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THE PREVENTIVE EFFECT OF POMEGRANATE PEEL EXTRACT AS A NATURAL ANTIOXIDANT ON SUNFLOWER OIL OXIDATIVE RANCIDITY

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ABSTRACT

Purpose: Pomegranate is a nutrient dense food rich in beneficial antioxidants. Our work was conducted to optimise the effect of pomegranate peel aqueous extract (PoP-Ax) as a natural antioxidant on sunflower oil oxidative rancidity compared to synthetic antioxidants (catachine, tannic acid and BHT).

Design and methodology: The total phenolic and antioxidant activity of PoP was determined by Folin-Ciocalteu, and 2,2- diphenyl-1-picrylhydrzyl (DPPH), respectively. High Performance Liquid Chromatograpy (HPLC) was used to investigate the major phenolic compounds and tocopherol in PoP-Ax. The pomegranate peel (PoP) antioxidant was extracted with water, ethanol, methanol and ethyl acetate.

Findings: The results showed that the antioxidant activity of PoP-Ax for different intervals during the storage period was evaluated and exhibited 86.03%, 88.82%, 96.91% and 98.68% at levels 0.01%, 0.02%, 0.05% and 0.1% PoP-Ax, respectively. The peroxide value (PV) and thiobarbituric acid (TBA) at 0.05% and 0.1% of POP-Ax gave the best results compared to synthetic antioxidants.

Research implications: Our study could be recommended to use PoP-Ax as natural antioxidants at 0.05% and 0.1% for protecting oils against rancidity.

Keywords: pomegranate peel; antioxidant extract; TBA; BHT; peroxide value.

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INTRODUCTION

One of the principal causes of food quality deterioration is lipid peroxidation (Gordon, 1991); lipids oxidation also occurs during raw material storage, processing, heat treatment and further storage of final products. The taste and aroma of a product can be the criteria for rejection of any kind of food if they differ significantly from what is expected by the consumer (Budzynska-Topolowska and Ziemlanski, 1992; Frankel, 1991). Products of lipid oxidation influence other food constituents; e.g. they interfere with the absorption of protein or folic acid and cause pathological changes in the mucous membrane of the alimentary tract, inhibit activity of enzymes, and increase the content of cholesterol and peroxides in blood serum, thus activating the process of atherosclerosis.

Lipid peroxidation results in the formation of reactive oxygen species and free radicals (Siddhuraju and Becker, 2003). Free radicals are major contributors to age-related and degenerative diseases such as cancer, cardiovascular disease, immune system decline, and brain dysfunction (Ames et al., 1990; Percival, 1996; Young and Woodside, 2001).

Antioxidants are the compounds that, when added to food products (especially to lipids and lipid-containing foods), can increase the shelf life by retarding the process of lipid peroxidation. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have restricted use in foods in industrial processing as these synthetic antioxidants are suspected to be carcinogenic (Madhavi and Salunkhe, 1995). Toxicologists and nutritionists have noted the noxiousness for a long time. Therefore, the importance of the search for and exploitation of natural antioxidants, especially of plant origin, has greatly increased in recent years (Jayaprakasha and Rao, 2000). Consumers generally perceive natural antioxidants as better than synthetic additives (Baardseth, 1989, Madsen et al., 1996, Pokorny, 1991 and Wang et al., 1996).

Pomegranate (Punica granatum L.) is an important tropical and sub-tropical fruit crop. The pomegranate fruit consists of three parts: seeds (about 3%), juice (about 30% of the weight of the fruit), and the peels (which include the husk and interior network membranes) (Prakash and Prakash, 2011). The pomegranate is an important source of bioactive compounds, and has been used for folk medicine for many centuries (Li et al., 2006; Elfalleh et al., 2012). Pomegranate peels (PoP) are characterised by an interior network of membranes comprising almost 26-30% of the total fruit weight, and are characterised by substantial amounts of phenolic compounds including flavonoids (anthocyanins, catechins and other complex flavonoids), and hydrolysable tannins (punicalin, pedunculagin punicalagin, gallic and ellagic acid). These compounds are concentrated in PoP and juice, which account for 92% of the antioxidant activity associated with the fruit (Afaq et al., 2005; Negi et al., 2003; Zahin et al., 2010; Fischer et al., 2011). Moreover, Farag et al. (2015) reported that the antioxidant activity of crude peels juice was higher than that of crude leaf juice, and demonstrated a positive correlation between polyphenolic content and the antioxidant activity of crude pomegranate juices.

