Carbohydrate Polymers 76 (2009) 100–109

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Effect of cooking on non-starch polysaccharides of hard-to-cook beans

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ARTICLE INFO

Article history:
Received 7 August 2007
Received in revised form 8 August 2008
Accepted 25 September 2008
Available online 10 October 2008

Keywords:
Beans
Phaseolus vulgaris
Common bean
Hard-to-cook
Non-starch polysaccharide

ABSTRACT

Experiments carried out to study changes induced by hard-to-cook (HTC) phenomenon in the non-starch polysaccharides of beans stored at 30 °C and 75% RH for 8 months showed that the development of HTC did not affect the amounts of soluble and insoluble fibre in cooked seeds but changed their carbohydrates physical properties. Aged beans non-starch polysaccharides presented lower water-solubility and underwent lower degradation of galacturonans and arabinose-rich polysaccharides when submitted to cooking. The decrease in non-starch polysaccharides water-solubility produced a shift in the polymers fractionation profile which resulted in an increase of weak and middle-alkali soluble polymers bulk as well as in their arabinose and uronic acid contents. Uronic acid contents were higher in polymers released by 1 M NaOH and in the cellulose-rich residues while the arabinose contents were higher in the mild-alkali soluble polymers of aged seeds. Methylation analysis showed no evident alterations in the xyloglucans and arabinans branching degree with beans ageing. However, both, the molecular mass of water-soluble pectins and CDTA-soluble pectins, increased. Even though changes in the non-starch polysaccharide solubility were not related to the decrease in the arabinan and xyloglucan degree of branching they may be related to the formation of new chemical interactions other than hydrogen bond. There was a correlation between acidic and neutral polysaccharides insolubilisation in beans ageing and probably in beans hardening. After processing, aged seeds present higher amounts of insoluble fibre when compared to normal beans.

1. Introduction

Bean is a nourishing food widely consumed by populations of developing countries (Bressani, 1993; Reyes-Moreno & Paredes-López, 1993). However, leguminous seeds submitted to long periods of storage at high temperature and humidity undergo gradual loss of nutritional and textural quality (Hincks & Stanley, 1986; Liu, 1995; Reyes-Moreno, Cárabez-Trejo, Paredes-López, & Ordorica-Falomir, 1994). This phenomenon, known as hard-to-cook (HTC) defect is characterised by extended cooking times for cotyledon softening (Liu, 1995). Defective seeds resistance to soften by cooking can be attributed to multiple mechanisms, such as lipid oxidation, insoluble pectates formation and middle lamella lignification (Liu, 1995).

Legume seeds and cereals are a good source of dietary fibre. Dietary fibre (DF) is a general term used to refer to components of food that are indigestible in the small intestine. In plant-based foods, DF includes complex polysaccharides derived from cell wall and protoplast (e.g. celluloses, hemicelluloses, pectins, gums and resistant starches) and non-carbohydrate components as such as lignin. Polysaccharides derived from plant cell wall are commonly defined as non-starch polysaccharides and comprise the majority of the dietary fibre.

The dynamic structure of plant cell wall is composed of complex polysaccharides, phenolic compounds and proteins stabilised by ionic and covalent linkages (Brett & Waldron, 1996; Carpita & Gibeaut, 1993). Polysaccharides, besides conferring rigidity, strength and shape to the cell are also responsible for textural properties in the plant-based foods (Bourne, 1983; Brett & Waldron, 1996; Jackman & Stanley, 1995). They perform an essential role in human health through their water-holding capacity and production of short-chain fatty acids after fermentation in the lower intestine (Oakenfull, 2001). Changes in their polymeric structure alter their viscosity, solubility and fermentation capability influencing their physiological properties as well as the type of microbial activity in the lower intestine (Eastwood, Brydon, Path, & Anderson, 1986). Structural alterations may also change the sensorial and nutritional characteristics of plant-based foods.

During cooking process, the high temperature affects the polysaccharides structure in many ways, mostly breaking linkages and

Abbreviations: AIF, alcohol-insoluble fraction; ASF, alcohol-soluble fraction; CDTA, *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′,-tetraacetic acid; CEL, cellulose; HTC, hard-to-cook; P1, P2 and P3, polymers obtained in the peaks 1, 2 and 3 of WSP anion-exchange chromatography; ROI, reactive oxygen intermediates; WIP, water-insoluble polysaccharides; WSP, water-soluble polysaccharides.

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doi:10.1016/j.carbpol.2008.09.035

promoting depolymerisation (Mattson, 1946, Del Valle & Stanley, 1995; Ilker & Szczesniak, 1990; Jones & Boulter, 1983). Cooking process softens the plant tissues, improves the texture and palatability of plant-based foods and helps to increase the access of digestive enzymes to the starch and protein present inside the cell. This process also promotes polysaccharides depolymerisation and changes the nutritional properties of the dietary fibre by increasing its water-solubility (Brett & Waldron, 1996).

At high levels, depolymerisation can cause a decrease in the DF content. At low levels it has a minor influence on the dietary fibre content, but may affect the functional and physiological properties of the fibre. Thus, solubility is important because of its influence on the nutritional properties of DF. While soluble fibre is commonly associated to the reduction in cardiovascular disease, insoluble fibre shows protective effects against colorectal cancer (Oakenfull, 2001).

According to the literature, changes in the cell wall polysaccharide structure and organisation seem to be the main cause of HTC (Hincks and Stanley, 1986; Liu, 1995). Studies in our laboratory also suggest a connection between non-starch polysaccharide insolubilisation and beans hardening (Shiga, Lajolo, & Filisetti-Cozzi, 2003, 2004). These alterations may affect the nutritional properties of the food by changing polysaccharides solubility. Hence a better knowledge of polysaccharides composition and structure in defective beans may help to understand the HTC phenomenon and its consequences to the nutritional quality of legume seeds.

The present study takes into account the occurrence of changes in the non-starch polysaccharides during storage, which not only may be responsible for beans hardening but can also affect the nutritional quality of beans stored at bad conditions. A better knowledge of cell wall polysaccharide composition and solubilisation patterns can lead to a better understanding of how the HTC phenomenon affects these compounds. Moreover, a detailed composition of soluble and insoluble non-starch polysaccharides in normal and defective beans helps to better understand the dietary fibre physical and physiological properties for human health.

