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# UTILIZATION OF NATURAL ANTIOXIDANT EXTRACTS OF POMEGRANATE PEELS AND GUAVA SEEDS IN VEGETABLE OILS STABILITY

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## ABSTRACT

Waste management is one of the most modern important trends in food technology to overcome the problems related to its accumulation, and in the same time to maximize its economical value. Pomegranate peels and guava seeds are resulted from food industry in big quantities, so this work is a tempt of utilization of these wastes as sources of natural antioxidants, which could be used in food industry.

Ethanolic extracts of both pomegranate peels and guava seeds, separately, with different concentrations (300 ppm, 600 ppm and 900 ppm), were examined for their antioxidant activity when added as well as BHT (200 ppm) to preheated RBD soybean and sunflower oils, separately, comparing to control samples (without any addition).

Peroxide value, UV absorbency (K232 and K270), Free fatty acids and the changes in fatty acids composition as parameters for evaluation of stabilization efficacy of the ethanolic extracts of pomegranate peels and guava seeds on both soybean and sunflower oils in accelerated conditions (Schaal oven test).

The obtained results showed that, each of pomegranate peel extractsand guava seeds extracts with 600 and 900 ppm have higher antioxidant effects than that of BHT (200 ppm), while 300 ppm extract concentration has lower antioxidant effect than BHT.

These results showed the possibility of using these wastes in retarding the oxidation of vegetable oils in industrial scale.

## INTRODUCTION

Marketing of sunflower and soybean oils is widespread in Egypt as many countries around the world. Vegetable oils are the ideal cooking media of the day because they are beneficial and popular due to their cholesterol lowering effects, but they are more susceptible to oxidation in comparison to animal fats, which predominantly contain saturated fatty acids and hence do not react readily with other chemicals specially oxygen (Matalgyto and Al-Khalifa, 1998).

To prevent the lipid peroxidation in fats and oils, synthetic antioxidants have been used as food additives for over 50 years (Cuvielier *et al.,* 1994). Addition of synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT) and tertiary-butyl hydroquinone (TBHQ), has been one of the most effective and popular methods to prevent oxidation and development of off-flavors (Halliwell and Gutteridge, 1985).

Nowadays, there are safety concepts and health risks associated with the use of synthetic antioxidants, which may cause health problems (Siddhuraju and Becker, 2003).On the other hand, natural materials based on

botanical origin have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcenogens (Dillard and German, 2000).

Pomegranate and Guava fruits are predominant in Egypt and they are consumed fresh or after processing, leading to huge quantities of wastes (including Pomegranate peel and Guava seeds) which could be used as sources of natural antioxidants (Iqbal *et al.,* 2008 and El Bedawey *et al.,* 2010).

The aim of this investigation is studying the possibility of using different extracts of Pomegranate peel and Guava seeds (as natural sources of antioxidants) in retarding oxidative deterioration of Refined, Bleached and Deodorized (RBD) sunflower and soybean oils.

## MATERIALS AND METHODS

**1- Samples:**

Refined, Bleached and Deodorized (RBD) sunflower and soybean oils were obtained from Sila Company for edible oils, El-Fayum, Egypt.

Pomegranate and Guava fruits were obtained from Maghara research station – Desert Research Center

**2- Extraction of total phenolics:**

Pomegranate peels and Guava seeds were dried in an oven at 55 C for 3 h. the dried samples were ground 3 times. About 25 g of each source were extracted with 100 ml of methanol, ethanol and acetone, separately. The mixtures were kept for 24 h at room temperature without shaking, then filtered and concentrated to 25 ml, and the extracts were freezed until further use (Kiralan *et al.,* 2008).

**3- Determination of total phenolics content :**

Total phenolics content of all studied extracts were determined spectrophotometricallyusing the Folin-Ciocalteu's reagent, according to the method described by Singleton and Rossi (1965) using gallic acid as a standard

**4- Oil sample preparation:**

Ethanolic extracts of Pomegranate peels and Guava seeds were added separately, to preheated (50 ◌ْC for 3 h) RBD sunflower and soybean oils separately, at concentrations of 300, 600 and 900 ppm , also, BHT (synthetic antioxidant) was added at its legal limit of 200 ppm to each oil (Iqbal *et al.,* 2008).

**5- Oxidation stability:**

All the oil samples (49 samples for each oil) were placed in dark brown bottles without stoppers and stored in an oven at fixed temperature of 65 ◌ْC. control samples (7 samples for each oil, without any antioxidants) were also placed under the same conditions. Oxidation was accelerated in a forced draft air oven set at 65 ◌ْC for up 14 days. During storage period, the samples were withdrawn every two days for analysis and the progress of the oxidative deterioration was followed by peroxide value and UV absorption characteristics (K232 and K270) (Kiralan *et al.,* 2008).

**6-** Peroxide value (PV), free fatty acids content, conjugated dienes (K232)and conjugated trienes (K270)were determined according to A.O.A.C. (2007).

**7- Fatty acids composition:**

Fatty acids composition was determined by gas chromatography according to A.O.A.C. (2005**)**.

**8- Statistical analysis:**

All determinations were carried out in triplicate (except fatty acid composition) and data is reported as mean. Significant differences ($p<0.05$) were calculated using Duncan's multiple range test, followed the method reported by Steel and Torrie, (1980).