Pomegranate peel extract (PoPx) had markedly higher antioxidant capacity than the pulp extract in scavenging or preventive capacity against superoxide anion, hydroxyl and peroxyl radicals, as well as inhibiting $CuSO_4$ induced 4LDL oxidation. The large amount of phenolic contained in PoPx may cause its strong antioxidant ability (Li et al., 2006). Therefore, PoPx has recently attracted interest because of its potential use as natural food preservatives and nutraceuticals (Qu et al., 2010).

The purpose of this work is to investigate phenolic compounds and tocopherol in pomegranate peel by using HPLC to extract pomegranate peel antioxidants. We do this using four solvents then estimating the stabilising efficiency of pomegranate peel aqueous extract against oxidative deterioration of sunflower oil.

MATERIALS AND METHODS

Materials

Pomegranate fruits were obtained from a local market in Dammam, Saudi Arabia.

Methods

Preparation of Pomegranate Peels

PoP were separated and dried in the air at room temperature. Then, it was ground using a blender (Waring 2-Liter Laboratory Blender- The Lab Depot Inc).

A B

Figure 1 Pomegranate peel (A) and pomegranate peel powder (B)

Source: (A) http://www.stylishwalks.com; (B) http://2beingfit.com

Determination of Total Polyphenol Content (TPP)

TPP were estimated by the Folin-Ciocalteu method, as reported by Elfalleh et al., (2012). Add 0.5ml of Folin-Ciocalteu (Prolabo) reagent to 0.5ml of aqueous solution from each sample. Then, add 4ml of sodium carbonate (1M) solution. The tubes were laid for 5 minutes in a water bath at 45°C and then put in a cold water bath. The absorbance was measured at 765nm using a spectrophotometer (Beckman, DU 7400 USA). TPP of each fraction were converted into mg gallic acid equivalents per g dry weight (mg GAE/g DW).

Determination of Hydrolysable Tannin Content (HT)

HT was determined using Çam and Hişil's (2010) method. Add 5ml of 2.5% KIO_3 and 1ml of 10-fold diluted extracts into a vial and vortex for 10 seconds. The absorbance of red coloured mixture was determined at 550nm versus the prepared water blank. Optimum reaction was defined as the time to gain maximum absorbance value; this was determined to be 2 minutes for pomegranate peel extract and 4 minutes for standard solutions of tannic acid. For calibrations we used tannic acid solutions at different concentrations (100mg/l to 1600mg/l). The final results were expressed as mg tannic acid equivalent per g of DW (mg TAE/g DW).

