FOOD PRESERVATIVE ACTIVITY OF PHENOLIC COMPOUNDS IN ORANGE PEEL EXTRACTS (*CITRUS SINENSIS L.*)

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Abstract

The objective of this study was to evaluate the preservative/antioxidant activity of orange peel extract in soybean oil after accelerated oxidation at 65˚C. Ethanol extracts of two varieties of Egyptian oranges (Baladi and Novel) were prepared, and their total phenolic and flavonoid contents, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were determined via standard colorimetric assays. Oil containing extract and butylated hydroxytoluene (BHT) or butylated hydroxylanisole (BHA) was stored at 65˚C for 7 days. Free fatty acid (FFA) content, Peroxide (POV), 2-Thiobarbituric acid (TBA), and P-anisidine (P-AV) values were determined every 24 hr. Total phenolic and flavonoid contents ranged from 559.3 to 591.8 mg tannic acid/100g peel and from 80.94 to 87.71 mg rutin/100g peel, respectively. DPPH scavenging activity was 72.33% and 65.05% for Baladi and Novel respectively. After accelerated oxidation, oil-containing extract showed significantly (P≤ 0.05) lower FFA (0.122, 0.120, 0.119, 0.117%), POV (35.18, 29.96, 20.77, 12.72 meq/kg), TBA (0.063, 0.061, 0.060, 0.055) and P-AV contents (6.31, 6.20, 5.97, 5.61) than the control (FFA 0.177%, POV 48.70 meq/kg, TBA 0.078, P-AV 10.65). BHT and BHA showed FFA contents of 0.129% and 0.134%, POV 34.36 and 35.46 meq/kg, TBA 0.066 and 0.650 and P-AV 7.24 and 7.39, respectively after 7 days. The inhibition of oil oxidation values (IO) were 32.01, 29.75, 30.32, 41.06, 59.92 and 76.45% for BHT, BHA, 400, 800, 1200, 1600 ppm extract, respectively. These results illustrate that orange peel extracts exhibit strong antioxidant activity. Therefore the use of these extracts in food is recommended to suppress lipid oxidation.

Key words: Orange peel; antioxidant; flavonoids; soy bean oil

INTRODUCTION

The world production of citrus is 122.09 million tons during 2008 [11]. Orange constitutes about 60% of the total citrus world production. In 2008, 3.23 million tons of citrus fruit was produced in Egypt contained 2.14 million tons of orange. A large portion of this production is addressed to the industrial extraction of citrus juice which leads to huge amounts of residues, including peel and segment membranes. Peels represent between 50 to 65%of total weight of the fruits and remain as the primary byproduct. If not processed further, it becomes west produce odor, soil pollution, harborage for insects and can give rise to serious environmental pollution [15], [16]. In Egypt and in many Mediterranean countries, major quantities of the peel are not further processed. Some attempts were made to use these residues as livestock feed, although their low nutritional value allowed only limited success [3]. Other applications included the extraction of pectin [8], [26], the recovery of essential oils, the production of clouding or thickening agents, and the removal of purification of carotenoids to obtain natural pigments suitable for food or juice coloring [2]. The antioxidant properties of plant extracts have been due to their polyphenol contents [4]. So plants containing high level of polyphenol have a great importance as natural antioxidants. The citrus peel and seeds are very rich in phenolic compounds, such as phenolic acids and flavonoids. The peels are richer in flavonoids than seeds [30]. Since a citrus fruit is peeled, peel and seeds are not used, it is necessary to

estimate these by-products as natural antioxidants in foods. Flavonoids of citrus have been show to be powerful antioxidant and free radical scavengers [4]. Synthetic antioxidant is used to suppress the development of rancidity in fat and oil. The synthetic antioxidant are know to have toxic and carcinogenic effects on human health [20]. Therefore, there is a strong need for effective antioxidant from natural sources as alternatives to prevent deterioration of fatty foods.

The objective of this study was to evaluate the preservative/antioxidant activity of orange peel extract in soybean oil after accelerated oxidation at 65˚C.

