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Effects of different hydration treatments on technological, physical, nutritional, and bioactive parameters of lentils

Efectos de diferentes tratamientos de hidratación sobre los parámetros tecnológicos, físicos, nutricionales y bioactivos de las lentejas

ABSTRACT

Regular lentil consumption may improve health and prevent certain chronic diseases. Lentils have high antioxidant activity and contain proteins, essential amino acids, fiber, minerals, and bioactive compounds. However, few studies have investigated the physicochemical characteristics of lentils when subjected to various soaking and cooking methods. We aimed to evaluate the effects of different hydration and cooking practices on the hydration coefficient, cooking time, primary metabolism compounds (lipids, proteins, fibers, carbohydrates), energy value, soluble protein, color and texture characteristics, amino acid and mineral profiles, bioactive compounds, antioxidant activity, and antinutritional factors (phytates and tannins) in lentils. Hydration water was preheated to initial temperatures of 25°C or 90°C. Lentils pre-hydrated with water at 90°C needed less cooking time, obtained greater softness, and had less decreases in amino acids, minerals, bioactive compounds, and antioxidant activity.

Keywords: Health; Hydration coefficient; Lentils; Nutritional value; Temperature.

RESUMEN

El consumo regular de lentejas puede mejorar la salud y prevenir ciertas enfermedades crónicas. Las lentejas tienen una alta actividad antioxidante y contienen proteínas, aminoácidos esenciales, fibra, minerales y compuestos bioactivos. Sin embargo, pocos estudios han investigado las características fisicoquímicas de las lentejas sometidas a varios métodos de remojo y cocción. El objetivo de este estudio fue evaluar los efectos de diferentes procesos de cocción e hidratación sobre el coeficiente de hidratación, el tiempo de cocción, los metabolitos primarios (lípidos, proteínas, fibras, carbohidratos), el valor energético, la proteína soluble, color y textura, los perfiles de aminoácidos y minerales, los compuestos bioactivos, la actividad antioxidante y los factores antinutricionales (fitatos y taninos) en la lenteja. El agua de hidratación se precalentó a temperaturas iniciales de 25°C o 90°C. Las lentejas prehidratadas con agua a 90°C necesitaban menos tiempo de cocción y obtuvieron una mayor suavidad, disminuyendo también la

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pérdida de aminoácidos, minerales, compuestos bioactivos y actividad antioxidante.

Palabras clave: Coeficiente de hidratación; Lentejas; Salud; Temperatura; Valor nutricional.

INTRODUCTION

Lentils are members of the Fabaceae family. They have significant nutritional value by being a source of proteins¹, essential amino acids, and considerable levels of carbohydrates, fiber, lipids, and minerals. Daily intake of lentils is associated with improved health and prevention of certain chronic diseases, including diabetes mellitus and cardiovascular diseases², especially because of its antioxidant activity³.

Legumes are widely used for human consumption in different regions of the planet, mainly for their nutritional importance⁴. The members of the Fabaceae family are, in general, widely appreciated and considered as staple foods in the nutrition of less economically favored societies, because of their energy and protein capacity⁵. It is known, however, that they also have antinutritional factors such as tannins, phytates, proteolytic enzyme inhibitors, lectin⁶ and trypsin inhibitors⁷.

One of the most produced fabaceous grains in the world is *Lens culinaris* L., known as lentils, cultivated and consumed beginning about 9,500 to 13,000 years ago⁸. Lentils are highly consumed by children⁹ and as a protein source for non-meat eating consumers¹, as it is a food source with high nutritional value. In addition to its nutritional value, lentils have an important phenolic composition, which has been associated with a reduction in the incidence of chronic degenerative diseases such as diabetes, cardiovascular diseases and cancers, being considered one of the best foods to have in the diet¹⁰.

However, although it is an important nutritional source, consumer preference for Fabaceae consumption is influenced by the physics and technological parameters like color of the raw grains and their texture following cooking¹¹.

In the global agricultural scenario, lentils are configured as one of the leguminous plants of greatest interest because they have significant representativeness both for human nutrition and for animal feed, also being an important economic agent in many countries. As in the American continent, lentils are an important source of economic maintenance for countries in Asia and Africa¹², with Argentina and Canada being important exporters to countries in Latin America, which relates to large financial amounts.

Although lentils are one of the most consumed grains in the eastern world, in the West, especially in South America, lentil consumption is still limited due also to lack of information on preparation and cooking procedures that guarantee maintenance of their nutritional qualities¹³. It is known that cooking heat may alter availability of the amino acids^{2,3,4,5,6,7,8,9,10,11,12,13,14} and some information is available on the anti-nutritional factors such as tannins and phytates⁴. However, little information is available on the chemical composition of lentils subjected to hydrothermal processing¹⁵, such as soaking in water at different temperatures before cooking.

Considering the economic and nutritional value of lentils, we aimed to study how different ways of processing and cooking lentils may affect nutritional properties. We evaluated the effects of presoaking on the hydration coefficient of lentils, time required for cooking, proximate composition, energy value, soluble proteins, colorimetric and texturometer characteristics, amino acid and mineral profiles, bioactive compounds, antioxidant activity, and anti-nutritional factors. This manuscript is an important source of information for lentil growers and consumers, especially those who wish to reduce their intake of meat and increase their intake of grain-derived proteins.

