J Food Sci Technol (April 2015) 52(4):2401–2407
DOI 10.1007/s13197-014-1277-y

ORIGINAL ARTICLE

Potential utilization of *Citrullus lanatus* var. Colocynthoides waste as a novel source of pectin

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Revised: 21 January 2014 / Accepted: 4 February 2014 / Published online: 15 February 2014

Abstract The *Citrullus lanatus* var. Colocynthoides is an ancestor type of watermelon. It was investigated as a new source of pectin. It was cultivated in Egypt for seeds only, while the remaining fruits are discarded as waste. Effect of different extraction conditions such as pH, solid: liquid ratio, temperature and extraction time on pectin yield of *Citrullus lanatus* var. Colocynthoides waste was investigated in the present study. The highest yield (19.75 % *w/w*) was achieved at pH 2, solid: liquid ratio1:15 and 85 °C, for 60 min. Methylation degree and galacturonic acid content of extracted pectin were 55.25 %, *w/w* and 76.84 %, *w/w*. The main neutral sugars were galactose followed by arabinose and rhamnose. In addition, glucose, xylose and mannose existed as constituents in the pectin hydrolysate. The results indicated that *Citrullus lanatus* var. Colocynthoide waste is a potential new source of pectin.

Keywords *Citrullus lanatus* var. Colocynthoides waste pectin · Extraction

Introduction

Pectin is a natural hydrocolloid material that exists in primary cell wall of higher plants. It consists of D-galacturonic acid, partially methylated galacturonic acid residues, and neutral sugars such as D-galactose, L-rhamnose, L-arabinose and D-xylose, whereas the kinds and proportions of neutral sugars vary with the source of pectin (Cho et al. 2001; Voragen et al. 2003; Kliemann et al. 2009). The physicochemical characteristics of pectin were highly influenced by the source of raw material and extraction conditions, mainly pH, temperature and extraction time (Levigne et al. 2002; Fishman et al. 1984; Kjoniksen et al. 2005; O'Donoghue and Somerfield 2008). Main methods for pectin extraction involve treating the plant materials with acid solutions from hydrochloric, sulfuric, nitric, acetic, and citric acid at temperatures ranged from 80 to 100 °C for 0.5–6 h (Rolin 2002; Levigne et al. 2002; Canteri-Schemin et al. 2005; Singthong et al. 2005). Due to the multifunctional properties of pectin, it has valuable applications in food industries as a gelling factor, emulsifier, thickener and stabilizer, (Bottger 1990; Endress and Christensen 2009; Willats et al. 2006). Moreover, pectin has abundant physiological functions and healthy usefulness such as macrophage induction (Iacomini et al. 2005), hypoglycemic (Wang et al. 2005), hypolipidemic (Brown et al. 1999) and anticancer (Nangia-Makker et al. 2002).

The major commercial pectin sources were citrus peel, apple pomace and pulp from sugar-beet (Yapo et al. 2007). With increasing demand for pectin for different industrial purposes, the needs of pectin continue to grow although the pectin production was far less from the demand. Consequently, there is a need for vigorous research with the view to discover domestic new affordable pectin sources particularly in developing countries (Jiang et al. 2012).

Citrullus lanatus var. Colocynthoides (C. var. Colocynthoides) is a wild type of watermelon. It belongs to family Cucurbitaceae (Ziyada and Elhussien 2008). It is locally known as seed watermelon or "Gorma". Seed watermelon is cultivated in Egypt in a large scale for using its seeds as snacks. A rising demand for C. var. Colocynthoides seeds and a desire for new cash crops have stimulated interest in cultivating large acreages of C. var. Colocynthoides. As described above, the seeds were only utilized while the total de-seeded

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fruits are thrown into nature as waste, causing environmental problems. This waste can be used as a raw material for the production of useful biomaterials and intermediate chemical products. Hence this study aimed for the first time to investigate the potential utilization of C. var. Colocynthoides waste as a new source of pectin in terms of the impact of different extraction conditions such as pH, S: L ratio, temperature and extraction duration on pectin extraction yield, and its chemical characteristics.

Materials and methods

Samples and reagents

C. var. Colocynthoides 10 fruits (about 50 kg) were obtained from a private farm located in Behera governorate north Egypt.

Fruits were washed and cut into halves to remove seeds using a stainless steel knife, chopped into small pieces and ground with blender “moulinex model 570, France”. The mash was washed in tap water to remove soluble solids then dried in a hot air oven at 50 °C to a constant weight. Then, it was milled to a fine powder and stored in sealed plastic bags at 4 °C for further analysis. All used chemical reagents were of analytical or chromatographic quality.

