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Research Article

Utilization of Bitter Orange Seeds as by-Product to Produce Natural Antioxidants

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Abstract

The present study is conducted to evaluate the antioxidative activity of Bitter Orange seeds extract on stability of rice bran oil. Ethanolic extract of Bitter Orange seeds was added to rice bran oil (200 and 300 ppm) as natural antioxidant compare with Tertiary Butylhydroxy Quinone (TBHQ) as synthetic one. Oxidative stability and thermal process for 8 hours at 180°C for treated rice bran oil with different antioxidants were estimated. Obtained results indicated that the total phenolic content being 51.94 mg of GAE/g in ethanolic extract of bitter orange seeds, while total flavonoids being 39.67mg RE/g in the same extract. Bitter orange seeds ethanolic extract DPPH activity was nearly to TBHQ. Identification and fractionation of phenolic compounds using HPLC cleared that ten phenolic compounds (371.53 and 11.25 mg/100g) were separated. E-vanillic the most abundant phenolic compound in Bitter Orange seeds extract was, while Hesperidin was the most abundant flavonoids compound in the same extract 929.91 mg/100 g. Results for thermal process of treated rice bran oil by Bitter orange seeds ethanolic extract at the concentration of 300 ppm were recorded the lowest values of hydrolysis, oxidation and rancidity parameter after 8 hours heating. So, addition of Bitter Orange seeds extract to rice bran oil showed a positive effect on the oxidative and thermal stabilities of such raw material and could be recommended as an alternative natural antioxidant from by – products in oil.

Keywords: Bitter Orange Seeds; Antioxidants Activity; Total Phenolic; Flavonods

Abbreviations

TBHQ: Tertiary Butylhydroxy Quinone; PV: Peroxide Value; DPPH: 2,2 Diphenyl1-Picrylhydrazyl; TBA: Thiobarbituric Acid Value; AV: Acid Value; BOS: Bitter Orange Seeds; FFA: Free Fatty Acids; RBO: Rice Bran Oil

Introduction

Citrus processing industry produces huge amount of agro-waste, mainly composed of peel, pulp and seeds. The citrus waste is composed of highly bioactive substances and phytochemicals, including essential oils ascorbic acid, sugars, carotenoids, flavonoids, dietary fiber, polyphenols, and a range of trace elements. Moreover, these functional foods play an important role in treating various disorders, including antidiabetic, anti-carcinogenic, anti-allergenic, anti-oxidative and anti-inflammatory [1].

Nowadays, the high volume of waste produced marks food industry. According to the recent research conducted by FAO, about 1.3 billion tons of food has been wasted worldwide per year, which represents one-third of the total food industry production [2]. The largest amount of loss is verified by fruits and vegetables, representing 0.5 billion tons. In developing countries, fruit and vegetable losses are severe at the agricultural stage but are mainly explained by the processing step, which accounts for 25% of losses. Due to the increasing production of food in the world with consequent increase of the production of waste, the importance of developing researches for its use is noticed [3].

The most abundant flavonoids in Citrus aurantium seeds are hesperidin, neohesperidin, naringin, and narirutin. Flavonoids were reported to be the major components (56%) in C. aurantium seeds at the mature stage, whereas phenolic acids were found at more moderate levels (22%), while Phenolic acids included gallic acid, vanillic acid, syringic acid, rosmarinic acid, p-coumaric acid, and trans-2-hydroxycinnamic acid [4].

Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones; thus, they are more readily acceptable to the modern consumers [5]. At high temperatures the formation of new compounds is very rapid, the oxygen pressure is reduced and the hydro-peroxides decompose rapidly and are practically absent above 150 ºC indicating that the decomposition of hydro-peroxides becomes faster than their formation [6].

In this study was conducted to investigate the antioxidative effectiveness of bitter orange seeds extract as natural antioxidant in compared with these of synthetic one namely TBHQ. In thermal stability of rice bran oil which treated with bitter orange seeds extract were studied.

Materials and Methods

Materials

Bitter orange seeds (Citrus aurantium seeds) were obtained from Best - Egyptian Canning factory in Aga city, Mansoura, Egypt. Rice Bran Oil was purchased From Hayber Market, 6th October City, Cairo, Egypt. Tertiary Butylhydroxy Quinone (TBHQ) was obtained from Arma Company for Oils at 10 th of Ramadan City, Cairo, Egypt.

All chemicals and reagents were purchased from El-Gomhouria Pharmaceutical Company, El- Mansoura City, El-Dakhaleia Governorate, Egypt.

Methods

Sample preparations

Preparation of bitter orange seeds powder

Seeds were washed using distilled water then dried in oven dryer at 45 ºC for 8 hours with air circulation dryer model (Officine specializzate, GARBUIO, Essiccatioi, TREVISO, ITALY). Dried materials were ground in a domestic mill (Braun, German). Then packaged in plastic air tight polyethylene until the extraction were performed.

