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EXTRACTION AND CHARACTERIZATION OF PECTIN FROM FRESH GLOBE ARTICHOKE AND CANNED ARTICHOKE WASTE

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Abstract

The pectin contents of fresh globe artichoke (stem, receptacle, and bract) and waste of artichoke canning industry were investigated. The highest pectin amount was found in the stem part of fresh globe artichoke (6.42%) with the highest amount of anhydrogalacturonic acid (AGA) and anhydrouronic acid (AUA) content. The pectin yields of receptacle and bract parts were found to be 5.31 and 4.55%, respectively. The pectin yield from the industrial waste was the lowest, 4.43%. The highest ash content (5.65 %) along with the lowest anhydrouronic acid amount (73.28%) indicated the lowest purity for the industrial waste. The degrees of esterification for the pectin obtained from the stem, receptacle and bract parts were 55.26%, 52.26%, and 56.17%, respectively indicating the presence of high methyl-esterified (HM) pectin. The pectin from the industrial waste had the lowest degree of esterification (46.02%). The FTIR results indicated that acid processing affected the structural properties of pectin from the industrial waste with higher methoxyl content and esterification degree.

Keywords: Globe artichoke, pectin, industrial waste, FTIR

TAZE ENGİNAR VE KONSERVE ENGİNAR ATIĞINDAN PEKTİN EKSTRAKSİYONU VE KARAKTERİZASYONU

Abstract

Bu çalışmada taze enginar (gövde, tabla ve yenilebilir yaprak) ile konserve enginar endüstrisinde çıkan atığın pektin içerikleri incelenmiştir. En yüksek pektin içeriği % 6.42 olarak taze enginarin gövde kısmında bulunmuştur. Pektin içeriğine ek olarak en yüksek anhidrogalakturonik asit ve anhidrouronik asit miktarları da gövde kısmında bulunmuştur. Tabla ve yenilebilir yaprak kısımlarında ise pektin verimi sırasıyla % 5.31 ve % 4.55 olarak bulunmuştur. En düşük pektin verimi % 4.43 ile endüstriyel atıktan elde edilmiştir. En yüksek kül içeriğine (% 5.65) ek olarak en düşük anhidrouronik asit miktarı (% 73.28) en yüksek safsızlığın endüstriyel atık suyunda olduğunu göstermektedir. Gövde, tabla ve yenilebilir yaprak kısımlarındaki esterleşme derecesi sırasıyla % 55.26, % 52.26 ve % 56.71 olarak elde edilmiştir. Bu yüzdeler yüksek metil esterleşmiş pektin olduğunu göstermektedir. % 46.02 ile en düşük esterleşme derecesi endüstriyel atıktan elde edilmiştir. FTIR sonuçlarına göre asit prosesiyle pektinin yapısal özelliklerinin değiştiği ve endüstriyel atıklarda daha yüksek metoksil içeriği ve daha yüksek esterleşme derecesi elde edildiği gözlenmiştir.

Anahtar kelimeler: Enginar, pektin, endüstriyel atık, FTIR

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INTRODUCTION

Pectin is a biopolymer which is a mixture of polysaccharides occurring naturally. Primary cell walls of many plant cells contain pectin. The uses of pectin are rapidly increasing in the pharmaceutical and biotechnology industry (1, 2). Conventionally, it is used in preparation of jams in order to exploit their gelling and thickening property. Recently, pectin has been used in drug delivery systems and other biotechnological applications. It is also used as a matrix material for entrapment of drugs, proteins and cells. In addition to these examples, several studies have shown that using pectin for diet can reduce levels of serum total cholesterol and low density lipoprotein cholesterol, and moderate the glucose response (3). Several investigations have been carried out to utilize industrial wastes as a pectin source. In addition, several plant materials were also reported as potential sources of pectin (4- 6).

Globe artichoke (*Cynara scolymus* L.) is a large thistle-like perennial plant that belongs to Asteraceae (*or Compositae*) family, and native to the Mediterranean basin (7). Today, globe artichoke is widely cultivated all over the world; however, it represents a pivotal component of the Mediterranean agricultural economy. The produced artichoke is consumed both in fresh and canned. The leaves of artichoke have been widely used in herbal medicine for treatment of digestive complaints (e.g., nausea, abdominal pains, dyspepsia, loss of appetite), hepatobiliary dysfunction, and for the inhibition of cholesterol absorption (7, 8). Artichoke canning also produces its industrial wastes mostly left unused or used as an animal feed. Therefore it is very important to utilize the waste as a source of pectin.

