Faba bean hulls as a potential source of pectin

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Abstract This study aimed for the first time to assess the potential use of faba bean hulls as a source of pectin. The study involved extracting pectin under various conditions of pH, temperature and extraction time and determining how these conditions affected pectin yield and its characteristics. The maximum yield of extracted pectin did not coincide with the highest degree of esterification since the maximum yield (15.75 %) was recorded at pH 1.5 and at a temperature of 85 °C for an 80-min extraction period and solid to liquid (1: 25) ratio, while the highest degree of esterification (54.62 %) occurred at pH 2.5 and at temperature of 90 °C for a 60-min extraction period. The composition of the pectin varied according to the extraction conditions: the neutral sugars galactose, arabinose and rhamnose increased under milder extraction conditions while glucose, mannose and xylose sugars predominated under harsher extraction conditions. The results indicated that faba bean hulls contains adequate amount of pectin, suitable for commercial utilization.

Keywords Faba bean hulls · Pectin · Extraction · Characteristics

Introduction

Pectin is a polysaccharide extracted from the cell walls of plants (Ngouemazong et al. 2012) and composed of linear

chains of partly methylesterified galacturonic acid linked to neutral sugars such as L-rhamnose, D-galactose, L-arabinose and D-mannose (May 2000; Vincken et al. 2003). The pectin extraction method is a multi-stage physicochemical process where pectin molecules are hydrolyzed and solubilized from the cell walls and middle lamella of the plant tissues, it primarily focus on breaking the bonds between pectin and other compounds, with minimum damage to the chemical structure of pectin (Endress and Christensen 2009). The methods commonly used for extracting pectin are based on exposing the plant tissues to diluted acid solutions at specific temperatures for defined time periods (May 2000). The physical and chemical characteristics of pectin are highly affected by the extraction conditions and the plant source (Kjoniksen et al. 2005). Because of its versatile gelling properties, pectin is used to form the structure in a broad spectrum of food and pharmaceutical products (Tamaki et al. 2008; Endress and Christensen 2009). In addition, pectin has been found to have many therapeutic effects: reducing hyperglycemia (Zhao et al. 2006), hyperlipidemia (Brown et al. 1999), obesity (Kumar and Chauhan 2010), diarrhea (Triplehorn and Millard 2002) and cancer rates (Glinsky and Raz 2009). The raw materials for pectin production have been mostly from food processing byproducts such as citrus peel and apple pomace (Voragen et al. 1995). However, several other food byproducts have been investigated as potential sources of pectin: soybean hull (Monsoor and Proctor 2001), Akebia trifoliolate peel (Jiang et al. 2012), passion fruit peel (Kulkarni and Vijayanand 2010), peach pomace (Faravash and Ashtiani 2008), banana peel (Happi Emaga et al. 2008) and sugar beet pulp (Levigne et al. 2002). At present, the global demand for pectin far exceeds its production due to the development of more industries requiring pectin (Yeoh et al. 2008). Consequently, more research is needed to discover new affordable sources of pectin, particularly in developing countries. Faba bean (*Vicia faba*) is one of the most important grain legumes in the world,

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used for providing cheap protein for a large sector of the population worldwide (Al Barri and Shtaya 2013). It is considered as a popular food consumed by all income groups in Saudi Arabia; however the local production from the central and west regions (Al-Suhaibani 2009; Alghamdi 2009) cannot meet the demand, so large quantities are imported every year. In 2011, Saudi Arabia was ranked as the third largest importer of faba beans in the world after Egypt and Sudan (FAO 2011). The decortication process for faba bean generates 14.28 % of the total weight of the dry beans as hulls (Güzel and Sayar 2012) which, at present, are disposed of as waste or used for animal feed. As no information is available on extracting pectin from faba bean hulls, the present study aims to provide this data for exploring the potential use of faba bean hulls as a novel source of pectin. To achieve this, the influence of extraction conditions such as pH, temperature and process time on pectin yield and its chemical characteristics will be investigated.

Materials and methods

Sample preparation

Faba bean hulls (5 kg) were obtained from a local market in Jeddah, Saudi Arabia, dried in a drying oven (Model UT6, Heraeus, Japan) at 60 °C to a constant weight. They were then ground to a fine powder using a grinder (Type MCU 1A, Moulinex, France) and stored in sealed plastic bags at 4 °C until analysis. All chemical reagents used were of chromatographic quality.

