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Evaluation of oxidative stability of sunflower oil at frying temperature in presence of butylated hydroxytoluene and methanolic extracts of medicinally important plants of Pakistan

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Abstract

An experimental study was carried out regarding oxidative stability of refined, bleached and deodorized (RBD) sunflower oil (SFO) at 180 ± 2.0°C to evaluate the antioxidant potential of methanolic extracts of *Althea rosea* L, *Chenopodium album* L, *Cichorium intybus* L and *Fumaria indica* L in camparison with butylated hydtoxytoluene (BHT). Initial screening of all plant extracts was carried out by DPPH assay on TLC. Peroxide value (PV), Iodine value (IV), conjugated diene (CD), conjugated triene (CT) and P-anisidine value of SFO were determined to analyze the extent of oxidation in comparison with blank SFO and BHT containing SFO. Plant extract of *Fumaria indica* L was found to be most potent source of natural antioxidants by extensive inhibition of lipid oxidation parameters. Total flavonoid and phenolic contents of all plants were also determined.

Introduction

Deep frying in vegetable oils at high temperature of 150°C to 190°C is one of the popular and oldest methods for food processing. Deep fat frying is associated with physiochemical reactions such as hydrolysis, polymerization and thermo oxidation due to moisture and oxygen resulting in deterioration of frying oils (Yamsaengsung and Moreira, 2002). Primary and secondary oxidation products not only associated with poor quality of vegetable oils but also involved in various diseases. Reactive oxygen species play an important role in lipid oxidation resulting in ageing, cardiovascular disorders and cell damages (Andrikopoulos *et al.*, 2002; Siddhuraju and Beeker, 2003). To encounter the reactive oxidation species, synthetic antioxidants are added in adequate amount to vegetable oils. These antioxidants enhance the oxidative stability of lipids and lipid containing foods by minimizing the phenomenon of oxidation. Use of synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and Tertiary butylhydroquinone (TBHQ) is common in oil and fat industry. Toxicity of BHA and BHT is an established fact and a health safety issue in consumers as well as scientific community (Ku and Mun, 2007).

Plants being the richest source of safe natural antioxidants are under consideration since last two decades. Bioactive components such as phenolics and flavonoids of plants tend to exhibit free radical scavenging, so can be exploited in searching of novel antioxidants (Sultana *et al.*, 2008; Othman *et al.*, 2007). The antioxidant activities of plants are due to presence of these biologically energetic compounds. Major identified natural antioxidants of plant origin include tocopherols, carotenoids, vitamin C and phenolics (Vichi *et al.*, 2001). That is the logic behind the use of plants and herbs not only in medicines but also to increase shelf life of oil and fat containing foods (Hulin *et al.*, 2002; Raza *et al.*, 2009).

Current study is designed to investigate the antioxidant potential of crude methanolic extracts of some medicinally important aromatic plants of Province Punjab, Pakistan in Refined bleached and deodorized (RBD) sunflower oil (SFO) at frying temperature. *Althea rosea* L (Malvaceae), *Chenopodium album* L (Chenopodiaceae), *Cichorium intybus* L (Asteraceae) and *Fumaria indica* L (Fumiraceae) were selected due to intensive use in folk medicines (Majeed *et al.*, 2011). Oxidative stability of SFO was determined by measuring Peroxide value (PV), Iodine value (IV), Conjugated diene (CD), Conjugated triene (CT) and P-anisidine value at frying temperature.

Material and Methods

Chemicals and reagents

1,1-Diphenyl-2-picryl hydrazyl (DPPH, $C_{18}H_{12}N_5O_6$) from Wako chemicals, Japan, BHT

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($C_{15}H_{24}O$), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH) and Folin-Ciocalteu reagent from Sigma-Aldrich USA. All the chemicals were of analytical grade.

Collection of plant material

Plant materials were collected from District Gujrat and District Jhelum of Punjab, Pakistan in October 2012. These plants were at peak of their growth in October. All the plants were identified from the botanical museum of Government college university Lahore, Pakistan.

Extract preparation

Forty grams of air dried and ground whole plant materials were extracted using methanol on Soxhlet apparatus separately for each plant. Extracts were filtered and dried under reduced pressure at 40°C using rotary evaporator. Finally a 2 mg/ml stock solution of each extract was prepared in methanol (Sanchez-Moreno, 2002).