MATERIALS AND METHODS

Preparation of Extract

Orange fruit were washed and peeled with a sharp knife. Fresh peel were extracted with 95% ethanol at a ratio of 1part of peel: 2parts of 95% ethanol W/V using Waring Blendor, Model B-4, Waring products Co., Winsted CONN. The extraction was filtered through cheese cloth and the residue was re-extracted two times under the same conditions. The combined of filtrate for each sample was divided into two parts one part was treated with the same volume of hexane (three times) to remove its lipid content. All extracts were evaporated in a rotary evaporator below 40˚C and freeze dried in a freeze dry system (Model 77530-00, Labconco Corporation, Missouri). The extract obtained after freeze drying was used as natural antioxidant.

Proximate Composition of Orange Peel

Moisture, protein, fat and ash were determined according to [24].

Determination of Total Phenolic Content

A 35% saturated sodium carbonate solution was prepared by dissolving 35 g of anhydrous Na_2CO_3 in 100ml of pure water. Heat and stirring were added overnight in order to facilitate complete dissociation. Additional water was added to prevent super saturation. Once solution was prepared it was left in low heat during analysis to prevent precipitation as solution was used. Analysis was performed by adding 3.5ml of deionized water, 50µL of sample extract and Folin-Ciocalteu reagent and 300µL of sodium carbonate to cuvette. The reaction was left for 15 minutes and then the absorbance was measured in triplicate at 730nm using a UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan).

The blank consisted of all reagents excluding the sample extract. A standard curve was fashioned using Tannic acid at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0mglmL diluted in ethanol. Total phenolic concentration was expressed as mg of tannic acid equivalents via the standard curve [27].

Determination of Total Flavonoids Content

The total flavonoids content of orange peel extract was determined use a colorimetric method described by [31]. A 0.5 ml aliquot of appropriately diluted sample solution was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a 5% NaNO2 solution. After 6 min, 0.15 mL of a 10% AlCL3 solution was added and allowed to stand for 6 min, then 2 mL of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 mL, then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus a prepared water blank. Rutin was used as standard compound for the quantification of total flavonoids. All values were expressed as milligrams of rutin equiv per 100 gram of fresh peel. Data were reported as means (SD) for three replications.

Determination of Total Antioxidant Activity

Total antioxidant activity was determined following the method of [25]. A 100µM 2.2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dilution of 32mg of DPPH with 800mL of ethanol. 500µL of sample extract was added to 3.0mL of DPPH solution in a cuvette. After 10 minutes the absorbance of the reaction mixture was measured in

triplicate at 517nm in a spectrophotometer. The control solution was prepared by adding 500µL of ethanol to the DPPH solution and ethanol was used as blank. The antioxidant activity (%) was determined by the following formula:

Activity (%) = {(Abs $_{control}$ – Abs $_{sample}$) / Abs $_{control}$ } *x100*

Where Abs. is the absorbance at 517nm. A 1.4mg/mL quercetin dehydrate standard was prepared for comparison.

Application of Orange peel Extract to Soy Bean Oil

Refined soy oil, free of additives, was used as the substrate for oxidation studies. Soy bean oil samples (100mL) containing 400, 800, 1200 and 1600 ppm orange peel extract (ethanol) were separately prepared and placed in 250ml glass beakers. Synthetic antioxidants (BHT) and (BHA) were mixed in soy bean oil for a comparative study at their legal limit of 200 ppm. Control samples without antioxidant were also placed under identical conditions. Each beaker was covered with aluminum foil, placed into an electric oven and subjected to accelerated oxidation at 65°C ± 2 for 7 days. All oil samples of each treatment were prepared in triplicate. Oil samples were withdrawn every 24hr to assess the antioxidant activity of orange peel extract.

Antioxidant Activity Testing

Official methods [1] were used to determination of Free fatty acids (FFA) content (% oleic acid) (method Ca 5a-40), 2-thiobarbituric acid (TBA) (method Cd 19-90) , P-anisidine value (P-AV) (method Cd 18- 90) and peroxide value (POV) that can be used as indicator of the primary oxidation of oils was estimated (method cd8-53). The inhibition of oil oxidation (IO) was expressed as follows:

IO % = 100- (Peroxide value increase) $_{sample}$ / (Peroxide value increase) $_{control}$ x100 [19].

