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Effect of antioxidants on stability, nutritional values of refined sunflower oil during accelerated storage and thermal oxidation in frying

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Abstract

Untreated sunflower oil (without antioxidants) was much more affected during long storage as well as by the thermal oxidation in 4 hours frying process. The shelf life of oil was increased both during storage and in frying process at elevated temperature by the addition of synthetic antioxidants. TBHQ (Tertiory butyl hydroquinone) BHT (Butylated hydroxyl toluene) BHA (Butylatd hydroxyl amine) and the mixture of BHA and BHT used in the present study in 0.02, 0.02, 0.02 and 0.01+0.01 amount respectively to observe the stability of refined sunflower when stored at 30^{o}C for 21 weeks in transparent PET bottles. The stored oils (treated and untreated) when underwent in the process of frying at the temperature of 180^{o}C further deterioration of oils happened. The antioxidant activities and protective effects in stabilization of sunflower oils during storage and in frying process measured in terms of POV (peroxide), FFA (free fatty acids), p-AnV (p-Anisidine value), Colour Index, RI (Refractive index), and fatty acid profile. Result indicated that TBHQ exhibited stronger antioxidant activity during storage and gave maximum protection against thermal oxidation when oil subjected in the process of multiple frying for continous four hours at elevated temperature. The results were indicated the effectiveness of antioxidants in the order like TBHQ > BHT > BHA > BHA+BHT.

Key words: Antioxidants, Thermal oxidation, Nutritional values.

Introduction

The oxidative stabilities in the vegetable oils are vital for the shelf life and nutritional value of oil itself and for the food in which they are used alongwith their applicability in industrial situation. High level of unsaturation of fatty acids contained in the oils of plant origin is appreciated by nutrionists (Ascherio. 1999) but it cause severe technological problems because of their greater susceptibility of oxidation. Oxidation of fats degrades the organoleptic quality of food, So reduce its nutritional value (Johnsen. 1985). The products developed as a result of oxidation weather it is auto or thermal participated in the aging of organism and in the aetiology of cardiovascular diseases and cancer Lipids protected against uncontrolled oxidation by the addition of antioxidants. Antioxidants have the ability to remove free radicals and reactive oxygen species that damage cellular and tissue structure (Bartosz 1978). Antioxidants occurred naturally in plants with minute quantity. Mostly synthetic antioxidants used due to the stability of the compound. Many scientistis fortified natural antioxidants to observe the changes and compare it with the synthetic added antioxidants (Byrd. 2001). Generally lipid oxidized products such as free radicals, peroxides, aldehydes and ketones are harmful to human health (Hereberg *et al* 1998) because the long term exposure to destructive chemical entities in body (free radicals) quenched or stabilized by free radicals (Park *et al* 2002). So antioxidants are usually added to fats, oils and food containing fat inorder to inhibit the development of off flavours arising from the oxidation of unsaturated fatty acids. The safety of consumer health made the synthetic synthetic antioxidants more important, so many study conducted on antioxidants activity of medicinal plant and edible plants and their application to food preservatives (Tian and White 1994). The use of natural compounds to prevent degedation during frying has been suggested by many scientists (Gorden and Kourisam 1995). In previous year certain vitamins also used as antioxidants but heat and thermal waste the fortified antioxidants vitamin (Yanhliev 1997).

Sunflower oil more susceptible to oxidation because of higher contents of unsaturated acid i,e linoleic acid. Charley in his study mentioned that fat contained more unsaturation undergo oxidation rapidly (Elliott 1990). The lipid oxidation of sunflower oil not only can produce rancid odors, unpleasant flavor and discolouration but can also decrease nutrition-

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al quality and safety due to degredation products. The condition become more worst when frying done in such oil and fat. Repeated frying at high temperature in presence of oxygen from air and water from food result a series of degradation reaction produced variety of decomposed compounds alongwith aldehyde and ketones (Anon1996). Different antioxidants used in food and oils/fats are TBHQ, BHA, BHT, CA, Lascorbic acid, Tocopherol Prophyl gallate Ascorbyl palmitate and extracts of essential oil in different concentration (Farhoosh and Moosavi 2007).