To the best of our knowledge, only a few studies have been carried out on the determination of pectin content in globe artichoke (5, 9). In addition, the discrete parts of the artichoke have never been scrutinized in this context. In the present study we isolated and characterized the pectin content of three structural sections (stem, receptacle, and bract) of globe artichoke and the industrial waste sample.

MATERIALS AND METHODS

Materials

Fresh globe artichokes were obtained from various grocery stores located in İzmir, Turkey, from January to March. Artichoke waste samples were obtained from a local canning factory. These wastes consisted of "liquid" materials which were used for blanching fresh artichokes and "processed artichokes" mainly composed of outer and inner bracts of the plant material. All the reagents used in the experiments were of analytical grade and used without further purification.

Preparation of plant raw materials

Fresh artichokes were dissected into four discrete parts including bracts (both outer and inner), receptacle, filament and stem. All the plant parts and solid wastes were dried at 40 °C in oven for four consecutive days-resulting in an average drying loss of 88%. Drying temperature was chosen as 40 °C. The dried samples were finely ground with a grinder. The ground samples were stored in light-protected glass bottles for further analyses.

Extraction and purification of pectin

The extraction method of pectin from fresh and industrial artichokes waste samples was adapted from the method as described (10), and duplicate extractions were carried out in each run. 10 grams of dried and ground plant material were transferred to a 500-ml screw-capped glass bottle containing 400 ml of distilled water. The pH of solution was adjusted to different pH values of 1.5, 3.0 and 4.5 with 1N HCI and heated with shaking at 90 °C for one hour. The extract was rapidly pre-filtered through a 53-µm metal sieve to remove the coarse particles. It was then filtered using Whatman No.4 filter paper. The filtrate was cooled rapidly in an ice bath, and mixed with 95 % ethanol at a volume ratio of 1:1. It was further stirred for 30 minutes, and allowed to stand overnight at 4 °C. The precipitate was centrifuged at 1844 x g for 20 minutes, and washed with 400-ml portions of 70 % ethanol until the precipitate is

essentially chloride ion-free (pH > 4). The precipitate was eventually dried *in vacuo* at 40 °C. The isolated pectin was used for further analysis.

Determination of pectic substances

The colorimetric method (10) was used to determine the pectin content in the artichoke samples. 0.1 gram of dried pectin was weighed, made into solution and diluted to 100 ml with 0.05 N NaOH. The solution was allowed to stand for 30 minutes to de-esterify the pectin. 2 ml of this solution was diluted to 100 ml with distilled water. 1 ml of pectin solution was mixed with 0.5 ml of 0.1% carbazole reagent in a tube. A white flocculent precipitate was formed. 6 ml of concentrated sulfuric acid was added with constant agitation. The tube was closed with rubber stopper and allowed to stand for 10 minutes for the color to develop. 15 minutes after adding the concentrated sulfuric acid, the absorbance of the sample was measured at 525 nm by setting the instrument to 100 % transmittance with the blank. The standard curve of anhydrogalacturonic acid (AGA) was plotted and the percentage content of the AGA was found in the samples. The method is based on the reaction of galacturonic acid, the basic structural unit of pectin molecule, with carbazole in the presence of H_2SO_4 and measurement of the color intensity at 525 nm. It is desirable to report the results as anhydrogalacturonic acid (AGA), since it is the basic structural unit of pectin (Equation 1).

$$\text{AGA\%} = \frac{(\mu g \text{ of AGA found in aliquot})\,(\text{dilution})\,100}{(\text{ml taken for estimation})\,(\text{weight of pectin sample})\,1{,}000{,}000} \quad (1)$$

The percentage of anhydrouronic acid content was calculated according to Equation 2 (10) where m.e. = milli equivalents.

$$\text{AUA (\%)} = \frac{(\text{m.e. alkali for free acid} + \text{m.e. Alkali for saponification} + \text{m.e. Titratable ash})(176)(100)}{\text{Weight of sample}} \quad (2)$$

Ash content and alkalinity of the ash

Pectin sample (1 gram) was weighed into a tared crucible and ignited slowly, then heated for 4 hours at 600 °C. The crucible was cooled to room temperature in a desiccator and weighed. To determine the alkalinity of ash, the ash was dissolved in 25 ml of 0.1 N HCI. The mixture was heated gently to boiling and cooled. It was then titrated with 0.1 N NaOH using phenolphthalein as indicator. A blank titration using 25 ml of the HCI was also carried out.