Extraction parameters

The strategy adopted was to optimize one particular parameter at a time and then include it at its optimum value in the next optimization step.

Extraction pH

The ground hulls were treated with diluted solutions of HCl (Sigma-Aldrich, USA) which were adjusted to pH values of 1.0, 1.5, 2.0 and 2.5. The extraction was conducted under reflux with gentle agitation in a shaking water bath (SW23, Julabo, Germany). The extraction temperature (90 °C) and time (60 min) were kept constant throughout this experimental step.

Extraction temperature

The ground hulls were subjected to extraction under gentle agitation at a series of temperatures: 80, 85, 90 and 95 °C for 60 min and at a previously optimized pH value of 1.5.

Extraction time

The extraction of pectin was conducted at time periods of 60, 70, 80 and 90 min at the previously identified optimal pH, 1.5, and optimal temperature, 85 °C.

Extraction solid to liquid (S: L) ratio

Pectin was extracted at various ratios of hulls to solvent, 1:15, 1:20, 1:25, and 1:30 whereas other conditions, pH, temperature and time were constant at 1.5, 85 °C and 80-min respectively.

Pectin extraction

Pectin was extracted as previously described by Jiang et al. (2012) with a slight modification. The extraction of samples was conducted at specific values of pH, temperature and time period. The extract was filtered through a 200-mesh filter cloth. The filtrate was centrifuged at 4800 rpm in a centrifuge (Eppendorf 3810 R, Hamburg, Germany) for 20 min to remove any solid particles. The pectin was precipitated with two volumes of 95 % ethanol (Sigma-Aldrich) and filtered after 1 h. The precipitate was washed three times with 65, 85 and then 100 % ethanol and the extracted pectin was dried in an air-circulation oven (Heraeus) at 50 °C to a constant weight. The dried pectin was ground and stored in airtight bags for further analysis. The yield was calculated as weight of dried pectin (g) per 100 g of dried hulls.

Monosaccharide and galacturonic acid analysis

Pectin samples (25 mg) from each extract were hydrolyzed with 2 M H₂SO₄ (2.5 ml) at 100 °C for 4 h. The hydrolysates were neutralized with NH₄OH (14 M). As an internal standard, 2-desoxy-D-glucose (Sigma-Aldrich) was used with neutral sugars (Happi Emaga et al. 2012). To adjust for the degradation of sugars and galacturonic acid during hydrolysis, the system calibration was based on a standard mixture of sugars treated in parallel with the sample. The hydrolysate was then filtered through a 0.45 µm PTFE membrane syringe filter (Sigma-Aldrich) and then analyzed by HPLC (Beckman System Gold, Fullerton, CA, USA). The neutral sugars were separated and quantified using an Aminex HPX-87P column 300×7.8 mm (Bio-Rad, USA) and a refractive index (RI) detector. Elution was carried out with ultra-pure deionized water at a flow rate of 0.6 ml/min at 80 °C. The galacturonic acid was isolated and estimated using an Aminex HPX-87H column 300×7.8 mm (Bio-Rad) at 60 °C eluted with 5 mM H₂SO₄ at a flow rate of 0.6 ml/min.

Determination of degree of methoxylation

Pectin samples from each extract were treated with 0.2 M NaOH for 2 h at 4 °C. Succinic acid was added as the internal standard. The methoxy group liberated after saponification was isolated, measured by Aminex HPX-87H at 30 °C and eluted with 5 mM H₂SO₄ solution at a flow rate of 0.6 ml/min. The degree of methoxylation was calculated as the percentage molar ratio of methanol (MeOH) to galacturonic acid content (Happi Emaga et al. 2008).

Statistical analysis

All measurements were done in triplicate. The data were presented as the mean values±standard deviation. Significant differences between means ($P<0.05$) were analyzed with ANOVA using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

Results and discussion

Effect of pH on extraction of pectin

The results in Table 1 showed that the yield obtained was significantly affected by the extraction pH. The yield of extracted pectin increased with increasing extraction pH to a maximum of 12.55 % at pH 1.5, then started to decrease to 9.57 % at pH 2.5. In agreement with Garna et al. (2007), who worked with apple pomace, pH 1.5 was the optimum value for pectin extraction. The reduced yield at a lower pH may be due

to the partial destruction of pectin molecules and poor sedimentation using alcohol during extraction (Kliemann et al. 2009). In contrast, the reduced pectin yield at a higher pH was perhaps due to the incomplete liberation of pectin from the cell walls of the hulls (Voragen et al. 2003; Faravash and Ashtiani 2008). The trend of the degree of methylation did not correspond with the extracted yield with respect to pH value. The degree of methylation increased significantly as the pH increased, which could have been due to ester hydrolysis as a result of higher acidity (Mort et al. 1993).