Determination of Pomegranate Peel Antioxidant by HPLC

Pomegranate peel phenolic compounds were determined by HPLC (HP 1050) according to the method described by Waksmundzka-Hajnos et al. (2007). Tocopherol was extracted and analysed by HPLC according to the method described by Kalogeropoulos et al. (2007). Tocopherol was isolated from the PoP sample by extracting a 0.5g sample five times, with 5ml hexane containing 20mg/kg butylated hydroxytoluene. The extracts were combined: hexane was evaporated to dryness under vacuum and the residue was dissolved in a mixture of chloroform: isopropanol (3/1, v/v). Aliquots of 20µl of these solutions were subjected to reversed phase high performance liquid chromatography (RP-HPLC) analysis by an HPLC system (Agilent Technologies, model HP 1050, Waldbronn, Germany) combined with auto-sampler, diode array detector HP-1050. A quaternary solvent system was used, consisting of water acidified with *o*-phosphoric acid at pH 3, methanol, acetonitrile and propanol-2, with gradient elution on a Nucleosil C18 100–5 (125mm×4.6mm) (MZ, Wöhlerstr., Mainz, Germany) at a flow rate of 1ml/ min as follows: initially 90% methanol/10% water (pH 3) in 10 minutes; 100% methanol in 10 minutes; isocratic for 5 minutes; 20% acetonitrile/20% methanol/60% propanol-2 in 1 minute; isocratic for 10 minutes; and finally to initial conditions in 4 minutes. A 10 minute post-run for the system equilibration was used. Quantification was carried out by a reference curve constructed by analysing standard α-tocopherol solutions.

Determination of Percentage Antioxidant Extracts

A weighed portion (5g) of dried PoP was extracted with 50ml of water, ethanol, methanol and ethyl acetate for 24 hours. The extracts of ethanol, methanol and ethyl acetate antioxidant were filtered and dried to dryness at room temperature. At the same time, the extract of water antioxidant was filtered and evaporated at 40°C to dryness in a rotary evaporator (RE 300/MS). The percentage of antioxidants was evaluated using the following equation:

$$\text{percentage of extracte (\%)} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100$$

Preparation of Antioxidant Aqueous Extract

A weighed portion (10g) of dried PoPx was extracted with 50ml of water for 24 hours. The extract was filtered and evaporated at 40°C to dryness in a rotary evaporator (RE 300/MS). The dried extract obtained from PoP was stored in an airtight container at 4°C until further use.

Schaal Oven Test

Triplicate samples of sunflower oil (100g) with added synthetic antioxidants (catachine, tannic acid, BHT), and POP-Ax as natural antioxidants

(0.01%, 0.02%, 0.05% and 0.1%), were heated at 80˚C/5 hours daily for 7 days. Samples (10g) were removed periodically for analysis (Zandi and Gordon, 1999).

Evaluation of the Intensity of Hydrolytic and Oxidative Activity in the Lipid

Peroxide value: The recommended method of the Association of Official Analytical AOAC (1999) was applied to determine the level of peroxide value. A known weight of the oil (ca. 2g) was dissolved in a mixture of glacial acetic acid: chloroform (30ml, 3:2 v/v).

Thiobarbituric Acid (TBA) Assay

The degree of oxidation of oil was measured by TBA assay as described by Ohkawa et al. (1979). The reacted solution (1ml) mentioned above was incubated with 0.2% (w/v) thiobarbituric acid (3ml) and 0.05M sulfuric acid (2.5ml) for 30 minutes in a 95˚C water bath. The coloured substances were extracted with 4.0ml of 1-butanol, and its absorbance was measured at 532nm. A calibration curve was constructed by using malondialdehyde bis (diethyl acetal) and results were expressed as malondialdehyde equivalents.

Determination of Antioxidant Activities

Scavenging activity of PoP-Ax, catachine, tannic acid and BHT extracts against DPPH radicals were assessed according to Rajan et al.'s (2011) method. The PoP-Ax, catachine, tannic acid and BHT extracts of different concentrations were mixed with an aliquot of DPPH (1ml, 0.004% w/v). The mixture was vigorously shaken and left to stand for 30 minutes in the dark at room temperature. The concentration of the remaining DPPH was determined at 517nm. The radical scavenging activity was calculated as a percentage inhibition using the following formula:

$$\text{Inhibition (\%)} = (\text{A control} - \text{A sample}) / \text{A control} \times 100.$$

Where: A control = the absorbance of the control reaction. A sample = the absorbance of the PoP-Ax, catachine, tannic acid and BHT extracts.

Statistical Analysis

All determinations were carried out in triplicate, and data were subjected to statistical analyses using Microsoft Excel Data Analysis. Significant differences between means were determined by Duncan's (1955) multiple range tests; they were considered to be significant when $P \leq 0.05$.

