Malting process has minimal influence on the structure of arabinan-rich rhamnogalacturonan pectic polysaccharides from chickpea (*Cicer arietinum* L.) hull

Shakuntala Sathyanarayana¹ · Keelara Veerappa Harish Prashanth¹

Revised: 18 January 2019 / Accepted: 21 January 2019 / Published online: 28 January 2019
© Association of Food Scientists & Technologists (India) 2019

Abstract The objective of the study was to determine the changes brought about by malting/germination on the pectic polysaccharides (PP's), the major components of soluble fibres present in chickpea (*Cicer arietinum* L.) hull. Chickpea hull PP's were extracted sequentially using ammonium oxalate (AO) and ethylenediaminetetraacetic acid (EDTA), and a comparative study was conducted in native (unprocessed, N–PP) and after subjecting to 48 h malting process (M–PP). Malting process did not show a significant change in the respective yields of AO and EDTA extracted pectic polysaccharides. The degree of esterification of N–PP–EDTA through Fourier transform infrared spectroscopy was found to be five times ($\sim 21\%$) more than N–PP–AO ($\sim 4\%$). AO isolated PP's have more complexed xylogalacturonan with relatively more galactan side chains compared to EDTA isolated PPs. Proton (^1H) nuclear magnetic resonance result further suggested the occurrence of arabinan rich rhamnogalacturonan in chickpea hull and malting process showed no significant changes in structure.

Keywords Chickpea hull · Pectic polysaccharides · Malting · Arabinan · Rhamnogalacturonan · NMR

Abbreviations

MHDP	Metahydroxydiphenyl
AO	Ammonium oxalate
PP	Pectic polysaccharides
N	Native
M	Malted
BSA	Bovine serum albumin
TDF	Total dietary fibre
DE	Degree of esterification
NSP	Non-starch polysaccharides

Introduction

Pulses are a good source of dietary fibre along with other vital nutrients such as proteins, minerals, β -carotene and unsaturated fatty acids. Chickpea (*Cicer arietinum* L.) generally known as Bengal gram, is a legume of the family Fabaceae. Chickpea is not only a healthy and relishable dietary component but also a valuable source of prebiotics and fibre. Chickpea is a largely produced food legume in South Asia and accounts for the third largest legume produced globally with extensive geographical distribution. The world's total production of chickpea hovers around 8.5 million metric tons annually and is grown over 10 million hectares of land approximately. India is the largest producer of chickpea and accounts for $2/3^{\text{rd}}$ of its global production (Vasishtha and Srivastava 2013).

The majority of chickpea processing wastes include chickpea hull which is an excellent source of nondigestible pectic polysaccharides and hence it is exploited as additives in the food industry. The pectic polysaccharides derived from chickpea can be utilized in nutraceuticals. Generally, dietary fibres from pulses are gaining importance all over the world due to their various health benefits.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13197-019-03600-4>) contains supplementary material, which is available to authorized users.

✉ Keelara Veerappa Harish Prashanth
harish@cftri.res.in

¹ Functional Biopolymer Lab, Department of Biochemistry,
CSIR-Central Food Technological Research Institute,
Mysore, Karnataka 570 020, India

The dietary fibre mainly consists of cellulose, hemicellulose, lignin, gums, mucilages, oligosaccharides and pectin (Devries 2004).

Pectin is principally found in the cell wall and middle lamellae of plants where it plays an important role as a hydrating agent and cementing material for the cellulosic network. Pectins are one of the most important dietary fibre constituents which are high molecular weight, branched, anionic, acidic polysaccharides. They are categorised under soluble dietary fibre which can alleviate disease symptoms of diabetes, colon cancer and atherosclerosis. The molecular weight may vary from 50 to 150 kDa. It is a heteropolysaccharide mainly composed of three structural elements, i.e. homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II). Homogalacturonan consists of a backbone of D-galacturonic acid (GalA) units linked together by α -(1 $\rightarrow$ 4) glycosidic bonds which can be partially esterified with a methyl group at C-6 and acetylated at O-2 and/or O-3. Rhamnogalacturonan-I is a polymer made up of chains containing alternate units of α -(1 $\rightarrow$ 2)-rhamnose- α -(1 $\rightarrow$ 4)-galacturonic acid, where arabinan, galactan and arabinogalactan branches can be attached. Rhamnogalacturonan-II is a complex polymer composed of GalA, rhamnose, arabinose, xylose, galactose.

In India, chickpea is used both in native and processed forms. Germination is an effective processing method for improving nutritional quality, reducing anti-nutritive compounds, boosting the level and digestibility of free amino acids and available carbohydrates, increasing mineral bio-availability and improving the functional properties of pulses (Lopez-Martinez et al. 2017). During this process, due to the induction of several hydrolytic enzymes, various biochemical changes take place both in starch and non-starch polysaccharides which results, an increase in reducing sugars along with the release of insoluble phenolic compounds covalently bound to cell wall polysaccharides (Nirmala et al. 2000). Germination induces biochemical changes in carbohydrate of cereals, which reduce energy values compared to raw and roasted samples (Rumiyati et al. 2012). Malting can increase protein, dietary fibre, vitamin concentrations, bioavailability of minerals and reduce tannin, phytic acid contents (Khataak et al. 2007). The content and composition of bioactives vary with respect to storage time and germination period of seeds.