MATERIALS AND METHODS

Lentils were stored at 15 ± 1 °C in a biological oxygen-demand (BOD) incubator until analyzed. As all analyses were performed after 15 days, samples were stored in the BOD at a controlled temperature. This was important because raw lentils exposed to temperature variations could undergo great stress, bringing distorted results, especially for secondary metabolism compounds, such as phenolic derivatives and antioxidant activity. For cooked grains, storage in the BOD was necessary to avoid contamination and/or proliferation of microorganisms, and also to keep them in the same conditions, preventing one more factor from interfering with the results. Tests were performed at the Post-harvest, Industrialization, and Grains Quality Laboratory of the Federal University of Pelotas in Capão do Leão, Brazil, and at the Chemical Laboratory of the Sul-Rio-Grandense Federal Institute, Pelotas, Brazil.

Physicochemical, nutritional, and technological parameters were measured for raw lentils, cooked lentils without hydration, and after four different hydration treatments described below. Lentils were hydrated in distilled water (1:4, v/w) at an initial temperature of 25 ± 2 °C for 4 h and divided into two portions. The hydration water of the first portion was removed and replaced with an equal volume of distilled water for cooking purposes (LSA25). For the second portion, the hydration water was retained and used for cooking purposes (LCA25). A second group was soaked in distilled water (1:4, v/w) at an initial temperature of 90 ± 2 °C for 3 h and divided into two portions. The first portion was cooked after the soaking water was replaced with an equal volume of distilled water (LSA90). The second portion was cooked in the same hydration water (LCA90).

Lentils were pressure cooked at 203kPa and 116 °C. Cook time was determined by the tactile method proposed by Vindiola et al¹⁶, where cooking is considered complete when an amount $\geq 90\%$ of the grains is defined as softened after tactile pressure with the thumb and forefinger. Pre-hydrated lentils cooked in 46.67% less time than non-hydrated lentils. It was decided to keep the times different based on the fact that if the hydrated lentil remained cooking for the same amount of time they would disintegrate. This fact would also bring unreliable texture results and would produce destruction of countless lentil compounds, creating results that are not representative of reality. The cooking time of unhydrated lentils was not reduced, due to the fact that the grain was not completely cooked. Although we did not conduct a sensory evaluation, we considered fully cooked as an acceptable standard, thinking that the consumer does not cook until the grain disintegrates, but, on the other hand, does not consume it rigidly. All analyses except texturometer measurements were conducted on both raw and cooked lentils. Texturometer measurement was performed on cooked lentils only because lentils are not consumed raw and texture is an important technological

parameter for indicating palatability, that is, cooking point for consumption. Another factor that makes clear that lentil shouldn't be consumed without cooking is the presence of antinutritional factors such as phytates and tannins, which, when ingested, reduce the absorption of minerals and proteins, with these factors reduced with increasing temperature.

Hydration curves

Hydration curves were plotted to establish the times at which the lentil was fully hydrated using water at initial water temperatures of 25 ± 2 °C or 90 ± 2 °C.

Hydrothermal behavior of the raw lentils was determined using a method developed at the Post-harvest, Industrialization and Grains Quality Laboratory of the Federal University of Pelotas, as described below. For the soaking treatments, the samples were taken from the water and placed in a capsule in the centrifuge, which contained 3 cm of cotton in the bottom every 15 minutes. Samples were centrifuged for 1 min at 1000 rpm, after which samples were weighed. This procedure was repeated until all the samples obtained constant weight. The moisture percentage (%m/m) of each sample was measured based on the time to total hydration in a climate-controlled environment at 25 °C with 65% relative humidity. Hydration curves were plotted by using polynomial regression. Hydration coefficients (HC) were calculated for the duration of time required for maximum hydration¹⁷ using the formula 1 below, where PGH represents lentil weight before hydration and PAH represents lentil weight after hydration.

$$HC = \frac{PGH}{PAH} \times 100 \quad (1)$$

The determination coefficient (R^2) was calculated to determine the best fitting regression line¹⁸.

The objective was not to perform mathematical modeling of the water absorption kinetics on the part of the lentil, but rather to show which hydration type would be most efficient in reducing cooking time and ensure the maintenance of the technological, physical, nutritional and bioactive quality of the lentil.

Primary metabolism compounds, energy content, and water-soluble proteins

The primary metabolism compounds (moisture, lipids, proteins, fibers, carbohydrates) were quantified according to AOAC¹⁹ methods. Water-soluble proteins were determined as described previously²⁰.

Colorimetry and texturometry

The colorimetric parameters L^* , a^* , and b^* were determined as described by Lawless²¹ using a Minolta colorimeter (CR-300; Konica Minolta, Chiyoda, Tokyo, Japan). It was used, as determined by the CIE system (International

Lighting Commission), standard observer 10° and illuminant C. The Hue angle (°H) and the chroma (C) were calculated according to Equations 2 and 3, respectively²².

$$^{\circ}H = \arctan \frac{b^*}{a^*} \quad (2)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

Texturometric analysis was performed on 20 replicates of individual lentils using a texture analyzer (Model TA.XTplus; Stable Micro Systems, Godalming, UK)²³. A compression of 80% was used with a cylindrical probe 40 mm in diameter and test speed of 1 mm.s⁻¹, in two cycles, using a 5 kg load cell.

