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RESEARCH ARTICLE

Valorization of Citrus Peel Waste

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Abstract

Citrus sinensis commercially known as an orange tree has a high pectin ratio and valuable essential oils. The huge amount of orange peel is generated in industries and they can be used as a raw material for essential oils and pectin. To obtain essential oils from orange peels distillation method is used and collected oil is analyzed to determine its d- limonene amount, also the yield of the essential oil production is calculated. The essential oil yield was found as 0.19%. GC results revealed that the sample had 94% d-limonene, and the data from the literature indicated that d-limonene amount of orange essential oil may vary between 32% and 98% depending on the variety of orange. In addition to essential oil, pectin was also extracted from the orange peels using two types of acids. First one was hydrochloric acid (HCl) as an inorganic acid, and the other was tartaric acid (TA) as an organic acid. The purpose of selecting the several types of acid was to observe the effects of acid type on pectin yield. The pectin amount obtained with HCl usage (4.72 % g pectin/orange fresh peel) was higher than that with TA usage (4.037 % g pectin/orange fresh peel). The experimental results for pectin contents of samples were also confirmed with the colorimetric test results. According to these results, concentrations of galacturonic acid in the samples obtained with HCl (0.509 mg galacturonic acid/mg) was found to be higher than the concentrations of galacturonic acid in the samples obtained with TA (0.103 mg galacturonic acid/mg).

Keywords: Essential oils, valorization, extraction of pectin, orange peel

Introduction

Citrus is one of the most important fruit crops in the world. Production of citrus fruits has increased enormously in the last few decades, going from an average of 62 million tons a year in the period 1987–1989 to about 100 million tons in the year 2010. Citrus is grown in more than 100 countries all over the world, mainly in tropical and subtropical areas, where favorable soil and climatic conditions prevail for citrus cultivation. Citrus fruits are marketed mainly as fresh fruit or as processed juice. During processing of citrus fruits, a huge amount of peels is generated as a by-product, which does not add value to the product as these are discarded or dumped. The potential use of citrus peels as value-added products has been widely studied because it contains numerous biologically active compounds including natural antioxidants such as phenolic compounds (Hayat et al, 2010).

Citrus waste includes more than half of the whole fruit when processed for juice extraction and mainly consists of:

• waste generated by the juice manufacturing industry, consisting of peel and pressed pulp

• fruit discarded for commercial reasons (damaged fruit, as an example)

• fruit discarded due to regulations that limit production

All these materials considered as waste because they are not part of food chain. In juice extraction process produces 500 tons of waste per 1000 tons of fruit processed. The percentage of fruit discarded due to commercial or regulatory issues are more difficult to calculate, but it ranges from 2% to 10% depending on the type of citrus considered and environmental aspects, such as weather conditions. Citrus waste generally

has a low pH (3-4), high water content (80-90%) and high organic matter content (95% of total solids) (Ruiz and Flotats, 2014).

Orange waste is produced in high quantities all over the world. In orange juice production, only half of the fresh orange weight is transformed into juice. Generating excessive amounts of residue (peel, pulp, seeds, orange leaves and whole orange fruits that do not reach the quality requirements), which accounts for the other 50% of the weight of the fruit and has a moisture content of approximately 82 g /100 g.

These wastes cause contamination in areas adjacent to the production locations, for its final use as a raw material in animal feed, or else it is burned. Management of this waste is important. One alternative to improve the management of these residues is the implementation of new processes for their recovery, for instance, through the production of organic fertilizers, pectin, bio-oil, essential oils, and antioxidant compounds, or as a substrate to produce several compounds with high added value, such as microbial proteins, organic acids, ethanol, enzymes and biologically active secondary metabolites and adsorbent materials. These are excellent alternatives to avoid environmental pollution and to add value to these substances (Rezzadoria et al., 2012).

