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Sunflower and soybean oil stabilized with natural extracts of turnip's peel

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Turnip (*Brassica rapa*) is considered to be one of the oldest cultivated vegetable since prehistoric times. Antioxidant activity of methanolic extracts from turnip's peel (TP) at variable concentrations (250 ppm, 500 ppm, 1000 ppm) was determined by total phenolic contents, total flavonoid contents, ferric reducing antioxidant power assay, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation scavenging activity and β-carotene linoleic acid emulsion system. The highest antioxidant activity of turnip's peel was observed at 1000 ppm. Stabilization of two edible oils *i.e*. sunflower and soybean using these extracts was evaluated under ambient conditions. Butyl-hydroxyanisole (BHA) and butyl-hydroxytoluene (BHT) at 200 ppm were used as synthetic reference antioxidants. Parameters like peroxide values (PV), free fatty acid values (FFA) and iodine values (IV) demonstrated that antioxidant potential of turnip's peel extract is relatively more than BHA and slightly less than BHT.

Keywords: *Brassica rapa*, Antioxidant activity, Sunflower oil, Soybean oil.

INTRODUCTION

Antioxidants are the substances that terminate oxidation, the absence of which effectuates membrane lipids, deoxyribonucleic acid (DNA), proteins and carbohydrates damage and in some cases even lead to cell death. In order to cope this, synthetic antioxidants such as butyl-hydroxyanisole (BHA) and butyl-hydroxytoluene (BHT) are widely utilized as food additives to stop such oxidative deterioration. However, they are quite toxic as well expensive. In this context, there is a rapid need to replace synthetic antioxidants with natural antioxidants for safety concerns. Natural antioxidants have opened a floor of great applications in nutraceuticals for disease prevention [1]. It is well established in the literature that several fruits, vegetables, plants, nuts, oilseeds and many other materials containing natural antioxidants have scavenging abilities [2] and can hence be employed for stabilization of vegetable oils.

Turnip (*Brassica rapa*) belongs to family *Cruciferae* or *Brassicaceae* and usually cultivated in different regions particularly possessing temperate climates [3]. It contains vitamin C, E, phenolic compounds, β-carotene and flavonoids and therefore can scavenge free radicals production and oxidative reactions. Search through accessible literature reveal that not so much work with reference to antioxidant potential of turnip has been reported except; chemical and antioxidative assessment of dietary turnip [4], phenolic compounds in brassica vegetables [5], growth and antioxidant response of *Brassica rapa* (turnip) irrigated with different compositions of paper and board mill (PBM) effluent [6], nutritional facts and antioxidant activity of turnip by 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) method [7]. These studies demonstrated that turnip have good antioxidant properties. However, it was surprising to be noted in the accessible literature that no work describing the stabilization of sunflower and soybean oil with natural antioxidants extracted from turnip's peel has been yet reported. Therefore in this paper we describe antioxidant potential of turnip's peel extract (turnip collected from local market) as well as its efficiency to stabilize the sunflower and soybean oil.

EXPERIMENTAL

Materials

Refined, bleached and deodorized (RBD) sunflower and soybean oil were obtained from local refinery located in Layyah, Pakistan. Turnips were 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.

Preparation of extract

Turnip (*Brassica rapa*) was peeled off, thoroughly washed with deionized water and finally dried at room temperature for one week. The dried

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peel was then pulverized to fine powder. 5 g of peel was soaked in 150 mL of methanol and then stirred well on shaker at room temperature and then filtered. Methanol has been recommended to be best solvent for extraction of antioxidants [8]. The resulting filtrate was evaporated at room temperature to yield semi solid extract which was used for further analysis.

Evaluation of antioxidant activity of extract

Following different assays were performed to determine the antioxidant activity of turnip's peel.

Determination of total phenolic content

The total phenolic content of turnip's peel extract were determined by an already reported method [9]. Absorbance at 765 nm was measured and the total phenolic contents in turnip's peel were calculated from the standard curve of gallic acid. The results are reported in GAE (mg/100 g) of dry weight.

Determination of total flavonoids

Total flavonoids were measured spectrophotometrically using the method described by [10]. Absorbance of the mixture was determined at 510 nm and calibration curve was prepared by using catechin at concentrations of 0.2 to 1 mg/mL in methanol. The results were reported as catechin equivalent (CE) as mg/100g of dry extract.

