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CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY AND STRUCTURE OF PECTIN AND EXTRACTS FROM LEMON AND ORANGE PEELS

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ABSTRACT: The study was conducted on lemon and orange peels to study the chemical composition of these residues by determining each of carbohydrate, protein, fat, ash, and fiber contents. The pectin was also separated and purified and studied using HPLC (High Performance Liquid Chromatography) and IR (Infrared Radiation) techniques. Finally studied the activity of antioxidant activity of pectin and methanol, ethanol and acetone extracts; Lemon peel contents of crude protein, ash, fiber, fat and carbohydrate were (7.89%, 5.30%, 15.40%, 2.50% and 68.91%, respectively) monosaccharides and disaccharides and their derivatives found in pectin were arabinose, galactose and glucose. Through HPLC analysis, and by IR analysis in the region from (1619: 2049) and from (3046: 3235), there were a groups of (COOH and CH₃) and pectin sugar was presented in the hexagonal ring structure. The area (616) nm and the antioxidant activity showed that the extract of methanol for lemon peel was the highest antioxidant as well as pectin orange peel contents of crude protein, ash, fiber, fat and carbohydrate were (5.26%, 4.30%, 9.90%, 2.75%, and 77.79% respectively) monosaccharides and disaccharides and their derivatives were found in pectin of orange were arabinose, galactose, glucose and mannose. Through HPLC analysis and by IR analysis in the regions (2050: 2359) and (3036: 3234) containing the presence of COOH and CH₃ Pectin sugar is presented by investigation at (615nm) in the hexagonal ring structure.

Key words: Antioxidant activity, lemon peels and orange peels pectin, monosaccharide HPLC and IR

INTRODUCTION

Lemon (*Citrus Limon*) is the third most important species of citrus after orange and mandarin, with a production totaling more than 4.4 million tons during the 2001/2002 season. Argentina with 1.2 million tons is currently the world's largest producer of lemon and oranges.

The peels is a by-product of lemon and orange juice processing, with a high potential use. Two different tissues are found in what is colloquially called lemon and orange peels, flavedo and albedo (Agust, 2003). Flavedo is the peel's outer layer, whose colour varies from green to yellow. It is a rich source of essential oils (Janati and Beheshti, 2012), which have been used since ancient times by the flavour and fragrance industry (Vekiari *et al.*, 2002).

Albedo is the major component of lemon and orange peels, and is a spongy and cellulosic layer laid under flavedo. The thickness of the albedo fluctuates according to several variables, among them variety and degree of ripeness. Furthermore, the presence of associated bioactive compounds (flavonoids and vitamin c) with antioxidant properties in fresh lemon and orange albedo involves healthier benefits than other sources of dietary fiber (Martin *et al.*, 2002). Citrus by products also represent a rich source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Ebrahimzadeh and Gayekhle, 2004; Fernandez-Lopez *et al.*, 2005; Jayaprakasha and Patil, 2007).

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Pectic substances are complex plant hetero polysaccharides. In 1917, Ehrlich announced that the basic building block of these substances is D-galacturonic acid (D-GalA) (Georgieu *et al.*, 2012) and later Henglein and Schneider (1936) proposed that the linkages between the building units in poly galacturonic acid are (1→4). Nowadays it is well-known that pectic polysaccharides are composed of several structural regions. The most studied and simply arranged structural fragment is called homo galacturonan. It is composed of (1→4)-linked α -D-GalA residues. The carboxyl groups are methyl esterified to different extent, which results in low methoxylated (< 50%) and high methoxylated (>50%) pectins. Some hydroxyl groups are partly acetylated at O-2 and/or O-3 (Schols and Voragen, 2003). This mode of esterification is critical for pectic functional properties. The second fragment is rhamno galacturonan I, constituted of linear repeating structure [\rightarrow 2)- α -L-Rhap-(1→4) - α -D GalpA-(1→]. Some of α -L-rhamnose (Rha) residues are substituted at O-4 and rarely at O-3 with neutral side chains, such as β -D-galactans, (1→5)- α -L-arabinans and arabinogalactans (Voragen *et al.*, 2009). There are arabinogalactan type I (arabino-1, 4- β -D-galactans) and II (arabino-3,6- β -D-galactans), which are among the most important pectic fragments responsible for different biological activities (Morris, 2009). Some α -L-arabinose (Ara) and D-galactose (Gal) residues could be feruloylated (Ralet and Thibault, 2005). The third famous reported structure is rhamno galacturonan II. It represents a homo galacturonan with four side chains at O-2 or O-3, composed of rare sugars like D-apirose, L-fucose, 2-keto-3-deoxy-D-manno-2-octulosonic acid, deoxy-D-lyxo-2-heptulosaric acid (Dha), aceric acid, their methoxylated analogs and others (Yapo, 2011).