Citrus Essential Oils (CEO) are liquids that contain, among other components, the volatile aroma compounds of citrus plants. The essential oils present in small vesicles which located in the flavedo (the upper shell of fruit) or exocarp of citrus fruit. Antimicrobial properties of CEOs are discovered in old times and citrus essential oils are used as natural healing (Ruiz and Flotats, 2014). Citrus fruits have oval, balloon-shaped oil sacs, glands or vesicles, the diameter of which varies from 0.4 to 0.6 mm. The essential oil presents in that. Ductless, and without communication with surrounding cells or the exterior, they have no proper walls but are simply bounded by the debris of degraded tissue (Board, 2011). Citrus essential oils have many components (more than 200) including terpenes, sesquiterpenes, aldehydes, alcohols and esters, and can be described as a mixture of terpene hydrocarbons, oxygenated compounds, and non-volatile residues.

Terpenes are unsaturated compounds that readily decay by light, heat, and oxygen. Removing of terpenes avoids unpleasant flavours; they make up about 80-98% in most citrus peel oils (Diaz et al., 2005). Limonene is the main volatile component of CEOs because of this the chemical, physical and biological properties of this compound influence the properties of the essential oil. Its concentration in the essential oil may vary between 32% and 98%, depend on the variety: 32-45% in bergamot, 45-76% in lemon and 68-98% in sweet orange (Ruiz and Flotats, 2014).

In addition to the citrus essential oils, *citrus sinensis* has high pectin content. Pectin, which is a valuable product, can be used for different areas for different purposes. It has long been used for its gel-forming, thickening and stabilizing properties in a wide range of applications from food to the pharmaceutical and cosmetic industries.

Pectin is naturally found in the structure of cell walls of all higher plants. The outer surface of plants is especially rich in pectin. Fruit peels are well known and used as pectin sources for industrial applications. Fruit peels are a rich source of rough dietary fibers. Pectin, hemicellulose, tannins, gum can be given as examples of these fibers. The fiber compounds give bulkiness into the food and help preventing constipation by reducing gastrointestinal transit time. Besides, they link to toxin chemicals in the food, in this way they protect them contacting with gut mucosa and thereby help cut-down colon cancer risks. Moreover, they link tightly to bile salts, which are produced from cholesterol, and eliminate the salts from the gut, thus, accordingly help lower serum LDL cholesterol levels (Joye and Luzio, 2000).

Pectin is a complex mixture of acidic structural polysaccharides in cell walls of all land plants, which is mainly formed of d-galacturonic acid and neutral sugars, such as l-rhamnose, l-arabinose, and d-galactose. D-galacturonic acid units that are partially esterified with methanol or acetic acid at the carboxylic acid (BeMiller, 1986). In this study, the valorization of orange peel wastes for the recovery of essential oil and pectin were aimed. Extraction of pectin in orange peel was studied using two different acids including hydrochloric acid and tartaric acid. Pectin yields were compared. The obtained pectin samples were further characterized.

Materials and Methods

Materials

The fresh oranges (*Citrus sinensis*) were bought from local grocery stores in Izmir/Turkey, in January to March 2016. All the reagents used in the experiment were in analytical grade and used without further purification. Ethanol %96 (Analiz Kimya, Turkey), HCl %37 (Sigma-Aldrich), tartaric acid (Sigma-Aldrich), H_2S0_4 %98 (Merck, Germany), D-(+)-Galacturonic acid (Sigma – Aldrich), 3-Phenylphenol, 85% (Aldrich), NaOH (Aldrich), sodium tetraborate (Borax) (Merck, Germany).

Methods

Preparation of orange peel for hydrodistillation

Experimental set up was insulated before to reduce heat losses. Orange peel waste was subjected to hydrodistillation. Distillation was performed twice to enhance the recovery of volatile fraction at the end of the process. In the first run, distillate was collected then collected distillate was distilled again.

In the product emulsion of water and essential oil was formed and after a short period of time essential oil and water were separated into two distinct phases. The amount of essential oil was measured with the help of Clevenger apparatus's measurement section. D-limonene amount in the essential oil was determined by using Gas Chromatography (GC) equipment (Agilent 7890A) with a FID. The samples (20µl) were injected into the injection port. A capillary column HP-5 (30m x 320µm x 0.25 µm film thickness) (Agilent) was used for chromatographic separation. The used temperature program was 5 min at 50 °C isothermal and an increase of 5 °C/min to 200 °C. Helium was used at 2ml/min as the carrier gas. The temperature of injector and detector was 250 and 270 °C, respectively.