Determination of minerals and amino acids

Minerals were quantified according to Souza et al²⁴, in which 0.2 g of sample were weighed and 2 ml of nitro-perchloric acid mixture (2: 1) (65% nitric acid and 70% perchloric acid) was added, slowly warming until 210 °C, and after, the sample was digested for 3 hours. The sample was cooled to 25 °C, transferred to 25 ml volumetric flask and made up to volume with deionized water. It was stored in amber flasks and taken to the flame atomic absorption spectrophotometry on a GBC AAS 932 Plus Atomic Absorption Spectrophotometer. Three readings of water only (control) were taken after cooking in order to reach conclusions about possible metal contamination. The reading of the control sample (water) was necessary in order to detect any species of metal contamination in the sample, since if there were, it could result in spurious findings. No water readings (control) showed metal contamination, therefore, the mineral evaluations exposed in this work were exclusively the result of the lentil.

Amino acids were identified according to Ravindran et al²⁵ where 40 mg of proteins were hydrolyzed with 10ml of hydrochloric acid at 110 ± 4 °C for 24h in vacuum sealed glass ampoules, and were quantified in an ion exchange High Performance/Pressure Liquid Chromatography (HPLC) system.

Determination of antioxidant capacity, phenolic compounds, and antinutritional factors (phytates and tannins)

Antioxidant capacity was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH method) described by Brand-Williams et al²⁶ and the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; ABTS) method by Re and Philip²⁷. Total and individual phenolic compounds were determined as described by Nasar-Abbas et al²⁸. Total anthocyanins were measured according to Abdel-Aal and Huc²⁹.

Tannins were quantified according to the method of Nasar-Abbas et al²⁸ in which 100 mg of Polyvinyl Pyrrolidone (PVPP) was weighed and placed in falcon 15 mL conical centrifuge tubes, adding 1 mL of deionized water and 1 mL of sample extract. Samples were stirred for 15 seconds and held at 5 °C for 15 min. Then they were centrifuged for 15 min at 7500 x g. The supernatant was collected and the non-tannic phenolic compounds were determined. Tannins were determined by

subtracting the total phenols from the non-tannic compounds.

Phytic acid (phytate) content was determined by the methodology proposed by Haug & Lantzsch³⁰. To carry out the methodology, homogenization of 0.015 g samples with 2 mL of 0.2 M hydrochloric acid for 30 minutes was conducted. Subsequently, centrifugation was performed at 17200 rpm for 15 minutes at 24 °C. 0.5mL of the supernatant was collected and transferred to Eppendorf by adding 1 mL of FeCl₃. The Eppendorf was kept in a water bath at 90 °C for 30 minutes. After this period at high temperature, centrifugation was performed for 15 minutes at 3.000 g at 24 °C. Subsequently, a 0.5 mL aliquot of the supernatant was transferred to another Eppendorf, to which 0.75 mL of bipyridine was added. The samples were read in a spectrophotometer with a 515 nm wavelength.

Statistical analyses

A completely randomized design in a bifactorial scheme was used in the present study. Atypical values (outliers) were identified and removed from the database³¹. The data were analyzed for normality using the Shapiro–Wilk test. Homoscedasticity was identified by the Hartley’s test. Independence of the residuals was determined by graphical analysis and subjected to ANOVA and the F-test ($P\leq0.05$). Treatment effects were compared by the Tukey’s test ($P\leq0.05$). The effect of hydration was compared by the Dunnett’s test ($P\leq0.05$).

RESULTS

Effect of water temperature on the hydration curve

The hydration curve for raw lentils is shown in figure 1. For maximum hydration, lentils required 240 min or 180

min soaking in water at 25 ± 2 °C or 90 ± 5 °C, respectively. Therefore, hydration time decreased with increasing the initial water temperature.

Lentils without pre-hydration required 15 min to be cooked. In contrast, hydrated lentils needed only 8 min to be fully cooked. Soaking/hydration optimized lentil preparation for cooking.

Effects of hydration on primary metabolism compounds, energy, and water-soluble protein content

Values in % dry basis are summarized in table 1. Temperature elevation and discarding the hydration water reduced total protein content. Raw lentils had the lowest soluble protein content. For this reason, they required prolonged hydration to ensure the highest availability of soluble protein. LCSH had relatively low soluble protein content, and differences between samples were statistically significant. That result was likely due to the fact that lentils had not been previously hydrated, reducing the mobilization of water-soluble proteins out of the lentil. As proteins are closely linked to starch, when lentils begin cooking, the starch gelatinized, making it difficult to mobilize soluble proteins to the cooking water.

It was observed that LCA90 lentils obtained lower caloric values, indicating that exposure to high hydration temperature promotes maintenance of starch integrity inside of the lentil.

The highest ash content was observed in LCA90 (3.4%), but the lowest was observed in LSA90 (2.3%), likely promoted by excluding the soaking water for cooking.

Figure 1: Moisture percentage of raw lentils during hydration.

Effects of hydration on the colorimetric and texturometric parameters

Lentils visually presented with a green color ($a^* < 0$); nevertheless, only raw lentils experimentally showed a negative a^* value (Table 2). Samples showed a yellow tint, with Hue angle falling between 79.68°H and 99.7°H. Untreated raw lentil had relatively higher brightness ($L^* = 86.55$) than the treated lentils. Cooking significantly darkened lentil tonality as indicated by the chroma values (range: 14.35–23.60).

Table 2 summarizes the texturometric parameters for cooked lentils. The lowest hardness values were obtained for cooked lentils after soaking (LCA25 and LCA90). Hydration ensured proper water permeation within lentils and tenderness after cooking.