Preparation of bitter orange seeds extract

Ethanolic extract was prepared according to the method described by [7].

Preparation of oil samples treated with different antioxidants

Rice Bran oil samples were treated with 200 and 300 ppm from bitter orange seeds extract in compared with those of TBHQ (200 ppm) as synthetic one (maximum legally permitted level) according to [8].

Evaluation of bioactive component

Determination of total phenolic compounds and total flavonoids

Folin-Ciocalteu method was used to estimate total phenolic compounds (as gallic acid equivalent) using standardized spectrophotometric according to [9] and Flavonoids were extracted and estimated by the method of [10] at Food Tech. Res. Institute, Agricultural Research Center, El-Giza, Egypt.).

Fractionation and identification of total phenolic compounds and total flavonoids

Phenolic and flavonoids compounds were determined using HPLC according to [11].

Determination of Antioxidants Activity

2,2 diphenyl1-picrylhydrazyl (DPPH %) assay was carried out according to the method of [12].

Thermal process

All rice bran oil samples were heated at 180 ºC for 0, 2,4,6 and 8 hours according to [13] in oven Model WT Binder then oil samples were refrigerated and storage at 5 ± 1ºC till further analysis were carried out.

The oxidative stability process

Rancimat test: The oxidative stability of all oil samples was determined by using automated Rancimat (Metrohm Ud. CH 9100 Herisau, Switzeland, model 679) [11] at Food Tech. Res. Institute, Agricultural Research Center, El-Giza, Egypt.

Physical and chemical properties of oil samples

Colour, viscosity, acid value (AV), free fatty acids (FFA%) and peroxide value (PV) were determined according to the methods described by [14]. Thiobarbituric acid (TBA) value was determined using spectrophotometer, model: SPECTROUV- VISAUTO, UV-2602 and absorbance was measured at 530nm. TBA value was expressed as mg/malonaldhyde/kg oil, according the method described by [14] Using the following equation

TBA =7.8 x O.D.

O.D. = Optical density at 530 nm

Results and Discussion

Radical scavenging activity (DDPH)of bitter orange seeds extracts

Radical scavenging activity were evaluated by DPPH technique which depend on donate hydrogen to free radical and inhibiting the propagation stage in lipids oxidation pathway. Radical scavenging activity of bitter orange seeds extracts depends greatly on the concentration of active compounds as resulted in Table 1.

Antioxidants / Parameters	TBHQ	Bitter Orange Seeds Extracts
Antioxidant activity %	89.45	80.18
Total phenolic Compounds (mg of GAE/g)	-	51.94
Total flavonoids (mg RE/g)	-	39.67

Table 1: Total flavonoids, Total phenolic compounds and radical scavenging activity (DPPH) % of bitter orange seeds extracts.

Phenolic and flavonoids compounds have several biological properties such as anti-oxidant properties and trapping free radical's properties. These compounds prevent or delay oxidative damage to fats and other important molecules and prevent cancer and coronary heart disease [15].

Results in table (1) showed the percentage of total phenolic content in bitter orange seeds extract which was 51.94 mg/g as gallic acid, while the antioxidant activity which was evaluated using the DPPH method reached to 80.18%.

These obtained results were in full agreement with those reported by [16] who stated that the percent of phenolic compounds from the plants depends on the nature of the solvent used in the extraction process of the plant. Also, there were a positive relationship between phenolic content of the examined extracts and their abilities as antioxidant factors.

Also, results in the same table showed that total flavonoids content of the extracts were expressed as rutin equivalents (RE) in milligram per gram dry extract. Results in table (1) showed that bitter orange seeds ethanolic extract was 39.67.

Total phenolic compounds of bitter orange seeds ethanolic extract were separated and identified by HPLC and the results were shown in figure 1, it could be noticed that ten phenolic compounds were separated. E-vanillic acid was the most abundant phenolic compound in bitter orange seeds extract (371.53 mg/100g) followed by Iso-ferulic (112.95 mg/100g) and Benzoic acid was (108.42 mg/100g). Moreover, Cinnamic, Ellagic and Vanillic were also detected in medium amounts. On the other hand, the Coumarin, Chlorogenic acid, Epicatechein and P.OH Benzoic acid were also detected in small amounts.