Preparation of orange peel for extraction

Fresh oranges were washed with deionized water and then dried. Cleaned and dried oranges were peeled off. After that, orange peels were diced into small and fine pieces. The diced pieces were treated with 96% ethanol, which was preheated to 65 °C, in the ratio of 1:2.5 (w/v). In this treatment procedure, firstly, the diced orange peels and 96% ethanol are mixed in a beaker for one hour at 65 °C. Then, the diced peel – ethanol mixture was kept at room temperature overnight. After that, the mixture was filtered by hand through muslin cloth, after which the insoluble materials were washed twice with warm 96% ethanol. The remaining solids or alcohol insoluble solids (AIS) given in Figure 1 were dried at 60 °C in an oven and stored until use.

Figure 1. The picture of prepared alcohol insoluble solids (AIS)

Extraction and purification of pectin

The extraction of orange peel and its purification methods was adapted from the method for isolation, characterization and modification of citrus pectin, as described in the literature (Georgiev et al.,2012).

The extraction steps were done in two parts. The first part was done with water, and then the second part was done with selected acid, which was either hydrochloric acid or tartaric acid. Firstly, AIS was treated with hot deionized water (1:25), (w/v) at 82 °C for 1 hour with continuous stirring and then filtered. Then, the retentate was treated with hot deionized water (1:10), (w/v) at 82 °C for 10 minutes with continuous stirring and then filtered.

To obtain water extracted pectin (WEP), the solutes from water extracted operation was purified. Since pectin dissolves in hot water, the solutes were coagulated with cold acidic 96% ethanol (0.5% HCl), in the ratio of 1:2 (v/v). The precipitated crude pectin was separated by filtration, washed once with 100 mL of 70% acidic ethanol, then with 70% ethanol to a neutral pH and finally with 100 mL of 96% ethanol. Pectin samples were dried at room temperature in fume hood. Thus, water extracted pectin (WEP) was obtained.

Also, for the recovery of acid extracted pectin (AEP), similar steps for the recovery of WEP were followed. Acid-extracted pectin continued with the solutes obtained from water extraction. The residue was treated with 0.5% acid (HCl and TA), with 1:20 ratio (w/v) at 82 °C for 50 min and continuously stirred at pH 1.7. Then, the acidic mixture was filtered and the solid retentate was treated with 0.5% HCl (1:8), (w/v) at 82 °C for 10 min and continuously stirred at pH 1.7. The only difference in that the ethanol in purification method was not acidic. Since the solutes came from the acidic extraction step, there was no need to acidify the ethanol in Figure 2 end products of extracted and purified pectin was given.

