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Activity of Phenolic Antioxidants on the Storage Ability of Soybean Cooking Oil

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Abstract: An objective procedure and technical study has been carried out that measure the degree of oxidation of vegetable oil samples stabilized with phenolic antioxidants. Traditionally used antioxidants were added with in the maximum permissible limit (200 ppm) and samples of refined, bleached and deodorized Soybean oil were stored at ambient conditions for a period of one year. Analysis was performed periodically after each sixty days. Various chemical and instrumental methods were applied to test the oil deterioration that permit the distinction of oxidative alteration level during storage. Quantitative measurements revealed significantly higher rate of oxidative alteration and degradation in untreated samples during the early period of six to seven months as compare to the stabilized and treated samples. Treated samples were found to show more extensive degradative changes in the ending period of four to five months as compare to early period.

Key words: Synthetic antioxidants, lipid oxidation, soybean oil, chemical analysis

Introduction

Oxidation of fats and oil is a degradation process which causes deterioration in the quality of the food products and also led to potential economic losses (Min, 1998; Sherwin, 1978). Minor autoxidised products are the most important contributors to the rancid and unpleasant flavors in oxidised oils, making them unacceptable to the consumers (Miyashita *et al*., 1991). Oxidation can cause other degrading effects such as discoloration, vitamin destruction, decomposition of essential fatty acids, that led to organoleptic failure and a decrease of nutritive value (Sherwin, 1978). Now, due to awareness and consciousness of scientifically balanced diet, the use of synthetic antioxidants in food-stuff, especially in oils and fats and oil containing products has become customary. In various countries restrictions exist concerning the use of synthetic antioxidants (Andrikopoulos *et al*., 1991) due to the perceived carcinogenic potential of these phenolic compounds (Razali *et al*., 1997; Min *et al*., 1982). Very recent reports have changed the status of oxidant and dietary antioxidants in human due to Helicobacter pylori (H. pylori) infection and cardiovascular disease risk (Khaled and Sarker 1998; Folsom, 1998). There have been very little findings about the effective period and stability of such substances. Also, phenolic antioxidants are found to be ineffective in inhibiting the "flavor reversion" a unique form of oxidation (Sherwin, 1978). So, their study has become an important objective and a diagnostic feature. Various chemical methods and analysis have been available that measure the degree of lipid oxidation and are widely used for routine analysis (Shahidi and Udaya, 1998; Lampi *et al*., 1997). Some earlier studies showed that it is necessary to use several analytical methods to compare and characterize oxidizeability of oil and fats (Lampi *et al*., 1997). This paper reports a comprehensive, technical evaluation study and chemical analysis, that measure the differences in the antioxidative activities of different phenolic compounds on the oxidation rate of edible oil. A number of chemical and instrumental tests such as peroxide value, free fatty acid, p-anisidine value, absorbances as 232 nm and 268 nm and oxidised polar lipid content by silica column chromatography have been carried to evaluate the differences between untreated oil and the same oil treated with phenolic compounds.

Materials and Methods

Samples of refined, bleached and deodorized soybean oil were collected from a local industry. All the solvents used were of E. Merck. Food-grade antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) were purchased from Sigma. Tertiary butyl hydroquinone (TBHQ), α-Tocopherol and trihydroxy butyrophenone (THBP) were got from (Eastman Chemical International A.G; Zug, Switzerland).

Sampling Method: The antioxidants in the oil to be stabilized were proportioned into a pipe line (i.d. 2 inch, length 100 feet) through which hot oil (70°C) was circulated. A stainless steel pump was used to meter the antioxidant into the oil at the rate of 200 ppm. The antioxidants solutions were fed into the inlet of the circulating pump and thoroughly mixed for uniform dispersion. Treated and the controlled samples were filled in two liter cleaned glass bottles and preserved at ambient conditions.

