Journal Tikrit Univ. For Agri. Sci. Vol. (18) No.(4) – 2018
ISSN-1813-1646

Extracting Carotenoids Pigments from Citrus Peel and Studying Their Functional Properties

Shahba Rafie Abdullah[1] and Rafid Khalil Abdul Razaq
Department of Food Science- college of Agriculture- Tikrit Uni.

Key Words:
pigments ,carotenoids, citrus peel, extracting.

Corresponding author:
Shahba R. Abdullah
E-mail:
shabaaa1992@gmail.com

Received: 13/9/2017
Accepted: 11/2/2018

ABSTRACT

This study was conducted in the graduate laboratories of the Department of Food Science at the College of Agriculture and the Chemistry Department at the College of Education for Pure Sciences at the University of Tikrit and included the extraction of carotenoids pigments from citrus peel (Turkish orange peel, Iraqi orange peel, Turkish Tangerine peel, Iraqi Tangerine peel , Iraqi Bitter orange peel). The study showed that the Turkish orange peel was characterized by moisture, ash, protein, fat and carbohydrates by 72.5%, 4.6%, 5.7%, 0.26% and 16.58%, respectively. While Iraqi orange peel in its carbohydrate content was 19.24%. The Turkish orange peel was the best in the extraction of dyes compared to the rest of the samples by carotenoids of 3.57 mg /L. The petroleum ether was distinguished on both distilled water and ethanol in extraction. The results showed. also the extraction ratio (10: 1) (weight: volume) at 30 ° C and 72 ° extraction time and only two extraction times, all IR, UV and HPLC techniques were used to diagnose dyes and identify substances in citrus peel extracts, and to use some chemical reagents to diagnose the active compounds found in these extracts and all samples studied. It was found that the retention percentage of the organic extract in dyes increased in the neutral and basal medium and increased the degradation of dyes in the acid medium. The highest retention rate of 98.57% was recorded at pH 7. The results showed that the use of natural antioxidant alpha-tocopherol was better than the use of industrial antioxidant BHT In the conservation of pigments, and showed the diagnosis of organic extracts, alcohol and water technology HPLC contain:

apo-B-cartene-10-ols, Dihdroxy epoxides, 10 -apo-B-cartene-10-als,(10−

apo-B-cartene-10-al, 8-apo-B-cartene-10-Ol, Zeaxanthin, B-cryptoxathin)8−

The use of HPLC showed that the organic extract is the highest in the percentage of for containing these pigments. Some chemical reagents showed that the three extracts (organic, water, alcoholic) were contained in the studied samples: (Flavonoids, steroids, phenols, resins, alkaloids, Saponins , tannins, volatile oils, Comarin and GIycosides).

استخلاص صبغات الكاروتينويدات من قشور الحمضيات ودراسة خواصها الوظيفية

شهباء رافع عبدالله ورافد خليل عبدالرزاق
قسم علوم الاغذية – كلية الزراعة– جامعة تكريت

الخلاصة

أجريت هذه الدراسة في مختبرات الدراسات العليا/ قسم علوم الأغذية في كلية الزراعة وقسم الكيمياء في كلية التربية للعلوم الصرفة في جامعة تكريت، وتضمنت استخلاص صبغات الكاروتينويدات من قشور الحمضيات (قشور البرتقال التركي، قشور البرتقال العراقي، قشور اللالنكي التركي، قشور اللالنكي العراقي، قشور النارنج العراقي) وبينت الدراسة تميز قشور البرتقال التركي في محتواه من الرطوبة والرماد والبروتين والدهن والكربوهيدرات بالنسب 72.5% و4.6% و5.7% و 0.26% و16.58 % على التوالي. في حين تميزت قشور البرتقال العراقي في محتواها من الكربوهيدرات بنسبة 19.24 % . و كانت قشور البرتقال التركي الأفضل في استخلاص الصبغات

الكلمات المفتاحية:
صبغة الكاروتينويدات، استخلاص، قشور الحمضيات.

للمراسلة:
شهباء رافع عبدالله
البريد الالكتروني:
shabaaa1992@gmail.com

الاستلام: 2017/9/13
القبول: 2018/2/11

[1] The research is based on a master's thesis for the first researcher.

مقارنة مع باقي العينات بنسبة كاروتينات بلغت 3.57 ملغم/لتر، وتميز الايثر البترولي على كل من الماء المقطر والإيثانول في الاستخلاص وأظهرت النتائج كذلك تمييز نسبة الاستخلاص (10:1) (وزن: حجم) عند درجة حرارة 30 °م ومدة استخلاص 72 ساعة و الاقتصار على مرتين من الاستخلاص،استخدمت جميع تقنيات ال IR و U.V و HPLC في تشخيص الصبغات وتحديد المواد الموجودة في مستخلصات قشور الحمضيات ، و استخدام بعض الكواشف الكيميائية في تشخيص المركبات الفعالة الموجودة في هذه المستخلصات ولجميع العينات المدروسة. ووجد أن نسبة احتفاظ المستخلص العضوي بالصبغات تزداد في الوسط المتعادل والقاعدي وزيادة الانحلال للصبغات في الوسط الحامضي وقد سجلت أعلى نسبة احتفاظ مقدارها 98.57% عند الرقم الهيدروجيني 7 ، كما أظهرت النتائج أن استخدام مضاد الأكسدة الطبيعي الألفا– توكوفيرول هو افضل من استخدام مضاد الأكسدة الصناعي BHT في المحافظة على الصبغات، واظهر تشخيص المستخلصات العضوية والكحولية والمائية بتقنية HPLC احتوائها على:
-apo-B-cartene-10- ,10 -apo-B-cartene-10-ols, Dihdroxy epoxides, 8-apo-B-cartene-10-al, 8-apo-B-cartene-10-Ol, Zeaxanthin , B-(10als cryptoxathin) تقنية HPLC فأظهرت ان المستخلص العضوي هو ألاعلى في نسبة احتوائه على هذه الصبغات، فأظهرت نتائج الكواشف الكيميائية احتواء المستخلصات الثلاثة (العضوية، المائية، الكحولية) في العينات المدروسة على: (التربينات ، الفلافونات ،الستيرويدات ،الفينولات ،الراتنجات ،القلويدات، الصابونيات ،التانينات ،الزيوت الطيارة ،الكومارينات ، الكلايكوسيدات).

Introduction:

The residues of manufacture of citrus impose a heavy problems on food factories and cause numerous environmental problems because it form(45 – 50%) of the weight of the original citrus and the peels form about 30% of the total weight (Shukla *et al.*, 2015).

The researchers found that peels have a better biologic activity than other parts of citrus and they are used in food products such as jelly as natural addition also used as antioxidant, in recent day the researchers increase their interest in natural sources of biologically active compounds and Functional foods. (Shukla *etal*.,2015,Sharma and Moon, 2009). Citrus fruit belongs to the Rutaceae family and consists of 140 species.

Many species include orange, tangerine, lemon, grapefruit, and other citrus (Kamal *et al.*, 2011).They are rich sources of ascorbic acid (vitamin C), other organic acids, and citrus are used primarily fresh In the concentrated juice industry and by-products of citrus fruits include peels , pulp and seeds are rich in dietary fiber, phenolic compounds, carotenoids, flavonoids, aromatic oils and others (Hue, 1999). Global production of citrus fruits reached about 82 million tons during 2009-2010. Orange production reached 50 million tons (US Department of Agriculture, 2010). 34% is used in juice production and the proportion of fruit crusts as secondary products is about 44% (Li, 2006). Peels are divided into epoicarp (internal), flavedo (External Colored Terminal) and albedo. The peels contain , phenols, amino acids, essential oils, carotenoids, pectin, flavonoids, sugars, vitamins and minerals and makes them protective against many diseases (Wang *et al*., 2014). Citrus peels have the most complex carotenoids compared with other fruits and are also characterized by high content of epoxides and flavonoids and constitute 70% of total carotenoids (Bailey and Curl, 1961). Extract from peels are used to treat diabetes, cardiovascular disease, stroke, ophthalmology, dermatitis and lung cancer. The reduction of clinical risk is reduced total cholesterol, triglyceride, high cholesterol and low density lipoprotein (LDL). Citrus peel is a major source of vitamin C, which is an organic acid dissolved in water and anti-oxidant. (Cioroi: 2007, Hacizevki ,2009). Oranges are the most productive citrus and 33% of the total production is processed artificially. This leads to the production of 15 million tons of secondary residues consisting of (peel - membrane - juice vesicles - seeds), a good source for the extraction of pigments such as carotenoids, pectin,

etc, and industrial treatments were very important to find new ways to benefit from waste and increase profit and disposal of environmental pollution and also useful as food additives and in medicine and pharmaceuticals (Verontica *et al*., 2008).
The purpose of the study was to extract natural dyes from citrus peel and to study their functional properties and their possible use as additives with certain foods **.**

Materials and methods:

Preparation of citrus peel :

The Iraqi and Turkish citrus were obtained from the local markets of Baghdad, Tikrit, Balad and Mikashifa, and were washed then peeled and dried at laboratory temperature (25 – 30° C). The dried peels were then grinded by the laboratory mill, then sifted with a 1 mm sieve to obtain homogeneous powder and then stored in sealed glass containers in a dry place until use according to Shukla *et al*., (2015).

