

## Effect of chemical composition and thermal properties on the cooking quality of common beans (*Phaseolus vulgaris*)

### Efecto de la composición química y propiedades térmicas en la calidad de cocción de frijol común (*Phaseolus vulgaris*)

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The relationship between chemical composition and thermal properties as it affects the degree of cooking in six varieties of common beans was investigated. The degree of cooking (DC) and the hardness of cooked beans were estimated by differential scanning calorimetry (DSC) and texture analysis, respectively. The total protein of bean seeds varied between 19.7 and 23.6%. However, the fractions of albumin, glutelin, and globulin showed significant differences among varieties. The amylose content varied from 18.4 to 36.0%. The first endothermic peak was associated with inactivated enzymatic activity, denaturation of low-protein fractions, and starch gelatinization, while the second endothermic peak was associated with the denaturation of heat-resistant protein fractions. The high values of minerals and chemical composition among bean varieties affected thermal behavior and was associated with greater hardness and a lower degree of cooking.

**Keywords:** *Phaseolus vulgaris*; chemical composition; denaturation temperature; amylose content; degree of cooking; texture

La relación entre la composición química y propiedades térmicas afectaron el grado de cocción en las seis variedades de frijol. El grado de cocción (DC) y la dureza de frijoles cocidos, fueron estimados mediante calorimetría diferencial de barrido (DSC) y análisis de textura. El contenido de proteína total en semillas de frijol varió entre 19.7 y 23.6%. Sin embargo, las fracciones de globulina, albúmina y glutelina mostraron diferencias significativas entre las variedades. El contenido de amilosa varió de 18.4 a 36.0%. El primer pico endotérmico se asoció con la inactivación de la actividad enzimática, desnaturalización de fracciones de proteína de bajo peso molecular y la gelación del almidón, el segundo pico endotérmico fue asociado con la desnaturalización de las fracciones de proteína resistentes al calor. El elevado contenido de minerales en las semillas de frijol afectó el comportamiento térmico de los frijoles cocidos. La composición química entre las variedades afecta el comportamiento térmico y fueron asociados con alta dureza de los frijoles y bajo grado de cocción.

**Palabras clave:** *Phaseolus vulgaris*; composición química; temperatura de desnaturalización; contenido de amilosa; grado de cocción; textura

### 1. Introduction

Dry common beans (*Phaseolus vulgaris*) are an important source of protein, starch, fiber, vitamins, phytochemicals, and minerals such as calcium, iron, copper, zinc, phosphorus, and magnesium, all of which provide potential health benefits in the human diet (Lim, 2012; Ulloa et al., 2013). Bean cropping is an important economic activity in Mexico – annually about 1000 million tons of grains are produced (González-Rentería et al., 2011). Legumes commonly do not soften easily and remain hard even after cooking via boiling for two or more hours. These textural defects have been classified as: (1) hard-shell, when the seeds do not absorb sufficient water during cooking and therefore do not soften when cooked, and (2) hard-to-cook (HTC), when the seeds absorb enough water but fail to soften upon cooking (Hohlberg & Stanley, 1987; Kyriakidis, Apostolidis, Papazoglou, & Karathanos, 1997). Several mechanisms are proposed for the HTC defect in legume seeds, including: (1) lipid oxidation and/or polymerization; (2) autolysis of cytoplasmic organelles, poor plasmalemma integrity, and lignification of the middle lamella; (3) phytin catabolism and pectin demethylation with subsequent formation of insoluble pectate; (4) interaction of proteins and polyphenols and polymerization of polyphenolic

compounds; and (5) inadequate postharvest handling and storage techniques (Hohlberg & Stanley, 1987; Kyriakidis et al., 1997; Maurer, Ozen, Mauer, & Nielsen, 2004; Pirhayati, Soltanizadeh, & Kadivar, 2011; Segura-Campos, Ruiz-Ruiz, Chel-Guerrero, & Betancur-Ancona, 2013). These studies have indicated that both storage conditions and age can have negative impacts on the cooking process, affecting the product's nutritional quality. The cooking process softens legumes, improves texture and palatability, and helps to increase the access of digestive enzymes to starch and protein within the cell (Sasikala, Ravi, & Narasimha, 2011; Shiga, Cordenunsi, & Lajolo, 2009). The HTC defect in seeds causes an increase in cooking time, which leads to an increase in the amount of energy (fuel) consumption needed for cooking. It also reduces the nutritional value, changes sensorial quality, and reduces the levels of phytochemical bioactives (García & Lajolo, 1994; Ramírez-Jiménez, Reynoso-Camacho, Mendoza-Díaz, & Loarca-Piña, 2014). Commonly, physical testing is used to evaluate textural properties (elasticity, chewiness, and hardness), as these play a primary role in how a product is perceived by the consumer. From these textural properties, the final hardness of common beans ( $HN_f$ ) is commonly associated with the degree of cooking (DC) and typically it is the initial cause for rejection by a discerning palate. From a scientific

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perspective, the physicochemical changes experienced by seed beans during the cooking process are interrelated with configurational and conformational changes of the biomolecules, principally to starch (gelatinization) and protein (denaturalization). Both phenomena make up the food matrix. Thus, the irreversible changes to common beans caused by the cooking process can be followed by texture analysis (Siqueira, Vianello, Fernandes, & Bassinello, 2013), chromatographic techniques (Mayolo-Deloya, Martínez, & Rito-Palomares, 2012), or by differential scanning calorimetry (DSC). From the latter, DC could be associated to the endothermic transitions in cooked beans produced during the cooking processes. These endothermic transitions are related to the chemical composition of beans (i.e., protein and starch concentration, types of protein, amylose content, and minerals among others). The significance of our study lies in providing relevant information about the relationship of chemical composition to thermal properties of six different common, commercially available beans, and determines their influence on the degree of cooking and final texture of the cooked product.

## 2. Materials and methods

### 2.1. Materials

Six varieties of common bean (*P. vulgaris* L.) were chosen because of high consumption and their pleasant taste according to consumers in the occident of Mexico: Azufrado, Flor de Mayo, Mayocoba, Negro Jamapa, Ojo Negro, and Pinto. Common bean seeds, harvested in February 2010, were collected directly from farmers in the municipality of Santiago-Ixcuintla, Nayarit, Mexico (geographical coordinates: 21°81'47" north, -105°20'42" west). The amylose/amylopectin kit used in the study was purchased from Megazyme Ireland International, Ltd (Bray, Ireland). The reagents used for protein extraction and Bradford procedure were purchased from Sigma-Aldrich (St. Louis, MO). The bovine serum albumin was sourced from Bio-Rad (Hercules, CA).