In the present work chemical composition, structure of polysaccharides and antioxidant activities of lemon and orange peels were studied.

MATERIALS AND METHODS

Materials

All chemicals and solvents were of analytical reagent grade. Lemon and orange peels were collected from the local market (in Zagazig, Sharkia Governorate, Egypt). After collection,

the peels were shade dried at room temperature (30-35°C). 100 g of peels of each of lemon and orange were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was dried in an oven at 40°C for 24 hr.

Preparation of Different Solvent Extracts

Lemon and orange peels powder were extracted with different solvents such as ethanol, methanol, chloroform and diethyl ether. 10 g of peels powder of each of lemon and orange peels were suspended in 200 ml of each solvent. Extraction was done using soxhlet apparatus for 5 hours at a specific temperature of each solvents but not exceeding the boiling point (Saadi and Abbas, 2003).

The attained extract was filtered through syringe filter and the solvent was removed by evaporation using Buchi Rota vapor under reduced pressure at 45°C with 5 bar to get a constant mass and concentration of 1 g. The resulting crude extract was then stored at 4°C until use (Subhashini *et al.*, 2010).

Methods

Chemical composition

Chemical composition of both lemon and orange peels were determined such as (protein, ash, fiber) according to AOAC (2005) and total carbohydrates and total soluble carbohydrates, reducing sugars and non-reducing sugars were extracted as described by Berfeld (1955) and Miller (1959) and color optical density of the reacted mixture was measured on absorbance (spectrophotometer at 540 nm).

Extraction of pectin

Alcohol-insoluble solids (AIS, 50g) from lemon and orange peels were separately treated with 1250 ml deionized water (dH₂O) at 82°C for 1 hr., with continuous stirring and then filtered as show on Fig. 1 each retentate was treated in the same way with 500 ml dH₂O for 10 min and then filtered. The crude filtrates with water extracted pectins (WEP) were obtained. The crude extracts were precipitated with two volumes cold 96% ethanol and left for an hour. Finally precipitant was washed with 100 ml of 96% ethanol. Pectin were dried at 60°C in a laboratory dryer Scheme for preparation of (AIS).

