

# Starch, Functional Properties, and Microstructural Characteristics in Chickpea and Lentil As Affected by Thermal Processing

Yolanda Aguilera, Rosa M. Esteban, Vanesa Benítez, Esperanza Mollá, and María A. Martín-Cabrejas\*

Departamento de Química Agrícola, Facultad de Ciencias, Universidad Autónoma de Madrid (UAM), 28049 Madrid, Spain

Changes in starch, functional, and microstructural characteristics that occurred in chickpea and lentil under soaking, cooking, and industrial dehydration processing were evaluated. Available starch in raw legumes represented 57–64%, and resistant starch (RS) is a significant component. As a result of cooking, available starch contents of soaked chickpea and lentil were significantly increased (21 and 12%, respectively) and RS decreased (65 and 49%, respectively) compared to raw flours. A similar trend was exhibited by dehydration, being more relevant in lentil (73% of RS decrease). The minimum nitrogen solubility of raw flours was at pH 3, and a high degree of protein insolubilization (80%) was observed in dehydrated flours. The raw legume flours exhibited low oil-holding capacities, 0.95–1.10 mL/g, and did not show any change by thermal processing, whereas water-holding capacities rose to 4.80–4.90 mL/g of sample. Emulsifying activity and foam capacity exhibited reductions as a result of cooking and industrial dehydration processing. The microstructural observations were consistent with the chemical results. Thus, the obtained cooked and dehydrated legume flours could be considered as functional ingredients for food formulation.

KEYWORDS: Chickpea; lentil; starch; functional properties; thermal processing.

# INTRODUCTION

Food legumes have been well recognized as valuable sources of dietary proteins and an important constituent of daily diet in many countries. They are also a good source of carbohydrates, calories, minerals, and vitamins; however, their protein digestibility is limited due to protein structure and the presence of antinutritional factors (1). Starch is the most abundant carbohydrate (22-45%) in the legume seeds (2) and also represents the major source of available carbohydrate in the human diet. The rate of starch digestion in legumes is lower, both in vitro and in vivo, than that in the cereals. In vivo, starch is hydrolyzed by salivary and pancreatic  $\alpha$ -amylase (3). However, a proportion of starch in starchy foods generally escapes to complete digestion. This fraction is called "resistant starch", which shows properties similar to those of fermentable fibers. Thus, the role of legumes as therapeutic agents in the diet of healthy vulnerable populations (diabetes, metabolic disorders ...) is actually of great interest (4).

Among legumes, chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* L.) are most commonly used in many countries due to their ideal cell wall polysaccharide composition and starch properties. Foods based on these legumes are prepared by a wide range of recipes and preparation methods. To improve their palatability and nutritional quality, heat processing is a well-established method for inactivating protease inhibitors, owing to

their effect on inhibitor (usually protein) conformation (2). However, it has also been reported to be quite influential on the protein functional properties, the extent, of course, depending on the level of protein denaturation. Limited denaturation could be beneficial, whereas extensive protein denaturation has been reported as being detrimental to functional properties, especially in relation to surface properties (5). In this sense, dehydration is a technology classified as a high-temperature process to produce a variety of foods and ingredients (6, 7) and offers numerous advantages including prolonged preservation time, high productivity, and high quality of resulting products (8). Therefore, an improved utilization of legumes can be obtained through the implementation of diverse processing strategies to facilitate the development of economically viable alternative products.

Extensive literature has been reported related to the nutritional improvement of lentil and chickpea by thermal processing. However, there is scarce information about a dehydration process after the traditional procedure (soaking and cooking). Presumably, lentil and chickpea undergo ultrastructural changes during this thermal processing that would affect their functional characteristics and can influence some of the physiological and metabolic properties of these legumes, such as their low glycemic response and hypocholesterolemic effect (4). Hence, the objective of this investigation was to consider the influence of soaking, cooking, and dehydration treatment on starch and functional properties in lentil and chickpea and also to evaluate the changes in microstructural characteristics that occur during

<sup>\*</sup>Author to whom correspondence should be addressed (telephone +34 91 4978678; fax +34 91 4973830; e-mail maria.martin@uam.es).

#### Article

the processing, with a view to providing useful information toward effective utilization of these legumes in various food applications.

#### MATERIALS AND METHODS

**Samples.** Seeds of chickpea (*C. arietinum* L.) variety Sinaloa and lentil (*L. culinaris* L.) variety Pardina were used in the present study. They were obtained from the agri-food industry Vegenat S.A. (Badajoz, Spain). From each legume there were batches of 250 g of raw and processed samples.