## RESULTS AND DISCUSSION

**1- Effect of different solvents on extraction of antioxidants:**

Figure (1) shows the efficiency of polyphenols extraction by different studied solvents (acetone, ethanol and methanol).

From the mentioned figure, it could be noticed that, pomegranate peels have high polyphenols content comparing to Guava seeds when extracted by acetone, ethanol and methanol separately.

Also, the mentioned data, indicated that the efficiency of polyphenols extraction either for pomegranate peels or guava seeds could be ordered as follow : methanol > ethanol > acetone, where it has been reported that, the antioxidant activity of polar solvent extracts is markedly greater than those of less/non-polar solvent extracts (Iqbal *et al.,* 2008).

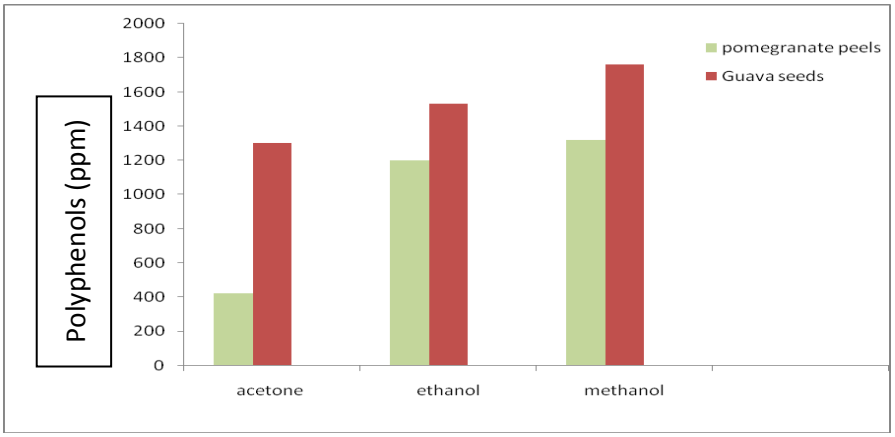


**Figure (1): Polyphenols extraction efficiency by different solvents from pomegranate peels and guava seeds**

**2- Effect of different extracts on soybean oil stability:**

**Acidity (F.F.A. %):**

Formation of free fatty acids might be an important measure of rancidity of foods (Zhang *et al.,*2010).

The acidity development during oven test of different soybean oil samples was determined and the results were tabulated in Table (1).

**Table (1): The acidity (F.F.A.% as oleic acid) development of different treatments during oven test of soybean oil**

| Treatments / Holding time | Control S.O | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 0.160 | | | | | | | |
| 2 | $0.167^{a}$ | $0.163^{b}$ | $0.163^{b}$ | $0.164^{ab}$ | $0.163^{b}$ | $0.163^{b}$ | $0.163^{b}$ | $0.164^{ab}$ |
| 4 | $0.190^{a}$ | $0.180^{b}$ | $0.180^{ab}$ | $0.181^{ab}$ | $0.181^{ab}$ | $0.180^{ab}$ | $0.175^{b}$ | $0.173^{b}$ |
| 6 | $0.252^{a}$ | $0.200^{cd}$ | $0.210^{b}$ | $0.193^{d}$ | $0.195^{cd}$ | $0.205^{bc}$ | $0.195^{cd}$ | $0.182^{e}$ |
| 8 | $0.320^{a}$ | $0.235^{c}$ | $0.250^{b}$ | $0.207^{e}$ | $0.202^{e}$ | $0.240^{c}$ | $0.225^{d}$ | $0.205^{e}$ |
| 10 | $0.410^{a}$ | $0.280^{c}$ | $0.298^{b}$ | $0.245^{e}$ | $0.230^{f}$ | $0.278^{c}$ | $0.260^{d}$ | $0.225^{f}$ |
| 12 | $0.505^{a}$ | $0.315^{b}$ | $0.340^{b}$ | $0.285^{f}$ | $0.260^{g}$ | $0.320^{c}$ | $0.290^{e}$ | $0.250^{h}$ |
| 14 | $0.650^{a}$ | $0.360^{d}$ | $0.390^{b}$ | $0.330^{e}$ | $0.300^{g}$ | $0.370^{c}$ | $0.325^{f}$ | $0.285^{h}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) S.O. soybean oil**

The tabulated results showed that, acidity percentage increased by increasing of the period of oven test for all studied treatments, but the highest increment was observed for control sample, which increased from 0.160 % (as oleic) in zero time to 0.650 % after 14 days in oven at 65°C. The same trend was observed for all other treatments, but the increasing rate was smaller than that of control sample. The best effect on retarding acidity development (comparing with control sample) was recorded for pomegranate peels extract (900 ppm) followed by Guava seeds extract (900 ppm), then pomegranate peels extract (600 ppm) and Guava seeds extract (600 ppm).

On the other hand, the lowest effect on aciditydevelopment (comparing with control sample) was recorded for Guava seeds extract (300 ppm) followed by pomegranate peels extract (300 ppm) and then BHT.

These results were in agreement with those of Zhang *et al.,* (2010), who found that addition of antioxidants caused significant reduction in FFA% of sunflower oil during 21 days of storage at 60°C, as the content of antioxidant (Carnosic acid) increased , inhibitory effects on FFA % also, increased considerably.

**Peroxide value:(PV)**

The first commonly used method for measuring the oxidation status of any oil or fat is the formation of peroxides which determined by peroxide value.Peroxide value is the measure of degree of initial oxidation of oils and fats.

