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Oxidative stabilization of corn oil with spinach extract

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Abstract

The present study was aimed to analyze the antioxidant activity of spinach (*Spinacia oleracea*) extract in oxidative stabilization of refined, bleached and de-odorized (RBD) corn oil (CO). Corn oil was supplemented with three different concentrations 600, 1200 and 1800 ppm of methanolic spinach extract and then stored at 25°C and 60°C. Different oxidation measuring parameters such as peroxide value (PV), free fatty acid (FFA) value, iodine value (IV), conjugated dienes (CD), conjugated trienes (CT) and para-anisidine value (PAV) were examined. The potency of spinach extract as natural antioxidant was found to be as effective as the synthetic antioxidants BHA and BHT used as standards and highest stabilization was observed in the order CO-1800>CO-1200>CO-BHT> CO-BHA>CO-600>CO-control. The results showed a significant effect of spinach extract in enhancing the oxidative stability of corn oil.

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Introduction

The shelf life of plant derived oils is greatly dependent on their oxidative stability which is a serious concern for their practical applications (Gertz *et al*., 2000). Oxidative deterioration of these oils results in the formation of free radicals and reactive oxygen species badly effecting not only the quality of these oils but also posing serious health threats (Nosratpour *et al*., 2017; Kanner, 2007). In order to enhance the oxidative stability of oils various methods are employed such as genetic modifications, compositional changes and use of additive materials such as antioxidants (Shahidi and Zhong, 2010). Use of many synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), ter-butyl hydro quinone (TBHQ) is common as potential inhibitors of lipid peroxidation in the oils and fats (Aluyor and Ori-Jesu, 2008; Ullah *et al*., 2003; Azeez *et al*., 2013). However, the probable health deleterious effects of these synthetic antioxidants limit their use in the edible oils.

Exploration of antioxidants of natural origin is increasing now a days (Rajendran *et al*., 2014; Augustyniak *et al*., 2010). Owing to the health promoting properties, natural antioxidants obtained mainly from fruits and vegetables are gaining the attention (Aladedunye, 2014; Li *et al*., 2014). Many studies revealed that people consuming more fruits and vegetables enriched with the natural antioxidants such as flavonoids, polyphenols, vitamin C are at a lower risk of chronic and cardiovascular diseases (Kris-Etherton *et al*., 2002). Various plant based natural antioxidants are explored and well characterized and their role as a potential substitute of synthetic antioxidants has also been well proven (Tundis *et al*., 2017). The use of natural antioxidants from different plant sources in stabilization of many vegetable oils has also been well studied (Redondo-Cuevas *et al*., 2017; Aydeniz and Yilmaz, 2016; Taghvaei and Jafari, 2015).

Corn oil is commonly used both for edible purposes as well

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as in many industrial applications. Presence of large content of unsaturated fatty acids and residual tocopherol contents after refining makes it more vulnerable to oxidation (Naz *et al*., 2005; Naz *et al*., 2004). Several investigations using this potential oxidative substrate was carried out to explore the antioxidant effectiveness of many plant extracts. In a study, Navas *et al.* (2006) reported the effectiveness of black tea, garlic and onion on stability of corn oil at different temperatures. In addition to plant extracts, seed oil of black cumin and coriander when added to corn oil was also proved to be effective at 60^{o}C temperature for 15 days storage (Ramadan and Wahdan 2012). Thermal stability of corn oil was assessed at different heating temperatures using thyme flower extracts and it was shown that thyme flower enhanced the stability of corn oil (Karoui *et al*., 2011). Sultana *et al*. (2008) investigated the antioxidative efficacy of different agro-waste extracts and found them as potent source for enhancing the oxidative stability of corn oil. In another study, they utilize the corncob extract for the stabilization of corn oil under microwave heating and found good results (Sultana *et al*., 2007). Anwar *et al*. (2003) observed the antioxidant potential of methanolic extracts of rice bran, guava leaf, roasted wheat germ, rosemary, coffee beans, basil and peppermint in stabilizing refined corn oil at heating conditions of 65^{o}C concluding all these extracts to be very effective as natural antioxidants. Some plant extracts were also investigated for enhancing the oxidative stability of corn oil under high pressure accelerated oxidation conditions (Bandak *et al*., 2011).

Spinach(*Spinacia oleracea*) is one of the most peculiar green leafy vegetable famous for its nutritional contents and also enriched with a number of phytochemical compounds giving it superb antioxidant activities (Ko *et al*., 2014; Lee *et al*., 2002). Utilization of spinach extract in oxidative stabilization of refined, bleached, and de-odorized corn oil was investigated in the present work. The results from all the parameters used to estimate the oxidation of oil clearly depicts the potency of spinach extract as a natural antioxidant in improving the stability of corn oil.

