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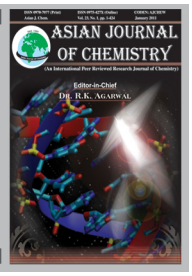

Extraction and Characterization of Pectin Obtained from Quince Fruits (*Cydonia vulgaris pers*) Grown in Turkey

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This paper reports a study of the extraction of pectin from quince (*Cydonia vulgaris pers.*) at different extracting conditions of temperatures (70, 80 and 90 ºC) and time (60, 90, 120 min) in 0.01 M HCl solutions at pH 2. The pectin content, composition and chemical characterization were studied. Highest yields of 1.83 % (dried pectin g/100 g quince pulp) were obtained at 90 ºC extraction temperature and 90 min 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. The higher galacturonic acid and lower ash contents of pectin are two criteria for its purity. The polysaccharides present in the extracts were characterized by FT-IR spectroscopy in the region between 4000-400 cm^{-1}.

Key Words: Quince, Extraction, Pectin, Characterization, Methoxylation.

INTRODUCTION

The quince or *Cydonia oblonga*, is the sole member of the genus *Cydonia*, the three shrubby quinces previously included are now classified in Chaenomeles. Quince has previously been classified as Pyrus cydonia and Cydonia vulgaris[1]. Cydonia vulgaris fruits, also known as Quince. C. vulgaris Pers. (sin. C. oblonga Mill.) is a small shrub belonging to the same family as apples and pears (Rosaceae)[2-4]. This species is the sole member of the genus. It is a small tree with bright golden yellow pome fruits, when mature. The native region of the quince is not precisely known, but it is probably wild only in parts of Asia including Armenia, Azerbaijan, Georgia, Turkistan, Iran and Saudi Arabia.

It has been cultivated in Mediterranean regions for millennia and has become naturalized in many parts; the fruit was highly regarded by the Greeks and Romans. It is a small deciduous tree, growing 5-8 m tall and 4-6 m wide, related to apples and pears and like them has a pome fruit. Fruits are light golden-yellow, green or orange, usually pear shaped (but sometimes round and apple-shaped) and very fragrant. The fruit pulp is firm, aromatic and always contains gritty cells. Individual fruits can weight up to 0.5 Kg (1 lb) or more and ripen late in the autumn. Fruits contain seeds which are poisonous. Quinces contain high levels of pectin, which ensures that any jelly made with them sets easily. Quinces have long been used as a herbal medicine, as an infusion to treat sore throat, diarrhoea and haemorrhage of the bowel. Turkey is the number one producer of Quince. Fruits of Turkish origin are large to very large, furrowed, oblong/pear-shaped, golden-yellow, very aromatic. Flesh mild, tender, light yellow, excellent quality. They are also high in pectin, a natural gelling agent that makes them ideal for use in jams, jellies and preserves. Pectin has many uses in the food industry, for example as a texture modifier and for setting jam, however the relationship between the structure of pectins and their performance as a food ingredient is poorly understood. Plant cells are surrounded by a cell wall composed principally of cellulose, but also of other compounds, including pectin, a complex polysaccharide. The cell walls of many flowering plants contain 30-40 % pectin and this molecule is thought to be important in maintaining the structure of the cell wall.

Chemically, pectins are a mixture of complex polysaccharides, homogalacturonan being the main component. This is a linear polymer made up of repeated units of α-(1-4)-linked D-galacturonic acid, to form a long polygalacturonic chain.

In their molecular structure, the carboxylic acids of galacturonic monomers may or may not be esterified with methanol or even acetic acid, in which case the percentage of esterified groups is expressed in the degree of methoxylation (DM) and degree of acetylation, respectively. Degree of methoxylation may reach the equivalent of 14 % methoxyl, which means esterification between 50 and 80 %. These are known as high grade methoxyl pectins, whilst those with a maximum of 7 % or a degree of esterification below 50 % are regarded as low-grade methoxyl pectins[5].

Food industries traditionally uses citrus peels and apple pomace as raw materials for pectin production. Alternative sources are currently investigated. Pectin extraction technology is being studied continually, because pectin is a commercially important product.

On an industrial scale, pectins are usually extracted using hot water acidified with a strong mineral acid [the so-called conventional (acid) extraction] under pH, temperature and duration conditions generally in the range of 1.3-3.0, 60-100 ºC and 20-360 min, respectively. Hot dilute solutions of strong mineral acids are indeed capable of releasing from pectin-rich cell wall materials. Acid-extraction generally enriches in GalA (homogalacturonic regions), pectin solubilized from cell wall materials, following substantial degradations of neutral sugar side chain-containing rhamnogalacturonic regions[6].

