

EFFECT OF QUINCE VARIETY ON THE QUALITY OF PECTIN: CHEMICAL COMPOSITION AND CHARACTERIZATION

Alev Akpınar Borazan* and Caglayan Acikgoz

Bilecik Seyh Edebali University, Faculty of Engineering,
Chemical & Process Engineering Department, 11210 Bilecik/ Turkey.

ABSTRACT

In this study, the extraction of pectin from two variety of quince (*C. vulgaris maliformis* and *C. vulgaris piriformis*) were performed at different extracting conditions of temperatures (70, 80, 90 °C) and time (60, 90, 120 min) in 0.01 M HCl solutions at pH 2. The highest yields were obtained 1.65 and 2.86% (dried pectin g/100g quince pulp) by extraction at 90 °C and 90 minutes. The degree of esterification is a key factor to determine conformation and rheological properties of pectin. Galacturonic acid content, degree of esterification and ash contents in pectin samples of quince were found 97.8- 72.1%; 85.27-78.22% and 2.7- 2.3%, respectively. The polysaccharides present in the different extracts were characterized by FT-IR spectroscopy in the region 400-4000 cm⁻¹. SEM images of pectic substances surface were obtained using an EVO-50XVP. Chemical composition was determined using the Genesis 4000EDX detector.

Keywords: Characterization, Composition, Extraction, Pectin, Quince.

INTRODUCTION

The genus *Cydonia*, known as quince (*Cydonia oblonga* Mill.), belongs to the family Rosaceae and the subfamily Maloideae, which includes pome fruits like apple (*Malus* spp) and pear (*Pyrus* spp). The Maloideae subfamily contains approximately 1000 species in 30 genera, all of which have 17 chromosomes^{1,2}. In Turkey, quince has been cultivated for a long time, and different types and cultivars of quince are grown in different parts of Anatolia. Most of the economically important quince cultivars (Limon, Demir, Ekmek, and Eşme) are in Turkey^{3, 4}. Quince tree and fruit morphological properties are very similar to each other, which makes distinction for reliable classifications difficult. General differentiation characteristics have been based on fruit shape, as apple shaped (*C. oblonga* var. *maliformis*) and pear shaped (*C. oblonga* var. *pyriformis*)⁵.

Nowadays, Turkey is the leading grower of quince in the world. Worldwide, there are about 106,000 acres (43,000 hectares) of quince in production with a total crop of 596,532 metric tons. Turkey is the largest producer with about 25% of world production. 31 quince types and varieties (*Cydonia oblonga* Mill.) were cultivated

throughout Turkey, especially in western Anatolia. In the 1972, the fruit characteristics of quince have been described for eight quince cultivars and for five other quince cultivars in Western Turkey by J.T.Sykes⁶. Later, Ercan and Özkarataş determined the phenological and pomological characters of 31 quince types and varieties which were selected from the west region of Turkey in the 2005. 33 characteristics were used to select the best quince types. Productivity, difficulty of swallow, juiciness of the flesh, aroma, flavour of flesh, texture of the flesh, firmness of the flesh, fruit size, fruit shape, shininess of skin and amount of russet were used to select most promising quince types⁷.

The extraction technology of pectin is being studied commonly, because pectin is a commercially important product. There are many processes for extraction of pectin and there are many uses for these products. Pectin form gels under certain conditions and this property is used in jellies, jams and marmalade, acid mild products. Traditionally, citrus peels and apple pomace are used for pectin production as raw materials in the industry.

Pectins are known as pectic polysaccharides, are rich in galacturonic acid. In nature, around 80

percent of carboxyl groups of galacturonic acid are esterified with methanol. The behavior of pectin in food applications is determined with the ratio of esterified to non-esterified galacturonic acid. Pectin is classified according to the degree of methoxylation (DM) as high methoxyl pectin (DM >50) and low methoxyl pectin (DM <50). The degree of methoxylation influences the properties of pectin, especially the solubility and the gel forming characteristics.