Determination of fruit physicochemical properties

The fresh weight of the fruits, seed contents (*w/w*) and diameter were determined using average measures of 3 mature fruits. Moisture, ash, crude fat and crude protein (N×6.25) contents in raw material and extracted pectin were determined according to the standard methods of (AOAC 2000).

Extraction conditions

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 dried C. var. Colocynthoides waste was subjected to extraction in acidified water with HCl at pH values 1.0, 1.5, 2.0, 2.5 and 3.0, whereas the S: L, extraction period and temperature were 1:20, 50 min and 98 °C, respectively. All extractions were done under shaking (100 rpm) using shaking water bath SW23, Julabo, Germany.

Extraction solid to liquid (S: L) ratio

Pectin was extracted at various ratios of dried waste to solvent, 1:10, 1:15, 1:20 and1:25, whereas other conditions, pH, temperature and time were constant at 2, 98 °C and 50 min respectively.

Extraction temperature

Extraction of pectin was conducted at series of temperatures 75, 85, 95 and 98 °C at constant conditions of pH2, S: L 1:15 and time 50 min.

Extraction time

Pectin extraction process was carried out at different time periods of 50, 60, 70 and 80 min under constant conditions: pH 2, S: L 1:15, temperature: 85 °C.

Pectin extraction

The extraction of pectin was according to Jiang et al. (2012) with slight modification. Samples were subjected to extraction at tested extraction parameters of adopted various pH values, S: L ratios, series of temperatures and time intervals. The resulting extract was filtered through a 200-mesh filter cloth. The filtrate was centrifuged at 4,800 rpm for 20 min to remove the solid particles. The pectin was precipitated with two volumes of 95 % ethanol and kept for 1 h prior to filtration. The precipitate was washed three times with 65 %, 85 % and 100 % ethanol, and the produced pectin was dried in an air-circulate oven (Heraeus, Model UT6, Japan) at 45 °C for 12 h. The dried pectin was ground to fine powder and stored in hermetically sealed bags for further analysis. The yield was calculated as g (dried pectin) /100 g (dried waste).

Characterization of extracted pectin

The pH of (1 % pectin solution) was determined at 25 °C using Jenway pH meter Model 3505, England. For monosaccharide and galacturonic acid analysis sample 25 mg was hydrolyzed with 2 M H_2SO_4 (2.5 ml) at 100 °C for 4 h. The hydrolysate was neutralized with NH_4OH (14 M). As internal standard, 2-desoxy-D-glucose was used with neutral sugars (Happi Emaga et al. 2012). To adjust for sugars and galacturonic acid degradation during hydrolysis, system calibration were based upon standard mixture of sugars and galacturonic acid standard solution were treated in parallel with sample, then hydrolysate was filtered through a 0.45 μm PTFE-membrane syringe filters, analyzed by HPLC (Beckman System Gold, Fullerton, California).

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.

Table 1 general physicochemical characteristic of C. var. Colocynthoides fruit

Parameter	Measurement
Fruit yield(ton)/ hectare	17.51±0.58
Fruit weight (kg)	4.99±0.19
Fruit diameter (cm)	20.97±1.42
Seed content (% *w/w*, fresh basis)	2.54±0.34
Moisture (%)	92.80±1.37
Crude protein (%, dry basis)	6.32±0.17
Crude fat (%, dry basis)	1.94±0.07
Ash (%, dry basis)	3.73±0.07
Fruit juice pH	4.91±0.08

Each value was expressed as mean ± standard deviation of triplicate measurements

Galacturonic acid was isolated and estimated by an Aminex HPX-87H column 300×7.8 mm (Bio-Rad, USA) at 60 °C eluted with 5 mM H_2SO_4 at a flow rate of 0.6 ml/min.

For determination of methoxy group content, pectin samples were treated with 0.2 M NaOH for 2 h, at 4 °C. Succinic acid was added as internal standard. The methoxy group which liberated after saponification was isolated and measured by Aminex HPX-87H at 30 °C, eluted with 5 mM H_2SO4 solution at a flow rate of 0.6 ml/min. Degree of methoxylation (DM) was calculated as the percent molar ratio of methanol (MeOH) to the galacturonic acid (GalA) content (HappiEmaga et al. 2008).

