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The effect of storage on the solubilization pattern of bean hull non-starch polysaccharides

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ABSTRACT

The storage of Carioca bean at 30 °C and 75% relative humidity for eight months altered the solubilization pattern of hulls non-starch polysaccharides. The polysaccharide physicochemical pattern changed, resulting in a shift in the composition of water-soluble and water-insoluble polysaccharides caused by the insolubilization of galacturonans and xyloglucan. Hulls make up 10% of whole beans, which showed an increase of about 5% in water-insoluble polysaccharides and a decrease of about 1% in water-soluble polysaccharides with aging. These values suggest that cotyledons and hulls together account for an increase of about 2 g of water-insoluble polysaccharides and a decrease of 1.5 g of water-soluble polysaccharides per 100 g of beans. This change in the polysaccharide composition may produce a considerable difference in the dietary fiber profile. The alterations observed in bean hull non-starch polysaccharide composition were similar to those previously observed in the cotyledon.

1. Introduction

The consumption of grains and vegetables is important to a healthy lifestyle because they are rich in dietary fibers, which are well-known to protect against degenerative diseases. The frequent consumption of these compounds helps reducing the risk of cardiovascular diseases and colorectal cancer and controls obesity by promoting laxation and reducing post prandial glycemia (Tungland & Meyer, 2002). Dietary fiber is mainly composed of non-digestible components of plant cell walls, mainly complex polysaccharides, which are resistant to digestive enzymatic degradation. These polysaccharides are partly composed of water-insoluble material, which has adsorptive and water-holding properties that contribute to stool softening and shorten the intestinal transit time. The soluble polysaccharides, in turn, are mainly fermented in the cecum, causing an increase in bacterial biomass and production of short-chain fatty acids (SCFA), which contribute to a delay in intestinal transit and maintaining gut integrity (Eastwood & Morris, 1995).

The plant cell wall is made up of complex polysaccharides, phenolic compounds and proteins stabilized by ionic and covalent linkages. This structure performs a variety of functions in living plants and is responsible for the sensorial and nutritional characteristics of plant-based foods (Bourne, 1983; Brett & Waldron, 1996; Gibeaut & Carpita, 1993; Jackman & Stanley, 1995). The solubilization and degradation patterns of cell wall polysaccharides are important because they affect the physiological properties of the dietary fibers.

Beans are consumed widely throughout the world and are a staple food in tropical, developing countries. They provide a rich source of energy, nutrients and dietary fiber. However, in leguminous seeds, long-term storage at high temperature and humidity causes a gradual loss of nutritive components and the development of the textural defect known as hard-to-cook (HTC), which causes the seeds to be resistant to softening during cooking (Hincks & Stanley, 1986; Liu, 1995; Reyes-Moreno & Paredes-López, 1993). This textural defect is related to several mechanisms that involve lipid oxidation and alterations in the cell wall composition, structure and organization (Hincks & Stanley, 1986; Liu, 1995).

The development of HTC may change dietary fiber composition and solubility, thus affecting its fermentability in the large intestine. Studies of bean cell walls suggest a connection between non-starch polysaccharide insolubilization and bean hardening (Shiga, Lajolo, & Filisetti-Cozzi, 2003; Shiga, Lajolo, & Filisetti, 2004). The HTC defect causes the insolubilization of the cotyledon cell wall polysaccharides in common beans, leading to a decrease in galacturonan and arabinose-rich polysaccharide depolymerization during cooking (Shiga, Cordenunsi, & Lajolo, 2009). Considering that hulls account for about 10%, which is composed of 67% insoluble non-starch polysaccharides and 4% soluble fiber, the changes in polysaccharide composition and structure may result in different physiological responses in the intestine. Moreover, bean hulls are rich in phenolic compounds, which are susceptible to polymerization, contributing to their impermeabilization (Liu, 1995).

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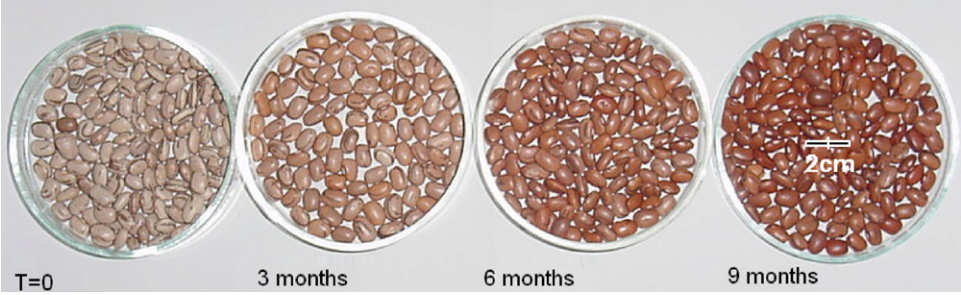

Fig. 1. Progress of bean hull discoloration during aging.

The aim of this study was to better understand bean hull cell wall polysaccharide composition and solubilization patterns and to understand the influence of aging on dietary fiber composition and organization.