The cultivar Carioca-Pérola, developed and widely cultivated in Brazil, was chosen for this work because of its higher commercial qualities (larger seeds with shorter cooking time and good taste that yield a thick broth after cooking), higher productivity and disease-resistant characteristics and mainly because of its higher proneness to develop the HTC phenomenon.

2. Materials and methods

2.1. Plant material

Common beans seeds (*Phaseolus vulgaris* L. c.v. Carioca-Pérola), grown in Goiatuba county (GO, Brazil) and harvested in September (1999) were kindly provided by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

2.2. Determination of cooking time

The control and aged seeds were soaked overnight and the cooking time was performed according to Mattson (1946). Cooking time was the meantime over four replications, when 50% of the beans were cooked, as indicated by plunger dropping, penetrating each bean. At the end of the accelerated ageing the cooking time increased by a factor of 5 (from 33 to 156 min).

2.3. Samples preparation

2.3.1. Control sample

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

2.3.2. Aged samples

Portions of 100 g of whole seeds were stored in hot chamber at 30 °C and 75% relative humidity (RH) for 8 months. At the end of storage the cooking time was determined and the cotyledons were frozen in liquid N_2 and freeze-dried.

2.3.3. Control-cooked and aged-cooked samples

Portions of control and aged beans cotyledons were soaked for 5 h in cold deionised water (seed:water, 1:3, w/w) and cooked in the soaking water for 30 min (cooking time of 30 min began from the time the water boiled). At the end of 30 min cooking the cotyledon of both samples softened producing a paste that was freeze-dried. These samples were, respectively, named control-cooked and aged-cooked samples. The aim was to evaluate the susceptibility of normal and hard beans non-starch polysaccharides to the cooking process.

2.4. Proximate composition

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

2.5. Extraction of the non-starch polysaccharides

The water-soluble polysaccharides (WSP) and water-insoluble polysaccharides (WIP) were isolated according to Shiga and Lajolo (2006). The WIP was fractionated with chelating agent (CDTA solution) and alkali gradient (0.01–4 M NaOH) as described in Shiga and Lajolo (2006). All polysaccharide fractions were treated with *Trichoderma* sp. endo-1,4-β-glucanase (Megazyme International Ireland Ltd.; EC 3.2.1.4.) to hydrolyse xyloglucans (XG),[1] according to the procedure described in Shiga and Lajolo (2006). Pectins were precipitated adjusting the reaction mixture to 80% EtOH (v/v).

2.6. Size exclusion chromatography (SEC)

The molecular mass determination was performed according to Shiga and Lajolo (2006).

2.7. Ion-exchange chromatography

Anion-exchange chromatography of WSP was performed in order to separate neutral and acidic polymers. The WSP was fractionated on a column packed with Q-Sepharose FastFlow (20 mm × 2.6 cm; Amersham Pharmacia Biotech, Uppsala, Sweden) according to Shiga and Lajolo (2006). Polymer fractions were named according to elution time as P1, P2 and P3, being P1 the first peak to be eluted and P3 the last one. The P1 fraction was basically composed of neutral glucose and xylose-rich polymers and P2 and P3 fractions composed of acidic polymers containing neutral domains.

2.8. Carbohydrate composition and linkage analysis

The carbohydrate composition and linkage analysis was obtained by GC-FID and GC–MS, according to Carpita and Whittern (1986) and Gibeaut and Carpita (1991).

[1] *Carbohydrate abbreviations:* RG, rhamnogalacturonan; XG xyloglucan; XGA, xylogalacturonan; UA, uronic acid; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; The numbers and *t* notation previous to the sugar name means, respectively, the C-linkage and terminal linkage (e.g. *2,5*-ara*f* and *t*-ara*f* means, respectively, an arabinose linked by carbon 2 and 5 and terminal arabinose). The "*p*" and "*f*" notation means, respectively, pyranose and furanose.

3. Results and discussion

3.1. Proximate composition and ageing process

Protein and ash contents decreased 7% and 15%, respectively, with seeds ageing (Table 1). The amount of soluble fibre in aged seeds was about 60% lower than that in the control (Table 1). After cooking only minor differences were noticed between the proximate compositions of aged and control beans. Fat content decreased (60%) in aged-cooked beans when compared to that in control-cooked. No other significant differences were found between cooked samples (Table 1).

The decrease of soluble dietary fibre in aged samples was not accompanied by increase of insoluble fibre (Table 1). This apparent incoherence in the results may be credited to the methodology commonly used for the determination of soluble and insoluble fibre, since the decrease of water-soluble polysaccharides (WSP) in aged samples reflected in an increase of the water-insoluble polysaccharides (WIP) (Table 2). The dietary fibre analysis simulates a digestive process and results in a product that contains starch and non-starch polysaccharides as well as other undigestible components (such as, fructo-oligosaccharides, protein and resistant starch). The WSP and WIP extraction was harsher and intended to obtain no other components but cell wall polymers.

Berrios, Swanson, and Cheong (1999) showed that the content of crude protein, ash and crude fat of beans stored at room temperature for 2 years showing quality factors characteristic of HTC beans, were not significantly different from those found in beans prior to storage. This study shows that even though the beans proximate composition changed with ageing, there were no great differences between fresh and aged beans after cooking.

3.2. Relative percentage of bean non-starch polysaccharide fractions

The yields of pectin-rich fractions (WSP and CDTA) in aged seeds were 34% and 42% lower than those in the control, respectively (Table 2). This decrease reflected in the amount of water-insoluble polymers (WIP) which increased 18% in aged seeds. In contrast, hemicelullose-rich fractions (0.01 M and 0.5 M) and cellulose-rich fractions increased 0.4, 1.7 and 0.2 times, respectively (Table 2).