Determination of total flavonoid contents (TFCs) and total polyphenol contents (TPCs)

The TFCs of all plant extracts were determined by following the method of Zhishen *et al.* (1999). Equal quantities of each methanolic extract (0.5 ml) were mixed with 2 ml of double distilled water followed by addition of 0.15 ml of 15% $NaNO_2$ solution and allowed to stay for five minutes. Then 0.15 ml of 10% $AlCl_3$ solution was added to each extract and allowed to stand for 5 minutes followed by 2 ml of 4% NaOH solution. Final volume of 5 ml was made by adding double distilled water and stayed for 15 minutes at ambient lab temperature. Absorbance of test mixtures was taken on UV-1700, Shimadzu, Japan Spectrophotometer at 510 nm using water as blank. Results expression was µg lutin trihydrate/g dried extract. All the tests were carried out in triplicate.

Folin-Ciocalteu method (Slinkard and Singleton, 1977) was utilized to determine TPCs of all extracts with Gallic acid as an internal standard. Dilutions of extracts for each plant were made by adding 45 ml of distilled water to 0.5 ml of extracts followed by addition of 1 ml of Folin-Ciocalteu reagent. After three minutes stay 3 ml of 2% Na_2CO_3 was added. The mixture was allowed to stand for 120 minutes with occasional shaking. After shaking absorbance was taken at 760 nm and concentration of TPCs in all extracts was expressed as µg of Gallic acid equivalent per gram of dry matter on spectrophotometrically on UV-1700, Shimadzu, Japan. All the tests were performed in triplicate.

TLC screening for antioxidant activity

DPPH assay on TLC was utilized to initially screen out the idea of antioxidant activity for further experimental proceeding. According to Bektas Bektas *et al.* (2005), dilutions (1:10) of all methanolic plant extracts were made in methanol. Five µl of each dilution was applied to TLC plates and developed with a mixture of methanol and ethyl acetate (1:1). Each TLC plate was sprayed with 0.2% of DPPH reagent in methanol and allowed to stand for 30 minutes. Bleaching of purple color of DPPH reagent by yellowish spots indicated positive antioxidant activity.

Antioxidant activity in sunflower oil

Refined, bleached and deodorized (RBD) sunflower oil (SFO) were procured to assess the effects of BHT and plant extracts on oxidative stability of SFO at elevated temperatures. To 400 ml of SFO, 250 ppm of BHT and methanolic plant extracts were added separately and heated at 180 ± 2.00°C for 15, 30, 45 and 60 mints. A blank SFO (without any additive) was also examined under same conditions. Selection of SFO was made due to its high degree of unsaturation and wide use in cooking. Peroxide value (PV), Iodine value (IV), conjugated dienes (CD), conjugated trienes (CT) and P-anisidine value were monitored for every SFO sample at all treatments in triplicate. PV and IV were determined following the recommended methods of AOCS (AOCS, 1989). For CD and CT, IUPAC (IUPAC, 1987) methods were followed. Samples were diluted with iso-octane and absorbance was measured at 232 nm and 268 nm respectively for CD and CT using spectrophotometer (Hitachi, U-2001, model 7400, Tokyo, Japan). To determine P-anisidine value, SFO samples were treated with P-anisidine reagent to generate colored compound and absorbance was measured at 350 nm (Hitachi, U-2001, model 7400, Tokyo, Japan) by standard method of IUPAC (IUPAC, 1987). All the measurements were carried out in triplicate and standard deviation was applied.

Results and Discussion

The TPCs and TFCs contents were represented in table 1. *Fumaria indica* L possessed the highest values for both TPCs and TFCs among all plants under investigation. Antioxidant activities of most of plants are due to phenolics present in them. Many repots emphasized on the fact that antioxidant activities of plants are due to phenolics and other biologically active compounds present in plants (Raza *et al.*, 2009; Rashid *et al*., 2009; Radulović *et al.*, 2007)

Table 1. Total phenolic (TP) and total flavonoids (TF) in mg/g dried plant extract

