Asian Journal of Chemistry; Vol. 26, No. 19 (2014), 6674-6678

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17590

Extraction and Characterization of Pectin from Red Hawthorn (*Crataegus* spp.) Using Citric Acid and Lemon Juice

SELMA YARLIGAN UYSAL* and EMRE YILDIRIM

Department of Chemistry, Faculty of Arts and Sciences, University of Osmangazi, Eskisehir, Turkey

*Corresponding author: Fax: +90 222 2393578; Tel: +90 222 2393750, Ext. 2869; E-mail: yarligan@ogu.edu.tr

Received: 12 March 2014; *Accepted*: 14 May 2014; *Published online*: 16 September 2014; AJC-15985

A new generation of pectin was extracted from red hawthorn fruit. Pectin extraction was optimized by varying pH, temperature and time as well as the type of extraction solvents, *i.e.*, lemon juice or citric acid. Accordingly, the maximum extracted yield of hawthorn pectin occured when extracted at pH 1.5/60 °C/120 min and pH 2.76/90 °C/120 min citric acid or lemon juice as an extraction solvent, respectively. Moreover, the characteristics measured at these optimized conditions are as follows: pectin yield 16.75 and 7.32 % of dry matter, anhydrouronic acid content 122.90 and 86.21 %, degree of esterification 54.16 and 53.34 % and acetyl groups 0.68 and 0.76 %, respectively. The optimum extraction condition ranges for obtaining desirable physico-chemical parameters of hawthorn pectin, were 60 and 90 °C for 120 min using acidic methods. Pectin obtained using both lemon juice and citric acid was a higher purity than commercial apple pectin.

Keywords: Hawthorn, Pectin, Extraction yield, Physico-chemical properties.

INTRODUCTION

The genus Crataegus, also referred to as hawthorn fruit, belongs to the Rosaceae family. These small trees are distributed throughout East Asia, eastern North America and Europe[1,2]. Hawthorn is represented by 17 different species in Turkey[3]. However, despite Turkey's naturally conducive environment to growth the full potential value of hawthorn fruit has not been fully explored. The characteristics of some Crataegus species have been described in earlier studies focusing on their anti-oxidant, antinociceptive and anti-inflammatory activities in Turkey[4,5]. However, the use of red hawthorn fruits as a source of pectin has not been reported. In Turkey, the flowers and fruits of hawthorn have several traditional medicinal uses for the treatment of different ailments. Hawthorn fruits are called crabapples and their original name was Aliç, they are consumed fresh.

Pectin is a biopolymer composing of α-D-galacturonic acid linked at the (1, 4)-positions and partially substituted by methoxyl ester. The galacturonic acid residues can be methyl-esterified at C-6, which determines the degree of esterification (DE); high-methoxyl pectin is explained to have a (DE = 50 %), whereas low-methoxyl pectins[6] has DE < 50 %. High-methoxyl pectin needs sucrose at a concentration higher than 55 w/w % and acidic conditions (usually a pH range from 2 to 3.5) to form gels, whereas low-methoxy pectin needs Ca^{2+} ions to form gels within a pH range from 2 to 7 regardless of the sugar content[7]. However, the degree of acetylation, which is the proportion of acetyl groups in relation to the total galacturonic acid units of the pectin is also relevant[8]. Both the degree of esterification and degree of acetylation have a substantial impact on the functional properties of pectin, influencing solubilization and gelation[9]. Furthermore, the anhydrouronic acid (AUA) content indicates the purity of the extracted pectin. The suggested minimum value of anhydrouronic acid is 65 % and pharmaceutical standards must contain[7,10] at least 74 %.

Pectin is frequently applied in the food industry, nutrition, cosmetics and pharmaceuticals. It is traditionally used as a thickening agent, such as in the production of jam and jelly[11]. In medicine, pectin is used for treating injuries, lowering blood cholesterol levels and reducing the risk of cardiovascular diseases[12]. In addition, it prevents spontaneous cancer metastasis[13].

Numerous studies have concentrated on the extraction method, chemical composition and gelling properties of pectin[14,15]. We hypothesize, hawthorn might contain many dietary fibers composed of pectic polysaccharides. Hence, the utilization of this residue increase its economic value and provide a wide range of suitable applications.