Cohesiveness differed among samples, ranging from 0.17 to 0.30. The lowest values were obtained for the hydrated lentils.

Chewiness was comparatively higher in non-hydrated lentils (1.26 Nmm⁻¹) than soaked lentils. Therefore, less energy was needed to chew hydrated lentils. In lentils, this parameter was strongly correlated with hardness ($r = 0.98$).

Table 1. Effects of hydration on the primary metabolism compounds, energy value, and water-soluble protein content of raw and cooked lentils.

Treatments	Water-soluble								
	Moisture	Proteins	proteins	Lipids	Carbohydrates	Energy		Total fiber	Ash
	(%)	(%)	(%)	(%)	(%)	(kcal)	(kJ)	(%)	(%)
Raw lentils	11.4±0.013c ^{1/}	25.9±0.022ab	74.8±0.761d	1.8±0.052a	65,6±0.016bc	382,2±0.011a	1599±0.011a	3.83±0.017c	2.87±0.014b
LCSH	77.5±0.011a	24.6±0.021ab	83.6±0.083c	1.3±0.063b	66.4±0.112b	375,7±0.122cd	1571±.213c	4.80±0.110ab	2.90±0.015b
LCA25	75.9±0.021±a ^{ns}	26.6±0.043a*	91.7±0.032a*	1.6±0.055bc ^{ns}	64.0±0.141c*	376,8±0.031c ^{ns}	1576±0.031bc ^{ns}	4.86±0.022ab ^{ns}	2.94±0.037b ^{ns}
LCA90	76.8±0.1141a ^{ns}	24.0±0.720bc ^{ns}	90.6±0.034ab*	1.1±0.087bc ^{ns}	66.4±0.021b ^{ns}	371,5±0.140e*	1554±0.002d*	5.10±±0.221a ^{ns}	3.40±0.024a*
LSA25	71.2±0.080b*	23.6±0.014c ^{ns}	90.9±0.040ab*	0.9±0.072d*	68,6±0.133a*	364,9±0.113f*	1526±0.113e*	4.60±0.033b ^{ns}	2.30±0.018c*
LSA90	72,7±0.1310b*	23.1±0.263c ^{ns}	89.0±0.942b*	1.0±0.013cd*	69,2±0.120a*	378,2±0.100b*	1582±0.041b*	4.67±0.294b ^{ns}	2.03±0.016d*

^{1/} Mean of three replicates ± standard deviation. Same letter in a column indicates differences are not significant according to Tukey's test ($P > 0.05$). * or ^{ns}, significant or not significant compared with control LCSH (lentils cooked without hydration) according to Dunnett's test ($P \leq 0.05$). LSA25 and LCA25 refer to cooked lentils without or with hydration water at an initial temperature of 25°C, respectively; LSA90 and LCA90 refer to cooked lentils without or with hydration water at an initial temperature of 90°C, respectively.

Table 2. Effects of hydration on technological parameters.

Evaluations	Treatments	Raw Lentil	LCSH	LCA25	LCA90	LSA25	LSA90
Texturometric parameters	Hardness (N)	Not evaluated ⁺	6.74±0.894a	3.52±1.00b*	2.15±0.881b*	1.89±0.056b*	1.08±0.133b*
	Adhesiveness (gs ⁻¹)	Not evaluated	-2.132±0.384NS	-2.167±0.075NS	-2.179±0.641NS	-2.312±0.090NS	-2.404±0.251NS
	Elasticity (mm)	Not evaluated	0.392±0.033NS	0.336±1.00NS	0.372±0.085NS	0.434±0.082NS	0.467±0.128NS
	Cohesiveness	Not evaluated	0.309±0.039a	0.287±0.080a ^{ns}	0.213±0.010b*	0.237±1.00b*	0.170±0.025c*
	Gumminess (N)	Not evaluated	0.787±0.117a	0.572±1.12b*	0.450±0.164bc*	0.361±0.099cd*	0.240±0.027d*
	Chewiness (Nmm ⁻¹)	Not evaluated	1.26±0.072a	0.424±0.077b*	0.167±0.055b*	0.145±0.081b*	0.106±0.023b*
	Resilience	Not evaluated	0.052±0.017ab	0.044±0.089abc ^{ns}	0.063±0.018a ^{ns}	0.024±1.00c*	0.030±0.017bc ^{ns}
Colorimetric parameters	L*	86.5±0.506a ^{1/}	59.6±0.974d	54.2±1.00e*	61.1±0.731c*	67.2±0.057b*	60.9±0.813c*
	a*	-3.994±0.135d	2.99±0.177a	2.78±1.00ab*	2.24±0.158c*	2.57±1.02b*	2.26±0.106c*
	b*	23.2±0.754a	17.5±0.386c	15.3±0.041d*	16.8±0.527c*	14.1±0.086e*	20.4±0.162b*
	°H	99.7±0.200a	80.3±0.047d	79.7±0.016de*	82.4±0.208c*	79.7±0.041e*	83.7±0.305b*
	Chroma	23.6±0.076a	17.8±0.305c	15.6±0.042e*	16.9±0.053d*	14.4±1.00f*	20.6±0.108b*

^{1/}Mean of six replicates ± standard deviation. Same letter in a column indicates differences are not significant according to Tukey's test ($P > 0.05$). * or ^{ns}, significant or not significant compared with control LCSH (lentils cooked without hydration) according to Dunnett's test ($P \leq 0.05$). LSA25 and LCA25 refer to cooked lentils without or with hydration water at an initial temperature of 25 °C, respectively; LSA90 and LCA90 refer to cooked lentils without or with hydration water at an initial temperature of 90 °C, respectively. + Raw lentils were not evaluated because the grain is only consumed after cooking.

