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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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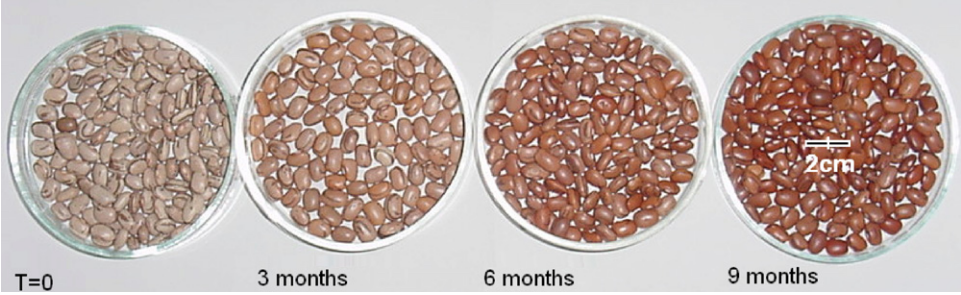

Fig. 1. Progress of bean hull discoloration during aging.

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.

Table 2
Comparison of dietary fiber profile in water-soluble (WSP) and water-insoluble (WIP) non-starch polysaccharides from control and aged bean hulls.

Sample	Dietary fiber (g 100 g^{-1} FW)			Non-starch polysaccharides (g 100 g^{-1} FW)		
	Insoluble	Soluble	Total	WIP	WSP	Total
Control	65.09 ± 5.14^{a}	3.98 ± 0.97^{a}	69.06 ± 5.23^{a}	71.1 ± 0.6^{a}	3.4 ± 0.2^{a}	74.5 ± 0.6^{a}
Aged	66.84 ± 3.68^{a}	5.10 ± 1.13^{a}	71.94 ± 3.85^{a}	75.8 ± 0.3^{b}	2.6 ± 0.2^{b}	78.3 ± 0.4^{b}

Different letters indicate significant differences.

3. Results and discussion

Recently harvested Carioca bean hulls ($T = 0$) are light brown with dark brown stripes (Fig. 1). The storage conditions did not change the moisture content (Table 1) and did not result in fungus development on the seeds' surface. However, storage did cause a visible discoloration of the hulls, turning them a bright dark brown color (Fig. 1), and increased the cooking time by a factor of five (Shiga et al., 2009). Hull darkening is evidence of phenolic compounds polymerization, which, along with protein cross-linking leads to bean hardening and hull impermeabilization, likely due to a lignification-like mechanism (Stanley & Aguilera, 1985; Hincks & Stanley, 1986; Srisuma et al., 1989).

Proximate composition and polysaccharides composition. Carioca beans are composed of about 89% cotyledon, 1.5% epycotyl and 10% hulls. The hulls are composed of about 70% dietary fiber and minor amounts of protein (~9%), ash (~5%), fat (~0.6%) and moisture (~2.5%). No considerable changes in this proximate composition were observed with aging (Table 1), in contrast to what had observed with bean cotyledon (Shiga et al., 2009).

While no differences were observed in the dietary fiber amounts with aging (Table 1), changes occurred in the amounts of non-starch polysaccharide. Before aging, hulls were composed of 71% water-insoluble polymers (WIP) and 3.4% water-soluble polymers (WSP) (Table 2); with aging, WIP increased by 5% while WSP decreased by 0.8%.

In whole beans, hulls account for 7% of all WIP, only a little less than cotyledons, which accounts for about 10% (Table 2). The cotyledon and the hulls together account for an increase of about 2 g of WIP and a decrease of 1.5 g in WSP per 100 g of beans, which may produce a significant difference in the non-starch polysaccharide profile and consequently in the dietary fiber profile (Table 2).

Non-starch-polysaccharide composition of hulls. The monosaccharide composition of both fractions (WIP and WSP) was determined in order to find changes in the polysaccharide solubilization pattern. According to the results, rhamnosyl residues and the uronic acid content increased in WIP (about 2 times and 30%), suggesting galacturonan insolubilization (Fig. 2).

Xylosyl residues decreased markedly in WIP (48%), likely due to a lignification-like mechanism that may make xylans less susceptible to acidic hydrolysis remaining unhydrolysed by TFA together with cellulose. Several cross-links in seed hull components may cause acidic and neutral polysaccharide insolubilization, inhibiting their hydrolysis and posterior quantification. It is well-known that aging causes phenolic compound polymerization and that their binding to pectin can increase by a factor of two in hard beans (Garcia, Filisetti, Udaeta, & Lajolo, 1998; Liu, 1995). Ferulic acids esterified to pectin can form diphenyl or ether bonds between the hydroxyl groups of phenolic compounds and the hydroxyl groups on polysaccharides. The structural proteins can also form covalent, interpolymeric linkages with ferulic acids, reinforcing polysaccharide structure (Brett & Waldron, 1996; Fry, 1983; Ishii, 1997). Moreover, there is a correlation between xylan degradability and lignin content. For example, tissues with higher lignin content show a decrease in xylan degradation (Grabber, Panciera, & Hatfield, 2002).