Statistical analysis

Data were analyzed with GLM (General Linear Model) program using statistical analysis system [22]. Mean values were compared by Duncan's Multiple Range Test. Differences were considered significant at ($P \leq 0.05$). All experiments were repeated three times

RESULTS

Proximate composition, total phenols and flavonoids of orange peel.

Novel orange peel has significantly ($P \leq$ 0.05) higher value of moisture and lower value of hexane extract than Baladi orange peel (Table 1). No significant differences were found in protein ash, and carbohydrate content of Baladi and Novel orange peel.

Total phenolic content of Baladi and Novel peel orange ranged from 559.32 ± 1.99 to 591.77 ± 2.20 mg/100g fresh peel (Tannic acid equivalents). No significant different was found in total phenolic content of Baladi and Novel orange peel (Table 2). Oil free extract of two varieties orange peel had higher total phenolic content than that of whole extract. The citrus peels are very rich in biologically active compounds such as flavonoids and phenolic acids [17], [28]. [18] reported that the total phenolic content of citrus peel (*Citrus reticulata*) was ranged from 13.4 ± 2.1 to 19.0 ±1.0 mg/g dried weight. Total flavonoids ranged from 80.93 ± 2.04 to 87.72 ± 2.16 mg/100g fresh peel rutin equivalents) (Table 2). Whole extract from Novel orange peel has higher total flavonoids content than that of Baladi. No significant different was found in total flavonoids contents .of oil free extract from Baladi and Novel orange peel. Total flavonoids of sour orange peel was 22.30mg/g dry matter [5]. In addition total flavonoid content ranged from 32.7 ± 1.06 to 49.2 ± 1.33 mg/g dried base (rutin equivalents) for eight varieties of citrus fruits peel [29].

Free radical scavenging activity

Free radical scavenging is the accepted mechanism for antioxidant to inhibit lipid oxidation. The method of scavenging stable DPPH free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts in short time [6]. In Figure 1 DPPH scavenging activities of

Baladi peel extracts were higher than those of Novel orange peel extracts. Oil free extracts for both varieties had DPPH scavenging activities ranged from 65 to 72%. The flavonoids compounds are powerful antioxidant against free radicals, because they act as "radical-scavengers". This activity is due to their hydrogen-donating ability. The phenol groups of flavonoids serve as a source of a readily available "H" atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure [7].

Table 1. Proximate composition of Baladi and Novel orange peel.

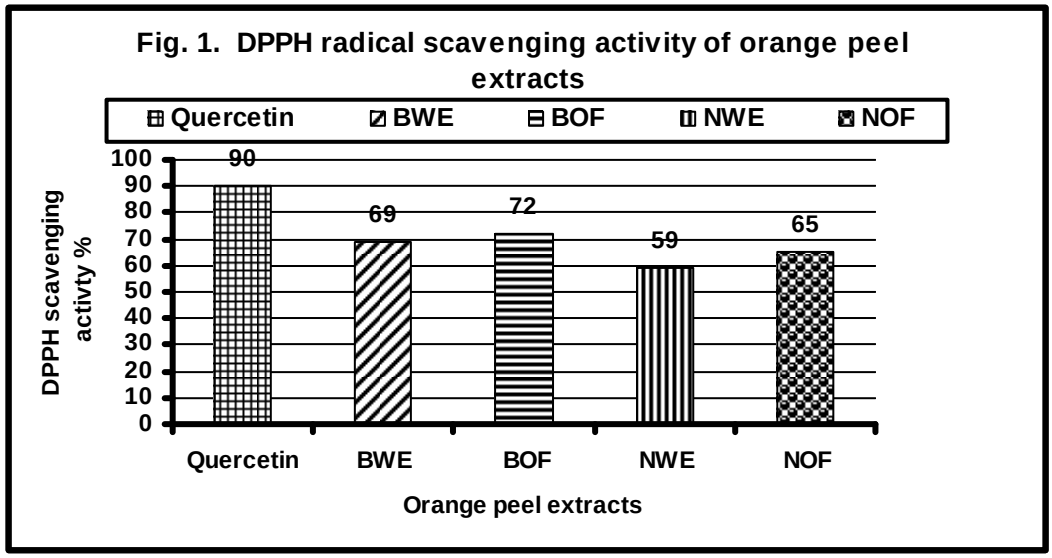
Orange peel	Moisture %	Protein %	Hexane extract %	Ash %	Carbohydrate* %
Baladi	71.87^{b} ±0.75	1.73^{a} ±0.05	2.26^{a} ±0.11	1.03^{a} ±0.02	23.04^{a} ±0.72
Novel	73.74^{a} ±0.32	1.83^{a} ±0.15	1.97^{b} ±0.06	0.88^{a} ±0.11	21.52^{a} ±0.60

