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Conversion of Processed Citrus Wastes into Nutritional Components

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Abstract

Food processing wastes may impose heavy burden on factories and cause enormous environmental problems. Citrus wastes typically are about 45-50% of the weight of citrus original and the percentage of waste to 30-50% for vegetables and fruits in general. Natural color plays a significant role in determining the degree of consumer acceptance of the product. In addition, carotenoids (vitamin A precursor) have high nutritional values which are important for human nutrition. The efficiency of different organic solvents such as acetone 85%, hexane, petroleum ether, ethyl acetate and ethanol 90% in the extraction of pigments from citrus peel was studied. Ethyl acetate is the best solvent in extracting carotenoids from citrus peel, followed by ethanol 90%. HPLC was used to identify the extracted pigments and their components. The extracted natural pigments were mixed with different carriers such as starch, lactose, dextrin, Arabic gum, and it was noted that lactose is the best one, followed by starch compared with different tested carriers. We also found that Alpha-tocopherol was relatively more stable than butylated hydroxy toluene (BHT) antioxidant (an artificial compound). Natural extracted pigments were used in food product (e.g. jelly) evaluations and gave the better values for the color, flavor and taste compared to commercial samples with artificial additives.

Keywords: Citrus wastes; Carotenoids; HPLC; Organic solvents; Artificial additives

Introduction

Orange is commonly consumed in world. About 50% of the processed oranges turned out in the form of orange peels. It is almost consumed as fresh, on the other hand, some factories are using sour orange (*Citrus aurantium*) in processing in juice and jam. However the outer layer usually called, flavedo in comparison with the inner layer of the peels usually called albedo contains considerable amounts of the natural carotenoids. Such pigments are widely utilized as natural colorants in foods [1-7]. The color is one of the most important factors affecting quality and palatability of the foods among different consumers [8]. Therefore, food quality and flavor are closely associated with color. So, liking or disliking a food is conditioned by its color, attractive foods are sought out as pleasure giving, while unattractive foods avoided as undesirable [9,10].

Francis [11] stated that carotenoids play a very important role as for protection of health against cancer, cardiovascular and eye disease and as antioxidant. Recently, natural colorants (Carotenoids) are preferred than synthetic coloring materials. Today a large number of industrially produced foods such as beverages, dairy products, confectionery margarine, pasta etc., which contain beta carotene as a colorant and as a nutrient. Colors may be added to foods for several reasons. Therefore, Natural colorants, (carotenoids) are widely used by most of the developed countries as natural colorants for foods instead of artificial ones which proved to have harmful effects on human health [8,12]. Many organic solvents were used by many investigation for the extraction of carotenoids from orange flavedo. Badr [10] suggested that caretoniod could be extracted with a mixture of isoproanol: petrolium ether. Ting and Hendrickson used acetone for extraction of carotenoids followed by hexane. Weissenberg et al. [13] reported that, diethyl ether and methanol were used sequentially as extraction solvents for carotenoid extraction from Sour orange.

Breithaupt [14] reported that, there is a wide variety of carotenoid analysis methods, but the most used are high pressure liquid chromatography (HPLC) and thin layer chromatography TLC. HPLC gives more detailed results, TLC is reliable, inexpensive, portable and readily carried out by nontechnical operators and, thus, often preferred. Francis and Isaksen [15] separated carotenoid of some different sources including paprika by thin-layer chromatography on silica layers using petroleum ether containing tert-butanal or tret-pentanol. They found that, this system gave chromatograms with improved separation of oxygenated carotenoids compared with acetone -petroleum ether system.