The ash content of pectin samples was calculated according to Equation 3 (10).

$$\text{Ash content (\%)} = \frac{(\text{Weight of ash})(100)}{(\text{Weight of sample})} \quad (3)$$

The alkalinity of ash is determined from the equation 4 (10).

$$\text{Alkalinity (\%)} = \frac{(\text{Blank - Titre})\,(\text{Normality of alkali})(60)(100)}{(\text{Weight of ash})(1000)} \quad (4)$$

Equivalent weight

Equivalent weight is used for calculating the anhydrouronic acid content and the degree of esterification. 0.5 gram of pectin sample was weighed into a 250-ml Erlenmeyer flask and moistened with 5 ml of pure ethanol. 1 gram of sodium chloride was added to sharpen the end point. 100 ml of carbon dioxide-free distilled water was added. It was ascertained that the entire pectin sample was dissolved and no lumps were retained on the sides of the flask. The solution was titrated slowly to avoid possible deesterification with 0.1 N NaOH by using automatic potentiometric titrator to the end point at pH 7.5. The neutralized solution was used for methoxyl determination. The equivalent weight of the samples was calculated from the equation 5 (10).

$$\text{Equivalent weight} = \frac{(\text{Weight of sample})(1000)}{(\text{Volume of alkali})\,(\text{Normality of alkali})} \quad (5)$$

Methoxyl content

To the neutral solution titrated for equivalent weight, containing 0.5 gram of pectin sample, 25 ml of 0.25 N NaOH was added. The mixture was shaken thoroughly and allowed to stand for 30 minutes at room temperature in a stoppered flask. 25 ml of 0.25 N HCI was added and titrated with 0.1 N NaOH by using automatic potentiometric titrator to the end point at pH 7.5. The methoxyl content of pectins was calculated according to the equation 6 (10).

$$\text{Methoxyl content (\%)} = \frac{(\text{Volume of alkali})\,(\text{Normality of alkali})(31)(100)}{(\text{Weight of sample})(1000)} \quad (6)$$

The degree of esterification was calculated from methoxyl and anhydrouronic acid contents by using the equation 7 (17).

$$\text{Degree of esterification (\%)} = \frac{\dfrac{(\text{Methoxyl content \%})}{(176)(100)}}{\dfrac{(\text{Anhydrouronic acid content \%})}{(31)}} \qquad (7)$$

FTIR Analyses

The pectin samples were lyophilized in a freeze drier (Labconco, FreeZone 18-l freeze dry system, USA) overnight to remove water. The pectin powder was mixed with dried potassium bromide (KBr) (Sigma-Aldrich, USA) in a mortar (at a ratio of 1:100). The mixture was then pressed to form a pellet. The spectral analysis was carried out using a Perkin-Elmer spectrometer equipped with MIR TGS detector (Perkin Elmer Inc., Norwalk, CT, USA). FTIR spectra of the samples were recorded between 4000 and 450 cm^{-1}. The interferograms were averaged for 20 scans at 4 cm^{-1} resolution.

RESULTS AND DISCUSSION

Effect of pH on the amount of extracted anhydrogalacturonic acid content (AGA)

The pH of the pectin extraction media is one of the essential parameters determining the pectin yield. To investigate the effect of pH on the pectin yield, a series of experiments were carried out by extracting the bract part of globe artichoke at three different pH values of 1.5, 3.0 and 4.5. The percentage of anhydrogalacturonic acid content (AGA) was determined and the results are presented in Figure 1. The anhydrogalacturonic acid content increased linearly with decreasing pH of extractant. Similar behavior has been reported for extraction of pectin from different plant materials (11-15). Hence, the maximum pectin yield was obtained at pH 1.5. Therefore the rest of the pectin extraction procedures were conducted at this pH value. Similarly the pectin yield was also determined at the same pH values and similar results were obtained indicating highest pectin extraction at pH value of 1.5.