Results and Discussions

Rancid oil forms harmful free radicals in the body, which are known to cause cellular damage; they can also cause digestive distress and deplete the body of vitamins B and E. Rancid oils may produce damaging chemicals and substances such as peroxides and aldehydes; these can damage cells and contribute to atherosclerosis. Free radicals produced by rancid oil can also damage DNA in cells. They are produced by toxins as well as by normal bodily processes; they can cause damage to arteries as well act as carcinogens - substances that can cause cancer. High levels of malondialdehyde are found in rancid foods. Malondialdehyde is a decomposition product of polyunsaturated fatty acids. This chemical has been reported to be carcinogenic and a potential health hazard does exist. Eating rancid oil will expose you to accelerated aging, raised cholesterol levels, obesity and weight gain. Daily consumption increases the risk of degenerating diseases such as cancer, diabetes, Alzheimer's disease, and atherosclerosis, a condition in which artery walls thicken due to a build-up of fatty materials. Natural antioxidants present in the diet increase resistance towards oxidative damages and they may have a substantial impact on human health. Dietary antioxidants such as a sorbates, tocopherols and carotenoids are well known and there is a surplus of publications related to their role in health. Pomegranate peel extract (PoPx) contains large amount of phenolic compounds, it may cause its strong antioxidant ability and potential use as natural food preservatives and nutraceuticals.

Table 1 Mean value of TPP and HT of pomegranate peel

TPP (mg/g dw)	*HT (mg/g dw) µg/ 100g*
54.4±2.72	181.9±9.09

Each value in the table is represented as mean ± SE (n=3).

**p-values* were determined by Fisher's exact test, significantly different (P≤0.05); NS, not significant.

Source: Devised by author.

Data in Table 1 show the amount of TPP and HT in PoP. The results indicated that TPP and HT in PoP are 54.4 and 181.9mg/g dw. This result is in agreement with Elfalleh et al. (2012) who reported that TPP and HT in pomegranate peel were 53.65, 62.71mg/g dw in aqueous and 85.60, 139.63mg/g dw in MeOH, respectively. Ali et al. (2014) stated that the methanol extract gave the highest content of TPP (103±2.77 mg/g dw). Similar results were also obtained by Farag et al. (2015).

Table 2 Analysis of the HPLC for pomegranate peel phenolic compounds and tocopherol

Chemical Constituents	*Pomegranate peel (mg/100g)*
Ferulic acid	12.204
Syringic acid	19.296
Cinnamic acid	5.834
Protocatechuic acid	11.472
Coumarin	2.400
Caffic	0.801
ρ- Coumaric acid	2.435
Resorcinol	0.919
Salicylic acid	10.628
Quercetin	0.645
Kaempferol	7.977
Tocopherol	21.193

Source: Devised by author.

HPLC Analysis of Main Phenolic Compounds in Pomegranate Peel

Results in Table 2 indicate the major phenolic compounds in pomegranate peel. The compounds are ferulic acid, syringic acid, cinnamic acid, protocatechuic acid, coumarin, caffeic, ρ-coumaric acid, resorcinol, salicylic acid, quercetin and kaempferol. In previous studies, Cai et al. (2004) reported that pomegranate peel included some phenolic compounds, e.g. quercetin and vanillic acid. Also, caffeic acid, p-coumaric acid, quercetin, and vanillic acid in pomegranate peel are revealed by Mansour et al. (2013).

HPLC in the present study shows that syringic acid was the most abundant phenolic acid in PoP, constituting 19.296% of total extracted compounds. On the other hand, quercetin was the lowest abundant phenolic compounds in PoP, constituting 0.645% of total extracted compounds. Vanillic acid and quercetin were present only in small quantities, equal to 1.0±0.7 and 1.9±0.5mg 100g-1 (Mansour et al., 2013). In the previous studies, many authors proved the presence of quercetin, vanillic acid, ferulic acid and kaempferol in pomegranate peel (Padmaja and Prasad, 2011; Van Elswijk et al., 2004). Table 2 also shows the concentration of tocopherol (21.193%) (Padmaja and Prasad, 2011).

