Pol. J. Food Nutr. Sci., 2022, Vol. 72, No. 4, pp. 361–370
On-line ISSN: 2083-6007
Print ISSN: 1230-0322
DOI: 10.31883/pjfns/155362
http://journal.pan.olsztyn.pl

Original article
Section: Food Technology

Hydration Kinetics of Nixtamalized White Bitter Lupin (*Lupinus albus* L.) Seeds

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Key words: nixtamalization, bitter white lupin, hydration kinetics

Nixtamalization is usually performed on grains by cooking in an alkaline solution to improve the final product characteristics. White bitter lupin (*Lupinus albus*) seeds were nixtamalized at various concentrations of calcium hydroxide in the range of 0.16–3.33% at 50, 70, and 90°C for 35 min and steeped for 0, 8, 16, and 24 h, and the moisture uptake was determined to model seed hydration kinetics. Moisture uptake increased with increasing nixtamalization temperature regardless of calcium hydroxide concentration. The Page and Weibull models adequately described white bitter lupin hydration kinetics during nixtamalization. Model parameters K_p (Page model) and α (Weibull model) ranged from 80.2 to 410.03 and from 88.21 and 93.96, respectively, for nixtamalization at different calcium hydroxide concentrations, and from 58.55 to 662.88 and from 68.74 and 132.99, respectively, for nixtamalization at different temperatures. The alkaloid content of raw lupin flour was 1.08 g/100 g and it gradually decreased as a result of nixtamalization in increasingly concentrated calcium hydroxide solutions and higher temperatures. The cracks were visible in the microstructure of nixtamalized seed coats. Their number and size increased with the increase of processing temperature, calcium hydroxide concentration, and steeping duration. Overall, the presented results may be useful in optimizing the industrial nixtamalization of lupin seeds and increasing the possibility of their use as a valuable food ingredient.

INTRODUCTION

Lupin (*Lupinus* spp.) belongs to the Fabaceae (Leguminosae) family [Pastor-Cavada *et al*., 2009]. There are more than 400 species from the genus *Lupinus*; from which only four (*i.e.*, *L. albus* L.: white lupin, *L. angustifolius* L.: blue or narrow-leafed lupin, *L. luteus* L.: yellow lupin, and *L. mutabilis* L.: pearl or Tarrwi lupin) are of agronomic interest [Annicchiarico *et al*., 2010; Chiofalo *et al*., 2012; Gulisano *et al*., 2022]. The first three species originate from the Mediterranean area, while the native environment for *L. mutabilis* is South America. Lupin seeds are employed as a protein source for animal and human nutrition in various parts of the world [Chiofalo *et al*., 2012; Prusinski, 2017]. Lupin is cultivated not only due to the nutritional value of its seeds, but also to its adaptability to marginal soils and climates. Human consumption of lupins has increased in recent years [Lucas *et al*., 2015].

Lupins are high protein crops with an average protein content in the white lupin seeds ranging from 30.6 to 37.6 g/100 g [Martínez-Villaluenga *et al*., 2006; Sujak *et al*., 2006]. Furthermore, lupin has a minimum content of proteins with anti-nutritive properties (allergenic proteins) compared to other legumes (*i.e.*, peas, soybean, and bean) [Kurlovich *et al*., 2002]. The mean value of crude fat in *L. albus* of different varieties is 13 g/100 g [Martínez-Villaluenga *et al*., 2006]. As well as lupin seeds and seed coats contain various types of carbohydrates, mainly non-starch ones [Malekipoor *et al*., 2022]; which are the most abundant in seeds. Most carbohydrates are represented by soluble or insoluble fiber accounting for about 39.42 g/100 g of dry matter [Keller *et al*., 2022; Martínez-Villaluenga *et al*., 2006]. Starch content of lupin is very low and according to Borek *et al*. [2011] mature air-dried white lupin seeds are starch free.

The presence of non-nutritional compounds is considered a limiting factor for lupin consumption. The main non-nutritional substances found in lupin seeds are various alkaloids of the quinolizidine group [De Cortes Sánchez *et al*., 2005; Estivi *et al*., 2022; Sujak *et al*., 2006]. Such alkaloids are usually removed by either selecting genotypes with a low content of these components or through post-harvest treatments including germinating, cooking, soaking, fermentation and extraction [Estivi *et al*., 2022; Prusinski, 2017]. Therefore, alkaloid removal would enhance lupin consumption.

Nixtamalization is a pre-Columbian era process in which corn kernels are cooked in a calcium hydroxide solution [Ramírez-Araujo *et al*., 2019]. Nixtamalization is also applied to other grains and seeds including amaranth [Valdez-Niebla *et al*., 1993], sorghums [Díaz González *et al*., 2019; Gaytán-Martínez *et al*., 2017] and beans [Santiago-Ramos *et al*., 2018a]. This process improves the rheological properties

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Submitted: 15 May 2022
Accepted: 12 October 2022
Published on-line: 14 November 2022

of final products, which include viscoelasticity [Santiago--Ramos *et al*., 2018b], significantly increases the content of calcium and other minerals [Santiago-Ramos *et al*., 2018a; Vega Rojas *et al*., 2017] as well as affects the protein quality [Rincón-Aguirre *et al*., 2021]. Additionally, it was found that nixtamalization resulted in a discount of mycotoxin content and a reduction in the potential toxicity of maize contaminated with fumonisin [Voss *et al*., 2017]