Characterization of pectin isolated from different parts of artichoke

The pectin from three different structural parts, stem, receptacle and bract of globe artichoke and an industrial waste were characterized in terms of pectin yield, anhydrogalacturonic acid content, anhydrouronic acid content, equivalent weight, total ash content and alkalinity of ash, methoxyl content and degree of esterification. The properties of pectin from different plants are dependent on the source (16). Similarly pectin from different structural parts of globe artichoke appears to have different physicochemical properties.

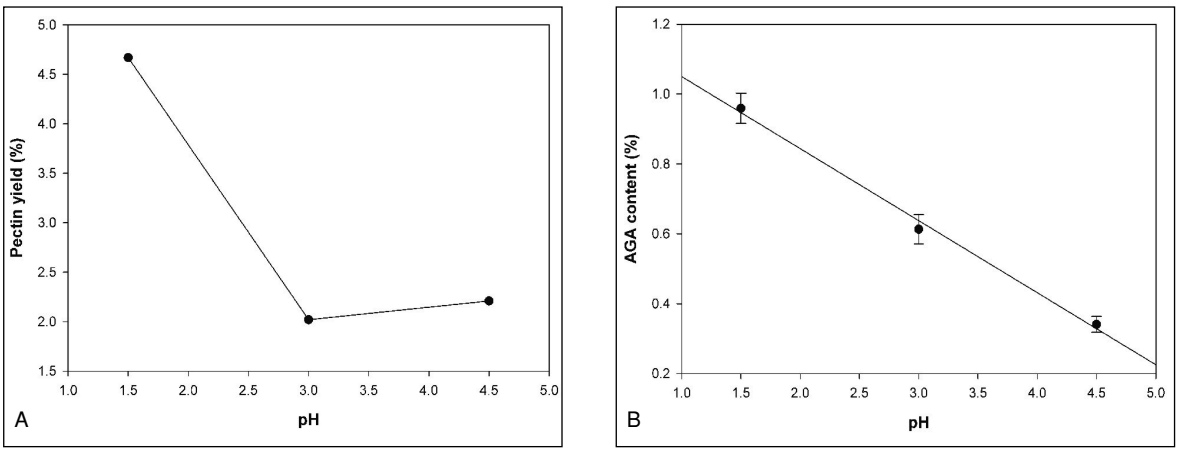

Figure 1. The effect of pH on the a) yield % and b) anhydrogalacturonic acid (AGA) % content of the pectin extracted from bract part of globe artichoke.

Pectin yield and total anhydrogalacturonic acid content

The yield of pectin results for different parts of artichoke are shown in Figure 2 a. As the results suggest the maximum pectin yield was obtained in the stem (6.42 ± 0.41 %). The pectin yields of receptacle and bract parts were found to be 5.31 ± 0.17 and 4.55 ± 0.25 %, respectively. The pectin obtained from the industrial waste yielded the lowest, 4.43 ± 0.19 %. The pectin yields were obtained slightly higher (7.12 %) or lower (2.4 %) then in earlier reports on the pectin from globe artichoke (5, 9). The differences between the pectin yields can be explained due to the methods followed in each study. Orlovskaya et al (9) used a method based on a mixture of oxalic acid and ammonium oxalate in different process parameters. Elwell and Dehn (5) used a consecutive sequence of water and sulfuric acid treatments with different process parameters such as extraction temperature and time. In our study we followed a method based on hydrochloric acid extraction at 90 °C for 1 hour (10).

Anhydrogalacturonic acid (AGA) content is an essential parameter in pectin extraction and characterization, since it is the basic structural unit of pectin molecule. The percentage of anhydrogalacturonic acid (AGA) in all artichoke samples are demonstrated in Figure 2 b. The results showed that the highest amount of AGA%, 1.01 %, was found in the stem part. The higher the AGA% is found in the sample, the higher the amount of pectin is represented in that sample. The lowest amount of AGA% was found in the industrial waste as it was anticipated. The bract and receptacle sections of globe artichokes possess lower amounts of anhydrogalacturonic acid in their plant tissues than that of the stem section.