Materials and methods

Sample

Samples of refined, bleached and de-odorized (RBD) corn oil were procured locally.

Preparation of spinach extract

Spinach samples were first washed thoroughly to remove soil and then macerated. The crushed sample (10g) was then extracted using 25ml methanol by shaking well at room temperature overnight. The pure extract was collected after subjecting to Soxhlet apparatus for about 3hrs. The extract was dried and measuring yield stored at 4^{o}C until used for further analysis.

Estimation of antioxidant potential of spinach extract

Antioxidant potential of spinach extract was estimated by total phenolic contents, DPPH free radical scavenging activity, β-carotene linoleate method, ferric reducing antioxidant power and ferric thiocyanate method as described in our previously reported work (Tehseen *et al*., 2014). Methanolic extracts of canal water irrigated wavy leaves spinach sample has shown the maximum antioxidant potential which are employed for this study.

Estimation of stability of corn oil using spinach extract

The prepared spinach extract was added to RBD corn oil at three different concentrations 600ppm, 1200ppm and 1800ppm. Samples were agitated vigorously for uniform mixing. Standard samples were prepared using synthetic antioxidants BHT and BHA at their safe limits of 200ppm. Beside control samples without any antioxidant was also taken. All these stabilized and control samples in triplicate were stored in amber glass bottles at 25^{o}C and 60^{o}C for 40 days and then analyzed further for oxidative degradation. Peroxide value (PV), Free fatty acid Value (FFA), Iodine value (IV), Conjugated dienes (CD) and trienes (CT) of both stabilized and control samples were measured according to the standard methods of IUPAC (IUPAC, 1987)and *p*-Anisidine value was analyzed as described by Saha *et al*. (2008).

Statistical analysis

All the oxidation parameters were performed in triplicate and data was analyzed statistically by using one-way ANOVA (SPSS ver. 8.0) and significant differences ($P< 0.05$) among different parameters were calculated (Steel *et al*., 1997).

Results and discussion

Peroxide value (PV)

Peroxide value represent the extent of formation of primary oxidation products during oxidative degradation of oils and fats. Hence the measurement of peroxide value can be used to depict how much an antioxidant inhibit the formation of these oxidation products. Peroxide values of corn oil stabilized with spinach extract, BHA, BHT and control at 25^{o}C and 60^{o}C for a storage period of 40 days is presented in Table I and II. The amber glass bottle was used for storage

Table I. Relative effect of storage conditions on PV of corn oil at 25°C

Storage time (Days)	Peroxide value (PV) (meq/kg)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	0.21±0.06	0.21±0.06	0.21±0.06	0.21±0.06	0.21±0.06	0.21±0.06
10	5.12±0.12	3.41±0.18	1.69±0.12	1.12±0.09	1.61±0.09	1.09±0.13
20	21.72±0.17	10.09±0.12	7.23±0.16	5.81±0.13	6.02±0.11	5.78±0.08
30	32.61±0.09	14.62±0.09	10.34±0.13	9.33±0.08	10.35±0.08	9.32±0.11
40	46.02±0.07	23.55±0.16	19.26±0.11	16.2±0.06	18.94±0.13	15.61±0.11

Table II. Relative effect of storage conditions on PV of Corn oil at 60°C

Storage time (Days)	Peroxide value (PV) (meq/kg)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	0.21±0.06	0.21±0.06	0.21±0.06	0.21±0.06	0.21±0.06	0.21±0.06
10	10.61±0.03	3.96±0.11	2.68±0.09	1.86±0.05	2.69±0.21	1.82±0.04
20	36.73±0.05	12.01±0.05	7.91±0.16	6.42±0.03	7.73±0.19	6.39±0.09
30	56.54±0.03	17.22±0.09	14.53±0.13	10.67±0.06	12.56±0.09	10.62±0.16
40	69.76±0.08	28.07±0.07	23.46±0.08	18.06±0.07	19.88±0.16	17.92±0.08

Free fatty acid value (FFA)