**Processing Conditions.** Legumes were subjected to an industrial dehydration process carried out in Vegenat SA. The processing consisted of the following steps: Raw material was soaked in tap water (1:10 w/v) for 16 h at 20 °C. After the soaking water had been drained, the soaked legumes were cooked by boiling for 70 min in the case of chickpeas and for 30 min in the case of lentils. The soaked–cooked seeds were dehydrated in a forced-air tunnel at  $75 \pm 3$  °C for 6 h. Samples were taken at each step, immediately frozen in liquid nitrogen, freeze-dried, sieved (0.5 mm), and stored at -20 °C until analyzed. Samples were named as follows: S (soaked legumes), S + C (soaked and cooked legumes), and S + C + D (soaked, cooked, and dehydrated legumes). The seeds were milled to flour and passed through a 250  $\mu$ m sieve.

**Starch Determination.** Starch content was determined from the residue obtained after soluble carbohydrate extraction according to the method of Li et al. (9) as modified by Vidal-Valverde et al. (10) using a procedure based on enzyme digestion of starch to glucose for 3 h for total starch and for 30 min for available starch. Resistant starch (RS) was calculated by the difference between total and available starch.

**Physicochemical and Functional Properties.** pH. The pH was measured on a slurry prepared with 10 g of legume flour in 40 mL of boiled, deionized water according to the official AOAC procedure (11).

**Protein Solubility.** The pH-dependent protein solubility was determined according to the method of Were et al. (12). Twenty-five milligrams of cotyledon flour was blended with 25 mL of distilled water, and the pH of the solution was set between 2 and 10 using 0.5 M NaOH and HCl. The solution was mixed using a magnetic stirrer (1 h at 20 °C) and centrifuged (12000g for 20 min at 4 °C). The supernatant was filtered through glass wool, and nitrogen was estimated by using Kjeldhal's method (11). The soluble protein (percent) profile was determined.

solubility (%) = 
$$\frac{\text{amount of N in the supernatant}}{\text{amount of N in the flour}} \times 100$$

*Bulk Density*. According to the Chau and Huang (13) method, this property was determined using a graduated cylinder (10 mL), previously weighed, and filled with sample to 10 mL by constant tapping, until there is no further change in volume and the content is weighted. The content was weighed, and from the difference in weight, the bulk density of sample was calculated as grams per milliliter.

*Water-Holding Capacity* (*WHC*). According to the method of Chau and Huang (I3), with slight modifications, 1 g of sample was stirred in 10 mL of distilled water for 24 h in a centrifuge tube at room temperature. After samples were centrifuged (2500g, 30 min), the supernatant was transferred to a graduated cylinder of 10 mL, where the volume was measured. The WHC was expressed as milliliters of water held per gram of sample.

*Oil-Holding Capacity (OHC)*. According to the method of Chau and Huang (13), with slight modifications, 1 g of sample was mixed with vegetable oil (1:10). The mixture was stirred for 30 min at room temperature. After samples were centrifuged (2500g, 30 min), the supernatant was transferred to a graduated cylinder of 10 mL, where the volume was measured. The OHC was expressed as milliliters of vegetable oil held per gram of sample.

Water Absorption Capacity (WAC). The WAC was determined essentially according to the method of Beuchat (14). One gram of flour sample was mixed with 10 mL of distilled water in a centrifuge tube for 1 min in a vortex and then centrifuged at 3000–5000g for 30–45 min depending on the availability of facility for this purpose. After separation of the content, the volume of supernatant was recorded and used for determination of water absorption; the results are expressed as grams per milliliter of sample. *Swelling Capacity*. According to the method of Robertson et al. (*15*), 100 mg of flour sample was hydrated in a known volume of distilled water (10 mL) in a calibrated cylinder at room temperature. After equilibration (18 h), the bed volume was recorded and swelling capacity expressed as volume occupied by sample per gram of original sample dry weight.

swelling capacity (%) = 
$$\frac{V_2 - V_1}{N} \times 100$$

 $V_1$  = volume of flour sample before soaking,  $V_2$  = volume of soaking flour sample, N = grams of flour sample.

*Emulsifying Activity* (*EA*). This property was evaluated following the method of Yatsumatsu et al. (*16*). One gram of flour sample was mixed with 20 mL of distilled water during 30 min. The mixture was made up to 25 mL, followed by the addition of 25 mL of corn oil, and homogenized for 3 min. The resulting emulsion was centrifuged at 2000g for 5 min, and the emulsion volume was measured. EA was expressed as percentage of the emulsified layer volume of the entire layer in the centrifuge tube.

Foaming Capacity (FC). This foaming property was determined according to the method of Bencini (17). One g of flour was dispersed in 50 mL of distilled water and whipped using a homogenizer (Polytron model Brinkman Instruments) at 5000 g for 5 min. The volumes were recorded into a 50 mL graduated cylinder. The volumes were recorded before and after whipping and the percentage volume increase was calculated.

Gelation Capacity. This property was evaluated using the method of Chau and Cheung (18). Suspensions were prepared in distilled water with concentrations of 4, 8, 12, and 14% (w/v). Aliquots of these suspensions (5 mL) were transferred to tubes and put in a water bath for 60 min at 100 °C and then put in an ice bath for 60 min. The least gelation concentration (LGC) was detected when the sample from the inverted test tube did not fall or slip.