Preparation of organic extract:

The organic extract was prepared according to Alwan (2017) by stirring 20 g of dried powder with 200 ml of petroleum ether (concentration of 40 – 60%) for 72 hours using the magnetic stirrer. The extraction solution was filtered using Whatman NO .1. filter paper and the filtrate was concentrated by rotary evaporator. The extract stored in dark containers in a cool dry place until use.

Preparation of the alcoholic extract:

The alcoholic extract was prepared according to the method of Alwan (2017) by mixing 20 gm of dried powder with 200 ml of ethanol (concentration of 95%) for 72 hours using the magnetic stirrer. The solution was filtered using Whatman NO .1. filter paper and the filtrate was concentrated by rotary evaporator, the extract stored in dark containers in a cool dry place until use.

Preparation of the water extract:

The water extract prepared according to Hernandezi *et al.*, (1994) by mixing dried 20 gm of powder with 200 ml distilled water for 72 hours using the magnetic stirrer, after that the solution was sprayed with several layers of medical gauze to remove plankton, and then the solution was filtered using Whatman NO .1. The excess solvent was disposed of by rotary evaporator, the extract was then stored in dark containers at a cool dry place until use.

Determination of optimal solvent:

Several solvents were used in the extraction of dyes (petroleum ether, distilled water and ethanol) and the weight of the resulting dye were calculated and the quality of pigments for the best sample were determined by HPLC device (Veronticaics *et al*., 2008).

Estimation of optimal temperature:

Different temperatures ranging from 10 up to 40° Cwere used in the extraction process, the solvent selected in the preceding paragraph was used , the quantities produced on these different temperature were then calculated to determine the optimum temperature.

Determination of optimal extraction time:

Different times ranged from 12-96 hours were used in the extracting process by the weight of the pigments extracted for each period were calculated using optimum condition. The extraction was carried out according to the circumstances specified previously.

Determination of optimal extraction ratio :

Carotene dyes were extracted from citrus peel using petroleum ether at 30 ° C for 72 hours and using ratios (1:5, 1:8,1:10) (weight / volume).

Determination of extraction times:

After Estimation of determining the optimal conditions, the carotenoids was extracted several times for the purpose of determining the optimum number of extraction process.

Determination of chemical and physical properties for citrus peels :

The content of moisture, fat, protein , ash, and total carbohydrate were determined according to AOAC(2000).

Diagnosis of carotenoids by (IR) Infra - red radiation :

The three extracts (organic extract, water extract and alcoholic extract) were identified by using IR according to jabamabiraj *et al*., (2015).

Diagnosis of carotenoids by Ultraviolet spectrum :

we used a wavelength of (360-460 nm) to investigate the presence of carotenoids in the extracts and then diagnose the carotenoids by HPLC according to Veronticas. *et al* (2008).

Diagnosis of carotenoids by High Pressure Liquid Chromatography(HPLC) :

The content of carotenoids in the organic, water and alcoholic extracts of orange peel was determined by HPLC by injecting (3 mL) of extract into the column of the device whose mobile phase of acetone and water consists of (9: 1) (volume : volume) and reading absorbance at wavelength 450 nm at 40 ° C and the sample passing rate of 1.5 ml / min in the instrument column and then separating and diagnosing the types of carotenoids and their concentration in each extract. The concentration of the dye was calculated as following law (Veronticaics *et al*., 2008).
Sample concentration (µm /mL) = sample area / standard area x standard concentration x dilution factor.

Diagnosis of carotenoids by chemical reagents :

The detection for Saponins, Phenoles, Resins, Flavones, Steroids, Terpenes, Glycosides, Tannins, Volatile oils, Alkaloids,and Comarines were carried out According to methods used by Shinata (1951), Harbone (1973), Cannell (1988), ALAbid (1958), Geismsan (1962), Dalali and AL- Hakim(1987) ,AL- Sheikhly *et al*. (1993).

Stability of carotenoids pigments evaluation:

Effect of PH Value: 1 g of powder extracted was taken from Turkish orange peel at 100% concentration. After a week of refrigerated storage at 4 ° C, Determined from PH (2-9) using HCl solution (0.1N) and NaOH (0.1N). The percentage of the retention of dyes was calculated by spectrophotometer at a wavelength of 450 nm from the extraction after 1 week of storage at 4 ° C. The percentage of pigments retention was calculated according to (Shukla *et al*,2015).

(% retentin =A2/A1 x 100)

A1: the value of absorption before exposure

A2: the value of absorption after exposure and storage for one week at a temperature of 4° C.

Effect of oxygen : 1 g of powder extracted was taken from Turkish orange peel at 100% concentration.It divided into three groups and studied the stability of the carotenoids, namely the control group and the organic extract powder group, which were treated with natural oxidation

agent (alpha-tocopherol), and the organic extract powder group with artifical antioxidant (BHT), And then the spectrophotometer was measured by a wavelength of 450nm. The previous operation was repeated for the three groups but subjected to nitrogen gas for 4 hours. and compare the results between them, according to (Shukla *et al*, 2015).

Results and Discussion:

Table (1) Main Ingredients of some Turkish and Iraqi citrus peel

Samples	Moisture %	Ash %	Protein %	Fat %	Carbohydrate %
orange peel Turkish	72.50	4.60	5.70	0.26	.1658
orange peel Iraqi	70.50	4.25	5.50	0.21	19.24
Tangerine peel Turkish	72.00	4.40	5.60	0.25	17.45
Tangerine peel Iraqi	71.40	4.10	5.30	0.22	18.69
Bitter orange peel Iraqi	71.85	4.35	5.20	0.23	18.07

Table (1) shows the chemical composition of different citrus peels. As noted the Turkish orange peel was higher in the percentage of moisture, ash, protein, and fat, compared to other peels. at 72.5%, 4.6%, 5.7%. 0.26% respectively compared to other scales, also noted superiority of Iraqi orange peel in the Carbohydrate of 19.24 % compared with other peels. The results close to those were reached by Almnai (2008), Ojha and others (2016), Asim and others (2015). the percentage of ash in the Turkish orange peel was 73.9%, the ash content was 4.4%, the protein content was 5.6%, the fat percentage was 0.28%, and the carbohydrate content in the orange peel was 19.78%.Differences may be attributed to several factors, including variety different environmental conditions, as well as the method of handling or preparing these peels.

optimal extraction peels types :

Five different types of different Turkish and Iraqi Peels were used for the purpose of determining the optimal type. Figure (1) shows the superiority of the Turkish orange peel by 3.57 mg / L, followed by the Turkish Tangerine peel with 3.05 mg /L, (3.00) mg / L, then the Iraqi orange peel by (2.97) mg /L, then the Iraqi Tangerine peel by (2.88) mg /L. These results are similar to those of Shukla *et al*., (2015), Wilaf *et al*., (2015), chairman (2016). Of carotene was 3.56 mg / L, 3.18 mg / L, 3.10 mg / L, 3.00 mg / L, 2.92 mg /L. In Turkish orange peel, Tangerine peel Turkish , Iraqi orange peel, Iraqi Bitter orange peel, Iraqi Tangerine peel, Respectively, and were lower in the studied samples because of the different method of preparation of peels and the method and conditions of extraction.