Effect of Solvent on Yield of PoPx

Figure 2 shows the percentage yield of PoPx in different extraction media. The extract yield ranged from 0.79-33.65% among different solvent systems. The highest yield was observed for methanol, followed by water, ethanol and ethyl acetate, respectively. The findings were supported by Iqbal et al. (2008); they reported that PoP methanol extract gave the highest yield compared to ethanol, acetone, chloroform, and ethyl acetate. Also, Iqbal et al. (2005) reported that methanol is usually recommended for the extraction of antioxidative compounds. Of the six solvent extracts, methanolic extracts exhibited the highest yield and antioxidant activity.

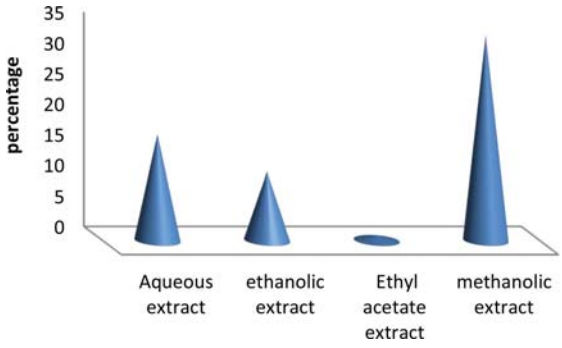

Figure 2 Percentage of pomegranate peel of different extracts

Source: Devised by authors

Evaluation of the Intensity of Hydrolytic and Oxidative Activity in the Lipid

It is important to assess the oxidative degradation of fats and oils in the food industry, because free radical initiated oxidation is one of the main causes of rancidity.

Peroxide Value (PV)

PV is the measure of degree of initial oxidation of oils and fats. In the light of our present knowledge,

using PoP-Ax as a natural antioxidant offers the most likely extracts for oils protection from rancidity compared to synthetic antioxidants (catachine, tannic acid, BHT). In our investigation, we added PoP-Ax and synthetic antioxidants at different levels (0.01%, 0.02%, 0.05%, 0.1%). PoP-Ax and synthetic antioxidants at 0.01% were added to sunflower oil; the oil was then heated at 80˚C for 7 days. PV was determined daily; the results are reported in Figure 3 and show that after 7 days the PV was not completely inhibited. The PV increased from 0.0100 at zero time to (2.5863) and (2.7599, 2.9319, 2.2614) by PoP-Ax and catachine, tannic acid and BHT, respectively. A control experiment was also carried out and showed a high level of PV (10.1460). It could be said that PoP-Axat 0.01% is not more effective for inhibiting PV.

Another experiment was carried out by adding synthetic antioxidants and PoP-Axat 0.02%. The results are recorded in Figure 4, and show that 0.02% PoP-Ax, is not more effective for inhibiting PV in the control experiment (10.1460). Adding 0.05% from PoP-Ax showed a high effect on sunflower oil compared with experiment control and synthetic antioxidants, as reported in Figure 5. On the other hand, BHT gave the best results (0.8320) compared to tannic acid (0.9470) and catachine (0.9580) as synthetic antioxidants.

The final experiment was carried out by adding synthetic antioxidants and PoP-Axat 0.1%. The results are reported in Figure 6 and show that PoP-Ax gave results (0.3329) near to its results at 0.05% (0.3370). The results show that the increase in PV in both experiments was very limited and close to each other. This decrease in PV after reaching to a maximum may be correlated with the removal of some of the hydroperoxide decomposition products during long heating treatments (Shahidi et al., 1992). The peroxide value was in the range of 64.21–147.34meq/kg for pomegranate peel extract stabilised samples, while it was 78.13meq/kg for the BHT stabilised sample after 24 days storage. It was 170.12meq/kg for control on the 20th day, which decreased to 156.25meq/kg up to the 24th day.