Scanning Electron Microscopy (SEM). SEM was performed on raw and processed chickpea and lentil. Lyophilized flour particles were placed on double-stick adhesive tape mounted on aluminum stubs. The samples were subjected to a nitrogen stream to remove unattached particles and then covered with gold in a vacuum evaporator SC 502 sputter coater. Scanning electron micrographs were taken using a Philips XL30 microscope that operated at an accelerating voltage of 20 kV and was coupled to an EDAX DX4i analyzer.

Statistical Analysis. Results were analyzed using Duncan's multiple-range test (DMRT) (19). Differences were considered to be significant at  $p \le 0.05$ .

#### **RESULTS AND DISCUSSION**

Thermal processing can change the physicochemical characteristics of legumes, due to the variations of their components. The starch profiles of raw and processed legumes are summarized in **Table 1**. Total starch content was 53.4 g 100 g<sup>-1</sup> of dry matter (DM) for raw chickpea and 46.3 g 100 g<sup>-1</sup> of DM for raw lentil, similar to those found in the literature (20-23). Bioavailability of native starches from grain legumes is known to be relatively poor compared to most cereal starches because they are relatively rich in amylose and very resistant due to its higher crystallinity. The available starch of the studied legumes represented 57–64% of the total starch; therefore, the RS is a significant component of this carbohydrate in raw samples (19.3 and 19.9 g 100 g<sup>-1</sup> of DM in chickpea and lentil, respectively).

After the soaking process, the level of total starch decreased around 8-11%, due to the loss of amylopectin solubilized by  $\alpha$ -amylase action from the legume seed and also to differences in seed size, membrane permeability, and starch structure (21,22,24). Soaking affects the available starch content to different extents in the studied legumes, decreasing in the case of lentil (10%). However, the concentration of RS is not much altered in the case of lentil, but in the case of chickpea a significant decrease of RS (28%) was observed. Different results are also found in the literature (21), showing that changes in starch content during

Table 1. Starch Profile of Raw and Processed Legumes (Grams per 100 g of Dry Matter)<sup>a</sup>

legume	raw	soaked	soaked + cooked	soaked $+$ cooked $+$ dehydrated
total starch				
chickpea	$53.4\pm2.4\mathrm{b}$	$47.7 \pm 2.7  \mathrm{a}$	$47.6\pm2.2\mathrm{a}$	$45.4 \pm 3.4$ a
lentil	$46.3\pm2.6\mathrm{b}$	$42.6\pm2.9\mathrm{b}$	$36.7\pm2.7\mathrm{a}$	$34.5 \pm 2.5  a$
available starch				
chickpea	$34.0\pm2.5\mathrm{a}$	$33.8\pm2.1\mathrm{a}$	$40.7\pm2.5\mathrm{b}$	$38.9\pm2.2$ b
lentil	$26.4\pm1.6\mathrm{b}$	$23.6\pm1.2a$	$26.5\pm1.2\mathrm{b}$	$29.1\pm1.1$ c
resistant starch				
chickpea	$19.3\pm1.2\mathrm{c}$	$13.9\pm0.7\mathrm{b}$	$6.8\pm0.5a$	$6.5\pm0.6\mathrm{a}$
lentil	$19.9\pm1.7\mathrm{c}$	$19.0\pm0.8\mathrm{c}$	$10.2\pm0.7b$	$5.4\pm0.5\mathrm{a}$

<sup>a</sup> Mean values of each row followed by different letters significantly differ when subjected to DMRT (p < 0.05).

Table 2. Physicochemical and Functional Properties of Raw and Processed Legume Flours	Table 2.	Physicochemical and	I Functional Pro	perties of Raw a	and Processed	Legume Flours <sup>a</sup>
---	----------	---------------------	------------------	------------------	---------------	----------------------------