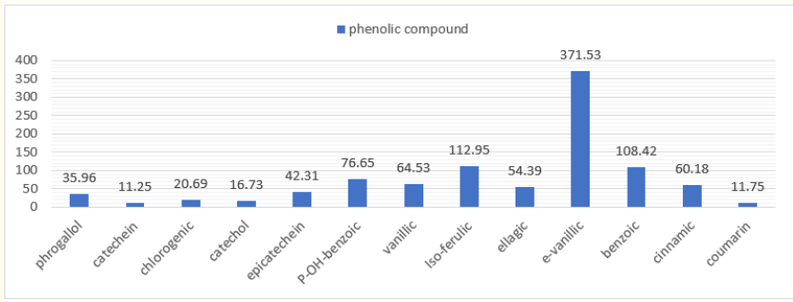

Figure 1: Fractionation and identification of some phenolic compounds in bitter orange seeds extract as mg/g.

Flavonoids were one of the most widely studied class of polyphenols with respect to their antioxidant and biological activities. They have powerful antioxidant activities *in vitro*, being able to scavenge a wide range of reactive oxygen species [17,18]. As recorded in figure (2), a total of 9 major flavonoids including flavanones, flavone and PMFs.

Among flavanones identified from the tested sample, Hesperidin was the most dominant flavanone followed by Naringin and Eriocitrin.

Figure 2: Fractionation and identification of some flavonoids in in bitter orange seeds extract as mg/100g.

The Hesperidin content was 929.91mg/100g. while Naringin content was 867.31mg/100g. Also, results in the same figure were shown that Eriocitrin content was 668.69mg/100g while Neohesperidin value was 562.77mg/100g.

In the same figure, it was resulted that the highest total of PMFs content was above 1000mg/100g. Among the 11 PMFs identified, nobiletin, tangeretin, and sinensetin were three dominating PMFs the highest levels of nobiletin 1137.05 mg/100g, tangeretin 536.75 mg/100g, sinensetin 297.99 mg/100g, while 5-OH-6,7,8,3′,4′-pentamethoxyflavone, iso-sinensetin and 5,6,7,4′-tetramethoxyflavone results were (211.38 mg/100g), (104.58 mg/100 g), and (105.40 mg/100 g) respectively.

Evaluation of stability process

The stability index (induction period) was defined as the point of maximum change of the rate of oxidation [19]. Both peroxide values and stability tested values by rancimat were used to given indication about the early stage of oil oxidation where hydro peroxides were formed [20].

Lipid oxidation is a degradation process considered to be a major cause of quality deterioration of spreadable fat products. It imparts rancid and unpleasant flavors to the products and thus decreases their organoleptic value. Hence, oxidative stability of shortenings, margarine and oils was a concern to bakers and snack food fryers. Therefore, the oxidative stability of lipid extracted from tested spreadable fats was determined immediately [21].

Oil samples	Control	Treated samples with BOS extract		TBHQ 200ppm
		200ppm	300ppm	
Induction period (Hour)	22.78	27.22	37.76	39.04

Table 2: Oxidative stability of rice bran oil treated with bitter orange seeds extract.

BOS: Bitter Orange Seeds

The rancimat time indicated that induction period of oil was 27.22 and 37.76 hours in oil samples treated with 200ppm and 300ppm of BOS extract. It was clear that addition of BOS extract with different concentration due increasing in induction period, which related to its active phenolic and flavonoid compounds composition.

Treated rice bran oil with BOS ethanolic extract at the concentration of 200 and 300 ppm showed the highest stability time in compare with control sample. These results could be to containing RBO of nutrient composition and also ethanolic extract could effectively dissolve the essential compounds. These results were nearly in agreement with [22].

Physical and Chemical properties of control and treated oil samples (with BOS extract)

The composition of vegetable oil samples and chemical and physical parameters have relationship and it has effect on their properties. Chemical and physical properties could be important to concurrence all treated samples for monitoring during exposure to high temperatures.

Colour is one of the main factors for determining quality of oils. Vegetable oils have minimum values of colour index are more suitable for edible purposes [23].

From data presented in table (3) it could be observed that the light yellow colour was in control sample, it is primarily due to B-carotene content, it was higher in treated samples (1.367 at 440 mm for treated sample with 200ppm and 1.095at 440 mm for treated sample with 300ppm) after 8 hours of heating.

This may be due to the effect of temperature which was used in different steps of heating. Results in table (3) were showed that control and treated sample with 200ppm (BOS extract) showed the highest viscosity values (0.620, and 0.600 P., respectively), while treated sample with 300 ppm (BOS extract) exerted lower value (0.491P.,) nearly to sample treated with TBHQ (0.480P) at the end of heating period. A similar trend was found by [24].

