± 0.1 ^{abc}	6.7 ± 0.1 ^{abc}	T4s	15.7 ± 1.02	14.6 ± 0.4 ^{a-e}	15.3 ± 0.8 ^{abc}	6.5 ± 0.5 ^{a-e}	6.3 ± 0.5 ^{a-e}	6.8 ± 0.5 ^{abc}
T5	14.4 ± 0.6 ^{a-d}	13.8 ± 0.7 ^{a-d}	14.6 ± 0.7 ^{a-d}	6.5 ± 0.1 ^{abc}	5.9 ± 0.1 ^{cd}	6.7 ± 0.1 ^{abc}	T5s	15.2 ± 0.8 ^{a-d}	15.1 ± 0.7 ^{a-e}	15.2 ± 0.5 ^{a-d}	6.5 ± 0.5 ^{a-e}	6.0 ± 0.4 ^{def}	6.8 ± 0.5 ^{abc}
T6	14.2 ± 0.8 ^{a-d}	14.0 ± 0.5 ^{cd}	14.5 ± 0.6 ^{a-d}	6.7 ± 0.1 ^{abc}	6.1 ± 0.1 ^{a-d}	6.8 ± 0.1 ^{ab}	T6s	15.3 ± 0.9 ^{abc}	15.1 ± 0.6 ^{a-e}	15.6 ± 0.8 ^{ab}	6.6 ± 0.4 ^{a-e}	6.3 ± 0.6 ^{a-e}	6.7 ± 0.5 ^{a-d}
T7	14.1 ± 0.8 ^{a-d}	14.0 ± 0.5 ^{bcd}	13.9 ± 1.0 ^{cd}	6.8 ± 0.1 ^a	6.3 ± 0.1 ^{a-d}	6.8 ± 0.1 ^a	T7s	14.8 ± 0.4 ^{a-e}	15.2 ± 0.6 ^{a-e}	15.3 ± 0.7 ^{abc}	6.4 ± 0.2 ^{a-e}	6.3 ± 0.3 ^{a-e}	6.6 ± 0.4 ^{a-d}
T8	14.9 ± 0.5 ^{a-d}	14.6 ± 0.6 ^{a-d}	14.2 ± 0.8 ^{a-d}	6.6 ± 0.1 ^{abc}	6.5 ± 0.1 ^{abc}	6.8 ± 0.1 ^{ab}	T8s	15.4 ± 0.5 ^{ab}	15.0 ± 0.6 ^{a-e}	14.6 ± 0.5 ^{a-e}	6.9 ± 0.1 ^a	6.4 ± 0.3 ^{a-e}	6.8 ± 0.4 ^{ab}
T9	14.5 ± 0.5 ^{a-d}	14.2 ± 1.0 ^{a-d}	14.2 ± 1.0 ^{a-d}	6.7 ± 0.1 ^{abc}	6.7 ± 0.1 ^{ab}	6.8 ± 0.1 ^a	T9s	15.4 ± 0.5 ^{ab}	14.8 ± 0.6 ^{a-e}	14.5 ± 0.5 ^{b-e}	6.9 ± 0.4 ^a	6.6 ± 0.5 ^{a-e}	6.8 ± 0.2 ^{ab}
T10	14.7 ± 0.7 ^{a-d}	14.2 ± 0.6 ^{a-d}	14.8 ± 0.6 ^{a-d}	6.9 ± 0.1 ^a	6.4 ± 0.1 ^{abc}	6.7 ± 0.1 ^{ab}							
T11	14.4 ± 0.4 ^{a-d}	14.4 ± 0.8 ^{a-d}	14.3 ± 0.3 ^{a-d}	6.6 ± 0.1 ^{abc}	6.4 ± 0.1 ^{abc}	6.6 ± 0.1 ^{abc}							
T12	14.5 ± 0.5 ^{a-d}	14.4 ± 0.7 ^{a-d}	14.2 ± 0.27 ^{a-d}	6.7 ± 0.1 ^{abc}	6.4 ± 0.1 ^{abc}	6.5 ± 0.1 ^{abc}							
Variety (V)	0.07			< 0.0001			Variety (V)	0.002			< 0.0001		
Treatment stages (TS)	< 0.0001			< 0.0001			Treatment stages (TS)	< 0.0001			< 0.0001		
Interaction V*TS	0.1024			0.301			Interaction V*TS	0.0267			0.3003		

Values followed by different letters within a column denote significant differences ($P < 0.05$). Means ± standard deviations ($n = 10$).