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Effect of antioxidant on thermal stability of vegetable oils by using ultrasonic studies

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Abstract

The effect of repeated heating process on edible oils namely, sesame oil (SO), sunflower oil (SFO) and palm oil (PO) with and without antioxidant, tertiary butyl hydroquinone (TBHQ) have been evaluated using ultrasonic velocity. The changes in physico-chemical properties of edible oils has been studied during repeated heating at 180 ± 5ºC for 4 hrs/day and for 4 days. The effect of antioxidant, in edible oils on the thermal oxidation behavior has been studied for repeated heating process. The thermal degradation of oils have been evaluated for physical properties such as ultrasonic velocity, viscosity, density and secondary parameters like adiabatic compressibility, intermolecular free length, relaxation time and acoustic impedance. Chemical changes were studied by free fatty acid, total polar compounds and the changes in the unsaturation level of fatty acids were confirmed by gas chromatographic (GC) analysis. The repeated heating process had effect on the physical properties of oil, which is due to the decrease in unsaturated fatty acid composition and other chemical changes. It is observed that all three control oils properties significantly deteriorate on repeated heating. The oils with TBHQ don't cause significant changes on the physico-chemical properties and ultrasonic properties. This result clearly indicates that oils with 200 ppm TBHQ are found to be more thermally stable.

Introduction

Heat treatment is widely used process in food processing industries. As heat-processed foods are much appreciated, analytical studies that can assess the changes caused by heating have been demanded from researchers (Lambelet *et al.*, 2003). During food processing, oils and fats present in food, suffer thermal oxidation and produces compounds such as peroxides. The peroxides turn into aldehydes, ketones, epoxides, dimers and polymers, undermining the quality of food (Rossell, 2001). In order to minimize such effects, the food industry makes use of antioxidant (Litwinienko *et al.*, 1999). The important antioxidants used in the food industries are butylated hydroxylanisol (BHA), butyl-1,4-hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propylgallate (PG). Among them, TBHQ has been found to be the most effective synthetic antioxidant (de Guzman *et al.*, 2009; Pimpa *et al.*, 2009).

According to USDA Code of Federal Regulations, 'antioxidants' are substances used to preserve fats, oils and foods by retarding deterioration, rancidity or discoloration due to oxidation. The activity of an antioxidant can be estimated quantitatively by estimation of primary or secondary products of auto oxidation (Kishk, 2007) of fats, oils and foods or by monitoring other variables. Normally, this occurs via the delay in hydro peroxides formation or production of secondary products of auto oxidation by chemical or sensory methods (Choe and Min, 2006). The active oxygen method (AOM), Schaal oven test, oxygen uptake/absorption, oxygen bomb calorimetry and Racimat apparatus are used to estimate the activity of an antioxidant by quantitative determination of primary or secondary products of auto oxidation of fats, oils and foods.

The formation of hydro peroxides is measured by iodometric titration and is expressed as the peroxide value (PV). Of the available methods for measuring hydro peroxides decomposition products, determination of aldehydic compounds (e.g., spectrophotometric determination of malonaldehyde using the 2-thiobarbituric acid (TBA) test or p-anisidine test) (Gulla and Waghray, 2011), measurement of total carbonyls or selected carbonyl compounds and assessment of off-flavors due to the formation of volatile decomposition products of

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hydro peroxides by objective and subjective means are used extensively. Most sophisticated analytical tools such as NMR, FT-IR, NIR (Barison *et al*., 2010) though meet the requirements, they are cost intensive. Therefore a cost effective and simple instrumental method needs to be developed to meet the above requirements.

Ultrasonic method is simple, instantaneous and cost effective method to study the physical characteristics of various products for quality control purposes. Ultrasonic velocity has been used to understand the molecular interactions in binary liquid mixtures by many scientists (Ali and *et al*., 2000; Ernest and Kavitha, 2011) and have explained the increases in values like isentropic compressibility and intermolecular free length may be attributed to the presence of molecular interactions between the molecules. Also interactions in the quaternary mixtures have been studied (Pandey, 2005). Although limited information is available on the determination of physical properties of triglycerides by using ultrasonic interferometry technique (Benedito *et al*., 2007; Izbaim *et al*., 2010). There is a lack of literature on ultrasonic properties of repeatedly heated oils with or without antioxidants and the molecular interaction between lipid and antioxidant molecule. So an effort has been made to evaluate the stability of thermally processed edible oils such as sesame oil (SO), sunflower oil (SFO) and palm oil (PO) by using ultrasonic velocity and the effect of synthetic antioxidant (200 ppm), tertiary butyl hydroquinone (TBHQ) on all three oils.