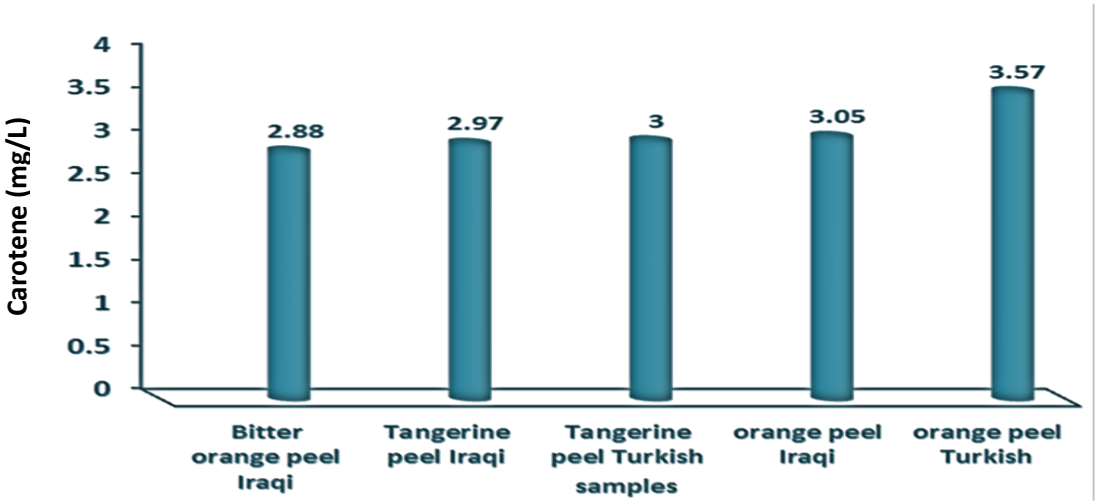


Figure (1) Percentage of carotene (mg /L) in different types of citrus peel at 30 ° C and 72 hours extraction

Determination of optimal solvent extraction:

Figure (2) shows the results of the extraction using several types of solvents. It pointed out that ether is better than distilled water and ethanol. the extraction percentage was 15.65 mg / L of petroleum ether and 6.81 mg / L for distilled water and 15.35 mg / L for ethanol. This difference is due to the polarity of solvents used for extraction. Results with Das and Bera (2013). indicated that the extraction ratio in petroleum ether ranging (4.28 ± 20.35) mg/L, and in distilled water the extraction rate has increased (5.21 ± 10.45) , and in ethanol the extraction rate has increased (5.11±20.29) mg/L in Turkish orange peel.

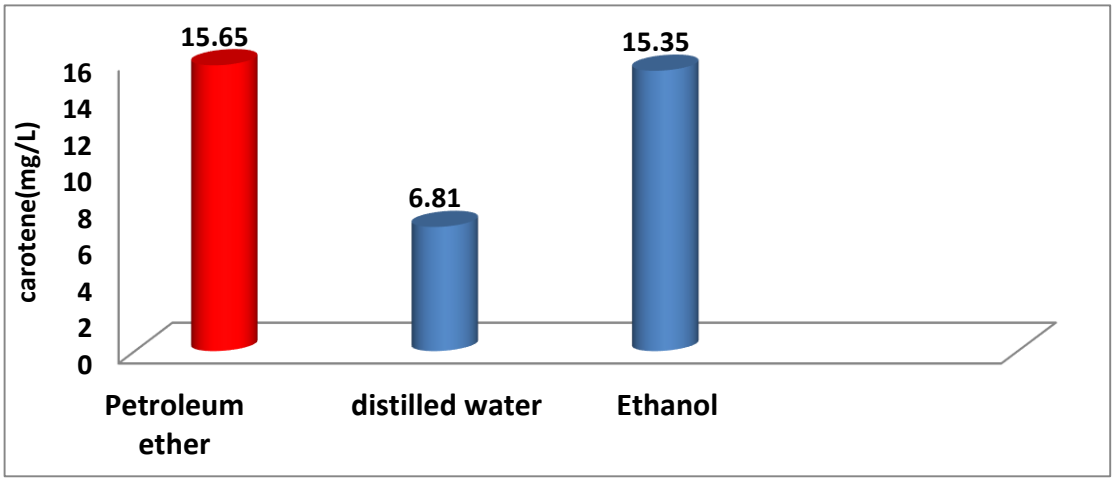


Figure (2) Effect of solvent type on pigment extraction from Turkish orange Peel

Determination of optimal extraction temperatures:

Figure (3) shows the effect of different temperatures on the extraction ratio of the carotenoids of the Turkish orange peel, where the high absorption is observed with high temperatures and the temperature of 30 ° C, as the optimal temperature in the extraction process. While the high temperature recovery from 40 ° C and exposure to light leads to the decay of carotene and this leads to the reduction of the effectiveness of the biologics and the breakdown of parts of the tissue, although the heat facilitates the arrival of the solvent to all parts of the fabric.

Aman *et al*., (2005). at low temperatures (30,20,10 ° C) the carotene extraction ratio was very low and then increased at 40 ° C with a carotene (1.92 mg / L). It is higher than carotene extracted from Turkish orange peel (1.78 mg / L). therefore, the optimal temperature of the extraction represents an important step in obtaining the largest amount of carotene with the least damage. **(**wang, 2006, schethinia , *et al*., 2013).

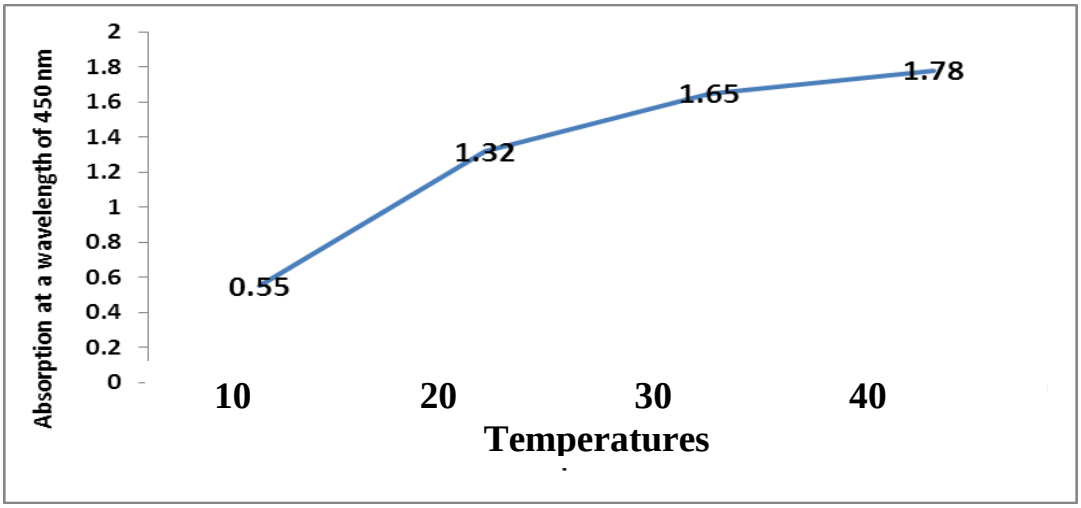


Figure (3) Effect of different temperatures on carotene from Turkish orange peel at 30 ° C and within 72 hours extracted by petroleum ether

Determination of optimal extraction time:

Figure (4) shows the effect of extracting time on the extraction ratio of the Turkish orange peel using petroleum ether. The optical density was measured along a wavelength of 450 nm for the extract and 72 hours were determined, which is optimal in extracting the highest percentage of carotenoids. As Calvo *et al*., (2007) point out, in increasing time, the beta-carotene is converted from all-trans to cis-isomers, causing changes in the composition of pigments and the appearance of new compounds that may be harmful to human health.