Chemical Analysis: Chemical and technical analysis of the samples was done Bi-monthly. Determination of peroxide value, free fatty acids and p-anisidine value was carried out by various standard laboratory methods (Paquot, 1979). The content of total polar components were determined by means of column chromatography (Column Silica Cartridge 19 mm i.d), containing 20 grams of silica gel deactivated with 5% water, following the method proposed by IUPAC (Paquot, 1979) with slight modification (Dobarganes and Perez-Camino,1988). Briefly, samples of oil were diluted (0.5g/9ml) with petroleum ether and transferred to chromatographic column. First elution was made with hexane:ethyl ether (92:8 v/v) to elute the non polar fraction. A final elution of the samples was made by methanol instead of ethyl ether that improved the recovery of polar components which concentrates altered products of the triglyceroids. The modification was made in need that a sharper separation is important in this study. It has been reported that (Dobarganes and Perez-Camino, 1988), some very high polar components are not eluted from the column with ethyl ether so, later se of methanol permitted the increased

Table 1: Relative Increase in Peroxide Value (meq/kg) of the Samples During Storage

Storage Period	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G
02 Months	1.00 ± 0.04	0.62 ± 0.04	0.70 ± 0.04	0.80 ± 0.03	0.50 ± 0.04	0.64 ± 0.03	0.60 ± 0.04
04 Months	1.90 ± 0.041	1.00 ± 0.05	0.94 ± 0.03	1.20 ± 0.04	0.94 ± 0.03	0.97 ± 0.04	1.07 ± 0.03
06 Months	3.04 ± 0.051	1.59 ± 0.05	1.67 ± 0.04	1.70 ± 0.06	1.48 ± 0.04	1.76 ± 0.04	1.80 ± 0.05
08 Months	4.20 ± 0.03	2.50 ± 0.03	2.30 ± 0.04	2.74 ± 0.02	2.19 ± 0.02	2.61 ± 0.03	2.40 ± 0.04
10 Months	5.10 ± 0.04	3.47 ± 0.03	3.62 ± 0.03	3.92 ± 0.03	3.30 ± 0.05	4.00 ± 0.06	3.58 ± 0.06
12 Months	6.08 ± 0.03	4.71 ± 0.04	4.80 ± 0.05	5.10 ± 0.04	4.37 ± 0.04	4.80 ± 0.05	4.93 ± 0.05

P.V (meq/kg) for fresh sample = 0.20

Table 2: Relative Increase in P-anisidine Values of the Samples During Storage

Storage Period	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G
02 Months	05.08 ± 0.450	3.90 ± 1.00	04.00 ± 0.500	4.33 ± 0.630	4.13 ± 0.700	3.94 ± 0.53	4.08 ± 0.41
04 Months	07.32 ± 0.700	5.11 ± 0.80	04.97 ± 0.600	5.09 ± 0.400	5.00 ± 0.480	4.86 ± 0.420	5.14 ± 0.50
06 Months	10.0 ± 0.620	6.55 ± 0.470	7.10 ± 0.580	7.23 ± 0.300	6.50 ± 0.720	6.98 ± 0.640	7.00 ± 0.46
08 Months	15.20 ± 0.500	8.42 ± 1.230	8.62 ± 0.640	9.20 ± 0.710	8.30 ± 0.640	8.87 ± 0.500	9.01 ± 0.70
10 Months	17.65 ± 1.101	1.20 ± 0.901	0.02 ± 0.801	3.58 ± 0.421	0.32 ± 1.001	1.07 ± 0.601	2.20 ± 0.10
12 Months	21.00 ± 0.801	4.07 ± 1.001	4.30 ± 0.501	7.87 ± 0.801	3.10 ± 0.671	5.10 ± 0.701	6.00 ± 0.62

Table 3: Relative Increase in Free Fatty Acids(%) of the Samples During Storage

Storage Period	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G
02 Months	0.11 ± 0.01	0.09 ± 0.02	0.10 ± 0.03	0.09 ± 0.04	0.09 ± 0.03	0.10 ± 0.03	0.10 ± 0.03
04 Months	0.12 ± 0.01	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.03	0.11 ± 0.03	0.11 ± 0.04
06 Months	0.13 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.12 ± 0.04	0.11 ± 0.02
08 Months	0.15 ± 0.04	0.11 ± 0.03	0.11 ± 0.01	0.12 ± 0.03	0.12 ± 0.04	0.13 ± 0.03	0.13 ± 0.03
10 Months	0.16 ± 0.03	0.12 ± 0.04	0.11 ± 0.03	0.13 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.13 ± 0.03
12 Months	0.17 ± 0.03	0.14 ± 0.02	0.13 ± 0.02	0.14 ± 0.03	0.14 ± 0.02	0.15 ± 0.04	0.15 ± 0.02