Ferric-reducing antioxidant power assay (FRAP)

FRAP assay was performed according to well documented reported method [10]. 10 µl of extract, 300 µl of FRAP reagent was added in 30 µl of distilled water and its absorbance was measured at 593 nm. $FeSO_4.7H_2O$ at different concentrations (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 $FeSO_4.7H_2O$.

Determination of ABTS radical cation scavenging activity

The ABTS (2,2'-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid) radical cation assay was carried out by the procedure reported in the literature [11]. The absorbance of sample at 734 nm was measured against control and the results were expressed by the following formula.

$ABTS^{\bullet+}$ radical scavenging (%)=((Ac-At)/Ac) x 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.

β-carotene linoleic acid emulsion system

Antioxidant activity of extract was determined by the β-carotene linoleic acid emulsion system by an already reported method [12]. Absorbance was recorded at 470 nm after every 15 minutes for 1 hour.

STABILIZATION STUDIES

Sample preparation

Methanolic extracts of turnip's peel (250, 500 and 1000 ppm concentrations) were added to RBD sunflower and soybean oil. Synthetic antioxidants, BHT and BHA (200 ppm) were also added in both oils for comparative study [13]. Parallel control sample of both oils *i.e*. without any antioxidants were also prepared. All the samples were stored at room temperature for 45 days. On first day, after 15 days, 30 days, 45 days analysis was carried out to check the stabilization of oils by antioxidants.

Determination of free fatty acid value (FFA), peroxide value (PV) and iodine value (IV)

Antioxidant potential of turnip's peel extract for stabilization of sunflower and soybean oil was evaluated for free fatty acid, peroxide and iodine values. Each prepared oil sample was tested for antioxidant activity after regular interval of 15 days till 45 days by following the AOAC official methods [14].

Statistical analysis

All the samples were set in triplicate and results were reported as mean ± standard deviation. Significant differences of data ($P < 0.05$) were tested by using one way ANOVA.

RESULTS AND DISCUSSION

Measurement of antioxidant activity of extract

Total phenolic and total flavonoid contents of methanolic extract of turnip's peel are 54.6 and 25.4 mg/100g, respectively.

The presence of such phenolic and flavanoid compounds in turnip's peel may impart antioxidant potential to it. It is reported that antioxidant activity of phenolic compounds is mainly due to redox properties, hydrogen donors, singlet oxygen quenches and metal chelators [15].

In FRAP assay, the reduction of methanolic extract of turnip's peel was measured. The FRAP assay showed the reducing ability of extract 0.359 ± 0.01 mM/L of $FeSO_4$ which indicates that there is a presence of antioxidative potential in turnip's peel. $ABTS^{\bullet+}$ method shows free radical scavenging

activity of turnip's peel methanolic extract with the decolorization of its blue color. Literature reveals that reactions of phenols with ABTS radical cations are rapid, hence more will be the presence of phenolic content in extract more will be the free radical scavenging ability of extract [16]. Antioxidant activity of turnip's peel extract determined by this method was compared with synthetic antioxidants (BHA and BHT) at different concentration such as 0.2, 0.4 and 0.6 mg/mL Fig.1. This figure depicts that by increasing concentration of turnip's peel extract (0.6 mg/mL), radical scavenging activity increases and becomes almost equal to that of BHA. However it is still lower than BHT.

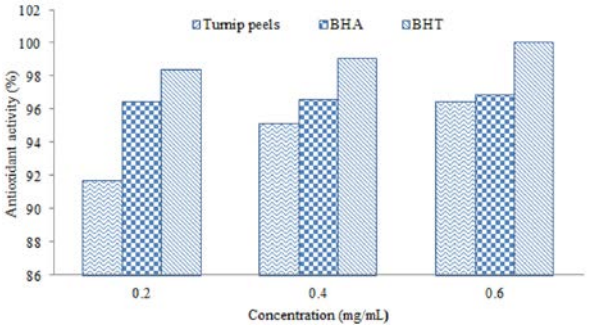

Fig. 1. Comparison of antioxidant activity (%) of turnip's peel extract with BHA and BHT.

β-Carotene linoleic acid emulsion system is also a useful method to assay the antioxidant potential [17]. It is observed and reported that the presence of antioxidants increases with the reduction of decolorization rate of β-carotene. Fig. 2 show the graph plotted between absorbance of samples at 470 nm and time required by the sample to decolorize β-carotene.