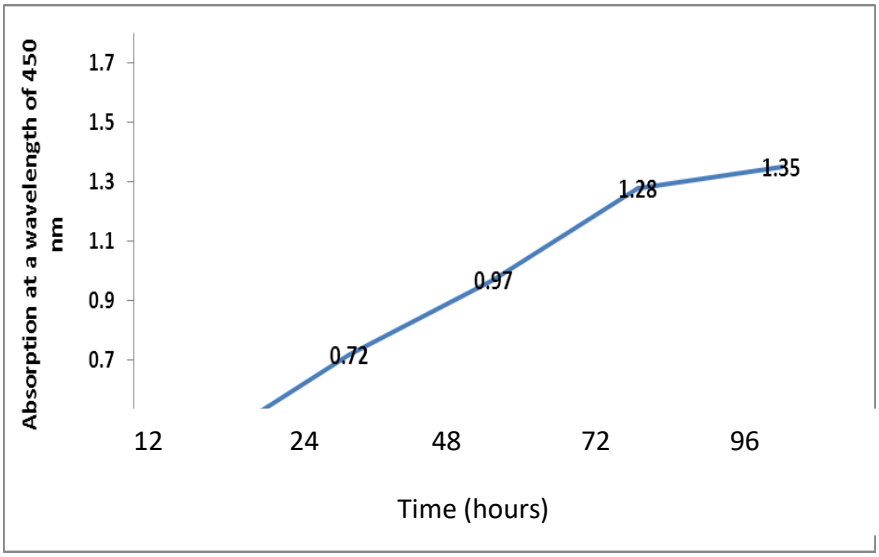


Figure (4) Effect of using different times in extraction from Turkish orange peel

Optimal extraction ratio :

Figure (5) shows the effect of extraction ratio (powder: solvent) on the efficiency of extracting carotenoids from Turkish orange peel. The figure shows that the ratio (5: 1) was 15.28 mg / L, as compared to 8: 1 (15.37 mg / L, compared to 10: 1) of 15.65 mg / L. The ratio of the solvent

gradually. Tan *et al.*, (2011), and the results agreed with Das and Bera (2013). pointed out that the ratio of extraction (5: 1) gave the ratio of (5.63 ±20.11) mg / L and the ratio of extraction (8: 1) gave the ratio of (4.88 ±20.71) mg / L, and the extraction ratio (10: 1) gave the ratio of (5.46± 20.82)mg / L in the best sample of Turkish orange peel.

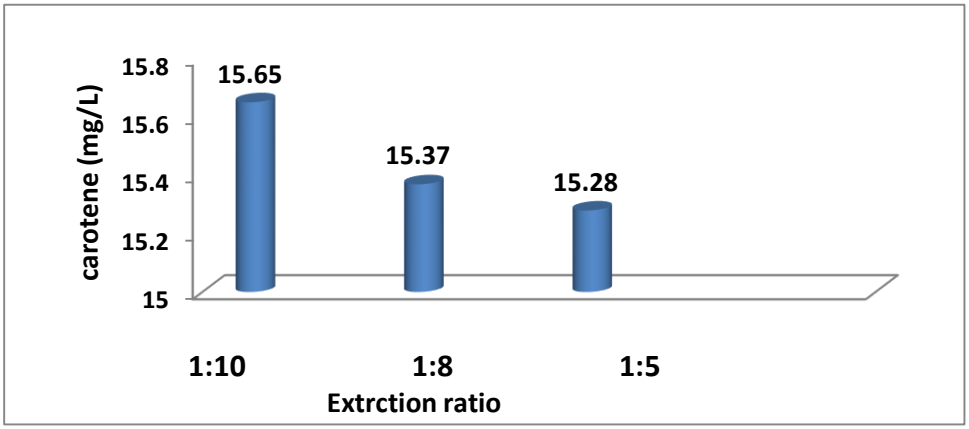


Figure (5) Effect of solvent extraction volume in carotene ratio of Turkish orange peel at 30 ° C and 72 hours extraction

Determine the optimal extraction times:

Figure (6) shows the number of extraction times in the extraction efficiency of carotenoids, showing the high percentage of extraction by increasing the number of extraction times for three, where the percentage of (15.36) mg / L in the first phase of extraction and in the second phase gave the proportion of pigments (15.66) mg / In the third phase, the percentage of pigments was (15.94) mg / L. The results show that the difference is small between the first and second extraction stages. The results were agreed with Norshazila et al ., (2017). Which indicated that in the first phase the extraction rate was(4.63 ±17.64) mg / L, in the second phase it was(5.64 ±18.55) mg / L, and in the third phase was (4.88±20.49) mg / L. Therefore, only two times of extraction was economically significant, as it represented a reduction in both the amount of solvent and the time of extraction for the consumer.

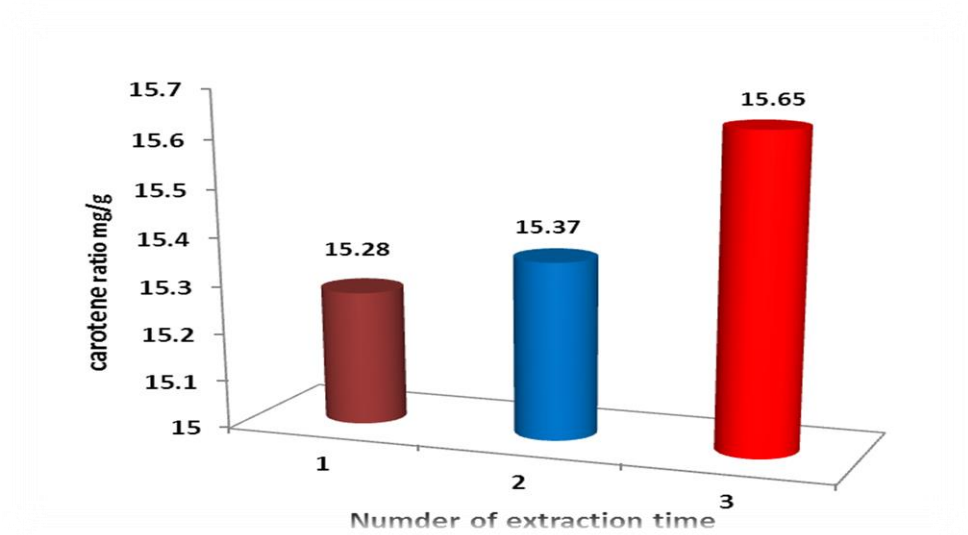


Figure (6) The effect of the number of extractions in the amount of carotenoids (mg /L) Using the petroleum ether at 30 ° C and within 72 hours extraction extracted from the Turkish orange peel

Detection of carotenoids by (IR)device :

Figures(7), (8), and (9) illustrate the FT-IR detection patterns using the IR spectra, the infrared has an important role in the detection and identification of the structure of organic molecules complex structure and give information only on the functional groups characteristic, using a very small sample which is a quick analysis of the diagnosis and ensure the presence of carotenoids.

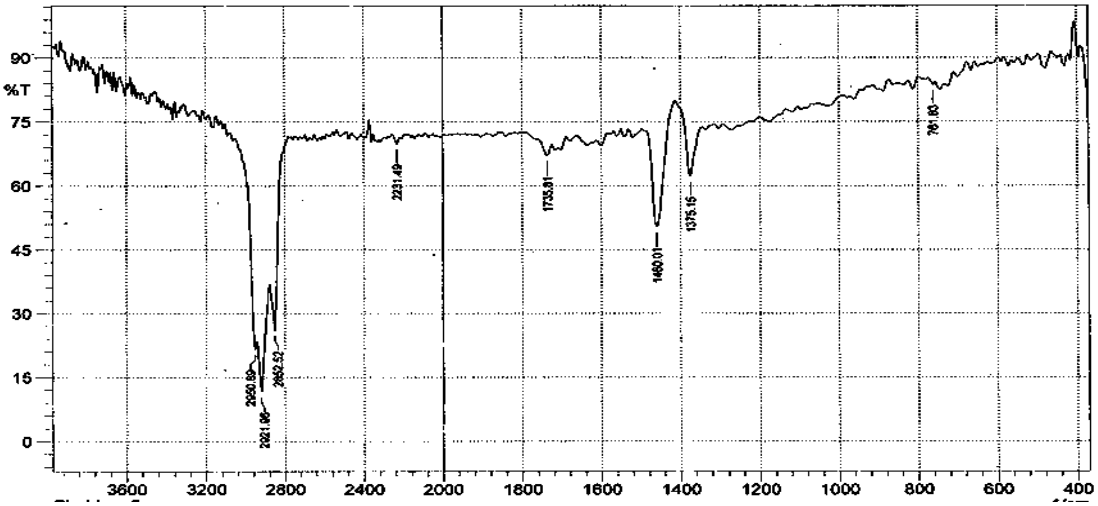


Figure (7) IR- Infra Organic of Turkish orange peel extract

The spectral chart in (400-4000)cm -1 for infrared of the organic extract showed that it contains several overlays of CH3 and CH2. The organic extract of the Turkish orange peel showed that in the package 761.83 the presence of abo -carotenoids and carotenoids and 1375.1 indicates that The main hydrocarbon series is(C-C) stretch, and in the package 1460.01 showed that it contains several overlays of CH3 and CH2. and in package (1735.81) contains C=O, C=C , and in the last packets indicate the presence of a group of (2950.89 − 2921.96 −2852.52 −2231.49) aromatic compounds with strong tensile strength in addition to the aldehyde group.