a-b Mean values followed by different letters in the same column are significantly different ($P \leq 0.05$).
* Carbohydrate calculated by difference

Table 2. Total phenolic and flavonoids of Baladi and Novel orange peel extract.

Extracts	Total phenol mg Tannic acid/100g fresh peel	Total flavonoids mg Rutin/100g fresh peel
Baladi whole extract	559.32^{b} ±1.99	84.03^{b} ±1.02
Baladi extract oil free*	591.69^{a} ±1.97	80.93^{b} ±2.04
Novel whole extract	560.55^{b} ±2.40	87.72^{a} ±2.16
Novel extract oil free*	591.77^{a} ±2.20	83.49^{b} ±1.85

a-b Mean values followed by different letters in the same column are significantly different ($P \leq 0.05$).
* Extract was treated with hexane



BWE: Baladi whole extract BOF: Baladi oil free
NWE: Novel whole extract NOF: Novel oil free

Peroxide value, fatty acids, ansidine value and TBA during storage of soy bean oil at 65˚C

Production of free fatty acids and increases of peroxide values are the best predictors of fat deterioration, which could be used to monitor the extent of oil spoilage. The oxidation rate of soy bean oil was reduced by adding of orange peel extracts. Inhibition oxidation of orange peel extract increased with increasing its concentration in treated soybean oil (Fig. 2). Orange peel at 400ppm concentration had the same

inhibition oxidation of BHT and BHA. Orange peel extract (1200, and 1600 ppm) inhibited peroxidation of soy bean oil at 65˚C two times more than BHT and BHA (200 ppm). These results confirm the findings of [9] who observed that methanolic extracts of peanut hulls (used at 480 and 1200 ppm) inhibited peroxidation of both soy bean and peanut oils more efficiently than did BHA used at 200 ppm. Phenolic compounds are known to act as antioxidants not only due to their ability to donate hydrogen or electron but also attributed to their stable radical intermediates, which prevent the oxidation of various food ingredients particularly fatty acids and oil [14].

Development of rancidity in soy bean oil was affected by temperature and storage time. A gradual increase in peroxide value of treated soy bean oil was observed during storage for 7 day at 65˚C. The peroxide value of control sample increased from 1.25 to 21.51 after 4 days of storage at 65˚C. The peroxide values of orange peel extract (1200 and 1600 ppm) had significantly ($P \leq 0.05$) (higher inhibition of soy bean peroxidation than that of synthetic antioxidants (Table 3). These concentrations extended the induction period to reach a peroxide value of 20 meq/kg in soybean oil under tested conditions (65˚C) over 7 days and BHT and BHA 5 days. However, there was no distinct difference between synthetic antioxidants (BHT and BHA 200ppm) and orange peel extracts 400 and 800 ppm in inhibition of soy bean oil peroxidation. These results confirm the findings of [20] who found that methanolic extract of citrus peel exhibited very strong antioxidant activity, which was almost equal to synthetic antioxidant (BHT and BHA). The antioxidant activity of orange peel ultra-filtered molasses attributed to the presence of phenols, including numerous flavanones, flavone glycosides, polymethoxylated flavones, hydroxyl cinamates and other miscellaneous phenolic glycosides and amines [13].