Statistical analysis

All determinations were carried out in triplicate and results were expressed as the mean value ± standard deviation of three measurements. Significant differences between means ($p<0.05$) were compared using SPSS 13.0 (SPSS Inc., IL, USA).

Results and discussion

Physicochemical characteristics of C.var.Colocynthoides fruit

As presented in Table 1, the seeds represented a 2.54 % of the total weight of the fruit, while 97.46 % of the fruit weight was discarded as a waste. Thus, each hectare cultivated with C.var.Colocynthoides generats~17.06 t of such waste. Chemical composition of the fruit showed that crude protein, crude fat and ash constituents represented 11.99 % while carbohydrates were the main component, showing that the waste is a rich carbohydrate source for further utilization.

Effect of extraction pH on the yield and characteristics of pectin

The results presented in Table 2 indicated that, the extraction pH had a significant impact on the pectin yield from C. var. Colocynthoides waste. The maximum yield of pectin was obtained at extraction pH 2.0. This in agreement with the findings of many investigators who found that, the highest yield of pectin was liberated at pH 2 from passion fruit peel (Kulkarni and Vijayanand 2010), young sugar palm (Rungrodnimitchai 2011) and apple pomace (Chakraborty and Ray 2011). However, increasing or decreasing the pH of the extraction media from the optimal value (pH 2) resulted in decreasing pectin yield. This may be due to increasing acid strength (pH<2) that could over hydrolyze and degrade C. var. Colocynthoides pectin to uncollected small pectin particles resulting in increased pectin-particle solubility and consequently pectin precipitation with alcohol is hindered (Kalapathy and Proctor 2001; Kliemann, et al. 2009). On the other hand, at lower acid strength, pectin molecules can be partially solubilized without degradation leading to difficulty in extractability of some pectin fractions due attachment to other cell wall components (Voragen et al. 2003).

The highest degree of methylation value of extracted pectin was obtained at pH 3 and the methylation degree decreased

Table 2 Effect of extraction pH on the yield and characteristics of C. var. Colocynthoides waste pectin

Chemical parameters	pH				
	1.0	1.5	2.0	2.5	3.0
Yield (% *w/w*)	11.43±0.17^{d}	14.77±0.36^{b}	15.33±0.23^{a}	13.57 ±0.15^{c}	11.32±0.35 ^{d}
Degree of methylation (% *w/w*)	50.81±0.70^{d}	53.11±0.44^{c}	54.10±0.22 ^{b}	54.83±0.20 $^{a\ b}$	55.40±0.35 ^{a}
Galacturonic acid (% *w/w*)	70.18±0.164 ^{d}	75.47±0.36 ^{c}	76.72±0.25 ^{a}	76.69±0.18 ^{a}	75.99±0.15 ^{b}
Protein (% *w/w*)	0.43±0.05 ^{b}	0.67±0.04 ^{a}	0.66±0.06 ^{a}	0.72±0.12 ^{a}	0.63±0.06 ^{a}
Ash(% *w/w*)	1.62±0.10 ^{a}	1.49±0.01 $^{a\ b}$	1.31±0.07 ^{b}	0.53±0.24 ^{c}	0.39±0.01 ^{c}
pH of 1 % solution	3.36±0.23 ^{a}	3.19±0.20 ^{a}	2.99±0.02 ^{a}	3.13±0.32 ^{a}	2.95±0.07 ^{a}

Mean values from triplicate measurements ± standard deviation. Values in the same linecarry different superscripts are significantly different ($p<0.05$). Extraction condition: S: L 1:20, Temperature 98 °C, time 50 min

Table 3 Effect of S: L ratio on the yield and characteristics of C. var. Colocynthoides waste pectin

Chemical parameters	Solid/ liquid ratio			
	1:10	1:15	1:20	1:25
Yield (% *w/w*)	13.88±0.24[b]	15.87±0.31[a]	15.27±0.11[a]	15.37±0.48[a]
Degree of methylation (% *w/w*)	54.28±0.53[a]	54.73±0.73[a]	53.95±0.49[a]	54.19±0.17[a]
Galacturonic acid (% *w/w*)	75.87±0.65[a]	76.52±0.54[a]	76.68±0.22[a]	76.33±0.48[a]
Protein (% *w/w*)	0.56±0.12[b]	0.66±0.19[b]	0.68±0.16[b]	1.24±0.25[a]
Ash (% *w/w*)	0.50±0.12[b]	0.93±0.49[a b]	1.01±0.37[a b]	1.47±0.07[a]
pH of 1 % solution	3.09±0.12[a]	3.03±0.16[a]	2.98±0.12[a]	3.12±0.22[a]

Mean values from triplicate measurements ± standard deviation. Values in the same linecarry different superscripts are significantly different ($p<0.05$). Extraction condition: pH 2, Temperature 98 °C and time 50 min

gradually with increasing the acidity of extraction media. This may be due to partial deesterification of pectin (Mort et al. 1993). Similar trend was reported during extraction of pectin from passion fruit peel (Kulkarni and Vijayanand 2010). It could be concluded that the maximum yield of pectin is not associated with its quality.