Oil properties	Heating time	Control sample	RBO treated with BOS extract		RBO treated with TBHQ (200ppm)
			200ppm	300ppm	
Physical properties	0	0.540	0.540	0.540	0.540
	2	0.667	0.578	0.555	0.548
Colour	4	0.801	0.705	0.634	0.591
	6	0.985	0.911	0.812	0.700
	8	1.610	1.367	1.095	1.000
Viscosity	0	0.312	0.312	0.312	0.312
	2	0.460	0.389	0.322	0.319
	4	0.488	0.422	0.368	0.337
	6	0.556	0.511	0.401	0.400
	8	0.620	0.600	0.491	0.480
Chemical properties	0	0.520	0.520	0.520	0.520
	2	0.533	0.525	0.520	0.520
Acid value	4	0.676	0.638	0.590	0.566
	6	0.731	0.715	0.610	0.587
	8	0.975	0.775	0.677	0.620
Free Fatty Acids%	0	0.330	0.330	0.330	0.330
	2	0.366	0.354	0.330	0.330
	4	0.574	0.354	0.342	0.336
	6	0.600	0.562	0.411	0.390
	8	0.635	0.580	0.498	0.465

Peroxide value	0	2.700	2.700	2.700	2.700
	2	3.011	2.843	2.711	2.700
	4	3.876	3.012	2.944	2.912
	6	5.098	3.432	3.021	3.011
	8	5.344	4.444	3.290	3.243
TBA	0	0.542	0.542	0.542	0.542
	2	0.603	0.557	0.551	0.542
	4	0.811	0.593	0.579	0.555
	6	0.888	0.617	0.600	0.584
	8	0.981	0.734	0.645	0.622

Table 3: Physical and Chemical properties of control and treated oil samples (with BOS extract) during heating 180°C.

RBO: Rice Bran Oil

It noticeable from chemical properties that acid value (AV) and free fatty acids (FFA %) value were used as a measure of the formation of acidic compounds and secondary products that were formed during oxidation [25].

Results in table (3) showed control rice bran oil sample exhibited the highest amount of acid value being 0.520, 0.533, 0.676,0.731 and 0.975 mg KOH/kg oil during all hours of thermal process, while the oil samples treated with synthetic antioxidant (TBHQ) showed the slowest. BOS antioxidant ethanolic extract with concentration (300ppm) had the highest antioxidant activity after 8h of thermal process at 180 °C. It was found that the BOS extract had the antioxidant activity which was significantly equal TBHQ in rice bran oil.

In the same table, it was showed that the changes in FFA% were in parallel with the changes in acid values. All treated oil samples with different antioxidants decreased gradually in compare with control one. Treated oil samples with BOS extract at the concentration of 200 and 300 ppm exhibited the lowest amount of FFA% in compare with control up to 8 hours of thermal treatment at 180°C. The changing rates in FFA% in compare with control oil sample after 8 hours of thermal process indicated addition of antioxidant at the concentration of 300 from BOS extract could lowering the change rate in FFA% to 0.498% followed by treated oil samples with BOS extract (200ppm) 0.580%.

Peroxide value (PV) used as an index for the early stage of oxidation process and degree of lipids oxidative rancidity and the formation of hydro-peroxides compounds [26]. Results in table (3) showed that, during the first 6 hours of thermal process at 180° C, PVs of the oil with added antioxidant (200 and 300ppm concentrations) were increased to 3.432, 3.021 ml. eqv./kg oil in compare to 5.098 ml .eqv/kg oil in control oil sample, the increase in PVs occurred more progressively throughout thermal process up to 8 hours. The values of peroxides reached 5.344in control oil sample, while in treated oil samples reached 4.444 and 3.290 ml. eqv./kg oil at the end of process.

Thiobarbutiric acid (TBA) test is considered the most common method of measurement of oxidative changes in food products and biological samples [27]. Determination of TBA value is more dependable than PV in examination of lipids deterioration as it is considered the secondary stages of oxidation or assemblage of secondary products [28].

From the same table (3), the changes in TBA values were increased in control sample more than which added antioxidants. It could be also observed that samples that were treated with BOS extract (200 and 300ppm concentration) had less values of TBA content and were more stable formation followed by the control one. It was showed that the treated sample by natural antioxidant with concentration 300ppm which was 0.645 mg malonaldhyde/kg oil is the nearest sample to that treated with TBHQ which was 0.622 mg malonaldhyde/kg oil after 8h.The increase in TBA throughout thermal process of control rice bran oil samples might be attributed to the formation of malonaldhyde products namely aldehydes and ketones and the free radicals from unsaturated fatty acid decomposition. These results are in agreement with those of [29].

Conclusion

In conclusion, Bitter Orange Seeds extract is the one effective natural antioxidant as by-product which Its use is economical and safe from a health point of view, as well as reducing the incidence of pollution. On other hand, adding it to rice bran oil showed a positive effect on the oxidative and thermal stabilities of such raw material and could be recommended as an alternative antioxidant in oil conservation.

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