Scavenging Activity of Grapefruit Peel and Seed Extract, a Natural Source of Antioxidant for the Stabilization of Soybean and Sunflower Oil

SONIA RASHEED[1], MUHAMMAD IMRAN[1], SUMMIA REHMAN[1], AMNA FAROOQ[1], IMTIAZ BEGUM[1], FARAH KANWAL[1], LIVIU MITU[2]*

[1]Institute of Chemistry, University of the Punjab, Lahore-54590, Pakistan

[2] University of Pitesti, Department of Chemistry, 1 Tg. din Vale, 110040, Pitesti, Romania

Grapefruit is source of anticancer and antioxidant reagents. Extracts of grapefruit peel (Gf-P) and seeds (Gf-S) were screened for their antioxidant capabilities at room temperature to stabilize soybean and sunflower oil for a storage period of 45 days. β-carotene linoleic acid system, FRAP assay, ABTS method were performed for the measurement of antioxidant potential of Gf-P and Gf-S. Oil samples containing extracts of Gf-P and Gf-S at 400 ppm, 800 ppm, 1200 ppm concentrations as well as synthetic antioxidants (BHT and BHA) at 200 ppm concentration were prepared. Free fatty acid (%) and peroxide (meq/Kg) values of all these prepared samples were evaluated. Results were statistically analyzed and it was observed that Gf-P has better antioxidant potential than Gf-S.

Keywords: Antioxidants, grapefruit, soybean oil, sunflower oil

During storage, oil deterioration is affected by several factors such as temperature, light,

compositions of fatty acid, *etc* [1]. Antioxidants reduce the oxidation of oils by donating hydrogen which combine with free radicals and convert them into non radical forms [2]. It is well established in the literature that fruits, vegetables, herbs and cereals are rich sources of natural antioxidants [3, 4]. In the world main producer of grapefruit is Florida. The main constitutent of grapefruit *i.e. Naringin* (flavanone-7-O-glycoside) acts as an antioxidant and free radical scavenger [5] while the other constituent *i.e. Naringenin* (flavanone) acts as an anticancer agent [6]. Both these constituents are present in higher concentration. Moreover, grapefruit is a source of limonoids which are cancer inhibitors [7]. The grapefruit can also be used as supplement for disease preventing diets [8]. In the literature antioxidant activity of seed extract of grapefruit on vegetable oil (soybean and sunflower oil) is reported [9]. But peel extract of grapefruit for the stabilization of edible oil has not been reported yet. The aim of present study is to describe the antioxidant potential of grapefruit peel and seed extracts. Moreover a comparative study of both (Gf-P and Gf-S) extracts to stabilize soybean and sunflower oil during storage is also part of this manuscript.

Experimental part

Sunflower and soybean oil used during stabilization studies were obtained from Layyah, Pakistan. These oils were refined, however collected before addition of synthetic antioxidants. Grapefruit was purchased from local market of Lahore, Pakistan. All the chemicals and reagents were of analytical grade and were used as received. Synthetic antioxidants butyl hydroxytoluene (BHT) and butyl-hydroxyanisole (BHA) were procured from Fluka.

Extract preparation

Grapefruits were peeled off, grapefruit's peel (Gf-P) and seeds (Gf-S) were dehydrated at room temperature (22°C) for 10 days. Dehydrated grapefruit's peel and seeds were powdered. Methanol was used as a solvent as it is reported to be the best solvent for extraction [10]. Ratio of sample to solvent was kept 1:30. The extracts were kept to shaking at room temperature for 3 days. The extracts were filtered and filtrates were evaporated till a semi solid extract was obtained. After this, yields were calculated and extracts were stored in refrigerator for further analysis.

Antioxidant potential

Estimation of total phenolic contents

By using Folin-Ciocalteu (FC) reagent, total phenolic contents of Gf-P and Gf-S were determined as per reported method [10]. Precisely, 0.2 mL of diluted extract was added in 0.8 mL of freshly prepared FC reagent (10 % v/v). After 5 min shaking, 2.0 mL of Na_2CO_3 (7.5 %) and 7 mL of distilled water were added. The reaction mixture was kept in dark and absorbance was noted at 765 nm against blank using gallic acid as a standard. The results are reported in GAE (mg/100g) of dry weight.