Table III. Relative effect of storage conditions on FFA of Corn oil at 25°C

Storage time (Days)	Free fatty acid value (FFA) (% oleic acid)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
10	0.14±0.04	0.05±0.08	0.03±0.06	0.02±0.04	0.03±0.01	0.02±0.07
20	0.19±0.03	0.06±0.08	0.04±0.04	0.02±0.02	0.04±0.03	0.03±0.06
30	0.24±0.01	0.09±0.06	0.07±0.03	0.04±0.03	0.06±0.02	0.05±0.05
40	0.35±0.02	0.14±0.09	0.10±0.04	0.06±0.02	0.09±0.01	0.07±0.02

to prevent UV light deterioration as UV light may enhance oxidation of lipid that destabilized the activity. The result shows a significant reduction in peroxide values of the samples with spinach extract and standard antioxidants as compared to control. Spinach extract at maximum concentration of 1800 ppm showed highest reduction in peroxide value ($P<0.05$) comparable with that of the standard antioxidants which indicates the efficacy of spinach extract in stabilization of corn oil at both storage temperatures. Sultana *et al*. (2007) reported the efficacy of corncob extracts in lowering the PVs of corn oil during microwave incubation. Navas *et al*. (2006) investigated the efficacy of black tea, onion and garlic extracts in stabilizing corn oil at heating temperatures of 55 and 140°C. Black tea was found to be most effective in enhancing corn oil stability. They also showed that determining PVs is rather less feasible at high heating temperatures as the peroxides formed earlier decompose or evaporate at high temperatures.

Free fatty acid value (FFA)

Free fatty acids are the degradation products formed by hydrolysis of fats or oils. They are more prone to oxidation and causes rancidity of fats and oils hence representing a key feature in predicting the extent of deterioration. Increase in FFA value during storage indicates more oxidative damage. In the present study FFA values of RBD corn oil stabilized with synthetic antioxidants and spinach extract was estimated at 25°C and 60°C

Table IV. Relative effect of storage conditions on FFA of corn oil at 60^{o}C

Storage time (Days)	Free fatty acid value (FFA) (% oleic acid)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
10	0.17±0.04	0.06±0.07	0.05±0.05	0.03±0.05	0.04±0.05	0.03±0.03
20	0.21±0.06	0.09±0.09	0.07±0.03	0.04±0.03	0.05±0.03	0.06±0.05
30	0.33±0.02	0.13±0.07	0.08±0.02	0.07±0.05	0.07±0.01	0.08±0.04
40	0.42±0.03	0.18±0.06	0.12±0.04	0.10±0.04	0.09±0.02	0.12±0.03

Table V. Relative effect of storage conditions on IV of corn oil at 25^{o}C

Storage time (Days)	Iodine value (IV)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	142±2.01	142±2.01	142±2.01	142±2.01	142±2.01	142±2.01
10	140±2.02	140±2.22	141±2.07	141±2.06	141±2.09	142±1.09
20	138±1.09	139±2.06	139±2.06	141±2.02	140±1.14	141±1.14
30	133±1.06	138±2.06	138±2.87	140±2.08	140±1.19	141±2.01
40	121±2.02	137±2.04	138±1.98	139±1.94	139±2.12	140±2.09

for 40 days storage (Table III and IV). The increase in FFA values was less in the samples stabilized with spinach extract which is comparable with the synthetic antioxidants. Spinach extract was found efficient to slow down the rate of formation of FFA as compared to the control samples which showed maximum increase in FFA. A significant variation ($p \leq 0.05$) in FFA contents of both stabilized and control samples was observed. The results are in close agreement with the previous studies on stabilizing the sunflower oil with extracts of waste cake which showed significant reduction in FFA values (Abd-El-Ghany *et al*., 2010). Ali *et al*. (2016) also studied the FFA values as an indicative tool for depicting the oxidative stabilization of cooking oil blends with added *Eucalyptus citriodora* leaf extract and showed that there is less increase in value of FFA during the induction period.

Iodine value (IV)

Degree of unsaturation of an oil or fat is measured in terms of its iodine value. Oil and fats with more unsaturation are more vulnerable to oxidation and become rancid. A significant decrease in IV of both stabilized and non-stabilized (control) corn oil samples was observed during the storage period and the maximum decrease was recorded after a 40 day induction period. However, this decrease in oil unsaturation was more in control corn oil samples. The results presented in Table V and VI indicate that a relatively small decrease in oil unsaturation was recorded in corn oil that were stabilized with spinach extract which is similar to that stabilized with BHT and BHA. These IVs for corn oil in the present study were correlated to the values reported in sunflower oil blended with olive waste cake extract (Abd-El-Ghany *et al*., 2010) and also in agreement with the observations that IV of blend oil samples show less decrease when stabilized with *Eucalyptus citriodora* leaf extract (Ali *et al*., 2016).