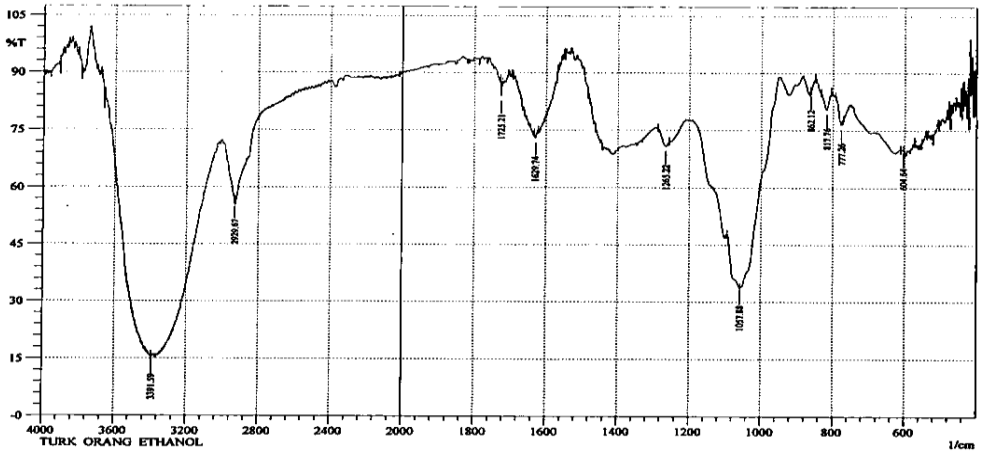


Figure (8) IR- Infra alcoholic of Turkish orange peel extract

Figure (8)showed the infrared spectral pattern of the alcoholic extract of the Turkish orange peel and the figure showed the complex nature of the compounds examined and its containment in beams (604.64-777.26) on abo-carotenoids and carotenoids in bulk and containing it in bundle on (C-C) In package (817.7 - 862.12) , and in package 1057.88 content group of nitro N-H stretch , and in package 1265.74 (C=C) ,and in package C=O, C=C (1629.74-1725.21) they are the main hydrocarbons and tensile compounds in pack (3391.59-2929.67) are aromatic compounds.

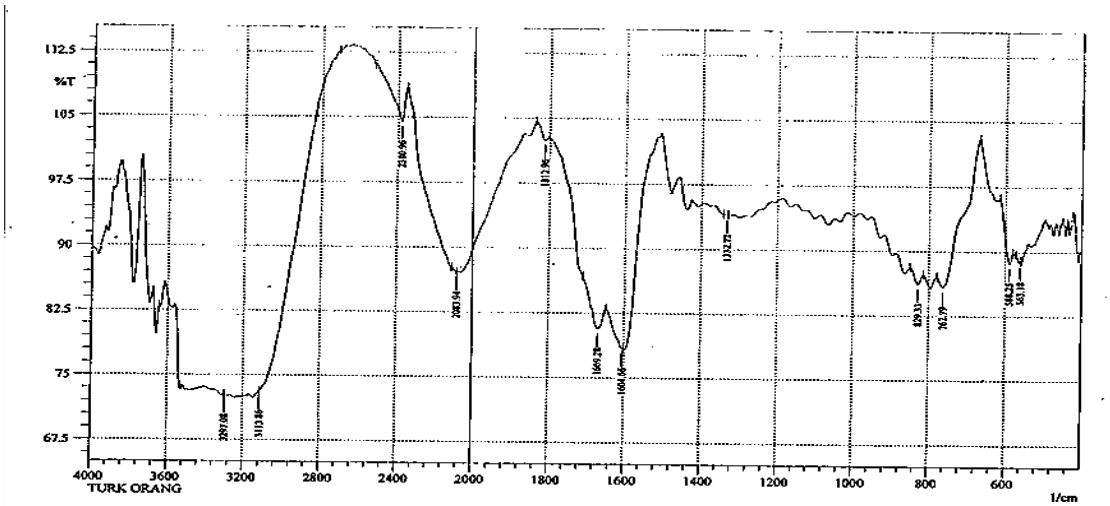


Figure (9) IR- Infra of water Turkish orange peel extract

Figure (9) shows the spectral pattern of the water extract of the Turkish orange peel, where a number of adsorption peaks are observed indicating the complex nature of the compounds in it. The large and intensive peaks indicate the absorption of the water molecules produced in the bonds, abo-carotenoids at 762.29 in C = O stretch and 829.33 in C= C in the two packages (563.18 - 588.25) and containing C=O, C=C, which are the main hydrocarbons in package(−1669.28− 1604.66− 1332.721812.96) and the aromatic aliphatic compounds in the packages (2380.96 - 3113.86 - 3297.08). According to Tomas *et al*.,(2001).

**Note that the results were compared with a standard model.

Detection of carotenoids by (HPLC)Technology in Turkish orange peel:

Table (2) HPLC analysis of Stander carotenoids for Turkish orange peel

Subject in Concentration 25 mg/ml	Retention time(min)	Area
10-apo-B-carotene-10-als	1.65	106244
10-apo-B-carotene-10-ols	2.48	124546
Dihdroxy epoxides	4.09	190244
8-apo-B-carotene-10-al	5.04	92136
8-apo-B-carotene10-Ol	5.96	141432
Zeaxanthin	6.75	93939
B-cryptoxathin	7.45	114676

Table (3) Diagnosis carotenoids organic extract in Turkish orange peel by device (HPLC)

PEAK	Time (min)	Area	Conc.
1	1.697	167923	10.4791
2	2.495	266401	16.6245
3	4.138	120574	7.5243
4	5.038	176685	11.0259
5	6.008	246572	15.3871
6	6.737	164192	10.2462
7	7.457	100585	6.2769