Total Flavonoids contents

Extracts of Gf-P and Gf-S (1.0 mL) were added in H_2O. Then, 0.3 mL of $NaNO_2$ (5 %), after 5 minutes 0.3 mL of $AlCl_3$ (10%) and at 6th min 2 mL of NaOH (1M) were added in flasks. The solution was diluted with water (2.4 mL) immediately and then mixed thoroughly. Absorbance was noted at 510 nm and catechin was used to develop a standard curve [11]. The results were reported as catechin equivalent (CE) as mg/100g of dry extract.

β-carotene linoleic acid system

Antioxidant potential of Gf-P and Gf-S was measured according to previously established protocol [12]. Briefly, β-carotene (2 mg) was added in chloroform (10 mL). Then, 2 mL of this solution was taken in round bottom flask. Linoleic acid (40 mg) and Tween 20 (400 mg) were then added in flask after removal of chloroform. 5 mL of this emulsion and 0.2 mL of Gf-P and Gf-S extracts were taken in test tubes. The absorbance was measured at 470 nm after maintaining 50°C at 0, 15, 30, 45 and 60 min against blank.

4. 2,2'-*azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay*

ABTS assay was done using a previously reported protocol [13]. According to this method, potassium

* email: ktm7ro@yahoo.com; Phone: 0040/725160304

persulphate (2.45 mM) and 2,2'-azinobis-3-ethyl-benzothiazoline- 6-sulphonic acid (ABTS) (7 mM) were mixed with same ratio. This mixture was left for 4 to 16 h until its color changed to blue green and then diluted with ethanol. 0.9 mL of this solution was mixed with 0.1 mL of sample and absorbance was measured at 734 nm. Results were expressed by using the following formula:

$$ABTS^{\bullet+} \text{ radical scavenging } (\%) = ((Ac - At)/\ Ac) \times 100$$

where, Ac is the absorbance of only $ABTS^{\bullet+}$ solution and At is the absorbance of the sample which reacted with $ABTS^{\bullet+}$ solution.

Ferric-reducing antioxidant power (FRAP) assay

25 mL acetate buffer (300 mmol/liter) was added in 2.5 mL 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (10 mmol/liter in 40 mmol/liter HCl). Then 2.5 mL $FeCl_3 \cdot 6H_2O$ (20 mmol/liter) was added for the preparation of FRAP reagent. 0.3 mL of freshly prepared FRAP reagent and 0.01 mL of extracts were mixed along with 0.03 mL of water. Absorbance was recorded at 593 nm against blank [14]. $FeSO_4 \cdot 7H_2O$ (0.2 mM/L to 1 mM/L) was used to develop the standard curve and the antioxidant activity was expressed as concentration of antioxidants having ferric reducing ability equivalent to mM/L of $FeSO_4 \cdot 7H_2O$.

Stabilization of soybean and sunflower

Soybean and sunflower oil samples containing 400 ppm, 800 ppm and 1200 ppm concentration of Gf-P and Gf-S were prepared. Similarly oils containing synthetic antioxidants BHT and BHA (200 ppm) were also prepared. These samples were kept for 45 days. Free fatty acid (FFA) and peroxide value (PV) of these samples were measured after 15 days interval [15]. FFA was expressed as (%) oleic acid and PV as meq/kg oil.

Statistical analysis

All the determinations were carried out in triplicate and data is presented as mean ± standard deviation. Significant differences ($p < 0.05$) were calculated by using one way ANOVA.

Results and discussions

The percentage yield of methanolic extracts of grapefruit's peel (Gf-P) and seeds (Gf-S) were 14.73 ± 0.11 and 13.67 ± 0.115 respectively. Methanol was used as solvent keeping in view its better extraction efficiency compared to other common solvents as reported in literature [10]. Flavonoids are phenolic substances which are present in plants and act as antioxidants [16]. Total phenolic and flavonoids contents as measured by using FC reagent in Gf-P and Gf-S are given in table 1.

FRAP is a quantitative assay for the determination of antioxidant potential. In this method, Fe^{3+} is initially reduced by antioxidants to Fe^{2+}. As a result of reduction a dark blue colored product is formed which is measured at 593 nm. 0.526 ± 0.01 mM/L of $FeSO_4$ concentration of Gf-P and 0.515 ± 0.21 mM/L of $FeSO_4$ concentration of Gf-S were measured by calibration curve.