The aims of this study are intended (i) to optimize the impact of the processing conditions (pH, temperature, time) and the type of acidic extracting solvent on the yield; (ii) to assess and amend the functional properties of the new extracted hawthorn pectin in model and real food systems. The results obtained could serve as a foundation for further research on the potential bioactivity of hawthorn pectin in relation to its polymer structure.

EXPERIMENTAL

Fresh red hawthorn fruits were used in this study. They were purchased from the herbal market in Eskisehir (Turkey). Analytical-grade hydrochloric acid, ethanol, sodium hydroxide, citric acid and magnesium sulfphate (Sigma-Aldrich, Chemical Co., Milan, Italy) were used without further purification.

Extraction of pectin: The red hawthorn fruits were first cleaned in deionized water and dried in a cool place and the seeds were removed manually. Then, the seedless fruit was ground for a few minutes in a domestic coffee grinder and sieved. The percentage yield of pectin from the initial wet peels was then determined on both a wet and dry weight basis. The relative humidity was 78 % before chemical analysis. The powdered samples were oven-dried at 40 °C to obtain constant weight. The moisture content of the pump used to dry the samples extracted from pectin was 16 %. The extracted samples were stored in polyethylene bags and left in a dry, dark place at 4 °C until used.

Isolation of pectin: To determine the best conditions for isolating pectin from red hawthorn fruit, different extraction conditions were applied. The dried pulp produced was re-suspended in water at a ratio of 1 g:10 mL of water. The pH was adjusted to 1-4 with 70 % (v/v) citric acid solution. The mixture was then heated to 60-90 °C and the extraction was performed with continuous stirring for 60-120 min[16]. Similarly, pectin was extracted in lemon juice in a pH range of 2.28-3.8 at 90 °C and 120 min. Deionized water was used to dilute both extraction solvents.

The hot acid extract was vacuum filtered through filter paper and the filtrate was cooled down to 4 °C. The filtrate was supplemented with one-half volume of 96 % ethanol. The system was instantly mixed and left to rest at 4 °C overnight. The mixture was centrifuged (Beckman Coulter Avanti J-25, California, USA) at 10000 rpm for 15 min at 10 °C and subsequently washed three times with 50, 75 and 96 % ethanol and distilled acetone. The alcohol-insoluble residue (AIR) was again centrifuged at 5000 rpm for 10 min at 10 °C. Eventually, the obtained pectin was dried at 40 °C until reaching constant weight and ground in a mortar. The pectin extraction yield was described as the % yield (grams dried pectin per dried peels). Each set of extraction conditions was undertaken in triplicate. The obtained pectin was used for the following analysis.

Characterization of pectin powder: The pectin, extracted from the two different extraction solvents, was examined according to diverse physico-chemical parameters, *i.e.*, ash and moisture contents, equivalent weight, methoxyl content, anhydrouronic acid, degree of esterification and acetyl value[17,18].

Structure analysis: FT-IR spectra of hawthorn pectin were recorded on a FT-IR-ATR spectrophotometer (Perkin Elmer 100 FT-IR Spectrophotometer, Berlin, Germany). The lyophilized dry powder was mixed with KBr and pressed into pellets. FT-IR spectra were obtained in a wavelengths range of 4000-400 cm^{-1}.

Data analysis: All values are expressed as the mean ± standard deviation of three replicate experiments. The data were analyzed by one-way analysis of variance (ANOVA). The level of confidence was 95 %. Significant differences among means were determined by the SPSS 20 (IBM Corporation, New York, USA) system software.

RESULTS AND DISCUSSION

The yield and properties of pectin are rely on both the nature of the source and the extraction process. The process of extraction of hawthorn pectin was optimized on the basis of three different conditions *e.g.*, pH, extraction time and solvent[15]. The extraction conditions were determined after comparing the result of articles that used different extraction conditions to obtain pectin from natural sources. Lemon juice and citric acid were applied to see the differences in terms of yield and other physico-chemical properties. The properties of enriched hawthorn pectin were used to evaluate the physico-chemical characteristics of samples extracted in citric acid and lemon juice.