Figure 2. The picture of extracted and purified pectin samples

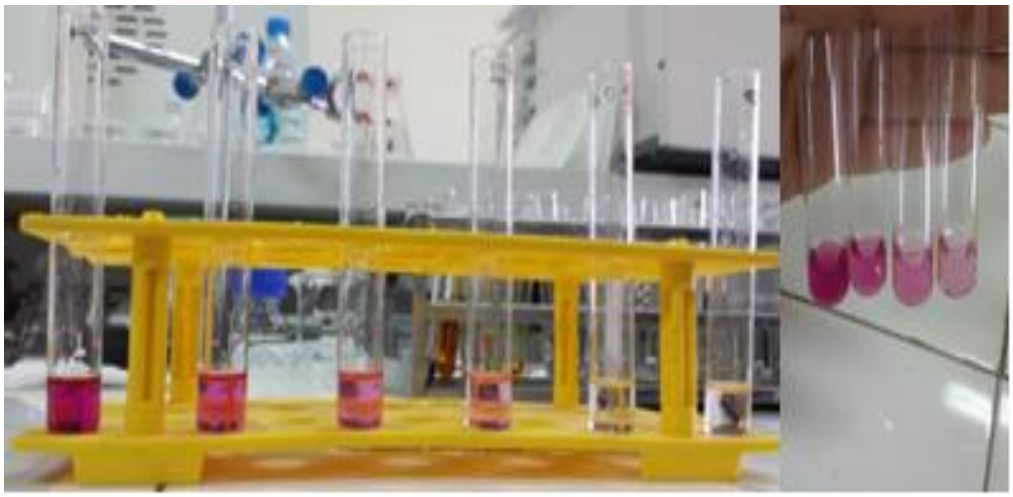
Determination of pectin substances

To determine the pectin content of extracted orange peels, the colorimetric method was used. The principal of this method is based on the reaction of the galacturonic acid with a reagent material, m-hydroxydiphenyl. If there is galacturonic acid in the sample, the reagent gives a pinkish color to the sample. Due to this reaction, pectin content of the samples can be determined by measuring the absorbance values using a spectrophotometer.

In the experiments, anhydrogalacturonic acid (AGA) content was determined by the m-hydroxydiphenyl method, using D-GalA as a standard.

Firstly 0.2 ml of sample, which contained 0.5 to 20 µg uronic acids, was prepared. Then, 1.2 ml of sulfuric acid/tetraborate was added. The tubes were refrigerated in crushed ice. The mixture was shaken regularly, and the tubes were heated in a water bath at 100°C for 5 min. Then, the tubes were cooled in a water-ice bath. After the tubes are cooled, 20 µl of the m-hydroxydiphenyl reagent was added into each of them. The tubes were shaken properly, and within 5 min, absorbance measurements made at 525 nm in a UV spectrophotometer. As carbohydrates produce a pinkish chromogen with sulfuric acid/ tetraborate at 100 °C, a blank sample was run without addition of the reagent, which was replaced by 20 µl of 0.5% NaOH. So, the absorbance of the blank sample was subtracted from the total absorbance (Blumenkrantz and Asboe-hansen, 1973).

Figure 3. Different samples of colorimetric methods.



FT-IR analysis

The pectin from orange peel was further investigated by using FT-IR analysis and the resulting spectrum was studied in order to understand the functional groups present. The dried pectin samples were ground with KBr at a 1/100 ratio (w/w). The powders were pelletized, and then the infrared spectra were obtained. The spectra of the samples were recorded in the 4000 - 650 cm^{-1} region at room temperature.

Results and Discussion

Determination of yield of essential oil

One of the aims of valorization of orange peel waste was obtaining essential oil from orange peel with a high yield as much as possible. From 73 g fresh orange peel and 200 ml water, 0.14 ml orange oil was obtained.

The yield was calculated as 0.19%. Calculated yield value was in the range of values reported by other researchers in the literature.

Yield depends on the season of harvesting, plant variety, the plant parts sampled, and the conditions under which the plant is grown. So, obtained yield can be affected by all these parameters. Also, composition of essential oil can vary with these parameters.