Though there are several reports available on isolated pectins from various other sources, the information on the isolation of pectic polysaccharides and its biochemical changes upon germination from chickpea hull has not been reported. Hence, this investigation was undertaken to study the effect of germination on structural aspects using gas chromatography (GC), FTIR and NMR of pectic

polysaccharides isolated sequentially using ammonium oxalate and EDTA between native and malted chickpea hull.

Materials and methods

Materials and chemical reagents

Chickpea was purchased from the local market in Mysore, India. Sugar standards: rhamnose, arabinose, xylose, mannose, galactose, glucose, galacturonic acid, inositol, methoxydiphenyls (MHDP), sodium tetraborate, Bradford reagent, ammonium oxalate, ethylenediaminetetraacetic acid, sodium borohydride, Amberlite IR 120-P (8% cross-linked, 16–50 mesh), *p*-nitrophenyl β -D-xylopyranoside, *p*-nitrophenyl α -L-arabinofuranoside, *p*-nitrophenyl α -D-galactopyranoside, *p*-nitrophenyl β -D-galactopyranoside, *p*-nitrophenyl acetate were purchased from Sigma-Aldrich Co. Ltd., St. Louis, MO, USA. All other chemicals used in this study were of analytical grade.

Malting/germination of chickpea seeds

Malting process was carried out as follows: Chickpea seeds (100 g) were cleaned, surface sterilized with sodium hypochlorite (0.02%) for 1–5 min and steeped for 16 h and germinated under controlled conditions on moist cloth at 25 °C in a biochemical oxygen demand incubator (BOD) at different time intervals (12, 24, 36, 48 and 60 h) and dried in an air oven at 50 °C for 12 h and vegetative growth portions were removed by hand brushing. Devegetated seeds were weighed, powdered and used for the extraction of the enzyme (Nirmala et al. 2000). Malting loss was calculated by using the below formula.

$$\text{Melting loss} = \frac{(W1 - W2)}{W1} \times 100$$

where, W1 = initial weight of the seeds and W2 = final weight of the seeds after malting followed by removing growth portions.

Enzyme assays

Native (N) and malted (M) chickpea flours (2.5 g each) were extracted with different buffers (20 mL each) for obtaining different enzymes; i.e., sodium phosphate buffer (0.1 M, pH 5.7) for obtaining arabinofuranosidase, xylopyranosidase, α -D-galactopyranosidase and β -D-galactopyranosidase, sodium potassium phosphate buffer (0.2 M, pH 6.5) for obtaining acetyl esterase, acetate buffer—I (0.1 M, pH 5.0) for obtaining polygalacturonase, acetate buffer—II (0.2 M, pH 5.0) for obtaining pectin

methyl esterase. Extractions were performed at 4 °C for 2 h. The samples were centrifuged at 3000 g for 20 min, the supernatant was dialyzed for overnight against the same buffer and assayed for the presence of various enzyme activities using appropriate substrates and respective conditions.

β -D-Xylopyranosidase, α -L-arabinofuranosidase, α -D-galactopyranosidase and β -D-galactopyranosidase activities were determined by monitoring the release of *p*-nitro phenol from respective substrates i.e. *p*-nitrophenol glycosides (0.5 mL of 2 mM substrate in sodium phosphate buffer, 0.1 M, pH 5.7) by incubating them with sample (0.1 mL) for 1 h at 37 °C (Beldman et al. 1996). Reaction was stopped by adding a saturated solution of sodium tetraborate (0.5 mL). Absorbance was read at 400 nm. One unit of activity was defined as the amount of enzyme required to liberate 1 μ mol *p*-nitro phenol/min under assay conditions. For determining acetyl esterase activity, the substrate (1 mL of saturated solution of *p*-nitro phenyl acetate in sodium–potassium phosphate buffer, 0.2 M, pH 6.5) was incubated with sample (0.1 mL) for 30 min at 25 °C. Reactions in the above enzyme assays were stopped by adding a saturated solution of sodium tetraborate (0.5 mL). Absorbance was read at 400 nm (Chung et al. 2002). One unit of activity was defined as the amount of enzyme required to liberate 1 μ mol of *p*-nitro phenol/min under assay conditions. For determining polygalacturonase activity, substrate (2 mL of citrus pectin in acetate buffer, 0.1 M, pH 5.0) was incubated with sample (0.1 mL) for 1 h at 50 °C. The reaction was stopped by the addition of DNS (1 mL) followed by boiling for 10 min. Absorbance was read at 550 nm (Miller 1959). One unit of activity was defined as the amount of enzyme required to liberate 1 μ mol galacturonic acid/min under assay conditions (Miller 1959). The method for assaying pectin methyl esterase (PME) was based on determination of the pH change due to an increase in nonesterified carboxyl groups by using the pH indicator bromothymol blue (Hagerman and Austin 1986).