Fig. 2. Inhibtion of soy bean oil oxidation by synthetic antioxidant and orange peel extracts

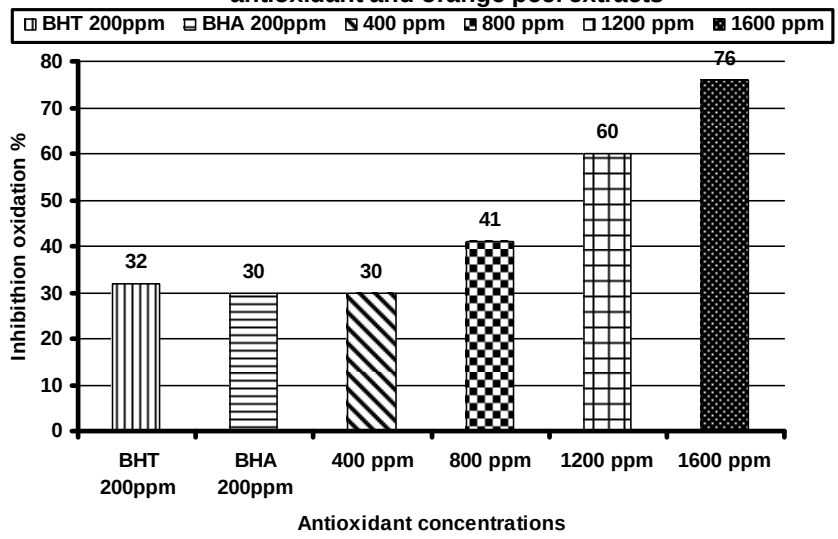


Table 3. Effect of synthetic antioxidant and orange peel extract on peroxide value (meq/kg) of soy bean oil stored at 65°C for 7 days

Storage day	Control	BHT 200 ppm	BHA 200 ppm	Orange peel extract			
				400 ppm	800 ppm	1200 ppm	1600 ppm
0	1.25[h]	1.25[g]	1.25[g]	1.25f	1.25[e]	1.25[e]	1.25[d]
1	7.21[g]	2.56[f]	2.26[e]	1.71[e]	1.53[d e]	1.60[d]	1.52[c d]
2	11.16[f]	2.60[f]	2.46[e]	1.90[e]	1.72[d e]	1.71[d]	1.54[c d]
3	16.26[e]	4.58[e]	2.69[e]	2.05[e]	1.79[d]	1.75[d]	1.57[c d]
4	21.51[d]	11.79[d]	7.07[d]	2.69[d]	1.98[d]	1.78[d]	1.60[c d]
5	30.33[c]	18.69[c]	14.02[c]	10.01[c]	8.38[c]	2.73[c]	1.85[c]
6	38.92[b]	27.91[b]	23.40[b]	21.58[b]	19.52[b]	6.56[b]	3.46[b]
7	48.70[a]	34.36[a]	35.46[a]	35.08[a]	29.96[a]	20.77[a]	12.72[a]

a-h Mean values followed by different letters in the same column are significantly different ($P \leq 0.05$).

Table 4. Effect of synthetic antioxidant and orange peel extract on free fatty acid (% oleic acid) of soy bean oil stored at 65°C for 7 days

Storage day	Control	BHT 200 ppm	BHA 200 ppm	Orange peel extract			
				400 ppm	800 ppm	1200 ppm	1600 ppm
0	0.032[g]	0.032[g]	0.032[e]	0.032[f]	0.032[f]	0.032[e]	0.032[f]
1	0.033[f]	0.057[f]	0.056d	0.065[e]	0.060[e]	0.065[d]	0.049[e]
2	0.081[f]	0.067[e]	0.060[d]	0.067[d e]	0.062[d]	0.066[d]	0.064[d]
3	0.094[e]	0.081[d]	0.069c	0.068[d]	0.064[d]	0.080c	0.065[d]
4	0.137[d]	0.091[c]	0.092b	0.079[c]	0.080[c]	0.083[c]	0.079[c]
5	0.149[c]	0.103[b]	0.111[a]	0.089[b]	0.106[b]	0.111[b]	0.110[b]
6	0.169[b]	0.110[b]	0.114[a]	0.113[a]	0.111[a]	0.113[b]	0.113[a]
7	0.177[a]	0.129[a]	0.134[a]	0.122[a]	0.120[a]	0.119[a]	0.117[a]

a-h Mean values followed by different letters in the same column are significantly different ($P \leq 0.05$).