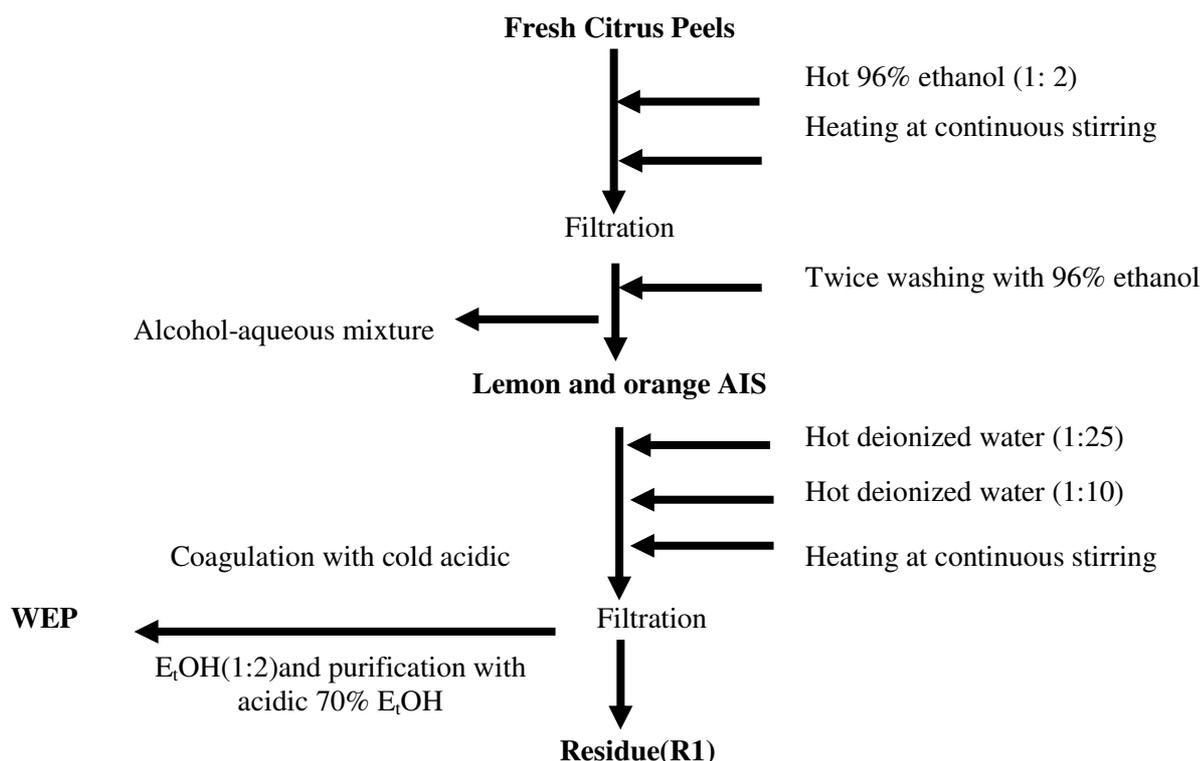


Fig. 1. Pectin extraction from lemon and orange peels

AIS = alcohol-insoluble solids

WEP = water -extracted pectins

Determination of monosaccharide composition and molecular weight

The crude polysaccharides (20 mg) were hydrolyzed with 2 M trifluoroacetic acid (TFA, 10 ml) for 1 hr., and 3 hr., for neutral sugar and uronic acid determination. Hydrolyzates were analyzed for neutral sugars on HPLC system (Agilent LC 1220, USA) with Zorbax Carbohydrate column (4.6x150 mm, 5 µm), and Zorbax Reliance Cartridge guard-column. The mobile phase was aceto nitril : H₂O (80/20) at 1.0 ml/min. The eluate was monitored at 35°C, using a refractive in dex-detector (RID 1260). Uronic acids were determined on HPLC (Waters, Milford, MA, USA) with Aminex HPX-87H column and 4 mM H₂SO₄ as eluent at 30°C. The elution rate was 0.8 ml/min and the eluate was monitored using a refractive index detector (R401, Waters). Monosaccharides were identified by their retention times (Yan *et al.*, 2004).

Molecular weights of the polysaccharides (2 mg/ml) were determined on HPSEC (Waters)

with Ultrahydrogel[®]120 and Ultrahydrogel[®]500 columns (7.8 × 300 mm, Waters). Elution was carried out at 25°C with 0.1 M NaNO₃ at 0.6 ml/min. The columns were calibrated, using Shodex standard P-82 (Showa DENKO, Tokyo, Japan). The standard kit contains eight pullulan standards with molecular weights in the range 0.59×10^4 – 78.8×10^4 Dalton.