### 2.2. Proximate chemical analysis

Bean seeds were cleaned, milled, and sieved through 40 mesh (0.425 mm) to obtain bean flour. Bean seeds and flour were stored at 4°C until required for use, to avoid the HTC phenomenon. The proximal chemical composition of bean flours was determined using AOAC methods (AOAC, 2000): moisture, total ash, crude protein ( $N \times 6.25$ ), crude fiber, minerals, and crude fat. The megazyme amylose/amylopectin assay procedure, utilizing a commercial kit, was followed according to the manufacturer's recommendations.

### 2.3. Protein extraction and fractionation procedure

Bean seed protein extraction was conducted according to the procedure proposed by Silva-Sánchez et al. (2008), with some modifications. The bean flour was defatted with hexane by stirring the 100 g/L (w/v) suspension for 4 h at 4°C. The defatted flour was then air-dried at room temperature. Defatted flour was stored at 4°C until used. Extraction of the albumin fraction was carried out on defatted flour using distilled water as the extracting agent. A suspension of defatted flour/extracting agent (1:10 w/v) was stirred for 40 min at 4°C and centrifuged at 13,000 g for 20 min and the supernatant was collected. The resulting pellet was used for 7 S globulin extraction, resuspended

in 0.1 M NaCl, 10 mM  $K_2HPO_4$  at pH 7.5, and 1 mM EDTA, stirred, and centrifuged as before. The new pellet was used for 11 S globulins, resuspended in 0.8 M NaCl, 10 mM  $K_2HPO_4$  at pH 7.5, and 1 mM EDTA, stirred, and centrifuged as before. The 7 S and 11 S fractions were considered total globulins. The prolamin fraction was extracted from the previous pellet with 60% aqueous ethanol and glutelin fraction with 0.1 M NaOH. Protein concentrations of the samples were determined according to the method of Bradford (1976) using bovine serum albumin as the standard.

### 2.4. Thermal transitions on bean flour

Thermal transitions on defatted bean flour were investigated using a differential scanning calorimeter DSC Q2000 (TA Instruments, New Castle, DE) according to the procedure reported by Espino-Sevilla et al. (2013). Briefly, 10 mg of each defatted flour variety was placed in a hermetic aluminum pan with 20  $\mu$ L of distilled water, mixed, sealed, and left at rest for 24 h at 20°C. The sample and empty pan were placed in a DSC chamber and heated from 20 to 115°C at 10°C/min under a  $N_2$  atmosphere (50 mL/min). The DSC was calibrated for temperature using indium and distilled water as standards. The peak temperature ( $T_p$ ) and enthalpies were calculated using Universal Analysis 2000, software version 4.7A (TA Instruments).

### 2.5. Cooking

Dry beans (150 g) were soaked in 1 L of distilled water for 24 h at 20°C. The soaked water was then removed and the soaked beans were placed in an aluminum pot with 1 L of fresh water and cooked in an open pot at 93°C (the boiling temperature of water in the city of Guadalajara) for 120 min. The evaporated water was replenished with hot water. Samples were recollected every 10 min during the cooking process. The samples were cooled with ice and placed in refrigeration for 24 h at 4°C before analysis.

### 2.6. Degree of cooking

DC was estimated by DSC analysis. Approximately 20 mg of beans collected at different cooking times were finely cut and placed inside a hermetic aluminum pan (Tzero, TA Instruments) with 20  $\mu$ L of water. The pans were sealed and stabilized for 1 h at 20°C. The sample and an empty pan were heated in the DSC chamber from 20 to 115°C at 2.5°C/min under a  $N_2$  atmosphere (50 mL/min). The enthalpy of cooked beans was analyzed using the Universal Analysis 2000 software. DC was calculated as follows:  $DC(\%) = [(\Delta H_0 - \Delta H_t) / \Delta H_0] \times 100\%$ , where  $\Delta H_t$  is the enthalpy of cooked beans at time t, and  $\Delta H_0$  is the enthalpy of the uncooked bean flour.

### 2.7. Hardness of cooked beans

Hardness of cooked beans collected at different cooking times was estimated using a texture analyzer TA-XT2 (Stable Micro Systems, Surrey, UK). A compression test was performed using a probe with cylindrical tip of 2 mm diameter. The penetration rate was set at 2 mm/s and 3 mm of penetration into the beans. Twenty cooked beans were used in each test, and the maximum penetrating force required was recorded (Kyriakidis et al., 1997).

## 2.8. Statistical analyses

All measurements were performed in triplicate and reported as mean  $\pm$  standard deviation, being evaluated during March 2010. Simple classification variance analysis was applied and whenever appropriate, Tukey's test was used in order to determine differences from the mean ( $p < 0.05$ ). Statistical analyses were performed with Statgraphics centurion XVI program version 16.1.17 (StatPoint Technologies, Inc., Warrenton, VA).

## 3. Results and discussion

### 3.1. Proximate analysis

Proximate chemical compositions of six varieties of common beans are presented in Table 1, and are in agreement with the results reported in literature (Carmona-García, Osorio-Díaz, Agama-Acevedo, Tovar, & Bello-Pérez, 2007; Lewu, Adebola, & Afolayan, 2010; Oomah, Blanchard, & Balasubramanian, 2008; Pinheiro, Baeta, Pereira, Domingues, & Ricardo, 2010; Rui, Boye, Ribereau, Simpson, & Prasher, 2011; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010).

### 3.2. Protein content

Legume seeds contain storage proteins that are tightly packed into the protein bodies of the parenchyma cells until germination, when they are degraded by endogenous proteases (Carbonaro, Maselli, Dore, & Nucara, 2008). Different bean varieties may have dissimilar protein profiles, a phenomenon that impacts their bioactivity

and functionality, which further affects applications in the food industry, and their cooking level (Ramírez-Jiménez et al., 2014; Rui et al., 2011). Table 2 shows the protein content extracted according to the procedure proposed by Silva-Sánchez et al. (2008). The albumin and globulin fractions were predominant in the common beans analyzed. The Mayocoba variety showed the highest proportion of albumin ( $43.8 \pm 1.4$  g/100 g), while Ojo Negro and Negro Jamapa showed the highest proportion of total globulin ( $41.5 \pm 1.4$  g/100 g) and glutelins ( $43.3 \pm 0.2$  g/100 g), respectively. In regard to prolamins, no significant differences were found between varieties yielding the lowest protein values ( $0.6$ – $1.4$  g/100 g) compared with other fractions.