Orange peel extracts caused significant ($P \leq 0.05$) reduction in free fatty acids (FFA) of soy bean oil during 7 days of storage at 65˚C (Table 4). Soy bean oils treated with synthetic antioxidant and orange peel extracts had lower FFA levels than that of control. These results are consistent with findings of [21] who reported that after 60 days storage at 45C, soy bean oil , containing 1600 and 2400 ppm potato peel extracts showed lower FFA (0.12 and 0.109%) than the control sample (FFA 0.32%). In fat deterioration, the first initiating step is the formation of fatty acids which are susceptible to oxygen attack in the presence of light, resulting in the formation of many organic compounds and free fatty acids which are responsible for development of rancidity and off flavors in fatty food materials [23].

Secondary oxidation products, measured as ansidine value. Soy bean oil containing orange peel extracts had lower ansidine values than those containing BHT and BHA and control. The ansidine value of control sample reached 10.65 and those containing synthetic antioxidant reached (7.24 – 7.39) or orange peel extracts reached (5.61-6.32) (Table 5). These results confirm the findings of [12] who found that the ansidine value of sunflower oil (control) was 33 and that of sunflower oil containing rosemary extract was 25 after 8 days of storage at 60C.

TBA values gradually increased with an increase in storage period for all soy bean oil samples (Table 6). Soy bean oils treated with antioxidants had lower TBA values than control. However, the addition of orange peel extract (1600 ppm) lowered the final TBA value after 7 days from 0.078 as by control sample, to 0.055. TBA measure the formation of secondary oxidation products, which may contribute to the off-flavor of oxidized oil. [10] reported that lower TBARS (Thiobarbituric acid-reactive substances) during accelerated oxidation of soy bean oil at 60C in the presence of mung bean hulls extract (100 ppm) than with the same concentration of BHA after 10 days of storage.

CONCLUSIONS

Based on these results obtained, A significant different was found in moisture and hexane extract content of Baladi and Novel orange peel. No differences (P >0.05) in total phenol and total flavonoids content were observed between Baladi and Novel orange peel whole extracts. Orange peel extracts treated with hexane had higher ($P\leq$ 0.05) phenolic content than whole extract. Baladi orange peel extract had higher ($P\leq$ 0.05) DPPH radical scavenging activity than Novel peel extract. Soy bean oil treated with orange peel extract had higher ($P\leq$ 0.05) inhibition oxidation than synthetic antioxidants. Orange peel extract and synthetic antioxidants reduced ($P\leq$ 0.05) free fatty acid, TBA and P-anisidine values of soy bean oil during storage at 65°C for 7 days. Orange peel extended the induction period to reach a peroxide value of 20 meq/kg in soy bean oil under tested conditions to over 5 days for BHT, BHA, and 400ppm, 6 days for both 800ppm and 1200 ppm, and 7 days for 1600 ppm. Orange peel extracts are a potential source of natural antioxidants and can safely be used instead of synthetic antioxidant to prolong the shelf life of fats and oils.

Table 5. Effect of synthetic antioxidant and orange peel extract on p-anisidine of soy bean oil stored at 65°C for 7 days