Many researchers are focused to reveal the relationship between the chemical structure of the various classes of polysaccharides and their corresponding functional properties. Such knowledge is essential for food industry to come to improved processes and products and to extend the use of agricultural products in food and feed application⁸.

There are numerous papers on the structure of citrus and apples and the quality of pectin extracted from these fruits. Alternative sources are currently investigated. However, there are a few papers about other sources of pectin, as sugar beet pulp^{9, 10}, banana^{11, 12}, peach^{13, 14}, mango¹⁵⁻¹⁷, chicory¹⁸, cabbage^{19, 20}, sunflower²¹⁻²⁴, yellow passion fruit²⁵⁻²⁸, and quince²⁹⁻³³ in the literature.

The objective of this research was to (1) investigate the effect of temperature and extraction time on pectin yield and purity for pectin production from two variety of quince (*C. vulgaris maliformis* and *C. vulgaris piriformis*) cultivated in western of Turkey (2) to study the characterization of the quince pectins.

MATERIALS AND METHODS

MATERIALS

The fruits were collected from commercial orchards in the city of Bilecik located in the west of Turkey. Two variety of quince SK13 (*C. vulgaris maliformis*) and KK26 (*C. vulgaris piriformis*) used as the source of pectin. Seeds were removed and the fruits were sliced, and frozen immediately after picking. All chemicals and solvents used were of analytical reagent grade.

Pectin extraction

Frozen quince samples (78.5% moisture) were washed using hot water (75°C) for 15 minutes to inactivated the peptic enzymes, washed extensively with water to remove sugars and flavonoids, filtered through a Buchner funnel into which silk filter cloth was placed. In this step, 500 ml deionized water was used for 100 g quince pulp. After filtration, washed pulp was extracted with 400 ml of 0.01M HCL (pH 2) preheated to at different temperature and times

in the water bath. Our preliminary study, increasing the extraction temperature to 100°C also didn't affect the pectin yield and even caused the decreasing of pectin yield. Therefore, the slurries were shaken in water heater bath for the ranges of 70°C-90°C and 60-120 min, respectively.

After extraction, the liquid phase was separated from the pulp by filtering. Concentrate pectin solution was precipitated with acidified ethanol (96 %). Formed gel was allowed to precipitate for 24hrs at +3°C. The gelatinous precipitate was filtered through a G4 sintered glass. The coagulated pectin obtained was washed with ethanol 60 (w/w, %) to remove mono and disaccharides.

The pectin was dried in a vacuum oven at 60°C to constant weight and the finely ground to pass a 30 mesh sieve and packaged under airless conditions. The yield of raw pectin was determined gravimetrically in this study.

Analyses

Pectin samples extracted at 90 °C and 90 minute were the only ones selected for analysis in the present study, because of the yields at this extraction temperature and time were among the highest.

The ash content of pectin samples was determined by ashing at 660 °C for 8h. Moisture contents of pectin samples were determined using an air oven method. Weight loss due to drying at 110 °C for 12h was reported as moisture content.

Galacturonic acid was determined by titration with NaOH. The degree of esterification was determined by titration with NaOH, based on free carboxyl group³⁴.

Gel strength was measured by the Ridgelimeter method. Briefly, at the end of boiling, the gel preparation was completely filled in a Ridgelimeter glass and the surface was covered with a waxed paper disc (to minimize evaporation) and left undisturbed at room temperature for 2 h before aging for a further 22 h in an incubator at 30 °C. The gel was then carefully demoulded undamaged onto a Ridgelimeter glass plate. After exactly 2 min of standing, the pointer of the apparatus was carefully lowered until it touched the gel surface and the percentage of sagging under its specific gravity was measured, from which the gel strength (sag) was calculated using an appropriate correction factor from established standard tables. Gel preparations contained 65.0% soluble solids (sucrose) and 0.70 wt% pectin at pH 2.3 (fine-tuned with a citric acid solution³⁵).