Hydration kinetics of grain is a complex phenomenon and its exact mechanisms are largely not understood. The analysis of kinetics hydration is useful for process design and optimization [Augusto & Miano, 2017]. The hydration kinetics of lupin seeds was studied by Solomon [2007]. The author soaked the seeds in water at 20, 30, 40, and 50°C for up to 12 h and further fitted the Peleg model equation adequately to describe the hydration characteristics. In other study, Solomon [2009] determined the hydration kinetics of roasted lupin seeds soaked in water at 25, 35, 45, or 55°C for up to 8 h. The author also used Peleg model in addition to the first-order kinetic model, and hydration kinetics was described adequately. On the other hand, Miano *et al*. [2015] fitted a sigmoidal model when they studied the hydration kinetics and mathematical modeling of Andean lupin (*L. mutabilis* Sweet) seeds. There are limited studies on the hydration kinetics of bitter lupin seeds and – to the best of our knowledge – there are no studies on the effects of nixtamalization of lupin seeds on their hydration kinetics. Therefore, this work was initiated to evaluate the hydration kinetics of white bitter lupin seeds under various nixtamalization conditions (*i.e.*, nixtamalization temperature and calcium hydroxide concentration) and to determine changes in the microstructure and content of seed alkaloids as a result of the process conditions.

MATERIALS AND METHODS

Materials

Bitter lupin seeds (*i.e.*, Egyptian origin of the crop year 2020) were procured from a local market in Amman, Jordan. Before conducting the tests, seeds with defects, including breakage and color damage, were discarded and not included in testing to ensure seeds' uniformity in terms of maturity. The initial moisture content of lupin seeds was determined by grinding the seeds into flour before drying using a convection oven at 105°C (±2°C) until constant weight. The initial moisture content of lupin seeds was 6.47 g/100 g.

Nixtamalization of lupin seeds

The nixtamalization of lupin seeds was carried out using solutions of $Ca(OH)_2$ in different concentrations, *i.e.*, 0.16, 0.33, 0.50, 0.66, 1.33, 2.00, 2.66, and 3.33%. Parallel, seeds were treated in water (0% $Ca(OH)_2$). Seeds were cooked at 50°C, 70°C, and 90°C before being steeped in the same calcium hydroxide solutions or water for 0, 8, 16, and 24 h. A total of 216 samples ((9×3×4)×2) was achieved. For nixtamalization, 100 g of bitter lupin seeds were placed in a previously heated calcium hydroxide solution (or water for control) at the adequate temperature (*i.e.*, 50, 70 or 90°C) and cooked for 35 min. Beakers were covered during the process to prevent water evaporation and to maintain the exact calcium hydroxide concentration.

After steeping for the required duration, nixtamalized seeds were washed with distilled water to remove the excess calcium hydroxide. The water remaining on the surface of the seeds was removed using a paper towel. Moisture uptake by lupin seeds (M) was measured as the weight difference after and before the nixtamalization process using an analytical balance (BTD-323, Phoenix Instrument, Blomberg, Germany).

Mathematical modeling of hydration kinetics

Non-linear regression models (the Peleg, Lewis, Page, Weibull, Henderson, Kaptso-Njintang-Komnek-Hounhouigan-Scher-Mbolung, and Ibarz-Augusto models) were used to define the hydration kinetics of bitter lupin seeds [Augusto & Miano, 2017]. A list of models and their respective equations used in this study is presented in Table 1. Root mean square error (RMSE) and coefficient of determination (R^2) were determined to fit each model to the experimental data. The average percentage difference between the experimental and predicted values, also known as the mean relative deviation modulus (PMRD), was calculated according to Equation (1) and used as a measure of model adequacy. The model with the highest R^2 (that must be as close as possible to the one) and the least RMSE and PMRD (that must be as close as possible to zero) was chosen as the best [Saleh & Meullenet, 2013].

$$PMRD = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{MR\ actual - MR\ predicted}{MR\ actual}\right) \quad (1)$$

where: n represents observations, i represents n=1, and MR is the rate of moisture uptake, given by Equation (2).

$$MR = \frac{M_t - M_z}{M_0 - M_z} \quad (2)$$

where: M_0 is the initial moisture content of the bitter lupin seeds, M_t is the moisture content of nixtamalized bitter lupin seeds at time t, and M_z is the final moisture content of nixtamalized bitter lupin seeds.

Microscopic analysis

The lupin seeds nixtamalized using the extreme and middle concentrations of calcium hydroxide solutions (3.33% and 0.66%, respectively), and water only (0% $Ca(OH)_2$) of the three temperatures and all steeping durations were photographed. The pictures were taken using an EZ4HD Leica microscope (Leica Microsystems, Singapore) and LAS EZ-V2-1 program.

Alkaloid content determination

The alkaloid content of selected samples was determined according to the method described by Shamsa *et al*. [2008] with some modifications. Alkaloids were extracted from dried (moisture content around 6 g/100 g) and ground into flour nixtamalized lupin seeds (10 g) with methanol (100 mL) in a water bath for 2 h, then sonicated for 30 min and left overnight at room temperature. The mixtures were filtered and methanol was evaporated under vacuum at 45°C. The dried extracts were dissolved in 2 M HCl and then filtered. One mL of the filtrate was washed twice with chloroform using a separatory funnel

TABLE 1. Equations of models used to fit bitter lupin seed hydration kinetics.