legume	pН	bulk density (g/mL)	OHC (mL/g)	WHC (mL/g)	WAC (mL/g)	swelling capacity (%)	EA (%)	FC (%)	LGC (%)
chickpea									
raw	6.57	$0.71 \pm 0.05  a$	$1.10 \pm 0.10~{ m a}$	$2.10\pm0.10\mathrm{a}$	$2.20 \pm 0.10$ a	$1.70 \pm 0.10  a$	$22.9\pm0.10~\mathrm{c}$	$24.0\pm0.20\text{b}$	$8.0\pm0.10\mathrm{a}$
S	7.15	$0.74 \pm 0.05  a$	$1.00\pm0.10\mathrm{a}$	$2.20\pm0.10\mathrm{a}$	$2.20\pm0.10\mathrm{a}$	$1.80\pm0.10\mathrm{a}$	$11.1\pm0.10\mathrm{b}$	$24.0 \pm 0.20  a$	$8.0 \pm 0.10 \ a$
S + C	7.39	$0.79 \pm 0.06  a$	$1.15 \pm 0.10  a$	$4.80\pm0.20\text{b}$	$3.20\pm0.10\text{b}$	$3.10\pm0.15\mathrm{b}$	$7.2 \pm 0.10  a$	$8.0\pm0.10\mathrm{a}$	$12.0\pm0.10\text{b}$
S + C + D	7.05	$0.81\pm0.04a$	$1.15 \pm 0.10  a$	$4.90\pm0.10\text{b}$	$3.80\pm0.20\mathrm{c}$	$3.50\pm0.10\text{b}$	$6.9\pm0.10\mathrm{a}$	$8.0\pm0.10\mathrm{a}$	$12.0\pm0.10\text{b}$
lentil									
raw	6.51	$0.91\pm0.05a$	$0.95\pm0.10a$	$3.20\pm0.10a$	$1.80\pm0.10\mathrm{a}$	$2.30\pm0.10a$	$47.4\pm0.30\mathrm{c}$	$40.0\pm0.20\mathrm{c}$	$8.0\pm0.10\mathrm{a}$
S	7.38	$0.91\pm0.05\mathrm{a}$	$0.90\pm0.10a$	$3.50\pm0.20\mathrm{a}$	$2.30\pm0.10\text{b}$	$3.30\pm0.10\text{b}$	$5.3\pm0.10\text{b}$	$32.0\pm0.20\text{b}$	$8.0\pm0.10\mathrm{a}$
S + C	7.19	$0.92\pm0.05a$	$0.95\pm0.10~\text{a}$	$4.70\pm0.10b$	$3.10\pm0.10~\text{c}$	$3.50\pm0.15\mathrm{b}$	$5.3\pm0.10\text{b}$	$20.0\pm0.10a$	$12.0\pm0.10\text{b}$
${\tt S}+{\tt C}+{\tt D}$	7.03	$1.02\pm0.07a$	$0.90\pm0.10a$	$4.80\pm0.10\text{b}$	$3.60\pm0.20\text{d}$	$5.10\pm0.20\mathrm{c}$	$1.8\pm0.10\;a$	$20.0\pm0.10a$	$13.0\pm0.10~\text{b}$

<sup>a</sup> Mean values of each row followed by different letters significantly differ when subjected to DMRT (p < 0.05).

soaking seem to depend not only on the type of solution employed but also on the legume studied.

After cooking of soaked legumes, total starch content was not affected in chickpea, whereas decreases of 14 and 21% were observed in lentil compared to soaked and raw legumes, respectively. The extent of variations in starch content after cooking may be due not only to the type and physical characteristics of legumes but also to the influence of previous treatment (24) and different boiling times. Available starch contents of soaked chickpea and lentil were further increased as a result of cooking (21 and 12%, respectively), and resistant starch contents decreased significantly, falling 65% in chickpea and 49% in lentil with respect to raw seeds. Hence, part of the resistant starch is modified by heat during cooking and is converted into digestible starch. A similar trend and similar RS values in conventionally processed legumes have been found (21, 25, 26). Processing treatments are known to enhance starch digestibility in legumes. Soaking, cooking, and autoclaving of legumes reduce the levels of starch but to lesser extents than antinutrients (phenolic compounds, phytate, amylase inhibitor ...) and other soluble components (24, 25). Thus, the improvement in starch digestibility after soaking and thermal treatments is due to gelatinization of starch granules and, probably, to the decreasing levels of antinutritional factors in the seeds. In fact, partial removal of tannins and phytic acid probably created a large space within the matrix, which increased the susceptibility to enzymatic attack and consequently improved the digestibility of protein and starch after thermal processing (27, 28).

Few data have been documented on the influence of industrial dehydration on starch. The effect of dehydration was not significant ( $p \le 0.05$ ) in total starch content in comparison with soaked—cooked legumes, but it is notable that dehydrated lentil showed higher starch digestibility than cooked lentil (10% increase), accompanied by a large decrease of RS (47%). However, dehydrated chickpea exhibited no significant decreases in either available or resistant starch. The effect of processing on starch fractions is controversial. Osorio-Díaz et al. (29) and

Mahadevamma and Tharanathan (30) verified that thermal processing induces an increase in RS values, suggesting amylose retrogradation as the major mechanism behind the reduction in digestibility. However, Almeida-Costa et al. (31) reported that RS amounts in raw samples were almost twice higher than those found in processed samples. The variations in the results of RS can be due to the use of several in vitro procedures for the quantification of resistant starch and consequently the resistant starch content will vary according to the procedure used (3,31). In addition, the freeze-dried treatment used for the sample preparation could have modified the amount and type of starch of different samples analyzed (raw, soaked, soaked + cooked, soaked + cooked + dehydrated).