Table 3. Effects of hydration on mineral composition of raw or cooked lentils.

Treatments	Fe (mg.100g ⁻¹)	Zn (mg.100g ⁻¹)	Cu (mg.100g ⁻¹)	P (mg.100g ⁻¹)	Mg (mg.100g ⁻¹)	Mn (mg.100g ⁻¹)	K (mg.100g ⁻¹)	Ca (mg.100g ⁻¹)
Raw lentils	8.47±0.023a ^{1/}	1.04±0.154ab	0.743±0.194NS	226±0.027d	92.2±0.022bcd	1.36±0.023b	432±0.027d	121±0.100ab
LCSH	8.38±0.029bc	1.05±0.439ab	0.610±0.041NS	253±0.029c	94.8±0.023bc	1.04±0.020e	493±0.023b	129±0.013a
LCA25	8.46±0.064ab*	1.12±0.078a ^{ns}	0.648±1.00NS	279±1.00b*	114±0.095a*	1.29±0.090c*	516±1.00a*	127±0.098ab ^{ns}
LCA90	8.39±0.037bc ^{ns}	0.984±0.270bc ^{ns}	0.719±0.136NS	299±0.016a*	103±0.020ab ^{ns}	1.48±0.021a*	512±0.028a*	129±0.019a ^{ns}
LSA25	8.35±1.00c ^{ns}	0.903±0.074cd*	0.626±0.098NS	222±0.077d*	86.3±0.098cd ^{ns}	1.41±0.083b*	442±1.02c*	119±1.02ab ^{ns}
LSA90	8.16±0.027d*	0.826±0.066d*	0.587±0.113NS	193±0.029e*	80.9±0.107d*	1.18±0.020d*	266±0.021e*	115±0.204b*

^{1/}Mean of three replicates ± standard deviation. Same letter in a column indicates differences are not significant according to Tukey's test ($P > 0.05$). * or ns, significant or not significant compared with control LCSH (lentils cooked without hydration) according to Dunnett's test ($P \leq 0.05$). LSA25 and LCA25 refer to cooked lentils without or with hydration water at an initial temperature of 25°C, respectively; LSA90 and LCA90 refer to cooked lentils without or with hydration water at an initial temperature of 90°C, respectively.

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Effects of hydration on mineral and amino acid composition

Table 3 shows that lentil processing significantly changed the availability of all inorganic compounds. Hydration water at the 90±2 °C increased mineral leaching into the soaking water. LCA90 contained relatively higher Cu, P, Mg, Mn, and Ca levels. Discarding the hydration water reduced the mineral content as observed in LSA90, which presented with comparatively lower Fe, Zn, Cu, P, Mg, K, and Ca levels.

Table 4 shows that glutamic and aspartic acids were the most abundant of amino acids in the lentils. Hydration increased the availability of aspartic acid, glutamic acid, glycine, arginine, threonine, methionine, leucine, and isoleucine, especially in samples cooked in the hydration water preheated to 90±2 °C (LCA90).

Positive correlations were observed between amino acids (Table 4) and bioactive compounds (Table 5); between individual phenolic compounds and glycine ($r = 0.92$), proline ($r = 0.89$), or lysine ($r = 0.94$); between total phenolic compounds or DPPH and proline ($r = 0.93$; $r = 0.95$, respectively); and between anthocyanins, and between total or individual phenolic compounds and lysine ($r = 0.85$; $r = 0.92$; $r = 0.97$, respectively). Thus, potential interactions existed between bioactive compounds and amino acids in lentils.

Effects of hydration on antioxidant activity, phenolic compounds, and antinutritional factors

As shown in table 5, cooking and eliminating the hydration water significantly reduced both bioactive compounds and antioxidant activity. However, individual phenolic compounds in LCSH were conserved possibly because they are heat-resistant and were not eliminated in the hydration process. Because anthocyanins are water-soluble and cannot resist high temperatures, they leached into the hydration water and were discarded with it. Consequently, LSA90 presented low anthocyanin content (1.55 mg.100 g⁻¹). ABTS analysis of antioxidant activity generated significantly different values among treatments. LCSH had the highest ABTS content (1988.5 µM Troloxg⁻¹).

Table 5 indicates that phytate and tannins leached into, and may have been discarded with, hydration water. An important but negative effect of phytates is their binding to nutritionally important metal cations such as iron, magnesium, and calcium, effectively sequestering and reducing bioavailability. The lowest values were measured for LSA25 and LSA90 and these values significantly differed from those obtained following other treatments.

A negative correlation was found between tannin content and soluble protein ($r = -0.92$) and between tannin content and carbohydrate levels ($r = -0.95$).

Table 4. Effects of hydration on amino acid composition of raw or cooked lentils.