The functional properties and some applications of hawthorn pectin were then compared with those of commercial apple pectin in the food system. The calculated yield and physico-chemical properties of hawthorn pectin are presented in Tables 1-5 and Fig. 1.

Extraction of pectin: Pectin extraction was evaluated at pH values between 4 and 1.5 at different times (60-120 min) and different temperatures (60-120 °C) as well as using different extraction solvents (lemon juice and citric acid). Table-1 displays the extraction yields of pectin from hawthorn under different conditions. The yield of the extracted red hawthorn fruit pectin ranged from 4.56 to 16.75 % of the dried peels, depending on the extraction conditions used. The maximum extractable pectin yield was obtained at pH 1.5/60°C/90-120 minutes in citric acid. The highest pectin yields, 16.75 ± 0.21 and 15.76 ± 0.42 %, were acquired under the following conditions: pH 1.5/60°C/90 and 120 min, respectively, in citric acid. For this reason, the extraction at pH 1.5/60°C/120 min will be used as a reference dataset for the recovery of pectin from red hawthorn. In this study, citric acid was selected based on reports that showed that citric acid is more effective for pectin extraction than any other acids in terms of physico-chemical properties and yield of pectin.

However, lemon juice was also selected as an alternative to citric acid in a suitable pH range to ameliorate the extraction yield using environmentally friendly extraction conditions. The yield of the extracted red hawthorn fruit pectin ranged from 3.91 to 7.32 % of the dried peels in lemon juice. The highest pectin yield (7.32 %) was obtained from the extraction under the conditions of pH 2.76/90°C/120 min in lemon juice. The extraction with lemon juice resulted in 7.32 % pectin, whereas, extraction with citric acid yielded 4.63 % pectin under the same conditions: 90 °C and pH 2.28 with an extraction time of 120 min. There was a notable difference between the pectin yields using lemon juice and citric acid in a given pH range. The results showed that extraction with lemon juice was more effective than citric acid, with the exception of pH 2.28 and 3.8. However, the use of citric acid, as an extractant, yielded

TABLE-1
YIELD OF PECTIN FROM HAWTHORN AT DIFFERENT pH, TEMPERATURE AND EXTRACTION TIMES USING LEMON JUICE AND CITRIC ACID AS EXTRACTION SOLVENTS

pH	Temp. (°C)	Extraction time (min)	Lemon juice yield (%)	Citric acid yield (%)
1.5	60	60		14.28 ± 0.26[a]
1.5	60	90		15.67 ± 0.42[a]
1.5	60	120		16.75 ± 0.21[a]
1.5	90	60		13.72 ± 0.42[a]
2.03	60	90		3.53 ± 0.24[a]
2.28	90	120	7.28 ± 0.09[b]	10.19 ± 0.22[a]
2.76	90	120	7.32 ± 0.05[a]	4.63 ± 0.14[b]
2.85	90	120	3.91 ± 0.11[a]	3.57 ± 0.05[a]
3.02	70	120	2.71 ± 0.06[a]	2.87 ± 0.08[a]
3.5	75	90	5.64 ± 0.03[a]	4.02 ± 0.08[b]
3.8	60	90	3.96 ± 0.13[a]	4.56 ± 0.15[a]

[a,b]Mean values in a column with different superscripts differ signify-cantly (P < 0.05). All results were obtained in triplicate

a higher amount of pectin than lemon juice. A pH value of 1.5 resulted in the highest extraction yield at 60 °C, therefore, this value was used in subsequent experiments. An extraction time of 120 min was also applied, as there were small differences in pectin yield between 90 and 120 min. Hence, pH 1.5 for 120 min at 60 °C was used to extract pectin. Using lemon juice as a solvent, the highest pectin yield was obtained at pH 2.76, 90 °C and 120 min reaction. Therefore, it could be assumed that an extraction time of 120 min was beneficial for the extraction of high quality pectin and would be the most suitable extraction time for obtaining good quality pectin. Most importantly, in citric acid, the pectin yield, from red hawthorn fruit was 16.75 % on a dry matter basis (w pectin/w red hawthorn), which is the same as values reported for main sources of pectic substances, such as apple fruit (16 %) the pectin obtained in our experiments, had a yield greater than 10 %, which makes it feasible for chemical use[19].