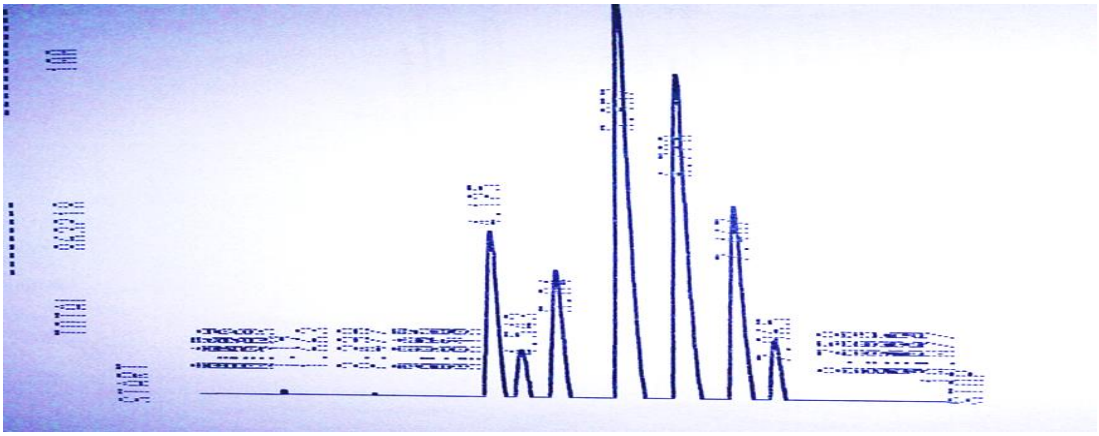
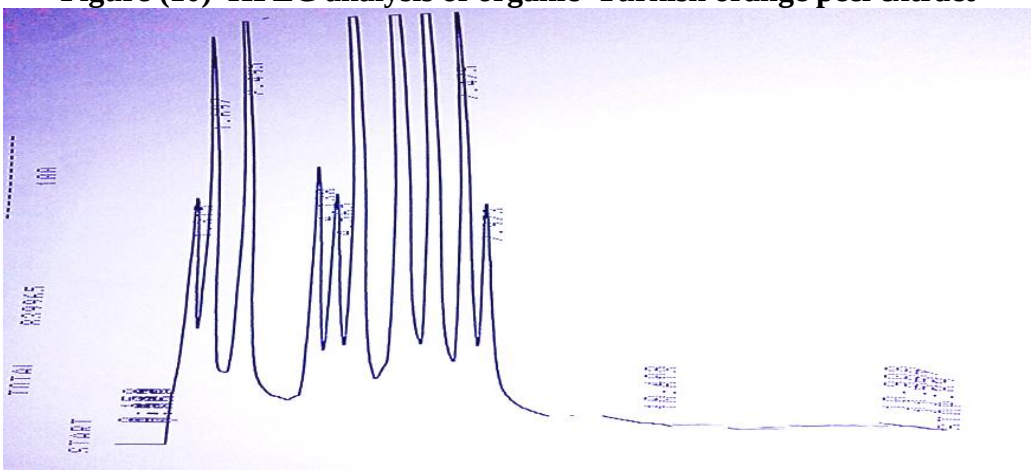


Figure (10) HPLC analysis of organic Turkish orange peel extract



Figure(11) HPLC analysis of alcoholic Turkish orange peel extract

The major pigments and peaks appearing in the alcoholic extract correspond to short-chain (abo-carotene)dyes at the time (1.69 and 2.49) min and concentrations (102.04-193.47). It is derived from beta-carotene (10 -apo - B - carotene - 10 - als , and 10 - apo - B - carotene -10 –ols) , which is (1,1dimethyl, 2 methyl, 2-cyclohexenal) also derivatives from beta-carotene is (8 - apo - B - carotene - 10 - al, 8 - apo- B-carotene - 10 – Ol), which is: (1,1 dimethy l, 2 methy, 3, prop – 2 cyclohexenel) and at time (5.03and 6.00) min .the vehicles respectively are concentrations(322.94-365.96) was also found in time(7.45, 4.13), and 6.73 min Concentrations respectively are (162.18-133.67 – 136.80), the following compounds are : Dihydroxy epoxides. Zeaxanthin, and B – cryptoxathin .

Table (4) Diagnosis carotenoids alcoholic extract in Turkish orange peel by device (HPLC)

PEAK	Time (min)	Area	Conc.
1	1.697	167923	5.1627
2	2.495	266401	11.4751
3	4.138	120574	12.1109
4	5.038	176685	16.0569
5	6.008	246572	21.7509
6	6.737	164192	7.2558
7	7.457	100585	7.4691

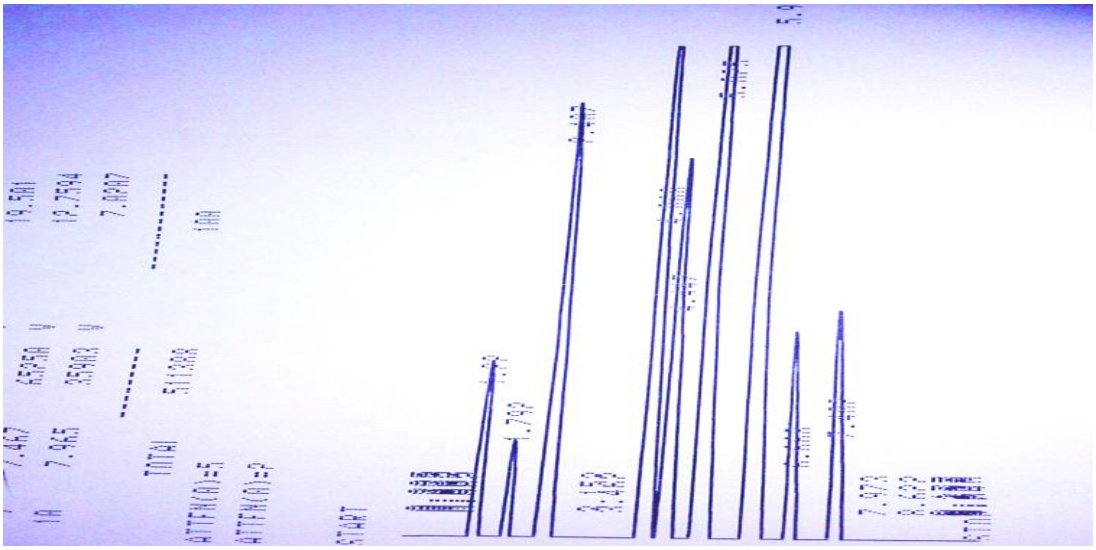


Figure (12) HPLC analysis of water Turkish orange peel extract

The main pigments in the water extract also correspond to short pigments(apo-carotene) dyes at the time (1.69 and 2.49) min and concentrations(15.93-14.37), It is derived from beta-carotene (10- apo-B-cartene-10-als and 10- apo-B-cartene-10-ols), which is (1,1 dimethyl, 2 methyl, 2-cyclohexenal) and also derived from beta-carotene (8- apo-B-carotene-10-al, 8-apo-B-carotene-10-ol) , and at time (6.00 and 5.03) min in the two concentrations (110.85 – 72.33) which is : (1,1dimethyl, 2 methy, 3, prop-2 cyclohexenel) .also found at time (6.73, 4.03 and 7.45) min and in concentrations (132.7- 36.6- 61.31). The following compounds are Dihydroxy epoxides Zeaxanthin and B-cryptoxathin .

Table (5) Diagnosis carotenoids water extract in Turkish orange peel by device (HPLC)

PEAK	Time (min)	Area	Conc.
1	1.697	167923	2.3887
2	2.495	266401	3.1041
3	4.138	120574	10.8944
4	5.038	176685	10.4262
5	6.008	246572	24.526
6	6.737	164192	19.501
7	7.457	100585	12.7594

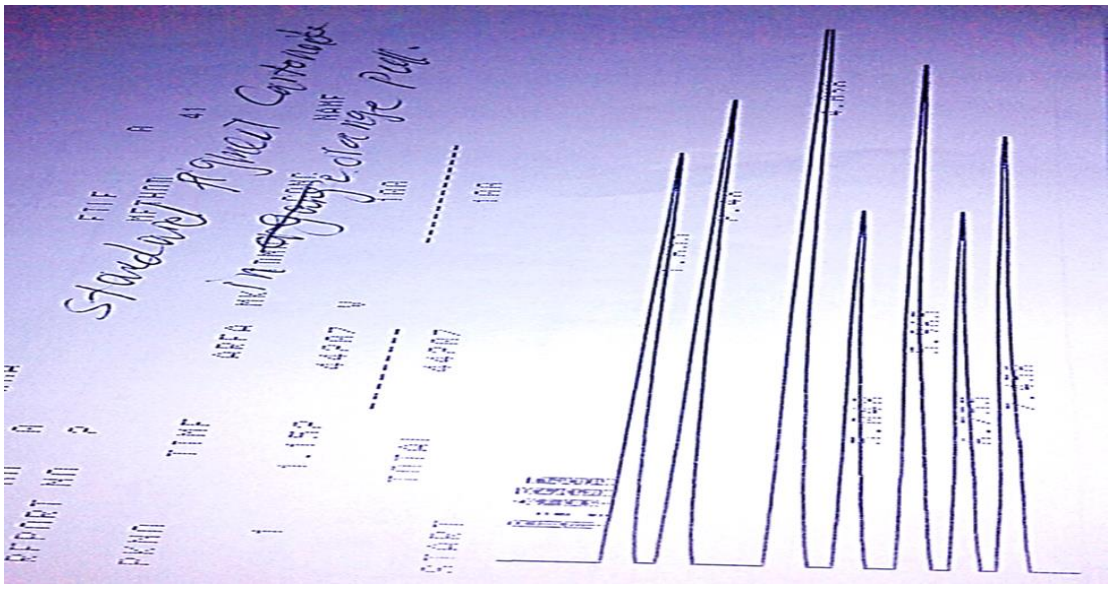


Figure (13) HPLC analysis Stander of Turkish orange peel

The dyes found in the organic, alcoholic and water extracts of Turkish orange peel were diagnosed with HPLC and Table (6) represented the proportion and types of carotenoids in the three extracts.