The highest galacturonic acid content and yield did not coincide at tested extraction pH values. The galacturonic acid content increased progressively with the increase of extraction pH values: similar observations were found in pectin extracted from chicory roots (Robert et al. 2006) and apple pomace (Garna et al. 2007). Yapo et al. (2007) disagreed with this: they found that the galacturonic acid content of pectin extracted from sugar beet pulp decreased as the pH value of the extraction media increased: this may be attributed to the different origins of plant tissue (Garna et al. 2007; Happi Emaga et al. 2008). The increased galacturonic acid content of pectin at a lower pH may be because at lower pH values non-pectic materials solubilized and consequently co-precipitated with pectin, taking into account that galacturonic acid content is indicative of pectin purity (Garna et al. 2007).

The neutral sugar contents of the extracted pectin were significantly influenced by the pH value of the extraction solutions. The galactose content was higher than the arabinose content, while the rhamnose content was lowest at all extraction pH values. These results disagreed with those of Levigne et al. (2002) who found that pectin extracted from sugar beet pulp at pH 1 contained more rhamnose than arabinose, while at pH 2 the pectin contained more arabinose and less rhamnose. Other investigators have shown that both galactose and arabinose contents decreased as the pH increased in pectins extracted from banana peel while rhamnose content increased with increased pH (Happi Emaga et al. 2008). It can be suggested that pectin is extracted with different proportions of neutral sugars as pH changes (Levigne et al. 2002; Iagher et al. 2002; Ralet and Thibault 1994). Glucose, xylose and mannose contents increased as acidity increased. This may be due to the co-extraction of materials other than pectin (cellulose and/ or hemicellulose) and therefore these saccharides came predominately from these sources (Happi Emaga et al. 2008).

Effect of temperature on extraction of pectin

Table 2 shows that an extraction temperature 85 °C released the maximum yield of pectin (13.79 %) while higher (90 and 95 °C) or lower (80 °C) temperatures resulted in decreased yields of pectin. The lower extraction temperatures may have led to incomplete liberation of pectin from the cell wall, while higher extraction temperatures may have caused cracking of

Table 1 Yield and chemical characteristics of faba bean hulls pectin extracted at different pH values

Parameter (%,w/w)	pH			
	1.0	1.5	2.0	2.5
yield	11.24±0.18 ^b	12.55±0.14 ^a	10.83±0.06 ^b	9.57±0.19 ^c
DM	47.52±0.2 ^d	51.59±0.21 ^c	53.17±0.05 ^b	54.62±0.17 ^a
GA	58.11±0.11 ^d	61.54±0.14 ^c	65.67±0.18 ^b	67.41±0.18 ^a
Rha	1.07±0.08 ^d	2.18±0.13 ^c	3.47±0.11 ^b	4.29±0.14 ^a
Ara	2.16±0.18 ^d	6.18±0.18 ^c	10.22±0.08 ^b	11.99±0.18 ^a
Xyl	14.68±0.16 ^a	14.6±0.17 ^a	12.93±0.06 ^b	12.34±0.3 ^b
Man	1.91±0.15 ^a	1.08±0.03 ^b	0.83±0.09 ^{bc}	0.74±0.05 ^c
Gal	3.41±0.12 ^d	8.54±0.04 ^c	11.51±0.11 ^b	12.99±0.08 ^a
Glu	12.71±0.07 ^a	12.57±0.17 ^a	10.37±0.14 ^b	10.23±0.04 ^b

Each value is expressed as mean±standard deviation of triplicate measurements. Values in the same row with different superscripts are significantly different ($p<0.05$). Extraction condition: S/L 1:20, temperature 90 °C and time 60 min. Degree of methylation (DM); Galacturonic acid (GA); Rhamnose (Rha); Arabinose (Ara); Xylose (Xyl); Mannose (Man); Galactose (Gal); Glucose (Glu)