Anhydrouronic acid content

Determination of percentage of anhydrouronic acid (AUA) content is crucial to verify the purity of extracted pectins, to determine degree of esterification, and to estimate their physical properties (10). Degree of purity and esterification degree are two important parameters in the use of pectin in jams and jellies as gelling agents used in food industry (10) which are closely related with AUA%. In this study the percentage of anhydrouronic acid (AUA) amount in all artichoke samples are demonstrated in Figure 2c. The results showed that the highest amount of AUA% (78.31 %) was found in the stem samples. The lowest amount of AUA% was found in the receptacle 72.47 % ± 2.52. The bract samples had the AUA% amount of 75.48 % ± 1.70 %. The industrial waste had an AUA value of 73.28 % ± 0.92 %. Therefore the stem had the pectin

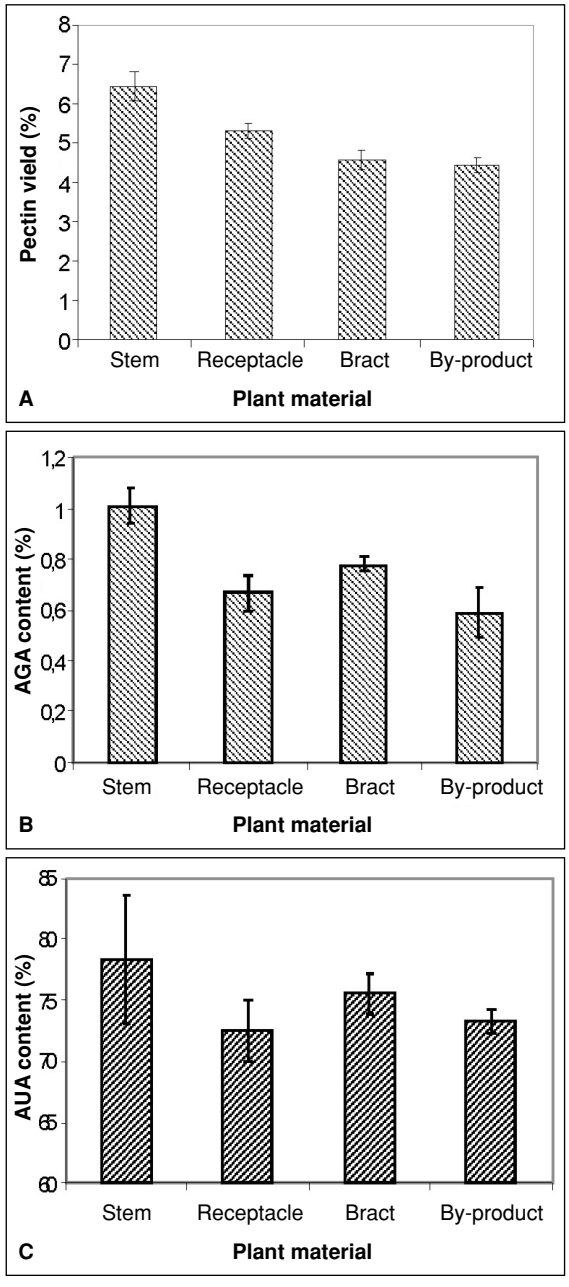

Figure 2. The a) yield percentage, b) percentage of anhydrogalacturonic acid (AGA) and c) anhydrouronic acid (AUA) content of pectin extracted from different parts of globe artichoke samples and industrial waste (By product).

content with the higher purity levels when compared with the other parts of the globe artichoke.

It was reported that the anhydrouronic acid content of pectin extracted from golden delicious pomace, golden delicious partially ripe apple, and that of commercial pectin was 74.1 %, 73.9 %, and 70.5 %, respectively (17). Anhydrouronic acid contents of pectin extracted from artichoke samples were found to be higher than that of commercial samples.

Determination of ash content and alkalinity of the ash

The results of ash content and alkalinity of ash are given in Figure 3a for all of the artichoke samples. The industrial-waste had the highest ash and alkalinity contents when compared with those of the artichoke parts. This indicated the lower purity of pectin in the industrial-wastes.

The ash content of the pectin samples determines its purity along with anhydrouronic acid (AUA%) value (18). Varying amounts of ash percent from several plants have been reported. The highest ash amount found in the industrial waste indicated the presence of minerals possibly added during the processing of globe artichoke wastes. The ash content of pectin samples was found to vary between 2.40 % and 5.65 %. The lowest ash content was found in the bract samples (2.40 %) and the highest ash content was found in the waste samples (5.65% ± 0.35%).