Table (6) Types of carotenoids and its presence in the three extracts of Turkish orange peel

Types of carotenoids	Alcoholic Extract mg/ml	Water Extract mg/ml	Organic Extract mg/ml
10- apo-B-carotene-10-als	102.04	14.37	395.13
10- apo-B-carotene-10_ols	193.47	15.93	534.74
Dihdroxy epoxides	133.67	36.6	158.44
8 -apo-B-carotene-10-al	365.96	72.33	479.41
8 -apo-B-carotene-10-Ol	322.94	110.85	435.84
Zeaxanthin	162.18	132.7	436.96
B-cryptoxathin	136.80	61.31	219.28

It is clear from the table (6) shows that the organic extract contains the highest concentration of these dyes compared to the water and alcoholic extracts and figures (10), (11), (12) and (13), and Tables 2,3,4 and 5, The carotenes of the three extracts show that the diagnosis of the organic extract of Turkish orange peel using by HPLC analysis.The main pigments and peaks appearing in the extract correspond to Short-chain (apo-carotene) dyes and beta-carotene derivatives(10-apo-B-carotene -10-als and 10-apo- B- carotene -10-ols), where at time (1.69 and 2.49)min. and concentrations (395.13 and 534.74).which is :(1.1, dimethyl,2 methyl, 2-cyclohexenal). and also derived from beta-carotene (8-apo-B-carotene-10-al, 8-apo-B-carotene-10-Ol) which is: (1,1, dimethyl, 2 methy, 3, prop-2 cyclohexenel) .and at time (5.03 and 6.00) min and in concentrations (479.41and435.84). It was also found that at time (6.73, 4.09 and 7.45) min and in concentrations (219.28, 158.44 and 436.96) the following compounds, Dihdroxy epoxides ,Zeaxanthin and B-cryptoxathin.

Active compounds in citrus peel :

Table (2) Detection of active compounds in Different citrus peel

Reagents	Samples	Organic Extract	Water Extract	Alcoholic Extract
1. flavones	orange peel (Turkish)	**(-)**	**(-)**	(+)
	orange peel(Iraqi)	**(-)**	**(-)**	(+)
	Tangerine peel(Turkish)	**(-)**	**(-)**	(+)
	Tangerine peel(Iraqi)	**(-)**	**(-)**	(+)
	Bitter orange peel(Iraqi)	**(-)**	**(-)**	(+)
2. Terpenes	orange peel (Turkish)	(+)	(+)	(+)
	orange peel(Iraqi)	(+)	(+)	(+)
	Tangerine peel(Turkish)	(−)	(−)	(+)
	Tangerine peel(Iraqi)	(−)	(−)	(+)
	Bitter orange peel(Iraqi)	(+)	(−)	(+)
3. Steroids	orange peel (Turkish)	(+)	(+)	(+)
	orange peel(Iraqi)	(+)	(+)	(+)
	Tangerine peel(Turkish)	(+)	(−)	(+)
	Tangerine peel(Iraqi)	(+)	(−)	(+)
	Bitter orange peel(Iraqi)	(+)	(+)	(+)
4. Phenoles	orange peel (Turkish)	(−)	(−)	(−)
	orange peel(Iraqi)	(−)	(+)	(−)
	Tangerine peel(Turkish)	(−)	(+)	(−)
	Tangerine peel(Iraqi)	(−)	(+)	(+)
	Bitter orange peel(Iraqi)	(−)	(+)	(+)
5. Resins	orange peel (Turkish)	(+)	(−)	(−)
	orange peel(Iraqi)	(+)	(+)	(−)
	Tangerine peel(Turkish)	(+)	(+)	(−)
	Tangerine peel(Iraqi)	(+)	(+)	(+)
	Bitter orange peel(Iraqi)	(+)	(+)	(+)
6. Alkaloids	orange peel (Turkish)	**(+)**	**(+)**	(−)
	orange peel(Iraqi)	**(+)**	**(+)**	(−)
	Tangerine peel(Turkish)	**(+)**	**(+)**	(−)
	Tangerine peel(Iraqi)	**(+)**	**(+)**	(−)
	Bitter orange peel(Iraqi)	**(+)**	**(+)**	(−)
	orange peel (Turkish)	(−)	(+)	(−)

Reagents	Samples	Organic Extract	Water Extract	Alcoholic Extract
7. Saponins	orange peel(Iraqi)	(−)	(+)	(−)
	Tangerine peel(Turkish)	(−)	(+)	(−)
	Tangerine peel(Iraqi)	(−)	(+)	(−)
	Bitter orange peel(Iraqi)	(−)	(+)	(−)
8. Tannins	orange peel (Turkish)	(−)	(+)	(−)
	orange peel(Iraqi)	(−)	(+)	(−)
	Tangerine peel(Turkish)	(−)	(+)	(−)
	Tangerine peel(Iraqi)	(−)	(+)	(−)
	Bitter orange peel(Iraqi)	(−)	(+)	(−)
9. volatile oils	orange peel (Turkish)	(+)	(−)	(−)
	orange peel(Iraqi)	(+)	(−)	(−)
	Tangerine peel(Turkish)	(+)	(−)	(−)
	Tangerine peel(Iraqi)	(+)	(−)	(−)
	Bitter orange peel(Iraqi)	(+)	(−)	(−)
10. comarines	orange peel (Turkish)	(−)	(+)	(+)
	orange peel(Iraqi)	(−)	(+)	(+)
	Tangerine peel(Turkish)	(−)	(+)	(+)
	Tangerine peel(Iraqi)	(−)	(+)	(+)
	Bitter orange peel(Iraqi)	(−)	(+)	(+)
11. Glycosides	orange peel (Turkish)	(+)	(−)	(−)
	orange peel(Iraqi)	(+)	(−)	(−)
	Tangerine peel(Turkish)	(+)	(−)	(−)
	Tangerine peel(Iraqi)	(+)	(−)	(−)
	Bitter orange peel(Iraqi)	(+)	(−)	(−)

(+) Indicate the presence of active substances, and (-) indicate the absence of active substances

It noted from Table (2) shows that all samples contain flavones in the extract of alcoholic and there is no in the organic and water extracts and the containment of Turkish orange peel and Iraqi orange peel on the terpenes in the three extracts, while the table and the absence of terpenes in the organic and water extracts of Turkish tangerine peel, The containment of the organic and alcoholic extracts on the terpenes in the Iraqi Bitter orange peel. and the results showed that all samples in the organic extract are free of resins, the water extract of the Turkish orange peel was free of resins and the rest of the samples were contained in its water extracts and the Turkish orange peel and the Iraqi orange peel and the Turkish tangerine peel were not included in the alcoholic extract on the resins and contain the Iraqi tangerine peel on the resins in the alcoholic extract, and all samples were alkaloid-containing in the organic and water extracts and were not found in the alcoholic extract. also, all the samples contain the Saponins in the water extract only and not found in the organic extract and

alcoholic, and not containing the organic extract in all samples on the tannins and found only in the water extract and in all samples and contain all the samples also on the volatile oils in the organic extract only and not containing in the extract water and alcoholic, and contain all the samples on the comarines in the water extract and the extract of alcoholic and all samples free of the comarines in the organic extract and contain all the samples on the glycosides n the organic extract and not found in the extract of water and alcoholic and these results are consistent with Sharma *et al*., (2014) and Gwacham (2015).