Isolation of pectic polysaccharides from native and malted chickpea hull

Chickpea seeds were milled to obtain the endosperm and hull. Pectic polysaccharides (PP) were isolated from both native and malted chickpea hull sequentially by using 0.5% AO and 0.5% EDTA respectively (Muralikrishna and Tharanathan 1994). Initially, the chickpea hull was extracted with 0.5% AO (1:10 w/v $\times$ 3) at room temperature for 2 h with continuous stirring followed by centrifugation (5000 g, 10 min). The residue was re-extracted with 0.5% AO (2 h, 85 °C) and centrifuged (5000 g, 10 min). The combined supernatants of 0.5% AO, were

concentrated and precipitated with three volumes of ethanol. The resulting precipitate was dissolved in water and dialyzed extensively against water, and then freeze-dried, which yielded AO extracted pectins (N–PP–AO for native and M–PP–AO for malted). The insoluble residue obtained after 0.5% AO extraction was further extracted with 0.5% EDTA followed by centrifugation. Precipitation of the supernatant with three volumes of alcohol followed by dialysis and lyophilization yielded EDTA extracted pectins (N–PP–EDTA for native and M–PP–EDTA for malted) respectively. The pectic polysaccharides thus isolated from the above extractions were evaluated for total sugar, uronic acid and protein as follows.

Chemical analysis

Carbohydrate content in PPs was determined by a phenol–sulfuric acid method using D-glucose as the standard (McKelvy and Lee 1969). The uronic acid content was determined by an MHDP method using D-galacturonic acid as standard (Blumenkrantz and Asboe-Hansen 1973). The amount of protein in the polysaccharides was determined according to the Bradford method using BSA as the standard (Bradford 1976).

Monosaccharide composition analysis

The pectic polysaccharides (10 mg) was hydrolysed with 2 N sulphuric acid for 10 h, followed by neutralization with barium carbohydrate and deionization with Amberlite (IR 120-H⁺) resin. Sodium borohydride was added to reduce the monosaccharides into alditols. Excess sodium borohydride was removed by adding 2 N acetic acid and co-distilled with methanol to remove the boric acid formed. Acetylation was carried out by adding equal volumes (1 mL each) of acetic anhydride and pyridine. Excess acetylating reagents were removed by co-distillation with water and toluene (2 mL $\times$ 3). The alditol acetates were extracted with chloroform (1 mL) filtered through glass wool and dried by flushing with nitrogen. The derivatives were taken in known amount of chloroform (Selvendran and O'Neill 1987; Sawardeker et al. 1965) and analyzed on RTX 2330 column by GLC (Shimadzu GLC system, 2010 Plus).

Viscosity

0.1 g of pectic polysaccharide was weighed precisely and dissolved in 50 mL of water and stirred for 2 h. 0.8 g of NaCl and 0.2 g of sodium hexametaphosphate in 15 mL distilled water was added to the above solution and stirred for one h. Adjust the pH with dilute acid or alkali to 6.0 ± 0.2 . The electrode of the pH meter rinsed into the

solution and transferred to a 100 mL volumetric flask and made up the volume. The viscosity was measured within an hour using a Rheometer (coupled with a thermostatic bath) HAAKE rheostress 6000 and thermo scientific K35 circulating water bath. The temperature of all the analysis (25 °C) were controlled with a conventional refrigerated cooling system with the accessories cup and bob/rotor (Z20DIN) (Wang et al. 2016).

Fourier transform infrared spectroscopy (FTIR)

IR spectral study of pectic polysaccharide (10 mg) samples was performed by using a Perkin-Elmer spectrum 2000 spectrometer, in an absorbance mode at a resolution of 4 cm⁻¹ with wavenumber range 4000–400 cm⁻¹.

Proton (¹H) NMR spectroscopy

¹H NMR of pectic polysaccharides were carried out by taking samples in D₂O. The deuterium resonance was used as a field frequency lock and the shifts were referenced to external TMS. The spectra were recorded in a Bruker AQS 500 MHz NMR spectrometer (5 mm BBO probe) operating at 27 °C for 4 h using spectral width of 10,330 Hz was set with offset frequency of 2351.4 Hz with 64 scans.

Scanning electron microscopy (SEM)

The dry sample, spread on a double sided conducting adhesive tape, pasted on a metallic stub, was coated with gold in a sputter coating unit for 2 min and observed in a LEO-435-VP (LEO Electron Microscopy Ltd, Cambridge, UK) electron microscope at 20 kV.

Statistical analysis

The entire experiments were performed with three replications, and data were presented as mean ± standard deviation (SD). All statistical analysis were performed using GraphPad Prism statistical software package version 5 to find out the significance of treatments.

Results and discussion

Malting of chickpea

Chickpea was malted for 60 h and changes in the pectic polysaccharides and their degrading enzymes were monitored. A comparative study pertaining to the changes (brought about by malting) in pectic polysaccharides and their respective degrading enzymes in native and malted chickpea hull was undertaken. Malting loss increased with

increase in time and was maximum (20.1%) at 60 h of germination (Supplement data 1). Malting the whole seeds for longer than 48 h would results in further dry matter loss without much improvement in nutritional quality (Nirmala et al. 2000). The loss in dry weight is mainly due to the increase in metabolic activity and the partial degradation of carbohydrate material.