Characterization of extracted pectin by physico-chemical parameters: Three extraction conditions were compared to determine the characteristics of the hawthorn fruit in citric acid. The moisture of all pectins, including enriched pectin, was quite high (10.51-10.7 %) and did not show any significant differences (Table-2). Pectin should have as low a moisture content as possible for safe storage and to inhibit the growth of microorganisms that can affect the quality of pectin due to the production of pectinase enzymes[17].

Table-1 shows that citric acid-extracted pectin contained almost as much ash content as the commercial apple pectin. Low ash content is good for gel formation. The maximum allowable ash content to meet good quality gel criteria[17] is 10 %. The equivalent weight obtained was used to calculate the anhydrouronic acid content and degree of esterification. Methoxyl content is a main factor in the determination of pectin functionality. The spreading quality and gelling degree of pectin depend on its methoxyl content[18]. The hawthorn fruit pectin produced in this study can be categorized as high methoxyl pectin because it has a degree of esterification (57.19-51.19 %) higher than 50 %, with methoxyl content values of (11.63-8.82 %) for all extraction conditions in citric acid at pH 1.5 (Table-2).

The acetyl content of hawthorn pectin was (0.68-0.13 %) in citric acid (Table-2). The properties of pectin in cell walls are sometimes modified by low levels of hydroxyl esterification in acetyl groups. The acetyl groups have been reported in the literature[8] to approach 4 %. The presence of acetyl groups in pectin inhibits gel formation. For this reason, the low acetyl content of red hawthorn, recommends the usefulness of red hawthorn fruit in making jam, jelly and other types of food.

TABLE-2
PHYSICO-CHEMICAL PROPERTIES OF HAWTHORN IN THE PRESENCE OF CITRIC ACID, AND OF COMMERCIAL APPLE PECTIN

pH 1.5	60 °C		90 °C	Commercial apple pectin
	90 min	120 min	60 min	
Moisture (%)	10.07 ± 0.13[a]	10.51 ± 0.21[a]	10.22 ± 0.05[a]	7.02 ± 0.21[b]
Ash (%)	1.71 ± 0.02[a]	1.86 ± 0.04[a]	1.76 ± 0.04[a]	1.16 ± 0.01[b]
Equivalent weight	470.63 ± 0.45[b]	390.51 ± 0.41[c]	326.11 ± 0.22[d]	1030.92 ± 0.69[a]
Methoxyl content (%)	8.82 ± 0.7[b]	11.63 ± 0.14[a]	10.10 ± 0.16[a]	5.32 ± 0.07[c]
DA (%)	0.26 ± 0.05[c]	0.68 ± 0.04[a]	0.13 ± 0.03[d]	0.50 ± 0.02[b]
DE (%)	57.19 ± 1.06[a]	54.16 ± 0.08[b]	51.53 ± 0.75[c]	42.68 ± 0.19[d]
AUA (%)	87.35 ± 0.27[c]	122.90 ± 0.15[a]	111.28 ± 0.24[b]	70.50 ± 0.29[d]

[a-d]Mean values in a column with different superscripts differ signify-cantly (P < 0.05). All results were obtained in triplicate; DA: degree of acetylation; DE: degree of esterification; AUA: anhydrouronic acid

The anhydrouronic acid content of the citric-acid-extracted pectin was found (122.90-70.50 %) and is given in Table-2. Based on these anhydrouronic acid values, the pectin obtained from hawthorn fruit could be considered of high purity, indicating its suitability for its use in the pharmaceutical industry[10]. Furthermore, some of the characteristics of the pectin extracted with lemon juice, such as the methoxyl content (15.11-5.62 %), anhydrouronic acid content (136.09-58.43 %) and degree of esterification (63.03-54.41 %) are comparable to corresponding values of 5.3, 70.50 and 42.68 % in commercial apple pectin in a suitable pH range[20]. These results show that the physico-chemical values of hawthorn pectin, in both types of extraction solvents, are higher than the physico-chemical values of commercial apple pectin. Table-3 demonstrates that the highest anhydrouronic acid content of (136.09 %) was observed in the pectin obtained from lemon juice, while citric-acid-extracted-pectin contained lower amounts of anhydrouronic acid (122.90 %). Hence, pectin was more effectively extracted from red hawthorn in lemon-juice, compared with citric acid, at a given pH range and both types can be classified as high purity pectin when compared with the anhydrouronic acid content of commercial apple pectin.