Heat-treatment affects the dietary fibre in different ways. At high temperatures there is a breakage of glycosidic linkages and of weak bonds among polysaccharide chains, changing and influencing the functional and nutritional characteristics of the food. When normal seeds were cooked an extensive non-starch polysaccharides breakdown occurred, resulting in decrease of WIP (32%), as well as in their pectin-rich and hemicelullose-rich subfractions (CDTA, 0.01 M, 0.5 M, 1 M and 4 M) (Table 2). However, when aged beans were submitted to cooking, only a negligible depolymerisation was produced, which resulted in high amounts of insoluble non-starch polysaccharides in aged-cooked beans which can be seen by their higher amounts of WIP (70% bigger than those of control-cooked beans) and lower amounts of WSP and CDTA fractions (Table 2). These results show that the degradation of non-starch polysaccharides was impaired in aged samples.

Table 2
Relative percentage on non-starch polysaccharides. Values correspond to the percentage obtained from the dry whole cotyledon flours. Values are means of six determinations.

Fractions	Control	Aged	Control-cooked	Aged-cooked
	(w/w)%			
WSP	6.2 ± 0.97^{a}	4.1 ± 0.23^{b}	5.7 ± 0.47^{a}	5.6 ± 0.48^{a}
WIP	10.9 ± 0.55^{a}	12.9 ± 0.75^{c}	7.4 ± 0.31^{b}	12.7 ± 1.37^{ac}
Total	17.1 ± 1.12^{a}	17.0 ± 0.78^{a}	13.1 ± 0.56^{b}	18.3 ± 1.45^{a}
WIP				
CDTA	1.2 ± 0.26^{a}	0.7 ± 0.05^{b}	0.7 ± 0.04^{b}	0.6 ± 0.08^{b}
0.01M	0.7 ± 0.11^{a}	1.0 ± 0.17^{b}	0.5 ± 0.10^{a}	1.1 ± 0.12^{b}
0.5M	0.7 ± 0.18^{a}	1.9 ± 0.48^{b}	0.6 ± 0.08^{a}	3.2 ± 0.66^{c}
1M	0.6 ± 0.04^{a}	0.7 ± 0.07^{a}	0.5 ± 0.03^{b}	1.7 ± 0.20^{c}
4M	5.3 ± 0.52^{a}	5.9 ± 0.45^{a}	3.7 ± 0.17^{b}	4.2 ± 0.52^{b}
Residue	2.3 ± 0.16^{a}	2.7 ± 0.22^{c}	1.4 ± 0.08^{b}	1.9 ± 0.30^{a}

WIP, water-insoluble polysaccharides; CDTA, polymers extracted by CDTA solution; 0.01–4 M, polymers extracted by 0.01–4 M NaOH; WSP, water-soluble polysaccharides. Means within a line followed by different letters are significantly different.

Non-starch polysaccharides breakdown is important because the cell wall polymers work as a barrier to the starch digestion by the gut enzymes. Moreover, the breakdown which occurs during cooking also changes the polysaccharides physiological properties making them more soluble in water. The presence of soluble dietary fibre to the detriment of insoluble fibre is desirable because the former is correlated to increase of stool bulk as well as the production of short-chain fatty acids in the large intestine (Eastwood et al., 1986). This study shows that after processing, aged seeds present higher amounts of insoluble fibre when compared to normal beans.

3.3. Non-starch monosaccharide composition

Changes in non-starch polysaccharides depolymerisation also results in modification of dietary fibre monosaccharide composition. This alteration may produce different fermentation patterns in the lower intestine.

The WIP monosaccharide composition of aged and control samples was similar between them (left side of Fig. 1). However, the arabinose contents of pectin-rich fractions (WSP and CDTA) were, respectively, 50% and 45% lower in aged seeds when compared to those in the control. Uronic acid (UA) contents in WSP and CDTA of aged seeds were also 60% lower when compared to those in the control (Fig. 1). This suggests that the amount of acidic polymers released by water and chelating agent decreased in aged beans. On the other hand, higher arabinose contents were found in the 0.01 M and 0.5 M fractions (~60% and 90%, respectively). Both fractions also showed low neutral sugar/uronic acid ratios (NS/UA) suggesting higher proportions of acidic polymers in both fractions. According to the results, the galacturonans became less

Table 1
Beans proximate composition of control and aged beans (35 °C/75% RH). Data expressed on a dry-weight basis. The cotyledon moisture content in the control seeds was of 7.5 ± 0.15%. Values are means of four determinations.

Samples	Protein	Ash	Fat	Total	Dietary fibre (%)		
	%				Soluble	Insoluble	Total
Control	23.9 ± 0.58^{a}	4.8 ± 0.05^{ad}	2.0 ± 0.06^{a}	30.6 ± 0.58^{a}	3.9 ± 0.13^{a}	19.0 ± 0.25^{a}	22.9 ± 0.28^{a}
Aged	22.2 ± 0.10^{d}	4.1 ± 0.17^{bc}	2.0 ± 0.11^{a}	28.2 ± 0.23^{b}	1.4 ± 0.02^{c}	16.2 ± 0.39^{bc}	17.6 ± 0.39^{b}
Control-cooked	25.0 ± 0.19^{bc}	3.9 ± 0.02^{bc}	2.0 ± 0.42^{a}	30.9 ± 0.46^{a}	5.7 ± 0.21^{b}	16.6 ± 0.23^{b}	22.3 ± 0.31^{c}
Aged-cooked	24.6 ± 0.30^{ac}	5.0 ± 1.02^{bcd}	0.8 ± 0.48^{b}	30.4 ± 1.16^{a}	5.1 ± 0.41^{b}	15.7 ± 0.34^{c}	20.8 ± 0.53^{d}

Means within a column followed by different letters are significantly different.