Figure 4. Gas Chromatography-Mass Spectrometry of Orange Peel Essential Oil

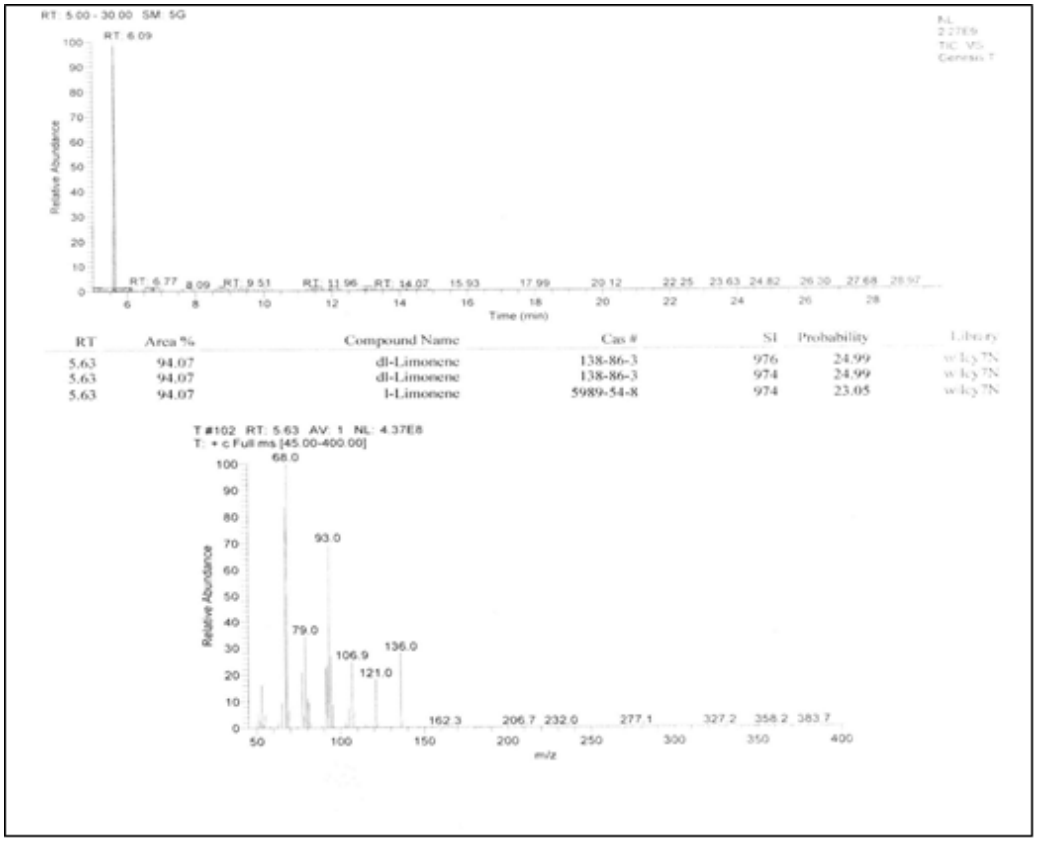
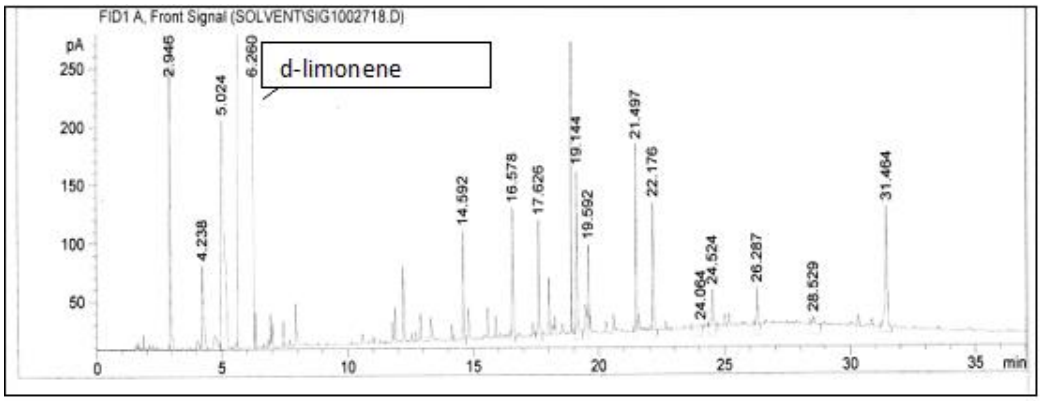


Figure 5. Gas chromatogram of Orange Peel Essential Oil



Results show that sample has 94% d-limonene, and from the literature survey d-limonene amount of orange oil may vary between 32% and 98% depending on variety of the orange.

The size of the waste peel was changing a parameter in the experiment, in the first run peels were cut into small pieces but results were ineffective. Because oil sacs place on the peel and when the peels were cut,

they were destroyed, and oil would escape. With this reason, the remaining runs of the experiment, peels were separated from white part and the orange outer surface was used as possible. Results were better than the first run.