### 3.3. Thermal transitions in defatted bean flour

Several researchers have reported the thermal transitions of isolated protein and starch fractions of common beans. Nevertheless, defatted bean flours have a more complex chemical composition (protein, amylose/amylopectin ratio, minerals, etc.), thus showing a more complex thermal profile than those reported for isolated systems. In defatted bean flour, the removed of lipids inhibits the formation of lipid-amylose and lipid-protein complexes. For this reason, we expect that the thermal transitions observed are related to protein denaturation and starch gelatinization. In general terms, all beans studied demonstrated two important endothermic processes that differ in enthalpy ( $\Delta H$ ) and peak temperature ( $T_p$ ) (Table 3 and Figure 1). Enthalpy is associated with the energy necessary for a transition, while  $T_p$  is considered as the temperature at which 50% of the molecules

Table 1. Proximate composition of whole seed samples from flour of six common bean varieties (*P. vulgaris* L.).

Tabla 1. Composición proximal de seis variedades de muestras harina de frijol (*P. vulgaris* L.) de semillas enteras.

Variety	Azufrado	Flor de Mayo	Mayocoba	Negro Jamapa	Ojo Negro	Pinto
Moisture (g/100 g)	6.4 $\pm$ 0.1 <sup>a</sup>	6.7 $\pm$ 0.2 <sup>a,b</sup>	7.6 $\pm$ 0.2 <sup>d</sup>	7.0 $\pm$ 0.1 <sup>b,c</sup>	8.9 $\pm$ 0.1 <sup>c</sup>	7.1 $\pm$ 0.0 <sup>c</sup>
Total protein (g/100 g)	20.4 $\pm$ 0.7 <sup>a</sup>	22.5 $\pm$ 0.0 <sup>b</sup>	22.6 $\pm$ 0.9 <sup>b</sup>	22.5 $\pm$ 1.1 <sup>b</sup>	22.1 $\pm$ 0.6 <sup>a,b</sup>	21.8 $\pm$ 0.1 <sup>a,b</sup>
Ash (g/100 g)	4.5 $\pm$ 0.0 <sup>a</sup>	4.5 $\pm$ 0.0 <sup>a</sup>	4.2 $\pm$ 0.0 <sup>a</sup>	5.3 $\pm$ 0.0 <sup>a,b</sup>	5.2 $\pm$ 0.9 <sup>a,b</sup>	7.2 $\pm$ 1.7 <sup>b</sup>
Crude fiber (g/100 g)	1.4 $\pm$ 0.2 <sup>a,b</sup>	1.5 $\pm$ 0.2 <sup>a,b</sup>	1.0 $\pm$ 0.1 <sup>a</sup>	1.5 $\pm$ 0.3 <sup>a,b</sup>	1.3 $\pm$ 0.0 <sup>a,b</sup>	1.7 $\pm$ 0.0 <sup>b</sup>
Lipid (g/100 g)	1.2 $\pm$ 0.1 <sup>a</sup>	1.8 $\pm$ 0.1 <sup>b</sup>	1.2 $\pm$ 0.2 <sup>a</sup>	1.7 $\pm$ 0.3 <sup>b</sup>	1.0 $\pm$ 0.0 <sup>a</sup>	0.9 $\pm$ 0.1 <sup>a</sup>
Total carbohydrate (g/100 g)	66.0 $\pm$ 0.8 <sup>a</sup>	63.0 $\pm$ 0.6 <sup>b</sup>	63.3 $\pm$ 0.7 <sup>b</sup>	61.9 $\pm$ 1.1 <sup>b</sup>	61.8 $\pm$ 0.7 <sup>b</sup>	61.3 $\pm$ 1.5 <sup>b</sup>
Amylose (%)	29.9 $\pm$ 1.1 <sup>a</sup>	24.3 $\pm$ 1.2 <sup>b</sup>	22.9 $\pm$ 1.1 <sup>b</sup>	34.4 $\pm$ 1.6 <sup>c</sup>	19.3 $\pm$ 0.9 <sup>d</sup>	22.5 $\pm$ 1.1 <sup>b,d</sup>
Zn (mg/kg)	28.6 $\pm$ 0.1 <sup>a</sup>	30.7 $\pm$ 1.3 <sup>b</sup>	25.7 $\pm$ 0.0 <sup>c</sup>	30.7 $\pm$ 1.3 <sup>b</sup>	28.6 $\pm$ 0.0 <sup>a</sup>	25.7 $\pm$ 0.1 <sup>c</sup>
Fe (mg/kg)	54.0 $\pm$ 0.8 <sup>a</sup>	48.4 $\pm$ 1.6 <sup>b</sup>	61.3 $\pm$ 2.5 <sup>c</sup>	58.3 $\pm$ 1.5 <sup>c,d</sup>	56.1 $\pm$ 0.0 <sup>a,d</sup>	59.3 $\pm$ 1.6 <sup>c,d</sup>
Ca (mg/kg)	1110 $\pm$ 30 <sup>a,b</sup>	1055 $\pm$ 15 <sup>a</sup>	737 $\pm$ 7 <sup>c</sup>	1730 $\pm$ 40 <sup>d</sup>	696 $\pm$ 6 <sup>c</sup>	1155 $\pm$ 5 <sup>b</sup>
Mg (mg/kg)	1190 $\pm$ 10 <sup>a,b</sup>	1260 $\pm$ 20 <sup>b</sup>	1120 $\pm$ 0 <sup>a</sup>	1430 $\pm$ 20 <sup>c</sup>	2180 $\pm$ 10 <sup>d</sup>	1805 $\pm$ 65 <sup>c</sup>
P (mg/kg)	515 $\pm$ 2 <sup>a</sup>	316 $\pm$ 5 <sup>b</sup>	400 $\pm$ 5 <sup>c</sup>	316 $\pm$ 5 <sup>b</sup>	515 $\pm$ 2 <sup>a</sup>	400 $\pm$ 5 <sup>c</sup>
Na (mg/kg)	17,500 $\pm$ 707 <sup>a</sup>	17,400 $\pm$ 1131 <sup>a</sup>	17,950 $\pm$ 3889 <sup>a</sup>	17,400 $\pm$ 1131 <sup>a</sup>	14,950 $\pm$ 354 <sup>b</sup>	19,750 $\pm$ 778 <sup>c</sup>
K (mg/kg)	53.8 $\pm$ 0.2 <sup>a</sup>	50.7 $\pm$ 1.8 <sup>b</sup>	61.2 $\pm$ 8.1 <sup>a,c</sup>	50.7 $\pm$ 1.8 <sup>b</sup>	74.0 $\pm$ 7.1 <sup>c</sup>	71.6 $\pm$ 5.5 <sup>c</sup>

Note: <sup>a,b,c,d,e</sup>: average ( $\pm$ S.E.,  $n = 3$ ) sharing the same letter in any row are a homogenous group ( $p > 0.05$ ) according to Tukey's HSD test.