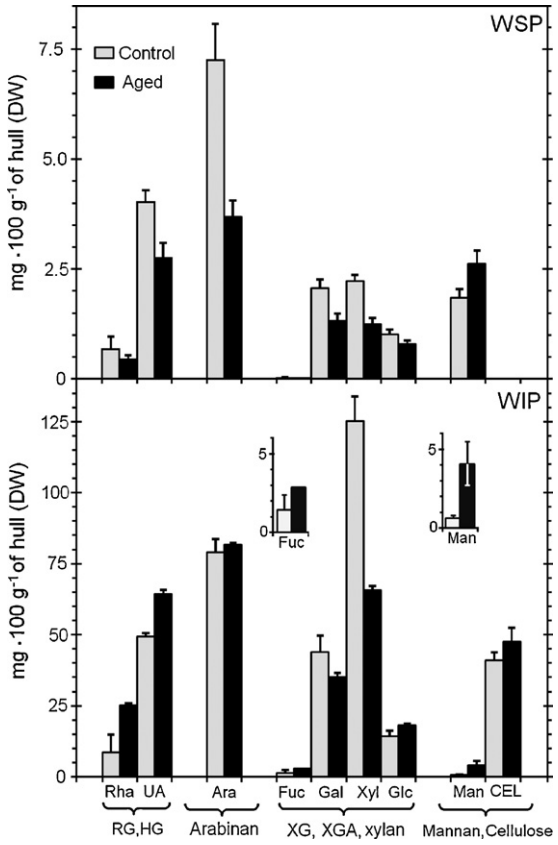

Fig. 2. Bean hull water-soluble polysaccharide (WSP) and water-insoluble polysaccharide (WIP) monosaccharide composition. UA: uronic acid content; CEL: glucose derived from cellulose; RG: rhamnogalacturonan; HG: homogalacturonan; XG: xyloglucan; XGA: xylogalacturonan; DW: dry weight. Values are means of three determinations ±SD.

Arabinosyl residues decreased 49% in WSP and may be correlated to a decrease in arabinan degradation (Fig. 2). Xylose and galactose contents also decreased in the WSP (44% and 56%, respectively), suggesting xyloglucan (XG) and xylogalacturonan (XGA) insolubilization (Fig. 2). Mannose content, in turn, was higher in WSP of aged seeds (41%), probably caused due to the decrease in pectin and ratios, since the extraction enzymes are mannose-rich (Fig. 2). Hence, the identification of neutral and acidic polysaccharides of WSP as well as their ratio may reveal which cell wall component had become less soluble due to aging.

Anion exchange chromatography. The WSP anion exchange chromatography produced two peaks, one composed mainly of neutral pectins (Pool 1) and another containing mostly acidic pectins (Pool 2) (Fig. 3). The proximate areas under Pools 1 and 2 of aged and soft seeds were calculated and its ratios (area of Pool 2/area of Pool 1) calculated. The results revealed that in aged seed WSP elution profile there was an increase in the area under Pool 2 and a shift of these compounds to a more acidic regions. This indicates an increase of acidic polymers in the WSP of aged seeds (Fig. 3). The monosaccharide compositions of both fractions (Pools 1 and 2) are consistent with an increase of acidic polymers in the WSP of aged seeds (Fig. 4).

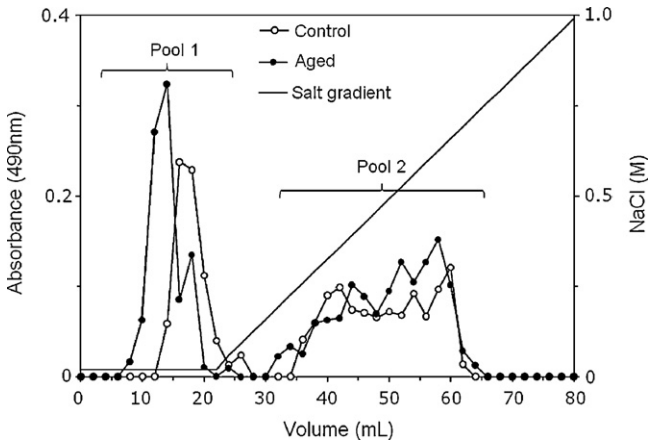

Fig. 3. Anion exchange chromatography of hull water-soluble polysaccharides (WSP).