Materials and Methods

Chemicals and reagents

Fresh sesame and sunflower oils were obtained from M/s. Sri Murugan Oil Mills, Mandya, India. PO was obtained from Pentagon Overseas India Limited. Tertiary butyl hydroquinone (TBHQ) was purchased from Sigma Aldrich and other chemicals of AR grade were used for all other determinations.

The edible oils such as sesame oil (SO), sunflower oil (SFO) and palm oil (PO) and their mixture with 200 ppm of TBHQ (SOQ, SFOQ and POQ) were repeatedly heated at 180 ± 5°C for 4 h per day and for 4 days and its effect on oil properties has been studied. In this study, 200 ppm of TBHQ (Akhtar, *et al*., 2012; Choe and Min, 2006) was used to formulate the vegetable oils as per FDA norms (Codex 1995).

SO, SFO and PO were stored in a fridge (8 – 10^0C) after they were extracted, then the day before the experiments they were kept in ambient condition to reach room temperature. Initially the oil was heated up to 180 ± 5°C and kept at this temperature for 4 h. Every day the oils were heat treated for 4 h. After every heating, the required samples were drawn, allowed to cool to room temperature and all the physico-chemical properties were determined. The process was repeated for four consecutive days. So oils exposed for 0, 4, 8, 12 and 16 h of heating at 180 ± 5°C, were then cooled to room temperature and characterized. All measurements of heat treated oils were carried out in triplicate and an average values are reported.

Techniques

The density (ρ) of the oils has been measured using a 10 mL specific gravity bottle and distilled water was used as the reference. The viscosity (η) of the oil and oil-antioxidant mixtures were measured using an Ostwald's viscometer immersed in a temperature controlled water bath (Advance Technocracy Inc.; Ambala; India).

Ultrasonic velocity

The velocity (U) of ultrasonic waves in the oils were measured using an ultrasonic interferometer (Mittal Enterprises; New Delhi, India), working at a fixed frequency of 2 MHz with a tolerance of ± 0.005%. The measuring cell was a specially designed, double-walled vessel to provide a constant temperature. The instrument was calibrated by using pure water and carbon tetrachloride (Kannappan and Santhi, 2005).

The adiabatic compressibility (β) is defined as the decrease of volume per increase of pressure when no heat flows in or out. Such a change is related to the compressibility of the medium by the thermodynamic relation as follows;

$$\beta = -\frac{1}{v}\left[\frac{\delta_v}{\delta_p}\right] \qquad (1)$$

where, V is the volume, δ_v is the relative change in volume and δ_p is the relative pressure change. It can also be calculated from the ultrasonic velocity (U) and the density of the medium (ρ) using Newton Laplace (Priya *et al*., 2010) equation as follows;

$$\beta = \frac{1}{U^2 \rho} \qquad (2)$$

The adiabatic compressibility of liquid can be expressed in terms of intermolecular free length (L_f), which is the distance between the surfaces of the neighboring molecules and is given by the following equation;

$$L_f = K_T \, \beta_{ad}^{\frac{1}{2}} \qquad (3)$$

where, K_T is the temperature-dependent constant, 201.1209 × 10^{-8} at 303 K.

The relaxation time is the time taken for the excitation energy to appear as transitional energy and it depends on the temperature and impurities. The dispersion of the ultrasonic velocity in a mixture reveals information about the characteristic time of the relaxation process that causes dispersion. The relaxation time (τ) can be calculated using formula (Priya *et al.*, 2010);

$$\tau = \frac{4}{3}\beta\ \eta \qquad (4)$$

The specific acoustic impedance (Z) is as follows (Priya *et al.*, 2010);

$$Z = U\rho \qquad (5)$$

where, U and ρ are the velocity and density of the oil respectively.

Fatty acid composition by gas chromatography

Fatty acid methyl esters (FAME) of the oils were prepared by transesterification, according to AOCS method No.: Ce 1-62, 1998 (Firestone 1998). FAMEs were analyzed by gas chromatography (GC) (Fisons 8000, Co., Italy), equipped with a hydrogen flame ionization detector (FID) and a fused silica capillary column (100 m 0.25 mm i.d.), coated with 0.20 µm SP2560 (Supelco Inc., Bellefonte, PA) as the stationary phase. The oven temperature was programmed from 140 to 240 at 4 /min with an initial hold at 140 for 5 min. The injector and FID were maintained at 260 . A reference standard, FAME mix (Supelco Inc.) was analyzed under the same operating conditions to determine the peak identity. The FAMEs was expressed as relative area percentage.