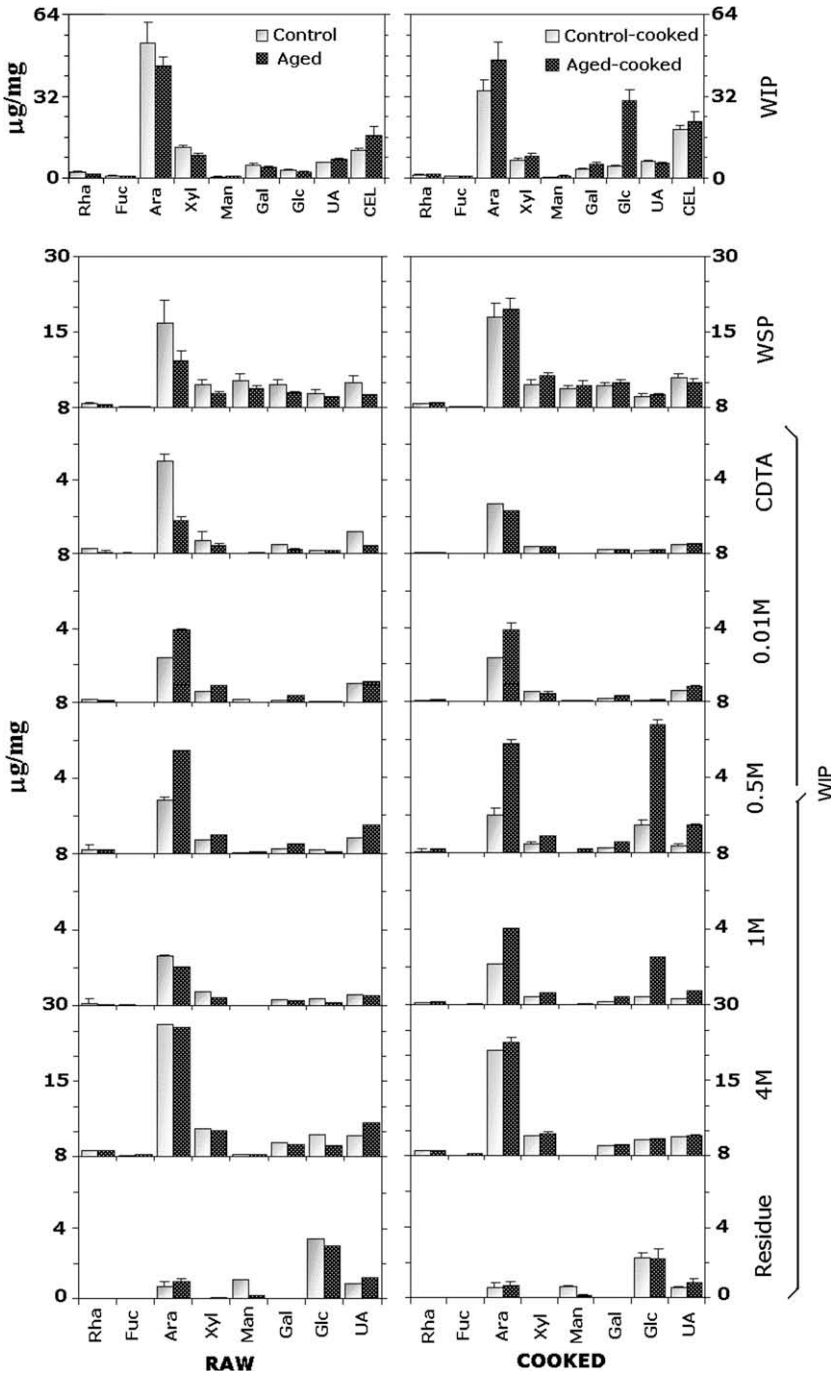

Fig. 1. Neutral sugar and uronic acid (UA) levels of cotyledon cell wall polymers of control, aged (30 °C/75% RH for 8 months), control-cooked and aged-cooked beans. WSP, water-soluble polysaccharides; WIP, water-insoluble polysaccharides; CDTA, polymers extracted by CDTA solution; 0.01–4 M, 0.01–4 M NaOH extracted polymers; Residue, cellulose-rich residue; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose, UA, uronic acids. The sugars were expressed as µg/mg of whole cotyledon (dry-weight), as a mean values of three determinations.

soluble in hot-water and chelating agent with beans ageing and could only be extracted with alkali solution. This shift in the polysaccharides solubility may have been caused by the creation of new interactions among the polymers.

Hydrothermal treatment affects pectins in the first instance due to their susceptibility to β-elimination. Hence, cooked samples may show whether changes in polysaccharides produced by ageing affected or not the depolymerisation during thermal processing.

Differences in WSP monosaccharide composition between aged-cooked and control-cooked samples were minimal (right side of Fig. 1). It can be assumed by these that the hydrothermal treatment was sufficient to produce a considerable polysaccharide degradation. However, the higher arabinose contents in the 0.01 M, 0.5 M and 1 M NaOH fractions of aged-cooked beans (0.6, 3.0 and 0.9 times higher than control-cooked, respectively) suggest that only lower amounts of neutral polymers were degraded in aged samples.

The bulk of WIP in aged-cooked beans was significantly bigger as well as its glucose content when compared to that in control-cooked samples. The glucose content was especially higher in the 0.5 M and 1 M subfractions. This increase in the glucose content may be attributed to resistant starch formation. With ageing, bean

starch shows gelatinisation enthalpy increase, resistance against amyloglucosidase attack. Its granules appear more birefringent under polarised light than those of control beans, making them more difficult to be removed (Garcia & Lajolo, 1994). However, this presumption requires further analyses.

The WIP of poor cooking lentils and beans have higher amounts of arabinose and the arabinan insolubilisation is correlated with beans ageing (Bhatty, 1990; Shiga et al., 2004). This study corroborates the literature showing that arabinose-rich polysaccharides, as well as the uronic acid-rich polymers lose their water-solubility and become less susceptible to depolymerisation during cooking.

3.4. Water-soluble polysaccharide (WSP) ion-exchange chromatography

The elution profile of the four samples were similar among them, despite the slight broadening of peak 2 (P2) observed in cooked samples (Fig. 2). With ageing raw seeds uronic acid contents in P2 and P3 increased in (56% and 35%, respectively). The mannose content also increased 2 times in the P3 of aged seeds (Fig. 2).

Cooking led to degradation of non-starch polysaccharide resulting in the production of short-chain fragments. Control seeds showed extensive depolymerisation of galacturonans during cooking, as it can be seen by the decrease in the uronic acid amounts in P1 and P3 fractions (Fig. 2). Aged seeds non-starch polysaccharides, in turns, were less soluble in water and their polymers underwent less degradation during cooking. Higher amounts of uronic acid in P2 and P3 confirm the aforementioned (values 3 and 4 times higher than that those of control) (Fig. 2).

