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Mechanistic insight into common bean pectic polysaccharide changes during storage, soaking and thermal treatment in relation to the hard-to-cook defect

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ABSTRACT

Different mechanisms responsible for the development of the hard-to-cook defect in common beans during storage, their soaking behavior and softening during thermal treatment have been previously suggested. However, these mechanisms have not been sufficiently confirmed by direct molecular evidence. This research aimed at gaining a detailed mechanistic insight into changes occurring in Canadian wonder bean pectic polysaccharides during storage, soaking and/or thermal treatment in different brine solutions in relation to the development and manifestation of the hard-to-cook (HTC) defect. Both fresh or easy-to-cook (ETC) and stored (HTC) bean samples were either soaked or soaked and thermally treated in demineralized water, solutions of Na_2CO_3 and $CaCl_2$ salts followed by extraction of cell wall materials. Pectic polysaccharide properties examined included sugar composition, degree of methylesterification (DM), extractability and molar mass (MM). The DM of pectin from ETC and HTC beans was similar but low (<50%). Upon (pre)treatment in a Na_2CO_3 solution, solubilization of pectic polysaccharides, especially the strongly bound chelator- (CEP) and Na_2CO_3- (NEP) extractable pectins was enhanced leading to increased amounts of water extractable pectin (WEP). Also, there was a decrease in high MM polymers paralleled by an increase in β-elimination degradation products. These observations are in line with the fast cooking behavior of beans (pre)treated in a Na_2CO_3 solution. In contrast, (pre)treatment in a $CaCl_2$ solution hindered softening leading to the failure of the beans to cook. The beans (pre)treated in a $CaCl_2$ solution showed increased high MM polymers and lack of cell wall separation. Therefore, it can be inferred that development of the hard-to-cook defect in Canadian wonder beans during storage and its manifestation during soaking and subsequent thermal treatment is largely reflected by the pectic polysaccharide properties in line with the pectin hypothesis. Our data suggest the release of Ca^{++} leading to pectin cross-linking and the increase or decrease of β-elimination depolymerization. However, the relatively high amounts of neutral sugars and strongly bound NEP in HTC seeds do not allow to rule out the possible existence of non-Ca^{++} based pectin cross-linking.

1. Introduction

Prolonged cooking times associated with legumes can be attributed to the hard-shell (HS) and/or hard-to-cook (HTC) defect. Both the HS and HTC defect develops when legumes are stored under relatively high temperature (>25 °C) and humidity (>65%) conditions (Garruti & Bourne, 1985; Liu et al., 1992). For the HS effect, seed coat properties restrict water imbibition during the soaking and subsequent cooking processes. The impermeability of the seed coat to water could be due to biochemical changes such as oxidation of tannins, formation of protein–tannin complexes or biophysical changes such as size reduction (Stanley, 1992; Martin-Cabrejas et al., 1997; Nasar-Abbas et al., 2008; Pirhayati et al., 2011). The HTC defect is a phenomenon that is characterized by failure of cotyledons to soften to a desired texture within a reasonable cooking time (Stanley & Aguilera, 1985; Shehata, 1992). Consequently, processing/cooking costs increase due to high energy consumption associated with longer cooking times. In addition, HTC also reduces the nutritional quality and changes the textural quality of legumes (Reyes-Moreno & Paredes-Lopez, 1993). Overall, acceptability and utilization of legume seeds are reduced (Garcia & Lajolo, 1994; Liu, 1995; Garcia et al., 1998; Pirhayati et al., 2011).