Tables 4 and 5 show the pectin yields and some chemical compositions of the extracted pectin types at constant time (120 min), temperature (60 °C) and pH values in citric acid. At constant time and temperature, the yield significantly increased with decreasing pH (or increasing acid strength) of the extraction[21]. Similarly, when the temperature was increased while pH and time remained constant, the pectin yield decreased. At constant pH and temperature, when the extraction time increased, with an expectation at 70 °C, the yield of pectin was significantly increased. Table-5 shows that the highest yield and anhydrouronic acid content were obtained in the range of pH 1.5. Different analysis divulged that pH was the

TABLE-3
COMPARISON OF PHYSICO-CHEMICAL PROPERTIES OF HAWTHORN WITH CITRIC ACID AND LEMON JUICE

pH	2.28		2.76		2.85	
	Lemon juice	Citric acid	Lemon juice	Citric acid	Lemon juice	Citric acid
Equivalent weight	394.86 ± 3.24[e]	372.28 ± 1.42[f]	470.71 ± 0.05[c]	437.48 ± 0.98[d]	660.16 ± 1.03[a]	597.83 ± 1.0[b]
Methoxyl content (%)	15.11 ± 0.1[a]	11.2 ± 0.67[b]	7.9 ± 0.13[c]	8.1 ± 0.12[c]	5.62 ± 0.09[d]	4.44 ± 0.05[e]
Degree of acetylation (%)	0.25 ± 0.02[d]	0.23 ± 0.01[d]	0.76 ± 0.03[a]	0.61 ± 0.03[b]	0.38 ± 0.02[c]	0.32 ± 0.02[c]
Degree of esterification (%)	63.03 ± 0.30[a]	57.36 ± 0.63[b]	54.52 ± 0.20[c]	53.34 ± 0.31[c]	54.41 ± 0.21[c]	46.11 ± 0.23[d]
Anhydrouronic acid (%)	136.09 ± 0.2[a]	110.86 ± 0.41[b]	82.26 ± 0.04[d]	86.21 ± 0.72[c]	58.43 ± 0.07[e]	54.63 ± 0.22[f]

[a-f]Mean values in a column with different superscripts differ significantly ($P < 0.05$). All results were obtained in triplicate

TABLE-4
EXTRACTION YIELDS (% DRY PEELS) OF HAWTHORN PECTIN IN CITRIC ACID
AT INCREASING pH, CONSTANT TEMPERATURE (60 °C) AND TIME (120 MIN)

pH	1.5	2	2.5	3	3.5
Yield (%)	16.75 ± 0.2[a]	3.58 ± 0.12[b]	3.08 ± 0.11[b]	1.97 ± 0.1[c]	2.02 ± 0.07[c]
Equivalent weight	390.51 ± 0.41[e]	430.43 ± 0.43[d]	488.48 ± 0.35[c]	723.54 ± 0.34[b]	824.41 ± 0.13[a]
Methoxyl content (%)	11.63 ± 0.14[b]	10.35 ± 0.22[b]	10.4 ± 0.12[b]	14.28 ± 0.22[a]	9.40 ± 0.08[b]
Degree of esterification (%)	54.00 ± 0.08[e]	58.95 ± 0.27[d]	62.15 ± 0.14[c]	76.90 ± 0.18[a]	71.42 ± 0.41[b]
Anhydrouronic acid (%)	122.90 ± 0.15[a]	99.65 ± 0.46[c]	94.96 ± 0.22[d]	105.51 ± 0.4[b]	76.90 ± 0.19[e]

[a-d] Mean values in a column with different superscripts differ significantly ($P < 0.05$). All results were obtained in triplicate

TABLE-5
COMPARISON OF THE EXTRACTION YIELD OF PECTIN AT pH 1.5 AT DIFFERENT TEMPERATURES AND EXTRACTION TIMES IN CITRIC ACID