However, the degree of purity of the final pectin isolates may be dependent on the used purification procedure. In general, the extracted cell wall fractions contain pectin as well as a considerable amount of contaminating free neutral components, which may include monomeric sugars, oligosaccharides and high molecular weight polysaccharides[7]. Commercial (apple or citrus) pectins are purified from filtered (and concentrated) aqueous acid extracts by means of an alcohol (methanol, ethanol or isopropanol) precipitation process, which may involve subsequent alcohol or acid-alcohol washings for removing salts, free sugars and other (acid-) alcohol soluble compounds and converting pectins to their free acid form in the latter case.

On a laboratory scale, alcohol-precipitation or dialysis is frequently used for the purification of pectins from aqueous medium-soluble cell wall extracts. Even if either process is believed to effectually remove small molecular weight sugar components, notably, monosaccharide and disaccharides, because of their high alcohol-solubility or ability to pass through dialyzing membrane pores, it could not be the case for non-pectin-incorporated longer neutral oligosaccharides and polysaccharides[8].

The aim of this investigation is to extract pectin from quince fruit and determine its compositional and gelling capability, and to study the characterization of the quince pectin. The degree of esterification is a key factor to determine conformation and rheological properties of pectin. As a result of this study quince is an interesting source of pectin regarding to its pectin content and quality. Quince could be used as new sources of pectin and also used for jam and jelly manufacturing because the ability of pectin to gel depends largely on degree of methylation.

EXPERIMENTAL

The fruits were collected from the orchard of the city of Bilecik located in the west of Turkey. All fruits were picked at the same developmental stage, when the seeds in the fruits had turned brown, indicating fruit maturity. Individual fruits, approximately 400 g, are light golden-yellow, round and apple-shaped and very fragrant. Seeds were removed and the fruits were sliced and frozen immediately after picking. Frozen samples were manually separated and weighed before further treatment.

Pectin production: Frozen quince pulp (78.5 % moisture) were washed using hot water (75 °C) for 15 min to inactivate enzymes, filtered through a Büchner funnel into which nylon filter cloth was placed. In this step 500 mL de-ionized water was used for 100 g frozen quince pulp. After filtration, washed pulp was extracted with 400 mL of 0.01 M HCl (pH 2) preheated at different temperature and times in the water bath. The slurries were shaken in water heater bath for the ranges of 70-90 °C and 1-2 h, respectively. The quince pectin was obtained by hydrochloride acid extraction procedure followed by alcohol precipitation. The liquid phase was separated from the fruit mass by filtering. 100 mL concentrate pectin solution was precipitated with 100 mL of ethanol 96 % (v/v). Formed gel was allowed to precipitate for 24 h at + 3 ºC. The gelatinous precipitate was filtered through a G4 sintered glass. The coagulated pectin obtained was washed with ethanol 60 % (v/v), washed again with pure ethanol to remove mono and disaccharides. The resultant pectin was dried in a vacuum oven at 60 ºC 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. The word "pectin" stands for pectin obtained in this study. Pectin yield and content were determined from triplicate measurements. Data were analysis of variance and means were separated by least significant difference when significant F ($p < 0.05$) values were observed.

Detection methods: The ash content of pectin samples was determined[9] by ashing at 660 ºC for 8 h. Moisture contents of pectin samples were determined using an air oven method. Weight loss due to drying at 110 ºC for 12 h was reported as moisture content[9].

The degree of amidation and methylation was determined by titration with NaOH, based on free carboxyl group[10]. Galacturonic acid was determined by titration with NaOH[10]. 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 ºC. 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 solution)[11].

The mixtures were prepared in a ratio of 100 parts KBr powder per part of pectin samples to obtain information on chemical structures The mixtures were pressed by means of a manually operated hydraulic press. Spectra were recorded in the absorbance mode from 4000-400 cm^{-1}, using a Perkins Elmer, FT-IR 2000 spectrometer. The IR-spectrum of the pressed pellets were recorded to find out the characteristic groups present in pectin samples and thereby to illuminate the quince pectin structure.

RESULTS AND DISCUSSION

Table-1 shows the pectin yields extracted from quince at the experimental temperatures and times assayed. The temperature and time had notable 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. Our preliminary study, increasing the extraction temperature to 100 ºC also didn't affected the pectin yield and even caused the decreasing of pectin yield. Therefore, all experiments were done in the ranges of 70-90 ºC.

TABLE-1
PECTIN YIELDS OF QUINCE PRODUCED BY EXTRACTION WITH 0.01 N HCI AT VARYING EXTRACTION TEMPERATURE AND TIME, FOLLOWED BY PRECIPITATION WITH ETHANOL

Temperature (ºC)	Time (min)	Pectin yield (% w/w)
70	60	1.27
	90	1.48
	120	1.44
80	60	1.38
	90	1.66
	120	1.63
90	60	1.59
	90	1.83
	120	1.66

The chemical compositions of the pectin obtained at 90 ºC and 90 min extraction conditions are given at Table-2.