Total polar compounds

The AOCS method Cd 20-91 was used to determine total polar compounds (TPC). A chromatogram column (21 mm i.d. and 450 mm long) was filled with about 30 mL of a mixture of light petroleum and diethyl ether (87:13, v/v). A wad of glass wool was introduced at the lower end of the column with the aid of glass rod. Slurry of 24 g of silica gel in about 80 mL of the solvent mixture was poured into the column. The elution solvent was drained through the column until its level was 10 cm above the silica gel. About 4 g of sea sand was added and the supernatant was drained down to the sand layer. For determination of TPC, 2.5 g of oil was dissolved in 20 mL of solvent mixture containing light petroleum and diethyl ether (87:13 v/v) at room temperature. The resulting solution was introduced into the column and drained off to the level of the sand layer. The non polar compounds were eluted with 150 mL of solvent mixture at a flow rate of 2.5 mL min^{-1}. TPC was calculated using the formula;

$$TPC\ (\%) = [m-m1] / 100 \qquad (6)$$

where, m1 is the mass (g) of the non polar fraction and m is the mass (g) of the oil containing 20 mL of solution added to the column.

Free fatty acid

The free fatty acid (FFA) content as the percentage of oleic acid was determined using AFNOR NF T 60-204 methods. Acid value was defined as the amount (mg) of KOH required to neutralize 1 g of oil sample dissolved in a mixture of diethyl ether and ethanol.

Statistical analysis

All determinations were carried out in triplicates and values were expressed as means ± standard deviation (SD). Significant statistical differences of investigated parameters were determined by Fisher's least significant differences (LSD) test at $p < 0.05$, after analysis of variance (ANOVA).

Results and Discussion

Density, viscosity and ultrasonic velocity

Figure 1[A] shows the variation of density of SO, SFO and PO with heating time at 180°C. It was noticed that the densities of the fresh oils differ slightly with one another. Such differences are common between different types of oils and are due to differences in composition (Formo *et al.*, 1979). It has been recorded that initially PO has low density as compared to that of SO and SFO. In the present work it observed that upon heating the density of all oils increases with heating time significantly. Similar kind of observation is reported elsewhere (Paul and Mittal, 1996; Tyagi and Vasishtha, 1996; Valantina *et al.*, 2010), but to the best of our knowledge not for palm or sunflower or sesame oil. During heating process, oxidation, polymerization and isomerization occurred in the oil, generating a multiple pyrolysed products (Belitz *et al.*, 2004). However, all oils with TBHQ (SOQ, SFOQ and POQ) showed increase in values is lower density with repeated heating processes compared to control oils. This result shows a sign of thermal stability of edible oils, which indicates the effectiveness of antioxidant activity.

The changes in the viscosity values of oils as a

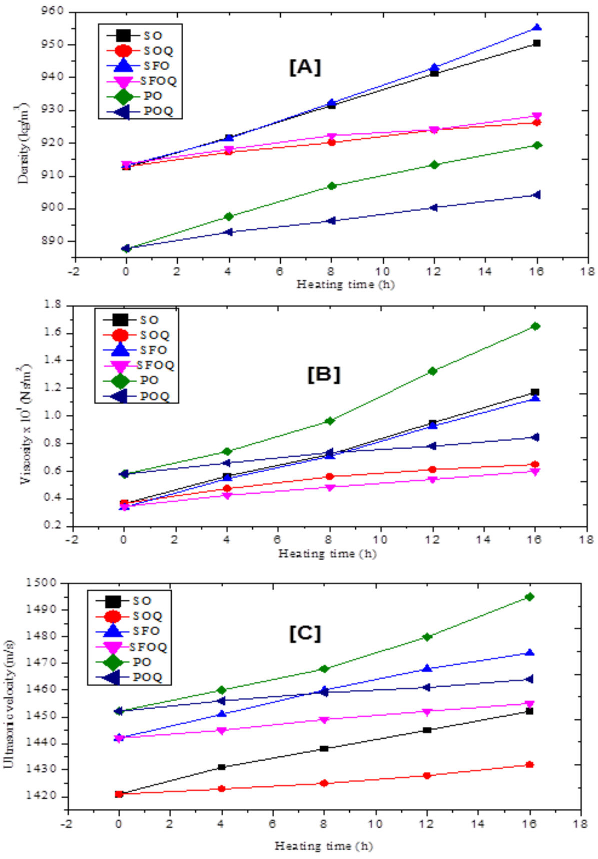

Figure 1. Variation of density, viscosity and ultrasonic velocity of the sesame, sun flower and palm oil with and without TBHQ as a function of heating time at 180^0C