Constant extraction time (120 min)		Constant temp. (60 °C)	
Temp. (°C)	Yield (%)	Extraction time (min)	Yield (%)
60	16.75 ± 0.20[a]	60	4.28 ± 0.26[b]
70	4.06 ± 0.07[a]	70	1.77 ± 0.20[b]
80	6.80 ± 0.12[a]	80	4.24 ± 0.14[b]
90	3.91 ± 0.4[b]	90	15.47 ± 0.40[a]

[a,b]Mean values in a column with different superscripts differ signify-cantly ($P < 0.05$). All results were obtained in triplicate

main parameter influencing the anhydrouronic acid content. The pectin-extracted at pH 1.5 contained more anhydrouronic acid than that obtained at other pH values, suggesting that the pectin content in anhydrouronic acid increased with decreasing pH (or increasing acid strength). These results also indicate that the pectin extracted at pH 1.5 was the purest compared with pectin obtained at other pH values at a given temperature and time.

FTIR analysis of Hawthorn fruit pectin: The fourier transform infrared (FTIR) spectra were analyzed to recognize the major functional groups of the extracted pectin in the range of 4000-400 cm^{-1} (Fig. 1). To confirm the identity of hawthorn pectin, the spectrum of apple commercial pectin was compared with the spectra for the enriched pectin samples extracted using lemon juice (pH 2.76/90 °C /120 min) and citric acid (pH 1.5/ 60 °C/120 min). The FTIR spectra of different pectin samples showed a good match with the spectrum of the commercial apple pectin spectrum. The broad peak at 3600-3200 cm^{-1} relates to OH groups in the pectin molecule. The characteristic peaks of ester and carboxylic (C-O) vibrations were observed at 1770-1760 and 1630-1600 cm^{-1}, respectively. According to previous studies, the relative intensity of the ester band proportionally expands with the degree of esterification (DE), whereas the intensity of the band, corresponding to carboxylic stretching, decreases[19]. This could be used to correlate the distinct types of pectin. Fig. 1 shows that hawthorn pectin shows a higher absorbance at 1750 cm^{-1} than at 1650 cm^{-1}, exhibiting

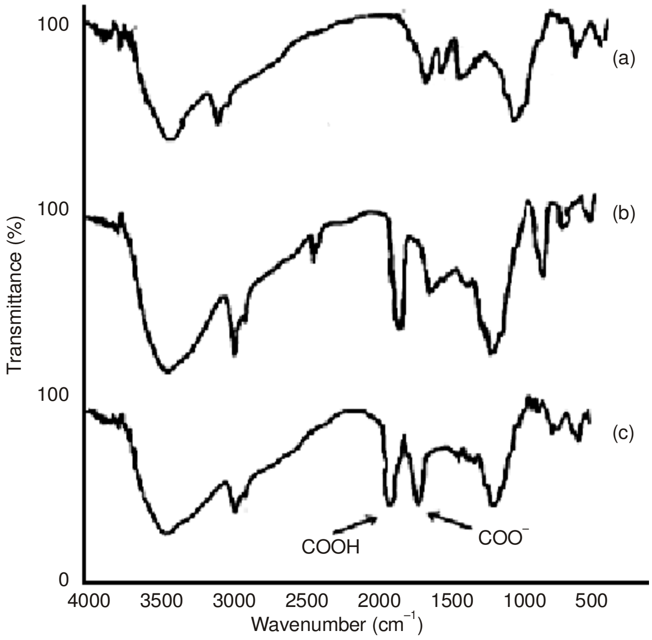

Fig. 1. FTIR spectra of pectins; (a) commercial apple pectin; (b) pectin extracted in lemon juice; and (c) c pectin extracted in citric acid

a high metoxyl pectin. The region between 1300 and 800 cm^{-1} is known as the fingerprint region; in particular, the peak at 1235 cm^{-1} indicates the presence of ($-O-CH_3$) groups.

Conclusion

The increasing industrial demand for pectin with varying ability to gel or stabilize products, boosts the need for pectin of various types or derivatives with tailored properties. In this study, red hawthorn fruit was subjected to some physical treatment to produce pectin in citric acid and lemon juice. The pectin, obtained from red hawthorn fruit, had a yield of approximately 18 %, a high degree of esterification (54.16 %) and a high anhydrouronic acid content (122.90 %) in citric acid, while enriched lemon-juice-extracted pectin yields values of approximately 8 %, (54.52 %) and (82.26 %), respectively.