DPPH radical-scavenging activity

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Ebrahimzadeh *et al.*, 2008). Different concentrations of each extracts were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamine C, BHA and quercetin were used as standard controls. IC₅₀ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Scavenging activity (%) = $(A_{517\text{control}} - A_{517\text{sample}}) / A_{\text{control}} \times 100$

RESULTS AND DISCUSSION

Chemical Composition of Lemon and Orange Peels

Table 1 presents the chemical composition and carbohydrates fractions of lemon and orange peels. It was observed that the lemon peels contained crude fat (2.5%), crude fiber (15.4%) and protein (7.89%). Ash content of lemon peel in this study was (5.3%), while total carbohydrates were (68.91%) total soluble carbohydrates (15.00%). The values of orange peel were crude fat (2.75%), protein (5.26%) and fiber (9.9%). Ash content of orange peel in this study was (4.3%) while total carbohydrates (77.97%) and total soluble carbohydrates (18.60%). Therefore with the value of ash reported in this study, lemon and orange peels may be suitable for animal feeds as the values are comparable to the values for varieties of animal feed reported by Egyptian national standard. Fat promotes the absorption of fat soluble vitamins hence it is very important in diets and contain the highest amounts of energy (**Akinhanmi and Akintokun 2008**).

Studies of antioxidant activity of lemon and orange peels by DPPH are shown in Table 2, the different extracts, which is an important limitation when interpreting the role of hydrophilic antioxidants. DPPH is a stable organic free radical with an absorption band around 515-528 nm which usually used as a reagent to measure free radical scavenging activity of antioxidants. It is sensitive sufficient to detect active concerning lemon and orange peels samples dried by air oven and extracted with methanol, ethanol and acetone. The DPPH (%) was found lower than of lemon peels as they realized 56.81, 60.86 and 68.91%, respectively. Regarding methanol extract, the microwave dried lemon and orange peels was higher than ethanol and acetone extract of lemon and orange peels. Noticeably, there are significant differences in the results of lemon and orange peels samples in cases of the three used extract solvents. The solvent plays a necessary role in extraction of the plant constituents. Methanol and ethanol are the highest polar amongst the solvents. Therefore, they include high yield of phenolic compounds and highest antioxidant activity (% DPPH scavenging activity) if compared to other solvents extracts (**Hegazy and Ibrahim,**

2012). Regarding DPPH in control, samples of lemon and orange peels valued 80.93, 53.11, 69.02 and 102.39 mg. The different levels obtained from these assays may indicate a relative difference in the ability of antioxidant compounds in the extracts to quench aqueous peroxy radicals (**Thaipong *et al.*, 2006**).

Table 3 presents the determined molecular weight of two different commercial citrus pectin between 8×10^3 - 1×10^6 Da by HPSEC MALLS. They concluded that the determinations were not affected by the eluent composition and its flow rate. **Berth (1988)** determined the molecular weight of commercial citrus pectin (DE = 60% and AUAC = 70%) by gel permeation chromatography. She calculated the molecular weight of crude pectin at about 3×10^6 Da, and after purification by ultracentrifugation at 1.54×10^5 Da, and ion-exchange chromatography at 1.65×10^5 Da. The molecular weight is important physicochemical parameter for pectin gelling properties and absorption in the intestinal tract.

HPLC Monosaccharide Composition of Lemon and orange pectin

Monosaccharide compositions of dry lemon and orange peels samples (weight, %) are summarized in Table 4 and Fig. 2. Rich Glucose content was found in orange then galactose, mannose and arabinose (43.61, 24.86, 23.29 and 8.22%), respectively. These findings are in general agreement with those of **Koubala *et al.* (2008)**. The highest arabinose content was found in lemon peels then, galactose, and glucose (60.75, 27.16 and 12.4%), respectively as show in Table 5 and Fig. 3.

FT-IR and HPLC of lemon and orange peels

As illustrated by IR analysis in lemon peel is in the region from (1618:2049) cm^{-1} and from (3046:3232) cm^{-1} , there was a group of (COOH and CH_3) and pectin sugar was present in the hexagonal ring structure. The area showed (616) nm.as show in Table 6 and Fig. 4.

As explained by IR analysis in orange peel It is in the region (2050: 2359) and (3063: 3234) showed the presence of (COOH and CH_3) Pectin sugar is present in the hexagonal ring structure. The investigation showed that pectin sugar is presented in the hexagonal ring structure the area showed (615) nm. as show in Table 7 and Fig. 5.