The peroxide values development during oven test of different soybean oil samples was determined and the results were tabulated in Table (2).

The results in Table (2), showed a continuous increase in PV with the increase of holding period in oven (65°C for 14 days) for all soybean oil studied samples, with slow rate till the eighth day and then it increased in accelerated rate.

**Table (2): Peroxide value (Meq $O_2$/kg oil)of different treatments during oven test of soybean oil**

| Treatments / Holding time | Control S.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 2.2 | | | | | | | |
| 2 | 4.4$^{a}$ | 2.8$^{c}$ | 3.0$^{bc}$ | 2.8$^{c}$ | 2.5$^{d}$ | 3.2$^{b}$ | 3.0$^{bc}$ | 2.8$^{c}$ |
| 4 | 8.2$^{a}$ | 4.2$^{d}$ | 5.4$^{b}$ | 4.6$^{c}$ | 4.0$^{d}$ | 4.6$^{c}$ | 4.0$^{d}$ | 4.0$^{d}$ |
| 6 | 11.4$^{a}$ | 6.4$^{c}$ | 7.2$^{b}$ | 6.2$^{cd}$ | 6.0$^{d}$ | 7.4$^{b}$ | 6.4$^{c}$ | 6.0$^{cd}$ |
| 8 | 19.2$^{a}$ | 10.0$^{c}$ | 12.0$^{b}$ | 9.2$^{d}$ | 8.2$^{e}$ | 12.2$^{b}$ | 9.5$^{cd}$ | 9.0$^{d}$ |
| 10 | 38.0$^{a}$ | 20.0$^{c}$ | 21.0$^{b}$ | 18.8$^{d}$ | 17.2$^{f}$ | 18.0$^{d}$ | 16.2$^{g}$ | 15.6$^{g}$ |
| 12 | 68.0$^{a}$ | 31.0$^{b}$ | 31.0$^{b}$ | 28.0$^{c}$ | 26.0$^{d}$ | 25.0$^{d}$ | 22.0$^{e}$ | 20.0$^{f}$ |
| 14 | 109.0$^{a}$ | 41.2$^{c}$ | 44.0$^{b}$ | 40.0$^{d}$ | 35.0$^{e}$ | 35.0$^{e}$ | 33.0$^{f}$ | 30.8$^{g}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) S.O. soybean oil**

From the presented results, it could be noticed that control sample had PV values ranged from 2.2 (at zero time ) to 109after 14 days of oven holding, while PV values of other treated samples after 14 days of oven holding ranged between 30.8 and 44. Also, the inhibitory effect on PV development could be ordered as follow:

P900 >P600>P300 =G900>G600> BHT>G300.

These findings agreed with those reported by Zhang *et al.,*(2010), who found that, using of different antioxidants either natural or synthetic antioxidants led to slow development rate of PV comparing to control sample. Also, Kiralan et al. (2008), reported that sunflower oil having 500 and 1000 ppm of nigella seeds extracts recorded PV 209.44 and 166.45 , respectively, after 12 days in oven at 60°C while control sample recorded PV 305.89 .

**UV Absorbency at 232 nm, K232:**

K232 is a good parameter related to the primary oxidation products of oils and fats, while the secondary oxidation products could be measured by K270 (Ranalli et al. 2000).

K232 values of all soybean oil studied treatments were determined and tabulated in Table (3).

Data presented in table (3), showed obviously that, addition of different concentrations of Guava seeds and pomegranate peels extracts as well as BHT to soybean oil led to retarding the primary oxidation process comparing to control sample. Where K232 of control sample increased from 0.23 at zero time to 5 after 14 days of holding in oven at 65 °C , this represents 3 folds at least of any other treatment.

From the same Table, at the end of the experiment, it could be noticed that, pomegranate peels extract (900ppm) had the best effect on retarding primary oxidation comparing to the other studied treatments, while the lowest effect was observed for guava seeds extract (300ppm).

**Table (3):UV absorbancy of K232 development of different treatments during oven test of soybean oil**

| Treatments / Holding time | Control S.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 0.23 | | | | | | | |
| 2 | $0.56^{a}$ | $0.31^{bc}$ | $0.33^{b}$ | $0.30^{bc}$ | $0.25^{d}$ | $0.32^{b}$ | $0.30^{bc}$ | $0.28^{cd}$ |
| 4 | $0.85^{a}$ | $0.50^{d}$ | $0.55^{c}$ | $0.50^{d}$ | $0.45^{e}$ | $0.58^{b}$ | $0.50^{d}$ | $0.45^{e}$ |
| 6 | $1.30^{a}$ | $0.65^{c}$ | $0.73^{b}$ | $0.63^{c}$ | $0.55^{d}$ | $0.75^{b}$ | $0.64^{c}$ | $0.55^{d}$ |
| 8 | $1.75^{a}$ | $0.79^{d}$ | $0.85^{c}$ | $0.79^{d}$ | $0.70^{f}$ | $0.90^{b}$ | $0.75^{e}$ | $0.67^{g}$ |
| 10 | $2.55^{a}$ | $0.90^{d}$ | $0.95^{c}$ | $0.89^{d}$ | $0.76^{e}$ | $1.10^{b}$ | $0.90^{d}$ | $0.75^{e}$ |
| 12 | $3.50^{a}$ | $1.11^{d}$ | $1.20^{c}$ | $1.09^{de}$ | $0.90^{f}$ | $1.40^{b}$ | $1.08^{e}$ | $0.88^{f}$ |
| 14 | $5.00^{a}$ | $1.40^{d}$ | $1.50^{b}$ | $1.35^{e}$ | $1.12^{g}$ | $1.45^{c}$ | $1.30^{f}$ | $1.05^{h}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) S.O. soybean oil**

**UV Absorbency at 270 nm, K270:**

The values of K270 of all studied treatments were determined and the obtained results were presented in Table (4) for soybean oil.