Physicochemical and functional properties of raw and processed legume flours are presented in Table 2. The pH values of the flours in water suspension are important because some functional properties (mainly related to protein) such as nitrogen solubility and emulsion properties are highly affected by pH changes. There were no significant differences in pH among raw legumes, whereas higher levels were shown in processed legumes. Nitrogen solubility for raw chickpea and lentil flours was pHdependent (Figure 1), being used increasingly as a guide to protein functionality. A sharp minimum solubility of nitrogen (27 and 29% for raw chickpea and lentil, respectively) was observed at pH 3.0, a level similar to that reported for minimum solubility in other legumes (32). Under neutral conditions, both legumes exhibited higher solubility (>80%), being maximum (100%) in alkaline conditions. However, at acidic pH (pH 2) nitrogen solubility was lower (60 and 75% in chickpea and lentil, respectively) than at basic pH. A high degree of protein insolubilization was observed in dehydrated legume flours (Figure 1), in which nitrogen solubility scarcely reached 20%. Along a pH gradient from 2 to 12, any increase in solubility was not significant, although an increasing trend was observed at pH > 10. A marked reduction in protein solubility has been reported after cooking of legumes up to pH 10.0 (13, 32), at which solubilization occurred, suggesting that it was dependent on



Figure 1. Nitrogen solubility of raw and processed legume flours.

deprotonation of lysine and arginine. Thus, the thermal processing may have denatured the protein, causing reduced solubility and affecting its applications in food formulations.

The bulk density exhibited by these legumes was comparable to that of other common legumes reported by Dzudie and Hardy (33), Prinyawiwatkul et al. (34), and Jood et al. (20). The flour prepared from lentil is significantly (p < 0.05) more dense than that prepared from chickpea. There were no significant differences in bulk density among flours made from processed lentil and chickpea. This property is important with regard to its packaging. In relation to oil holding capacity, the studied legume flours exhibited similar values, 1.10 and 0.95 mL/g in raw chickpea and lentil, respectively. The lipophilic tendency was less than that for soybean (1.93 mL/g) but higher than those reported for cowpea flours (0.69 mL/g) (34) and beans (0.80 mL/g) (35). The variations of OHC depend on the presence of nonpolar side chains, which bind the hydrocarbon side chain of oil. The OHC of flours prepared from lentil and chickpea did not show any differences by thermal processing. Due to their low OHC, these flours may be a potential ingredient in fried products because it would provide a nongreasy sensation.

With regard to water holding capacity, raw lentil flour exhibited a greater level than chickpea, and the results were similar to the literature (20, 33). The effect of processing was significant during cooking and dehydration, showing a drastic increase of WHC in both legumes, which reached similar levels (4.80-4.90 mL/g). It is probably due to protein denaturation and unfolding that exposes previously hidden peptide bonds and polar side chains, holding more water molecules (5). In addition, another factor influencing the WHC is the carbohydrate content such as starch, which gelatinizes, and dietary fiber, which absorbs water (36). The increase of insoluble dietary fiber previously observed (37) and available starch in these legume flours during cooking and dehydration processes may affect this functional property. The WHC is desirable in foods such as sausages, custards, and doughs because these are supposed to imbibe water without dissolution of protein, thereby attaining body thickening and viscosity. For this reason, processed lentil and chickpea flours could be used in the formulation of the above foods. In addition, the hydrophilic capacity was also measured by water absorption capacity and swelling capacity, which are initially surface phenomena, but higher hydration level absorption can occur inside the structure, leading to swelling and eventual solubilization. The results obtained were similar to the literature (20) and exhibited the same trend as WHC in processed samples.

Emulsifying activity, as shown **Table 2**, was higher in raw lentil flour than in chickpea, lentil levels being similar to those of the

mucuna bean, jackbean, and soybean EAs (18, 35). Data revealed that this property from soaked, cooked, and dehydrated legume flours was less than those from raw legume flours. These results confirm that the emulsion capacity response was very noticeable to heat treatment as has been previously reported (18, 35). Variations in EA during processing are possibly a result of interactions of different components of the flours that influence their properties. Our observations agree with the general correlation between EA and protein solubility as reported in previous works (19). Foam capacity was more stable in raw lentil flour than in chickpea flour. Nevertheless, the effect of thermal processing produced a reduction in the FC in both legumes, showing processed chickpea flours the highest reduction (67%). A similar tendency was found in processed beans (75% of reduction in FC during cooking) (36).

Gelation capacity, important in the preparation and acceptability of many foods, was studied using the least gelation concentration (LGC) as the index of gelation capacity; a low LGC means better gelation property. The LGC of these legumes was similar to that found in the literature (18, 35). Both raw and soaked legume flours exhibited the lowest LGC (8%), whereas the minimum concentration needed for the formation of the gel increased with the thermal treatment (12-13%). The variations in gelling properties have been associated with the relative ratio of different constituents such as proteins, lipids, and carbohydrates in legume flours. Gelation mechanism and gel appearance are fundamentally controlled by the balance between attractive hydrophobic interactions and repulsive electrostatic interactions (37). Likewise, LGC is influenced by a physical competition for the water between the protein gelling and the starch gelatinization (5, 37). The higher LGC in processed flours is due to the increase in the thermodynamic affinity of proteins for the aqueous solution, which decreased the interactions between proteins. When cooking is applied, protein denaturation and aggregation are produced and repulsive/attractive forces appear, caused by surface charges from various functional groups exposed by the thermal unfolding of the protein (5).