Table 1. Chemical composition and carbohydrates fraction of lemon and orange peels

| Parameter | Lemon peels (% W/W) | Orange peels (% W/W) |
|-----------------------------|---------------------|----------------------|
| Protein | 7.89 | 5.26 |
| Fiber | 15.40 | 9.90 |
| Ash | 5.30 | 4.30 |
| Fat | 2.50 | 2.75 |
| Total carbohydrates | 68.91 | 77.79 |
| Total soluble carbohydrates | 15.00 | 18.60 |
| In soluble carbohydrates | 53.91 | 59.19 |
| Reducing sugar | 7.87 | 9.92 |
| Non-reducing sugar | 7.13 | 8.68 |

Table 2. Antioxidant activity investigation of pectin and different extracts of lemon and orange peels by DPPH technique

| Scavenging activity (%) DPPH | | | | | | | | |
|------------------------------|-------------|---------|---------|--------|--------------|---------|---------|--------|
| Solvent | Lemon peels | | | | Orange peels | | | |
| | Methanol | Ethanol | Acetone | Pectin | Methanol | Ethanol | Acetone | Pectin |
| Zero | 2.25 | 2.31 | 2.21 | 2.21 | 2.19 | 2.11 | 3.8 | 2.56 |
| 30 Min | 56.81 | 56.15 | 6.90 | 59.22 | 69.0 | 68.85 | 8.0 | 71.81 |
| 60 Min | 60.86 | 59.36 | 7.21 | 62.61 | 73.0 | 71.07 | 9.3 | 74.79 |
| 120 Min | 68.91 | 65.71 | 8.92 | 68.72 | 81.0 | 75.31 | 11.6 | 80.81 |

Table 3. Molecular weight of lemon and orange pectins

| Pectin | Molecular weight (Da.) | (%) |
|--------|------------------------|------|
| WELP* | 4.3×10^6 | 100 |
| WEOP** | 2.6×10^2 | 16.2 |

*WELP (water extract lemon pectin)

**WEOP (water extract orange pectin)

Table 4. HPLC of orange peels

| Reten. time (min) | Response | Amount (mg/g) | Amount (%) | Compound name |
|-------------------|----------|---------------|------------|---------------|
| 1.117 | 1834.892 | 0.000 | 0.0 | |
| 1.767 | 45.033 | 0.000 | 0.0 | |
| 2.608 | 1.110 | 1.980 | 8.22 | Arabinose |
| 3.167 | 4.088 | 5.609 | 23.29 | Mannose |
| 3.758 | 4.882 | 5.986 | 24.86 | Galactose |
| 4.183 | 7.046 | 10.502 | 43.61 | Glucose |
| 4.708 | 5.920 | 0.000 | 0.0 | |
| 5.192 | 6.611 | 0.000 | 0.0 | |
| 5.733 | 28.565 | 0.000 | 0.0 | |
| 7.400 | 15.506 | 0.000 | 0.0 | |
| Total | | 24.077 | 100.0 | |