The presented data showed obviously that, K270 of control sample increased from 0.023 to 0.900 after holding in oven (at 65 ◌ّC) for 14 days,

**Table (4): UV absorbency of K270 development of different treatments during oven test of soybean oil**

| Treatments / Holding time | Control S.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 0.023 | | | | | | | |
| 2 | $0.028^{a}$ | $0.025^{c}$ | $0.025^{c}$ | $0.025^{c}$ | $0.025^{c}$ | $0.026^{b}$ | $0.025^{c}$ | $0.025^{c}$ |
| 4 | $0.035^{a}$ | $0.028^{bc}$ | $0.030^{b}$ | $0.028^{bc}$ | $0.028^{bc}$ | $0.030^{b}$ | $0.027^{c}$ | $0.028^{bc}$ |
| 6 | $0.050^{a}$ | $0.031^{d}$ | $0.039^{b}$ | $0.030^{e}$ | $0.030^{e}$ | $0.033^{c}$ | $0.030^{e}$ | $0.028^{f}$ |
| 8 | $0.100^{a}$ | $0.046^{d}$ | $0.052^{b}$ | $0.040^{e}$ | $0.036^{g}$ | $0.048^{c}$ | $0.038^{f}$ | $0.034^{h}$ |
| 10 | $0.220^{a}$ | $0.065^{c}$ | $0.070^{b}$ | $0.055^{d}$ | $0.043^{f}$ | $0.070^{b}$ | $0.052^{e}$ | $0.040^{g}$ |
| 12 | $0.500^{a}$ | $0.090^{d}$ | $0.100^{b}$ | $0.081^{e}$ | $0.060^{f}$ | $0.098^{c}$ | $0.080^{e}$ | $0.055^{g}$ |
| 14 | $0.900^{a}$ | $0.120^{d}$ | $0.140^{b}$ | $0.095^{e}$ | $0.078^{g}$ | $0.135^{c}$ | $0.093^{f}$ | $0.071^{h}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) S.O. soybean oil**

where, maximum value (0.900) represents 6-12 fold more than studied treatments. The most effective treatment on retarding secondary oxidation process was recorded for pomegranate peels (P) extract (900 ppm) and guava seeds (G) extract (900 ppm), while the lowest effective treatment was recorded for guava seeds extract (300 ppm) and pomegranate peels extract (300). The inhibitory effect on secondary oxidation progress could be ordered from the highest to the lowest as follow: P900 > G900 > P600> G600> BHT>P300 >G300 .

**Fatty acids composition:**

Fatty acids composition of different treatments was determined in zero time and after 14 days of oven test (at 65 ◌̊C) for soybean oil and the results were tabulated in Table (5).

The presented data in table (5) showed that, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) , linoleic acid (C18:2) and linolenic acid (C18:3), were the main dominant fatty acids in soybean oil samples. These results are in agreement with those of **Juarez et al.(2011**), who found that , these five fatty acids , represented the main fatty acids in soybean oil with the following percentages 10.3, 4.9,21.5, 53.4, and 5.1 %, respectively. Also, Farhoosh *et al.,* (2009), found that, these percentages as follow 11.9, 4.15, 25.8, 51.1 and 5.96%, respectively.

The previous data, also indicated that, linoleic acid was the most fatty acid affected by holding soybean oil samples at 65◌̊ C for 14 days, where its percentage decreased from 55.2 % in zero time to 50.34 % for control sample. While, it was decreased by 0.85 – 3.63 % for other treatments. The minimum degradation rate of linoleic acid was recorded for oil samples treated by 900 ppm for either pomegranate peels or guava seeds extracts.

**Table (5) :Fatty acids composition of different treatments after 14 days of oven test of soybean oil**

| Treatments / Fatty acid | 0 time | Control S.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| C16:0 | 9.13 | 10.80 | 10.04 | 10.77 | 10.11 | 10.07 | 10.68 | 10.03 | 9.16 |
| C16:1 | 0.06 | 0.16 | 0.09 | 0.09 | 0.03 | 0.10 | 0.33 | 0.68 | 0.04 |
| C17:0 | 0.07 | 0.17 | 0.09 | 0.10 | 0.16 | 0.20 | ND | 0.09 | 0.08 |
| C17:1 | 0.03 | 0.04 | 0.04 | 0.04 | 0.29 | 0.07 | ND | 0.04 | 0.01 |
| C18:0 | 4.02 | 4.96 | 4.64 | 4.92 | 4.65 | 4.55 | 4.69 | 4.37 | 4.23 |
| C18:1 | 24.05 | 25.09 | 24.27 | 24.29 | 24.31 | 23.91 | 24.13 | 24.77 | 24.74 |
| C18:2 | 55.20 | 50.34 | 52.68 | 51.57 | 53.18 | 54.31 | 51.82 | 53.40 | 54.35 |
| C18:3n6 | 1.66 | 1.59 | 1.66 | 1.47 | 1.54 | 1.15 | 1.60 | 1.57 | 1.50 |
| C18:3n3 | 5.07 | 4.60 | 5.03 | 4.91 | 4.94 | 5.03 | 4.96 | 4.94 | 5.00 |
| C20:0 | 0.77 | 0.76 | 0.53 | 0.52 | 0.25 | 0.22 | 0.58 | 0.28 | 0.26 |
| C20:1 | 0.25 | 0.66 | 0.47 | 0.43 | 0.16 | 0.23 | 0.66 | 0.22 | 0.37 |
| C22:0 | 0.29 | 0.73 | 0.46 | 0.89 | 0.38 | 0.16 | 0.55 | 0.11 | 0.26 |