Present study was carried out to investigate the effect of minute quantity of antioxidants TBHQ 0.02%, BHA 0.0%, BHT 0.02% and the combination of BHT and BHA 0.01+0.01 in sunflower oil refined for its storage stability and chemical evaluation of oil after frying the potatoes in treated and untreated stored sunflower oils. The study will help to increase the awareness of consumer about the role of antioxidants and selection of right antioxidant for particular purpose of particular oil. The study would also help the local industry and manufacturers of oils and fat about the selection and quantity of antioxidants for inventory and for frying oil preparation.

Material and Methods

Material

A refined sunflower oil without addition of any antioxidantsor preservative was supplied by a local factory (Hamza Vegetable ghee andindustry pvt ltd) in transparent PET bottles. TBHQ, BHT, BHA obtained from and used as antioxidant. Potatoes were purchased from local market washed, peel cut into cubics using a machine cutter to prepare thin cubics and soaked in water until frying.

Experimental

Fortification of antioxidants

The antioxidants TBHQ 0.02%, BHT 0.02%, BHA 0.02 and the combination of two BHT + BHA 0.01%= 0.01% were incorporated in refined sunflower oils by direct addition. First the oils heated at 70ºC agitated until the entire body is in motion then start addition of antioxidants slowly with continous agitation of oils. After completing addition agitation of oil continued for next 30 minutes for uniform and homogenized fortification of antioxidants in refined sunflower oils.

Sample Preparation

Four samples of oils with different antioxidant and one without any antioxidant (control) were prepared. The samples were placed at 30^{o}C temperature to investigate their shelf life with or without antioxidants for 21 weeks. After each three weeks physiochemical constant of each sample ware noted to measure the stability of oils.

Physicochemical values of the oil

The physicochemical values of the oils were determined according to the AOCS 1990 method (AOCS 1990, AOAC, 2002). Abbe's refractometer was used to determine the refractive index of the oil samples. The refractive index was recorded at 40^{o}C. "Lovibond tintometer" was used to note the colour. After each 3 weeks the samples with different antioxidants were drawn and tested for their colour to compare with the control one. The reading was calculated as formula Y+10R. Free fatty acid value were determined using alkali titration and calculated as oleic acid %age, peroxide value as meq/kg, p-anisidne value were determined according IUPAC 1987 (IUPAC 1992) and absorbance of solution measured at 350nm using Hitachi spectrophotometer after each three week during storage after the fortification of antioxidants.

Frying process of 21 week old oil

Frying experiments were conducted in five individual trials of 21 week old oils contained antioxidants and a control oil samples. The intial frying temperature was set at 180ºC and sliced potatoes (7.0 x 0.2 x 0.3 cm) submerged in water were fried in the oil samples. The process of frying continued for four hours after which the oils were taken for the evaluation of physicochemical constants. The control sample was heated at the same temperature for the same time without frying any items.

Esterification of oil

The methyl ester of the oils after 15 weeks were prepared with triflouride-methanol solution (Raie *et al* 1980). The methyl esters of the fatty acids so formed were purified by slicia gel TLC using hexane and diethylether (90:10) prior to gas chromatography (Muhammad *et al* 2009).

Gas chromatography

The fatty acid composition of oils were determined on Shimadzu GC 14 A gas chromatograph equipped with FID

and capillary column (25mx0.2mm, i.d) coated with PEG. A temperature programme for the column oven was 180ºC 5 mine-3ºC/min - 220ºC while the injector and detector temperature were main tained at 230ºC and 250ºC respectively. The peaks were recorded on Shimadzu C-R4A chrmatopac and identified by compiring their relative retention times with those of standards run under the same conditions (Akhtar *et al* 2006).

Statistical analysis

Experiments were replicated twice on different occasions. All analysis were carried out in triplicate (n=3) for each replicate which were reported as mean ± SD and ANOVA test.