Determination of equivalent weight

The equivalent weight of pectin extracted from the stem, receptacle, bract and waste were found to be 681.93, 576.60, 688.29 and 764.68, respectively as shown in Figure 3b. The equivalent weight of commercial pectin (i.e., extracted from apple) was found to be 1030.9 (17). The equivalent weight of the pectin extracted from artichoke samples was lower than those for the industrial waste sample.

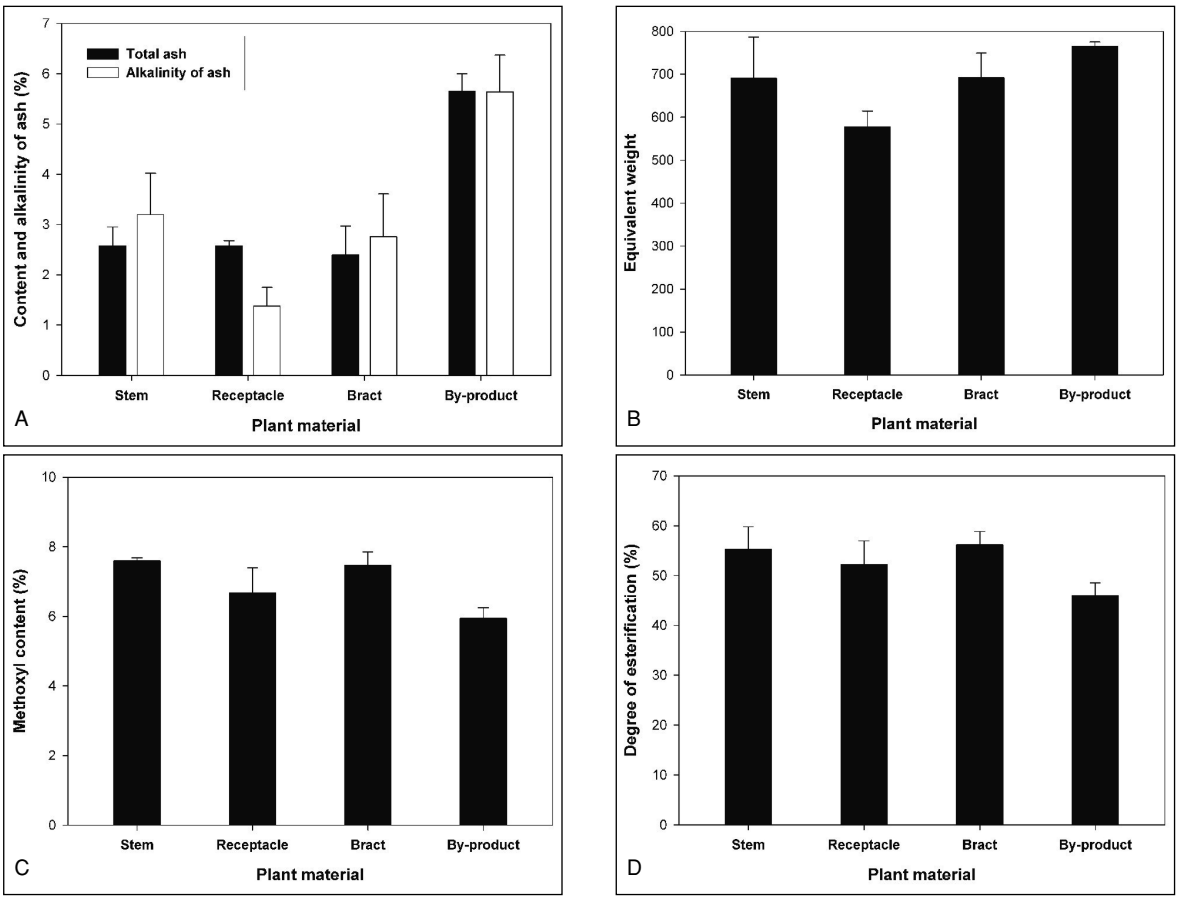

Figure 3. a) The content of total ash and alkalinity of ash, b) the equivalent weight, c) percentage of methoxyl content, d) the degree of esterification of pectin extracted from different parts of globe artichoke samples and the industrial waste (By-product).

Determination of methoxyl content

The methoxyl content is an essential factor governing the setting time of pectins, their gelling properties, and the sensitivity of pectins to polyvalent cations (10). According to the methoxyl content, the stem and bract samples had high methoxyl pectins (methoxyl content more than 7 %), and could form high sugar gels with the sugar amount of 65% and more (1086) as shown in Figure 3c. On the other hand, the receptacle and waste samples had low methoxyl pectins that could form gels with lower concentrations of sugar in the presence of polyvalent cations.