Model	Equation
Peleg	$M(t) = M_0 + \frac{t}{k_1 + k_2 \times t}$
Lewis	$\frac{M(t) - M_0}{M_\infty - M_0} = \exp(-k_l \times t)$
Page	$\frac{M(t) - M_0}{M_\infty - M_0} = \exp\,(-K_p \times t^n)$
Henderson	$\frac{M(t) - M_0}{M_\infty - M_0} = P_1 \times exp(-K_{H1} \times t) + P_2 \times exp(-K_{H2} \times t)$
Weibull	$\frac{M(t)}{M_\infty} = 1 - exp\left[-(\frac{t}{\alpha})^\beta\right]$
Kaptso-Njintang-Komnek-Hounhouigan-Scher-Mbolung	$M(t) = \frac{M_\infty}{1 + exp[-k_k \times (t - \tau)]}$
Ibarz-Augusto	$M(t) = \frac{M_\infty}{1 + \frac{M_\infty - M_0}{M_0} exp(-k_{IA} \times M_\infty \times t)}$

k_1, k_2, k_l, K_p, P_1, P_2, K_{H1}, K_{H2}, α, β, k_k, and k_{IA}: kinetic parameters of the mathematical models; M: product moisture content on dry basis, d.b. (g water/100 g solids); M_0: initial moisture content (g water/100 g d.b.); M_∞: equilibrium moisture content (g water/100 g d.b.); t: time (min); (τ): parameter that describes the time (s) of the inflexion point, related to the lag phase.

and neutralized with 0.1 M NaOH. Then, 5 mL of 0.1 mM bromocresol green solution and 5 mL of phosphate buffer (pH 4.7) were added. The complex formed was extracted with 10 mL of chloroform by vigorous shaking. After separation of the chloroform phase, its volume was adjusted to 10 mL in a volumetric flask with chloroform. The absorbance of the mixture was measured at 470 nm. Alkaloid content was quantified on the basis of atropine standard curve and results were expressed as g of standard equivalent per 100 g of seeds.

Statistical analysis

To determine the effect of temperature, calcium hydroxide concentration, and steeping duration, one-way analysis of variance (ANOVA) with least significant differences (LSD) post-hoc test at a 5% level of probability was carried out using JMP statistical software release 10.0 (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Lupin seed hydration

The hydration characteristics of nixtamalized lupin seeds in terms of moisture uptake at different calcium hydroxide concentrations, cooking temperatures, and steeping durations are presented in Table 2.

Discussing the effect of the temperatures used during the nixtamalization of bitter lupin seeds on moisture uptake, it can be mentioned that at the beginning of steeping (0 h), the water absorption of seeds treated at 50°C was lower ($p<0.05$) compared to that for seeds cooked at 90°C regardless of the calcium hydroxide concentration. Significant ($p<0.05$) differences were also noted between the nixtamalization at 50 and 70°C, but with the exception of processes carried out in 0.66%, 2.00%, and 2.66% $Ca(OH)_2$ solutions. No significant ($p\geq0.05$) differences were shown between treatments at 70°C and 90°C for low calcium hydroxide concentrations (0.16–0.66%). At a steeping duration of 8 h, nixtamalization in 0.16%, 0.33%, and 1.33% $Ca(OH)_2$ solutions showed no temperature effect on moisture uptake. In turn, water absorption of the samples treated without $Ca(OH)_2$ and at its concentrations of 0.50%, 0.66%, 2.66% and 3.33% were significantly ($p<0.05$) lower at 50°C than at 70°C and 90°C. In general, at the early stage of hydration, there was a difference in the moisture uptake by lupin seeds treated at different temperatures. Trends of increasing moisture uptake at higher temperatures could be due to the increase in seed coat pore size, which improved the water flow [Oliveira *et al*., 2013].

The results showing the effect of steeping duration on the hydration process are presented in Table 2. They indicated that the longer the steeping duration, the higher the moisture uptake. After 16 h of steeping, most of the samples reached their maximum moisture uptake (up to 130.9 g/100 g). Whereby, the highest rate of the water absorption occurred at the early stage of the process followed by a decrease in the rate. Similar trends have been reported for peas and beans [Ueno *et al*., 2015] and cereal grains [Thakur & Gupta, 2006]. In turn, the equilibrium moisture uptake of all the samples in our study was lower compared to the results reported by Solomon [2007] for hydrated lupin seeds (~140 to 170 g/100 g).

The decrease in moisture uptake of lupin seeds in some treatments after 24 h of steeping (Table 2) was attributed to the effect of calcium hydroxide, which softened lupin seed coat and increased the solid mass loss with the prolonged steeping. Similar results were reported by Castro-Muñoz & Yáñez-Fernández [2015] who found that the alkaline solution remaining after steeping of corn (that was not absorbed by grain) had a high content of soluble solids (polysaccharides, protein, lipids, as well as some bioactive compounds as polyphenols and carotenoids) and insoluble solids (including fiber fractions such as cellulose and hemicellulose).

TABLE 2. Moisture uptake of bitter lupin seeds (g/100 g, dry base) nixtamalized at different temperatures and calcium hydroxide concentrations, and steeped for different durations.