Scanning electron micrographs of raw and processed chickpea and lentil are presented in **Figures 2** and **3**, respectively. In both raw legumes, the starch granules, which as other SEM studies have also pointed out (26), were the most representative storage components. These starch granules exhibited similar shapes of starch granules, spherical—oval, although the size of the chickpea granule was slight smaller (17  $\mu$ m) than that of the lentil granule (22  $\mu$ m) (**Table 3**). In raw samples, starch granules were characterized by a smooth surface surrounded by well-defined protein bodies or fragments of protein matrix disrupted during milling



Figure 2. SEM of chickpea flours: (A) raw; (B) soaked; (C) soaked + cooked; (D) soaked + cooked + dehydrated. Bar size =  $20 \mu m$ .



Figure 3. SEM of lentil flours: (A) raw; (B) soaked; (C) soaked + cooked; (D) soaked + cooked + dehydrated. Bar size =  $20 \mu m$ .

(Figures 2A and 3A). These results agreed with those found in the literature (26, 38, 39).

The extent of the morphological changes occurring during processing is different depending on the legume type. During

Table 3. Effect of Processing on Starch Granule Size of Legumes<sup>a</sup>

legume	raw (µm)	soaked (µm)	soaked + cooked ( $\mu$ m)	soaked + cooked + dehydrated ( $\mu$ m)
chickpea	$17.1 \pm 2.9  \text{a}$	$22.3 \pm 2.1  {\rm a}$	$125.0 \pm 16.1$ c	$\begin{array}{c} 98.1 \pm 7.5  \text{b} \\ 89.4 \pm 8.0  \text{b} \end{array}$
lentil	22.0 $\pm$ 3.0 $ \text{a}$	77.7 $\pm 8.1  {\rm b}$	$121.1 \pm 3.7$ c	

<sup>a</sup> Mean values of each row followed by different letters significantly differ when subjected to DMRT (p < 0.05).

soaking, visible changes were produced within chickpea; starch granules appeared to maintain a regular structure (oval), and a slight increase of granules was observed in **Figure 2B**. However, more pronounced changes were detected in soaked lentil; starch granules became greater (3.5 times higher), rougher, and slightly eroded (**Figure 3B**). This fact could be mainly due to the water absorption of starch that occurred to a higher extent in lentil than in chickpea, and also protein bodies started to disappear in both flours due to activated proteolytic enzyme action.

Scanning electron micrographs of cooked chickpea and lentil are shown in Figures 2C and 3C, respectively. The action of cooking after soaking enlarged extensively the size of the starch granule, reaching similar sizes (121–125  $\mu$ m) in both legumes. These enlargements represent sizes 7.3 and 5.5 times greater than raw starch granules in chickpea and lentil, respectively. The granules still kept the internal integrity, however; their surface was flattened as an effect of heat action. Proteins were mostly disrupted and, in some cases, remnants of the protein matrix adhering to the starch granules could be found. Similar observations were also found in traditionally and microwave cooked chickpeas and beans (26). With regard to dehydration after soaking and cooking processing, scanning electron micrographs were similar to cooked legumes. However, they exhibited smaller starch granules compared to cooked seeds. The reductions of dehydrated starch granules with respect to cooked were relevant and similar in both legumes (21-26%), and a more pronounced flattened surface of starches was also observed in Figures 1D and **2D**. Thus, differences in starch granules after dehydration were due to the loss of holding water, but the starch granule integrity was kept. Amylose and amylopectin are modified during both processes and increase the amount of available starch versus total starch. Interestingly, the internal integrity exhibited by starch granules in the cooked and dehydrated flours was not detected in other processings such as fermentation or germination, which brought about endocorrosion of starch granules (38) and alteration in protein structure (40), respectively.

In conclusion, as a result of cooking and dehydration, there is an increase of available starch accompanied by a decrease of RS. However, the extent of these changes depended on the type of legume and the process applied. With regard to functional properties, the raw and processed legume flours exhibited low OHC, being a potential ingredient in fried products because it would provide a nongreasy sensation. In addition, the hydrophilic tendency of these samples showed a drastic increase as the effect of cooking and dehydration due to protein denaturation and also the carbohydrate content that influences the water holding. However, thermal processing may have denatured the protein, causing reduced solubility, emulsifying activity, and foam and gelation capacities. Thus, lentil and chickpea flours could be used as functional ingredients in food systems and incorporated into products such as bakery products, seasonings, and sausages among others. SEM provided information regarding the changes in structural characteristics that occurred in legumes under soaking, cooking, and dehydration processes. These microstructural modifications in the main components of flours are consistent with the increase of available starch of legumes after thermal processing.