**S.O. soybean oil**

These findings were in harmony with those reported by Juarez *et al.,*(2011), who found that , linoleic acid percentage was decreased from 53.4 % to 50.3 after uncontinuous thermal treatment process at 180 C for 14 h ( as total thermal process time), they also, reported that a significant decrease of polyunsaturated fatty acids resulting from the oxidation reactions occurred during thermal process, with the formation of polar compounds as observed by Machado *et al.,* (2008).

From the same table, it could be noticed that, palmitic acid and stearic acid contents were slightly increased after 14 days of holding at 65◌̊ C, for all studied samples, while lenolenic acid decreased at the same conditions for all studied samples.

These results agreed with those of Juarez *et al.,* (2011), who found that palmatic acid and stearic acid contents were increased from 10.3 and 4.9 % at zero time respectively, to 11.5 and 5.6 %, respectively, after thermal process of soybean oil at 180 ◌ْC for 14h. In same conditions they noticed that linolenic acid decreased from 5.1 % to 4.1%.

**3- Effect of different extracts on sunflower oil stability:**

**Acidity (F.F.A. %):**

Concerning the acidity development during oven test (65◌ْ C for 14 days) of sunflower oil treated samples presented in Table (6), it could be noticed that, holding oil samples at 65◌ْC for 14 days led to raising in acidity **(**F.F.A. %)percentages for all studied treatments. This may be due to that, the formation of free fatty acids% are usually formed during hydrolysis of triacyglycerols and as degradation products of oxidized triacyglycerols (Aladedunye and Przybylshi, 2013).

**Table (6): The acidity (F.F.A.% as oleic acid) development of different treatments during oven test of sunflower oil**

| Treatments / Holding time | Control Sf.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 0.241 | | | | | | | |
| 2 | 0.250$^{a}$ | 0.245$^{a}$ | 0.245$^{a}$ | 0.246$^{a}$ | 0.241$^{a}$ | 0.245$^{a}$ | 0.240$^{a}$ | 0.241$^{a}$ |
| 4 | 0.280$^{a}$ | 0.268$^{b}$ | 0.269$^{b}$ | 0.255$^{e}$ | 0.251$^{f}$ | 0.262$^{c}$ | 0.258$^{d}$ | 0.252$^{f}$ |
| 6 | 0.325$^{a}$ | 0.295$^{b}$ | 0.297$^{b}$ | 0.286$^{c}$ | 0.268$^{e}$ | 0.280$^{d}$ | 0.267$^{e}$ | 0.263$^{f}$ |
| 8 | 0.385$^{a}$ | 0.320$^{c}$ | 0.330$^{b}$ | 0.305$^{d}$ | 0.283$^{f}$ | 0.307$^{d}$ | 0.297$^{e}$ | 0.277$^{g}$ |
| 10 | 0.490$^{a}$ | 0.350$^{b}$ | 0.360$^{b}$ | 0.337$^{bc}$ | 0.305$^{c}$ | 0.329$^{bc}$ | 0.325$^{bc}$ | 0.300$^{c}$ |
| 12 | 0.600$^{a}$ | 0.385$^{c}$ | 0.400$^{b}$ | 0.365$^{d}$ | 0.330$^{g}$ | 0.361$^{e}$ | 0.358$^{f}$ | 0.320$^{h}$ |
| 14 | 0.760$^{a}$ | 0.420$^{c}$ | 0.445$^{b}$ | 0.400$^{d}$ | 0.357$^{g}$ | 0.395$^{e}$ | 0.390$^{f}$ | 0.342$^{n}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) Sf.O. soybean oil**

The mentioned results also, illustrated that significant differences ($p< 0.05$) in free fatty acids contents were observed between control sample and other sun flower oil samples treated with different antioxidants. However the inhibitory effect of BHT on FFA content was lower than those of pomegranate peels extracts (900,600 and 300 ppm) and guava seeds extracts (900 and 600 ppm), but better than that of guava seeds extract (300ppm).

**Peroxide Value:(PV)**

The peroxide value development during oven test of different sunflower oil samples was determined and the results were tabulated in Table (7).

A similar trend to soybean oil samples could be noticed for sunflower oil samples in relation to peroxide value, but with higher values comparing to soybean oil. Where control sample jumped from 9.2 in zero time to 290 after 14

days of holding on 65◌ْ C. while the PV jumped from 9.2 in zero time to 65, 85, 55, 50, 57, 48 and 45.2 (Meq $O_2$/kg oil)for BHT, G300, G600, G900, P300, P600 and P900 treatments , respectively.