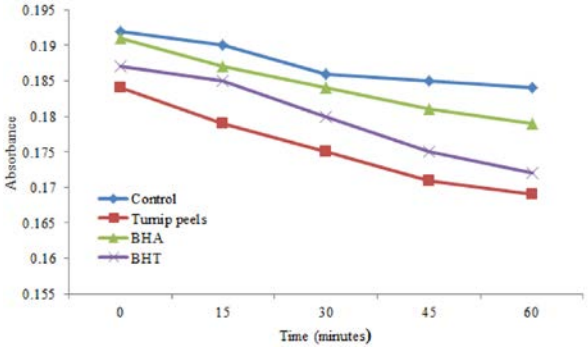

Fig. 2. Graph between absorbance of samples at 470 nm and time (minutes) required by the sample to decolorize β-carotene.

It can be concluded from these results that with increase of time, turnip's peel extract has maximum antioxidants as observed by the reduction of decolorization rate of β-carotene (absorbance 0.184 to 0.169). Similar trend for synthetic antioxidants was found to be BHA (0.191 to 0.179) and BHT (0.187 to 0.172). Least reduction was observed in control sample i.e. only linoleic acid (0.192 to 0.184). Thus β-carotene linoleic acid emulsion system proved that the turnip's peel extract have enough potential to be used as natural antioxidant.

Stabilization of sunflower oil (SFO) and soybean oil (SBO)

RBD sunflower and soybean oil were used as oxidative substrates. Stabilization of these oils was done using turnip's peel extracts. Comparison was made with synthetic antioxidants (BHA and BHT).

Peroxide value (PV)

PV determines the initial oxidation of fats and oils which occurred due to the formation of peroxides [8]. Fig .3 and Fig. 4 exhibit the gradual increase in PV during storage period at room temperature of treated sunflower and soybean oil samples. On 45th day, maximum enhancement in peroxide value was observed for all samples in both oils. Initially, PV of control sunflower oil sample was 9.85 ± 0.21 meqO_2/kg which reaches to 43.6 ± 0.28 meqO_2/kg of oil on 45th day, whereas PV of control soybean sample was observed as 2.1 ± 0.14 meqO_2/kg of oil which reaches to maximum value of 7.03 ± 0.23 meqO_2/kg on 45th day. Control sample showed maximum oil deterioration with increase of storage period as it was without antioxidants. Turnip's peel extract (1000 ppm) showed minimum PV value in both oils; initially in sunflower oil sample it was 9.85 ± 0.21 meqO_2/kg which raised to 19.9 ± 0.42 meqO_2/kg of oil on 45th day. Similarly in soybean sample it was 2.1 ± 0.14 meqO_2/kg on 0 day and 2.99 ± 0.01 meqO_2/kg of oil on 45th day which shows the antioxidant presence in methanolic turnip's peel extract preventing oil from deterioration. Our results are consistent with the findings of other workers who reported that lipid peroxides were significantly reduced by the addition of natural antioxidants in oils [18, 19]. PV for other concentrations of turnip's peel extract (250 ppm and 500 ppm) and BHA, BHT (200 ppm) were also measured and has been graphically represented in Fig. 3 and Fig. 4 for sunflower and soybean oil respectively. From these figures following trend has been concluded; for sunflower oil, the trend is: TP 1000 ppm > BHT 200 ppm > TP 500 ppm > TP 250 ppm > BHA 200 ppm > Control; For soybean oil, the trend is as follow: TP 1000 ppm > BHT 200 ppm > BHA 200 ppm > TP 500 ppm > TP 250 ppm > Control. Comparable results of TP extract at 1000 ppm with synthetic antioxidants BHA and BHT were observed in both oils.

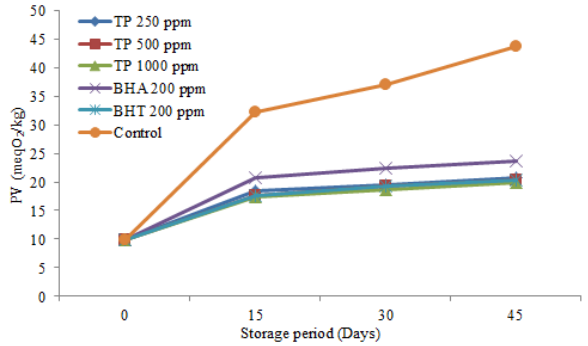

Fig. 3. Increase in PV (meqO_2/kg) during storage period of treated sunflower oil samples.