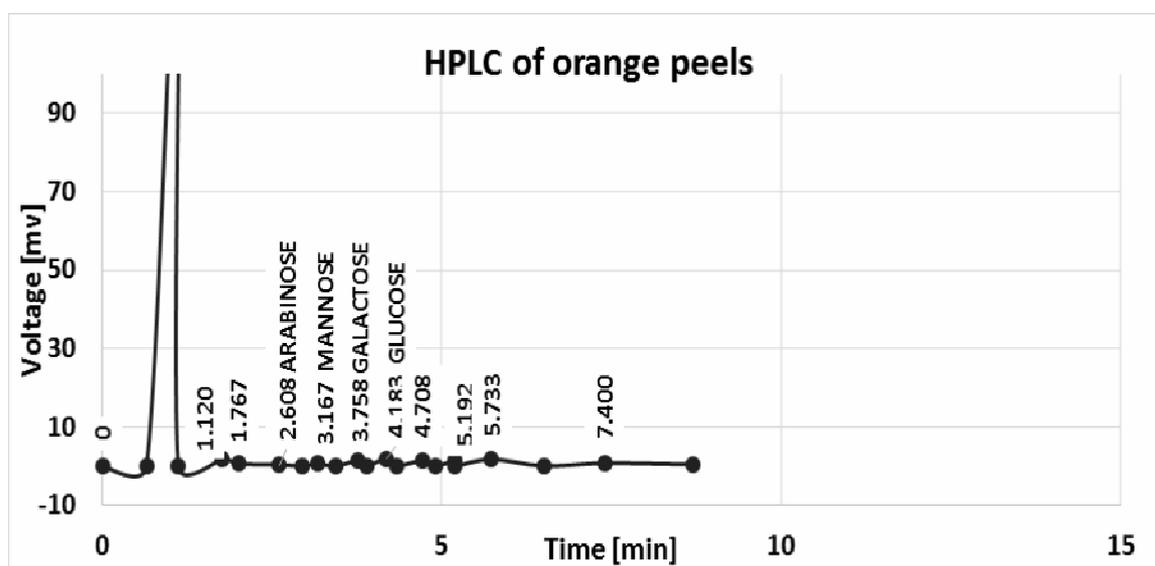


Fig. 2. HPLC of orange peels

Table 5. HPLC of lemon peels

| Reten. Time (min) | Response | Amoun (mg/g) | Amount (%) | Compound name |
|-------------------|----------|--------------|------------|---------------|
| 0.700 | 1.104 | 0.000 | 0.0 | |
| 0.942 | 4.134 | 0.000 | 0.0 | |
| 1.117 | 1830.876 | 0.000 | 0.0 | |
| 1.658 | 8.982 | 0.000 | 0.0 | |
| 2.167 | 47.641 | 0.000 | 0.0 | |
| 2.700 | 5.283 | 8.969 | 60.75 | Arabinose |
| 3.708 | 2.944 | 4.009 | 27.16 | Galactose |
| 4.033 | 1.258 | 1.785 | 12.04 | Glucose |
| 4.408 | 6.471 | 0.000 | 0.0 | |
| 4.925 | 2.467 | 0.000 | 0.0 | |
| 6.200 | 141.759 | 0.000 | 0.0 | |
| 10.850 | 68.108 | 0.000 | 0.0 | |
| Total | | 14.763 | 100.0 | |