FFA (%) for fresh sample0.08

Table 4: Relative Increase in the Total Polar Components (% based on mass) of the Samples During Storage

Storage Period	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G
02 Months	3.50 ± 0.30	2.05 ± 0.21	2.10 ± 0.10	2.88 ± 0.22	2.00 ± 0.30	2.45 ± 0.28	2.72 ± 0.32
04 Months	4.75 ± 0.40	2.70 ± 0.40	2.87 ± 0.30	3.65 ± 0.30	2.60 ± 0.24	3.48 ± 0.36	3.59 ± 0.28
06 Months	6.30 ± 0.32	3.25 ± 0.30	3.77 ± 0.44	4.41 ± 0.41	3.17 ± 0.40	4.08 ± 0.20	4.20 ± 0.36
08Months	7.60 ± 0.35	4.56 ± 0.52	4.59 ± 0.20	5.38 ± 0.30	4.20 ± 0.35	5.00 ± 0.31	5.12 ± 0.35
10Months	9.00 ± 0.21	6.18 ± 0.30	6.39 ± 0.38	7.00 ± 0.20	5.62 ± 0.42	6.87 ± 0.33	6.74 ± 0.44
12Months	10.20 ± 023	7.59 ± 0.30	8.00 ± 0.21	9.03 ± 0.30	7.10 ± 0.37	8.29 ± 0.40	8.43 ± 0.55

TPC (%) in Fresh Sample = 1.60

Table 5: Relative Increase in Conjugated Oxidation Products of the Samples During Storage

Storage Period	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F		Sample G	
	ε_1	ε_2	ε_1	ε_2	ε_1	ε_2	ε_1	ε_2	ε_1	ε_2	ε_1	ε_2	ε_1	ε_2
02 Months	0.88	0.76	0.50	0.44	0.60	0.50	0.65	0.47	0.47	0.38	0.56	0.43	0.60	0.50
04 Months	1.45	1.22	0.90	0.67	0.92	0.74	1.20	0.98	0.83	0.60	0.94	0.67	1.04	0.86
06 Months	2.64	2.00	1.61	1.24	1.55	1.30	1.93	1.60	1.36	1.00	1.60	1.39	1.55	1.45
08 Months	3.50	2.87	2.40	2.00	2.38	2.13	2.75	2.51	2.10	1.54	2.47	2.10	2.64	2.04
10 Months	4.48	3.49	3.35	2.69	3.44	3.02	3.49	3.18	3.00	2.02	3.71	2.80	3.65	2.65
12 Months	5.37	4.10	4.19	3.40	4.50	3.63	4.70	3.90	4.00	2.80	4.53	3.39	4.60	3.29

Fresh sample ε= (0.36), (0.20) at 232 nm and 268 nm X(3) = ± (0.09-0.25) $\varepsilon_1 = \varepsilon^{1\%}_{1cm(\lambda_{232})}$ $\varepsilon_2 = \varepsilon^{1\%}_{1cm(\lambda_{268})}$,

Sample A. (Blank) Contained no antioxidant
Sample B. Sample stabilized with (200 ppm) Tertiary butyl hydroquinone
Sample C. Sample stabilized with (200 ppm) Butylated hydroxyanisole
Sample D. Sample stabilized with (200 ppm) Butylated hydroxytoluene
Sample E. Sample stabilized with (200 ppm) α-Tocopherols
Sample F. Sample stabilized with (200 ppm) Propyl gallate
Sample G. Sample stabilized with (200 ppm) Trihydroxy butyrophenone

recovery of polar fraction. Control of the separation was done by Thin-layer Chromatography using 0.25 mm thick silica gel plates (20 × 20 cm) of E.Merck. A solvent mixture Hexane: ethyl ether (87:13 v/v) was used as developing solvent. Oil samples, polar and non polar fraction were diluted with n-hexane and applied as spots using a microsyringe. Plates were developed for thirty minutes, (17 cm) and spots were visualized by spraying with iodine vapours. The autoxidized products conjugated dienes and trienes were determined by Spectrometer, Perkin Elmer, Model Lambda 2. at 232 nm and 268 nm respectively (Yoon *et al.*, 1985). Samples were diluted with iso-octane to bring the absorbance with in the permitted limits (0.2-0.8) and absorptivity (ε 1cm(λ)) was calculated.