FT-IR spectroscopy was used to identify the chemical groups present in the pectin samples. The infrared spectrums of samples were recorded on Perkin Elmer Spectrum 100 FT-IR spectroscopy in the 4000–400 cm^{-1} wavelength range. The FT-IR spectrums of samples were obtained using the ATR technique (with a diamond protected Attenuated Total Reflectance crystal unit) with a resolution of 4 cm^{-1} after 100 scans. The IR- spectrums were used to find out the characteristic groups present in pectin samples and thereby to illuminate the quince pectin structure.

Samples for SEM analysis were sputter coated with Au/Pd using a Polaron SC7640 sputter coater. Scanning Electron Microscopy (SEM) images (SE and BSE) of the sample surface were obtained using an EVO-50XVP(Carl Zeiss SMT Ltd.) Chemical composition was determined using the Genesis 4000EDX detector (EDAX Inc.) All values were on dry weight basis and analyses were performed at least in duplicates. Data were analysis of variance and means were separated by least significant difference when significant ($p < 0.05$) values were observed.

RESULTS AND DISCUSSION

Table 1. shows the pectin yields extracted from two variety quince at the experimental temperatures and times assayed. The temperature and time had significant influence on the yield of extracted pectin. The results of studies indicated that while lower temperature or shorter extraction time resulted in lower pectin extraction yield, higher temperature resulted in higher pectin yield. However, longer extraction time caused lower pectin extraction yield.

According to Table 1. the highest yields were obtained at the highest temperature at 90 minutes conditions. And there is a notable difference between the two varieties of quince due to extraction yield. The available results in Table 1. and 2. indicate effect of variety on the degree of esterification and pectin content. Pectin esterification degree and galacturonic acid content KK26 were significantly lower than SK13.

A chemical characterization of peptic substances from a particular source material requires a determination of acetyl as well as determination of the methoxyl content because the poor gelling properties of pectin is related to its acetyl content. [36] This study shows that pectin containing over 90% pectin, with a yield of over 2.86% , could be produced from quince(*C. vulgaris maliformis*) using optimum acid extraction with 0.01N HCl at 90°C and 90 minute extraction conditions, followed by alcohol

precipitation. The pectin content, as percent galacturonic acid, and yield of quince pectin were similar to commercial pectin.

Forni et al. reported that the pectin yield from quince was on average 0.53% on fresh weight and the quince pectin had a high galacturonic content (about 78%), and a degree of methoxylation of about 59% corresponding to a medium-high methoxyl pectin²⁹. Thomas et al. stated that the pectin content in the fruits of two genotypes of Japanese quince was 1.4 and 1.3 g pectins/100 g fresh fruits of genotypes, respectively³⁰. The basic chemical characteristics of 22 quince (*Cydonia oblonga Mill.*) genotypes and cultivars were determined by Rop et. al .High contents of pectins were found out in the cvs Morava (3.07 g/100 g FW), Ironda (3.06 g/100 g FW), and Jurák (2.94 g/100 g FW), while the lowest one was determined in the cv. Kocurova (1.87g/100 g FW)³².

Our previous work³³ was the extraction of pectin from quince (*Cydonia vulgaris pers.*) cultivated and called "limon quince" in Turkey. As a result of the study, 1.83 % (dried pectin g/100 g quince pulp) was obtained at 90 °C extraction temperature and 90 minute extraction time. Galacturonic acid content, the degree of esterification, the gel strength and ash contents in samples were found 77.5, 79.70, 137.50 and 2.47 %, respectively.

FTIR spectra in the region between 4000- 400 cm^{-1} identified the major chemical groups in the pectin and provided structural information that could be used to compare two different types of pectin. FTIR spectra indicated that there were no major structural differences in pectin obtained from two varieties. A general view of FTIR spectra of pectin are presented in Figures. 1 and 2. , Table 3.