Table 2 Yield and chemical characteristics of faba bean hulls pectin extracted at different temperatures

Parameter (%,w/w)	Temperature, °C			
	80	85	90	95
yield	11.42±0.15 ^c	13.79±0.11 ^a	12.34±0.22 ^b	11.35±0.12 ^c
DM	53.59±0.21 ^a	52.55±0.1 ^b	51.6±0.11 ^c	49.23±0.18 ^d
GA	61.26±0.29 ^a	61.31±0.22 ^a	61.36±0.12 ^a	61.62±0.23 ^a
Rha	3.72±0.09 ^a	3.37±0.17 ^a	2.19±0.05 ^b	1.33±0.19 ^c
Ara	7.72±0.07 ^a	7.47±0.19 ^a	6.21±0.21 ^b	5.04±0.15 ^c
Xyl	13.4±0.15 ^c	13.41±0.21 ^c	14.6±0.17 ^b	16.88±0.01 ^a
Man	0.43±0.04 ^d	0.72±0.03 ^c	1.07±0.05 ^b	1.51±0.08 ^a
Gal	13.32±0.12 ^a	11.95±0.05 ^b	8.48±0.16 ^c	7.22±0.07 ^d
Glu	11.49±0.18 ^c	11.33±0.10 ^c	12.57±0.17 ^b	15.03±0.22 ^a

Each value is expressed as mean±standard deviation of triplicate measurements. Values in the same row with different superscripts are significantly different ($p<0.05$). Extraction condition: S/L 1:20, pH 1.5 and time 60 min

pectin molecule resulting in decreased pectin yield (Chang et al. 1994).

The degree of methylation values reduced significantly as the extraction temperature increased. At higher temperatures, catalysis of partial hydrolysis of the methyl ester-bonds in pectin molecule occurred (Kulkarni and Vijayanand 2010; Yapo et al. 2007). However, the highest degree of methylation (53.21 %) was not associated with the maximum yield (13.61 %). The galacturonic acid content was constant at all tested temperatures. Similar results were reported by Levigne et al. (2002) in pectin extracted from sugar beet pulp.

The results have revealed that pectin extracted at lower temperatures contained higher amounts of rhamnose, arabinose and galactose and lower amounts of xylose, mannose and glucose, and the opposite at higher temperatures. At higher temperatures non-pectic saccharides are extracted and co-precipitated with pectin resulting in increasing the contents of xylose, mannose and glucose in the pectin (Kulkarni and Vijayanand 2010; Monsoor 2005).

Effect of extraction time

Table 3 shows that the amount of pectin extracted increased with time up to the peak point (14.6 %) at 80 min then decreased thereafter. Such a decline in yield may be attributed to the destructive effect of both acidity and heat on the pectin molecule as extraction time increased (Pagan et al. 2001).

The highest value for the degree of methylation was observed for a 60-min extraction time then decreased with increasing extraction time. This is perhaps due to the partial cleavage of the ester bond as a result of acidic action on the pectin molecule. Similar findings have been reported with pectin extracted from the peel of passion fruit by Kulkarni

Table 3 Yield and chemical characteristics of faba bean hulls pectin extracted at different extraction periods

Parameter (%,w/w)	Extraction time, min			
	60	70	80	90
yield	13.33±0.11 ^b	13.41±0.11 ^b	14.6±0.16 ^a	13.18±0.06 ^b
DM	53.35±0.10 ^a	52.58±0.19 ^b	50.92±0.16 ^c	49.48±0.06 ^d
GA	61.51±0.45 ^a	61.88±0.09 ^a	61.35±0.15 ^a	61.50±0.25 ^a
Rha	3.49±0.18 ^a	3.37±0.15 ^a	2.52±0.11 ^b	1.91±0.10 ^c
Ara	10.13±0.09 ^a	7.47±0.19 ^b	5.20±0.20 ^c	4.19±0.08 ^d
Xyl	13.53±0.04 ^b	13.31±0.16 ^b	15.50±0.21 ^a	15.85±0.08 ^a
Man	0.44±0.09 ^c	0.78±0.09 ^{bc}	1.15±0.05 ^b	1.72±0.22 ^a
Gal	13.14±0.24 ^a	12.08±0.04 ^b	10.61±0.15 ^c	9.09±0.06 ^d
Glu	10.18±0.02 ^d	11.33±0.10 ^c	13.69±0.23 ^b	14.73±0.20 ^a