Hence, the solubilization of polymers in aged seeds is lower than in softer seeds and the polymers solubilized is more acidic.

Rhamnose, galactose and glucose decreased 3, 4 and 3% in Pool 1, respectively, while arabinose increased 9% (Fig. 4). Pool 2, in turn, showed an increase of rhamnose, galactose and glucose contents (3, 6 and 5%, respectively), while xylose and arabinose values decreased (4 and 12%, respectively) (Fig. 4). The decrease of rhamnosyl, galactosyl and glucosyl residues suggests that Pool 1 of aged seeds has less rhamnogalacturonans (RG), galactans and xyloglucans (XG), while the increase of arabinosyl and xylosyl residues suggests higher amounts of xylans, xylogalacturonans (XGA) and arabinans. The presence of XGA was also reported in the pectin of pea hulls by Renard, Weightman and Thibault (1997).

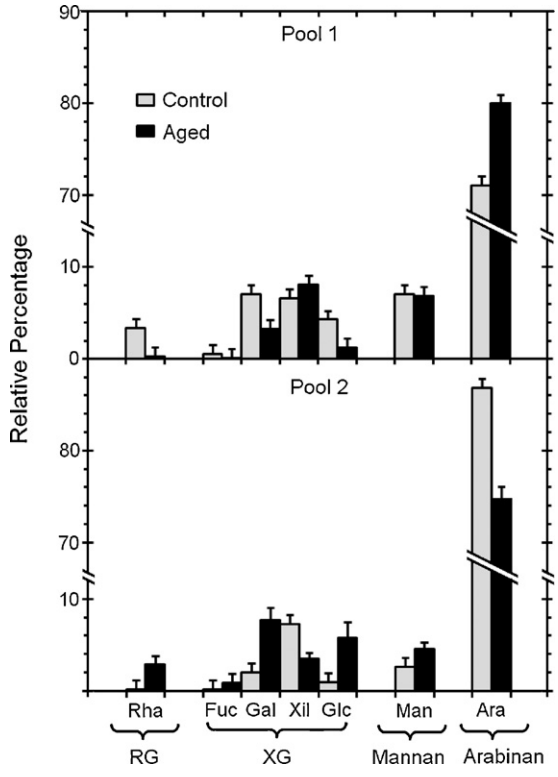

Fig. 4. Monosaccharide composition of water-soluble polysaccharide (WSP) fractions obtained from anion exchange chromatography. Pool 1: less acidic polymers; Pool 2: more acidic and more retained polymers; RG: rhamnogalacturonan; XG: xyloglucan.

Pool 2 of aged seeds showed higher amounts of RG, XG and galactans and lower amounts of XGA and xylans, suggesting separation of highly branched regions from less branched regions. The decrease in rhamnosyl residues along with the increase in arabinose content in Pool 1 of aged seeds corroborates the aforementioned (Fig. 4).

Linkage analysis. The most prominent change in the WSP of aged beans was the increase in the amounts of *t*-man*p* and 2-man*p* (15 and 3%) and the decrease in the 5-ara*f* levels (Fig. 5). Aged bean WSP contained lower amounts of 5-ara*f* residues (16%) which resulted in a 5% decrease arabinan content (Figs. 5 and 6). The decrease in 1,5-linked arabinofuranosyl residues suggests that the arabinans of WSP in aged seeds are shorter. On the other hand, the increase of mannosyl residues is, similar to the cotyledon, a consequence of the reduction in the amount of pectins in relation to the mannose-rich polymers derived from the extraction enzymes.

A degree of branching of the XG of control and aged beans was calculated from the amounts of 4-glc*p* and 4,6-glc*p* using the data from Fig. 5 (Fig. 6). Low branching values were found in the WIP for both control and aged samples (8 and 7%, respectively). In the WSP, no XG branching was detected in control seeds, while the degree of branching was 15% higher in aged seeds (Fig. 6). Using the same principle, the degree of branching for arabinan was also calculated. In the WIP of aged beans, arabinan degree of branching is 7% lower than in control. In the WSP, the arabinan degree of branching did not change with bean aging.