Degree of esterification

The degree of esterification values of pectins extracted from the various samples were found to be 55.26 %, 52.26 %, 56.17 %, and 46.02 % for the stem, receptacle, bract and industrial waste samples, respectively (Figure 3d). It was reported that the degree of esterification value for the industrial sample was 42.68 % (17). The degree of esterification for artichoke pectin was higher than the reported commercial value. Depending upon the degree of methyl esterification, the pectins extracted from stem, receptacle and bract sections of the artichoke are categorized as high methyl-esterified (HM) pectins with the degree of esterification value of higher than 50 %. On the other hand, the pectin extracted from artichoke waste was low methyl-esterified (LM) pectin with the degree of esterification value lower than 50 %.

FTIR Analyses

The spectral data can be analyzed with many different digital manipulations producing both qualitative and quantitative information considering shifts in peak positions, changes in bandwidths and band intensities to obtain structural and functional information about the systems analyzed (19). In this study, we examined structural features of isolated pectin from different sections of globe artichoke and the industrial waste using FT-IR spectroscopy.

Figure 4a shows the average FTIR spectra for the artichoke and industrial waste samples in different sections of the 3743-860 cm^{-1} spectral region. Since the FTIR spectrum of the pectin samples consists of several bands originating from the contribution of different functional groups of macromolecules the spectra were analyzed for the following regions: 3743-2791 cm^{-1} and 1804-1484 cm^{-1}, 1484-1183 cm^{-1}, and 1183-863 cm^{-1}. As seen in Figure 4a the band centering around 3287 cm^{-1} which was assigned to be O-H stretching of carbohydrates (20) shifted towards higher wave numbers in 3743-2791 cm^{-1} spectral region indicating the hydrogen bond breakage due to acid based heating step in the industrial waste samples (21, 22). The band is between 3436 and 3444 cm^{-1} for the artichoke samples but shifted up to 3544 cm^{-1} for the industrial waste samples as seen in Figure 4a.

The bands at 2956 cm^{-1} and 2938 cm^{-1} were assigned to the asymmetric stretching of methoxyl groups (23). The increase in the intensity of these bands indicated the increase in the methoxyl bands as seen in Figure 4b. Similarly, the band at 971 cm^{-1} was assigned to O-CH_3 groups of pectin and had higher intensity values for the industrial waste samples as shown in Figure 4d (24). The amount of methoxyl groups for the waste samples was found to be lower in the chemical analyses. The reason for this might be due to higher acid concentrations in the waste samples. Therefore FTIR results can be considered to be more accurate in the determination of the methoxyl amount in the processed artichoke samples. The band at 1323 cm^{-1} was assigned to C-H ringing and was found to have more intensity for the industrial waste samples as seen in Figure 4c.

Since FTIR method is also used for the analysis of degree of esterification in pectin samples (25), the method was used to analyze different parts of artichoke pectin and the industrial waste samples. For this purpose the intensity ratios of the band around 1740 cm^{-1} and the band around 1619 cm^{-1} were calculated (Figure 4b). The degree of esterification was found to be 0.4832, 0.4912, 0.5160 and 0.4949 for the bract, receptacle, stem and industrial waste samples respectively. In this research we tested the both methods for comparison reasons. The esterification degree for the waste samples was found to be slightly higher in the FTIR analyses when compared to those obtained with chemical methods as shown in the previous section. Again this might be due to possible effect of residual acid in the industrial waste samples. The degree of esterification obtained for the three parts of the globe artichoke were slightly lower than those obtained with the biochemical methods.