Hamed [16] demonstrated that, the natural carotenoids extracted from balady mango wastes were suspended on soluble starch as a good carrier in coloring water base food (e.g., ice cream or stick). Rizk et al. [17] studied the adsorption of concentrated yellow pigment (carotenoids from yellow carrot and pumpkin) on solid matrixes such as, lactose, dextrin, flour, starch and skim milk. They found that, the best carrier for adsorption of carrot carotenoids was starch followed by lactose, while lactose was the most effective adsorbent for carotenoids extracted from pumpkin followed by flour. Deli et al. and Kim and Min [18,19] investigated quantitatively changes in the carotenoid pigments of the *C. annuum* by HPLC. They reported that, in all of the chromatograms, 40 peaks were detected; 34 carotenoids were identified. The total carotenoid content of the ripe fruits was about 1.3g/100g of dry weight, of which capsanthin constituted 37%, zeaxanthin was 8%, cucurbitaxanthin was 7%, capsorubin constituted 3.2% and β-carotene accounted for 9%. They also found that, the remainder was composed of capsanthin 5,6-epoxide, capsanthin 3, 6-epoxide, 5, 6

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diepikarpoxanthin, violxanthin, antheraxanthin, β-cryptoxanthin, and several cis isomers and furanoid oxides.

Shea et al. [20] Investigated different factors affecting stability of carotenoids extracted from apricot processed wastes i.e pH value media , heat tolerance at different temperatures ranging from 0 to 170°C for 15 min. as well as the effect of oxidation and light by exposing the separated pigments to aeration and direct sun light. They found that, carotenoids were stable in alkaline solution pH value 9 and were not affected by high temperatures up to 100 °C for 15 min. They also observed that, carotenoids were very sensitive to light and oxygen.

Hanaa et al. [21] used the natural colors of carrots to improve the color of childrens' candy. Six treatments were investigated; three of them were prepared using fresh yellow carrot after blanching in water, blanching by steam and without blanching. Each sample of these three treatments was dried and grounded to a fine powder, which was added to the candy in 5%. The other three treatments were prepared using fresh red carrot, which was dried and grounded to a fine powder. Two of them were prepared by extracting the pigment from red carrot powder (10 grams /each) using 0.8 and 1.0% citric acid respectively. Meanwhile, the last treatment was prepared by adding 10 grams of powder to 500g candy. The candy samples analyzed directly after production and the organoleptic evaluation of different samples indicated that the yellow and red carrot could be good natural colorant for candy. Shea et al. [20] studied the suitability utilization of the separated natural pigments from apricot processed wastes in coloring cake (one of an important bakery products) in concentrations of 0.5, 1, 1.5 and 2 g /250g flour (without egg yolk) with one sample left without any addition of colorants (as control). The results of organoleptic evaluation indicated that the highest palatability of panelists for cake was achieved by increasing the concentration of the natural carotenoids. Chantaro and Chiewchan [22] separated antioxidant from carrot and beetroot pulp wastes and used for value addition in food formulations. This research was aimed to study the following main points:

1. Extraction, determination and identification of natural colorants from different citrus wastes (citrus peel)
2. Factors affecting the stability of extracted pigments.
3. Using specific carriers for the extracted pigments.
4. Study the possibility of using produced pigments in manufacturing of food product (Jelly).

Materials and Methods

Materials

Citrus waste (Citrus peels): Two species of citrus from local market in Al-Ahsa Government will use.

Sour orange (*Citrus aurantium*)

Grape fruit (*Citrus paradisi*)

Methods

Sample preparation: The outer layer called flavedo of two mature citrus varieties *i.e.* Sour orange and Grape fruit were prepared as method described by Askar and Treptow [23]. Each sample was divided into three parts; the first part was treated with 0.1% of α-tocopherol (w/w) as natural antioxidant. The second part was treated with 0.1% butylated hydroxy toluene (BHT, w/w) as artificial antioxidant. The third part was untreated sample (as control). The samples were dried in oven at 45°C for 72 hour and ground into fine powders in an electric grinder [13]. Three replicates per sample were taken for further analysis.