In the β-carotene linoleic acid system, the rate of b-carotene decolorization can be reduced in the presence of antioxidants. Figure 1 illustrates that as the time increases, the difference in initial absorbance of sample and final absorbance of sample also increases indicating that the presence of antioxidants reduced the decolorization of β-carotene. The minimum increase of absorbance difference by increasing time, showed higher antioxidant potential. The graph shows that BHT has higher antioxidant potential as compared to the others used during this study. The antioxidant activity of Gf-P, Gf-S, BHA and BHT were calculated by given formula:

$$\text{Antioxidant activity } (\%) = \left(\frac{Bc-Bs}{Bc}\right) \times 100$$

where, *Bc* is difference in initial and final absorbance of control and *Bs* is difference in initial and final absorbance of sample [17]. The antioxidant activity (%) was as follow; BHT 82.95 ± 0.04, BHA 70.82 ± 0.15, Gf-P 75.53 ± 0.50 and Gf-S 57.84 ± 0.7.

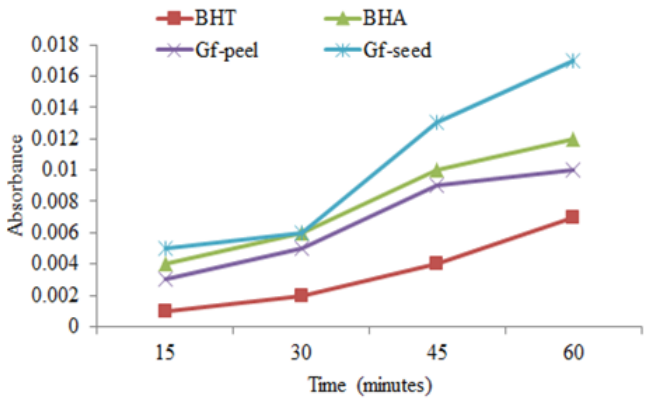

Fig. 1. Graph between absorbance (sample initial absorbance - final absorbance) and time (min)

ABTS radical scavenging method was also used for the determination of antioxidant potential. The percentage antioxidant activity was determined by the given formula:

$$\text{Antioxidant activity } (\%) = \left(\frac{Ac-As}{Ac}\right) \times 100$$

Ac and *As* are the absorbance of control and sample respectively [18]. The obtained results by this method are summarized in figure 2. According to this figure, BHT shows highest radical scavenging activity. The order of decrease in the antioxidant activity (%) was BHT ΔGf-P Δ BHA Δ Gf-S.

Stabilization of Soybean and Sunflower oil

Free Fatty Acid (FFA)

The changes in FFA (%) of soybean and sunflower oil have been graphically represented in (fig. 3,4) and (fig. 5,6) respectively. The increase in FFA value was observed in each sample with a maximum increase in control samples *i.e.* without antioxidant. Methanolic extracts of Gf-P and Gf-S showed a great effect in stability of soybean

Sample	Total Phenolic contents (mg/100g)	Flavonoids contents (mg/100g)
Grapefruit peel (Gf-P)	221.02 ± 0.02	130.95 ± 0.070
Grapefruit seed (Gf-S)	187.07 ± 0.24	11.5 ± 0.00

Table 1
TOTAL PHENOLIC AND FLAVONOIDS CONTENTS OF GF-P AND GF-S

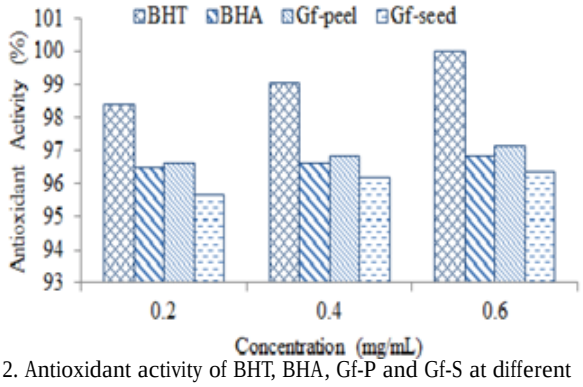

Fig. 2. Antioxidant activity of BHT, BHA, Gf-P and Gf-S at different concentrations