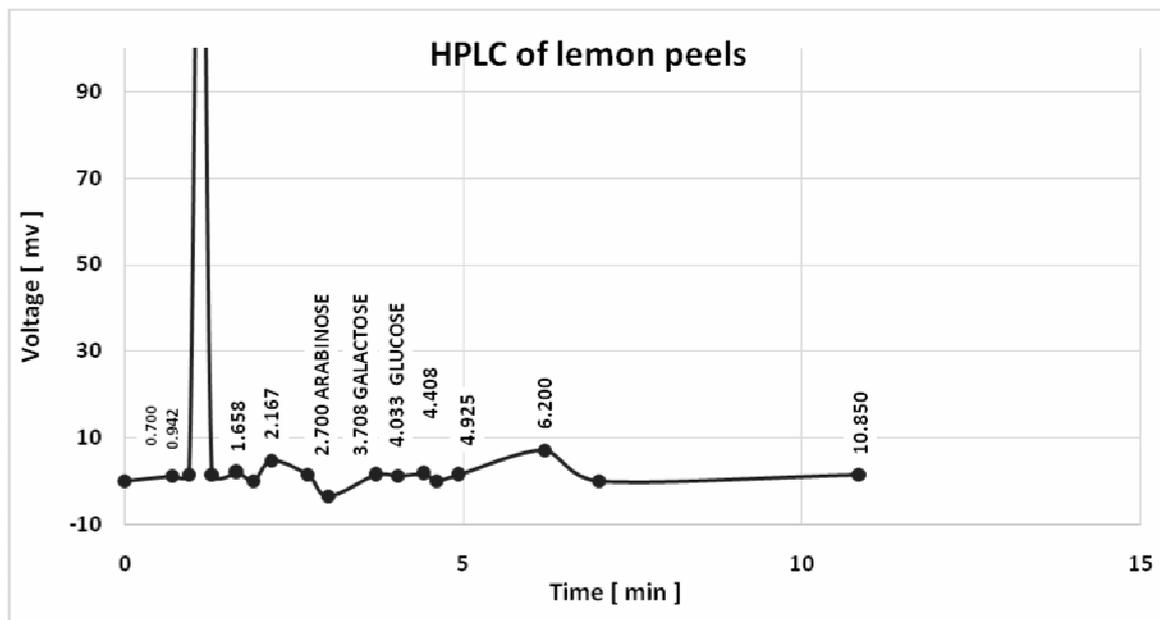


Fig. 3. HPLC of lemon peels

Table 6. IR of lemon peels

| No. | cm ⁻¹ | T (%) | NO | cm ⁻¹ | T (%) | NO | cm ⁻¹ | T (%) |
|-----|------------------|---------|----|------------------|---------|----|------------------|---------|
| 1 | 3232.97 | 58.7183 | 2 | 3046.01 | 64.4232 | 3 | 2049.96 | 92.1302 |
| 4 | 1618.95 | 81.8687 | 5 | 1414.53 | 49.9257 | 6 | 1095.37 | 37.9504 |
| 7 | 616.145 | 53.0946 | | | | | | |

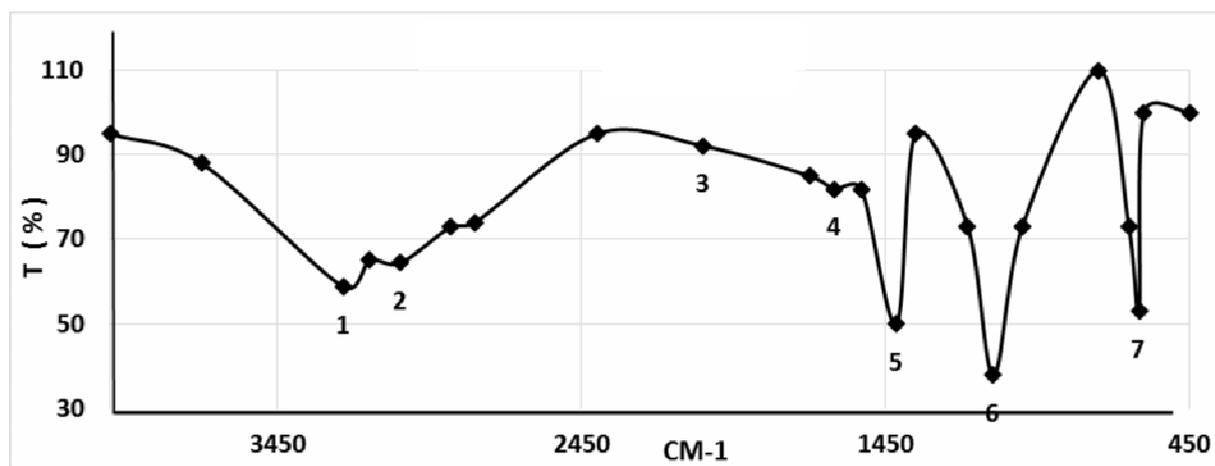


Fig. 4. IR of lemon peels

Table 7. IR of orange peels

| No. | cm ⁻¹ | T (%) | No. | cm ⁻¹ | T (%) | No. | cm ⁻¹ | T (%) |
|-----|------------------|---------|-----|------------------|---------|-----|------------------|---------|
| 1 | 3234.04 | 67.8863 | 2 | 3063.37 | 71.8383 | 3 | 2359.48 | 103.97 |
| 4 | 2050.92 | 95.4912 | 5 | 1614.13 | 82.5054 | 6 | 1407.78 | 62.2673 |
| 7 | 1101.15 | 54.4676 | 8 | 615.118 | 63.3696 | | | |