Extraction of natural pigments: Colorants were extracted from citrus samples according to the technique described by Askar and Treptow [23]. A known volume of some solvents (30 ml of each) (Acetone, petroleum ether, Hexane, Ethanol 90%, Ethyl acetate, Methylen chloride) was mixed with a known weight of dry samples (0.5 gm) in dark bottles and kept overnight and then filtered on paper whatman No.1. The filtrate was made up to a known volume (100 ml) by the same solvent. The absorbance was measured by spectrophotometer, model, Pharmacia LKB, NOVA SPEC II, at 440, 644 and 662 nm.

Identification of natural pigments: Carotenoid pigments extracted from citrus samples were identified by High Performance Liquid Chromatography (HPLC) apparatus (Hewlett Packard series 1050) according to the method reported by Hart and Scott [24].

Stability of pigments

Effect of pH: Degradation of carotenoids extracted from citrus samples caused by different pH values were measured according to the method described by Elbe et al. [25].

Effect of oxidation: Samples of the carotenoids pigment solution were divided into two groups, the first group was exposed to aeration for 4 hours, and the other was exposed to nitrogen gas for 4 h, then absorbance measurements were read by spectrophotometer.

The adsorption of concentrated pigments on solid supports: The collected extracts of carotenoids were concentrated by removal of solvent in a rotary vacuum evaporator at 40°C. The concentrated pigments were adsorbed to solid matrixes (starch, dextrin, flour, lactose and arabic gum) in different ratios (1:1, 1:2, 1:3 and 1:4 Carrier/pigment) and the mixtures were dried in oven at 40°C for 24 hours.

Jelly preparation: Jelly was prepared in the laboratory using the ingredients given in Table 1 as follows: sucrose was added to water with heating and stirring. Then 2% animal gelatin and 0.2% citric acid were added to sugar solution, citric acid was added on sugar weight basis. Mixture was stirred until complete dissolving and brought to boiling. After the complete dissolving of ingredients heating was stopped then investigated prepared yellow pigment were added by different ratios (0.0066, 0.0133, 0.0266%). Concerning to control Jelly it was purchased from local market in Alahsaa, Saudi Arabia in powder state and prepared as mentioned on package. Finally jellies were poured into glass and chilled for several hours until evaluation. The measurements were performed in triplicate, 20 hours after the Jelly preparation.

Organoleptic evaluation of jelly: Samples of jelly were subjected to organoleptic evaluation by panelists by a taste panel comprising of by a ten member panel at Department of Food and Nutrition Sciences, King Faisal University, Saudi Arabia according to Gadallah [26]. Panelists were asked to evaluate color (20), clarity (15), Flavor (10), taste (10), texture (15), Shape (10), and overall acceptability (20).

Ingredients	Weight (%)
Water	53.88
Sucrose	44.00
Gelatin	2.00
Citric acid	0.20
Synthetic flavor	0.02
Natural yellow pigment	by different ratios

Table 1: Formulation of jelly sample.

Statistical analysis of the experimental results

The results were analyzed by Two Way Anova by using Statistical 7.0 software (Stat Soft Inc., Tulsa, USA). Significant differences ($p<0.05$) were determined by Duncan's multiple range test.

Results and Discussion

Extraction of natural pigments

Carotenoid pigments content of Sour orange and Grape fruits are shown in Table 2. The tabulated results showed that, ethyl acetate was the most efficient solvent in extract of carotenoid pigments from Sour orange and Grape. In Sour orange samples the total carotenoids was 5.65, 4.63, 4.56, 4.45 and 3.22 mg/l for ethyl acetate, acetone 85%, petroleum ether, n-hexane and ethanol, while in Grape fruit samples, were 1.42, 1.26, 1.10, 0.62 and 0.61 mg/l for ethanol, respectively. These results are agreed with those reported by Zhou et al., Hamed and Schieber et al. [6,16,27].

In spite of the previous data, it could be concluded that, the higher extraction yield of carotenoid pigments in Sour orange referred to such samples could be considered as a good source of carotenoids comparing to grape fruit.

Determination and identification of carotenoids

The technique of HPLC has been widely applied to investigate the composition of carotenoids extracted with ethyl acetate method. HPLC is characterized by short analysis time, high resolution, good reproducibility, and little structural modification.