For a collection of essential oil, the distillate from first distillation run was fed back into the system and at the end of second distillation, oil droplets were observed clearly. After waiting for a brief period, oil droplets have created a layer on the water surface. Separation was achieved easily due to the density differences between oil and water.

Characterization of extracted pectin samples

In order to characterize the pectin samples, FT-IR spectra were obtained for acid and water extracted pectin material and results are given in Figure 6.

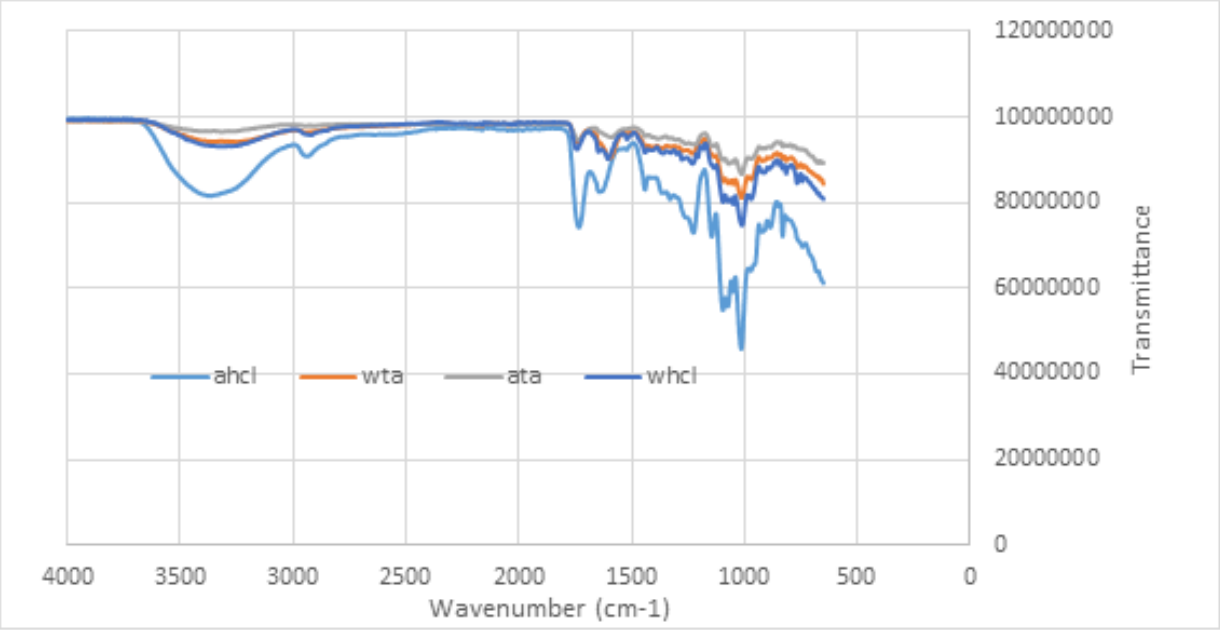


Figure 6. FT-IR Spectra of Extracted Pectin Samples: (ahcl: Acid Extracted Pectin with HCl; ata: Acid Extracted Pectin with TA; wta: Water Extracted Pectin with TA; whcl: Water Extracted Pectin with HCl)

The broad, strong area of absorption between 3600 and 2500 cm^{-1} refers to O-H stretching absorption due to intermolecular and intramolecular hydrogen bonds. The O-H stretching vibrations occur within a broad range of frequencies and indicate several features of a compound, including free hydroxyl groups stretching bands which occur in samples in the vapour phase and bonded O-H bands of carboxylic acid (Silverstein et al., 1991). In the case of pectin samples, absorption in the O-H region is due to the intermolecular and intramolecular hydrogen bonding of the galacturonic acid polymer. Finer bands appearing at the longer end of the O-H region indicate overtones and combination of tones. Bands around 2950 cm^{-1} (3000-2800 cm^{-1}) refer to C-H absorption. These include CH, CH_2, and CH_3 stretching and bending vibrations. Typically, two moderately intense bands are observed in the C-H region of aliphatic compounds (Gnanasambandam and Proctor, 2000).