The highest PV was observed for the control, followed by SFO-250, SFO-500, SFO-BHT and SFO-1000 respectively. At all the stages of the storage period, there was a regular pattern of an increase in PV for all the stabilised samples. Among the extract stabilised samples, SFO-250 exhibited the highest PV at all stages, while SFO-1000 showed the lowest. The PV of SFO-500 increased slowly initially, followed by a sharp increase after the 8th day. The PV for SFO-BHT was initially comparable to SFO-1000, which increased rapidly than SFO-1000 during later periods of storage; this suggests a higher stability of SFO-1000 over SFO-500. The maximum PV of SFO-500 after 16 days storage was found to be 64.54meq/kg, which is far less than that of sunflower oil stabilised by guava leaves (87.8meq/kg (Anwar et al., 2006). From the results of peroxide value, an optimisation may be deduced that PoP extract at 800-850ppm has a stabilising effect comparable to SFO-BHT at its legal limit (Iqbal et al., 2008). Pomegranate peel reduced the POVs from 58.5±0.13meqkg^{-}1 (control) to 22.1±0.04meqkg^{-}1, and FFA values from 9.50±0.04% (control) to 1.60±0.15%, respectively (Lutfullah et al., 2015).

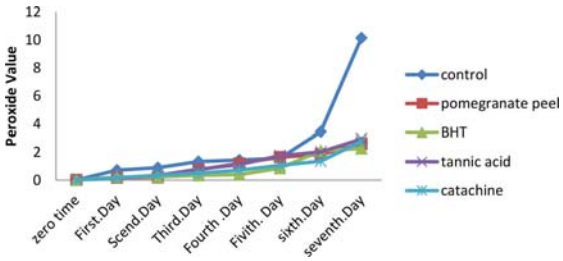

Figure 3 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.01% on PV during heating sunflower oil at 80°C/5 hours for 7 days. The experiment was carried out in triplicate

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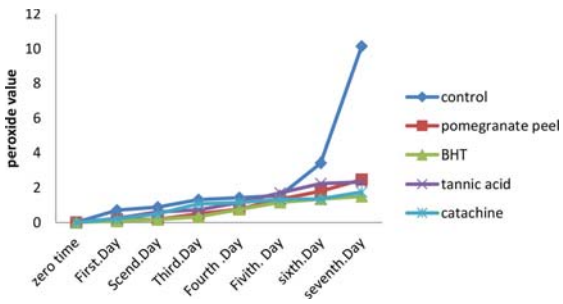

Figure 4 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.02% on PV during heating sunflower oil at 80°C/5 hours daily for 7 days. The experiment was carried out in triplicate

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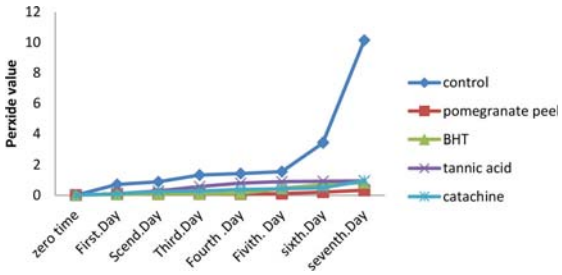

Figure 5 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.05% on PV during heating sunflower oil at 80°C/5 hours daily for 7 days. The experiment was carried out in triplicate

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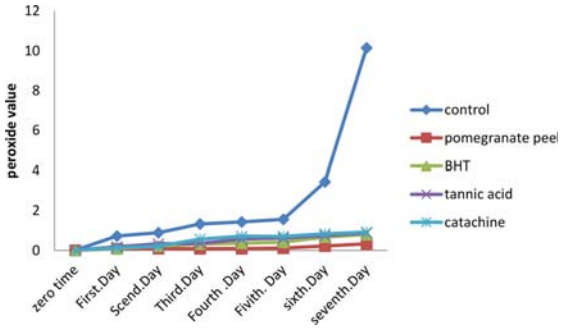

Figure 6 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.1% on PV during heating sunflower oil at 80°C/5 hours daily for 7 days. The experiment was carried out in triplicate

Source: Devised by authors

Thiobarbituric Acid (TBA)

The TBA value measures the formation of secondary oxidation products, mainly malondialdehyde, which may contribute to an off-flavour in oxidised oil.