Results and Discussion

Antioxidants are compounds which slow down or hinder the oxidation rate and in the presence of these compounds the formation of free radicals also become slow in the initiation step or by interruption, the propagation of the free radical chain. In the present study antioxidant TBHQ, BHT, BHA and a mixture of BHT & BHA was added in sunflower oil to see their effect on the storage stability of the oil. The oil was also stored for 21 week without antioxidants. The sample drawn after each 3 weeks were analyzed for their physico-chemical values. Frying is the most important part of cooking in the subcontinental countries so potatoes fried for four hour in 22 week old oils with or without added antioxidants to observe the changes in different parameters are shown in Tables I-IX.

Refractive index of different samples of sunflower of sunflower oil are shown in Table I. The variation of antioxidants at different interval of time does not show any significant effect on the refractive index of oil. But the change is seen in control sample (without antioxidant) as shown in Figure 1.

The refractive index of 21 week old 4 hours repeated fried oils showed significant change. aaaaaathe change may be due to the decomposition of the product fried in the oil BHA and BHT showed greater change and this may be due to elevated temperature of frying. Previous studies also strengthened the trend of result that antioxidants decomoposed or evaporate at elevated temperature .

The colour of oil with different antioxidants at different intervals of time are shown in Table II. The results show slightly change of colour in the sample, with out antioxidant, which indicates that addition of antioxidants maintains the colour of the oil. The change in colour of oil was particularly in the red colour in the oil out antioxidants it may be due to oxidation of some phenolic compounds present in the oil.

The Colour developed may be due to the oxidation of phenolic compound present in the oil which enhanced when the temperature elevated at 180ºC.

Table I: Refractive index of sunflower oil with different antioxidents

	Time duration						
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	1.4668	1.4668	1.4668	1.4669	1.4668	1.4669	1.4669
Sample-2 (BHT)	1.4669	1.4668	1.4669	1.4668	1.4669	1.4670	1.4670
Sample-3 (BHT and BHA)	1.4668	1.4670	1.4668	1.4669	1.4070	1.4670	1.4670
Sample-4 (BHA)	1.4670	1.4669	1.4668	1.4670	1.4668	1.4670	1.4670
Sample-5 Control)	1.4668	1.4668	1.4669	1.4668	1.4869	1.4671	1.4671

Table Ia: Refractive index of 21 week old 4 hours repeated fried oil with different antioxidants

Samples	21 weeks
Sample-1 (TBHQ)	1.4560
Sample-2 (BHT)	1.4540
Sample-3 (BHT and BHA)	1.4540
Sample-4 (BHA)	1.4541
Sample-5 Control)	1.4538

Formation of free fatty acids might be an important measure of rancidity of oils. FFA is formed due to hydrolysis of triglycerides and may get promoted by the reaction of oil with moisture (frega1999). Addition of antioxidant caused significant reduction in FFA of sunflower oil. Inhibitory effect of TBHQ and BHA were better than BHT. It is evident from these results that during 9 week storage at 30 C the change in FFA of the sample without antioxidant increased

Table II: Determination of colour of sun flower oil with different antioxidant

		Time duration						
Sample	Colour	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	Y	4.5	4.6	4.6	4.6	4.8	4.9	4.9
	R	0.1	0.1	0.2	0.1	0.3	0.4	0.5
Sample-2 (BHT)	Y	4.5	4.6	4.6	4.7	4.8	5.1	5.3
	R	0.1	0.2	0.3	0.3	0.4	0.6	0.9
Sample-3 (BHT and BHA)	Y	4.6	4.6	4.7	4.7	4.7	5.4	5.9
	R	0.1	0.2	0.3	0.3	0.4	1.0	1.1
Sample-4 (BHA)	Y	4.6	4.7	4.7	4.7	4.8	5.3	5.5
	R	0.1	0.3	0.4	0.5	0.5	1.1	1.3
Sample-5 Control)	Y	4.6	4.7	4.8	4.8	4.9	5.2	5.7
	R	0.2	0.4	0.8	1.0	1.1	1.0	1.3