During malting process various biochemical changes takes place both in starch and non-starch polysaccharides due to the induction of several hydrolytic enzymes. Chickpea showed slight decreasing trend in enzyme activity after 48 h of malting. Polygalacturonase was the major non-starch polysaccharide degrading enzyme appeared in the chickpea malt. Its activity increased with increase in time of malting and was found to increase approximately by two folds at 48th h. The pectin methyl esterase activity was shown to be modest (but detectable) and was much lesser when compared to acetyl esterase activity. The latter was found to be higher at the interval of 48 h (Fig. 1). Glycosidases namely, arabinofuranosidase and xylopyranosidase were found to be present, yet there was no significant change in their activity due to malting process till 60 h. α -galactopyranosidase activity was shown to be increasing steadily whereas its peak activity was found to be at the 48 h of malting process. Although β -galactopyranosidase enzyme activity shown to be increasing until 12 h, there was no further variation observed till 60th h of malting (Fig. 1).

Isolation of pectic polysaccharides from native and malted chickpea hull

As observed, the activity of cell wall degrading enzymes was moderately higher in 48 h malts. Hence 48 h time interval was considered as the condition to isolate pectic polysaccharides from malted chickpea hull and compared with native chickpea hull. Extraction of pectic polysaccharides from native (N-PP-AO and N-PP-EDTA) and malted (M-PP-AO and M-PP-EDTA) chickpea hull by sequentially using 0.5% ammonium oxalate (AO) and 0.5% EDTA gave 10.27, 3.08 (N) and 12.55, 2.54% (M) yields respectively (Table 1A). Ammonium oxalate extracted pectic polysaccharides increased upon malting, but not a very significant change in yield was noted. In general, malting causes loosening of cell wall which may facilitate improved solubility of various non-starch polysaccharides. Results from the previous study indicated a detectable increase in non-starch polysaccharide contents upon malting of ragi (Subba Rao and Muralikrishna 2001). The yields of EDTA extracted pectic polysaccharides did not change notably upon malting. AO extracted pectic polysaccharides from both native and malted chickpea was completely soluble, where as EDTA extracted pectic polysaccharides

Fig. 1 Graphs showing variations in the NSP degrading enzyme activities during malting of chickpea seeds. **a** Polygalacturonase; **b** methyl esterase; **c** β -D-xylopyranosidase; α -L-arabinofuranosidase; α -D-galactopyranosidase; β -D-galactopyranosidase; acetyl esterase

were only sparingly soluble in water. According to Benítez et al. (2013), the increase of total dietary fibre (TDF) content was due to synthesis of new polysaccharides during germination. Total dietary fibre was found to be significantly increased in kidney bean, mung bean, soy beans and peanuts, after germination followed by a significant

increase in soluble and insoluble dietary fibres (Megat et al. 2016). A contradictory finding was reported by Martín-Cabrejas et al. (2008) in cowpea, jack bean and mucuna beans where TDF was shown to be reduced after germination.

In similarity, germinated Dolichos and soy beans showed reduced content of total dietary fibre (Megat et al. 2016). Hence, the above studies indicate that germination process influence TDF content differently concerning legume types and conditions of the malting process. The increase in crude and dietary fibre content of chickpea upon germination process could be mainly attributed to the extensive use of seed components and degradation of protein to simple peptides. Ghumman et al. (2016), has showed in lentil and horse gram that germination process induces the breakdown of proteins and respective structural changes.

The results of evaluation of total carbohydrate and protein of these fractions are shown in Table 1A. N-PP-AO and N-PP-EDTA contained 36% and 40% of total carbohydrates, 6.47% and 3.58% protein respectively. The uronic acid content of N-PP-AO and N-PP-EDTA is 42.25% and 53.75% respectively. M-PP-AO and M-PP-EDTA contained 49.33% and 33.33% of total carbohydrates, 2.47% and 1.74% protein respectively. The uronic acid content of M-PP-AO and M-PP-EDTA was found to be 60.5% and 21.05% respectively. The present investigation shows that there was an increase in the total carbohydrate and uronic acid content of AO extracted pectic polysaccharide of malted chickpea when compared to the native chickpea. Metabolic changes occur during germination process of legume seeds in which carbohydrate stored in the form of starch and oligosaccharides are hydrolysed which contributes to the increase in sugar levels (Urbano et al. 2005). According to Martín-Cabrejas et al. (2008), α -galactosidase activity was increased during germination causing the breakage of α -1,6-galactosidic linkages and thereby increasing the amount of total sugar.