From the same Table, it could be observed that, among these treatments, pomegranate peels extract (900 ppm) remained the most effective antioxidant as it gave the lowest PV.

**Table (7): Peroxide values (Meq $O_2$/kg oil) of different treatments during oven test of sunflower oil**

| Treatments / Holding time | Control Sf.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 9.2 | | | | | | | |
| 2 | 17.0$^{b}$ | 16.4$^{c}$ | 18.0$^{a}$ | 15.6$^{d}$ | 15.0$^{e}$ | 16.2$^{c}$ | 15.4$^{d}$ | 15.0$^{e}$ |
| 4 | 29.8$^{a}$ | 19.8$^{c}$ | 21.0$^{b}$ | 18.2$^{e}$ | 17.4$^{f}$ | 19.0$^{d}$ | 18.2$^{e}$ | 17.4$^{f}$ |
| 6 | 45.0$^{a}$ | 25.2$^{c}$ | 28.2$^{b}$ | 24.6$^{d}$ | 23.4$^{e}$ | 25.0$^{d}$ | 22.6$^{f}$ | 21.0$^{g}$ |
| 8 | 90.2$^{a}$ | 31.6$^{c}$ | 36.0$^{b}$ | 30.0$^{d}$ | 27.2$^{f}$ | 30.0$^{d}$ | 28.0$^{e}$ | 25.0$^{g}$ |
| 10 | 140.2$^{a}$ | 41.0$^{c}$ | 50.0$^{b}$ | 35.0$^{d}$ | 29.6$^{f}$ | 34.0$^{e}$ | 30.0$^{f}$ | 27.0$^{g}$ |
| 12 | 201.0$^{a}$ | 50.0$^{c}$ | 65.0$^{b}$ | 42.0$^{d}$ | 36.0$^{f}$ | 40.0$^{e}$ | 36.0$^{f}$ | 33.0$^{g}$ |
| 14 | 290.0$^{a}$ | 65.0$^{c}$ | 85.0$^{b}$ | 55.0$^{e}$ | 50.0$^{f}$ | 57.0$^{d}$ | 48.0$^{g}$ | 45.2$^{h}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) Sf.O. soybean oil**

These results are in harmony with those reported by Iqbal et al. (2008), who found that , using pomegranate peel extracts at 250,500 and 1000 ppm and BHT (200ppm) led to lowering the PV development of sunflower oils in accelerated conditions (65°C) for different periods , comparing with control sample (without antioxidant).

Also, Roman et al.(2013), found that using α-tocopherol (as antioxidant) led to decrease PV development rate, which was raised from 0.1 to 18 and from 0.6 to 11.9 for sunflower oil control sample and treated sunflower oil sample with α-tocopherol, respectively, after 6 h of heating at 100°C.

**UV Absorbency at 232 nm, K232:**

Concerning the results of K232 of sunflower oil presented in Table (8). Which approximately, took the same trend of soybean oil, it could be noticed that, K232 value of control sample was 0.90 and reached 9.0 after 14 days of oven holding at 65 °C (2-3 fold of all treatments). The mentioned data indicated that pomegranate peels extract (900 ppm) had the best effect on retarding primary oxidation process of sunflower oil followed by Guava seeds extract (900 ppm). While the lowest effect was recorded for Guava seeds extract (300 ppm), followed by pomegranate peels extract (300 ppm).

K232 results of both soybean and sunflower oils are in harmony with those reported by Iqbal et al. (2008), who found that using pomegranate peel extract (250,500 and 1000 ppm) and BHT (200ppm) , had positive effect on lowering conjugated dienes development compared to control sample.

Also, Kiralan *et al.,* (2008), found that using 500 and 1000 ppm of nigella seed extract led to retarding K232 development in sunflower oil after 14 days of oil storage at 60° C , comparing with control sample, while using of 2000 ppm of nigella seed extract had a negative effect , where, K232 increased more than control sample.

**Table (8): UV absorbency of K232 of different treatments during oven test of sunflower oil**

| Treatments / Holding time | Control Sf.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 0.90 | | | | | | | |
| 2 | 1.40$^{a}$ | 1.10$^{d}$ | 1.30$^{b}$ | 1.10$^{d}$ | 1.00$^{e}$ | 1.20$^{c}$ | 1.20$^{c}$ | 0.99$^{e}$ |
| 4 | 2.10$^{a}$ | 1.60$^{d}$ | 1.80$^{b}$ | 1.60$^{d}$ | 1.30$^{e}$ | 1.80$^{b}$ | 1.78$^{c}$ | 1.30$^{e}$ |
| 6 | 2.90$^{a}$ | 1.90$^{d}$ | 2.09$^{b}$ | 1.85$^{e}$ | 1.60$^{g}$ | 2.05$^{c}$ | 2.09$^{b}$ | 1.62$^{f}$ |
| 8 | 4.00$^{a}$ | 2.20$^{e}$ | 2.41$^{b}$ | 2.10$^{f}$ | 1.80$^{g}$ | 2.35$^{d}$ | 2.38$^{c}$ | 1.80$^{g}$ |
| 10 | 5.50$^{a}$ | 2.60$^{e}$ | 2.90$^{b}$ | 2.40$^{f}$ | 2.05$^{h}$ | 2.75$^{d}$ | 2.80$^{c}$ | 2.08$^{g}$ |
| 12 | 7.00$^{a}$ | 3.10$^{cd}$ | 3.50$^{b}$ | 2.75$^{d}$ | 2.35$^{e}$ | 3.30$^{bc}$ | 3.30$^{bc}$ | 2.33$^{e}$ |
| 14 | 9.00$^{a}$ | 3.80$^{d}$ | 4.20$^{b}$ | 3.20$^{f}$ | 2.80$^{g}$ | 3.95$^{c}$ | 3.50$^{c}$ | 2.75$^{g}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) Sf.O. soybean oil**

**UV Absorbency at 270 nm, K270:**

Regarding to K270 of sunflower oil samples in table (9), it could be concluded that, the higher the extracts concentration the higher the inhibitory effect on secondary oxidation process for either guava seeds extracts or pomegranate peels extracts comparing to control sample.