## ACKNOWLEDGMENT

We thank the Servicio Interdepartamental de Investigación of Universidad Autónoma de Madrid for technical support and, especially, Esperanza Salvador for expert help in SEM.

## LITERATURE CITED

- (1) Pusztai, A.; Bardocz, S.; Martín-Cabrejas, M. A. The mode of action of ANFs on the gastrointestinal tract and its microflora. In *Proceedings of the Fourth International Workshop on Antinutritional Factors in Legume Seeds and Oilseeds*; Muzquiz, M., Hill, G. D., Cuadrado, C., Pedrosa, M. M., Burbano, C., Eds.; EAAP: Wageningen, The Netherlands, 2004; pp 87–100.
- (2) Chavan, J. K.; Kadam, S. S.; Salunkhe, D. K. Handbook of World Food Legumes: Nutritional, Processing, Technology and Utilization; CRC Press: Boca Raton, FL, 1989.
- (3) Hoover, R.; Zhou, Y. *In vitro* and *in vivo* hydrolysis of legume starches by α-amylase and resistant starch formation in legumes – a review. *Carbohydr. Polym.* 2003, 54, 401–417.
- (4) Duranti, M. Grain legume proteins and nutraceutical properties. *Fitoterapia* 2006, 77, 67–82.
- (5) Singh, U. Functional properties of grain legume flours. J. Food Sci. Technol. 2001, 38, 191–199.
- (6) Vega-Mercado, H.; Góngora-Nieto, M. M.; Barbosa-Cánovas, G. V. Advances in dehydration of foods. J. Food Eng. 2001, 49, 271–289.
- (7) Aguilera, J. M.; Chiralt, A.; Fito, P. Food dehydration and product structure. *Trends Food Sci. Technol.* 2003, 14, 432–437.
- (8) Nickels, N. C. Process for manufacturing dehydrated precooked legumes. U.S. Patent 2006/0263509 A1, 2006.
- (9) Li, B. W.; Schuhmann, P. J.; Wolf, W. R. Chromatographic determinations of sugars and starch in a diet composite reference material. J. Agric. Food Chem. 1985, 33, 531–536.
- (10) Vidal-Valverde, C.; Frías, J.; Sotomayor, C.; Díaz-Pollán, C.; Fernández, M.; Urbano, G. Nutrients and antinutritional factors in faba beans as affected by processing. *Z. Lebensm. Unters. Forsch.* A 1998, 207, 140–145.
- (11) Official Methods of Analysis, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.
- (12) Were, L.; Hettiarachchy, L.; Kalapathy, U. Modified soy proteins with improved foaming and water hydration properties. *J. Food Sci.* **1997**, *62*, 821–824.
- (13) Chau, C.-F.; Huang, Y.-L. Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of *Citrus sinensis* L. cv. Liucheng. J. Agric. Food Chem. 2003, 51, 2615–2618.
- (14) Beuchat, L. R. Functional and electrophoretic characteristics of succinylated peanut flour protein. J. Agric. Food Chem. 1977, 25, 258–261.
- (15) Robertson, G. L.; Monredon, F. D.; Dysseler, P.; Guillon, F.; Amado, R.; Thibault, J.-F. Hydration properties of dietary fibre and resistant starch: a European collaborative study. *Lebensm.-Wiss. -Technol.* 2000, *33*, 72–79.
- (16) Yatsumatsu, K.; Sawda, K.; Moritaka, S.; Mikasi, M.; Tada, J.; Wada, T.; Ishi, K. Whipping and emulsifying properties of soybean products. *Agric. Biol. Chem.* **1972**, *36*, 719–727.
- (17) Bencini, M. C. Functional properties of drum dried chickpea flour. J. Food Sci. 1986, 51, 1524–1526.
- (18) Chau, C. F.; Cheung, P. C. K. Functional properties of flour prepared from three Chinese indigenous legume seeds. *Food Chem.* **1998**, *61*, 429–433.
- (19) Bender, F. E.; Douglass, L. W.; Kramer, A. Statistical Methods for Food and Agriculture; Food Products Press: New York, 1989.