function of heating time have been plotted in Figure 1[B]. The viscosity (η) gradually and substantially increased with an increase in the heating period. Adding 200 ppm of TBHQ into SO, SFO and PO led to small increments in the viscosity values during the heating period compare to oils without TBHQ. Figure 2 shows that the heat treated oils had the drastical increase in viscosity values from 0.36553 × 10^{-1} to 1.17236 × 10^{-1} Ns/m^2 in SO, from 0.33824× 10^{-1} to 1.12456 × 10^{-1} Ns/m^2 in SFO and 0.5785 × 10^{-1} Ns/m^2 to 1.6523 × 10^{-1} in PO. Furthermore, oil with 200 ppm of TBHQ had the lowest viscosity value changes, from 0.36793 × 10^{-1} Ns/m^2 to 0.64842 × 10^{-1} for SOQ, 0.34238 × 10^{-1} to 0.59871 × 10^{-1} Ns/m^2 for SFOQ and 0.5786 × 10^{-1} to 0.84419 × 10^{-1} Ns/m^2 for POQ. The obtained results were in good agreement with the results published elsewhere (Shaker, 2006; El Anany, 2007; Rehab, 2010). The viscosity of oil increases with duration of frying due to oxidation, isomerization and polymerization reactions. An oxidation reaction leads to the formation of carbonyl or hydroxyl groups bonded to a carbon chain resulting in flux among the molecules that in turn increases the viscosity (Priya *et al*., 2010). The larger changes in the viscosity of fresh oils may be due to formation of polymers of triacylglycerols, which are the most abundant degradation products. During food frying or simple heating, many reactions result in increasing saturation (Belitz *et al*., 2004); these reactions may also affect viscosity changes.

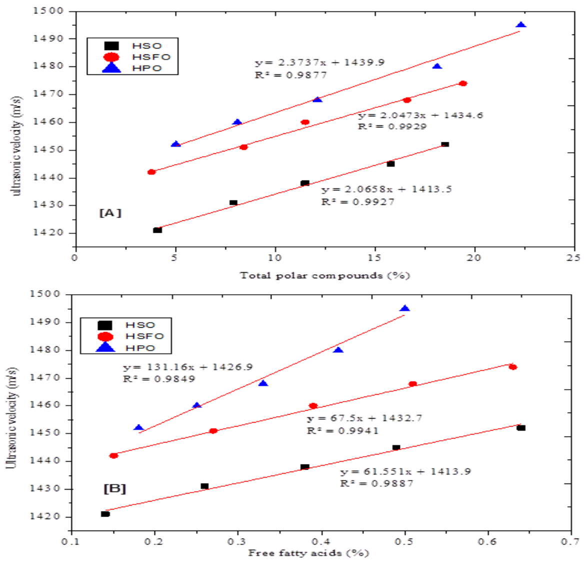

Figure 2. Plots of ultrasonic velocity as a function of polar compounds and free fatty acids for the HSO, HSFO and HPO at 30^0C

Sound velocity in the oil can be measured as like the other lubricant properties. Sound velocity in the liquid is lower than that of solid. So, it directly related to the bulk modulus or modulus of elasticity of the substances (Mia, 2010). The ultrasonic velocity and attenuation depends on the physico-chemical properties of the oil (McClements, 1997). Majority of the studies which used ultrasonic evaluation of food properties use ultrasonic velocity, as it is more reliable than attenuation, and related to the physico-chemical properties of the medium (Benedito *et al*., 2007).

Figure 1[C] displays the effect of physico-chemical changes on the ultrasonic velocity of all the oils. It was observed that the ultrasonic velocity increased in the same pattern of density and viscosity as the heating time was increased in all the three oils. The increased in ultrasonic velocity with heating period indicates a sign of deterioration. However, there was retardation in the degradation of oils when treated with TBHQ, which is indicated by smaller increments in the ultrasonic velocity values for the oils treated with TBHQ. The degradation effect is found to be more on unsaturated fatty acids than saturated fatty acids. The fatty acid has a marked effect on heating performance as well as on physico–chemical behaviour. The linoleic acid behaviour is more negative factor on stability of oil. All these changes affect on the ultrasonic velocity of the oil.