Stability of the organic extract:

Table (9) Effect of pH on the stability of organic extract dyes from Turkish orange peel

PH value	% Keep	Dissolution%
2	54.47	45.53
3	55.17	44.83
4	71.42	28.58
5	82.41	17.59
6	90.66	9.34
7	98.57	1.43
8	86.95	13.05
9	78.40	21.6

The data in above table (9) shows that the increase in the value of pH gave an increase in the percentage of retention of pigments .As noted PH 7 gave the highest retention rate (98.57%) and the lowest percentage of dissolution in the extract and decreased retention rate reached to 54.47% of PH 2 and 78.40% of PH 9, the dissolution of pigments also decreased from 45.53 to 21.6% with increasing the PH. in neutral medium was keep the pigments and become dissolution in PH basal medium. This is consistent with what Point out by Shukla. *et al*., (2015).

Oxygen effect:

Table (10) effect of exposure to Oxygen on the stability of organic extracts of Turkish orange peel

Transactions	Exposure to air for 4 hours		Under nitrogen gas for 4 hours	
	Keep %	Dissolution%	Keep %	Dissolution%
1. (control)	92.26	7.74	93.56	6.44
2. (alpha-tocopherol)	97.17	2.83	97.6	2.4
3. (BHT)	97.15	2.85	97.52	2.48

The results table (10) shows that the use of the industrial antioxidant BHT had less retention 97.52% after exposure to nitrogen gas for 4 hours compared to the retention rate 97.6% by using the natural antioxidant alpha – tocopherol ,while the retention percentage of the control sample was 93.56% The results showed that the use of alpha-tocopherol was the best for the stability of pigments consistent with Shukla *et al*., (2015).

References :

AL- Sheikhly,C. L. ; Benavente, O. and Castillo, J.(1993). Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. J. Agric. Food Chem. 56, 6185–6205.

AL-Abid, S. R. (1958). Analysis of Steroids and Terpenes in Green Tea consumption and liver disease systematic review . Liver,Interational,28(7):990-996.

AL-Dalali, B. K. and Hakim, S. H. (1987). Food analysis. Directorate of Dar Al Kutub for Printing and Publishing. University of Al Mosul. The Republic of Iraq.

Al-Manei, W. (2008). Extraction of Carotenoids and their Use in Food Coloring. Master Thesis, Faculty of Agriculture, King Abdulaziz University.

Alwan,H.R. (2017). Study of the effect of the extracts of alcohol and water leaves Alas Myrtus communis and Potontials tomentilla on the growth of some bacterial species, Tikrit University Agricultural Journal, 17(2):1. ISSN-1813-1646.

Aman, A. ; Schiebera, R. and Carle, A. (2005). Effects of heating and illumination on trans-cis isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. Journal of Agricultural and Food Chemistry, 53: 9512–9518.

A.O.A.C. (2000) ,Official Methods of Analysis ,Association of Official Analytical Chemists, Washington, D.C., USA.

A.O.A.C. (2006), Official Methods of Analysis ,Association of Official Analytical Chemists, Arlington, VA., USA.

Asim, E. A. ; Qotbi, A. ; Seidavi, F. W. ; Edens, V. and Tufarelli, V. (2016). Selected Plasma Constituents of Broller Chickens Feed Different Levels of Dried Sweet Orange (Citrus Sinensis) Peels :Oxford university press:pp.1-4.

Bailey, and Curl,A.H. (1961). Dietary supplement use in the united states, J. Nutr141, 261–266.

Calvo, M. M. ; Dado, D. and Santa-Maria, G. (2007). Influence of extraction with ethanol or ethyl acetate on the yield of lycopene, β-carotene, phytoene and phytofluene from tomato peel powder. European Food Research and Technology, 224: 567–571.

Cannell, A. D. (1988). food chemistry .Ed.3ed U.S.A.

Chairman, G.W. (2016). Dose phine ozioma Eza Kwesili and Ngozi− Chinese medicinal food ingredients in China. Plant Food Hum Nutr 61(2):57–65.

Cioroi,D.A. (2007). Ingredients of Citrus peel and their effectiness in food.

Curl, and Balley, R.B. (1961). The carotenoids of navel oranges. J.

Das, S. and Bera, D. (2013). Mathematical model study on solvent extraction of carotene from carrot. International Journal of Research in Engineering and Technology 2: 343-349.

Geismsan, A. V. (1962). Study on the use of natural color in candy, conference and exhibition, In, Ismailia 149-153.

Gwacham, C. N. (2015). Photo chemical analysis Using organic extract academy of Sciences 15(1);31-42.

Hacizevki,A.F. (2009). Chemical composition of some selected fruit peels.

Harborne, J. B. (1973), Plant secondary metabolites. J.Braz.Chem.S.,14:203−200 .

Hernandezi, C. K. D. ; Thomas, J. and paul, W. (1994). preparation of water extract for medicinal plants Medicinal Flora". Turk. J. Biol. Vol. 29, 41-47.

Hue,G.N. (1999). study on oxygenated constituents percentage existed in page mandarine peel oil during a special season. J Med Plant b; 4. (2):87-93.

jabamabiraj, A. ; Doraiaj, S. ; Yadav, S. A. and Bathrachalam, S. (2015). Diagnosis of carotenoids by IR Infrared radiation .Med (Baghdad), 44(2): 326 – 333 .

Kamal, D. V. ; Ronald, A. M. ; Hill, C. D. and Klaassen, D. C. (2011). Volatile constituents of Vietnamese pummelo, orange, tangerine and lime peel oils. Flavour Fragr J 17:169-174.

Kumar, S. p. ; Ahirwar, p. ; Man al shilpi, K. A. ; Shwakarma, V. and shaddha, P. G. (2014). Photo chemical analysis Using ethanolic extract .J of Ethanol pharmacology, 71: 365-376.

Li, H. K. and Luh, B. S. (2006). Effect of harvest maturity on carotenoids in pastes made from vf-145-7879 tomato,J,food sci,42;216-220 .

Norshazil, A. S. ; Koy, C. N. ; Rashidi, O. ; HO, L. H. ; Azrina, I. N. ; Zaizuliana, R.A. and Zarinah, (2017). The Effect of Time, Temperature and Solid to Solvent Ratio on Pumpkin Carotenoids Extracted Using Food Grade Solvents.

Ojha, R. ; Sofi, S. A ; Nadia, F. and Gulzar,A.H. (2016), Citrus peel as a source of functional ingredient: A review American Heart Associetion 42: 605-612 .

Schetinia, E. Anwar, F. ; Naseer, R. ; Bhanger, M. I. ; Ashraf, S. and Talpur, F. N.(2013). Effect of citrus fiber (albedo) incorporation in cooked pork sausages. In: Proceedings of International Food Technologists Annual Meeting. IFT Annual Meeting, New Orleans, Louisiana. 64 p.

Sharma, B. D. and moon,H.A. (2009). Photo chemical analysis Using ethanolic extract. medical journal, Vol. 46, No. 1, pp.

Shinata,A.R. (1951). Detection of Saponins and Resins in Medicinal plants.

Shukla, P. S. ; Gawande, S. S. and Thorat, A.V.(2015). Extraction, Identification and Utilization of Pigments Extracted from Citrus Wastes.

US Department of Agriculture,S.Z. (2010). Juice components of a new pigmented citrus hybrid Citrus sinensis (L.) Osbeck × Citrus clementinaHort. ex Tan. J Agric Food Chem 51(6):1611–6.

Verontica, S. C. ; Panaglotis, K. and Carmen, S. (2008). Two orange peel wastes (Valencia and Navel).

Wang,A.H. (2006). Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. Inter Food Res J; 18(4): 1275-1282.

Wang, L. J. ; Borradaile, N. M. ; Dreu, L. and Huff, M. W.(2014). Secretion of hepatocyte apo-B-carotene is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of Acat2 and Mtp. Res.;42:725–734.

wilaf, B. S. ; Anwar, F. ; Hussain, A. I. ; Sarri, N. and Ashraf, M. I. (2015). Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. Inter Food Res J; 18(4): 1275-1282.