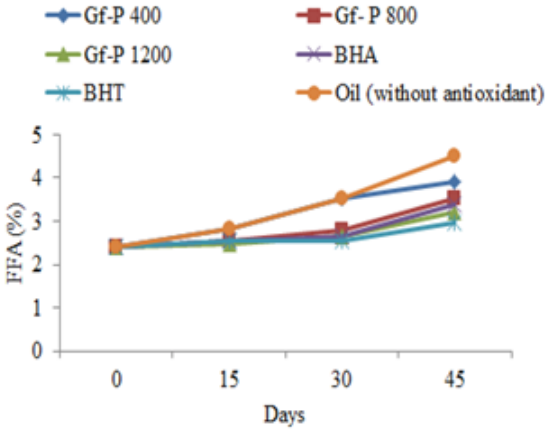

Fig. 3. Free Fatty acids (%) value of soybean oil (SB) containing natural antioxidant (Gf-P 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

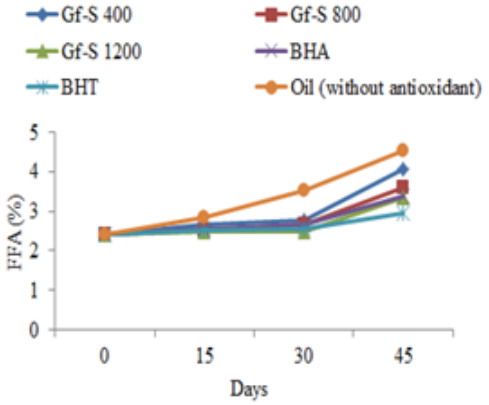

Fig. 4. Free Fatty acids (%) value of soybean oil (SB) containing natural antioxidant (GS-S 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

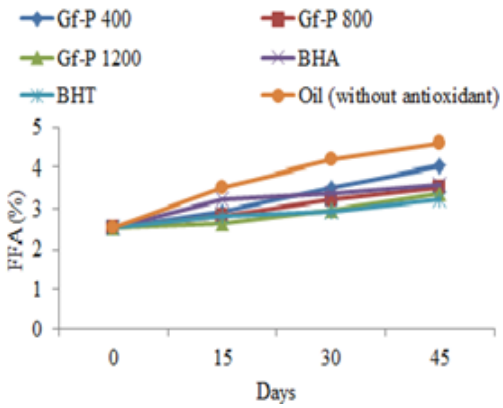

Fig. 5. Free Fatty acids (%) value of sunflower oil (SF) containing natural antioxidant(Gf-P 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

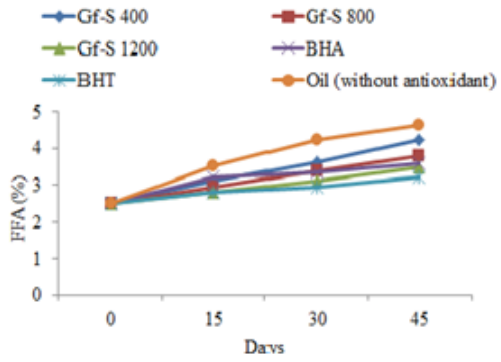

Fig. 6. Free Fatty acids (%) value of sunflower oil (SF) containing natural antioxidant (Gf-S 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

oil. The efficiency in retardation of rancidity development was observed as follow: BHT> Gf-P> Gf-S> BHA. At 30 day Gf-P effect on FFA value of oil was almost equal to the BHT. Similar trend was observed in case of sunflower oil at 30 day. Our findings are in agreement with the related literature [19].

Peroxide Value (PV)

Degree of initial oxidation of oil and fats is usually measured by peroxide value (PV). Generally an increase in PV of both oils during storage (45 days) was observed and is represented in (fig. 7-10). The PV (%) of soybean oil containing BHT, Gf-P, BHA and Gf-S was 3.1 ± 0.14, 3.25 ± 0.07, 3.4 ± 0.00, 3.55 ± 0.07 (meq/Kg) respectively which were less than PV of soybean oil without antioxidant (fig.