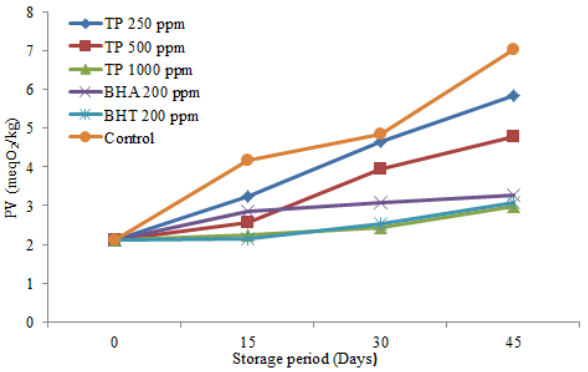

Fig. 4. Increase in PV (meqO_2/kg) during storage period of treated soybean oil samples.

Free fatty acid value (FFA)

When oils come in contact with moisture, hydrolysis of triglycerides may lead to the formation of free fatty acids [20]. Fig. 5 and Fig. 6 shows that FFA value goes on increasing during storage period at room temperature of treated sunflower and soybean oil samples respectively. Control exhibits highest FFA value in both oils. For instance; FFA of control sunflower oil sample from zero to 45th day was; 1.91 ± 0.08 % to 4.60 ± 0.07 %, whereas FFA of control soybean sample was 1.42 ± 0.01 % to 4.42 ± 0.07 %. Turnip's peel extract (1000 ppm) in sunflower oil exhibited FFA value 2.57 ± 0.05 % and in soybean it was 2.14 ± 0.05 %. Similar pattern of increase in FFA value during storage period due to decomposition products of hydroperoxides was reported by [21].
The FFA value for other concentrations of turnip's peel extract (250 ppm and 500 ppm) and for BHA, BHT (200 ppm) were also determined and are graphically represented in Fig. 5 and Fig. 6 for sunflower and soybean oil samples respectively. The comparison revealed following trend in sunflower oil samples: TP 1000 ppm > BHT 200 ppm > TP 500 ppm > TP 250 ppm > BHA 200 ppm > Control. In case of soybean oil samples, the trend is as follow: TP 1000 ppm > BHT 200 ppm > TP 500 ppm > TP 250 ppm > BHA 200 ppm > Control.

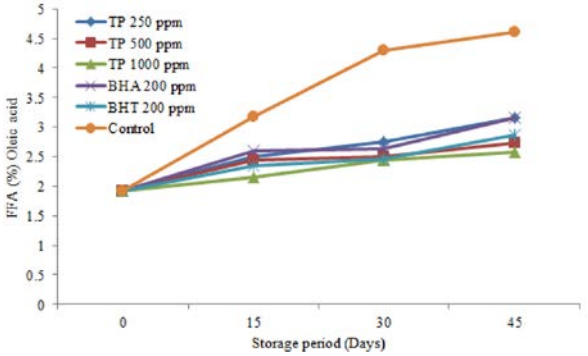

Fig. 5. Increase in FFA (%) value of treated sunflower oil samples during storage period.

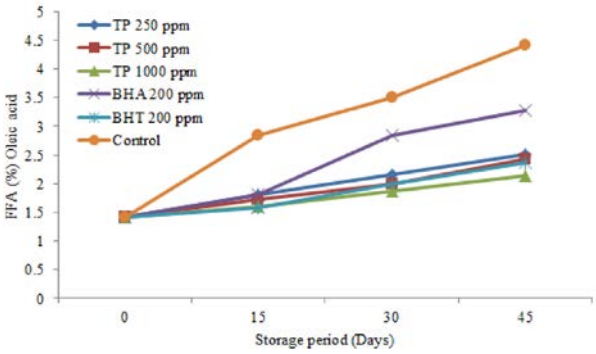

Fig. 6. Increase in FFA (%) value of treated soybean oil samples during storage period.