Conjugated dienes and trienes value (CD and CT)

Oxidation process of oils and fats results in the formation of conjugated dienes and trienes due to shifting of double bonds and showing a particular absorbance at 232 and 268nm respectively. Hence, both these values directly correlate with the extent of oxidation. Oxidative stability of oils decreases, if the CD and CT values increases in consequence of oxidative degradation. CD and CT values of corn oil sample stabilized with spinach extract, BHA, BHT and control is shown in Table VII, VIII, IX and X respectively. Supplementation with the spinach extract shows a significant reduction in CD and CT values as compared to control and the effect is also comparable to standard antioxidants used. The present findings are in close agreement with the other studies performed to evaluate the CD and CT contents of oils stabilized with different natural plant extracts. Sultana *et al*. (2008) measured CD and CT contents of corn oil stabilized with agro-wastes of different plant sources and showed that

Table VI. Relative effect of storage conditions on IV of corn oil at 60°C

Storage Time (Days)	Iodine value (IV)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	142±2.01	142±2.01	142±2.01	142±2.01	142±2.01	142±2.01
10	139±1.09	140±2.09	141±2.06	141±2.08	141±2.12	140±1.08
20	135±1.07	136±2.06	139±2.09	140±1.77	139±1.13	139±1.29
30	129±1.06	132±2.07	138±2.01	138±1.96	136±118	138±1.18
40	118±2.00	126±1.19	130±2.03	136±2.09	132±2.03	135±2.04

Table VII. Relative effect of storage conditions on CD value of corn oil at 25°C

Storage time (Days)	Conjugated dienes value (CD) (ε at λ_{232})					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	1.62±0.03	1.62±0.10	1.62±0.03	1.62±0.03	1.62±0.03	1.62±0.03
10	3.96±0.10	2.91±0.82	2.44±0.05	2.13±0.11	2.48±0.14	2.03±0.21
20	8.32±0.08	4.83±0.96	4.51±0.07	4.18±0.13	4.52±0.11	4.16±0.17
30	11.62±0.06	7.72±0.77	7.42±0.06	6.91±0.09	7.13±0.09	6.73±0.19
40	15.71±0.07	8.36±0.80	8.01±0.02	7.78±0.12	7.98±0.12	7.52±0.16

Table VIII. Relative effect of storage conditions on CD value of corn oil at 60°C

Storage time (Days)	Conjugated dienes value (CD) (ε at λ_{232})					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	1.62±0.03	1.62±0.03	1.62±0.03	1.62±0.03	1.62±0.03	1.62±0.03
10	4.24±0.04	3.49±0.05	3.11±0.04	2.83±0.13	3.01±0.15	2.81±0.11
20	9.82±0.15	6.05±0.11	5.82±0.06	5.39±0.11	5.81±0.13	5.32±0.13
30	13.53±0.10	9.86±0.09	9.27±0.05	8.98±0.13	9.14±0.08	8.84±0.09
40	17.65±0.09	11.23±0.08	10.93±0.04	10.31±0.12	10.65±0.11	10.09±0.06

Table IX. Relative effect of storage conditions on CT values of corn oil at 25°C

Storage time (Days)	Conjugated trienes value (CT) (ε at λ_{268})					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	0.38±0.11	0.38±0.11	0.38±0.11	0.38±0.11	0.38±0.11	0.38±0.11
10	1.92±0.09	1.62±0.09	1.08±0.13	0.73±0.07	1.01±0.06	0.72±0.06
20	2.61±0.12	2.16±0.06	2.01±0.14	0.93±0.11	1.82±0.02	0.91±0.07
30	3.84±0.14	3.27±0.07	3.11±0.12	1.27±0.13	2.36±0.13	1.23±0.12
40	5.09±0.15	4.35±0.13	3.86±0.11	1.49±0.09	2.87±0.16	1.46±0.13

Table X. Relative effect of storage conditions on CT values of corn oil at 60^{o}C

Storage time (Days)	Conjugated trienes value (CT) (ε at λ_{268})					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	0.38±0.11	0.38±0.11	0.38±0.11	0.38±0.11	0.38±0.11	0.38±0.11
10	2.07±0.16	1.76±0.12	1.61±0.09	0.81±0.17	1.09±0.12	0.76±0.14
20	3.56±0.17	2.91±0.17	2.73±0.07	1.09±0.12	2.16±0.19	0.98±0.12
30	4.92±0.12	4.87±0.09	3.56±0.02	1.46±0.15	2.54±0.09	1.32±0.17
40	6.13±0.09	5.29±0.08	4.01±0.12	1.88±0.14	3.01±0.16	1.63±0.13