Temperature (°C)	Calcium hydroxide concentration (%)	Steeping duration (h)			
		0	8	16	24
	0.00	$43.5\pm1.9^{\mathrm{cB}b}$	$117.5\pm0.1^{\mathrm{bA}b}$	$130.3\pm2.1^{\mathrm{aA}a}$	$126.5\pm0.3^{\mathrm{aA}b}$
	0.16	$31.5\pm1.8^{\mathrm{cD}c}$	$118.0\pm2.8^{\mathrm{bA}a}$	$125.7\pm0.6^{\mathrm{aBC}b}$	$125.5\pm1.6^{\mathrm{aAB}a}$
	0.33	$31.8\pm0.3^{\mathrm{cD}b}$	$114.2\pm2.4^{\mathrm{bB}a}$	$126.7\pm3.2^{\mathrm{aAB}a}$	$127.7\pm0.7^{\mathrm{aA}a}$
	0.50	$36.4\pm1.6^{\mathrm{cCD}b}$	$114.9\pm0.3^{\mathrm{bAB}b}$	$124.0\pm2.8^{\mathrm{aBCD}a}$	$126.7\pm0.8^{\mathrm{aA}a}$
50	0.66	$42.0\pm7.2^{\mathrm{cBC}b}$	$113.2\pm1.0^{\mathrm{bB}b}$	$121.4\pm1.3^{\mathrm{abD}b}$	$126.5\pm1.5^{\mathrm{aA}a}$
	1.33	$49.9\pm0.7^{\mathrm{cA}c}$	$113.5\pm0.7^{\mathrm{bB}a}$	$122.9\pm1.3^{\mathrm{aBCD}a}$	$121.1\pm1.4^{\mathrm{aC}a}$
	2.00	$47.4\pm2.1^{\mathrm{dAB}b}$	$111.9\pm0.7^{\mathrm{cB}b}$	$122.7\pm0.7^{\mathrm{bBCD}b}$	$128.0\pm1.3^{\mathrm{aA}a}$
	2.66	$42.4\pm1.3^{\mathrm{cBC}b}$	$113.9\pm1.2^{\mathrm{bB}b}$	$122.0\pm1.1^{\mathrm{aCD}b}$	$123.3\pm1.2^{\mathrm{aBC}b}$
	3.33	$41.5\pm1.1^{\mathrm{cBC}c}$	$112.4\pm1.1^{\mathrm{bB}c}$	$124.1\pm1.8^{\mathrm{aBCD}b}$	$125.8\pm1.6^{\mathrm{aAB}a}$
	0.00	$50.8\pm1.7^{\mathrm{cBC}a}$	$123.1\pm1.7^{\mathrm{bA}a}$	$130.0\pm0.1^{\mathrm{aA}a}$	$127.3\pm0.2^{\mathrm{aA}a}$
	0.16	$57.7\pm3.3^{\mathrm{bA}a}$	$123.5\pm3.2^{\mathrm{aA}a}$	$128.0\pm0.1^{\mathrm{aAB}a}$	$122.4\pm0.2^{\mathrm{aCD}a}$
	0.33	$55.2\pm3.9^{\mathrm{bAB}a}$	$121.2\pm1.0^{\mathrm{aAB}a}$	$124.4\pm1.6^{\mathrm{aCD}a}$	$122.4\pm1.2^{\mathrm{aCD}b}$
	0.50	$49.6\pm1.4^{\mathrm{cBC}a}$	$121.5\pm0.1^{\mathrm{bAB}a}$	$125.3\pm0.5^{\mathrm{bBC}a}$	$120.4\pm0.4^{\mathrm{aD}c}$
70	0.66	$51.5\pm2.0^{\mathrm{cBC}ab}$	$119.3\pm1.3^{\mathrm{bAB}a}$	$125.0\pm0.6^{\mathrm{aBCD}a}$	$122.3\pm2.1^{\mathrm{abCD}a}$
	1.33	$52.0\pm0.4^{\mathrm{cAB}b}$	$116.1\pm4.7^{\mathrm{bB}a}$	$122.1\pm1.2^{\mathrm{abD}a}$	$126.3\pm2.3^{\mathrm{aAB}a}$
	2.00	$49.4\pm3.6^{\mathrm{cBC}b}$	$118.9\pm3.5^{\mathrm{bAB}ab}$	$127.3\pm1.2^{\mathrm{aABC}a}$	$124.3\pm0.7^{\mathrm{abBC}b}$
	2.66	$45.7\pm4.4^{\mathrm{bC}b}$	$122.9\pm3.6^{\mathrm{aA}a}$	$127.4\pm1.9^{\mathrm{aABC}ab}$	$128.9\pm0.5^{\mathrm{aA}b}$
	3.33	$50.9\pm0.7^{\mathrm{cBC}b}$	$118.5\pm2.9^{\mathrm{bAB}b}$	$127.9\pm2.4^{\mathrm{aAB}ab}$	$123.3\pm1.8^{\mathrm{abCD}a}$
	0.00	$51.5\pm2.3^{\mathrm{cCD}a}$	$126.1\pm0.2^{\mathrm{bA}a}$	$129.6\pm0.6^{\mathrm{aAB}a}$	$126.2\pm0.2^{\mathrm{bA}b}$
	0.16	$46.1\pm0.7^{\mathrm{cD}b}$	$121.8\pm1.9^{\mathrm{bAB}a}$	$128.8\pm0.2^{\mathrm{aAB}a}$	$125.2\pm3.0^{\mathrm{abA}a}$
	0.33	$52.5\pm0.3^{\mathrm{cC}a}$	$120.9\pm3.5^{\mathrm{bB}a}$	$126.6\pm1.7^{\mathrm{aAB}a}$	$123.1\pm0.3^{\mathrm{abA}b}$
	0.50	$53.6\pm0.8^{\mathrm{cC}a}$	$121.8\pm1.3^{\mathrm{bAB}a}$	$126.8\pm1.4^{\mathrm{aAB}a}$	$123.6\pm0.2^{\mathrm{bA}b}$
90	0.66	$56.2\pm0.6^{\mathrm{cABC}a}$	$121.8\pm0.0^{\mathrm{bAB}a}$	$125.6\pm0.7^{\mathrm{aAB}a}$	$123.7\pm1.4^{\mathrm{abA}a}$
	1.33	$54.8\pm0.4^{\mathrm{bBC}a}$	$122.9\pm4.2^{\mathrm{aAB}a}$	$124.0\pm8.7^{\mathrm{aB}a}$	$123.6\pm2.4^{\mathrm{aA}b}$
	2.00	$61.2\pm3.4^{\mathrm{bA}a}$	$123.8\pm1.3^{\mathrm{aAB}a}$	$129.0\pm0.8^{\mathrm{aAB}a}$	$124.6\pm1.2^{\mathrm{aA}ab}$
	2.66	$59.9\pm6.0^{\mathrm{bAB}a}$	$123.3\pm1.2^{\mathrm{aAB}a}$	$132.3\pm4.4^{\mathrm{aA}a}$	$125.4\pm0.5^{\mathrm{aA}a}$
	3.33	$62.2\pm3.5^{\mathrm{cA}a}$	$125.1\pm0.8^{\mathrm{bAB}a}$	$130.9\pm0.6^{\mathrm{aAB}a}$	$125.6\pm0.2^{\mathrm{bA}a}$