TABLE-2
CHEMICAL COMPOSITIONS OF THE PECTIN OBTAINED AT 90 ºC AND 90 min EXTRACTION CONDITIONS

Composition (%, w/w)	Pectin
Yield[a]	1.83
Moisture	6.71
Ash	2.47
Galacturonic acid	77.5
Degree of amidation[b]	4.56
Degree of methylation[b]	79.70
Gel strength	137.50

a: Yield was expressed as percentage of 100 g fresh quince pectin. b: Degree of methylation was calculated as molar ratio (%) of methanol. The values are mean ± SD of duplicate tests with at least three measurements.

The higher galacturonic acid and lower ash contents of pectin are the two criteria for its purity. Since anhydrogalacturonic acid is the fundamental unit or the backbone of pectin, its quantification is a primary method used to determine the pectin content in a sample. The degree of esterification is a key factor to determine conformation and rheological properties of pectin. A chemical characterization of peptic substances from a particular source material requires a determination of the methoxyl content.

This study shows that pectin containing over 70 % galacturonic acid, with a yield of over 1.83 %, could be produced from quince (*Cydonia vulgaris pers*.) using optimum acid extraction with 0.01 M HCl at 90 ºC and 90 min extraction conditions, followed by alcohol precipitation. The pectin content, as per cent galacturonic acid and yield of quince pectin were similar to commercial pectin.

FTIR spectra in the region between 4000-400 cm^{-1} identified the major chemical groups in the pectin and provided structural information of pectin. The spectral data obtained were analyzed by comparing the FTIR spectra in the following characteristic regions. O-H stretching band envelope 3600-3100 cm^{-1}, C-H stretching bands 3000-2800 cm^{-1}, the fingerprint region of spectra under *ca*. 2000 cm^{-1}, including the band contributing to resonant absorption energy of pyranose cycle vibrations 1200-950 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 1149, 1104, 1076, 1052, 1019 and 1016 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*. 1650-1600 and 1450-1400 cm^{-1}, respectively[12,13]. 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 1200-1100 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 polysaccharides[14,15].

A general view of FTIR spectra of quince pectin are presented in Fig. 1. The peak in 3600-3200 cm^{-1} region shows that there are too many OH^- groups in the pectin molecule. The band centered at 1749 cm^{-1} has been utilized to probe the DE in pectin. This band has been assigned to the C=O stretching vibration of methyl ester. There are two bands in the quince pectin spectrum within this region, a major one centered at 1617 cm^{-1} and a less intense one at 1384 cm^{-1}. These two band correspond, respectively, to asymmetrical and symmetrical

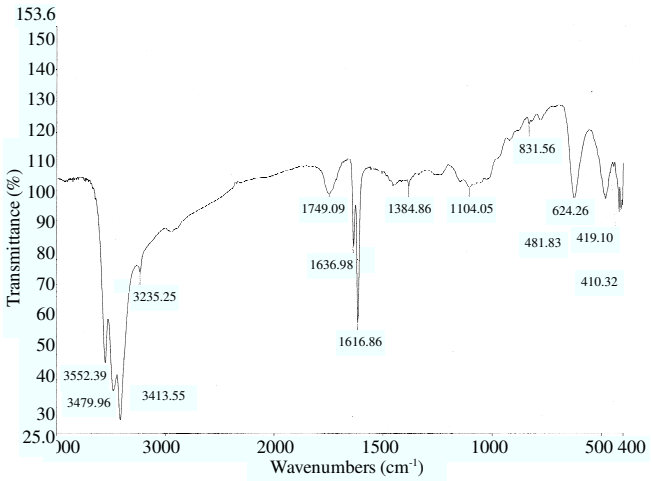

Fig. 1. FTIR spectra of pectin extracted from quince

stretching vibrations due to the COO^- group of polygalacturonic acid. The absorbances at 1104 and 1000 cm^{-1} are the galacturonic acid, because all peptic polysaccharides characterized mainly by these peaks.

Conclusion

The procedure for extracting fruit pectin was optimized in terms of pH, extraction time and temperature, thus allowing the most efficient and suitable, in terms of yield and degree of methylation, to be chosen. Degree of methylation and pectin yield are important factors in determining the firmness of the gel and, subsequently, the value and possible use of raw material in the food industry. Extraction that aims to obtain a higher yield and better characterization of pectins in terms of degree of methylation is a useful tool for technological purposes.

The extraction of pectin from quince fruit (*Cydonia vulgaris pers*.) was done extracting conditions of temperature (90 ºC) and time (90 min) in 0.01 M HCl solutions at pH 2. The yield of 1.83 % (dried pectin g/100 g frozen quince pulp) were obtained in this extracting conditions. 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. According to FAO, FCC and EU, the content of galacturonide expressed as galacturonic acid, $C_6H_{10}O_7$ (M_W 194.1) must be least 65 % on sugar, ash and moisture free basis[16]. It can be also concluded that the hot acid extraction, usually utilized for commercial pectins production, is highly suitable for the recovery of pectin from quince fruit (*Cydonia vulgaris pers*.) and the potential of quince as an alternate source for commercial production of pectin.

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