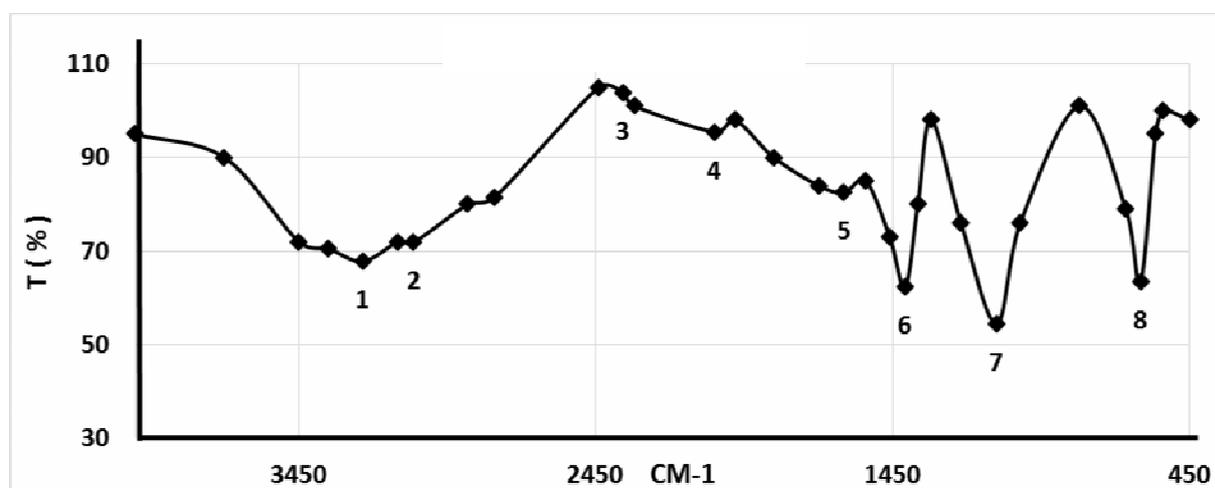


Fig. 5. IR of orange peels

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هذه الدراسة تمت على قشور الليمون والبرتقال عن طريق تحليل محتواهما من (الكربوهيدرات - البروتين - الدهون - الرماد - الألياف)، كما تم فصل وتنقية البكتين منهما ودراسة تركيباتهم باستخدام HPLC, IR كما تمت دراسة النشاط المضاد للاكسدة بواسطة البكتين ومستخلصات (الميثانول - الإيثانول - الأسيتون) لقشور الليمون والبرتقال، وأوضحت النتائج أن قشور الليمون تحتوى على البروتين (7.89%)، والرماد (5.3%)، والألياف (15.4%)، والدهون (2.5%)، وأن السكريات الأحادية والثنائية ومشتقاتها التي توجد في البكتين هي (أرا بينوز - جلاكتوز - جلوكوز) وذلك من خلال التحليل بواسطة HPLC، كما أوضح تحليل IR أنه يوجد في المنطقة (من 1619 إلى 2049 سم⁻¹ ومن 3046 إلى 3235 سم⁻¹) حيث تبين ان مشتقات هذه السكريات تحتوى على مجموعة COOH, CH₃ وأن سكر البكتين يتواجد في التركيب الحلقي السداسي كما أوضحت المنطقة 616 سم⁻¹ وأظهرت نتائج نشاط مضادات الأكسدة أن مستخلص (الميثانول لقشور الليمون) أعلى نشاطا في البكتين كمضاد للاكسدة، وفي قشور البرتقال وجد أن البروتين (5.26%)، والرماد (4.3%)، والألياف (9.9%)، والدهون (2.83%)، والسكريات الأحادية والثنائية ومشتقاتها التي توجد في البكتين هي (أربينوز - جلاكتوز - جلوكوز - مانوز) وذلك من خلال التحليل بـ HPLC، كما أوضح تحليل IR أنه يوجد في المنطقة (من 2050 إلى 2359 ومن 3036 إلى 3234) مما يدل على وجود مجموعة COOH, CH₃ وأن سكرات البكتين يتواجد في التركيب الحلقي السداسي كما أوضح الامتصاص في المنطقة 615 نانوميتر وأظهرت نتائج نشاط مضادات الأكسدة أن مستخلص (الميثانول لقشور البرتقال) أعلى من قيم البكتين كمضاد للاكسدة.

المحكمون:

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