The spectral data obtained were analyzed by comparing the FTIR spectra in the following characteristic regions. O-H stretching band envelope 3100-3600 cm^{-1} , C-H stretching bands 2800-3000 cm^{-1} , the fingerprint region of spectra under ca. 2000 cm^{-1} , including the band contributing to resonant absorption energy of pyranose cycle vibrations 950-1200 cm^{-1} , as well as the region 1200-1800 cm^{-1} featuring the state of carboxylic groups. For the pyranose cycle vibrations region, one should note almost identical spectral parts with five bands at 1016-1019-1052-1076-1104-1149 cm^{-1} characteristic for peptic substances. Most interesting is the region featuring the state of carboxylic groups 1750-1350 cm^{-1} . The band at ca. 1750 cm^{-1} is assigned to stretching C=O mode non-ionized methylated or protonated carboxyl. Ionization i.e. the formation of salts leads to its

disappearance, and two new band appear due to ant symmetric and symmetric stretching modes of COO- at ca. 1600- 1650 and 1400-1450 cm^{-1} , respectively³⁷. Thus, in principle, considering the relative intensities of bands in these regions, one may correlate them to the relative amount and degree of esterification of carboxylic groups. The absorption bands between 1100-1200 cm^{-1} were from ether (R-O-R) and ring C-C bonds in pectin molecules. The polysaccharides rich that contribute to this distinction are the peptic polysaccharides rich in GaIA and Xyl-rich hemicellulosic polysaccharides. The selection of the most important wave numbers, by two independent chemo metric techniques allowed to define the region between 1120- 990 cm^{-1} as the range for the spectral identification of GaIA in peptic polysaccharides³⁸.

Scanning Electron Microscopy (SEM) images of the pectic substance extracted from SK13 were given in the Figure.3.

Chemical composition was determined using the Genesis 4000EDX detector. Elemental spectrum was recorded in order to qualitatively characterize the sample. Figure 4 shows that the identification spectrum of these elements.

The atomic percent of these elements have been given in the Table3. In the pectin sample, C, O, B,

K, Mg, Ca, Cl are present. EDX spectrum of pectic substances obtained from quince showing strong O and C signals.

CONCLUSIONS

It can be concluded that the quince variety had some effect on the galacturonic acid content, the degree of esterification and extraction yield. Pectin extraction yield were also affected by the extraction temperature and time. The quantities of pectin obtained in this work are higher than those obtained for quince in the literature. The researchers have reported quince to contain 0.53 to 1.83 g pectin/ 100 g fresh quince. The yields of pectin obtained two variety of quince cultivated in western of Turkey (*C. vulgaris maliformis* and *C. vulgaris piriformis*) ranged from 2.86% and 1. 65 % (dried pectin g/ 100g quince pulp). Quince fruit cultivated in Turkey can be considered to be an interesting source of commercial pectin as same as apple and orange.

ACKNOWLEDGEMENTS

The author would like to thank the Anadolu University Research Foundation (Project No: 030933) for the financial support.

Table 1: Pectin yields of two varieties of quince produced by extraction with 0.01 N HCl at varying extraction temperature and time, followed by precipitation with ethanol

Temperature (°C)	Time (min.)	Pectin yield of SK13 (w/w, %)	Pectin yield of KK26 (w/w, %)
70	60	1.99	1.15
	90	2.32	1.34
	120	2.25	1.30
80	60	2.17	1.25
	90	2.60	1.50
	120	2.65	1.47
90	60	2.48	1.43
	90	2.86	1.65
	120	2.60	1.50

Table 2: Comparison of ash, moisture, esterification degree, galacturonic acid percentage and gel strength of pectin in two variety quince

Variety	Ash (%w/w)	Moisture (%w/w)	Esterification degree %	Galacturonic acid (%w/w)	Gel Strength
SK13	2.7	8.9	85.27	97.80	165.40
KK26	2.3	3.7	78.22	72.10	140.73