Results are expressed as mean ± standard deviation. Different lowercase letters in the same row indicate significant ($p<0.05$) differences between the values determined for the samples treated at different steeping duration. Different capital letters in the same column (separately for each temperature) indicate significant ($p<0.05$) differences between the values determined for the samples treated with different concentrations of $Ca(OH)_2$. Different lowercase italic letters in the same column (separately for each $Ca(OH)_2$ concentration) indicate significant ($p<0.05$) differences between the values determined for the samples treated at different temperatures.

The effect of calcium hydroxide concentration on the hydration of lupin seeds did not follow a specific trend (Table 2). At the beginning of steeping (0 h), the lupin seeds treated with calcium hydroxide at concentrations of 1.33% and 2.00% had the highest moisture uptake at 50°C, while the calcium hydroxide concentration of 0.16%, 0.33% and 1.33% allowed to obtain the highest moisture uptake at 70°C. At 90°C, the highest values were determined at $Ca(OH)_2$ concentrations of 0.66% and 2.00–3.33%. In turn, at the end of steeping (24 h), the highest moisture uptake was found in the seeds treated with a wide range of calcium hydroxide concentrations (0–0.66%, 2.00% and 3.33%) at 50°Cas well as at concentrations of 0%, 1.33%, and 2.66% at 70°C; whereas $Ca(OH)_2$ concentration had no effect on water absorption at 90°C. The calcium hydroxide solubility in water is limited to about 1.2 g/L at 25°C [Farhad & Mohammadi, 2005]. The non-soluble part of calcium hydroxide could influence the hydration kinetics indirectly by disposing on the seed surface, which supposedly

TABLE 3. Root mean square error (RMSE) and coefficient of determination (R^2) of Peleg, Lewis, Henderson, Kaptso-Njintang-Komnek-Hounhouigan-Scher-Mbolung (KNKHSM), and Ibarz-Augusto models used to fit bitter lupin seed hydration kinetics.

Calcium hydroxide concentration (%)	Peleg		Lewis		Henderson		KNKHSM		Ibarz-Augusto	
	RMSE	R^2	RMSE	R^2	RMSE	R^2	RMSE	R^2	RMSE	R^2
0.00	4.36	0.98	0.35	0.62	0.39	0.45	9.14	0.98	8.94	0.98
0.16	7.48	0.96	0.36	0.59	0.45	0.35	10.36	0.96	10.24	0.96
0.33	104.84	0.96	0.35	0.61	0.39	0.40	11.66	0.95	20.96	0.96
0.50	104.52	0.97	0.35	0.61	0.05	0.97	10.27	0.97	10.19	0.97
0.66	4.86	0.98	0.34	0.63	0.04	0.97	11.18	0.97	11.05	0.96
1.33	4.02	0.98	0.33	0.64	0.03	0.98	10.72	0.98	10.51	0.97
2.00	5.12	0.98	0.33	0.64	0.05	0.97	11.74	0.96	11.56	0.96
2.66	6.38	0.97	0.34	0.62	0.92	0.41	10.87	0.97	10.67	0.97
3.33	6.35	0.96	0.33	0.62	0.51	0.35	10.99	0.96	10.77	0.96
Temperature (°C)										
50	2.44	0.98	0.38	0.68	0.20	0.97	12.74	0.97	17.79	0.98
70	3.01	0.98	0.32	0.61	0.34	0.98	8.88	0.98	8.93	0.98
90	1.58	0.98	0.31	0.59	0.19	0.98	7.87	0.98	7.83	0.98

contributed to the softness of the seed coat. The formation of gummy and sticky pericarp due to the calcium hydroxide treatment was reported by Martínez-Bustos *et al*. [2001] who indicated a significant role of maize pericarp modifications on the moisture kinetics.