Table IIa: Colour index of 21 week old 4 hours repeated fried oil with different antioxidants

Sample	Colour	Time period
Sample-1 (TBHQ)	Y	9.8
	R	0.9
Sample-2 (BHT)	Y	17.6
	R	3.4
Sample-3 (BHT and BHA)	Y	19.3
	R	4.1
Sample-4 (BHA)	Y	17.1
	R	4.1
Sample-5 Control)	Y	21.7
	R	5.1

linearly but after 9 week FFA value accelerated and reached the maximum at the end of 21 week but in the case of the oil treated with TBHQ the acceleration of FFA value was not much high and this is because of the stability of antioxidant. Oils of 21 week old when underwent in the process of frying the FFA value reached it maximum but trerated oils (21 week old) with TBHQ the FFA value was not high as compared to BHT and BHA or even the combination of both. The reason might be that at high temperature the antioxidant decomposed. BHT and BHA decomposed and the resulting compounds have low density to inhibit the deterioration of oils but TBHQ when decompose at high temperature the product formed after the breakdown of the main compound may also be effective antioxidants. Lolos et al identified the oxidative stability of derivatives of TBHQ in his work when examined TBHQ in process od photooxidation (loles 1999).

Table III: Free acids of sunflower oil with different antioxidents

	Time duration						
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	0.06	0.08	0.09	0.11	0.13	0.15	0.19
Sample-2 (BHT)	0.07	0.09	0.12	0.15	0.19	0.21	0.24
Sample-3 (BHT and BHA)	0.07	0.10	0.11	0.15	0.19	0.22	0.27
Sample-4 (BHA)	0.08	0.10	0.12	0.18	0.20	0.24	0.3
Sample-5 Control)	0.09	0.12	0.17	0.29	0.36	0.4	0.79

Table IIIa: FFA of 21 week old 4 hours repeated fried oil with different antioxidants

Sample	Time duration (21 weeks)
Sample-1 (TBHQ)	4.2
Sample-2 (BHT)	4.9
Sample-3 (BHT and BHA)	7.1
Sample-4 (BHA)	7.9
Sample-5 Control)	11.9

Peroxide value is the measure of the concentration of peroxides and hydroperoxides formed in the initial stage of lippid oxidation. Peroxide value widely used to test the oxidative rancidity in oils/fats. (Camira and Kantor 1999). For this oxidation degree on sunflower oil samples were determined by measuring POV in the absence or presence of antioxidants at 30^{o}C for 21 weeks. The results showed the increase in antioxidant treatedoils and high acceleration in the oil with-

out antioxi dants. The result of fried 21 weeks old treated and untreated oil showed high POV and this is because. The presence of moisture in potatoes which help in boosting the oxidation process.

Smoke point showed rapid variation and this is because of thermal decomposition of oil during frying, free fatty acids, other volatile matter the degradation of oils leave the oils as gases and appear as smoke. The increase in FFA caused a considerable decrease in the smoke point of the fried oil

Table IV: Peroxide values of sunflower oil with different antioxidents

	Time duration						
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	3.82	4.55	5.26	6.82	7.81	8.9	9.7
Sample-2 (BHT)	4.15	4.85	5.82	7.60	8.12	10.1	12.7
Sample-3 (BHT and BHA)	4.22	5.02	6.20	8.10	9.05	10.3	12.4
Sample-4 (BHA)	4.35	5.22	7.85	9.16	9.25	10.1	12.9
Sample-5 Control)	4.42	8.54	12.32	12.45	32.65	38.76	44.05

Table IVa: Peroxide values of 21 week old 4 hours repeated fried oil with different antioxidants

Sample	Time duration (21 weeks)
Sample-1 (TBHQ)	17.9
Sample-2 (BHT)	21.4
Sample-3 (BHT and BHA)	21.3
Sample-4 (BHA)	27.5
Sample-5 Control)	59.4

although the oils treated with antioxidants. That's why scientists suggested that frying temperature should be kept low than their smoke point (Mcsavage.2001).