The decrease of galactosyl and glucosyl residues (2-gal*p* and 4-glc*p* and in 3 and 9%, respectively and 5-ara*f* and 3,5-ara*f*, both in 2%) in WIP suggests a decrease in XG amounts and the presence of low-branched arabinans in the WIP (Figs. 4 and 6). On the other hand, 4-xyl*p* + 2-xyl*p* values in the WIP of aged beans increased 10% and could be indicative of an increase in the xylan contents (Fig. 6). The increase of the UA and *t*-xyl*p* levels (5 and 1%, respectively) in the WIP (Figs. 2 and 4) suggests that XGA and or galacturonans insolubilization also occurred. Small amounts of XGA may also became less soluble, and the increase of hull darkening and galacturonans insolubilization (HG and XGA) (Figs. 1 and 5) may occur due to phenolic compound polymerization. The start of a lignification-like mechanism is suggested as one cause of seeds hardening in various studies conducted with leguminous seeds as described in Liu (1995). Hence, in the WIP there was a decrease of XG and a slight increase of xylan which, along with a substantial increase of arabinans contents, may explain the significative decrease in the total xylopiranosil ratios observed in Fig. 2.

The insolubilization of galacturonans is corroborated by the increase of WIP amounts in aged seeds.

The increase in the *t*-xyl*p* residues and decrease in the 4- and 4,6-glc*p* residues and 2,5-ara*f* and 3,5-ara*f* residues in the WIP of aged seed suggest that arabinans and XG are less branched and more fragmented in this fraction (Figs. 2 and 6). The decrease of arabinans (10%) and increase of highly branched XG (14%) in the WSP of aged seeds hulls, as well as the decrease in the WSP amounts suggests the insolubilization of straight regions of xyloglucans and the high molecular mass neutral pectic material in aged seed hulls. The acidic rhamnogalacturonans also became more branched (almost 11%) in the WSP compared to control and may be evidence of homogalacturonan insolubilization. Ageing caused a selective insolubilization of wall polysaccharides, resulting in an increase in the WIP fraction and a decrease in the WSP fraction. The WIP fraction of the aged beans was characterized by a higher content of low-branched acidic pectin (homogalacturonan) and lower content of branched neutral polysaccharides (XG and arabinans). The WSP fraction of the aged beans was composed of highly branched XG and rhamnogalacturonans (highly soluble polymers).