Also, BHT recorded higher inhibitory effect than both pomegranate peel extract (300 ppm) and guava seed extract (300 ppm), while it was lower than the other treatments of both guava seed and pomegranate peel extracts during all oven holding periods.

The development of K270 (secondary oxidation process measure) was very high in control sample which reached 1.200 after 14 days of oven holding at 65 ◌C, this value represents around 10 folds more than the highest value of all other treatments , approximately.

**Table (9): UV absorbency of K270 of different treatments during oven test of sunflower oil**

| Holding time / Control Bf.O. | Control Bf.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| 0 | 0.039 | | | | | | | |
| 2 | 0.044$^{a}$ | 0.040$^{a}$ | 0.042$^{a}$ | 0.040$^{a}$ | 0.040$^{a}$ | 0.043$^{a}$ | 0.040$^{a}$ | 0.040$^{a}$ |
| 4 | 0.058$^{a}$ | 0.050$^{a}$ | 0.054$^{a}$ | 0.046$^{a}$ | 0.045$^{a}$ | 0.056$^{a}$ | 0.043$^{a}$ | 0.043$^{a}$ |
| 6 | 0.077$^{b}$ | 0.063$^{d}$ | 0.070$^{c}$ | 0.056$^{e}$ | 0.053$^{f}$ | 0.079$^{a}$ | 0.050$^{g}$ | 0.048$^{h}$ |
| 8 | 0.120$^{a}$ | 0.073$^{d}$ | 0.081$^{c}$ | 0.062$^{e}$ | 0.058$^{f}$ | 0.085$^{b}$ | 0.059$^{f}$ | 0.055$^{g}$ |
| 10 | 0.235$^{a}$ | 0.085$^{d}$ | 0.090$^{c}$ | 0.075$^{e}$ | 0.067$^{f}$ | 0.094$^{b}$ | 0.068$^{f}$ | 0.062$^{g}$ |
| 12 | 0.555$^{a}$ | 0.097$^{d}$ | 0.102$^{c}$ | 0.087$^{e}$ | 0.074$^{h}$ | 0.110$^{b}$ | 0.084$^{f}$ | 0.079$^{g}$ |
| 14 | 1.200$^{a}$ | 0.110$^{d}$ | 0.125$^{c}$ | 0.099$^{e}$ | 0.081$^{h}$ | 0.130$^{b}$ | 0.091$^{f}$ | 0.084$^{g}$ |

**Values bearing the same superscript within the same row are not significantly different (P> 0.05) Sf.O. soybean oil**

The results of K270 for either soybean or sunflower oils agreed with those reported by Iqbal *et al.,* (2008), who reported that using of 250, 500 and 1000 ppm of pomegranate peels extract as well as BHT (200 ppm) led to decrease the formation of conjugated trienes during storing sunflower oil at 65 ◌C for 24 days comparing with control sample.

**Fatty acids composition:**

In relation to fatty acid composition results of sunflower oil samples presented in Table (10), illustrated that, the main dominant fatty acids in sunflower oil samples were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), which their contents were 6.06, 4.03, 23.55 and 64.15%, respectively. These results were in harmony with those reported by Roman *et al.,* (2013), who found that the content of these four fatty acids in sunflower oil were 5.92, 4.14, 26.04 and 62.27 % , respectively. The presented data indicated that, the most fatty acids affected by oven holding at 65° C for 14 days was linoleic acid, which decreased from 64.15 % in zero time to 60.75 % for control sample. While, it was decreased about 0.58 – 2.82 % for other studied treatments. The minimum degradation rate of linoleic acid was recorded for oil samples treated by 900 ppm for either pomegranate peel or guava seed extract. These results were in harmony with those of Kowalski, (2009), who found that polyunsaturated fatty acid content was decreased by heating sunflower oil at 90 °C for 120 h. He, also noticed that, the maximum decrease was recorded for control sample (5 % ), while sunflower oil samples with sliphumperfoliatum rhizome extracts , were characterized by higher content (from 2-5%) of linoleic acid compared with control sample with no extract addition.