p-Anisidine value (AnV) play an important role in the oxidation process of edible oil and edible fats. Results of AnV measurement showed significantly increased trend throughout the storage time in control sample while slightly low consistency in increasing in treated oil samples. TBHQ treated oil showed prominent inhibitory effect on AnV and this is

Table V: Smoke point of sunflower oil with different antioxidents

	Time duration						
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	398	398	398	398	398	398	398
Sample-2 (BHT)	398	398	398	398	398	398	398
Sample-3 (BHT&BHA)	398	396	396	396	396	396	396
Sample-4 (BHA)	395	395	396	396	396	396	396
Sample-5 Control)	398	390	390	374	370	36	360

Table Va: Smoke point of 21 week old 4 hours repeated fried oil with different antioxidants

Sample	Time duration (21 weeks)
Sample-1 (TBHQ)	370
Sample-2 (BHT)	350
Sample-3 (BHT and BHA)	350
Sample-4 (BHA)	355
Sample-5 Control)	310

may be due to two para hydroxyl group which made phenol more easily to donate hydrogen atoms to active free radicals to interrupt the chain reaction of antioxidant. Previous study strengthened the same observation (Jiang 2006 and Madhavi 1995).

The AnV reached maximum after frying the oil because frying process boost the secondary oxidation of oils/fats.

Table VI: p - Anisidine value of sunflower oil with different antioxidents

	Time Duration						
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	11.7	14.0	20.7	29.9	29.9	30.1	30.7
Sample-2 (BHT)	11.9	15.5	19.8	28.7	30.3	30.4	32.2
Sample-3 (BHT and BHA)	12.3	16.1	18.7	29.0	32.7	32.9	35.0
Sample-4 (BHA)	12.7	16.1	19.7	29.8	32.9	33.7	36.4
Sample-5 Control)	17.7	20.1	20.9	32.7	32.9	34.0	38.7

Table VIa. p-Anisidine value of 21 week old 4 hours repeated fried oil with different antioxidants

Sample	Time duration (21 weeks)
Sample-1 (TBHQ)	42.2
Sample-2 (BHT)	51.3
Sample-3 (BHT and BHA)	67.8
Sample-4 (BHA)	64.3
Sample-5 Control)	81.9

The variation of antioxidant at different intervals of time did not show any significant effect on iodine value of treated oil samples with antioxidants but control sample showed continous decrease in iodine value which strengthened the possibility of oxidation process in the oil when the oil storage time accelerated.

term of iodine absorption seen which was drastic in the oil without any oxidants. The result indicated that there was a chemical change occurred in the chain of fatty acids of oils which was indicated by the lowering value of iodine.

Saponification value is related to the molecular weight of the fat and therefore provides information of the mean molecular weight of the combined fatty acid. Saponification value of sunflower oil with different antioxidant at different interval of time is shown in Table VIII. The variation of antioxidants at different time intervals do not show any significant effect on this value of sunflower oil.

Like other values fried oils with and without fortification of antioxidants showed the prominent change.

Table VII: Iodine values of sunflower oil with different antioxidents

	Time duration						
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	122.12	122.22	121.62	123.15	123.72	122.5	124.9
Sample-2 (BHT)	122.15	122.35	121.15	123.20	121.92	120.75	120.60
Sample-3 (BHT and BHA)	122.60	122.54	122.35	123.15	121.90	119.8	118.3
Sample-4 (BHA)	122.62	123.52	123.60	122.54	122.62	121.7	120.3
Sample-5 Control)	123.42	125.25	123.75	122.18	122.92	121.72	120.35

But when the 21 weeks old oils both treated and non treated oil were subjected for frying huge change in the values in

Table VIIa: Iodine value of 21 week old 4 hours repeated fried oil with different antioxidants

Sample	Time duration (21 weeks)
Sample-1 (TBHQ)	115.7
Sample-2 (BHT)	111.9
Sample-3 (BHT and BHA)	103.7
Sample-4 (BHA)	101.8
Sample-5 Control)	95