Amino acids	Raw lentils	LCSH	LCA25	LCA90	LSA25	LSA90
Aspartic acid	3.74±0.025a ^{1/}	2.58±0.075b	3.36±0.033a*	3.98±0.028a*	3.28±0.041ab ^{ns}	2.90±0.011ab ^{ns}
Glutamic acid	4.02±0.048bc	3.67±0.024c	4.34±0.071b*	4.93±0.020a*	4.24±0.040b*	4.00±0.023bc*
Serine	1.00±0.013ab	1.24±0.016ab	1.24±0.089a ^{ns}	1.00±0.021ab ^{ns}	0.805±0.030b*	1.36±0.202a ^{ns}
Glycine	0.551±0.016c	0.931±0.019b	1.41±0.040a*	0.465±0.109c*	0.701±0.063bc ^{ns}	1.01±0.021b ^{ns}
Histidine	0.249±0.014NS	0.421±0.201NS	0.412±0.090NS	0.207±0.107NS	0.094±1.00NS	0.400±0.016NS
Arginine	1.94±0.023ab	2.04±0.301ab	2.38±0.051a ^{ns}	1.76±0.209b ^{ns}	1.70±0.081b ^{ns}	2.19±0.100ab ^{ns}
Threonine	1.51±0.109a	0.814±0.200b	1.64±0.044a*	1.40±0.104a*	0.796±0.140b ^{ns}	0.911±0.210b ^{ns}
Alanine	1.14±0.122NS	1.00±0.109NS	1.12±1.00NS	0.908±0.206NS	1.24±0.066NS	1.13±0.101NS
Proline	0.512±0.125c	1.02±0.407abc	1.10±0.028ab ^{ns}	0.679±0.117bc ^{ns}	1.31±0.065a ^{ns}	1.29±0.107a ^{ns}
Tyrosine	0.701±0.135NS	0.702±0.154NS	0.814±0.075NS	0.844±0.102NS	0.715±0.045NS	0.871±0.109NS
Valine	1.43±0.112NS	1.12±0.105NS	1.26±0.085NS	1.49±0.108NS	1.48±0.053NS	1.35±0.208NS
Methionine	0.211±0.108ab	0.110±0.025b	0.300±1.02ab*	0.438±0.134a*	0.214±1.02ab ^{ns}	0.162±0.023b ^{ns}
Cysteine	0.202±0.122NS	0.202±0.155NS	0.231±1.04NS	0.38±0.117NS	0.333±1.00NS	0.251±0.102NS
Isoleucine	1.04±0.171b	1.03±0.156b	1.20±0.265b ^{ns}	2.17±0.109a*	1.30±0.087b ^{ns}	1.13±0.400b ^{ns}
Leucine	1.00±0.190b	1.62±0.101	1.87±0.164a ^{ns}	2.08±0.200a*	2.04±1.00a*	1.91±0.141a ^{ns}
Phenylalanine	1.4±0.209ab	1.2±0.100b	1.49±0.500ab ^{ns}	1.30±0.201ab ^{ns}	1.62±1.06a*	1.37±0.100ab ^{ns}
Lysine	0.5±0.107b	0.9±0.104ab	0.941±1.00ab ^{ns}	0.401±0.101b*	1.20±1.02a ^{ns}	1.49±0.204a*

^{1/}Mean of three replicates ± standard deviation. Same letter in a column indicates differences are not significant according to Tukey's test ($P > 0.05$). * or ^{ns}, significant or not significant compared with control LCSH (lentils cooked without hydration) according to Dunnett's test ($P \leq 0.05$). LSA25 and LCA25 refer to cooked lentils without or with hydration water at an initial temperature of 25 °C, respectively; LSA90 and LCA90 refer to cooked lentils without or with hydration water at an initial temperature of 90 °C, respectively.

Table 5. Effects of hydration on antioxidant activity, phenolic compounds, phytates and tannins.

Treatments	Anthocyanins (mg.100g ⁻¹)	DPPH (μM.Troloxg ⁻¹)	ABTS (μM.Troloxg ⁻¹)	Total phenols (mg tannic acid.g ⁻¹)	Simple phenols (mg tannic acid.g ⁻¹)	Phytate (mg phytic acid.g ⁻¹)	Tannins (mg tannic acid.g ⁻¹)
Raw lentils	1.80±0.055a ^{1/}	2.36±0.041a	2023±0.708a	30.5±0.236a	4.06±0.022a	38.9±0.115a ^{1/}	26.9±0.038a
LCSH	1.76±0.030a	2.19±0.066b	1988±1.24b	25.9±2.24b	4.02±0.019a	38.8±0.082ab	21.1±0.017b
LCA25	1.75±0.046a ^{ns}	2.23±0.080b ^{ns}	1964±0.044c*	19.8±1.00c*	3.71±0.040b*	38.5±0.070b*	16.1±1.00c*
LCA90	1.70±0.028ab ^{ns}	2.26±0.083b*	1906±0.110d*	20.7±1.99c*	3.99±0.101a ^{ns}	38.6±0.066b ^{ns}	12.0 ±0.012cd*
LSA25	1.63±0.017bc*	2.19±0.071b ^{ns}	1297±1.00f*	8.83±0.484d*	3.39±1.02c*	37.1±0.083c*	5.44±0.070e*
LSA90	1.55±0.058c*	2.24±0.049b ^{ns}	1319±0.029e*	9.68±0.470d*	3.41±0.036c*	36.7±0.069d*	3.95 ±0.032e*

^{1/}Mean of three replicates ± standard deviation. Same letter in a column indicates differences are not significant according to Tukey's test ($P > 0.05$). * or ^{ns}, significant or not significant compared with control LCSH (lentils cooked without hydration) according to Dunnett's test ($P \leq 0.05$). LSA25 and LCA25 refer to cooked lentils without or with hydration water at an initial temperature of 25 °C, respectively; LSA90 and LCA90 refer to cooked lentils without or with hydration water at an initial temperature of 90 °C, respectively.