Data in Table 3 showed the carotenoid pigments composition of Sour orange and Grape fruits, which were separated based on their functional groups into nine fractions for the Sour orange and six fractions for Grap fruit. However, these results are approximately agreed with those obtained by Matus et al. and Rizk et al. [28,29].

Stability of pigments

The effect of pH value: The retention of carotenoids extracted from Sour orange and Grape fruit, after seven days of refrigeration at 4°C using different buffer solution with pH values ranged from 2 to 9 was calculated and the results are presented in Table 4. The tabulated results showed that, increasing in pH value increased retention of carotenoids (97.13%) until pH 7.0. However, the retention percentage was increased from 45.36 and 39.53 at pH 2.0 to 97.13 and 96.43 at pH 7.0 for Sour orange and Grape fruit, respectively. Then the retention percentage was decreased to 79.36 and 77.76 at pH 9.0 for the same samples, respectively. These results are in agreement with those obtained by Rizk et al. [29] who found that the highest retention rates were (95.98%) and (100%) for carotenoids extracted from yellow carrot and pumpkin , respectively at pH 7.0, then the retention rates were decreased by increasing in pH value.

Citrus peels	Solvents	Chlorophyll A	Chlorophyll B	Total carotenoids
Sour orange	Ethyl acetate	0.15	0.12	5.65
	Acetone 85%	0.15	0.12	4.63
	Petroleum ether	0.06	0.29	4.56
	n.hexane	0.11	0.03	4.45
	Ethanol 90%	0.02	0.31	3.22
Grape fruit	Ethyl acetate	0.08	0.03	1.42
	Acetone 85%	0.17	0.11	1.26
	Petroleum ether	0.23	0.02	1.10
	n.hexane	0.14	0.23	0.62
	Ethanol 90%	0.08	0.18	0.61

Table 2: Effect of some solvents on extraction of pigments (mg/L).

Type of Citrus peels	Identified carotenoids	%
Sour orange	Capsorubin	39.22
	Zeaxanthin	4.40
	Cryptoxanthin	5.53
	Unidentified	0.53
	Unidentified	0.71
	Lutein	3.76
	Canthaxanthin	5.20
	Capsanthin	34.88
	β-Carotene	5.20
Grape fruit	Antheraxanthin	4.75
	Violaxanthin	36.76
	Lutein	4.41
	Zeaxanthin	44.66
	Cryptoxanthin	3.75
	β-Carotene	5.30

Table 3: Identification of carotenoid pigments of Sour orange and Grape fruits.

pH value	Sour Orange Peels		Grape Fruit Peels	
	Retention%	Degradation%	Retention%	Degradation%
2	45.36	54.32	39.53	60.63
3	55.02	45.33	52.66	47.12
4	71.33	28.36	70.12	29.87
5	82.25	17.82	80.54	19.32
6	93.31	6.11	96.51	3.56
7	97.13	2.77	96.43	3.45
8	86.54	13.16	78.21	22.32
9	79.36	20.11	77.76	22.21

Table 4: The effect of pH value on the stability of carotenoids extracted from Sour orange and Grape fruit.

Citrus peels	Exposure to air for 4 h		Under nitrogen gas for 4h	
	Retained %	Degradation %	Retained %	Degradation %
Sour orange (Control)	90.36	9.44	99.88	0.02
Sour orange (α-tocopherol)	99.10	0.80	100	0.00
Sour orange (BHT)	98.87	0.13	100	0.00
Grape fruit (Control)	88.83	11.17	99.84	0.06
Grape fruit (α-tocopherol)	93.22	6.68	100	0.00
Grape fruit (BHT)	93.11	6.79	100	0.00

Table 5: Effect of oxygen on the stability of carotenoids extracted from Sour orange and Grape fruit.