Nota: <sup>a,b,c,d,e</sup> Promedios ( $\pm$ S.E.,  $n = 3$ ) que comparten la misma letra en el mismo renglón, son grupos homogéneos ( $p > 0.05$ ) de acuerdo a la prueba de Tukey's HSD.

Table 2. Protein content of defatted flour from selected bean varieties (*P. vulgaris* L.).

Tabla 2. Contenido de proteína en harina de las variedades de frijol desgrasada (*P. vulgaris* L.).

Variety	Azufrado	Flor de Mayo	Mayocoba	Negro Jamapa	Ojo Negro	Pinto
Albumin (g/100 g of protein)	36.4 $\pm$ 0.1 <sup>a</sup>	37.2 $\pm$ 0.2 <sup>a</sup>	43.8 $\pm$ 1.4 <sup>b</sup>	32.8 $\pm$ 4.6 <sup>a</sup>	32.9 $\pm$ 0.4 <sup>a</sup>	36.1 $\pm$ 1.0 <sup>a</sup>
Total globulin (g/100 g of protein)	29.7 $\pm$ 0.8 <sup>a</sup>	23.4 $\pm$ 0.4 <sup>b,c</sup>	28.7 $\pm$ 1.1 <sup>a,b</sup>	23.1 $\pm$ 4.3 <sup>c</sup>	41.5 $\pm$ 1.4 <sup>d</sup>	25.9 $\pm$ 0.4 <sup>a,b,c</sup>
Prolamins (g/100 g of protein)	0.6 $\pm$ 0.1 <sup>a</sup>	0.7 $\pm$ 0.2 <sup>a</sup>	1.4 $\pm$ 0.2 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>a</sup>	0.9 $\pm$ 0.1 <sup>a</sup>
Glutelins (g/100 g of proteins)	33.2 $\pm$ 0.9 <sup>a</sup>	38.6 $\pm$ 0.0 <sup>b</sup>	26.0 $\pm$ 0.3 <sup>c</sup>	43.3 $\pm$ 0.2 <sup>d</sup>	24.7 $\pm$ 0.9 <sup>c</sup>	37.1 $\pm$ 0.6 <sup>b</sup>

Note: <sup>a,b,c,d</sup>: average ( $\pm$ S.E.,  $n = 3$ ) sharing the same letter in any row are a homogenous group ( $p > 0.05$ ) according to Tukey's HSD test.

Nota: <sup>a,b,c,d</sup> Promedios ( $\pm$ S.E.,  $n = 3$ ) que comparten la misma letra en el mismo renglón, son grupos homogéneos ( $p > 0.05$ ) de acuerdo a la pruebas de Tukey's HSD.

Table 3. Thermal properties of defatted flour from common bean varieties (*P. vulgaris* L.).Tabla 3. Propiedades térmicas de las variedades de frijol desgrasada (*P. vulgaris* L.).

Variety	Azufrado	Flor de Mayo	Mayocoba	Negro Jamapa	Ojo Negro	Pinto
T <sub>p1</sub> (°C)	80.1 ± 0.90 <sup>a</sup>	79.3 ± 0.60 <sup>a,b</sup>	76.3 ± 1.20 <sup>c</sup>	79.1 ± 0.90 <sup>a,b</sup>	77.2 ± 1.50 <sup>b,c</sup>	81.3 ± 0.70 <sup>a</sup>
ΔH <sub>1</sub> (J/g)	0.82 ± 0.17 <sup>a,b</sup>	0.62 ± 0.07 <sup>a</sup>	0.69 ± 0.03 <sup>a,b</sup>	0.93 ± 0.14 <sup>b</sup>	1.35 ± 0.06 <sup>c</sup>	0.77 ± 0.08 <sup>a</sup>
T <sub>p2</sub> (°C)	94.3 ± 0.40 <sup>a</sup>	95.3 ± 0.30 <sup>a</sup>	90.5 ± 0.80 <sup>b</sup>	95.4 ± 0.70 <sup>a</sup>	84.3 ± 1.20 <sup>c</sup>	95.2 ± 1.00 <sup>a</sup>
ΔH <sub>2</sub> (J/g)	0.40 ± 0.01 <sup>a</sup>	0.54 ± 0.03 <sup>b</sup>	0.57 ± 0.02 <sup>b</sup>	0.39 ± 0.10 <sup>a</sup>	0.34 ± 0.03 <sup>a</sup>	0.71 ± 0.06 <sup>c</sup>

Note: <sup>a,b,c</sup>; average (±S.E., *n* = 3) sharing the same letter in any row are a homogenous group (*p* > 0.05) according to Tukey's HSD test.

Nota: <sup>a,b,c</sup> Promedios (±S.E., *n* = 3) que comparten la misma letra en el mismo renglón, son grupos homogéneos (*p* > 0.05) de acuerdo a la prueba de Tukey's HSD.

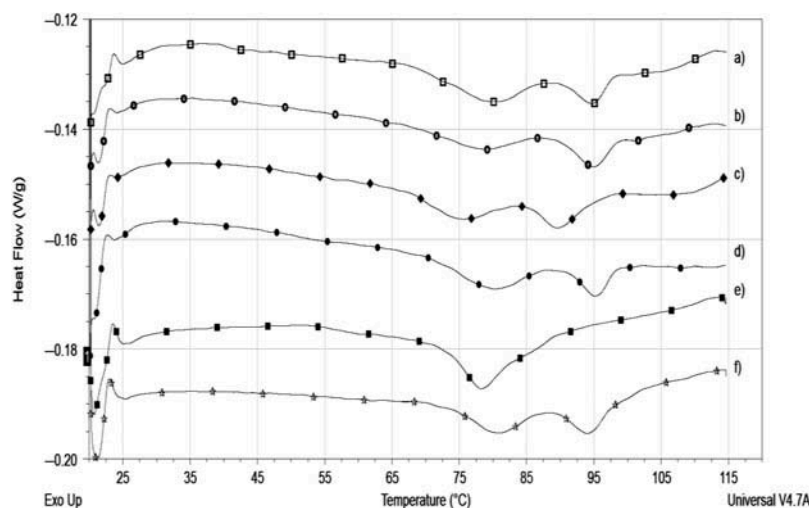


Figure 1. Thermal transitions of flour from common beans defatted by DSC: (a) Azufrado (□), (b) Flor de Mayo (○), (c) Mayocoba (◆), (d) Negro Jamapa (●), (e) Ojo Negro (■), and (f) Pinto (☆).