The lower proneness of acidic polysaccharide to degrade under hydrothermal treatment changed the chemical characteristics of the dietary fibre in aged samples, producing a meal with higher amount of insoluble galacturonans. Differences were not restricted to the solubility of acidic carbohydrates, since defective seeds also showed higher contents of arabinose (67% in P1 and 32% in P3, when compared to normal seeds) (Fig. 2).

Normal beans showed higher depolymerisation of galacturonans and arabinose-rich polysaccharide. The results are in agreement with the literature since the galacturonans insolubilisation was observed in HTC beans (Aguilera and Rivera, 1992; Garcia, Lajolo, & Swanson, 1993).

3.5. Distribution of molecular mass

Bean non-starch polysaccharides showed low polydispersity and high apparent molecular mass profile (from 1.6 to 3.5 MDa) (Fig. 3). The molecular mass of aged beans WSP was about 60% higher than that found in control, revealing lower polysaccharide degradation in defective seeds (Fig. 3).

In cooked samples there were no differences between aged and control beans WSP molecular mass. However, calcium pectate

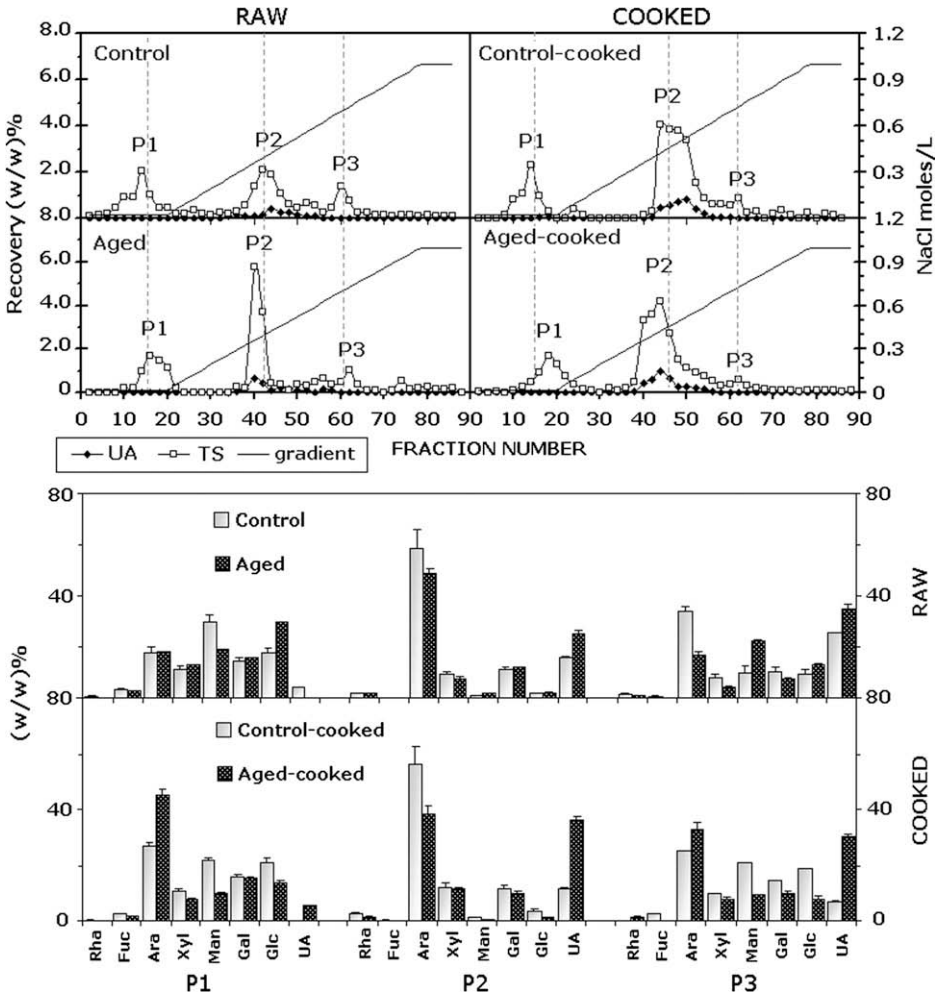

Fig. 2. Anion-exchange chromatography of the water-soluble polysaccharides (WSP) and neutral sugar and uronic acid levels. P1, P2 and P3 correspond to peaks 1, 2 and 3, respectively. Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acids. Values are means of three determinations.