Table 3: Functional group compositions of pectin samples

The spectral data obtained (SK13, KK26)	Groups and Class of compound
3414-3555 cm^{-1}	O-H stretching band envelope 3100-3600 cm^{-1}
2924-2934 cm^{-1}	C-H stretching bands 2800-3000 cm^{-1}
1747 cm^{-1}	carboxylic groups 1750-1350 cm^{-1} , ca. 1750 cm^{-1} is assigned to stretching C=O mode non-ionized methylated or protonated carboxyl
1617-1635 cm^{-1}	ant symmetric and symmetric stretching modes of COO- at ca. 1600- 1650
1445 cm^{-1}	appear due to ant symmetric and symmetric stretching modes of COO- at ca. 1400-1450 cm^{-1}
1000-1103 cm^{-1}	The pyranose cycle vibrations region, identical spectral parts with five bands at 1016-1019-1052-1076-1104-1149 cm^{-1} characteristic for peptic substances. 1120- 990 cm^{-1} as the range for the spectral identification of GalA in peptic polysaccharides [37,38].
831-775 cm^{-1}	O-H Aromatic groups
491-404 cm^{-1}	The far-infrared region were also detected and assigned to the C-O-C torsion deformation.

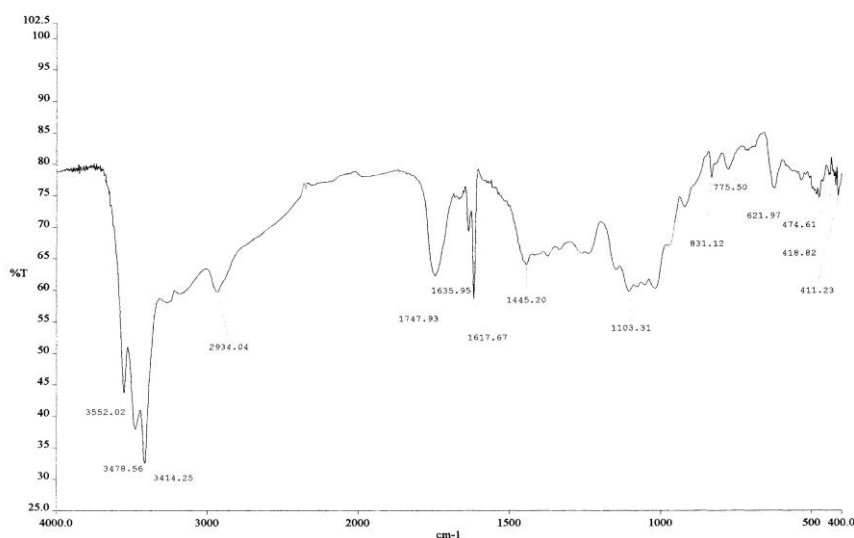


Fig. 1: FTIR spectra of pectin extracted from quince SK13

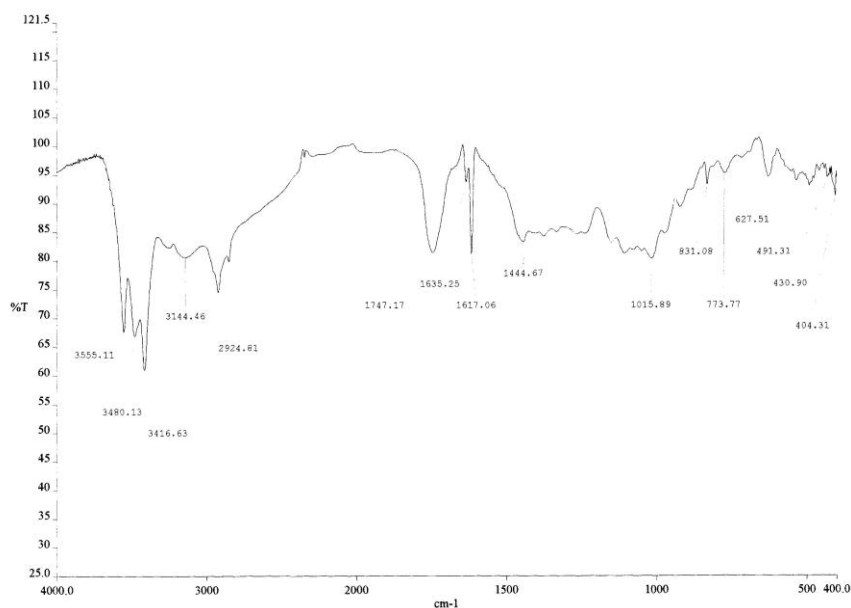


Fig. 2: FTIR spectra of pectin extracted from quince KK26

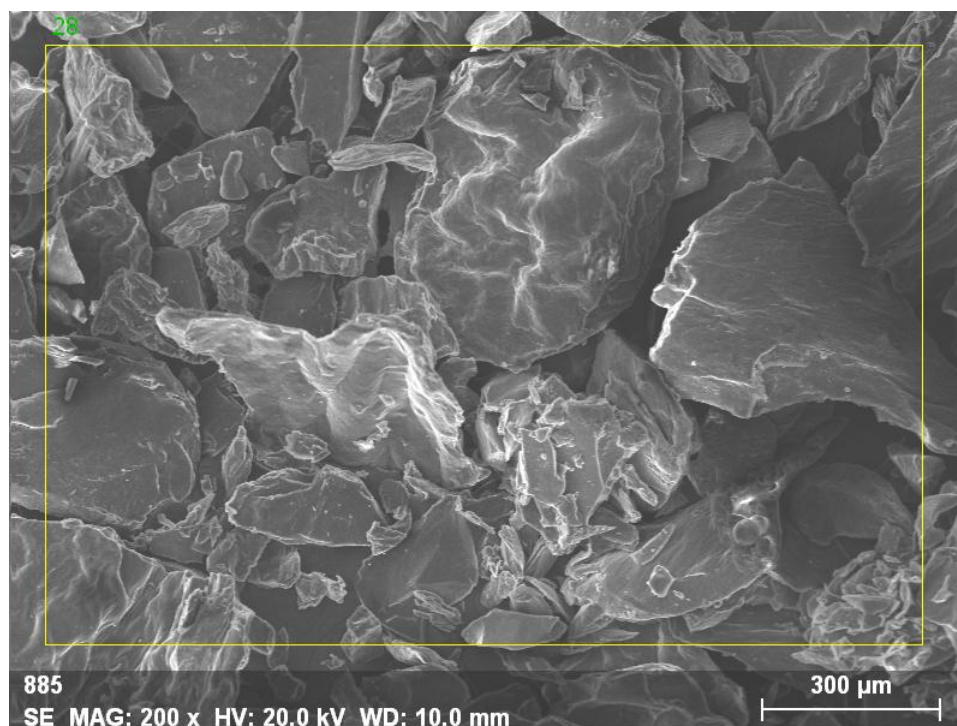


Fig. 3: SEM scan at 20kV of pectic substances obtained from SK13, Magnitude: 200 X, original with of picture was 300μ