This study also demonstrates that the pectin from red hawthorn fruit can be obtained using a lemon juice extraction method without chemical acids in an appropriate pH range. Because consumer cares about chemical additives have grown, this study can donate to the movement of the food and pharmaceutical industry into the natural area of eco-friendly processing technology without harmful chemicals. In addition, pectin form red hawthorn fruit is readily extracted and has a long history of food consumption in Turkey. Therefore, there is significant commercial potential for this new type of pectin.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. Hasan Togrul for his invaluable support and encouragement throughout the study. Thanks are also due to Cansel Tuncer and Harun Elci, Department of Chemistry, University of Osmangazi, Eskisehir, Turkey for their beneficial help with experimental analysis. The authors are also grateful for the assistance of the research group in the Department of Chemical Informatics, University of Szeged, Hungary.

REFERENCES

1. D. Kumar, V. Arya, A. Bhat and N. Ahmad, *Braz. J. Pharmacog*., **22**, 1187 (2012).
2. B.S. Virk and D.S. Sogi, *Prop*., **7**, 693 (2004).
3. A.A. Donmez, *Turk. J. Bot*., **28**, 29 (2004).
4. Z. Bor, R. Arslan and N. Bektas, *Turk. J. Med. Sci*., **42**, 315 (2012).
5. O. Caliskan, K. Gunduz, S. Serce, C. Toplu, O. Kamiloglu, M. Sengul and S. Ercisli, *Pharmacogn. Mag*., **8**, 16 (2012).
6. J. Singthong, S. Ningsanond, S.W. Cui and H. Douglas Goff, *Food Hydrocoll*., **19**, 793 (2005).
7. L. Liu, J. Cao, J. Huang, Y. Cai and J. Yao, *Bioresour. Technol*., **101**, 3268 (2010).
8. L.C. Vriesmann and C.L.O. Petkowicz, *Food Hydrocoll*., **33**, 58 (2013).
9. L.C. Vriesmann, R.F. Teofilo and C.L.O. Petkowicz, *Sci. Technol*., **49**, 108 (2012).
10. U. Sotanaphun, A. Chaidedgumjorn, N. Kitcharoen, M. Satiraphan, P. Asavapichayont and P. Sriamornsak, *Silpakorn Univ. Sci. Technol. J.*, **6**, 42 (2011).
11. J. Lim, J. Yoo, S. Ko and S. Lee, *Food Hydrocoll*., **29**, 160 (2012).
12. Y. Liu, H. Ahmad, Y. Luo, D.T. Gardiner, R.S. Gunasekera, W.L. McKeehan and B.S. Patil, *J. Agric. Food Chem*., **49**, 3051 (2001).
13. P.M. Moriarty, J. Backes, J.-A. Dutton, J. He, J.F. Ruisinger and K. Schmelzle, *J. Clin. Lipidol*., **7**, 140 (2013).
14. N. Arslan and H. Togrul, *J. Food Eng*., **27**, 191 (1996).
15. D.F. Torralbo, K.A. Batista, M.C.B. Di-Medeiros and K.F. Fernandes, *Food Hydrocoll*., **27**, 378 (2012).
16. M. Masmoudi, S. Besbes, F. Abbes, C. Robert, M. Paquot, C. Blecker and H. Attia, *Food Bioprocess Tech*., **5**, 687 (2012).
17. N. Ismail and N. Ramli, *Sains Malaysiana*, **41**, 41 (2012).
18. M. Jindal, V. Kumar, V. Rana and A. Tiwary, *Carbohydr. Polym*., **93**, 386 (2013).
19. V. Urias-Orona, A. Rascón-Chu, J. Lizardi-Mendoza, E. Carvajal-Millán, A.A. Gardea and B. Ramírez-Wong, *Int. J. Mol. Sci*., **11**, 3686 (2010).
20. A. Kumar and G.S. Chauhan, *Carbohydr. Polym*., **82**, 454 (2010).
21. B.M. Yapo, C. Robert, I. Etienne, B. Wathelet and M. Paquot, *Food Chem*., **100**, 1356 (2007).