Storage day	Control	BHT 200 ppm	BHA 200 ppm	Orange peel extract			
				400 ppm	800 ppm	1200 ppm	1600 ppm
0	2.85^{g}	2.85^{g}	2.85^{f}	2.85^{f}	2.85^{e}	2.85^{d}	2.85^{f}
1	3.60^{f}	3.18^{f}	3.59^{e}	3.24^{e}	3.96^{d}	4.41^{c}	5.01^{e}
2	4.62^{ef}	3.27^{f}	3.61^{e}	3.84^{d}	4.29^{cd}	4.89^{b}	5.19^{de}
3	6.30^{d}	3.44^{e}	3.89^{d}	3.96^{d}	4.36^{c}	4.91^{b}	5.25^{cd}
4	6.09^{d}	4.04^{d}	4.00^{d}	3.98^{d}	4.48^{c}	5.07^{b}	5.36^{c}
5	7.78^{c}	5.17^{c}	4.58^{c}	4.32^{c}	4.57^{c}	5.18^{b}	5.45^{b}
6	9.66^{b}	6.45^{b}	5.95^{b}	5.65^{b}	5.86^{b}	5.74^{a}	5.50^{b}
7	10.65^{a}	7.24^{a}	7.39^{a}	6.31^{a}	6.32^{a}	5.97^{a}	5.61^{a}

a-f Mean values followed by different letters in the same column are significantly different ($P\leq$ 0.05).

Table 6. Effect of synthetic antioxidant and orange peel extract on 2-Thiobarbituric acid (TBA) value of soy bean oil stored at 65°C for 7 days

Storage day	Control	BHT 200 ppm	BHA 200 ppm	Orange peel extract			
				400 ppm	800 ppm	1200 ppm	1600 ppm
0	0.027^{e}	0.027^{f}	0.027^{f}	0.027^{e}	0.027^{e}	0.027^{f}	0.027^{f}
1	0.047^{d}	0.038^{e}	0.037^{e}	0.036^{d}	0.039^{d}	0.032^{e}	0.032^{e}
2	0.060^{c}	0.042^{e}	0.039^{e}	0.037^{cd}	0.041^{cd}	0.034^{e}	0.034^{de}
3	0.060^{c}	0.049^{d}	0.040^{de}	0.040^{bc}	0.042^{c}	0.037^{d}	0.035^{cd}
4	0.061^{c}	0.051^{cd}	0.045^{cd}	0.042^{b}	0.043^{c}	0.039^{d}	0.036^{bcd}
5	0.062^{c}	0.056^{bc}	0.050^{c}	0.045^{b}	0.043^{c}	0.044^{c}	0.038^{bc}
6	0.069^{b}	0.058^{b}	0.056^{ab}	0.060^{a}	0.053^{b}	0.051^{b}	0.038^{b}
7	0.078^{a}	0.066^{a}	0.065^{a}	0.063^{a}	0.061^{a}	0.060^{a}	0.055^{a}

a-f Mean values followed by different letters in the same column are significantly different ($P\leq$ 0.05).

REFERENCES

[1] AOCS *Official Methods and Recommended Practices* of *the American Oil Chemists' Society* (fifth edn), American Oil Chemists' Society, Champaign, IL, USA, (1998)

[2] Aravantinos-Zafiris, G., Tzia, C., Oreopoulou, V., Thomopoulos, C. D. Fermentation of orange processing waste for citric acid production. J. Sci. Food Agric. (1994) 65 (1):117-120.

[3] Bampidis V. A., Robinson P. H., Citrus by-products as ruminant feeds: a review. Anim. Feed Sci. Technol. (2006) 128 (3/4):175-217.

[4] Benavente-Garicia O., Castillo J., Marin F. R., Ortuno A., Del Rio, J. A. Uses and properties of Citrus flavonoids, J.Agric and Food Chem. (1997) 45:4505-4515.