**Table (10): Fatty acid composition of different treatments after 14 days of oven test of sunflower oil**

| Treatments / Fatty acids | 0 time | Control Sf.O. | BHT | Guava seeds extract (G) | | | Pomegranate peels extract (P) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 300 ppm | 600 ppm | 900 ppm | 300 ppm | 600 ppm | 900 ppm |
| C16:0 | 6.06 | 6.89 | 6.81 | 6.88 | 6.93 | 6.96 | 6.71 | 6.61 | 6.39 |
| C16:1 | 0.01 | 0.14 | 0.04 | 0.12 | 0.03 | 0.05 | 0.15 | 0.02 | 0.08 |
| C17:0 | 0.03 | 0.03 | ND | 0.03 | 0.02 | 0.06 | 0.05 | 0.11 | 0.03 |
| C17:1 | 0.04 | 0.01 | ND | 0.02 | ND | 0.01 | ND | ND | ND |
| C18:0 | 4.03 | 4.88 | 4.52 | 4.88 | 4.68 | 4.53 | 4.82 | 4.50 | 4.42 |
| C18:1 | 23.55 | 24.92 | 24.65 | 24.63 | 24.59 | 24.07 | 24.68 | 24.11 | 23.90 |
| C18:2 | 64.15 | 60.75 | 61.67 | 61.33 | 62.11 | 63.47 | 61.42 | 63.10 | 63.57 |
| C18:3n6 | 0.45 | 0.24 | 0.17 | 0.19 | 0.14 | 0.15 | 0.29 | 0.30 | 0.41 |
| C18:3n3 | 1.29 | 0.58 | 0.65 | 0.54 | 0.78 | 0.85 | 0.68 | 0.81 | 0.88 |
| C20:0 | 0.14 | 0.44 | 0.36 | 0.25 | 0.15 | 0.05 | 0.38 | 0.18 | 0.13 |
| C20:1 | 0.12 | 0.24 | 0.30 | 0.16 | 0.27 | 0.07 | 0.22 | 0.05 | 0.02 |
| C22:0 | 0.13 | 0.88 | 0.83 | 0.97 | 0.30 | 0.13 | 0.60 | 0.21 | 0.17 |

**Sf.O. soybean oil**

Data in the same table demonstrated that, palmitic acid and stearic acid contents were slightly increased after 14 days of holding at 65 °C, for all studied treatments, while lenolenic acid decreased at the same conditions for all studied samples.

These results agreed with those of Juarez *et al.,* (2011), who found that palmatic acid and stearic acid contents were increased from 6.5 and 3.5 % at zero time, respectively, to 7.3 and 4 %, respectively, after thermal process of sunflower oil at 180 C for 14h. In same conditions they noticed a slight decrease in linoleneicacid content from 0.4% to 0.3%.

## CONCLUSION

From this investigation, it could be concluded that, extracts of pomegranate peels and guava seeds at 600 -900 ppm, may play an important role in replacing synthetic antioxidants such as BHT during the production of soybean and sunflower oils. Also, it must be noticed that, using of these extracts required more attention, where the concentration of used antioxidants, storage temperature and fatty acids composition of the oil play an important role and have great effects on the oxidation stability.

Also, besides using of pomegranate peels and guava seeds in stabilizing vegetable oils, it could be recommended that, pomegranate peel and guava seed powders could be used as food additives for different food systems, such as infants' foods, after many investigations.

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## الاستفادة من مستخلصات مضادات الأكسدة الطبيعية لقشور الرمان وبذور الجوافة فى ثبات الزيوت النباتية

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**وحدة التصنيع الزراعي – قسم الإنتاج النباتي – مركز بحوث الصحراء القاهرة**

إدارة المخلفات احد أهم الاتجاهات الحديثة في التصنيع الغذائي وذلك للتغلب على المشاكل المتعلقة بتراكمها وفى نفس الوقت لتعظيم قيمتها الاقتصادية. التصنيع الغذائي ينتج عنه كميات كبيرة من قشور الرمان وبذور الجوافة والتي يمكن الاستفادة منها كمصادر طبيعية لمضادات الأكسدة .

المستخلص الإيثانولى لكل من قشور الرمان وبذور الجوافة (بعد تجفيفها) كلا على حدي بتركيزات ٣٠٠، ٦٠٠ ، ٩٠٠ جزء فى المليون ، تم دراسة تأثيرها المضاد للأكسدة عند إضافتها لكل من زيت دوار الشمس وزيت الصويا (المكرر والمبيض ومنزوع الرائحة ، RBD) وكذلك تم إضافة بيوتيلاتيد هيدروكسى تولوين BHT (٢٠٠ جزء فى المليون) كمضاد أكسدة صناعي شائع الاستعمال ومقارنة الجميع بعينة كنترول بدون إضافات. وتم قياس كل من رقم البيروكسيد والامتصاص فى منطقة الأشعة البنفسجية (K232, K270)

والحموضة وكذلك التغيرات فى تركيبة الأحماض الدهنية لكل زيت ناتج من المعاملات السابقة وذلك تحت ظروف تسريع الأكسدة (اختبار الفرن). وأظهرت النتائج المتحصل عليها أن كل من مستخلصات قشور الرمان وبذور الجوافة عند تركيزات ٦٠٠ ، ٩٠٠ جزء فى المليون كانت لها فعل مضاد للأكسدة أكبر من BHT ، بينما كانت مستخلصات قشور الرمان وبذور الجوافة ذات التركيز ٣٠٠ جزء فى المليون اقل تأثيرا من BHT .

هذه النتائج أظهرت مدى إمكانية استخدام كل من هذه المخلفات كمصادر طبيعة هامة لمضادات الأكسدة المستخدمة في تأخير أكسدة الزيوت النباتية وزيادة عمرها التخزيني على نطاق صناعي ، بل يمكن استخدام مساحيق هذه المواد فى كثير من التطبيقات الغذائية بعد دراسة متأنية.