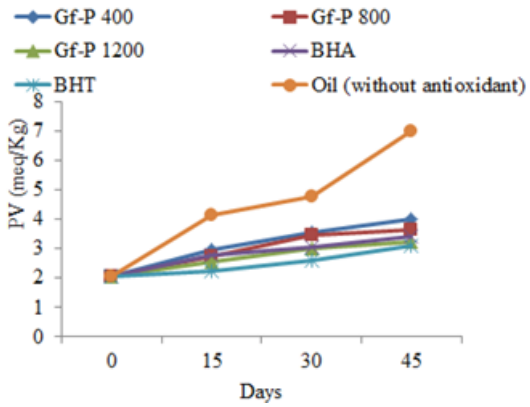

Fig. 7. Increase in PV of soybean oil (SB) containing natural antioxidant (Gf-P 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

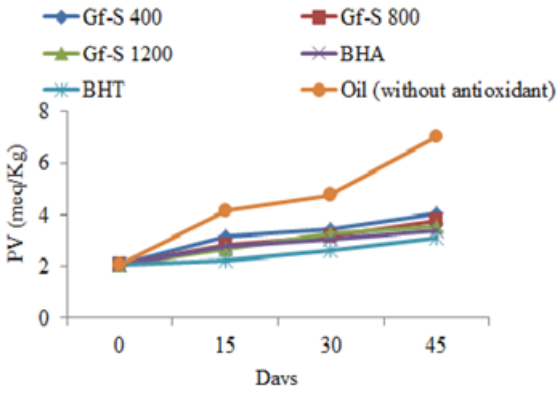

Fig. 8. Increase in PV of soybean oil (SB) containing natural antioxidant (Gf-S 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

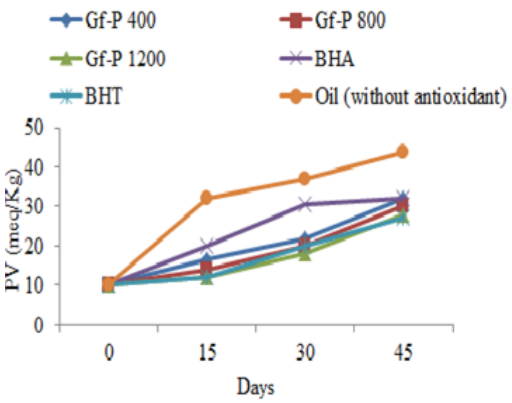

Fig. 9. Increase in PV of sunflower oil (SF) containing natural antioxidant (Gf-P 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

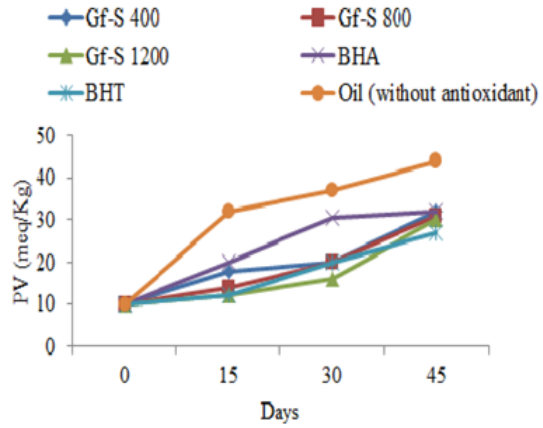

Fig. 10. Increase in PV of sunflower oil (SF) containing natural antioxidant (Gf-S 400, 800 and 1200), synthetic antioxidant (BHT, BHA) and only oil without antioxidant

7,8). In sunflower oil BHT showed minimum increase in PV but Gf-P and Gf-S presented better results than BHA. The results of PV of sunflower oil treated with synthetic and natural antioxidant ($P < 0.05$) have been shown in (fig. 9,10). Inhibition order of oxidation of sunflower oil is as follow: BHT > Gf-P (1200 ppm) > Gf-S (1200 ppm) > BHA.

Studies concerning the soybean and sunflower oils were published in [20].

Conclusions

It is concluded from the study that extracts of Gf-P and Gf-S exhibited appreciably good antioxidant potential when compared with recommended synthetic antioxidants like BHA, BHT frequently used for stabilization of edible oil. These are agriculture wastes and can be optimized into valuable products for retardation of oxidation of oil.

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