It is reported that soaking and germination significantly decreased protein and fibre contents, but increased carbohydrate contents of chickpea (Desalegn 2015). It is also noteworthy that protein contents of legumes (kidney bean, mung bean, soya bean and peanut) and rice were decreased after the germination (Megat et al. 2011). The reduction in protein content of cowpea during soaking may be attributed to protein denaturation by the heat generated or leaching of some of the water-soluble proteins and solubility of nitrogen into the soaking medium. The increase in uronic acid in malts could be due to the mobilization of uronic acid containing pectins during malting. Vasishtha and Srivastava (2013) reported soaking and germination had no significant effect on pectins of chickpeas. The monosaccharide composition of pectic polysaccharides

Table 1 (1A) The yields, total carbohydrate and protein content of pectic polysaccharides extracted from native and malted (48 h) chickpea hull. (1B) Ratio of monosaccharide composition and uronic acid (%) content of pectic polysaccharides from native and malted (48 h) chickpea hull

(1A)							
Fractions	Yield (%)			Total carbohydrate (%)			Protein (%)
N-PP-AO	10.27 ± 0.02			36.00 ± 0.02			6.47 ± 0.01
M-PP-AO	12.55 ± 0.03			49.33 ± 0.03			2.47 ± 0.01
N-PP-EDTA	3.08 ± 0.01			40.00 ± 0.01			3.58 ± 0.02
M-PP-EDTA	2.54 ± 0.01			33.33 ± 0.02			1.74 ± 0.03
(1B)							
Fractions	Rha	Ara	Xyl	Man	Gal	Glu	Uronic acid
N-PP-AO	1.68	3.46	4.42	1	2.57	1.48	42.25 ± 0.03
M-PP-AO	3.20	6.25	8.38	1	3.87	2.67	60.50 ± 0.01
N-PP-EDTA	5.49	18.88	4.63	1	7.92	2.30	53.75 ± 0.04
M-PP-EDTA	3.34	14.63	2.30	1	4.67	2.93	21.05 ± 0.02

Values represent mean ± standard deviation

Ratio of Rha, Ara, Xyl, Gal and Glc was determined relative to mannose content

Rha rhamnose, Ara arabinose, Xyl xylose, M mannose, Gal galactose, Glc glucose

from native (N-PP-AO and N-PP-EDTA) and malted (M-PP-AO and M-PP-EDTA) chickpea hull indicated rhamnose, arabinose, xylose, mannose, galactose, glucose and uronic acid in different ratios (Table 1B). Xylose, arabinose and galactose were the major neutral sugars while rhamnose, glucose and mannose were the minor sugars in both N-PP-AO and M-PP-AO. Upon malting change in the ratio of pentose (arabinose and xylose) to hexose (mannose, glucose & galactose) was observed. Increase in the ratio of the same indicated higher rate of degradation of hexoses like mannose and galactose. This characteristic finding might be accredited to the moderate induction of activity of hydrolytic enzymes such as galactosidases. Malting increased rhamnose content in AO extracted pectic polysaccharides. All the neutral sugars in the EDTA extracted pectic polysaccharides decreased upon malting. Chickpea, grouped into two types: desi (Indian origin) and Kabuli (Mediterranean and Middle Eastern origin) based on seed colour and geographic distribution. The present investigation supports the presence of more xylose, possibly in the form of xylogalacturonan similar to those found in pea (*Pisum sativum*) and bean (*Phaseolus vulgaris*) seed coats (Le Goff et al. 2001; Shiga and Lajolo 2006).

Pectins are responsible for gelling behaviour and texture in gels. The pectin used in fruit jam preparation determines the rheological flow behaviour and consequently the quality of the products. The viscosity of 1 g/100 mL AO and EDTA extracted pectic polysaccharides of native (N-PP-AO, N-PP-EDTA) and malted (M-PP-AO, M-PP-EDTA) chickpea at pH 6, was found to be 14.7, 14.9 and 13.4, 14.7 cp, respectively (Supplement data 2). The above results align with a previous study which suggests that the viscosities of unheated sunflower pectin ranged from 7 to 17 cp at pH 4 to 7 (Miyamoto and Chang 1992). There was

a slight reduction in viscosity of AO extracted pectic polysaccharide upon malting which is in accordance with the earlier study (Schmelter et al. 2002). The solubility factor influence the viscosity, hence substantial information may not be deduced for rheological preparation of PP in the present study.

FTIR spectroscopy analysis

To confirm the identity of chickpea hull pectin extract and estimate the degree of esterification (DE), the samples, N-PP-AO, M-PP-AO, N-PP-EDTA and M-PP-EDTA was analyzed by Fourier transform infrared spectroscopy (FTIR). The spectra were compared against each other and with standard commercial apple pectin (data not showed). Spectra of the above-mentioned preparations exhibited pectin “fingerprint” pattern suggesting the isolated samples are precisely pectin. The analysis of FTIR data showed that each polysaccharide has a specific band maximum in the 1200–1000 cm^{-1} region. This region is dominated by ring vibrations overlapped with stretching vibrations of (C–OH) side groups and the (C–O–C) glycosidic bond vibration. The FTIR spectrum of native chickpea hull pectin (N-PP-AO) isolated using ammonium oxalate as presented in Fig. 2a shows a very low absorbance at 1739 cm^{-1} than at 1585 cm^{-1} , indicating low methoxy pectin content. The degree of esterification (DE) of N-PP-AO was determined to be about 4.11% (Supplement data 2). Upon malting, M-PP-AO (Fig. 2b) showed a slight increase in esterification, (5.26%) (Supplement data 2).