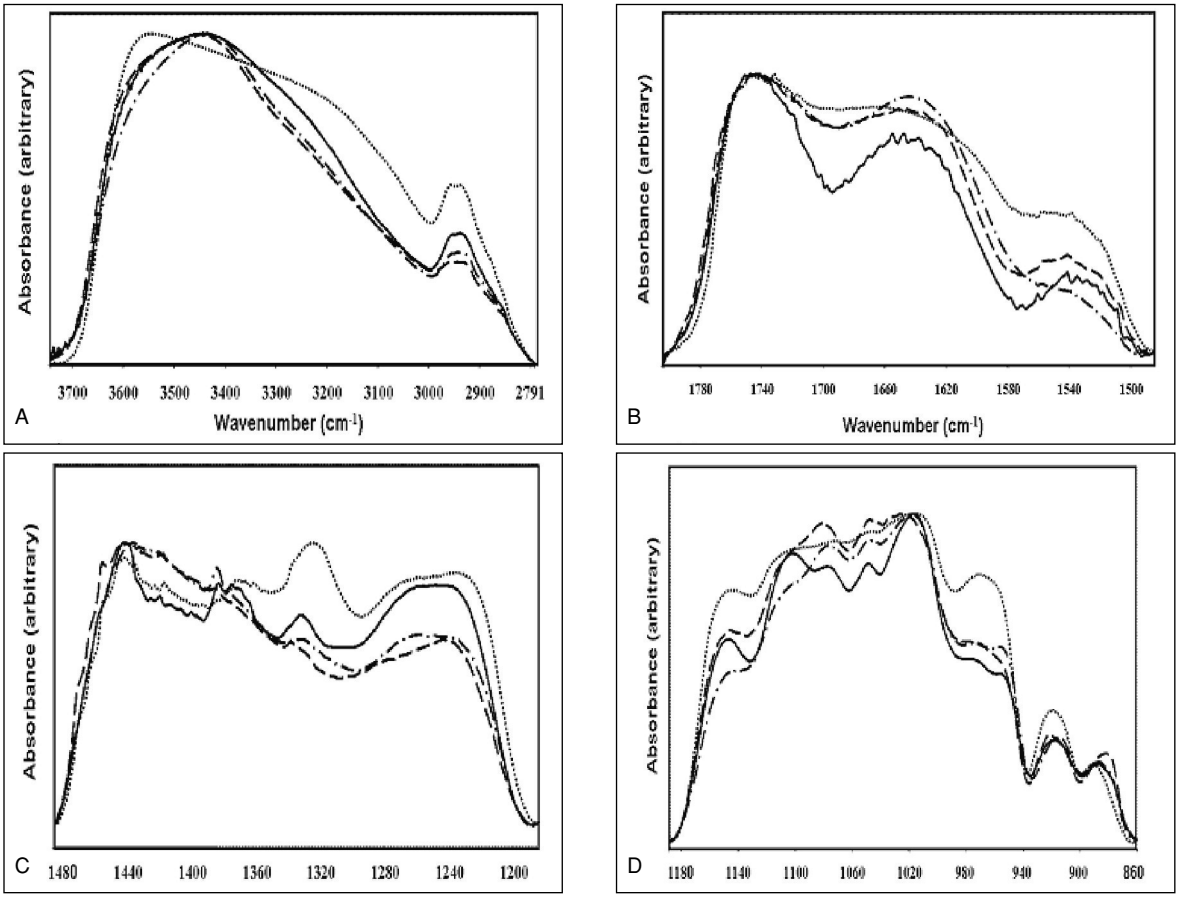

Figure 4. The FTIR analyses of different parts of globe artichoke and the industrial waste pectin samples in the wavenumber ranges of a) 3743-2791 cm^{-1}, b) 1804-1484 cm^{-1}, c) 1484-1183 cm^{-1}, and d) 1183-863 cm^{-1} (straight line (stem), line-dot-line (bract), line-space-line (receptacle), (dotted line (industrial waste)).

CONCLUSIONS

The results indicated that globe artichoke parts were important sources of pectin. The stem part of the plant had the highest pectin yield and anhydrogalacturonic acid content. The highest ash content and the total anhydrouronic acid contents of the industrial waste indicated the lowest degree of purity for the industrial waste. The degree of esterification for all the three different parts of the globe artichoke indicated that their pectin contents were of high methyl-esterified (HM) pectins. In parallel with this result the industrial waste had low methyl-esterified (LM) pectin. The results obtained with FTIR spectroscopy indicated that acid processing affected the structural properties of pectin obtained from the industrial waste with higher methoxyl content and esterification degree. The spectroscopic examination of the pectin samples indicated that chemical analyses should be compounded by FTIR spectroscopy as well for a better understanding of the process-dependent structural changes in the molecular structure of pectin molecules. As the results suggest globe artichoke can be employed as a source of HM pectin, and the industrial waste can be used as a source of LM pectin or it requires further purification to be used as HM pectin.

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