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# Food Research International

journal homepage: www.elsevier.com/locate/foodres



# Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes

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#### ARTICLE INFO

Article history: Received 2 August 2012 Accepted 30 September 2012

Keywords: Non-conventional legumes Germination Dietary fiber Starch Physicochemical properties

#### ABSTRACT

The objective of this study was to evaluate the impact of germination on dietary fiber composition, starch availability and physicochemical properties in four non-conventional legumes (*Vigna unguiculata*, *Canavalia ensiformis*, *Stizolobium niveum*, *Lablab purpureus*) in order to improve the carbohydrate supply and to optimize native products of developing countries. Germination promoted a significant decrease of resistant starch along with an increase of available starch percentage. Total dietary fiber contents increased during germination and improved insoluble/soluble dietary fiber ratio. This process produced an increase of total sugar content, mainly due to the rise of cellulosic glucose from metabolic reaction undergone during germination. Moreover, physicochemical properties of germinated legume flours were modified, improving oil holding, water holding, water absorption and gelation capacities, whereas decreases of emulsifying and foaming capacities were detected. In conclusion, germination provides non-conventional legume flours with higher nutritional quality and better physicochemical properties than the raw flours.

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#### 1. Introduction

Developing countries are facing an increasing demand for proteinrich food due to teeming population, cereal-based diet and scarcity of fertile land (Sridhar & Seena, 2006). Recent research efforts are being directed to identify and evaluate under-exploited legumes as alternative protein crops for the future (Martín-Cabrejas et al., 2008). Apart from proteins and energy, legumes show desirable characteristics such as abundance of carbohydrates, starch as the most abundant carbohydrate (22–45%); low fat content (except oilseeds); B complex vitamins and minerals (Sridhar & Seena, 2006). Furthermore, components like soluble and insoluble fibers, resistant starch and bioactive polyphenols make legumes suitable for a wide range of food applications. Many studies have been carried out to know the benefits of dietary fiber such as prevention of obesity, cardiovascular disease, type 2 diabetes and also the large intestine cancer (Champ, Langkilde, Brouns, Kettlitz, & Collet, 2003). Thus, the role of legumes as therapeutic agents in the diet of healthy vulnerable populations (diabetes, metabolic disorders, etc.) is actually of great interest (Duranti, 2006).

Moreover, the uses of legumes in food formulation are assuming a greater importance and have attracted the attention of food processors, marketers and consumers (Boye, Zare, & Pletch, 2010), since their physicochemical properties show a great impact on their utilization and are very important in the development of functional ingredients in some foods such as breads, cakes and biscuits (Anton, Ross, Lukow, Fulcher, & Arntfield, 2008; Han, Jann, & Gerlat, 2010).

However, the utilization of legumes is limited by the presence of antimetabolic/antiphysiological substances, such as protease inhibitors, non-protein amino acids, lectins, saponins and flatulence compounds (Adebowale, Adeyemi, & Oshodi, 2005). In this regard, germination has been identified as an inexpensive and effective technology to improve the quality of legumes, by enhancing their digestibility, increasing the content of amino acids (Chang & Harrold, 1988) and reducing the levels of antinutrients (Vidal-Valverde et al., 2002). The effect of germination on nutrients and antinutritional factors has been widely studied; however, very little information is found in the literature about the effects of germination on dietary fiber composition and physicochemical properties necessary to know the possible applications of legumes. Moreover, germination conditions and their effects on legume composition can vary with the plant species, seed varieties or cultivars (Paucar-Menacho, Berhow, Mandarino, Chang, & Mejia, 2010). Therefore, the objective of this study was to evaluate the effect of germination on dietary fiber composition, starch

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Abbreviations: Ara, Arabinose; BD, Bulk density; Celob, Cellobiose; DF, Dietary fiber; EA, Emulsifying activity; FC, Foaming capacity; Gal/Ram, Galactose/Rhamnose; Glc, Glucose; IDF, Insoluble dietary fiber; LGC, Least gelation capacity; Man, Mannose; NS, Neutral sugar; OHC, Oil holding capacity; RS, Resistant starch; SDF, Soluble dietary fiber; SWC, Swelling capacity; TDF, Total dietary fiber; UA, Uronic acids; WAC, Water absorption capacity; WHC, Water holding capacity; Xyl, Xylose.

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availability and physicochemical properties in the above mentioned non-conventional legumes in order to optimize native products of developing countries with high nutritional value.

#### 2. Material and methods

#### 2.1. Samples

Seeds of *Vigna unguiculata* (L.) Walp (cowpea), *Canavalia ensiformis* (L.) DC (jack bean), *Stizolobium niveum* (L.) DC (mucuna) and *Lablab purpureus* (L.) Sweet (dolichos) were grown and supplied by the Instituto de Ciencia Animal (La Habana, Cuba).