- (20) Jood, S.; Bishnoi, S.; Sharma, A. Chemical analysis and physicochemical properties of chickpea and lentil cultivars. *Nahrung/Food* **1998**, 42, 71–74.
- (21) Pujolà, M.; Farreras, A.; Casañas, F. Protein and starch content of raw, soaked and cooked beans (*Phaseolus vulgaris* L.). *Food Chem.* 2007, 102, 1034–1041.
- (22) Frías, J.; Vidal-Valverde, C.; Sotomayor, C.; Díaz-Pollán, C.; Urbano, G. Influence of processing on available carbohydrate content and antinutritional factors of chickpeas. *Eur. Food Res. Technol.* 2000, 210, 340–345.
- (23) Chung, H.-J.; Liu, Q.; Hoover, R.; Warkentin, T. D.; Vandenberg, B. *In vitro* starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food Chem.* **2008**, *111*, 316–321.
- (24) Rehman, Z. Domestic processing effects on available carbohydrate content and starch digestibility of black grams (*Vigna mungo*) and chickpeas (*Cicer arietium*). *Food Chem.* **2007**, *100*, 764–767.
- (25) Tovar, J.; Melito, C. Steam-cooking and dry heating produce resistant starch in legumes. *J. Agric. Food Chem.* **1996**, *44*, 2642–2645.
- (26) Marconi, E.; Ruggeri, S.; Cappelloni, M.; Leonardi, D.; Carnovale, E. Physicochemical, nutritional, and microstructural characteristics of chickpeas (*Cicer arietinum* L.) and common beans (*Phaseolus vulgaris* L.) following microwave cooking. J. Agric. Food Chem. 2000, 48, 5986–5994.
- (27) Negi, A.; Boora, P.; Khetarpaul, N. Starch and protein digestibility of newly released moth bean cultivars: effects of soaking, dehulling, germination and pressure cooking. *Nahrung/Food* 2001, 45, 251–254.
- (28) Martín-Cabrejas, M. A.; Aguilera, Y.; Pedrosa, M. M.; Cuadrado, C.; Hernández, T.; Díaz, S.; Esteban, R. M. The impact of dehydration process on antinutrients and protein digestibility of some legume flours. *Food Chem.* **2009**, *114*, 1063–1068.
- (29) Osorio-Díaz, P.; Bello-Pérez, L. A.; Sayazo-Ayerdi, S. G.; Benitez-Reyes, M. D. P.; Tovar, J.; Paredes-Lopez, O. Effect of processing and storage time on *in vitro* digestibility and resistant starch content of two bean varieties. *J. Sci. Food Agric.* **2003**, *83*, 1283–1288.
- (30) Mahadevamma, S.; Tharanathan, R. N. Processing of legumes: resistant starch and dietary fibre contents. J. Food Qual. 2004, 27, 289–303.
- (31) Almeida-Costa, G. E.; Silva, K.; Pissini, S. M.; Costa, A. Chemical composition, dietary fibre and resistant starch contents of raw and

cooked pea, common bean, chickpea and lentil legumes. *Food Chem.* **2006**, *94*, 327–330.

- (32) Carbonaro, M.; Cappelloni, M.; Nicoli, S.; Lucarini, M.; Carnovale, E. Solubility-digestibility relationship of legume proteins. J. Agric. Food Chem. 1997, 45, 3387–3394.
- (33) Dzudie, T.; Hardy, J. Physicochemical and functional properties of flours prepared from common beans and green mung beans. J. Agric. Food Chem. 1996, 44, 3029–3032.
- (34) Prinyawiwatkul, W.; Beuchat, L. R.; McWatters, K. H.; Philips, R. D. Functional properties of cowpea (*Vigna unguiculata*) flour as affected by soaking, boiling, and fungal fermentation. J. Agric. Food Chem. **1997**, 45, 480–486.
- (35) Chel-Guerrero, L.; Red-Flores, V. P.; Betancun-Ancona, D.; Vila-Ortiz, G. D. Functional properties of flours and protein isolates from *Phaseolus lunatus* and *Cannavalia ensiformis* seeds. J. Agric. Food Chem. 2002, 50, 584–591.
- (36) Granito, M.; Brito, Y.; Torres, A. Chemical composition, antioxidant capacity and functionality of raw and processed *Phaseolus lunatus. J. Sci. Food Agric.* 2007, 87, 2801–2809.
- (37) Wagner, J. R.; Guéguen, J. Surface functional properties of native acid treated and reduced soy glicinin 1. Foaming properties. J. Agric. Food Chem. 1999, 47, 2174–2180.
- (38) Sotomayor, C.; Frías, J.; Fornal, J.; Sadowska, J.; Urbano, G.; Vidal-Valverde, C. Lentil starch content and its microscopical structure as influenced by natural fermentation. *Starch/Staerke* 1999, 5, 152–156.
- (39) Milán-Carrillo, J.; Reyes-Moreno, C.; Armienta-Rodelo, E.; Carábez-Trejo, A.; Mora-Escobedo, R. Physicochemical and nutritional characteristics of extruded flours from fresh and hardened chickpeas (*Cicer arietinum* L.). *Lebensm.-Wiss. -Technol.* 2000, 33, 117–123.
- (40) Blaszczak, W.; Doblado, R.; Frías, J.; Vidal-Valverde, C.; Sadowska, J.; Fornal, J. Microstructural and biochemical changes in raw and germinated cowpea seeds upon high-pressure treatment. *Food Res. Int.* 2007, 40, 415–423.

Received for review March 25, 2009. Revised manuscript received September 18, 2009. Accepted October 20, 2009. We thank Vegenat SA for financial support.