Name of Plant	Total Phenolics (TP) (mg gallic acid/g dried extract)	Total Flavonoids (TF) (mg rutin trihydrate/g dried extract)
Althea rosea L	388 ± 2.00	219 ± 1.00
Chenopodium album L	397 ± 2.00	237 ± 2.00
Cichorium intybus L	424 ± 2.00	334 ± 3.00
Fumaria indica L	463 ± 3.00	404 ± 4.00

± indicates standard deviation for triplicate values

Table 2. Relative Increase in PV in meq/kg for various SFO samples at 180 ± 2.0°C

Heating Time in Minutes	Blank SFO sample	BHT containing SFO sample	*Althea rosea* L extract containing SFO sample	*Chenopodium album* L extract containing SFO sample	*Cichorium intybus* L extract containing SFO sample	*Fumaria indica* L extract containing SFO sample
00	0.22±0.10	0.22±0.11	0.22±0.10	0.22±0.12	0.22±0.12	0.22±0.10
15	0.89±0.14	0.28±0.11	0.39±0.10	0.38±0.11	0.40±0.10	0.33±0.11
30	1.52±0.12	0.35±0.13	0.62±0.11	0.59±0.12	0.66±0.11	0.39±0.11
45	2.12±0.10	0.41±0.12	0.81±0.10	0.80±0.10	0.85±0.13	0.47±0.13
60	2.32±0.13	0.46±0.11	0.93±0.12	0.92±0.12	0.95±0.14	0.53±0.12

± indicates standard deviation for triplicate values of PV

Table 3. Relative decrease in IV (gI2/100 g oil) for various SFO samples at 180 ±2.0°C

Heating Time in Minutes	Blank SFO sample	BHT containing SFO sample	*Althea rosea* L extract containing SFO sample	*Chenopodium album* L extract containing SFO sample	*Cichorium intybus* L extract containing SFO sample	*Fumaria indica* L extract containing SFO sample
00	143± 1.01	143± 0.55	143± 1.04	143± 1.14	143±0.99	143±1.00
15	139 ±1.03	142 ±1.01	140 ±1.02	140 ±1.03	141±1.15	141±1.06
30	133 ±0.92	140 ±1.03	135 ±1.14	134 ±1.10	135±1.01	138±0.95
45	128 ±1.01	138 ±1.11	130 ±1.25	130 ±1.05	131±1.31	135±0.71
60	125 ±1.05	137 ±0.75	127 ±1.01	127 ±1.05	126±1.25	131±0.65

± indicates standard deviation for triplicate values of IV

Table 4. Relative Increase in CD of SFO in terms of molar extinction coefficient [ε_{1cm} ($\lambda_{232\ nm}$)] at 180 ± 2.0°C

Heating Time in Minutes	Blank SFO sample	BHT containing SFO sample	*Althea rosea* L extract containing SFO sample	*Chenopodium album* L extract containing SFO sample	*Cichorium intybus* L extract containing SFO sample	*Fumaria indica* L extract containing SFO sample
00	0.11±0.02	0.11±0.01	0.11±0.05	0.11±0.01	0.11±0.02	0.11±0.01
15	0.36±0.02	0.16±0.02	0.35±0.02	0.31±0.03	0.32±0.02	0.24±0.02
30	0.89±0.05	0.22±0.01	0.69±0.02	0.59±0.01	0.60±0.03	0.35±0.02
45	1.04±0.03	0.25±0.02	0.83±0.02	0.72±0.02	0.70±0.02	0.41±0.02
60	1.12±0.05	0.27±0.01	0.90±0.01	0.81±0.02	0.77±0.01	0.53±0.01

± indicates standard deviation for triplicate values of CD

Table 5. Relative Increase in CT of SFO in terms of molar extinction coefficient [ε_{1cm} ($\lambda_{268\ nm}$)] at 180 ± 2.0°C

Heating Time in Minutes	Blank SFO sample	BHT containing SFO sample	*Althea rosea* L extract containing SFO sample	*Chenopodium album* L extract containing SFO sample	*Cichorium intybus* L extract containing SFO sample	*Fumaria indica* L extract containing SFO sample
00	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.02
15	0.26±0.01	0.16±0.02	0.23±0.02	0.21±0.01	0.21±0.02	0.18±0.02
30	0.49±0.02	0.20±0.01	0.44±0.02	0.42±0.01	0.41±0.01	0.24±0.02
45	0.68±0.02	0.24±0.02	0.53±0.02	0.51±0.02	0.50±0.02	0.29±0.02
60	0.79±0.02	0.26±0.01	0.60±0.01	0.57±0.02	0.55±0.01	0.32±0.01