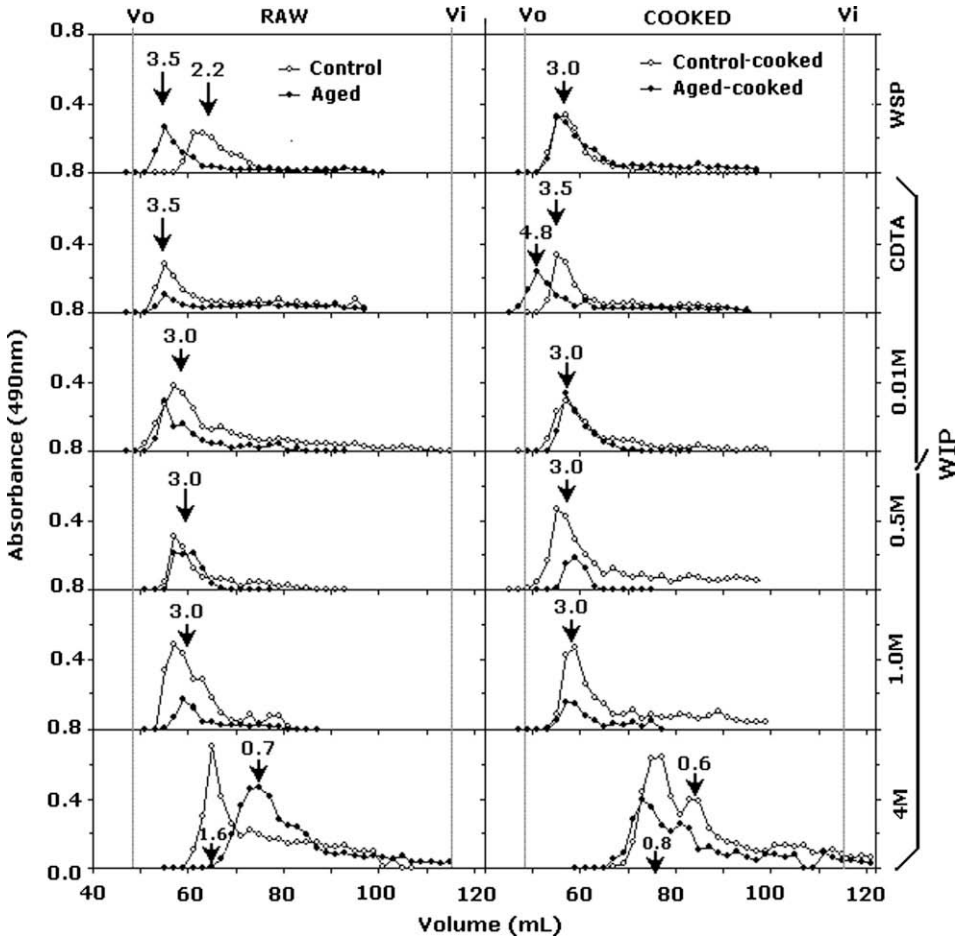

Fig. 3. Differences in the apparent molecular mass of beans polymers. Values over the arrows given in MDa.

Table 3
Glycosyl-linkage composition of the cotyledon cell wall polysaccharides. Values are means of three determinations.

Related polymer	Derivatives	Fractions (mol %)									
		4 M		0.01 M + 0.5 M + 1 M		CDTA		P2		WSP	
		Control	Aged	Control	Aged	Control	Aged	Control	Aged	Control	Aged
Arabinan	*t*-ara*f*	28	28	24	27	29	25	30	28	25	20
	5-ara*f*	24	22	30	28	32	27	34	31	26	22
	3,5-ara*f*	5	5	7	6	7	6	7	7	6	6
	2,5-ara*f*	5	5	7	6	7	6	6	5	3	4
	2,3,5-ara*f*	6	7	10	8	9	9	9	8	7	6
	DB (%)	40	43	44	42	42	44	39	39	38	42
XG XGA	*t*-glc*p*	3	2	2	1	2	2	–	1	2	2
	4-glc*p*	2	3	2	1	1	2	1	1	1	1
	4,6-glc*p*	5	6	1	1	1	2	–	1	2	3
	t-xyl*p*	13	13	12	14	7	14	7	12	9	11
	2-xyl*p* + *4*-xyl*p*	3	3	2	1	1	2	1	–	1	2
	t-fuc	1	1	–	1	–	–	–	–	1	1
	t-gal*p*	3	3	2	2	2	2	1	1	2	2
	2-gal*p*	1	1	1	–	0	1	–	–	1	1
	DB (%)	71	67	33	50	50	50	0	50	67	75
Galactans RG	*3*-gal*p*	–	–	–	–	–	–	1	1	1	1
	3,6-gal*p*	–	–	–	–	–	–	1	1	–	–
	4-gal*p*	–	–	–	1	1	1	1	1	–	1
	4,6-gal*p*	–	–	–	–	–	–	–	–	1	1
	6-gal*p*	1	1	1	1	1	1	1	1	1	1
	2-rha	1	1	1	1	1	1	1	1	1	1
	2,4-rha	–	–	1	1	1	1	1	1	1	1

(–), traces; DB, degree of branching. 4 M, polymers released by 4 M NaOH; 0.01 M + 0.5 M + 1 M, polymers solubilised by 0.01–1 M NaOH; CDTA, polymers released by CDTA solution; WSP, water-soluble polysaccharides; XG, xyloglucan; XGA, xylogalacturonan, RG, rhamnogalacturonan; P2, peak 2 obtained from WSP anion-exchange chromatography.

Table 4
Glycosyl-linkage composition of the cotyledon cell wall polysaccharides. Samples treated with cellulase and precipitated by adding 4 volumes of ethanol. Data correspond to alcohol-insoluble fractions (AIF). Values are means of three determinations.

Related polymer	Derivatives	Fractions (mol %)							
		4 M		0.01 M+0.5 M+1 M		CDTA		WSP	
		Control	Aged	Control	Aged	Control	Aged	Control	Aged
Arabinans	*t*-ara*f*	23	25	33	31	29	21	29	15
	5-ara*f*	42	50	37	33	45	53	34	52
	3,5-ara*f*	8	10	7	6	9	9	9	10
	2,5-ara*f*	6	6	4	6	3	3	4	2
	2,3,5-ara*f*	7	8	5	7	6	5	6	3
	DB (%)	33	32	30	37	29	24	36	22
XG, XGA	*t*-glc*p*	1	–	1	1	1	1	2	1
	4-glc*p*	1	–	1	2	–	1	2	2
	4,6-glc*p*	–	–	–	–	–	–	–	–
	t-xyl*p*	4	1	8	9	4	4	7	6
	2-xyl*p* + *4*-xyl*p*	1	–	–	–	–	–	1	1
	t-fuc	–	–	–	–	–	–	–	–
	t-gal*p*	3	–	2	1	1	2	1	5
	2-gal*p*	1	–	–	–	–	–	–	2
	DB (%)	0	0	0	0	0	0	0	0
Galactans, RG	*3*-gal*p*	–	–	–	–	–	–	1	–
	3,6-gal*p*	–	–	–	–	–	–	–	–
	4-gal*p*	–	–	–	1	–	–	1	–
	6-gal*p*	2	–	1	1	2	1	2	2
	2-rha	1	–	1	1	–	–	1	–
	2,4-rha	1	–	1	1	–	–	1	–

(–), traces; DB, degree of branching. 4 M, polymers released by 4 M NaOH; 0.01 M + 0.5 M + 1 M, polymers solubilised by 0.01–1 M NaOH; CDTA, polymers released by CDTA solution; WSP, water-soluble polysaccharides; XG, xyloglucan; XGA, xylogalacturonan; RG, rhamnogalacturonan; HG, homogalacturonan.