#### 2.2. Germination

Two portions of 150 g of seeds were washed with 0.7% sodium hypochlorite, soaked in 450 mL of distilled water at room temperature for 6 h, and shaken every 30 min. The water was then drained off, and the seeds were transferred to a separating funnel. The samples were germinated under 12 h of light daily (Díaz, Martín-Cabrejas, González, Torres, & Node, 2007). In all cases, the germination was carried out at 25 °C for 96 h and seeds were sprayed daily with distilled water in order to maintain an adequate hydration level. The sprouts and the seeds were ground and freeze-dried for analysis. The germination experiments were performed in duplicate.

#### 2.3. Starch determination

Starch content was determined from the residue obtained after soluble carbohydrate extraction according to Li, Schuhmann, and Wolf (1985) and modified by Vidal-Valverde et al. (1998) 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.

#### 2.4. Dietary fiber determination

Mes-Tris AOAC method 991.43 was used for dietary fiber (DF) determination (AOAC, 1995). The insoluble residues were isolated by filtration and soluble fiber was precipitated with ethanol. Determination of residual ashes and proteins (as Kjeldahl N 6.25) (AOAC, 1995) was carried out in the residues for corresponding corrections. Total dietary fiber (TDF) was calculated as sum of soluble and insoluble fractions.

#### 2.5. Chemical analysis of dietary fiber components

The composition of DF was determined after acid hydrolysis, as described by Jaime et al. (2002) of fiber residues obtained as mentioned above (AOAC, 1995). The acid hydrolysis released the different fiber components: neutral sugars and uronic acids. The neutral sugars were determined by HPLC and uronic acids were analyzed colorimetrically following the methods described by Jaime et al. (2002).

## 2.6. Physicochemical properties

#### 2.6.1. pH

pH was measured on a slurry prepared with 10 g legume flours in 40 mL of boiled, deionized water according to official AOAC procedures (AOAC, 1990).

#### 2.6.2. Bulk density

Bulk density (BD) was determined using a graduated cylinder (10 mL), previously weighed, which is filled with sample up to 10 mL by constant tapping, until there is no further change in volume.

The content is weighed and from the difference in weight, the bulk density of sample is calculated as grams per milliliter (Chau & Huang, 2003).

#### 2.6.3. Swelling capacity

Swelling capacity (SWC) was determined according to Robertson et al. (2000). The sample (100 mg of flour) 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 SWC expressed as volume (mL) occupied by sample per gram original sample dry weight.

#### 2.6.4. Water holding capacity

Water holding capacity (WHC) was determined according to the method of Chau and Huang (2003), with slight modifications. The sample (1 g of flour) was stirred in 10 mL distilled water for 24 h in a centrifuge tube at room temperature. After samples were centrifuged (2500 g, 30 min), the supernatant was transferred to a graduated cylinder of 10 mL, where volume was measured. The WHC was expressed as milliliter of water held by 1 g of dry sample.

#### 2.6.5. Water absorption capacity

Water absorption capacity (WAC) was determined essentially according to the method of Beuchat (1977). The sample (1 g of flour) was mixed with 10 mL of distilled water in a centrifuge tube for 1 min in a vortex and then centrifuged at  $3000-5000 \, g$  for 30-45 min depending on the availability of facility for this purpose. After separating the content, the volume of supernatant is recorded and used for determination of water absorption, and the results are expressed as mL/g sample.

#### 2.6.6. Oil holding capacity

Oil holding capacity (OHC) was determined according to the method of Chau and Huang (2003), 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 (2500 g, 30 min), the supernatant was transferred to a graduated cylinder of 10 mL, where volume was measured. The OHC was expressed as milliliter of vegetable oil held by 1 g of dry sample.

#### 2.6.7. Gelation capacity

Suspensions were prepared in distilled water with concentrations of 4, 8, 12, 14, 16, 18 and 20% (w/v). Aliquots of these suspensions (5 mL) were transferred to tubes and placed in a water bath for 1 h to 100 °C, and then placed in an ice bath for 1 h. The least gelation concentration (LGC) was detected when the sample did not slide when the tube is inverted (Chau & Cheung, 1998).

#### 2.6.8. Emulsifying activity

Emulsifying activity (EA) was evaluated following the method of Yatsumatsu et al. (1972). The sample (1 g of flour) was mixed with 20 mL of distilled water for 30 min. The mixture was made up of 25 mL, followed by the addition of 25 mL of corn oil and homogenized for 3 min. The resulting emulsion was centrifuged at 2000 g for 5 min and then emulsion volume was measured. Emulsifying activity was expressed as percentage of the emulsified layer volume of the entire layer in the centrifuge tube.

#### 2.6.9. Foaming capacity

Foaming capacity (FC) was determined according to the method of Bencini (1986). The sample (1 g of flour) was dispersed in 50 mL of distilled water and whipped using a homogenizer (Polytron model Brinkmann Instruments) at 2000 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.

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