± indicates standard deviation for triplicate values of CT

Table 6. Relative increase in P-anisidine value [$\varepsilon_{1c}(\lambda_{350\ nm})$] at 180 ± 2.0°C

Heating Time in Minutes	Blank SFO sample	BHT containing SFO sample	*Althea rosea* L extract containing SFO sample	*Chenopodium album* L extract containing SFO sample	*Cichorium intybus* L extract containing SFO sample	*Fumaria indica* L extract containing SFO sample
00	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
15	0.09±0.01	0.02±0.01	0.07±0.01	0.07±0.01	0.06±0.02	0.04±0.01
30	0.19±0.01	0.04±0.01	0.15±0.01	0.15±0.01	0.14±0.01	0.07±0.02
45	0.30±0.02	0.06±0.01	0.27±0.01	0.25±0.01	0.24±0.01	0.10±0.01
60	0.36±0.02	0.07±0.01	0.30±0.01	0.31±0.02	0.29±0.01	0.14±0.01

± indicates standard deviation for triplicate values of CT P-anisidine value

TLC screening for antioxidant activity

The DPPH assay on TLC plate indicated the positive antioxidant activity by all plant extracts by bleaching purple color of DPPH reagent by yellow spots. Such screening on initial basis led to further investigation of antioxidant activity in SFO at frying temperatures for various time oriented protocols.

Antioxidant activity in sunflower oil

Increase in PV is an index of primary lipid oxidation products and represents the formation of hydro peroxides during oxidation. PV is widely monitored by scientists working on antioxidants to assess the extent of oxidation (McGinely, 1991; Gulcan and Bedia, 2007; Raza *et al.*, 2009). The trends in PV are given in Table 2. Comparison of PV for all SFO indicated that only plant extract of *Fumaria indica* L was major contributor in resistance to wards increase in PV. Increase in PV is due to formation of unstable hydro peroxides (Mariod *et al*., 2006). Although other plant extracts exhibited antioxidant activity but plant extract of *Fumaria indica* L was the nearest to BHT. Variation in IV is shown in table 3. Similar trend was observed in case of IV study. IV is the measure of degree of unsaturation present in vegetable oils and fats (Erickson, 1990). Phenomenon of oxidation reduces the unsaturation sites within molecule. A decrease in IV was found in case of all plant extracts having SFO as well as BHT containing SFO sample. A clear cut mark of difference existed among blank SFO and all other SFO samples. Maximum inhibition in decrease of IV was targeted by BHT in SFO. While among plants *Fumaria indica* L was found to be most effective source of natural antioxidants.

Results of CD and CT values were given in table 4 and 5. CD and CT both are measurement of secondary oxidation products (Poiana, 2012). The CD values were higher than CT but the formation of both CD and CT was not so rapid showing that the secondary oxidation products are more stable than primary oxidation products. Role of BHT was most efficient followed by extract of *Fumaria indica* L. P-anisidine value is also a good indicator of lipid oxidation. Less stable hydro peroxides undergo further breakdown to form aldehydes and ketones (Abdulkarim *et al*., 2007) which are secondary oxidation products imparting off flavors and toxic effects in vegetable oils and fats. Increase in P-anisidine value of various SFO samples is represented in table 6. Trends regarding P-anisidine value were similar as compared to previous oxidation parameters. No plant extract was as successful as BHT in antioxidant and antiradical potential. However plant extract of *Fumaria indica* L was found to be a potent source of natural antioxidant among all plant extracts due to higher concentration of flavonoids and phenolics. The overall order of antioxidant potential of all plant extracts was *Fumaria indica* L > *Cichorium intybus* L > *Chenopodium album* L > *Althea rosea* L.

Conclusion

From results and discussion of study conducted, it can be concluded that *Fumaria indica* L plant marked incredible antioxidant potential and proved as a good source of novel antioxidants of great industrial importance. Further isolation, purification and identification of bioactive components from *Fumaria indica* L can lead us to potent natural antioxidants which will not only be supportive at manufacturing and processing but also to enhance shelf life of vegetable oils and fatty foods.

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