EDTA isolated pectin (N-PP-EDTA) spectrum presented in Fig. 2c indicates the presence of characteristic pairs of strong intensity bands between 1679 cm^{-1} and 1605 cm^{-1} which corresponds to carboxylic acid functional

Fig. 2 FTIR spectra of pectic polysaccharides from native and malted chickpea hull. **a** N-PP-AO; **b** M-PP-AO; **c** N-PP-EDTA; **d** M-PP-EDTA

group. The effect of shifting (1679 cm^{-1}) at higher wave numbers could be a resultant of the factors deriving from polymeric neighbourhood, where the COO^- groups of galacturonic acid indicating asymmetrical and symmetrical vibrations (Manrique and Lajolo 2002). The band that appears at about 1730 cm^{-1} can be assigned to C=O

stretching vibration of esterified carboxyl group. The DE of N-PP-EDTA (20.95%) was found to be five times more than N-PP-AO (Supplement data 2). Further upon malting (M-PP-EDTA), the DE was decreased to 16%. This change of net decrease of degree of esterification between N-PP-EDTA and M-PP-EDTA may be accredited to the shape of

the band around $1600\text{--}1690\text{ cm}^{-1}$, probably due to the complexity of the pectic carbohydrate groups (Kyomugasho et al. 2015). In the isolated pectins spectra, rhamnogalacturonan mixture bands between 1140 and 1015 cm^{-1} were strongest. Further for rhamnogalacturonan, the distinct band shapes with the main maxima were found at 1096 and 1013 cm^{-1} respectively.

¹H NMR spectroscopy analysis

Figure 3 depicts the ¹H NMR spectra of pectic polysaccharides from native and malted chickpea extracted sequentially using 0.5% ammonium oxalate (AO) and 0.5% EDTA were analyzed to determine their primary structure. According to the characteristic signals, the spectra of the polysaccharides were primarily summarized, and the respected chemical shift signals were assigned (Table 2). The chemical shift in ¹H spectra indicates that all the samples N-PP-AO, M-PP-AO, N-PP-EDTA and M-PP-EDTA contained mainly α 1,4-linked GalA residues. The spectra of the pectin samples contain a sharp signal at 3.68/3.69 ppm from methoxy group H-2 indicating esterified pectin (Winning et al. 2007). The degree of esterification (DE) was found in agreement with the estimated value through FTIR (Supplement data 2). The intensity at the higher field signal at 3.52 ppm resulted in good correlation (0.91) to DE as well (Winning et al. 2007). The H-1 of rhamnose was not visible, but methyl group around 1.1 and 1.2 ppm (doublet) indicated the presence of rhamnose along with ring protons of the rhamnosyl residues. The O-linked acetyl was also present around 2 ppm.

The ¹H NMR of N-PP-AO and M-PP-AO showed the presence of xylopyranoside residues. The spectrum for N-PP-AO showed a peak at around 4.45 ppm representing for anomeric proton of α -xylopyranose at the reducing end to non-esterified GalA. The EDTA isolated pectic polysaccharides do not show the presence of xylose residues indicating that AO isolated pectic polysaccharides have more complexed xylogalacturonan with relatively more galactan side chains (¹H, 5.1 ppm, and additional respective ring signals) compared to EDTA isolated PPs. This suggests the structure of side chains of pectins differed inexistence in the region of cell wall architecture in agreement with the previous study on tomato pectins (Orfila and Knox 2000).

Furthermore, ¹H spectra for all the isolated pectic polysaccharides N-PP-AO, M-PP-AO, N-PP-EDTA and M-PP-EDTA showed two peaks in its anomeric region. These signals are assigned to α -L-arabinofuranosyl residues. The ¹H chemical shifts were attributed to H-1 of residues of linear and branched α -L-arabinofuranose at 5.09/5.08 and 5.06/5.07 ppm respectively. This result implies the occurrence of arabinan moiety of an isolated

arabinan rich PP from the cell wall or hull of chickpea. Arabinogalactans are mostly found localized on the surface of plant cell walls and plasma membranes to form arabinogalactans-proteins (AGP) core-coat interface (Wood et al. 2014).

Difference in chickpea coat chemical composition and structure of pectins are still not fully explored as composition and structure elucidation may alter due to subtle variation in isolation process. To the best of our knowledge, such heterogeneity in chickpea soluble pectic polysaccharides structure has been proposed for the first time in the present study (Fig. 4).

Scanning electron microscopy

The micrographs of isolated pectic polysaccharides from native and malted chickpea hull are presented in Supplement data 3. N-PP-AO, M-PP-AO, N-PP-EDTA and M-PP-EDTA samples showed that the pectin particles were compact, flaky in shape and had a rough surface. A previous study on apple pomace pectin and mango pectin have demonstrated the pectin surfaces to be irregular and rough (Begum et al. 2017). Although slim differences in color and texture can be observed visually, the surface topology of both AO and EDTA isolated PPs indicated no significant morphological changes.