Table 1. Variation of Adiabatic compressibility, intermolecular free length, relaxation time, acoustic impedance of the sesame, sun flower and palm oil with and without TBHQ at different heating time

Heating Time in hrs	SO	SOQ	SFO	SFOQ	PO	POQ
	Adiabatic compressibility $(\beta) \times 10^{10}$ m^2/N					
0	5.4267	5.4196	5.2667	5.2635	5.3428	5.3424
4	5.3036	5.3866	5.1544	5.2162	5.2263	5.2836
8	5.2085	5.3634	5.0319	5.1637	5.1166	5.2416
12	5.1214	5.3255	4.9201	5.1326	4.9984	5.2033
16	5.0442	5.2939	4.8183	5.0883	4.8665	5.1598
Heating Time in hrs	Intermolecular free length $(L_f) \times 10^{10}$ m					
0	0.46851	0.46821	0.46155	0.46141	0.46488	0.46486
4	0.46317	0.46678	0.45661	0.45934	0.45978	0.46229
8	0.45901	0.46577	0.45115	0.45702	0.45493	0.46045
12	0.45514	0.46412	0.44611	0.45564	0.44964	0.45877
16	0.45172	0.46274	0.44147	0.45367	0.44367	0.45685
Heating Time in hrs	Relaxation time $(\tau) \times 10^{11}$ s					
0	2.6448	2.6586	2.3750	2.4028	4.1211	4.1214
4	3.9975	3.5305	3.7496	2.943	5.1741	4.6483
8	5.0083	3.9887	4.7449	3.3311	6.5651	5.1362
12	6.3509	4.3332	6.0724	3.6928	8.8315	5.4198
16	7.5126	4.5767	7.2111	4.0618	10.721	5.8152
Heating Time in hrs	Acoustic impedance $(Z) \times 10^{-3}$ kg^{-1} m^2 s^{-1}					
0	1296.79	1298.48	1316.7	1317.51	1289.02	1289.11
4	1317.61	1304.62	1337.05	1326.69	1310.53	1299.88
8	1335.13	1308.39	1361.17	1336.49	1331.34	1307.59
12	1351.26	1314.94	1384.5	1341.8	1351.77	1315.42
16	1365.33	1320.02	1408.01	1350.69	1374.47	1323.79

Values are Mean (n= 3) at $p < 0.05$

After each heating period, the ultrasonic velocity increases and thus it is possible to distinguish the two oils with and without thermal degradation (Izbaim *et al*., 2010). The ultrasonic velocity plays a major role in understanding the nature of intermolecular interactions in liquid mixtures (Voleišienė and Voleišis, 2008). The increased in ultrasonic velocity may be due to the pyrolysed products which are formed after collapsing the triglyceride structure of oils.

Table 1 illustrates the interaction between the ultrasonic waves and the composition of oil molecules. The adiabatic compressibility and free length are deciding factors of the ultrasonic velocity in liquid systems (Priya *et al*., 2010). The increase in the ultrasonic velocity was due to decrease in the free length and adiabatic compressibility. The adiabatic compressibility and free length were found to differ slightly for oils with 200 ppm of TBHQ as compared to the changes in the base oils. The decrease in the values of the free length indicates that the triglyceride molecule with unsaturated bonds is likely to collapse as a result of the oxidation process (Okoye *et al*., 2009). The structures of triglycerides are kept intact due to the presence of TBHQ in oil (de Guzman 2009). Hence, the values of free length which indicates the intermolecular distance didn't reduce as a result of using TBHQ.

The variation in the relaxation time of oils as a function of heating time is presented in Table 1. It was found that relaxation time is increased with the heating period. The base oils showed greater enhancement in relaxation time and its least increment were found in oil with 200 ppm of TBHQ. The dispersion of ultrasonic velocity in the system should contain information about the characteristic time, τ of the relaxation process that causes dispersion, where, τ is in the order of 1×10^{-11} s due to the structural relaxation process (Benedito *et al*., 2007) and in such a situation, the molecules get rearranged due to a co-operative process (Ali *et al.*, 2000).