In pectin samples, the C-H stretching and bending vibrations were seen, usually, as a band superimposed upon the broader O-H band that ranges from 2500 to 3600 cm^{-1}. This was observed with all pectin samples studied. In pectin samples, the weaker symmetric COO- stretching is followed by moderately intense absorption patterns between 1300 and 800 cm^{-1} collectively referred to as the “fingerprint” region that is

unique to a compound. These bands are usually difficult to interpret. As seen from the Figure 6 all the extracted pectin samples are compatible with each other and with the literature survey.

Determination of pectin content

For 500 grams fresh orange peel, 77 grams alcohol insoluble material (AIS) was obtained. For 100 g fresh orange peel, 15.4 g AIS was obtained. These values are in accordance with the results reported in the literature (Georgiev et al., 2012, Kaya et al., 2014).

At the end of the extraction and purification steps of each set, purified pectin samples were dried in a fume hood at room temperature. After drying process, pectin samples were used for the preparation of pellets. These pellets form of pectin samples were weighed, and with this weight measurement values, g pectin / g fresh orange peel ratio was calculated for each set. The data are tabulated in Table 1.

Table 1 Pelletized pectin samples prepared by using hydrochloric acid and tartaric acid.

	HCl	**TA**
WEP, g	1.773	1.360
AEP, g	4.124	3.685
Total Pectin, g	5.896	5.046
%(g pectin)/(orange fresh peel)	4.7170	4.0367

According to data given in Table 1, it was shown that amount of pectin extracted with HCl (4.72 % g pectin/orange fresh peel) was higher than amount of pectin extracted with TA (4.037 % g pectin/orange fresh peel). The colorimetric method was used to determine the amount of pectin substances in WEP and AEP samples in terms of galacturonic acid equivalent concentrations.

In the colorimetric method, the coloring reagent, which was m-hydroxydiphenyl, gives a reaction in the presence of galacturonic acid resulting in a pinkish color in the sample. The concentration of pectin substances in the samples was calculated by using a UV spectrophotometer. One μg AEP sample contains 0.509 μg galacturonic acid equivalent and 0.103 μg galacturonic acid equivalent for HCl and TA sets, respectively. Also, one μg of WEP sample contains approximately 0.383 μg galacturonic acid equivalent (gae). All these values determined as galacturonic acid equivalent are given in Table 2.

Table 2. Pectin substances content of pectin samples

	μg gae/ μg sample
WEP from HCl set	0.424
WEP from TA set	0.342
AEP from HCl set	0.509
AEP from TA set	0.103

The yields of extraction with inorganic acids were higher than those with organic acids. However, considering the undesired properties of inorganic acids, the results obtained with tartaric acid were comparable to the ones obtained with hydrochloric acid. In addition to the less harmful and toxic properties, tartaric acid which occurs naturally in many plants and can be recovered from various natural resources, mainly from winery byproducts. Other sources of tartaric acid are biotechnological processes or synthesis via the peroxidation of maleic anhydride (Kontogiannopoulos et al., 2016), another waste product of many industries. It is possible

to utilize the waste of food industry such as tartaric acid for the feasible production of value-added products such as pectin from waste peel of oranges in juice industry.

Conclusion

Produced waste amounts increase with urbanization, correspondingly consumption growth. Waste recovery is an important and urgent issue. Moreover, since fruit industry grows day by day, generated fruit wastes increase. Large amounts of fruit wastes are probably caused a pollution problem in the case of improper management of these wastes. If the wastes are effectively managed to produce value-added products economic benefits along with reduced environmental problems can be achieved. Orange juice is one of the big juice sectors. From these sectors, large amount of wastes especially orange peels are discarded. However, orange peel wastes can effectively be utilized for the economic production of value-added products such as pectin. Our results revealed that it was possible to produce value-added products from the orange peel wastes such as essential oil and pectin.

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