Table XI. Relative effect of storage conditions on *p*-anisidine value of corn oil at 25^{o}C

Storage Time (Days)	*p*-anisidine value (meq/kg)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	1.04±0.40	1.04±0.40	1.04±0.40	1.04±0.40	1.04±0.40	1.04±0.40
10	3.93±0.37	2.82±0.12	2.31±0.52	1.98±0.31	2.02±0.92	1.88±0.19
20	5.35±0.31	3.94±0.09	3.24±0.61	2.88±0.52	2.99±0.83	2.74±0.17
30	9.21±0.35	5.32±0.10	4.21±0.59	3.78±0.55	3.89±0.86	3.61±0.09
40	11.97±0.39	7.93±0.08	6.09±0.53	5.62±0.39	5.97±0.56	5.08±0.15

Table XII. Relative effect of storage conditions on *p*-anisidine value of corn oil at 60^{o}C

Storage time (Days)	*p*-anisidine value (meq/kg)					
	CO-Ctrl	CO-600	CO-1200	CO-1800	CO-BHA	CO-BHT
0	1.04±0.40	1.04±0.40	1.04±0.40	1.04±0.40	1.04±0.40	1.04±0.40
10	4.31±0.32	3.87±0.09	3.37±0.93	2.91±0.23	3.08±0.62	2.30±0.37
20	8.42±0.34	5.89±0.12	5.21±0.48	4.83±0.29	4.92±0.65	4.62±0.33
30	13.16±0.35	7.31±0.23	7.08±0.59	6.78±0.32	6.89±0.49	6.17±0.39
40	18.69±0.38	10.62±0.29	10.06±0.62	9.62±0.25	9.78±0.53	9.21±0.43

about 71% and 59% reduction occur. In another study they tested the efficacy of corncob extract in stabilization of corn oil and observed about 45% and 39% inhibition in CD and CT values as compared to control (Sultana *et al*., 2007). Siddiq *et al*. (2005) evaluated the antioxidant potential of methanolicextractof *Moringaoleifera* leave in sunflower oil stabilization by measuring both CD and CT values with a significant decline in the rise of both these values. CD and CT Parameters as indicative of the oxidative degradation was also investigated for sunflower oil stability using garlic extract (Iqbal and Bhanger 2007).

p-anisidine value

Many non-volatile carbonyl compounds mostly aldehydes formed as secondary oxidation products of lipid peroxidation are estimated by *p*-anisidine values. The concentration of aldehydes directly correlates with oxidative degradation of the oils, greater amount of these aldehydes in the oils corresponds to less oxidative stability. These aldehydes react with the *p*-anisidine reagent to form a coloured product, the absorbance of which is measured at 350nm on spectrophotometer. The *p*-anisidine values of the corn oil stabilized with spinach extract, BHT, BHA and control is shown in Table XI and XII. The results showed that the corn oil with the added spinach extract inhibited the rise in *p*-anisidine value as compared to control both at ambient and accelerated storage temperature. The rate of formation of carbonyl compounds is high in control sample as compared to those with the added spinach extract and standard antioxidants. The maximum reduction in *p*-anisidine value was observed for the spinach extract at CO-1800 concentration and these are also consistent with the decline in *p*-anisidine values for the samples with standard BHA and BHT. Sultana *et al*. (2007) represented the *p*-ansidine values for corn oil samples stabilized with corncob methanolic extract, BHT, and control. The corncob extract at 1000ppm concentration showed a significant decrease 41.8% as compared to control. The extracts of agro wastes of different plant materials was assessed in stabilizing corn oil and the *p*-anisidine values measured showed a reduction up to 59% with the added extract (Sultana *et al*., 2008). *p*-Anisidine values of soyabean oil also show a decline up to 24% when stabilized with the rice bran extract at 60^{o}C (Chatha *et al*., 2006).

Conclusion

Vegetable oils are more prone to oxidative deterioration during storage, hence use of synthetic antioxidants is common to enhance their oxidative stability. However, there is space for work on exploration of natural antioxidants which can be used to enhance the oxidative stabilization of vegetable oils. The present research is aimed to investigate the efficacy of spinach extract in stabilization of corn oil. The results of different oxidation parameters depicted that spinach is a good source of antioxidants and more potent in enhancing the oxidative stability of corn oil. Further investigations in the subject matter is needed to develop useful antioxidants of natural origin and to enhance their practical applicability in vegetable oils and other food products.

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