The maximum galacturonic acid values were obtained at pH ranged from 2 to 2.5. These may be attributed to that the solubility of cell walls components other than pectin at higher acidity extraction conditions (pH 1, 1.5) and present in pectin as impurities (Garna et al. 2007). The results are in accordance with those of Happi Emaga et al. (2008) who reported that the highest proportion of galacturonic acid in pectin extracted from banana peels was occurred at pH 2.

The ash level increased progressively with decreasing acidity. The ash content of pectin was detected at all extraction pH values in amounts less than mentioned by Ptichkina et al. (2008) and Jiang et al. (2012) in pectin extracted from pumpkin and Akebia trifoliata var. australis peel. This can be attributed to increasing minerals solubility at higher acid strength that eventually precipitated with pectin (Kalapathy and Proctor (2001). On the other hand, the protein content level seemed to be stable at all tested acid strength except for at pH 1. At this pH, the slight decrease in protein content could be attributed to the nature of proteinaceous compounds in the raw material. The data also exhibited that the pH values of 1 % solution of the extracted pectin samples was not influenced by different acid strength levels investigated.

Effect of solid to liquid ratio on the yield and characteristics of pectin

The results in Table 3 showed that the pectin yield significantly increased as the S: L ratio increased and was maximal (15.87 %) at ratio 1:15 with no further increase thereafter. On the other hand, the lowest pectin yield was liberated with ratio (1:10) due to the incomplete solublization 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 Monsoor and Proctor (2001).

The degree of methylation in extracted pectin samples was not significantly affected by variation of the S: L ratio. A typical feature was described by Kulkarni and Vijayanand (2010) with passion fruit peel pectin. No significant deference was detected in galacturonic acid content at all tested dilutions.

Table 4 Effect extraction temperature on the yield and characteristics of C. var. Colocynthoides waste pectin

Chemical parameters	Temperature, °C			
	75	85	95	98
Yield (% *w/w*)	15.74±0.23[b]	17.90±0.40[a]	15.99±0.46[b]	15.85±0.15[b]
Degree of methylation (% *w/w*)	56.04±0.36[a]	55.92±0.36[a]	54.29±0.82[b]	54.13±0.06 [b]
Galacturonic acid (% *w/w*)	76.69±0.46 [a]	76.65±0.83[a]	76.47±0.45[a]	76.53±0.47[a]
Protein (% *w/w*)	0.52±0.03[a]	0.49±0.11[a]	0.45±0.07[a]	0.66±0.19[a]
Ash (% *w/w*)	1.35±0.07[a b]	1.42±0.02[a]	1.39±0.01[a]	1.42±0.36[a]
pH of 1 % solution	3.02±0.17[a]	3.03±0.06[a]	2.94±0.08 [a]	3.03±0.05[a]

Mean values from triplicate measurements ± standard deviation. Values in the same line carry different superscripts are significantly different ($p<0.05$). Extraction condition: pH 2, S: L 1:15 and time 50 min

Table 5 Effect of extraction time on the yield and characteristics of C. var. Colocynthoides waste pectin

Chemical parameters	Extraction time, min			
	50	60	70	80
Yield (% *w/w*)	18.07±0.39^{b}	19.75±0.07^{a}	15.84±0.31^{c}	15.62±0.46^{c}
Degree of methylation (% *w/w*)	55.96±0.42^{a}	55.25±0.18^{b}	54.06±0.07^{c}	53.71±0.24^{c}
Galacturonic acid (% *w/w*)	76.65±0.83^{a}	76.84±0.20^{a}	75.13±0.11^{b}	74.94±0.11^{b}
Protein (% *w/w*)	0.57±0.10^{a}	0.60±0.17^{a}	0.72±0.07^{a}	0.73±0.09^{a}
Ash (% *w/w*)	1.42±0.13^{a}	1.56±0.05^{a}	1.54±0.12^{a}	1.58±0.07^{a}
pH of 1 % solution	3.13±0.26^{a}	3.01±0.02^{a}	3.07±0.13^{a}	3.04±0.06^{a}

Mean values from triplicate measurements ± standard deviation. Values in the same linecarry different superscripts are significantly different ($p<0.05$). Extraction condition: pH 2, S: L 1:15 and temperature 85 °C

All S: L ratios produced pectin with similar protein content except for the highest ratio 1:25 that produced pectin with more protein content. On the other hand, the ash content increased slightly as the S: L ratio increased. The pH of the extracted pectin was similar at all tested S: L ratio.