Chickpea hull is very rich in non-starch polysaccharides (NSP) as compared to the endosperm (Wood et al. 2014). Since the polysaccharide obtained was found to be rich in galacturonic acid and low in neutral sugars, it is likely that polysaccharides isolated may be predominantly pectic polysaccharides. Uronic acid was used as a measure of pectin content and was found to be approximately 10 times more in the seed coat than the cotyledon fractions (Wood et al. 2014). Pectins are one of the most important dietary fibre constituents and are grouped under soluble dietary fibre and are known to alleviate disease symptoms of diabetes, colon cancer and atherosclerosis, which emphasizes on importance of food legumes in health. It has been previously reported in similar to our observations that the NSP of endosperm undergoes more structural changes than the seed coat material upon malting (Woolard et al. 1977). The chemical composition analysis, chromatography and spectrometric methods collectively facilitated identification of some aspects of the pectin structure from chickpea hull. As the chickpea hull is mostly discarded as waste after endosperm utilization; there is a lot of scope to utilize pectic polysaccharides and bioactive oligosaccharides derived from it to develop products with nutraceutical importance. This preliminary attempt to evaluate the composition and structure of pectins can be extended to further analyse the structure–function relationship of pectic polysaccharides from chickpea hull.

Fig. 3 ^1H NMR spectra of pectic polysaccharides from native and malted chickpea hull. **a** N-PP-AO; **b** M-PP-AO; **c** N-PP-EDTA; **d** M-PP-EDTA

Fig. 3 continued

Table 2 Chemical shifts (ppm) of proton resonances of pectic polysaccharides from native and malted chickpea hull (^1H NMR data)

Fractions	Residue	Chemical shifts (ppm)					
		H-1	H-2	H-3	H-4	H-5	H-6
N-PP-AO	→ 4) α -D-GalpA-(1 →	4.96	3.68	3.88	4.35	4.52	–
	→ 3) β -D-Xylp-(1 →	4.50	3.15	3.22	3.46	3.68	–
	→ 2) α -L-Rhap-(1 →	–	3.93	3.77	3.36	3.66	1.11/1.12
	→ 5) α -L-Araf-(1 →	5.06	4.04	4.08	–	3.87	–
M-PP-AO	→ 4) α -D-GalpA-(1 →	4.98	3.69	3.89	4.35	4.52	–
	→ 3) β -D-Xylp-(1 →	4.49	3.18	3.29	3.50	3.67	–
	→ 2) α -L-Rhap-(1 →	–	4.01	3.78	3.36	3.64	1.15/1.20
	→ 5) α -L-Araf-(1 →	5.09/5.07	4.05	4.09	4.16	3.86	–
N-PP-EDTA	→ 4) α -D-GalpA-(1 →	4.97	3.69	3.89	–	–	–
	→ 2) α -L-Rhap-(1 →	–	3.96	3.77	–	3.65	1.11/1.12
	→ 5) α -L-Araf-(1 →	5.08/5.06	4.04	4.08	4.09	3.88	–
M-PP-EDTA	→ 4) α -D-GalpA-(1 →	4.97	3.68	3.89	–	–	–
	→ 2) α -L-Rhap-(1 →	–	4.00	3.76	–	3.66	1.11/1.12
	→ 5) α -L-Araf-(1 →	5.08/5.06	4.04	4.08	4.09	3.88	–

Fig. 4 Probable structural model for pectic polysaccharides from chickpea hull

Conclusion

In conclusion, the malting loss in ‘whole seeds’ of chickpea (*Cicer arietinum* L.) found increased upon germination. Polygalacturonase activity showed two folds increase at 48 h of malting. But malting did not significantly change the yield of sequentially (ammonium oxalate and EDTA) extracted pectic polysaccharides from chickpea ‘hull’. The

evaluation of FTIR and NMR data revealed the structure of arabinan-rich rhamnogalacturonan pectic polysaccharides from chickpea hull. The NMR complementing with GC data confirms the presence of high contents of α 1,4-linked GalA residues in the linear polymeric region. Further, ^1H NMR suggested the presence of arabinans as side chains with higher content of complex xylogalacturonan in AO isolated pectic polysaccharides with relatively more

galactan side chains compared to EDTA isolated PPs. Overall, this study reveals that germination process does not have much influence on chickpea hull's pectic polysaccharide structural complexity.

Acknowledgements The authors would like to thank the Director, Council of Scientific and Industrial Research-Central Food Technological Research Institute, Mysuru for the constant encouragement. Ms Shakuntala S., thanks Indian Council of Medical Research, New Delhi, for the Senior Research Fellowship.