Each value is expressed as mean±standard deviation of triplicate measurements. Values in the same row with different superscripts are significantly different ($p<0.05$). Extraction condition: S/L 1:20, pH 1.5 and temperature 85 °C

and Vijayanand (2010). The galacturonic acid content of extracted pectin was similar for all tested extraction times, a similar trend being reported for pectin extracted from fresh sugar beet and banana peel (Levigne et al. 2002; Happi Emaga et al. 2008). The results in Table 3 have shown that extending the extraction times resulted in extracting pectin with higher contents of xylose, mannose and glucose, which have been supposed to have a non-pectic origin (Zhang et al. 2011).

Effect of solid to liquid ratio

Table 4 shows that the yield of pectin was significantly increased as the S: L ratio increased to reach its maxim (15.75 %

Table 4 Yield and chemical characteristics of faba bean hulls pectin extracted at different extraction solid to liquid ratio

Parameter (%,w/w)	Solid: Liquid, w/v			
	1:15	1:20	1:25	1:30
yield	11.03±0.09 ^c	14.86±0.14 ^b	15.75±0.22 ^a	15.74±0.19 ^a
DM	51.09±0.36 ^a	50.97±0.03 ^a	50.96±0.31 ^a	51.05±0.10 ^a
GA	63.97±0.02 ^a	63.06±0.13 ^b	61.35±0.27 ^c	61.48±0.40 ^c
Rha	3.56±0.25 ^a	2.87±0.11 ^b	2.42±0.19 ^c	1.94±0.25 ^d
Ara	7.04±0.29 ^a	6.40±0.32 ^b	5.29±0.16 ^c	4.19±0.14 ^d
Xyl	15.33±0.14 ^a	15.44±0.39 ^a	15.50±0.35 ^a	15.44±0.39 ^a
Man	1.10±0.17 ^a	1.10±0.04 ^a	1.12±0.11 ^a	1.16±0.06 ^a
Gal	12.66±0.08 ^a	11.85±0.17 ^b	10.57±0.17 ^c	9.09±0.10 ^d
Glu	9.06±0.16 ^d	11.61±0.49 ^c	13.72±0.29 ^b	15.73±0.34 ^a

Each value is expressed as mean±standard deviation of triplicate measurements. Values in the same row with different superscripts are significantly different ($p<0.05$). Extraction condition: pH 1.5, temperature 85 °C and time 80 min

w/w) at 1:25 ratio, which is a ratio similar to that reported for the highest yield of pectin extracted from cocoa husks (Chan and Choo 2013). There was, however, no significant effect on increasing S: L on extracted pectin yield. On the other hand, at lower S: L ratios, lower pectin yields were liberated due to the incomplete solubilization of pectin as a result of inadequate solvent (Kulkarni and Vijayanand 2010). Similar results for the effect of S: L ratio on the pectin yield extracted from soy hull was reported by Korish (2014); Monsoor and Proctor (2001). From the Table 4, it can be concluded that, the highest extracted pectin yield was 15.75 % of the dry weight of the faba bean hulls. This value was generally higher than pectins extracted from cocoa husks (Chan and Choo 2013); sugar beet (Yapo et al. 2007); and banana peel (Happi Emaga et al. 2008). From the commercial point of view, each one-ton faba bean hulls can produce 157.5-kilogram pectin, under the above-optimized conditions. No significant deference was determined in the degree of methylation at all tested dilution ratios. Similar observation was indicated by Kulkarni and Vijayanand (2010). The amount of galacturonic acid declined significantly with increase of solvent ratio. This may be due to co-solubilization of impurities at higher solvent ratios. The results in Table 4 have shown that increasing the S: L ratios resulted in extracting pectin with higher contents of glucose, mannose and xylose, which have been supposed to have a non-pectic source (Zhang et al. 2011).

Conclusions

These results indicate that the yield and characteristics of pectin extracted from faba bean hulls varies greatly according to the extraction conditions. The present study has clearly demonstrated that faba bean hulls are a potential new source of pectin. Further studies are required for effective utilization of faba bean hulls as a source of pectin.

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