Figura 1. Transiciones térmicas del frijol desgrasado analizados por DSC: (a) Azufrado (□), (b) Flor de Mayo (○), (c) Mayocoba (◆), (d) Negro Jamapa (●), (e) Ojo Negro (■), (f) Pinto (☆).

have undergone thermal transition. The differences in thermal profiles observed in beans were associated with variation in the chemical composition, thermal properties, and quality of the protein fractions. Generally, in globular proteins, the denaturation temperature gradually increased with increasing ionic strength (Meng & Ma, 2001; Zhang et al., 2010). The higher concentration of minerals (principally Ca, K and Na) could impact the stability of proteins, shifting the denaturation process to slightly higher temperatures. For example, Ojo Negro seeds showed lower Na content and lower peak temperature, while Pinto seeds showed higher Na and K content and higher peak temperature. The first endothermic transition was associated with inactivated enzymatic activity, low protein fractions (65–70°C), and starch gelatinization (75–85°C), while the second apparent endothermic transition was associated with the presence of heat-resistant proteins such as globulins (82–105°C), lectins (89–95°C), and heterogeneous albumins (89–105°C) as previously reported by other investigators (Bernal-Lugo, Parra, Portilla, Peña-Valdivia, & Moreno, 1997; Chung, Liu, Peter Pauls, Fan, & Yada, 2008; Del Valle, Cottrell, Jackman, & Stanley, 1992; Hoover & Ratnayake, 2002; Meng & Ma, 2001; Paredes-López, Maza-Calviño, & González-Castañeda, 1989; Rocha, Genovese, & Lajolo, 2002; Rui et al., 2011; Tang, 2008). Thus, the correct interpretation of the thermal events involved requires a more detailed investigation and is beyond the reach of this work. However, it is important to know in a general point of view,

the influence of thermal events in the quality of cooking processes.

### 3.4. Degree of cooking

Figure 2 shows the thermal transitions of Azufrado beans for different cooking times. Soaked raw beans show a different thermal profile than that shown by defatted beans (Figure 2(a)). Soaking promotes swelling, leaching of polyphenols, and dissolution of salts and other ionic compounds present in the seeds, which induces changes in thermal profile. After 40 min of cooking (Figure 2(c)) the first endothermic peak has disappeared, indicating that the process of starch gelatinization and denaturation of low-molecular weight protein is complete. The second endothermic peak associated with the denaturation of heat-resistant protein fractions required about 120 min for completion (Figures 2(d)–(f)). Based on these profiles, DC was calculated from enthalpy change as a function of cooking time for the different common bean varieties, and showed a nonlinear behaviour with respect to cooking time (Figure 3). Particularly, the varieties Azufrado, Flor de Mayo, Negro Jamapa and Pinto showed higher T<sub>p2</sub> values than the cooking temperature (93°C). During cooking, protein–protein interactions (unfolding, aggregation, coagulation, or gelation) depend on the solubility and thermal stability of the seed proteins (Liu, McWatters, & Phillips, 1992). Protein fractions with a high denaturation temperature increase cooking time, resulting in the cooking process being time dependent. For example,



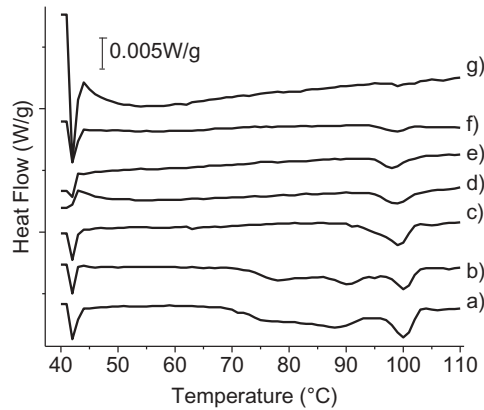


Figure 2. Thermal transitions of Azufrado beans for different cooking times: (a) 0 min, (b) 20 min, (c) 40 min, (d) 60 min, (e) 80 min, (f) 100 min, and (g) 120 min.

Figura 2. Transiciones térmicas del frijol Azufrado a diferentes tiempos de cocción: (a) 0 min, (b) 20 min, (c) 40 min, (d) 60 min, (e) 80 min, (f) 100 min, y (g) 120 min.

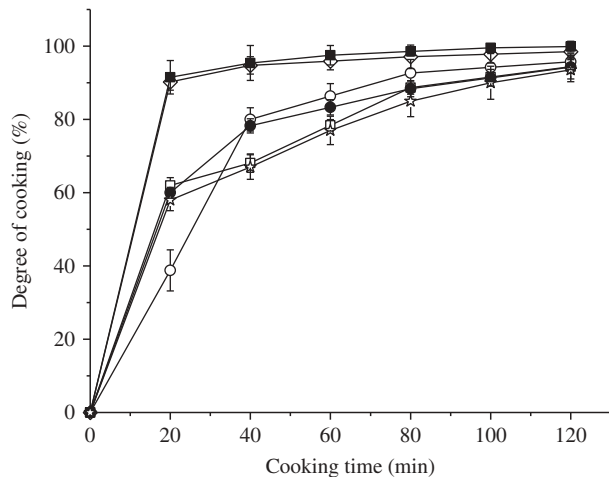


Figure 3. Degree of cooking as a function of cooking time according to DSC: Azufrado (□), Flor de Mayo (○), Mayocoba (◇), Negro Jamapa (●), Ojo Negro (■), and Pinto (☆). Bars indicate the standard deviation for three determinations.