Modeling the hydration kinetics of bitter lupin seeds

Water absorption data of the lupin treatments in terms of moisture uptake under the experimental conditions were fitted into several models by either concentration and/or by temperature (Table 1). The sigmoidal models (Kaptso-Njintang-Komnek-Hounhouigan-Scher-Mbolung and Ibarz--Augusto) that are used in the modeling of the hydration kinetics were not appropriate in describing the moisture uptake of bitter lupin seeds (Table 3). This inappropriate description by the sigmoidal models can be attributed to the effects of calcium hydroxide concentration that resulted in greater hydration. In a previous study on the hydration of Andean lupin seeds, it was found that the curve of hydration kinetics showed a sigmoidal shape [Miano *et al*., 2015]. The authors reported that the hydration lag phase was shortened as the temperature of the process increased, because at higher temperatures the minimum moisture content required for the seeds to change from glass to rubbery was reduced, resulting in increased water permeability.

In the exponential category, Page and Weibull models had the highest R^2 and the lowest values of RMSE and PMRD compared with other models evaluated (Table 3 and Table 4). The models adequately characterized the hydration behavior of bitter lupin seeds upon nixtamalization. The estimated parameters of Page and Weibull models for calcium hydroxide concentrations and temperatures, RMSE, R^2 and PRMD, are presented in Table 4. In the Page model, the value of the parameter related to diffusion coefficient and the sample geometry (K_p) ranged from 62.29 to 410.03 for different $Ca(OH)_2$ concentrations with the lowest value noted for the concentration of 2.00% and the highest one for the concentration of 0.16%. The n parameter of the Page model is related to the diffusion type and food microstructure [Miano & Augusto, 2017]. Our results showed a negative value of n for modeling the hydration kinetics of all samples regardless of the $Ca(OH)_2$ concentration. Since this model was originally developed for the drying process, this possibly explains the negative values of the n parameter in our study (*i.e.*, the water absorption being in the inverse direction to the drying process). The trend of the parameters of the Page model in function of temperature was very clear with K_p increasing with the increase in temperature while n had the invers function that reflects the diffusion phenomenon that increases with temperature elevation.

Weibull model is described by two parameters, *i.e.,* α – which is related to the reciprocal of the process rate constant, and the shape parameter β [Miano & Augusto, 2017]. The value of the α parameter determined for different calcium hydroxide concentrations ranged from 87.70 to 97.75 and for temperatures of 50, 70, and 90°C was 132.99, 81.73, and 68.74, respectively (Table 4); hence, its values increased along with decreasing temperature. In turn, the values of β determined for different $Ca(OH)_2$ concentrations and temperatures were below one and ranged from 0.65 to 0.81 for calcium hydroxide concentrations and from 0.70 to 0.79 depending on temperature. The correlations between the two parameters of each model determined to fit the hydration of lupin seeds at different calcium hydroxide concentrations and temperatures were analyzed. There was a high correlation between Page model parameters with R^2 of 0.93 (for $Ca(OH)_2$ concentrations)

TABLE 4. Equation parameters, root mean square error (RMSE), coefficient of determination (R^2), and mean relative deviation modulus (PMRD) of Page and Weibull models used to fit bitter lupin seed hydration kinetics.

Calcium hydroxide concentration (%)	Equation parameters*				RMSE		R^2		PMRD	
	Page		Weibull		Page	Weibull	Page	Weibull	Page	Weibull
	K_p	n	α	β						
0.00	213.74	-1.50	91.41	0.76	0.03	0.03	0.99	0.94	0.38	0.01
0.16	410.03	-1.66	93.05	0.81	0.06	0.05	0.97	0.91	2.57	2.42
0.33	126.28	-1.34	97.75	0.73	0.06	0.06	0.96	0.92	2.56	2.17
0.50	198.93	-1.47	92.82	0.77	0.05	0.04	0.97	0.93	1.37	1.02
0.66	80.02	-1.24	92.40	0.68	0.04	0.04	0.98	0.94	0.34	0.25
1.33	64.29	-1.19	87.70	0.65	0.03	0.03	0.98	0.95	0.11	0.65
2.00	62.29	-1.18	89.86	0.65	0.05	0.04	0.97	0.94	0.84	0.01
2.66	103.89	-1.30	93.96	0.71	0.05	0.04	0.97	0.94	0.59	0.10
3.33	98.70	-1.30	88.21	0.68	0.05	0.05	0.97	0.93	1.28	0.44
Temperature (°C)										
50	58.55	-1.08	132.99	0.70	0.04	0.03	0.85	0.99	0.14	0.00
70	208.65	-1.52	81.73	0.74	0.03	0.03	0.99	0.99	0.10	0.21
90	662.88	-1.86	68.74	0.79	0.03	0.03	0.98	0.99	0.08	0.30

*Equations of Page and Weibull models are shown in Table 1.

and 0.88 (for temperatures). In the Weibull model, the correlation between α and β was found only for modeling hydration at different temperatures with R^2 of 0.81.