Table 5
Glycosyl-linkage composition of the cotyledon cell wall polysaccharides. Samples were treated with cellulase and precipitated by adding 4 volumes of ethanol. Data correspond to alcohol-soluble fractions (ASF). Values are means of three determinations.

Related polymer	Derivatives	Fractions (mol %)							
		4 M		0.01 M + 0.5 M + 1 M		CDTA		WSP	
		Control	Aged	Control	Aged	Control	Aged	Control	Aged
Arabinan	*t*-ara*f*	17	10	19	16	32	16	20	12
	5-ara*f*	13	16	17	21	31	22	26	44
	3,5-ara*f*	3	5	4	5	6	5	5	9
	2,5-ara*f*	2	4	3	5	6	6	3	9
	2,3,5-ara*f*	3	8	3	8	7	8	3	13
	DB (%)	38	52	37	46	38	46	30	41
XG, XGA	*t*-glc*p*	6	4	4	4	2	2	1	–
	4-glc*p*	4	4	6	6	4	21	24	12
	4,6-glc*p*	14	14	10	9	2	1	2	–
	t-xyl*p*	13	11	15	10	5	3	6	–
	2-xyl*p* + *4*-xyl*p*	4	8	5	7	1	2	1	2
	t-fuc	2	2	2	–	1	–	–	–
	t-gal*p*	8	5	4	4	2	2	1	–
	2-gal*p*	11	9	9	7	2	7	7	–
	DB (%)	78	78	63	60	33	4.5	7.7	0
Galactans, RG	*3*-gal*p*	–	–	–	–	–	–	–	–
	3,6-gal*p*	–	–	–	–	–	5	–	–
	4-gal*p*	–	–	–	–	–	–	–	–
	6-gal*p*	–	1	–	–	1	1	1	–
	2-rha	–	–	–	–	1	–	–	–
	2,4-rha	–	–	–	–	–	1	–	–

(–), not detected; DB; degree of branching. 4 M, polymers released by 4 M NaOH; 0.01 M + 0.5 M + 1 M, polymers solubilised by 0.01–1 M NaOH; CDTA, polymers released by CDTA solution; WSP, water-soluble polysaccharides; XG, xyloglucan; XGA, xylogalacturonan, RG, rhamnogalacturonan; HG, homogalacturonan.

(CDTA fraction) of aged-cooked beans showed a molecular mass about 40% bigger than that of control-cooked. This result suggests lower degradation of calcium pectate in defective seeds after cooking (left side of Fig. 3) or, most probably formation of high amounts of calcium pectate in beans stored at high humidity and temperature.

The polymers of raw-aged beans 4 M fraction showed lower molecular mass when compared to those of control. However, when cooked samples were compared, aged and normal beans molecular mass showed no differences (Fig. 3). The higher amounts of WIP in aged-cooked beans may suggest that, as a rule, the polysaccharides of aged beans undergo lower degradation when under cooking.

Table 6
Summary table.

Data analysed	Fractions		Control	Aged	Conclusion
Fibre	Soluble		++++	++	Aged beans: soluble fibre ↓
	Insoluble		++++	+++	
	After cooking				
	Soluble		+++++	++++	Control beans: soluble fibre↑↑↑↑
	Insoluble		+++	+++	Aged beans: soluble fibre ↑↑
Non-starch polysaccharides (NSP) Amount	WSP		++++	+++	Aged and control: similar non-starch polysaccharides composition. Slight decrease WSP amount and increase in 0.5M fraction in aged samples.
	WIP		++++	+++++	
	WIP	CDTA	++	+	
	WIP	0.5M	+	++	
	WIP	1M	+	+	
	WIP	4M	++++	++++	
	After cooking				
	WSP		++++	++++	Control: higher solubilization and degradation of NSP. Aged: Lower degradation and solubilization of NSP. Higher amounts of alkali-soluble polysaccharides.
	WIP		++	++++	
	WIP	CDTA	+	+	
	WIP	0.5M	+	++	
	WIP	1M	+	++	
	WIP	4M	+++	++++	
Monosaccharide composition. neutral sugar/ uronic acid ratios (NS/UA ratio)	WSP		++++	+++++	Control: higher NS/UA ratio in the water-insoluble polymers, especially in alkali-soluble fractions. (lower amounts of acidic pectins) Aged: lower NS/UA in alkali-soluble polysaccharides. Higher NS/UA ratio in the water-soluble fraction. (higher amounts of acidic pectins in WIP and lower in WSP)
	WIP		++++++	++++	
	WIP	CDTA	+++	++++	
	WIP	0.01M	++	+++	
	WIP	0.5M	++++	++++	
	WIP	1M	+++++	++++	
	WIP	4M	++++++	++++	
	WIP	Residue	+++	++	
	After cooking				
	WSP		++	+++	Cooking decreased the amounts of neutral polysaccharides in the 1 and 4M fractions and in the WSP of control beans. Aged beans: low NS/UA ratio in WIP reveals lower degradation of acidic polymers when compared to control. Cooking also produce lower degradation of acidic pectins in aged beans WSP.
	WIP		++++++	++++	
	WIP	CDTA	+++	++	
	WIP	0.01M	+++	+++	
	WIP	0.5M	+++++	++++	
	WIP	1M	++++	++++	
	WIP	4M	+++	+++	
	WIP	Residue	+++	++	
WSP anion exchange chromatography (arabinose / UA)	P1		+++ / 0	+++ / 0	Higher arabinose / UA ratio in aged beans. Lower pectins solubilisation and galacturonan degradation
	P2		++++ / +	+++ / ++	
	P3		+++ / ++	++ / +++	
	After cooking				
	P1		+++ / 0	++++/ +	Control: similar profile after cooking
	P2		++++ / +	+++ / +++	
	P3		+++ / +	++++ / ++++	Aged: lower degradation of galacturonans
Molecular mass	WSP		+++	++++	Aged beans: slight increase in the water-soluble polysaccharides molecular mass
	CDTA		++++	++++	
	4M		++	+	
	After cooking				
	WSP		++++	++++	Aged beans: slight increase in the calcium pectate molecular mass
	CDTA		++++	++++++	
	4M		+	+	
Linkage analysis	Both were very similar				No apparent structural change

UA, uronic acid, 0.01, 0.5, 1 and 4 M, polymers solubilised by 0.01–4 M NaOH; CDTA, polymers released by CDTA solution; WSP, water-soluble polysaccharides; WIP, water-insoluble polysaccharides, P1, P2, P3, peaks 1, 2 and 3 named according to the order of elution.