Figura 3. Grado de cocción en función del tiempo de cocción por DSC: Azufrado (□), Flor de Mayo (○), Mayocoba (◇), Negro Jamapa (●), Ojo Negro (■) y Pinto (☆). Las barras indican la desviación estándar de tres determinaciones.

the varieties Ojo Negro and Mayocoba showed the lowest  $T_{p2}$ , as a consequence of the shortest cooking time, reaching ~98% DC after 80 min of cooking, while Pinto, Negro Jamapa, and Flor de Mayo yielded the highest  $T_{p2}$  values for the same cooking time, with DC ~80–85%. After 120 min of cooking, all beans had a pleasant texture with the following values of DC: Azufrado ( $94.4 \pm 1.9\%$ )<sup>a</sup>, Negro Jamapa ( $94.3 \pm 3.2\%$ )<sup>a</sup>, Pinto ( $95.3 \pm 1.7\%$ )<sup>a</sup>, Flor de Mayo ( $95.7 \pm 2.1\%$ )<sup>a</sup>, Mayocoba ( $98.5 \pm 2.1\%$ )<sup>a,b</sup>, and Ojo Negro ( $99.4 \pm 0.6\%$ )<sup>b,2</sup>. Bernal-Lugo et al. (1997) reported differences in cooking quality for uncooked flour of two common bean varieties (Ojo de cabra and Michigan 800) that showed different thermal profiles. Both varieties demonstrated the same enthalpy of starch gelatinization but different protein denaturation enthalpies. These results suggest that the denaturation temperature of heat-resistant

proteins ( $T_{p2}$ ) may be involved in the cooking quality of common beans. The effect of protein denaturation on bean softening was observed by Del Valle et al. (1992). Bean varieties showing endothermic transitions higher than the cooking temperature require longer cooking time to reach a proper degree of cooking.

### 3.5. Texture

In order to compare the results obtained by DSC analysis, the change in hardness of the different common bean varieties as a function of cooking time was evaluated (Figure 4). The penetrative force required in soaked raw beans was as follows: Negro Jamapa ( $16.2 \pm 1.7 \text{ N}$ )<sup>a</sup> > Pinto ( $15.5 \pm 3.8 \text{ N}$ )<sup>a,b</sup> > Azufrado ( $14.6 \pm 1.7 \text{ N}$ )<sup>a,b</sup> > Flor de Mayo ( $14.0 \pm 1.4 \text{ N}$ )<sup>b</sup> > Mayocoba ( $11.0 \pm 2.2 \text{ N}$ )<sup>c</sup> > Ojo Negro ( $9.8 \pm 0.5 \text{ N}$ )<sup>c</sup>.<sup>1</sup> After 10 min of cooking, the hardness of common beans was not significantly different ( $p < 0.05$ ) from that displayed at the commencement of cooking; however, the seeds had already absorbed more water and swollen (data not shown). The amylose/amylopectin ratio has a considerable influence on starch functions, especially those related to pasting, hydration, and product applications (Ambigaipalan et al., 2011). The degree of swelling was greater in Mayocoba, Ojo Negro, and Pinto, probably due to the low amylose content. It is well known that amylopectin content contributes to the swelling of starch granules, whereas amylose content inhibits swelling (Chung et al., 2008). After 10 min of cooking, the hardness of the beans had decreased as a function of cooking time. The loss of hardness is related to the gelatinization and denaturation processes of each bean variety (time/temperature dependent). It is important to highlight that some beans of the Ojo Negro and Mayocoba varieties showed evidence of overcooking after 60 min. Commonly, the final hardness of cooked beans is related to the ash, fiber, and calcium contents. Shiga et al. (2009) reported that the aging of beans is characterized by varying amounts of soluble and insoluble fiber, and that these differences disappeared after cooking. Shimelis and Rakshit (2005) related the hardness and quality of cooked beans to the calcium concentration and other cross-linking compounds present in common beans. Kyriakidis et al. (1997) and Pirhayati et al. (2011) indicated that a higher divalent cation content in water medium ( $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$ ) led to the formation of insoluble pectate, rendering the seed impenetrable to boiling water. The amylose/amylopectin ratio in cooked bean seeds can improve the final texture, apparently, at higher amylose, higher hardness in cooked common beans. Similar results were reported by Yu, Ma, and Sun (2009), who found that the hardness of cooked rice was affected by the amylose/amylopectin ratio: rice with a high ratio was harder while that with a lower ratio was softer.

## 4. Conclusions

The proximate chemical composition of common beans showed significant differences between varieties, principally in regard to protein, minerals (Zn, Fe, Ca, Mg, P, Na, and K), and the amylose/amylopectin ratio. The results show that certain elements of the cooking process of common beans (cooking degree and hardness) are associated with their proximal composition, principally the relationship amylose/amylopectin and type of protein. The DSC results showed the presence of two apparent endothermic processes related to protein and starch in defatted bean flour. The heat-resistant proteins ( $T_d > 93^\circ\text{C}$ ) demonstrated a slow denaturation process (time dependent), limiting the degree of cooking and texture quality.