The TBA number of sunflower oil was determined after adding PoP-Ax and synthetic antioxidants (catachine, tannic acid, BHT) at 0.01%, 0.02%, 0.05%, 0.1%, and heating at 80˚C/5 hours daily for 7 days. The results are recorded in Figures 7-11 and show that the TBA at 0.1% of PoP-Ax amounted to (0.0090). This result is relatively near to control at zero time (0.0050). On other hand, TBA numbers of the synthetic antioxidants (catachine, tannic acid, BHT) were increased from (0.0050) at zero time to (0.0280, 0.0380 and 0.0160), respectively, at 0.1% after 7 days. This is the most widely used method for the measurement of secondary oxidation products.

Results for the measurement of TBARS for the control and stabilised samples were as follows (Iqbal et al., 2008): SFO-250 and SFO-500 showed similar behaviour with a slow degree of increase in TBARS formation up to the 8th day of storage, after which the rate of formation of TBARS for SFO-250 increased substantially up to the 16th day followed by an increase up to 24 days, reaching a value of 2.47lmol/g oil, with a slightly reduced rate. The rate of TBARS formation for SFO-BHT was very slow up to the 12th day, and was slightly lower than SFO-1000, after which its rate increased. The TBARS for SFO-BHT became higher than SFO-1000 after the 16th day of storage, suggesting a higher stabilisation efficiency of PP extract at 1000ppm, in controlling the formation of secondary oxidation products after long period of storage, 24 days, under an accelerated set of conditions (Anwar et al., 2006).

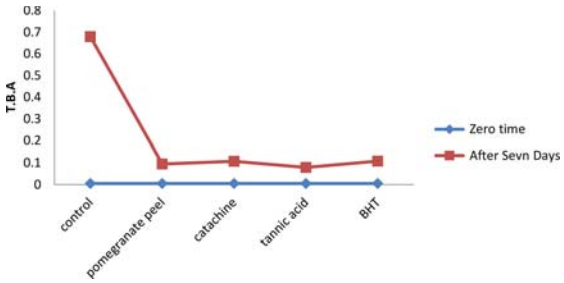

Figure 7 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.01% on TBA during heating sunflower oil at 80°C/5 hours daily for 7 days. The experiment was carried out in triplicate

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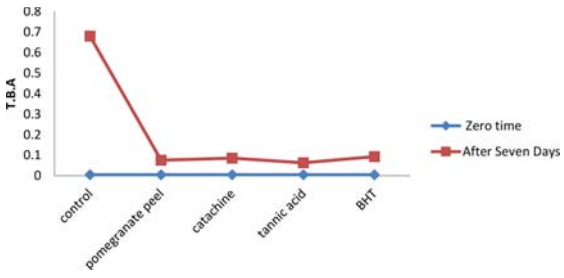

Figure 8 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.02% on TBA during heating sunflower oil at 80°C/5 hours daily for 7 days. The experiment was carried out in triplicate

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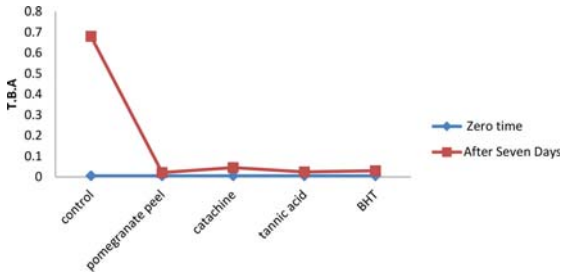