Results and Discussion

In the present study, phenolic compounds TBHQ, BHA, BHT, α-Tocopherols, PG and THBP have been evaluated for their relative antioxidant effects at ambient conditions. Table 1 and Table 2 showed the increase in peroxide value and p-anisidine values of the samples during storage. These two oxidation parameters were high in the untreated samples as compare to those with the phenolic additives. Highest peroxide value and p. anisidine value was observed in the untreated Sample 'A' i.e., (6.08 meq/kg) and (21.00) respectively. The treated samples were found to show significantly lower rate of deterioration during early period but in the ending period of four to five months the rate of increase was greater than earlier period.

Sample 'E' stabilized with α-Tocopherol showed least peroxide and p-anisidine values (4.37 meq/kg) and (13.10) respectively. Peroxide value may be less reliable for monitoring oil deterioration because of the decomposition of peroxide that are formed during primary oxidation (Sherwin, 1978; Al-Kahtani, 1991) However p-anisidine value is more reliable measure of the oxidative alteration as it measure the secondary stage of oxidation or accumulation of secondary products (Al-Kahtani, 1991). Table 3 showed the relative increase in the free fatty acid formed due to hydrolysis of the tryglyceroids and may promote by the reaction of the oil with moisture. No valuable differences were noted in the results of this parameter in case of both treated and untreated samples as all the preserved samples belonged to the same parent (deodorized oil) and were kept freed of moisture under controlled conditions. There have also been reports that regression analysis of the experimental data did not show a general correlation between FFA concentration and oxidative stability (Frega *et al.*, 1999). Table 4 and Table 5, showed increase in the total polar components and absorptivity at 232 nm and 268 nm due to conjuable oxidation products (COP), dienes and trienes respectively. It was found that most of the oil samples with higher TPC were higher in COP and the size of the polar fraction indicate the degree of oil deterioration. A lower degree of oxidative degradation was indicated by these measures for the Sample 'E' (sample treated with tocopherol) and 'B' (sample treated with TBHQ) with lowest figure in case of Sample 'E' i.e., Total polar components (7.10 %) and dienes, trienes (4.00), (2.80) respectively at the end of the experimental period (one year). Sample 'D' treated with BHT was found to show greater TPC (9.03%) and a high level of conjugated dienes and trienes i.e (4.70) and (3.90) respectively. While a highest level of these two parameters was noted in the untreated Samples 'A' i.e., TPC (10.20%) and conjuated dienes and triens (5.37) and (4.10). Results discussed in various tables for the treated and untreated samples showed that phenolic antioxidants retarded the mechanism of lipid oxidation quite fairly with in the early period of storage, up to five and six months. But their retarding efficiency varied from compound to compound in various preserved oil samples and the degree of inhibition was found to decrease in the following order; α-Tocopherol > TBHQ > BHA > PG > THBP > BHT. This may be due to their varied dispersion and formulation characteristics and their compatibility with fats and oils. Antioxidant (α-Tocopherol) was found to show highest resistance to lipid oxidation in this study i.e., up to period of seven and eight months as compare to other phenolic additives. But later on, in the ending months, it was found that the effectiveness of antioxidant compounds decreased and the rate of oil oxidation reaction increased more extensively. The untreated oil samples showed a more rapid increase in the early period followed by a nearly regular increase in the later ending period. On the basis of observed results from this technical study, we come to the conclusion, that various phenolic compounds used as antioxidants retarded lipid oxidation very well in the initial stages i.e., initiation step of this free radical mechanism. But with the passage of time, their efficiency decreases until a point may reach they become ineffective. Such antioxidants terminate or at least interrupt oil and fat deterioration in early stages and thus delay the one set of the reaction and are found to be efficient only up to specific period. It may be hypothesized that phenolic antioxidants check and retard lipid oxidation at the cost of their own life and thus decompose and deteriorate themselves with the course of time. Further study is in progress to evaluate more analytical investigations e.g their improved organoleptic behaviour, chemistry of their own decomposition products and their perceived correlation with nephritic disorders in human being. Now, with the opening of more biological windows, identification and isolation of new natural antioxidative compounds from botanical species and other natural sources (Liwei and Wang, 1997; Zhang *et al.*, 1997; Yanishlieva and Marinova, 1998; Zandi and Gondon, 1999; Abdalla and Roozen, 1999) has become a recent research concern for which field is quite empty.

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