The root mean square error (RMSE) of the two models describing the hydration of lupin seeds at different temperatures and $Ca(OH)_2$ concentrations was from 0.03 to 0.06 (Table 4). In turn, R^2 of the actual and estimated values ranged from 0.97 to 0.99 for calcium hydroxide concentrations following the Page model as well as from 0.91 to 0.95 when obtained using the Weibull model. Concerning temperatures, R^2 values were 0.85, 0.99, and 0.98 at 50°C, 70°C, and 90°C, respectively, following the Page model and 0.99 obtained by using the Weibull model at the three temperatures used in the present study. Thus, the Weibull model was more appropriate for describing the hydration kinetics in the function of temperatures for the nixtamalized bitter lupin seeds whereas the Page model proved more efficient in describing it in the function of $Ca(OH)_2$ concentrations.

PMRD which is the average percentage difference between the experimental and predicted values that is used as a measure of model adequacy was lower for most calcium hydroxide concentrations when analyzed using the Weibull model compared to the Page model (Table 4).

Microscopic pictures

Microscopic pictures of seeds nixtamalized at selected calcium hydroxide concentrations at 50°C, 70°C, and 90°C and steeped for 0–24 h are shown in Figure 1. From the pictures of nixtamalized seeds, it could clearly be noticed that with the increase in nixtamalization temperature, calcium hydroxide concentration and steeping duration, an increased number and size of cracks in seed coat were visible. The samples treated at 50°C without calcium hydroxide showed very small and countable cracks that were distributed on the edges of the seeds with a smooth surface. With the increase in calcium hydroxide concentration to 0.66%, the $Ca(OH)_2$ caused a small number of fissures with larger size on the surface of lupin seeds, which increased in size with the augmentation of steeping duration. For higher $Ca(OH)_2$ concentrations, the number of fissures increased and a great number of cleavages was noticed on the seeds treated in the 3.33% $Ca(OH)_2$ solution. Lupin seeds nixtamalized at 70°C debuted from very small cracks on the surface. The number of cracks and fissures increased with the increase of calcium hydroxide concentration from 0.66% to 3.33%. At 3.33% $Ca(OH)_2$ concentration, large fissures with a great number of lost parts were reported when the samples were steeped after nixtamalization for more than 16 h. Nixtamalization at greater temperature (*i.e.*, 90°C) resulted in similar trends of the seeds treated without $Ca(OH)_2$ to those nixtamalized at 70°C. However, with the increase in calcium hydroxide concentration from 0.66% to 3.33%, the number and size of cracks and fissures increased significantly. Our results are in accordance with the hydration of cowpeas that was reported by Sefa-Dedeh & Stanley [1979].

Alkaloid contents

The non-treated lupin seed flour had a total alkaloid content of 1.08 g/100 g. This value was lower than that obtained for *L. albus* L. seeds (14.4 g/kg) by Erbas [2010]. The alkaloid contents of the treated lupin seeds are presented in Table 5. For all those samples, the determined values were significantly