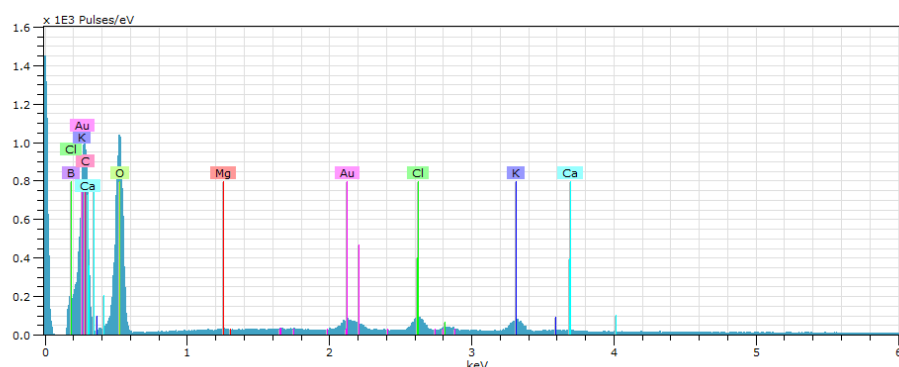


Fig. 4: EDX Spectrum of pectic substances obtained from quince

REFERENCES

1. Evans RC and Campbell CS. The origin of the apple subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes. *Am J Bot.* 2002;89:1478-1484.
2. Halász J, Hoffmann V, Szabó Z, Nyéki J, Szabó T and Hegedűs A. Characterization of quince (*Cydonia oblonga* Mill.) cultivars using SSR markers developed for apple. *Int J Horticult Sci.* 2009; 15:7-10.
3. Özbek S. Özel Meyvecilik. Ankara University Press, Ankara. 1978.
4. Yüksel C, Mutaf F, Demirtaş İ, Öztürk G, Pektaş M and Ergül A. Characterization of Anatolian traditional quince cultivars, based on microsatellite markers. *Genet Mol Res.* 2013;12:5880-5888.
5. Radović A, Nikolić D, Milatović D, Đurović D and Trajković J. Investigation of pollen morphological characteristics in some quince (*Cydonia oblonga* Mill.) cultivars. *Turk J Agric For.* 2016; doi:10.3906/tar-1511-76.
6. Sykes JT. A Description of Some Quince Cultivars from Western Turkey. *Econ Bot.* 1972;26:21-31.
7. Ercan N and Özkarakaş İ. Evaluation and adaptation of some quince (*Cydonia Vulgaris* Pers.) collected from Aegean region In Turkey, Anadolu. *Journal of Aegean Agricultural Research Institute.* 2005;15:27-42.

8. Schols H, Kabel M, Bakx E, Daas P, Alebeek JV and Voragen F. HPLC of oligosaccharides: New developments in detection and peak identification. 2000, http://www.pietdaas.nl/beta/pubs/pubs/AVH_Schols.pdf. Accessed 10 January 2017.
9. Levigne S, Ralet MC and Thibault JF. Characterization of pectin extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydr Polym.* 2002;49:145-153.
10. Turquois T, Rinaudo M, Taravel FR and Heyraud A. Extraction of highly gelling peptic substances from sugar beet pulp and potato pulp: influence of extrinsic parameters on their gelling properties. *Food Hydrocolloid.* 1999;13: 255-262.
11. Emagaa TH, Ronkart SN, Robert C, Wathelet B and Paquot M. Characterization of pectins extracted from banana peels (*Musa AAA*) under different conditions using an experimental design. *Food Chem.* 2008;108:463-471.
12. Li-Ping Q, Guang-Lei Z, Hui W, Lu J, Xiao-Feng L and Jun-Juan L. Investigation of combined effects of independent variables on extraction of pectin from banana peel using response surface methodology. *Carbohydr Polym.* 2010;80:326-331.
13. Pagan J and Ibarz A. Extraction and rheological properties of pectin from fresh peach pomace. *J Food Eng.* 1999;39:193-201.
14. Pagan J, Ibarz A, Llorca M, Pagan A and Barbosa GV. Extraction and characterization of pectin from stored peach pomace. *Food Res Int.* 2001;34:605-612.