[5] Bocco, A., Cuvelier, M. E., Richard, H., Berset, C., Antioxidant activity and phenolic composition of citrus peel and seed extracts. J. Agric. Food Chem. (1998) 46:2123-2129.
[6] Brand-Williams, W., Cuvelier, M. E., Berset, C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaftund Technologie, (1995) 28:25-30.
[7] Di Majo D., Giammanco M., La Guardia M., Tripoli E., Giammanco S., and Finotti E. flavanones in citrus fruit: Structure-antioxidant activity relationships. J. Rese. Intern. (2005) 38:1161-1166.
[8] Donaghy J.A., McKay A. M., Pectin extraction from citrus peel by polygalacturonase produced on whey, Bioresour. Technol. (1994) 45:25-28.
[9] Duh P. D., and Yen G-C. Antioxidant efficacy of methanolic extracts of peanut hulls in soy bean and peanut oils. J. Am. Oil Chem. Soc. (1997) 74:745-748.
[10] Duh P. D., Yen W. J., Du P. C., and Yen G-C. Antioxidant activity of mung bean hulls. J. Am. Oil Chem. Soc. (1997) 74:10591063.
[11] FAO http://faostat.fao.org/ Roma, 2010
[12] Hras A. R., Hadolin M., knez Z., and Bauman D Comparison of antioxidative and synergistic effects of rosemary extract with ∝ –tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chemistry (2000) 71:229-233.
[13] Johan I. M. Fractionation of orange peel phenols in ultra filtered molasses and mass balance studies of their antioxidant levels. J. Agric. and Food Chem. (2004) 52:7586-7592.
[14] Maillard M. N., Soum M. H., Boivia P., Berset C., Antioxidant activity of barley and malt : relationship with phenplic content. Lebensm. Wiss. Technol. (1996) 3:238-244.
[15] Mandalari G., Bennett R. N., Bisignano G., Saija A., Dugo G., Lo Curto R. B., Faulds C. B., Waldron K. W., Characterization of flavonoids and pectin from bergamot (*Citrus bergamia Risso*) peel, a major byproduct of essential oil extraction. J. Agric. and Food Chem. (2006) 54:197-203.
[16] Manthey J. A., Grohmann K., Concentrations of hesperidin and other orange pel flavonoids in citrus processing byproducts. J. Agric. Food Chem. (1996) (44):811-814.
[17] Manthey J. A., Grohmann K. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. J. Agric. Food Chem. (2001) (49):3268-3273.
[18] Ma, Y. Q., Chen, J. C., Liu, D. H., Ye, X. Q. Effect of ultrasonic treatment on the total phenolic and antioxidant activity of extracts from citrus peel. J. Food Sci. (2008) 73 (8):115-120.
[19] Moure, A., Franco, D., Sineiro, J., Dominguez, H., Nunez, M. J., Lema, J., Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. J. Agric. Food Chem. (2001) 48: 380- 3897.
[20] Rehman Z. U. Citrus peel extract – A natural source of antioxidant. Food Chemistry, (2006) 99:450-454.
[21] Rehman, Z. U., Habib, F., Shah, W. H. Utilization of potato peels extract as a natural antioxidant in Soy bean oil Food Chemistry, (2004) 85:215-220.
[22] SAS., SAS/STAT Guide for personal computers, Version 6 ed. SAS Institute, Cary, N.C. (1987)
[23] Sattar A., and Demen J. M. Effect of packing material on light induced quality deterioration of milk. J. of Canada Sci. and Technol. Aliment. (1973) 6:170-174.
[24] Singleton, V. L., Rossi, J.A., Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enology and Viticulture, (1965) 16: 144–158.
[25] Su, M., and Silva, J. L. Antioxidant activity, anthocyanins,and phenolics of rabbiteye blueberry (Vaccinium ashei) byproducts as affected by fermentation. Food Chemistry, (2006) 97:447–451.
[26] Tamaki Y., Konishi T., Masakuni T., Isolation and characterization of pectin from *Citrus tankan,* biosci. Biotechnol. Biochem. (2008) 72 (3):896-899.
[27] Ting, S. V., and Rouseff, R. L., Citrus fruits and their products-Analysis- Technology. Marcel Dekker, Inc, New York. (1986)
[28] Tripoli, E., La Guardia, M., Giammanco, S., Di Majo, D., Giammanco, M. citrus flavonoids: molecular structure, biological activity and nutritional properties: A review. Food Chemistry, (2007) 104:466-479.
[29] Wang Y, C., Chuang Y. C., Hus H. W., The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan, Food Chemistry, (2008) 106:277-284.
[30] Yusof S., Mohd Ghazali H., Swee King G., Naringin content in local citrus fruits. Food Chemistry, (1990) 37:113-121.
[31] Zhishen, J., Mengcheng, T., Jianming, W., The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. (1999) 64: 555-559.