Figure 9 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.05% on TBA during heating sunflower oil at 80°C/5 hours daily for 7 days. The experiment was carried out in triplicate

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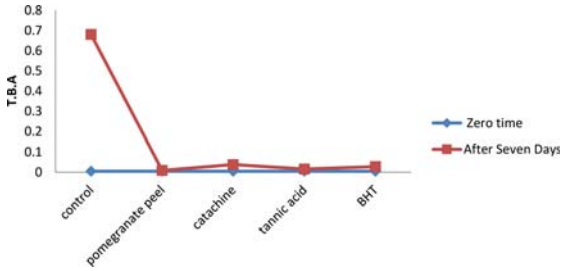

Figure 10 Effect of pomegranate peel aqueous extract and synthetic antioxidants at 0.1% on TBA during heating sunflower oil at 80°C/5 hours daily for 7 days. The experiment was carried out in triplicate

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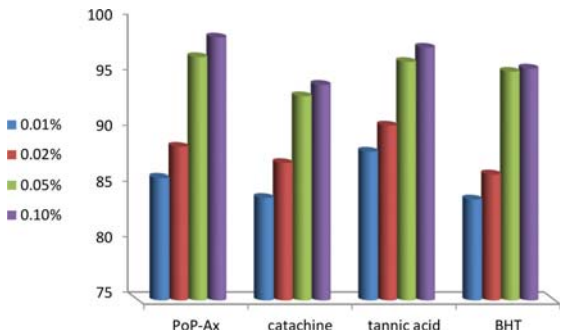

Figure 11 Antioxidant activity of pomegranate peel aqueous extract and synthetic antioxidants at 0.01%, 0.02%, 0.05% and 0.1% during heating sunflower oil at 80°C/5 hours daily for 7 days

Source: Devised by authors

The PPEs were at the 0.010%, 0.025%, 0.035% and 0.050% levels, and synthetic antioxidants (BHA and BHT) were added at 0.010% and 0.020%. The addition of natural and synthetic antioxidants to soybean oil affected, to differing degrees, the peroxide and TBA values during accelerated oxidation at 60°C for 12 and 13 days, respectively. All those samples with an added PPE level at 0.010-0.050% were more stable on heating at 60°C than the control, when assessed by the change in peroxide and TBA values. The antioxidant effect of PPE increased with concentration and, at a concentration of 0.050%, its antioxidant activity was higher and significantly different ($P<0.05$) from that of the synthetic antioxidant (BHA and BHT) at the 0.010% and 0.020% levels (Yasoubi et al., 2007).

Antioxidant Activity of PoP-Ax and Synthetic Antioxidants

The radical scavenging activity (RSA) of PoP-Ax and synthetic antioxidant was evaluated by a decrease in the peak area of the DPPH radical at 517nm. The RSA power of extracts was indicated by a decrease in absorbance. As a general trend, the RSA increased with increasing extract concentrations (Figure 11). PoP-Ax at 0.1% gave the highest antioxidant activity compared to catachine, tannic acid and BHT, respectively, as indicated by lower PV and TBA values. PoPx, as evaluated by a ferric reducing antioxidant power (FRAP) assay, was found to be the richest source of antioxidants among peel extracts of the most commonly consumed fruits (Okonogi et al., 2007; Tehranifar et al., 2011).

The free radical scavenging activity of PoP phenolics involves electron donation to free radicals that converts them to relatively more stable compounds. Studies have confirmed that the antioxidant activity of plant extracts depends on the concentration of phenolic compounds (Naveena et al., 2008; Negi and Jayaprakasha, 2003; Padmaja and Prasad, 2011). For most natural antioxidants, maximum antioxidant activity was achieved using a 0.05% concentration (Rossel, 1994). Padmaja and Prasad (2011) reported that pomegranate peel extract at 100ppm showed an RSA of 86.58%.

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