The fatty acid profile of fortified samples showed no significant change in the fatty acids of 21 week old oils which reflected that antioxidant addition in the unsaturated oils control the degradation of oils at with the addition of antioxidant but the fatty acid profile of sample 5 which was without antioxidant showed changes. The decrease in the amount of linoleic acid observed with the increase the concentration of oleic acid. Although both oleic and linoleic acid are essential fatty acids and their amount in such a good ratio lower the HDL while maintaining the LDL by reducing plasma lipoprotein by directing fatty acid metabolism away from the

Table VIII: Saponification value of sunflower oil with different antioxidents

	Time duration						
Samples	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks	18 weeks	21 weeks
Sample-1 (TBHQ)	188.00	188.95	189.50	188.25	190.52	190.78	191.89
Sample-2 (BHT)	188.90	189.75	190.10	190.95	191.72	192.82	193.75
Sample-3 (BHT and BHA)	189.15	190.25	190.85	191.75	192.13	193.73	194.85
Sample-4 (BHA)	189.25	190.75	190.25	190.25	192.13	193.92	195.05
Sample-5 Control)	190.25	191.9	191.35	192.35	193.25	194.5	195.8

Table VIIIa: Saponification value of 21 week old 4 hours repeated fried oil with different antioxidants

Sample	Time duration (21 weeks)
Sample-1 (TBHQ)	203.7
Sample-2 (BHT)	201.9
Sample-3 (BHT and BHA)	199.9
Sample-4 (BHA)	198.3
Sample-5 Control)	207

Fatty acid composition of the sunflower oil is shown in Table IX which clearly shows that oil synthesis and towards oxidation. It down regulate gene that control fatty acid and cholesterol synthesis (Kris 1999, Mensonk 1990).

The results of the present study conclude that all the antioxidants used in the study improved the storage stability of sunflower oil. The relative inhabition effect of antioxidants was in the following order TBHQ> BHT> BHT & BHA > BHA.

Table IX: Fatty acid composition of 21 week old sunflower oils with or without different antioxidant

		Time duration					
Short Hand Name	Name of fatty acie	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Fresh
Myrstic Acid	$C_{14:0}$	0.1	Traces	0.07	0.04	0.09	0.1
Palmitic Acid	$C_{16:0}$	6.8	7.0	6.3	6.2	6.1	6.4
Palmitoleic Acid	$C_{16:1}$	0.1	Traces	0.1	Traces	Traces	0.3
Stearic Acid	$C_{18:0}$	3.1	2.9	2.8	3.1	3.4	2.8
Oleic Acid	$C_{18:1}$	22.9	23.0	22.7	22.9	35.9	22.4
Linoleic Acid	$C_{18:2}$	22.9	23.0	22.7	22.9	35.9	22.4
Linolenic Acid	$C_{18:3}$	0.4	0.4	0.3	0.5	0.4	0.6
Arachidic Acid	$C_{20:0}$	0.3	0.5	0.4	0.7	1.5	0.4

Table IXa: Fatty acid composition of 21 week old fried sunflower oils with or without different antioxidant

		Time duration					
Short Hand Name	Name of fatty acie	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Fresh
Myrstic Acid	$C_{14:0}$	0.1	Traces	0.07	0.04	0.09	0.1
Palmitic Acid	$C_{16:0}$	6.8	7.0	6.3	6.2	6.1	9.6
Palmitoleic Acid	$C_{16:1}$	0.1	Traces	0.1	Traces	Traces	7
Stearic Acid	$C_{18:0}$	3.1	2.9	2.8	3.1	3.4	3.1
Oleic Acid	$C_{18:1}$	22.9	23.0	22.7	22.9	35.9	3.9.7
Linoleic Acid	$C_{18:2}$	59.7	59.9	60.9	61.7	52.1	63.1
Linolenic Acid	$C_{18:3}$	0.4	0.4	0.3	0.5	0.4	0.6
Arachidic Acid	$C_{20:0}$	0.3	0.5	0.4	0.7	1.5	0.4

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