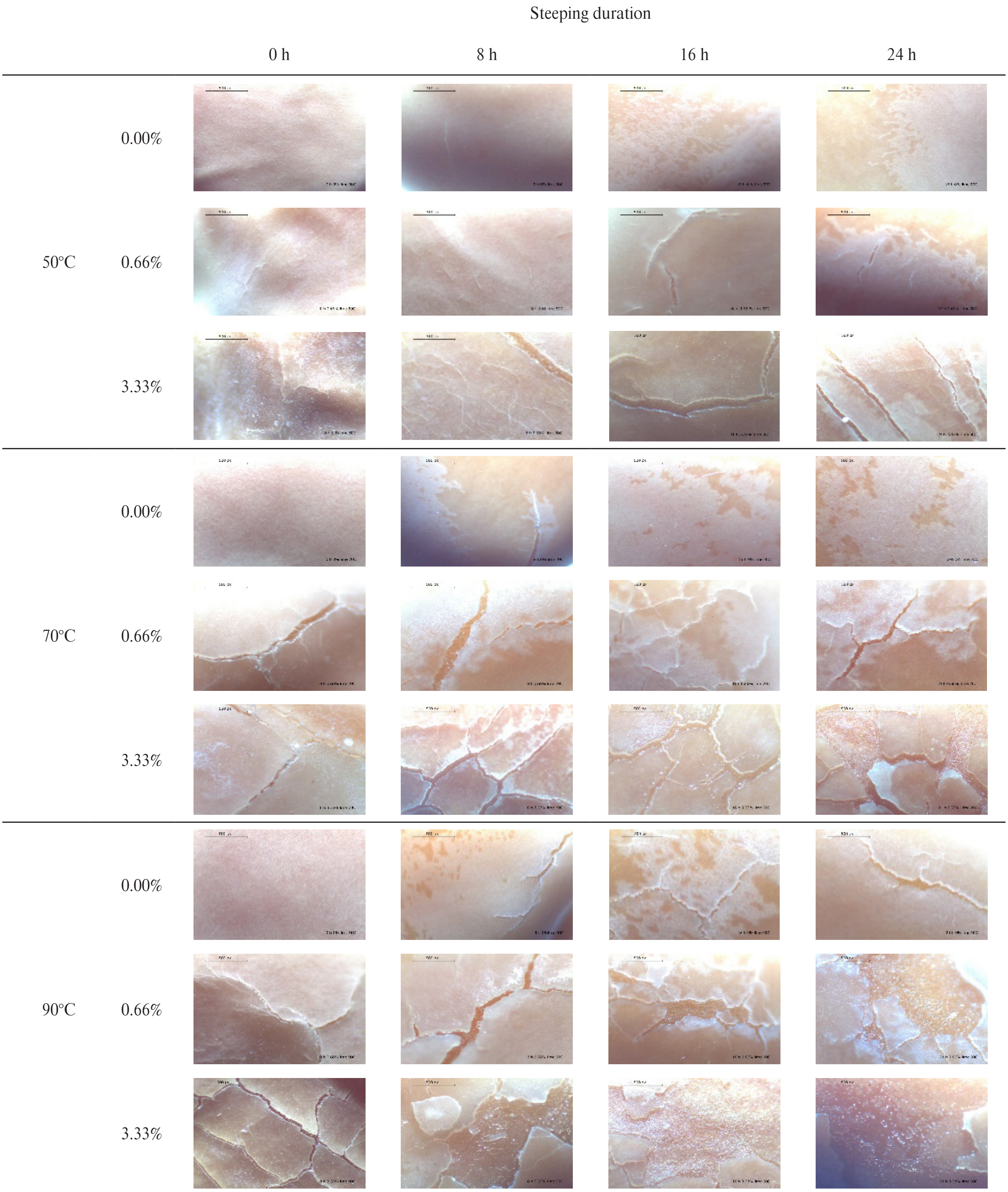

FIGURE 1. Microscopic pictures of bitter lupin seeds nixtamalized at different temperatures (50–90°C) and calcium hydroxide concentrations (0–3.33%) and steeped for durations of 0–24 h.

($p<0.05$) lower compared to the non-treated flour. The alkaloid contents of the nixtamalized seeds decreased significantly ($p<0.05$) with an increasing calcium hydroxide concentration and also with increasing cooking temperatures. Jiménez-Martínez *et al*. [2001] reported that the alkaline thermal treatment, in which the *L. campestris* seeds had a final alkaloid content of 0.002%, was more efficient than the aqueous thermal treatment. The steeping duration did not have a significant ($p<0.05$) effect on the elimination of alkaloids (Table 5). Their low content (0.39 g/100 g) was determined in the seeds treated at 90°C with 3.33% calcium hydroxide concentration and steeped for 16 h; however, it is still a high result within

TABLE 5. Alkaloid content of bitter lupin seeds (g/100 g) nixtamalized at different temperatures and calcium hydroxide concentrations, and steeped for different durations.

Steeping duration (h)	Calcium hydroxide concentration (%)	Temperature (°C)	
		50	90
0	0.00	0.99±0.00^{aAa}	0.86±0.01^{aAb}
	0.66	0.85±0.00^{bAa}	0.52±0.01^{bBb}
	3.33	0.70±0.00^{cAa}	0.40±0.01^{cAb}
16	0.00	0.99±0.02^{aAa}	0.69±0.02^{aBb}
	0.66	0.82±0.01^{bAa}	0.61±0.01^{bAb}
	3.33	0.68±0.02^{cAa}	0.39±0.01^{cAb}

Results are expressed as mean ± standard deviation. Different lowercase letters in the same column (separately for each stepping duration) indicate significant (p<0.05) differences between the values determined for the samples treated with different concentrations of $Ca(OH)_2$. Different capital letters in the same row indicate significant (p<0.05) differences between the values determined for the samples treated at different temperatures. Different lowercase italic letters in the same column (separately for each $Ca(OH)_2$ concentration) indicate significant (p<0.05) differences between the values determined for the samples treated with different steeping duration.

the range of 0.3% to 3% of bitter seeds that reported by Roberts & Wink [1998]. To eliminate more alkaloids from seeds, it is recommended to prolong the cooking duration with the addition of an excess amount of water.

CONCLUSIONS

The steeping duration of bitter white lupin seeds treated under the conditions of this study increases the hydration process until the equilibrium moisture content. The use of high nixtamalization temperatures accelerated the moisture uptake by seeds, and also, the high calcium hydroxide concentration increased the hydration, but the trend was not clear and needs more studies. A number of models were used to evaluate the shape of the hydration kinetics curve. Only two models, *i.e.*, Page and Weibull models, fitted to the experimental results of hydration of lupin seeds. Nixtamalization effectively reduced the content of alkaloids in the lupin seeds, which decreased with the increase of both the $Ca(OH)_2$ concentration and temperature applied during the process. On the other hand, steeping duration had no significant effect on the alkaloid content of the seeds. The microstructure of nixtamalized lupin seeds showed the increase in the number and size of cracks in the seed coat with the increase in the nixtamalization temperature, calcium hydroxide concentration, and steeping duration that accelerated the hydration process by the entrance of water through the hilum, cracks, and fissures.

This research may have practical application in the development of industrial production of lupin masa (*i.e.*, flour) and nixtamalized lupin flour with low alkaloid content for further use in bakery products or as a substitution for other flours. Furthermore, results may help optimize the industrial nixtamalization of lupin seeds and thus contribute to the reduction in energy costs of the process.

RESEARCH FUNDING

The study received no external funding.

CONFLICT OF INTERESTS

The authors declare that they do not have any conflict of interests.

ETHICAL REVIEW

The study was approved by the Ethical Committee, the University of Jordan. Project approval number 9180366/2020/2022. No human or animal trails were used in this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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