Several possible mechanisms through which the HTC defect may develop have been hypothesized. Among them is the mechanism involving pectic polysaccharides through the pectin–cation–phytate model. It is postulated that activity of pectin methylesterase (PME)

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leads to demethoxylation of pectin that consequently cross-links with Ca^{++} released due to the hydrolysis of phytates by phytase, to form insoluble or more thermally stable pectate compounds (Mattson, 1946; Jones & Boulter, 1983; Moscoso et al., 1984; Liu et al., 1993; Mafuleka et al., 1993; Reyes-Moreno & Paredes-Lopez, 1993; Liu, 1995; Garcia et al., 1998; Galiotou-Panayotou et al., 2008). Further, demethoxylation of pectin may lead to restricted pectin β-elimination depolymerization (Albersheim et al., 1960; Diaz et al., 2007). Other mechanisms involve changes in physical and/or chemical properties of starch and/or proteins (Garcia-Vela & Stanley, 1989; Del Valle et al., 1992; Garcia & Lajolo, 1994; Liu et al., 1992; Liu, 1995, 1997) and lignification (Stanley, 1992; Rivera et al., 1989; Srisuma et al., 1989; Bhatty, 1990; Reyes-Moreno & Paredes-Lopez, 1993; Nasar-Abbas et al., 2008).

Soaking of legumes prior to thermal treatment in different types of brine solutions has been practiced for a long period of time. It affects the cooking quality (Bhatty, 1989; Garcia-Vela et al., 1991; Aguilera & Rivera, 1992; De León et al., 1992; Kinyanjui et al., 2015, under review) and the nutritional quality of legumes (De León et al., 1992; Reyes-Moreno & Paredes-Lopez, 1993; Eyaru et al., 2009; Fernandes et al., 2010; Kruger et al., 2015). With regard to cooking quality, the type of cations and/or anions present in the soaking and/or cooking brine determines whether the cooking times could be shortened or prolonged. Soaking in bivalent salt in particular Ca^{++} solutions results in hardening while, soaking in monovalent salt (e.g. Na^{+}) solutions and solutions containing chelating agents (e.g. phytic acid or EDTA) leads to faster softening (Garcia-Vela et al., 1991; Aguilera & Rivera, 1992; Reyes-Moreno & Paredes-Lopez, 1993; Pirhayati et al., 2011, Kinyanjui et al., 2015, under review). The effects of soaking and thermal treatment on the cooking quality of legumes are often associated with the pectin hypothesis (Jones & Boulter, 1983; Moscoso et al., 1984; Kinyanjui et al., 2015, under review).

Pectin is a complex polysaccharide found in cell walls and generally consists of three domains: homogalacturonan (HG) (smooth region), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) (hairy regions) (Ridley et al., 2001; Vincken et al., 2003). Homogalacturonan is a linear homopolymer consisting of (1 → 4)-α-linked-D-galacturonic acid (GalA) (Voragen et al., 2009). The carboxyl moieties of the polymer are esterified to a certain degree with methanol at C-6 (Voragen et al., 2009). The ratio of the methanol to the GalA content is referred to as the degree of methyl-esterification (DM), which is an important parameter for pectin functionality. Rhamnogalacturonan I is an acidic pectic domain consisting of as many as 100 repeats of the disaccharide (1 → 2)-α-L-rhamnose-(1 → 4)-α-D-galacturonic acid. The rhamnosyl residues of RG I can be substituted at O-4 with neutral sugar side chains composed of galactosyl and/or arabinosyl residues (Voragen et al., 2009). Rhamnogalacturonan II is a highly complex macromolecule with a (1 → 4)-linked α-D-galacturonan backbone, partially methyl-esterified at C-6 of the GalA residues and substituted by four different side chains (Voragen et al., 2009).