2. Materials and methods

2.1. Plant material

Common bean seeds (*Phaseolus vulgaris* L. c.v. Carioca-Pérola), grown in Goiatuba county (GO, Brazil) and harvested in September were kindly provided by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

2.2. Sample preparation

Control sample. Recently harvested seeds were dehulled manually and freed from the germ. The cotyledons and hulls were frozen in liquid N_2 and freeze-dried.

Aged sample. Portions of 100 g of whole seeds were stored in a hot chamber at 30 °C and 75% relative humidity (RH) for eight months. At the end of storage, the cooking time was determined as described below. The seeds were dehulled, and cotyledons and hulls were frozen in liquid N_2 and freeze-dried.

Cooking time determination. Control and aged seeds were soaked overnight, and the cooking time was determined according to Mattson (1946). Cooking time was defined as the mean time, over four replications, when 50% of the beans were considered cooked, as indicated by plunger dropping, penetrating each bean. At the end of the accelerated aging, the cooking time increased by a factor of five (from 33 to 156 min).

Proximate composition. The Standard Association of Official Analytical Chemists (AOAC) (1995) methods were used to determine ash, crude fat, protein and dietary fiber contents. Moisture content was defined as weight loss after heating whole bean flour ($n = 4$) at 105 °C for 12 h.

Non-starch polysaccharide extraction. The water-soluble polysaccharides (WSP) and water-insoluble polysaccharides (WIP) were isolated according to Shiga and Lajolo (2006). About 1 g of tegument flour were incubated with 15 mL $CHCl_3$:methanol (1:1, v/v) at 45 °C for 30 min and centrifuged at 9000 × *g* for 15 min. The residue was washed with 15 mL methanol and 15 mL acetone and dried. The de-fatted flour was homogenized with 40 mL of 0.08 M phosphate buffer using a tissue homogenizer with a Teflon® pestle. The suspension pH was adjusted to 6.0 and 0.1 mL of α-amylase (Sigma–Aldrich Co., USA) was added and incubated for 30 min in boiling water. At the end, the pH was adjusted to 7.5 and the mixture incubated with 0.1 mL with a protease (5 mg/mL solution; Sigma–Aldrich Co., USA) for 1 h at 60 °C. The pH was readjusted to 4.3 and then, 0.3 mL of amyloglucosidase (Sigma–Aldrich Co., USA) was added and incubated for 1 h at 60 °C. The suspension was centrifuged for 9000 × *g* and the supernatants were dialysed for 48 h against distilled water, freeze-dried and named water-soluble cell wall polymers (WSP). The residue was washed exhaustively with distilled water and treated with 15 mL of 0.5 M sodium phosphate buffer, pH 7.2. The remaining residue was treated with 15 mL of 90% dimethyl sulfoxide (DMSO) for 20 min in an ultrasonic bath, washed with 15 mL of 90% DMSO and rinsed with distilled water. The final residues were suspended in water, freeze-dried and named water-insoluble polymers (WIP).

The WIP were fractionated with a chelating agent (CDTA solution) and alkali gradient (0.01–4 M NaOH) as described in Shiga and Lajolo (2006). The pectins were precipitated by adjusting the reaction mixture to 80% EtOH (v/v).

Ion exchange chromatography. Anion exchange chromatography of WSP was performed according to Shiga et al. (2009). The WSP were fractionated on a Q-Sepharose FastFlow column (20 mm × 2.6 cm; Amersham Pharmacia Biotech, Uppsala, Sweden), and polymer fractions were named according to elution time as Pool 1 (first peak to be eluted) and Pool 2 (last peak to be eluted).

Carbohydrate composition and linkage analysis. The neutral monosaccharide composition and linkage analysis was obtained by GC–FID and GC–MS, according to Carpita and Whittern (1986) and Gibeaut and Carpita (1991). Sugar standards were purchased from Sigma Chemical Co. (USA) and inositol was used as internal standard.

Uronic acid determination. WIP and WSP fractions were homogenized using Teflon® pestle, forming a fine suspension or dissolved in distilled water (0.5 mg/mL).The uronic acids content were determined according to Filisetti-Cozzi and Carpita (1991).

Table 1
Proximate composition of hulls of control and aged beans.

Sample		Proximate composition (g 100 g^{-1} FW)				
		Protein	Ash	Fat	Moisture	Total
Hulls	Control	8.93 ± 0.71^{a}	4.96 ± 0.44^{a}	0.58 ± 0.09^{a}	2.72 ± 0.30^{a}	17.19 ± 0.89^{a}
	Aged	9.10 ± 0.58^{a}	5.08 ± 0.30^{a}	0.73 ± 0.10^{a}	2.53 ± 0.19^{a}	17.44 ± 0.69^{a}
Cotyledon*	Control	23.9 ± 0.58^{a}	$4.8 \pm 0.05^{a,d}$	2.0 ± 0.06^{b}	7.52 ± 0.15^{b}	38.17 ± 0.60^{b}
	Aged	22.2 ± 0.10^{d}	$4.1 \pm 0.17^{b,c}$	2.0 ± 0.11^{b}	4.13 ± 0.08^{c}	32.35 ± 0.24^{c}

Data obtained from Shiga et al. (2009).
Different letters indicate significant differences.