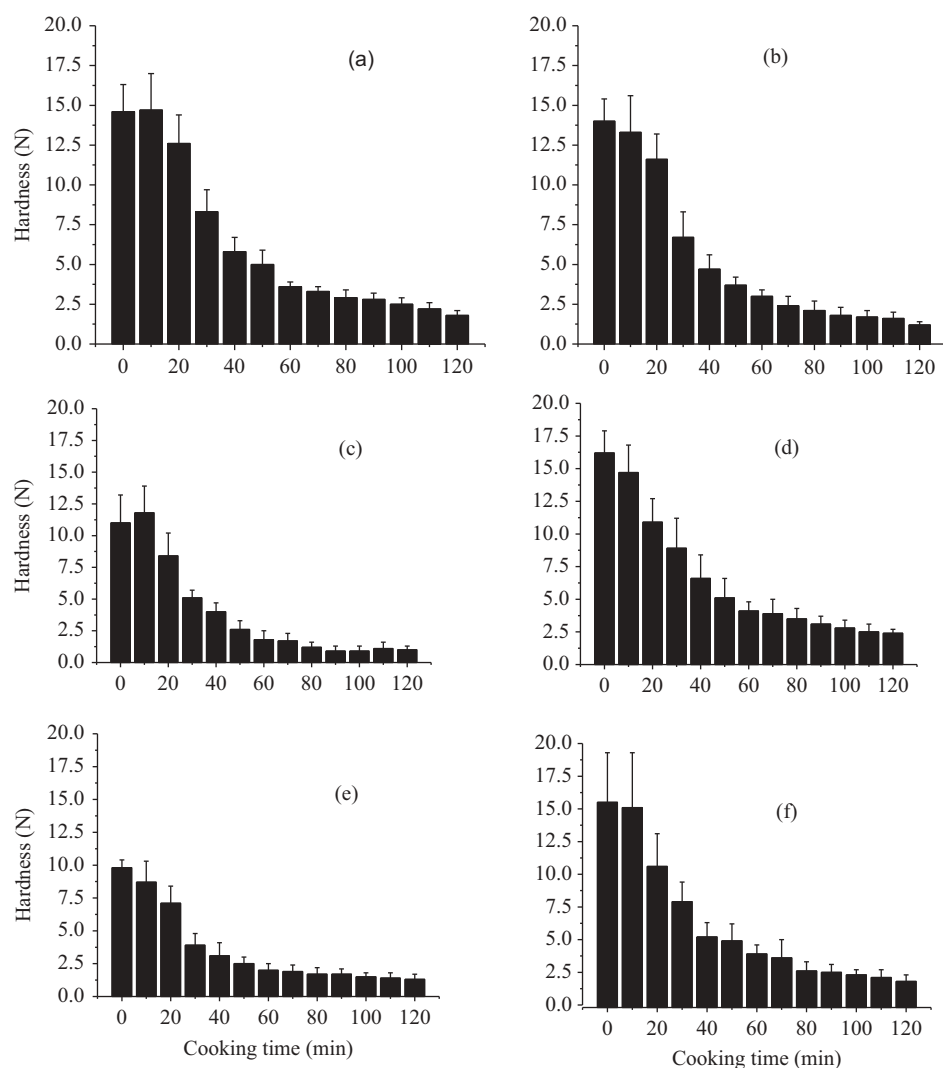


Figure 4. Change in texture of different bean varieties following the cooking process: (a) Azufrado, (b) Flor de Mayo, (c) Mayocoba, (d) Negro Jamapa, (e) Ojo Negro, and (f) Pinto. Bars indicate the standard deviation average of 20 measurements.

Figura 4. Cambio en la textura de las diferentes variedades de frijol a través del proceso de cocción: (a) Azufrado; (b) Flor de Mayo; (c) Mayocoba; (d) Negro Jamapa; (e) Ojo Negro; (f) Pinto. Las barras, significan la desviación estándar de promedio de 20 mediciones.

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## Notes

- <sup>a,b,c</sup> Homogeneous groups in hardness ( $p > 0.05$ ) according to Tukey's test.
- <sup>a,b</sup> Homogeneous groups on degree of cooking ( $p > 0.05$ ) according to Tukey's test.

## References

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., & Seetharaman, K. (2011). Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. *Food Research International*, 44(9), 2962–2974. doi:10.1016/j.foodres.2011.07.006

- AOAC. (2000). *Official methods of analysis* (17th, Vol. II). Washington, DC: AOAC International.
- Bernal-Lugo, I., Parra, C., Portilla, M., Peña-Valdivia, C. B., & Moreno, E. (1997). Cotyledon thermal behavior and pectic solubility as related to cooking quality in common beans. *Plant Foods for Human Nutrition*, 50(2), 141–150. doi:10.1007/bf02436033
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. doi:10.1016/0003-2697(76)90527-3
- Carbonaro, M., Maselli, P., Dore, P., & Nucara, A. (2008). Application of Fourier transform infrared spectroscopy to legume seed flour analysis. *Food Chemistry*, 108(1), 361–368. doi:10.1016/j.foodchem.2007.10.045
- Carmona-García, R., Osorio-Díaz, P., Agama-Acevedo, E., Tovar, J., & Bello-Pérez, L. A. (2007). Composition and effect of soaking on starch digestibility of *Phaseolus vulgaris* (L.) cv. 'Mayocoba'. *International Journal of Food Science & Technology*, 42(3), 296–302. doi:10.1111/j.1365-2621.2006.01218.x
- Chung, H.-J., Liu, Q., Peter Pauls, K., Fan, M. Z., & Yada, R. (2008). In vitro starch digestibility, expected glycemic index and some physicochemical properties of starch and flour from common bean (*Phaseolus vulgaris* L.) varieties grown in Canada. *Food Research International*, 41(9), 869–875. doi:10.1016/j.foodres.2008.03.013