Iodine value (IV)

Degree of unsaturation of given oil can be determined by iodine value. Greater iodine value gives information about better quality of oil [22]. Fig. 7 and Fig. 8 show the relative decrease of iodine value in sunflower and soybean oil respectively as storage period increases. IV (gI_2/100g of oil) for TP extract (1000 ppm) in sunflower oil was 153.17 ± 1.26 gI_2/100g to 64.73 ± 1.81 gI_2/100g which is higher than BHT (153.17 ± 1.26 gI_2/100g to 56.38 ± 1.02 gI_2/100g) and BHA (153.17 ± 1.26 gI_2/100g to 53.02 ± 1.40 gI_2/100g); while IV of TP 1000 ppm in soybean oil was 169.82 ± 1.48 gI_2/100g to 88.34 ± 1.11 gI_2/100g which is also higher than BHT (169.82 ± 1.48 gI_2/100g to 79.83 ± 1.64 gI_2/100g) and BHA (169.82 ± 1.48 gI_2/100g to 61.63 ± 1.02 gI_2/100g), respectively. Generally, control exhibited the lowest content of IV followed by TP 250 ppm, TP 500 ppm, BHA 200 ppm, BHT 200 ppm and TP 1000 ppm in sunflower oil while samples in soybean oil followed this pattern: control < TP 250 ppm < BHA 200 ppm < TP 500 ppm < BHT 200 ppm < TP 1000 ppm, respectively. Iodine value of all stabilized samples is higher than control of both oils indicating good antioxidant potential of turnip's peel extract under investigation.

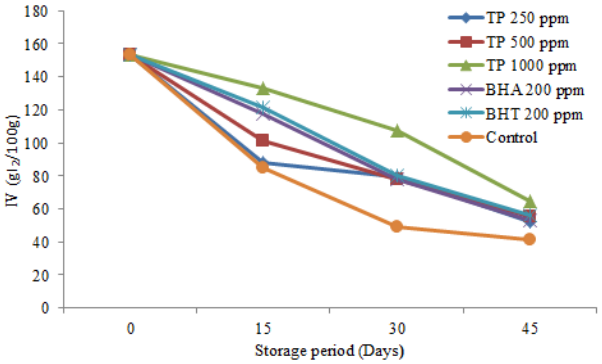

Fig. 7. Decrease in IV (gI_2/100g) of treated sunflower oil samples during storage period.

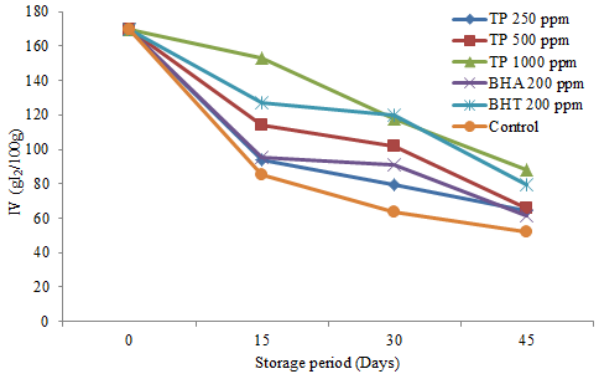

Fig. 8. Decrease in IV (gI_2/100g) of treated soybean oil samples during storage period.

CONCLUSIONS

The study for stabilization of sunflower and soybean oil revealed that turnip's peel extract having antioxidative potential can be safely employed as a better natural antioxidant when compared with synthetic antioxidants (BHT and BHA). Generally it was observed that turnip's peel extract has better stabilized oil samples than BHA, however its stabilization efficiency was slightly lower than BHT. It inhibits oxidative deterioration of both oils thus play role in preventing against diseases and can be utilized as food additive.

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СЛЪНЧОГЛЕДОВИ И СОЕВИ МАСЛА, СТАБИЛИЗИРАНИ С ЕСТЕСТВЕНИ ЕКСТРАКТИ ОТ КОРИ НА РЯПА

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(Резюме)

Ряпата (*Brassica rapa*) се смята за един от най-ранните култивирани зеленчуци още от пра-исторически времена. Антиоксидантните свойства на метаноловите екстракти от кората (TP) при различни концентрации (250 ppm, 500 ppm, 1000 ppm) са определени като общо съдържание на феноли, на флавоноиди, редукционна способност на тривалентно желязо, радикало-отстраняваща способност (ABTS) и β-каротин/линолова киселина емулсия. Най-висока антиоксидантна активност на корите от ряпа при 1000 ppm. Стабилизирането на две едливи масла (т.е. слънчогледово и соево масло), с помощта на тези екстракти е оценено при обикновени условия. BHA и BHT (200 ppm) са използвани като синтетични референтни антиоксиданти. Параметри, като пероксидно число (PV), свободни мастни киселини (FFA) и йодно число (IV) демонстрират, че антиоксидантният потенциал на екстрактите от кори от ряпа е относително по-висок отколкото на BHA и малко по-малко от този на BHT.