3.6. Linkage analysis of cotyledon non-starch polysaccharides

It is well known that polysaccharides water-solubility is correlated with molecular mass and degree of branching and that highly branched polysaccharides are water-soluble while linear structure favour their interaction and aggregation. Studies carried out with radish and soybean seeds showed that during germination and storage the seed coat produces reactive oxygen intermediates

(ROI) (Khan, Hendry, Atherton, & Vertucci-Walters, 1996; Liu, 1995; Schopfer, Plachy, & Frahry, 2001) associated with the loss of viability and with HTC development (Liu, 1995). These ROIs may also induce scission of XGs and pectins *in vitro* (Fry, 1998; Fry, Dumville, & Miller, 2001; Tabbi, Fry, & Bonomo, 2001). Miller and Fry (2001) used Fenton-derived ·OH to prove that the XG sidechains of tamarind seeds are more susceptible to the attack of ·OH than its backbone. Hence, the presumption that polysaccharide sidechains can be attacked by ROIs under adverse conditions and result in less soluble and thermally stable XG aggregates is, in theory, feasible.

Bean methylated derivatives suggest a cell wall composed of branched arabinans, xyloglucans (XG), galactans and rhamnogalacturonan type I (Table 3). With these data, the degree of branching for the XG was calculated from the amounts of *4*-glc*p* and *4,6*-glc*p* of Table 3. Likewise, arabinan degree of branching was calculated from the amounts of *5*-ara*f*, *2,3,5*-ara*f*, *3,5*-ara*f* and *2,5*-ara*f*. These calculations produce an estimation of values, although useful for practical purposes (Tables 3–5). The degree of beans xyloglucan branching was about 60–70% while in arabinan was about 40%.

3.7. Endoglucanase treatment

Arabinans predominated in the undigested alcohol-insoluble fraction (AIF) while the products of endoglucanase digestion showed predominately XG oligomers (Tables 4 and 5). Likewise current literature (Gooneratne, Needs, Ryden, & Selvendran, 1994; Huisman et al., 2001; Renard, Weightman, & Thibault, 1997) beans also revealed the presence of XGA due to their high amounts of *t*-xyl*p* residues in the AIF in the absence of *4,6*-glc*p*. Since the *t*-xyl*p* from XGA was not present in the alcohol-soluble fraction (ASF), a more reliable XG degree of branching was obtained after cellulase treatment. The degree of branching of XG oligomers and arabinans of ASF and AIF also showed values in a 60% range (Tables 4 and 5) and, except for CDTA in ASF (Table 5), no other polymer fraction showed a significant decrease of XG degree of branching. Hence, this study revealed no significant evidence that XG and arabinans branching degree decreased with ageing.

The summary of these results as well as the main conclusions of this study, presented on Table 6, show that beans ageing was characterised by a decrease in the amounts of soluble and insoluble fibre, and that the differences of dietary fibre amounts between soft and hard beans disappeared after cooking. In contrast, non-starch polysaccharides composition revealed changes in the neutral sugar/uronic acid ratios in each polysaccharide fraction of raw and cooked beans (Table 6). The lower NS/UA ratio in aged beans water-insoluble polysaccharides may be related to galacturonan insolubilisation. Table 6 also shows that in soft seeds, there is a higher solubilisation of galacturonans and arabinose-rich pectins while in aged seeds there is less solubility and less proneness to degradation under cooking. Apparently, the structure of beans polysaccharides did not change with ageing since the structural analysis did not reveal changes in the arabinans and the XG degree of branching (Table 6). Except for WSP (raw sample) and CDTA-soluble polymers (cooked sample) which showed higher molecular masses in aged seeds, all the other non-starch polysaccharide from raw and cooked beans showed no differences in their molecular masses.

Changes in dietary fibre properties and composition are important because they may affect the fermentation of these compounds in the large intestine and also prevent enzymes action during digestion. Their non-starch polysaccharide structure showed differences in their structure while their solubility and proneness to degrade under cooking decreased with ageing. Ageing may not have affected considerably the structure of beans cell wall polysaccharides, but certainly their solubility.

The result of this work leads to the presumption that the decrease in the solubility of acidic pectin and the increase of arabinose content in non-starch polysaccharides of aged-cooked beans may have a correlation with ageing and, eventually, with beans hardening.

4. Conclusion

Solubilisation and depolymerisation of galacturonans and arabinose-rich polysaccharides during aged beans the cooking process reached lower levels. The development of HTC did not affect the amounts of soluble and insoluble fibre in cooked seeds but changed the physical properties of its carbohydrates. The non-starch polysaccharides of aged beans showed lower water-solubilisation and lower degradation when submitted to cooking. Decrease in non-starch polysaccharides water-solubility produced a shift in the polymers fractionation profile which resulted in an increase of the weak and middle-alkali soluble polymers bulk as well as in their arabinose and uronic acid contents. The uronic acid levels were especially higher in the polymers released by strong alkali solution and in the cellulose-rich residues while the arabinose contents were higher in the mild-alkali soluble polymers. Methylation analysis showed no obvious alterations in the xyloglucans and arabinans degree of branching with beans ageing. Although changes in the non-starch polysaccharides solubility were not related to the decrease in the arabinan and xyloglucan degree of branching, they may be related to the formation of new chemical interactions other than hydrogen bond. Acidic and neutral polysaccharides insolubilisation were related with beans ageing and may also be related with beans hardening. The occurrence of changes in the dietary fibre composition may result in the production of different products after their metabolisation in the large intestine which, in return, may produce different physiological responses in the organism.

Acknowledgements

The authors acknowledge Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support and scholarship.

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