- Del Valle, J. M., Cottrell, T. J., Jackman, R. L., & Stanley, D. W. (1992). Hard-to-cook defect in black beans: The contribution of proteins to salt soaking effects. *Food Research International*, 25(6), 429–436. doi:10.1016/0963-9969(92)90167-4
- Espino-Sevilla, M. T., Jaramillo-Flores, M. E., Hernández-Gutiérrez, R., Mateos-Díaz, J. C., Espinosa-Andrews, H., Barba De La Rosa, A. P., & Lugo-Cervantes, E. C. (2013). Functional properties of Dityx heterantha proteins. *Food Science & Nutrition*, 1(3), 254–265. doi:10.1002/fsn3.34
- García, E., & Lajolo, F. M. (1994). Starch alterations in hard-to-cook beans (*Phaseolus vulgaris*). *Journal of Agricultural and Food Chemistry*, 42(3), 612–615. doi:10.1021/jf00039a002
- González-Rentería, S. M., Soto-Cruz, N. O., Rutiaga-Quiñones, O. M., Medrano-Roldán, H., Rutiaga-Quiñones, J. G., & López-Miranda, J. (2011). Optimización del proceso de hidrólisis enzimática de una mezcla de pajas de frijol cuatro variedades (Pinto villa, Pinto saltillo, Pinto mestizo y Flor de mayo). *Revista Mexicana De Ingeniería Química*, 10, 17–28.
- Hohlberg, A. I., & Stanley, D. W. (1987). Hard-to-cook defect in black beans. Protein and starch considerations. *Journal of Agricultural and Food Chemistry*, 35(4), 571–576. doi:10.1021/jf00076a033
- Hoover, R., & Ratnayake, W. S. (2002). Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry*, 78(4), 489–498. doi:10.1016/S0308-8146(02)00163-2
- Kyriakidis, N. B., Apostolidis, A., Papazoglou, L. E., & Karathanos, V. T. (1997). Physicochemical studies of hard-to-cook beans (*Phaseolus vulgaris*). *Journal of the Science of Food and Agriculture*, 74(2), 186–192. doi:10.1002/(SICI)1097-0010(199706)74:2<186::AID-JSFA785>3.0.CO;2-X
- Lewu, M. N., Adebola, P. O., & Afolayan, A. J. (2010). Effect of cooking on the mineral contents and anti-nutritional factors in seven accessions of *Colocasia esculenta* (L.) Schott growing in South Africa. *Journal of Food Composition and Analysis*, 23(5), 389–393. doi:10.1016/j.jfca.2010.02.006
- Lim, T. K. (2012). *Edible medicinal and non-medicinal plants*. Vol. 2, (D. Haank, M. Mos & P. Hendriks, Eds.). Heidelberg: Springer-Verlag GmbH.
- Liu, K., McWatters, K. H., & Phillips, R. D. (1992). Protein insolubilization and thermal destabilization during storage as related to hard-to-cook defect in cowpeas. *Journal of Agricultural and Food Chemistry*, 40(12), 2483–2487. doi:10.1021/jf00024a028
- Maurer, G. A., Ozen, B. F., Mauer, L. J., & Nielsen, S. S. (2004). Analysis of hard-to-cook red and black common beans using Fourier transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, 52(6), 1470–1477. doi:10.1021/jf035083s
- Mayolo-Deloisa, K., Martínez, L. M., & Rito-Palmares, M. (2012). Chromatographic techniques and their application to studies of conformational changes, stability and refolding of proteins. *Revista Mexicana De Ingeniería Química*, 11(3), 415–419.
- Meng, G. T., & Ma, C. Y. (2001). Thermal properties of *Phaseolus angularis* (red bean) globulin. *Food Chemistry*, 73(4), 453–460. doi:10.1016/S0308-8146(00)00329-0
- Oomah, B. D., Blanchard, C., & Balasubramanian, P. (2008). Phytic acid, phytase, minerals, and antioxidant activity in Canadian dry bean (*Phaseolus vulgaris* L.) cultivars. *Journal of Agricultural and Food Chemistry*, 56(23), 11312–11319. doi:10.1021/jf801661j
- Paredes-López, O., Maza-Calviño, E. C., & González-Castañeda, J. (1989). Effect of the hardening phenomenon on some physicochemical properties of common bean. *Food Chemistry*, 31(3), 225–236. doi:10.1016/0308-8146(89)90060-5
- Pinheiro, C., Baeta, J. P., Pereira, A. M., Domingues, H., & Ricardo, C. P. (2010). Diversity of seed mineral composition of *Phaseolus vulgaris* L. germplasm. *Journal of Food Composition and Analysis*, 23, 319–325. doi:10.1016/j.jfca.2010.01.005
- Pirhayati, M., Soltanizadeh, N., & Kadivar, M. (2011). Chemical and microstructural evaluation of 'hard-to-cook' phenomenon in legumes (pinto bean and small-type lentil). *International Journal of Food Science & Technology*, 46(9), 1884–1890. doi:10.1111/j.1365-2621.2011.02697.x
- Ramírez-Jiménez, A. K., Reynoso-Camacho, R., Mendoza-Díaz, S., & Loarca-Piña, G. (2014). Functional and technological potential of dehydrated *Phaseolus vulgaris* L. flours. *Food Chemistry*, 161(0), 254–260. doi:10.1016/j.foodchem.2014.04.008
- Rocha, M. G. P., Genovese, M. I., & Lajolo, F. M. (2002). Albumins from the bean *Phaseolus vulgaris*: Effects of heat treatment. *Journal of Food Biochemistry*, 26(3), 191–208. doi:10.1111/j.1745-4514.2002.tb00852.x
- Rui, X., Boye, J. I., Ribereau, S., Simpson, B. K., & Prasher, S. O. (2011). Comparative study of the composition and thermal properties of protein isolates prepared from nine *Phaseolus vulgaris* legume varieties. *Food Research International*, 44(8), 2497–2504. doi:10.1016/j.foodres.2011.01.008
- Sasikala, V. B., Ravi, R., & Narasimha, H. V. (2011). Textural changes of green gram (*Phaseolus aureus*) and horse gram (*Dolichos biflorus*) as affected by soaking and cooking. *Journal of Texture Studies*, 42(1), 10–19. doi:10.1111/j.1745-4603.2010.00263.x
- Segura-Campos, M., Ruiz-Ruiz, J., Chel-Guerrero, L., & Betancur-Ancona, D. (2013). Antioxidant activity of *Vigna unguiculata* L. walp and hard-to-cook *Phaseolus vulgaris* L. protein hydrolysates. *CyTA – Journal of Food*, 11(3), 208–215. doi:10.1080/19476337.2012.722687
- Shiga, T. M., Cordenunsi, B. R., & Lajolo, F. M. (2009). Effect of cooking on non-starch polysaccharides of hard-to-cook beans. *Carbohydrate Polymers*, 76(1), 100–109. doi:10.1016/j.carbpol.2008.09.035
- Shimelis, E. A., & Rakshit, S. K. (2005). Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *LWT – Food Science and Technology*, 38(4), 331–338. doi:10.1016/j.lwt.2004.07.002
- Silva-Sánchez, C., De La Rosa, A. P. B., León-Galván, M. F., De Lumen, B. O., De León-Rodríguez, A., & De Mejía, E. G. (2008). Bioactive peptides in Amaranth (*Amaranthus hypochondriacus*) seed. *Journal of Agricultural and Food Chemistry*, 56(4), 1233–1240. doi:10.1021/jf072911z
- Siqueira, B. D. S., Vianello, R. P., Fernandes, K. F., & Bassinello, P. Z. (2013). Hardness of carioca beans (*Phaseolus vulgaris* L.) as affected by cooking methods. *LWT – Food Science and Technology*, 54(1), 13–17. doi:10.1016/j.lwt.2013.05.019
- Tang, C.-H. (2008). Thermal denaturation and gelation of vicilin-rich protein isolates from three *Phaseolus* legumes: A comparative study. *LWT – Food Science and Technology*, 41(8), 1380–1388. doi:10.1016/j.lwt.2007.08.025
- Ulloa, J. A., Bonilla-Sánchez, C. R., Ortíz-Jiménez, M. A., Rosas-Ulloa, P., Ramírez-Ramírez, J. C., & Ulloa-Rangel, B. E. (2013). Rehydration properties of precooked whole beans (*Phaseolus vulgaris*) dehydrated at room temperature. *CyTA – Journal of Food*, 11(1), 94–99. doi:10.1080/19476337.2012.699104
- Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., & Gawalko, E. J. (2010). Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Research International*, 43(2), 589–594. doi:10.1016/j.foodres.2009.07.012
- Yu, S., Ma, Y., & Sun, D.-W. (2009). Impact of amylose content on starch retrogradation and texture of cooked milled rice during storage. *Journal of Cereal Science*, 50(2), 139–144. doi:10.1016/j.jcs.2009.04.003
- Zhang, Y.-H., Tang, C.-H., Wen, Q.-B., Yang, X.-Q., Li, L., & Deng, W.-L. (2010). Thermal aggregation and gelation of kidney bean (*Phaseolus vulgaris* L.) protein isolate at pH 2.0: Influence of ionic strength. *Food Hydrocolloids*, 24(4), 266–274. doi:10.1016/j.foodhyd.2009.10.002