15. Koubala BB, Kansci G, Mbome LI, Crépeau MJ, Thibault JF and Ralet MC. Effect of extraction conditions on some physicochemical characteristics of pectins from Améliorée and Mango mango peels. *Food Hydrocolloid.* 2008;22:1345-1351.
16. Malviya R, Srivastava P, Bansal M and Sharma PK. Mango peel pectin as a superdisintegrating agent. *J. Sci. Ind. Res.* 2010;69:688-690.
17. Berardin N, Knödler M, Schieber A and Carle R. Utilization of mango peels as a source of pectin and polyphenolics. *Innov. Food Sci Emerg.* 2005;6:442-452.
18. Robert C, Happi Emaga T, Wathelet B and Paquot M. Effect of variety and harvest date on pectin extracted from chicory roots (*Cichorium intybus L.*). *Food Chem.* 2008;108:1008-1018.
19. Westereng B, Michaelsen TE, Samuelsen AB and Knutsen SH. Effects of extraction conditions on the chemical structure and biological activity of white cabbage pectin. *Carbohydr Polym.* 2008;72:32-42.
20. Fuchigami M, Kato N and Teramoto A. High-Pressure-Freezing Effects on Textural Quality of Chinese Cabbage. *J Food Sci.* 1998;63:122-125.
21. Wiesenborn DP, Wong J, Chang CK and Schwarz JG. Comparison of continuous and batch processes for pectin extraction from sunflower heads. *Ind Crop Prod.* 1999;19:171-181.
22. Shi XQ, Chang KC, Schwarz JG, Wiesenborn DP and Shih MC. Optimizing pectin extraction from sunflower heads by alkaline washing. *Bioresource Technol.* 1996;58:291-297.
23. Iglesias MT and Lozano JE. Extraction and characterization of sunflower pectin. *J Food Eng.* 2004;62:215-223.
24. Wiesenborn DP, Wang J, Chang KC and Schwarz JG. Comparison of continuous and batch processes for pectin extraction from sunflower heads. *Ind Crop Prod.* 1999;9:171-181.
25. Pinheiro ER, Silva IMDA, Gonzaga LV, Amante ER, Teófilo RF, Ferreira MMC and Amboni RDMC. Optimization of extraction of high-ester pectin from passion fruit peel (*Passiflora edulis flavicarpa*) with citric acid by using response surface methodology. *Bioresource Technol.* 2008; 99: 5561-5566.
26. Yapo BM and Koffi KL. Yellow Passion Fruit Rind A Potential Source of Low-Methoxyl Pectin. *J Agr Food Chem.* 2006;54:2738-2744.
27. Kulkarni SG and Vijayanand P. Effect of extraction conditions on the quality characteristics of pectin from passion fruit peel (*Passiflora edulis f. flavicarpa L.*). *LWT - Food Sci. Technol.* 2010; 43:1026-1031.
28. Yapo BM. Biochemical Characteristics and Gelling Capacity of Pectin from Yellow Passion Fruit Rind as Affected by Acid Extractant Nature. *J Agr Food Chem.* 2009;57:1572-1578.
29. Forni E, Penci M and Polesello A. A preliminary characterization of some