Pectic polymers can interact with each other through several covalent and non-covalent linkages which influence the functional properties of pectin. They include among others the cross-linking with calcium ions according to the 'egg box model' i.e. negatively charged non-methyl-esterified GalA residues in the HG domain can ionically cross-link with divalent ions (Morris et al., 1982; Voragen et al., 2009) and the occurrence of ferulic acid dihydrodimers forming RG II dimers (Ishii et al., 1999; Voragen et al., 2009). In addition, pectic polysaccharides may also be cross-linked to other cell wall components such as hemicelluloses, phenolic compounds and proteins, which provide an added structural and functional complexity to the cell wall (Caffall & Mohnen, 2009). Furthermore, in legumes, decrease in pectin solubility and hence strengthening of the cell wall can occur during storage and soaking when pectin exchanges monovalent cations with divalent cations released from the degradation of phytin by phytase (Jones & Boulter, 1983; Mafuleka et al., 1993; Reyes-Moreno & Paredes-Lopez, 1993; Liu, 1995; Garcia et al., 1998). However, detailed insight into changes occurring at a molecular level in pectic polysaccharides during soaking and subsequent thermal treatment in different brine solutions for both easy-to-cook (ETC) and HTC common beans is still lacking. The objective of this research was to gain mechanistic insights into common bean pectic polysaccharide changes occurring during storage and (pre)treatments. The information would provide a better understanding of the effect of (pre)treatments on common beans pectic polysaccharides in relation to the development and manifestation of the HTC defect in common beans (*Phaseolus vulgaris*). Specifically, it was the goal to determine pectic polysaccharide changes of Canadian wonder beans occurring after soaking in demineralized water, sodium carbonate (Na_2CO_3) and calcium chloride ($CaCl_2$) solutions, whether or not followed by a thermal treatment, before (ETC) and after storage (HTC).

2. Materials and methods

2.1. Materials selection and (pre)treatment

Freshly harvested Canadian wonder (GLP-24) common beans (*P. vulgaris*) were sourced from the Kenya Agricultural and Livestock Research Organization (KALRO) on March 2013. A portion of the beans was kept at −20 °C immediately after harvesting (fresh/easy-to-cook) while the other was stored in perforated tray at 35 °C and 83% relative humidity (RH) for 6 months (HTC) in a storage chamber. The relative humidity was maintained during the storage period by the use of potassium chloride saturated solution. After the storage, bean samples were stored at −20 °C until the time of analysis. First, the cooking quality of beans was determined based on the cooking times for both fresh and stored samples in demineralized water. To determine the cooking times, bean seeds pre-soaked in demineralized water for 16 h at 25 °C were subjected to a thermal treatment at 96 °C in the same solution in a thermostated water bath (Memmert WBU-45, Germany). The number of cooked seeds was determined after every 30 min by finger pressing method (Vindiola et al., 1986; Kinyanjui et al., 2015, under review). In addition, to evaluate the effect of different salts on the cooking times, fresh and stored beans were soaked in 0.1 M Na_2CO_3 (monovalent ion and increased pH accelerating β-elimination reaction) and 0.1 M $CaCl_2$ (bivalent ion cross-linking pectin) salt solutions followed by thermal treatment and cooked seeds determination as described for demineralized water (pre)treatment. Statistical data analysis was performed using a Statistical Analysis System (SAS) statistical software package (SAS Enterprise Guide 4.3, USA). Since the cooking curves appeared sigmoidal in nature, a sigmoidal regression equation was used for their quantification (Kinyanjui et al., under review).

$$y = \frac{100}{1 + \exp^{b+cx}}$$

In the equation, y is the predicted value of cooked beans (%), x is the cooking time (min) and b and c are two theoretical parameters that completely describe the cooking curves. From the estimated parameters b and c, a parameter which is easily interpreted such as time required to cook 95% of the seeds i.e. total cooking time (time taken for all beans to soften) was generated. Significant differences in total cooking times of ETC and HTC beans soaked and thermally treated in demineralized water, sodium carbonate and calcium chloride solutions were examined using the post-hoc Tukey test at a significance level of $p \leq 0.05$.

To obtain samples for pectin analysis, both fresh and stored beans were either soaked or soaked and thermally treated. Specifically, for soaked samples, 10 g of bean seeds was soaked (1:5 w/v) in either demineralized water, 0.1 M Na_2CO_3 or 0.1 M $CaCl_2$ brine solutions for 16 h at 25 °C. For soaked and thermally treated samples, 10 g of seeds were presoaked in aforementioned brine solutions for 16 h at 25 °C followed by a thermal treatment, in the same brine solutions, at 96 °C for a time of 120 and 270 min, for fresh and stored seeds respectively,