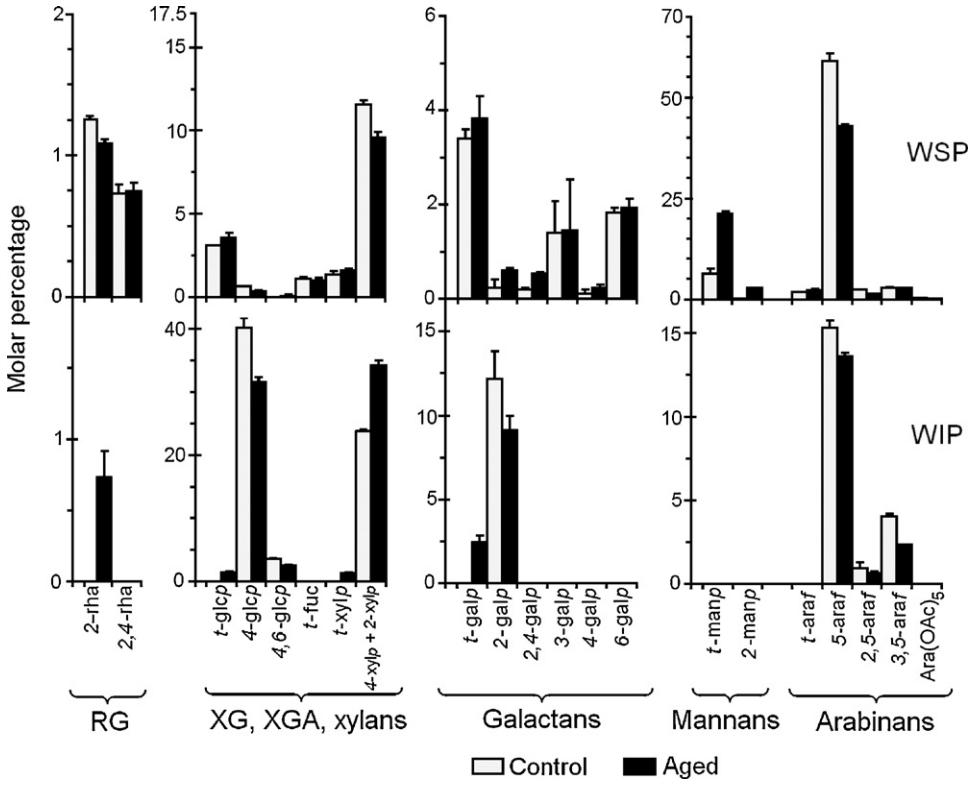

Fig. 5. Linkage analysis of bean hull cell wall polysaccharides and the related polysaccharides. WSP: water-soluble polysaccharides; WIP: water-insoluble polysaccharides; RG: rhamnogalacturonans; XG: xyloglucan; XGA: xylogalacturonans. Linkages were deduced from partially methylated alditol acetates; *t*-ara*f*: terminal arabinofuranose; *t*-xyl*p*: terminal-xylopyranose; 5-ara*f*: an arabinosyl residue containing a C-5 linkage; 2-xyl*p*: 2-linked xylopyranose; 3,5-ara*f*: arabinosyl residue containing C-3 and C-5 linkages.

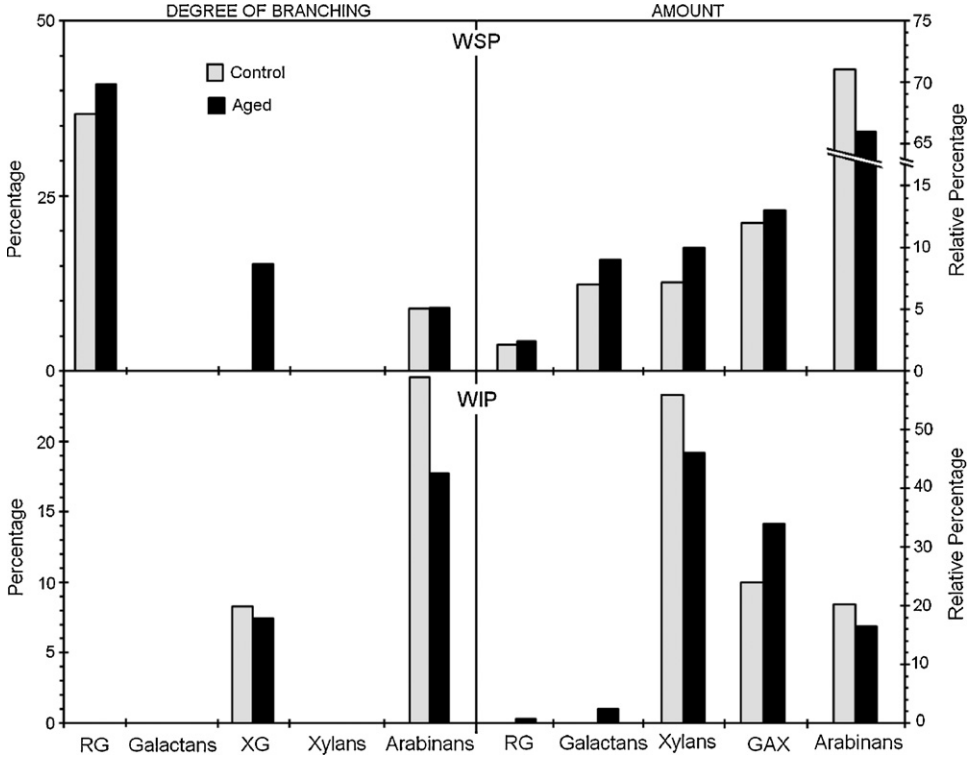

Fig. 6. Relative percentage of polymers related to the partially methylated monosaccharides and degree of branching of bean hull cell wall polysaccharides. WSP: water-soluble polysaccharides; WIP: water-insoluble polysaccharides; rhamnogalacturonans (RG; 2-rha, 2,4-rha); galactans (*t*-gal*p*, 3-gal*p*, 4-gal*p*, 6-gal*p*); xyloglucans (XG; *t*-glc*p*, 4-glc*p*, 4,6-glc*p*, t-fuc, 2-gal*p*, 2,4-gal*p*, t-xyl*p*); xylans (4-xyl*p* + 2-xyl*p*); arabinans (*t*-ara*f*, 5-ara*f*, 2,5-ara*f*, 3,5-ara*f*, Ara $(OAC)_5$). Linkages were deduced from partially methylated alditol acetates.

4. Conclusions

Aging caused changes in hull polysaccharide structure, solubility and hydrolysis, resulting in an increase in WIP and decrease in WSP with consequent changes in polysaccharide solubilization and degradation patterns. This study shows that aging changed the non-starch polysaccharide physicochemical properties, which may affect the metabolism of these compounds by gut bacteria, producing alterations in the physiological response of the organism.

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