The effect of oxygen on carotenoids stability: The results presented in Table 5 showed the effect of oxygen on stability of carotenoids extracted from Sour orange and Grape fruits treated with antioxidant (α-tocopherol as natural antioxidant and BHT as synthetic antioxidant) in solution of pH 7.0 after exposing to air for 4 h in comparison with that kept under nitrogen gas, then absorption spectra was measured.

Concerning the data of untreated samples showed in Table 5, the retention rate of carotenoid extracted from Sour orange was 90.36% followed 88.83% (Grape fruit) when these samples were exposed to air for 4 h. Meanwhile, this retention rate of Sour orange and Grape fruits carotenoids was 99.88 and 99.84% respectively, under nitrogen conditions. In other words, the degree of degradation in Sour orange carotenoids was lower than that of the Grape fruit; meanwhile there was no pronounced decrease in the content of these pigments when

kept under untreated conditions. These results could be confirmed with Khoo et al., Shea et al. and Matthaus [7,20,30], they reported that exposing natural carotenoids from apricot processed wastes to aeration for 4 h caused about 13.1% degradation in the content of these pigments.

Regarding to retention rate of carotenoids extracted from Sour orange treated with α-tocopherol and BHT, which presented in Table 5. The rate was increases from 90.46% (control sample) to 99.10% (α-tocopherol) and 98.87% (BHT), after exposing to air for 4 h. Meanwhile, this retained reached to 100% under nitrogen gas conditions for either α-tocopherol treatment or BHT treatment.

The retention rate of carotenoids extracted from Grape fruit treated with α-tocopherol and BHT presented in Table 5. After exposing to air for 4 h, it was increased from 88.93% (control) to 93.22% and 93.11% respectively. On the other hand, the retention rate of Grape fruit carotenoids recorded maximum value (100%) when nitrogen gas was used for both carotenoids treated with α-tocopherol or BHT. However, it can be concluded that α-tocopherol treatment had the highest desirable effect on the carotenoid stability followed by BHT treatment then control samples for Sour orange and Grape fruits. These results are approximately agreed with those obtained by Lin and Chen [31].

Adsorption of colorants: Trials were made to select a suitable carrier for coating the carotenoid to keep and store it in a way by which it could be easily used. The data obtained for the adsorption of carotenoids extracted from Sour orange as a natural food colorants on different carriers (lactose, starch, flour, dextrin and Arabic gum) at different ratios (1:1, 2:1, 3:1, 4:1 pigment: carrier w/w) are given in Table 6. These results indicated that, lactose adsorbent had the highest carotenoid concentrations (1650, 1940, 2260 and 2520 mg/100gm) at all studied ratios, respectively, followed by starch. Also, from the same table it could be noticed that Arabic gum had the lowest carotenoid concentrations at all studied ratios. Lactose was the most effective adsorbent coated carrier material for red colorant extracted from Sour orange followed by starch. These results are in agreement with those found by Kim and Min and Rizk et al. [19,17]. On the other hand, it could be observed from Table 6 that the adsorption of carotenoids extracted from Grape fruit recorded the maximum concentrations when starch was used as carrier, while the minimum concentrations were recorded with Arabic gum.

Generally, the obtained results proved that, the best carrier for yellow pigment extracted from Grape fruit was the starch, while the worst carrier was the Arabic gum. These results are in agreement with those obtained by Hamed (2000) who showed that, starch was best carrier for yellow pigments extracted from Orange peels.

Organoleptic evaluation: Sensory properties of jelly with different ratios (0.0066, 0.0133 and 0.0266%) of carotenoid pigments extracted from Sour orange peels were evaluated. Organoleptic characteristics of jelly samples were determined in respect to commercial jelly sample as control samples and the results were tabulated in Table 7. From the noticed data, it could be noticed that, addition of carotenoids by 0.0066% led to insignificant increase in recorded color value comparing to control sample while the second addition ratio had insignificant decrease in recorded color value comparing to control sample. On the other hand, only the third addition ratio had significant decrease in respect to control sample.