DISCUSSION

We observed that a higher temperature of hydration water reduced the length to completely hydrate the lentils. After soaking for 180 min, the hydration coefficients of pre-hydrated lentils at 25 ± 2 °C or 90 ± 2 °C, respectively, were 189.34% or 202.60%, higher than previously reported values³². Thus, hydration improves with increasing the initial temperature of the soaking water. Accordingly, high temperatures reportedly shorten the time to hydrate beans³³.

The time required for cooking was also reduced as a result of presoaking, because the high temperature of hydration water also promotes lentil precooking, optimizing preparation time.

Huma et al³³ and Joshi et al⁸ reported that soaking grains reduced their cooking time. Thus, reducing the soaking time is preferable because prolonged hydration may facilitate growth of pathogenic microorganisms⁸.

Lentils naturally have water in their composition (represented by the moisture content), thus, the increase of the external environment temperature promoted water vapor expansion inside the lentil, increasing the intragranular porosity, promoting empty spaces inside the lentil. Subsequently, the formed spaces were filled with hydration water, which at 90 °C also promoted the elasticity of the integument fibers, although it did not prevent rupture.

A certain proportion of the proteins in Fabaceae are used for storage, these are soluble proteins, representing the hydrophilic fraction of the total proteins of the grain³⁴.

The reduced total protein content after eliminating hydration water and the increasing soluble protein content by maintaining hydration water provides us with one of the most important data points when it comes to lentils, which is the solubility and large capacity to migrate to soaking water. Since lentils are classified as a proteic grain, this data is fundamental for understanding lentil behavior when submitted to the presoaking process. In technological matters the migration of proteins to hydration water and its maintenance in cooking promotes broth gelation and thickening, which is a feature appreciated by most consumers, especially associated with the reduced cooking time. Raw lentils had the lowest soluble protein content, strengthening what was previously reported. Huma et al³³ reported that high temperature negatively affects the total protein content of lentils. According to Rockenbach et al¹¹, the levels of soluble proteins derived from beans were similarly found to increase with increasing the temperature of the hydration water, since the increase of the hydration water temperature increases the leaching of compounds to the outside of the lentil because it presents increased intragranular porosity, facilitating the exit of compounds.

Legumes normally have high caloric values⁴, like those observed at table 1, but lower values (1995kJ) than these observed by Padovani et al³⁵ for North American *Lens culinaris* Medik. However, lentils soaked in water at an initially higher temperature yielded relatively higher energy values than those soaked in water at lower temperatures.

The minerals in the lentils were leached and dispersed in the soaking water. Therefore, discarding this water significantly reduced ash content.

In lentils soaked in hydration water at 90 ± 2 °C and cooked with this water (LCA90) a high ash content was observed, similar to what was reported by Toledo and Canniatti-Brazaca³⁶ who cooked samples with hydration water. However, the lowest value was observed in LSA90 (2.03%), exposing that discarding hydration water promotes reduction of minerals, because these elements are leached by the water, and disposed of. This result supports the findings reported by Wang et al¹⁵. Total ash content in the raw lentil (2.87%) was higher than that reported by Padovani et al³⁵ (2.61 and 2.67%), but lower than the 4.62% and 5.72% levels reported by Garcia et al³⁷ in cowpea, a grain belonging to the same taxonomic family.

A negative a^* value for raw lentils (Table 2) was observed, indicating green color. These values are similar to those reported by Zhang et al³⁸ in green lentil cultivars. Thermic processing leads to the development of a reddish-brown color ($a^*>0$) in lentils; this color has been proposed to be associated with the tannin content³⁹, as well as the degradation of the tegument pigmenting agents promoted by temperature increases. Another factor to consider is the disruption of the integumentary fibers causing elasticity and reducing lentil brightness. The Hue angle was close to 90 °H, and according to Del Bemet al⁴⁰, values near 90 °H indicate yellowish coloration. The integument accounts for the green color of lentils, but their cotyledons are yellow. If the proportion of cotyledon exceeds that of the integument, then the lentils appear yellow because their husks are separated from the endosperm during cooking. In raw lentils, the integument is thin and the yellow cotyledons are more readily visible. It was possible to verify that the thermal processing reduced the brightness of lentil, as observed in table 2, but in raw lentils, values are closer than those reported by Zhang et al³⁸ for a Canadian lentil cultivar ($L^*=85.15$). Chroma values, which are low or approach zero, are indicative of gray lentil tones.

Regarding the values of the texturometric parameters, in table 2 it is possible to observe that in general, lentil texture was dependent on hydration, because softness is related with the soaking time and the temperature of hydration water. The hydration process promoted distribution of water in the lentil and tenderness after cooking. Similar results were reported by Joshi et al⁸. The lowest values for cohesiveness were observed in hydrated lentils, thus it is possible to observe that the hydration process can guarantee adequate palatability. Even lower values were reported by Rockenbach et al¹¹ (0.14–0.16). Chewiness, lower in hydrated lentils, refers to the force and number of chews required to enable a food to be swallowed, and this parameter is strongly related to hardness. Both chewiness and hardness depend on presoaking and cooking of lentil.