- pectins from quince fruit (*Cydonia oblonga* Mill.) and prickly pear (*Opuntia ficus indica*) peel. *Carbohydr Polym.* 1994;23: 231-234.
30. Thomas M, Guillemain F, Guillon F and Thibault JF. Pectins in the fruits of Japanese quince (*Chaenomeles japonica*). *Carbohydr Polym.* 2003;53:361-372.
31. Thomas M and Thibault JF. Cell-wall polysaccharides in the fruits of Japanese quince (*Chaenomeles japonica*): extraction and preliminary characterization. *Carbohydr. Polym.* 2002;49:345-355.
32. Rop O, Balík J, Řezníček V, Juríková T, Škardová P, Salaš P, Sochor J, Mlček J and Kramářová D. Chemical Characteristics of Fruits of Some Selected Quince (*Cydonia oblonga* Mill.) Cultivars. *Czech J Food Sci.* 2011;29:65-73.
33. Acikgoz C. Extraction and Characterization of Pectin Obtained from Quince Fruits (*Cydonia vulgaris pers*) Grown in Turkey. *Asian J Chem.* 2011;23:149-152.
34. Food Chemical Codex. National Academy Press, Washington D.C. U.S.A. 1996;283-286.
35. National Research Council In food Chemical Codex 2nd Ed. National Academy of sciences, Washington, DC. 1973;577-582.
36. Kar F and Arslan N. Characterization of orange peel pectin and effect of sugars, L-ascorbic acid, ammonium persulfate, salt on viscosity of orange peel pectin solutions. *Carbohydr Polym.* 1999; 40:285-291.
37. Kamnev AA, Colina M, Rodriguez J, Ptitchkina NM and Ignatov VV. Comparative spectroscopic characterization of different pectins and their sources. *Food Hydrocolloid.* 1998;12:263-271.
38. Ferreira D, Barros A, Coimbra MA and Delgadillo I. Use of FT-IR spectroscopy to follow the effect of the processing in cell wall polysaccharide extracts of the sun dried pear. *Carbohydr Polym.* 2001;45:175-182.