Effect of extraction temperature on the yield and characteristics of pectin

The results in Table 4 show that the extraction temperature had a significant impact on the extraction of pectin from C. var. Colocynthoides waste. The pectin yield increased as the extraction temperature increased to reach its peak (17.90 %) at 85 °C. Further, increasing in the extraction temperature above 85 °C decreased the pectin yield. The decrease in the yield at higher temperature probably is due to break down of pectin molecules resulting in reduced pectin yield (Chang et al. 1994). These results are in agreement with those of Pagan et al. (2001) who reported that the temperature had a significant effect on the extraction of pectin from stored peach pomace. However, Yapo et al. (2007) found that the temperature did not alter the extraction characteristics of pectin from sugar beet pulp.

The present results indicated that methylation degree of the pectin declined significantly with increasing extraction temperature. Similar findings were reported by Kumar and Chauhan (2010); Kulkarni and Vijayanand (2010). This may be attributed to the deesterification of pectin at higher temperature (Mort et al. 1993; Monsoor and Proctor 2001).

The galacturonic acid content was not affected by the extraction temperatures. Similarly, Levigne et al. (2002) reported that galacturonic acid content of pectin extracted from fresh sugar beet was not impacted by extraction temperature.

No significant effect of increasing extraction temperature on the co-precipitation of protein during pectin extraction was detected, whereas ash content was slightly increased. Nonetheless, the pH value of extracted pectin (1 % solution) was not impacted by extraction temperature.

Effect of extraction time on the yield and characteristics of pectin

Amounts of pectin at different extraction periods are shown in Table 5. It was evident that, pectin yield was significantly affected by extraction time. Similar observation was reported by Pagan and Ibarz (1999); Joye and Luzio (2000); Faravash and Ashtiani (2008); Wai et al. (2010). The highest pectin yield was achieved at 60 min. Kulkarni and Vijayanand (2010); Pagan et al. (2001) reported similar results on the extraction of pectin from passion fruit peel and stored peach pomace, respectively. It was clearly evident that extending the extraction time longer than 60 min decreased pectin yield. Similarly, Pagan et al. (2001) observed that yield of pectin extracted from stored peach pomace reduced with lengthen of the extraction period. This could be due to the combined negative effect of acid and temperature for extended extraction time on the pectin molecule (Pagan et al. (2001). In addition, there was a significant decrease in the methylation degree with extending extraction intervals. These results are in agreement with those of Garna et al. (2007) and Happi Emaga et al. (2008) who extracted pectin from apple pomace and banana peels, respectively.

Table 6 Neutral sugars contents of pectin from C. var. Colocynthoides waste

Neutral sugar	Rha	Ara	Xyl	Man	Gal	Glu
Means (%, *w/w*)	2.54±0.06	4.88±0.11	0.56±0.02	0.22±0.01	9.07±0.07	2.52±0.07

Mean values from triplicate measurements ± standard deviation

The amount of galacturonic acid declined slightly with advancement of extraction time. On the other hand, the impurities content (protein & ash) of pectin were not affected by extraction time.

Neutral sugars composition

Concentrations of neutral sugars measured in a sample of pectin extracted under the determined optimal extraction conditions (pH 2, S: L 1:15 and 85 °C, for 60 min) are presented in Table 6. The neutral sugars galactose was predominant followed by arabinose and rhamnose. This perhaps indicated that the main structure of pectin could consist of a rhamnogalacturonan backbone and arabinan and/or arabinogalactan-rich side chains (Oosterveld et al. (2000). Other sugars glucose, xylose and mannose existed as constituents in the pectin hydrolysate, the source of these sugars may be due to the co-extraction of substances other than pectin such as cellulose and hemicellulose (Zhang et al. 2011).

Conclusion

The study presents, for the first time, the potential of C. var. Colocynthoides as a new source of high quality pectin for commercial utilization. Further research is needed to elucidate this material as a pectin source.

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