Discarding the hydration water reduced the mineral content of lentils (Table 3), corroborating previous

observations³⁶. Heat treatment reportedly diminishes the mineral content of lentils⁴. We found iron content in raw lentils to be 8.47 mg.100 g⁻¹ which was higher than that reported by Johnson et al¹ (54.6–68.3 mg.kg⁻¹). However, this value was similar to that reported by Wang et al¹⁵. LCA25 had 8.46 mg.100g⁻¹ Fe, which was significantly higher than that reported by Hefnawy²(6.1–7.0 mg.100 g⁻¹). Iron is essential for many biochemical reactions and physiological processes, including cell growth, energy supply, and oxygen transport.

Zinc content in raw lentils was 1.04 mg.100 g⁻¹. Different values have been reported previously; for example, Angelova et al⁴¹ reported 0.47 mg.kg⁻¹Zn. Hefnawy² and Lazarte et al⁴² reported 3–4 mg.100 g⁻¹Zn in cooked lentils.

Fabaceae have low levels of free phosphorus but substantial quantities of phytate. Relatively higher phosphorus content was found in LCA90 (299 mg.100 g⁻¹). The lowest phosphorus level was measured for LSA90 (193 mg.100 g⁻¹). Raw lentils contained 226 mg.100 g⁻¹phosphorus, which was below previously reported values^{2,3,4,5,6,7,8,9,10,11,12,13,14,15}.

LCA90 had a potassium content of 512 mg.100 g⁻¹which was equal to that reported by Hefnawy². Potassium levels significantly vary among species. Although potassium is not integrated into plant structures, it plays a key role in physiological and biochemical processes. Calcium content in the lentils ranged from 115 to 129 mg.100 g⁻¹which was similar to values reported by Lazarte et al⁴², but higher than those reported by Somavilla et al⁴³ (20.17 to 56.89 mg.100g⁻¹). Porres et al⁴⁴reported markedly lower calcium levels in Spanish lentils than those we found in this study.

Table 4 shows amino acids composition. It was observed that glutamic and aspartic acid were higher than all the other protein-derived nitrogen-containing compounds. According to Toledo and Canniatti-Brazaca³⁶, the available amino acid content was found to be relatively higher in pre-hydrated, cooked beans, possibly because lentils were exposed only to a high temperature for a short time. In this sense, Hefnawy² reported that heating reduces amino acid availability. Similar or higher amino acid content in LCA90 can be explained by degradation of both total and soluble proteins, resulting in the liberation of amino acids in the soaking water, which was used for cooking. The correlation between soluble protein and amino acids was significant ($r=0.98$).

We found that antioxidant activity and levels of bioactive compounds were reduced by cooking and discarding the hydration water, as also reported by Ranilla et al⁴⁵. Because antioxidant compounds in lentils' husk could be extracted with hot water³, hydration before cooking may liberate antioxidants, but the hydration water must be kept. Discarding the hydration water causes reduction of anthocyanins too, especially in lentils hydrated at high temperature. Importantly, anthocyanins are present in whole lentils and their fractions¹⁵ and are determinants of lentil quality as they are colored, ranging from red to purple.

Besides anthocyanins content, antioxidant activity determined by the ABTS method is reduced by the cooking

process. These results corroborate those of Silva et al⁴⁶ who reported that cooking beans reduced the antioxidant capacity compared to that of the grain in natura.

It was found that cooking and elimination of hydration water profoundly reduced levels of the antinutritional factors (phytates and tannins) (Table 5). Our results corroborate those of Vidal-Valverde et al³⁹, who reported that tannins and phytates were extracted by hydration water and that cooking effectively reduces levels of antinutritional compounds^{2,3,4,5,6,7,8,9,10,11,12,13,14,15}. Huma et al³³ reported significant reductions in phytate and tannin content in lentils after soaking and pressure-cooking. In a study with *Inga paterno* (Fabaceae), Sánchez-Mendoza et al⁴⁷ reported a tannin content in the range of 1.46–1.51 mg±catechin eq.g⁻¹ and a phytate content ranging from 2.04 to 2.66 mg.g⁻¹. These levels were lower than those determined for lentils.

Hydration lowers the content of anti-nutritional compounds by solubilizing them so that they may be discarded along with hydration water. Vidal-Valverde et al³⁹ reported that tannins can bind to proteins and carbohydrates reducing the nutritional quality of the legumes and their protein content³⁴.

CONCLUSION

We observed that lentil hydration and cooking time decreased with higher soakingwater temperature. Lentils cooked without soaking were stickier, more elastic, and gummier than those soaked and cooked, and required relatively greater chewing force. Soaking and disposal of the hydration water significantly reduced levels of anti-nutritional factors. The maintenance of nutritional parameters is extremely important for metabolic maintenance, ensuring that biochemical processes occur properly and are capable of establishing a positive relationship with the physiological state of individuals. Phytates bind to metal cations; tannins bind with proteins and carbohydrates in lentils. Both substances reduce the bioavailability of these nutrients.

Based on these results, we suggest that lentils be cooked in hydration water that has been pre-heated to an initial temperature of 90 °C. This will ensure favorable lentil texture and color. Moreover, levels of minerals, bioactive compounds, antioxidants, and amino acid smay be maintained in the lentils. It is worth mentioning that although laboratory conditions were used for this study, with distilled water and pressure control in cooking, consumers can find the same results at home. For that, the consumer must place soaking grains in water at room temperature (25 °C) or in boiled water (90 °C) and after, cook lentils in a domestic pressure cooker, according to the procedures used in this study.

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