Concerning jelly taste results (Table 7), it could be observe that, only the first addition ratio of sour orange peels carotenoids had significant improvement in jelly taste comparing to control sample. Significant disorders in jelly shape was observed at all studied ratios comparing with control sample. However, the lowest disorder was recorded for jelly sample prepared by addition of 0.0066% sour orange peels carotenoids.

For instance, insignificant reduction in overall acceptability was achieved for jelly sample prepared with the first addition ratio, while the two other studied ratios led to decrease the recorded value significantly from 17.41 in control sample to 15.08 and 13.58 for the samples prepared by the second and third addition ratios, respectively. However the results obtained are agree with the results of Kang et al. [5].

Generally, from the previous results, it could be noticed that no significant in most sensory characteristics of jelly sample prepared only

Pigment/carrier (w/w)	Applied carriers				
	Lactose	Starch	Flour	Dextrin	Arabic gum
	Sour orange mg/100gm				
1:1	1650	960	856	794	82.28
2:1	1940	1258	880	894	127.76
3:1	2260	1696	1260	1350	435.25
4:1	2520	2092	1448	1478	623.9
	Grape fruit mg/100gm				
1:1	350	378	372	314	84.8
2:1	708	760	688	566	122.4
3:1	1000	1136	982	728	163.8
4:1	1244	1490	1336	936	195.2

Table 6: Color concentration (mg/100gm carrier) of extracted carotenoids from Sour orange and Grape fruits with different carriers at different ratios (w/w).

Treatments	Color (20)	Clarity (15)	Flavor (10)	Taste (10)	Texture (15)	Shape (10)	Over all acceptability (20)
1	17.66 a	13.12a	7.62a	7.45 a	12.70a	8.62a	17.41a
2	17.75a	11.62b	7.83a	8.00 bc	11.50b	7.90b	16.83a
3	16.95ab	11.04b	7.5a	7.7ab	10.70c	7.54c	15.08b
4	16.16b	9.66c	7.62a	7.16c	10.50c	6.66d	13.58c

Means in the same column with different letters are significantly different ($P < 0.05$)
1, Jelly + synthetic yellow color (control); 2, jelly + 0.0066 % carotenoids; 3, jelly + 0.0133 % carotenoids; 4, jelly + 0.0266 % carotenoids

Table 7: Sensory evaluation of prepared jelly by adding extracted carotenoids from Sour orange peels.

by addition of 0.0066% of sour orange peels pigments was accrued. On the other hand, there were no significant differences between synthetic colored sample and natural colored samples for taste, texture and bleeding, when was carried out sensory evaluation of glazing jelly products.

Conclusion

Natural colors (carotenoids) play very important role in determining the acceptability food for consumer. Furthermore, some components of the carotenoids are precursors of vitamin A which is very important in human nutrition and food colorants, beside its anticancer properties. However, From the above mentioned results it can be concluded that:

The higher extraction yield of carotenoid pigments from Sour orange referred to such samples could be considered as a good source of carotenoids comparing to Grape fruit. Ethyl acetate was the most efficient solvent in extracting natural pigments from Sour orange, while, that of Grape fruit were ethyl alcohol. The highest carotenoid extracted from Sour orange was 97.13 at pH 7.0, while, the highest for the carotenoid extracted from Grape fruit was 96.43. Using nitrogen gas had losses approximately in carotenoid content for all studied samples either treated or untreated with antioxidants. The Sour orange is the best source of carotenoids due to its very rich carotenoid content and heat tolerance followed by Grape fruit. The antioxidant treatments (either by a-tocopherol or BHT) were relatively enhanced the carotenoids stability, but the highest effect was observed for the Sour orange. Lactose was the best carrier for the dispersion of Sour orange carotenoid, while, starch was more effective adsorbent coated carriers for pigments extracted from Grape fruit. Regarding to the overall acceptability of jelly samples, it could be showed that, slight insignificant reduction in overall acceptability was achieved for jelly sample prepared with the first addition ratio 0.0066%. However, no significant in most sensory characteristics of jelly sample prepared only by addition of 0.0066% comparing to control.

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