References

- Begum R, Yusof YA, Aziz MG, Uddin MB (2017) Structural and functional properties of pectin extracted from jackfruit (*Artocarpus heterophyllus*) waste: effects of drying. *Int J Food Prop* 20:190–201
- Beldman G, Osuga D, Whitaker JR (1996) Some characteristics of β -D-xylopyranosidases, α -L-arabinofuranosidases and an arabinoxylan α -L-arabinofuranohydrolase from wheat bran and germinated wheat. *J Cereal Sci* 23:169–180
- Benítez V, Cantera S, Aguilera Y (2013) Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. *Food Res Int* 50:64–69
- Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. *Anal Biochem* 54:484–489
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Chung HJ, Park SM, Kim HR (2002) Cloning the gene encoding acetyl xylan esterase from *Aspergillus ficuum* and its expression in *Pichia pastoris*. *Enzyme Microb Technol* 31:384–391
- Desalegn BB (2015) Effect of soaking and germination on proximate composition, mineral bioavailability and functional properties of chickpea flour. *Food Public Health* 5:108–113
- Devries JW (2004) Dietary fiber: the influence of definition on analysis and regulation. *J AOAC Int* 87:682–706
- Ghumman A, Kaur A, Singh N (2016) Impact of germination on flour, protein and starch characteristics of lentil (*Lens culinari*) and horsegram (*Macrotyloma uniflorum* L.) lines. *LWT Food Sci Technol* 65:137–144
- Hagerman AE, Austin PJ (1986) Continuous spectrophotometric assay for plant pectin methyl esterase. *J Agric Food Chem* 34:440–444
- Khattak AB, Zeb A, Bibi N, Khalil SA, Khattak MS (2007) Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts. *Food Chem* 104:1074–1079
- Kyomugasho C, Christiaens S, Shpigelman A (2015) FT-IR spectroscopy, a reliable method for routine analysis of the degree of methylesterification of pectin in different fruit- and vegetable-based matrices. *Food Chem* 176:82–90
- Le Goff A, Renard C, Bonnin E, Thibault JF (2001) Extraction, purification and chemical characterisation of xylogalacturonans from pea hulls. *Carbohydr Polym* 45:325–334
- Lopez-Martinez LX, Leyva-Lopez N, Gutierrez-Grijalva EP, Heredia JB (2017) Effect of cooking and germination on bioactive compounds in pulses and their health benefits. *J Fun Food* 38:624–634
- Manrique GD, Lajolo FM (2002) FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biol Technol* 25:99–107
- Martín-Cabrejas MA, Díaz MF, Aguilera Y (2008) Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. *Food Chem* 107:1045–1052
- Mckelvy J, Lee YC (1969) Microheterogeneity of the carbohydrate group of *Aspergillus oryzae* α -amylase 1, 2, 3. *Arch Biochem Biophys* 132:99–110
- Megat RMR, Noralizia CW, Azrina A, Zulkhairi A (2011) Nutritional changes in germinated legumes and rice varieties. *Int Food Res J* 18:705–713
- Megat RMR, Azrina A, Norhaizan ME (2016) Effect of germination on total dietary fibre and total sugar in selected legumes. *Int Food Res J* 23:257–261
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428
- Miyamoto A, Chang KC (1992) Extraction and physicochemical characterization of pectin from sunflower head residues. *J Food Sci* 57:1439–1443
- Muralikrishna G, Tharanathan RN (1994) Characterization of pectic polysaccharides from pulse husks. *Food Chem* 50:87–89
- Nirmala M, Rao MVSSTS, Muralikrishna G (2000) Carbohydrates and their degrading enzymes from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf-15). *Food Chem* 69:175–180
- Orfila C, Knox JP (2000) Spatial regulation of pectic polysaccharides in relation to pit fields in cell walls of tomato fruit pericarp. *Plant Physiol* 122:775–782
- Rumiyati R, James AP, Jayasena V (2012) Effect of germination on the nutritional and protein profile of Australian Sweet Lupin (*Lupinus angustifolius* L.). *Food Nutr Sci* 3:621–626
- Sawardeker JS, Sloneker JH, Jeanes A (1965) Quantitative determination of monosaccharides as their alditol acetates by gas liquid chromatography. *Anal Chem* 37:1602–1604
- Schmelzer T, Wientjes R, Vreeker R, Klaffke W (2002) Enzymatic modifications of pectins and the impact on their rheological properties. *Carbohydr Polym* 47:99–108
- Selvendran RR, O'Neill MA (1987) Isolation and analysis of cell walls from plant material. *Methods Biochem Anal* 32:25–153
- Shiga TM, Lajolo FM (2006) Cell wall polysaccharides of common beans (*Phaseolus vulgaris* L.)—composition and structure. *Carbohydr Polym* 63:1–12
- Subba Rao MVSST, Muralikrishna G (2001) Non-starch polysaccharides and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf - 15). *Food Chem* 72:187–192
- Urbano G, López-Jurado M, Frejnagel S (2005) Nutritional assessment of raw and germinated pea (*Pisum sativum* L.) protein and carbohydrate by in vitro and in vivo techniques. *Nutrition* 21:230–239
- Vasishtha H, Srivastava RP (2013) Effect of soaking and germination on dietary fibre constituents of chickpea (*Cicer arietinum* L.). *Legume Res* 36:174–179
- Wang W, Ma X, Jiang P (2016) Characterization of pectin from grapefruit peel: a comparison of ultrasound-assisted and conventional heating extractions. *Food Hydrocoll* 61:730–739
- Winning H, Viereck N, Nørgaard L (2007) Quantification of the degree of blockiness in pectins using ¹H NMR spectroscopy and chemometrics. *Food Hydrocoll* 21:256–266
- Wood JA, Knights EJ, Campbell GM, Choct M (2014) Differences between easy- and difficult-to-mill chickpea (*Cicer arietinum* L.) genotypes. Part I: broad chemical composition. *J Sci Food Agric* 94:1437–1445
- Woolard GR, Rathbone EB, Novellie L (1977) Studies on free sugars, water soluble gums and hemicelluloses from barley grains and malts. *J Sci Food Agric* 28:469–476

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.