The acoustic impedance is calculated using ultrasonic velocity and density and obtained data is tabulated in Table 1. It was found that the acoustic impedance was low in the oil with TBHQ and without heating. With increased heating time, the acoustic impedance increased noticeably in the base oils, but to a very little extent in the oil with 200 ppm of TBHQ. The excess parameters play a major role in understanding the nature of intermolecular interactions in liquid mixtures (Ernest, 2011). The intermolecular free length and acoustical impedance depend upon the intermolecular attractive and repulsive forces. Excess acoustical impedance may be due to the pyrolysis effect allowing the fitting of molecules of different sizes after collapsing the

Table 2. Fatty acid profile of fresh oils and heated oils with and without TBHQ using GC

Fatty acid	SO	HSO	HSOQ	SFO	HSFO	HSFOQ	PO	HPO	HPOQ
C14:0	ND	ND	ND	ND	ND	ND	1.7 ± 0.2	4.5±0.2	2.5±0.3
C16:0	11.2 ± 0.9	19.8±0.51	16.86±1.12	6.9 ± 0.9	17.8±0.42	11.3±0.5	44.3 ± 1.2	50.8±1.2	45.8±1.1
C18:0	5.7 ± 0.5	12.86±0.34	8.16±0.85	2.9 ± 0.3	9.8±0.62	6.9±0.4	4.8 ± 0.4	10.9±0.6	11.4±0.5
C18:1	38.9 ± 1.5	36.85±1.15	37.75±1.13	42.7 ± 1.6	40.6±0.8	42.3±0.8	38.4 ± 1.1	27.3±0.8	32.8±1.2
C18:2	44.2 ± 1.4	29.23±1.14	37.23±1.16	47.5 ± 1.9	31.8±0.9	39.5±1.1	10.8 ± 0.8	6.5±0.3	7.5±0.4
C18:3	0.35 ±0.01	ND	ND	ND	ND	ND	ND	ND	ND
Total	100	100	100	100	100	100	100	100	100
SFA %	16.9 ± 1.4	32.66± 1.4	24.84± 1.3	9.8 ± 1.2	27.60± 1.2	18.20± 1.2	50.8 ± 1.8	66.20±1.4	61.7± 1.5
MUFA%	38.9 ± 1.5	36.85± 1.4	37.75±1.13	42.7 ± 1.6	40.6±0.8	42.3±0.8	38.4 ± 1.1	27.3±0.8	32.8±1.2
PUFA %	44.2 ± 1.4	29.23± 1.2	37.23±1.16	47.5 ± 1.9	31.8±0.9	39.5±1.1	10.8 ± 0.8	6.5±0.3	7.5±0.4

C14:0, Myristic acid; C16:0, Palmitic acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; α-C18:3, α-linolenic acid, SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, ND - not detected

Values are mean ± S.D (n= 3) at $p < 0.05$

triglyceride structure.

The measured acoustic parameters such as β, τ, L_f and Z were correspondingly found to significantly change in the base oils. The addition of TBHQ didn't allow any breaking up of the molecular clustering in the oil. The interaction between the molecules of oil occurred to a lesser extent and hence, very small structural changes occurred during heating in the presence of an antioxidant. In the base oils, as the antioxidant activity is much less, there is a break up in the molecular clustering, releasing several dipoles for the interaction. In view of the greater interactions, larger changes have been occurred for heat treated oils in the adiabatic compressibility, intermolecular free length, relaxation time and acoustical impedance. It was found that the thermally stability of all three oils with antioxidant, TBHQ was better even at high temperature and repeatedly heating process (Okoye *et al*., 2009; Rehab, 2010).

Fatty acid composition

Gas chromatography (GC) is a useful analytical technique for studying the effect of different heating conditions on the changes in fatty acid composition of oils (Kowalski, 2007). Table 2 indicate the fatty acids composition of SO, SFO and PO before heating and HSO, HSFO and HPO after heating. Also the fatty acid profile changes for the three oils with TBHQ after heating are shown as HSOQ, HSFOQ and HPOQ. The results show generally that, in all the three heating systems, there was decrease in linoleic acid (C18:2) content, whereas palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) were increased with heating time. In particular, during heating, polyunsaturated fatty acid values (PUFA) were decreased and total saturated fatty acid values were increased. Many researchers (Arroyo *et al*., 1995; Jorge *et al*., 1997) have pointed out that the heat treatment of oils and fats induces modifications of fatty acids with two or three double bonds. In the current study, the level of PUFA tended to decrease, whereas that of saturated fatty acids increased. The estimated concentration of the unsaturated fatty acids by means of relative GC peak areas showed that SO and SFO had a good unsaturation level compared to that of PO. After 16 h of heating, unsaturation level decreases in all the three oils more in comparison with samples treated with 200 ppm TBHQ. The decrease in PUFA i.e., linoleic acid (18:2) is found to be 44.2 to 29.23% in SO, 47.5 to 31.8% in SFO and 10.8 to 6.5% in PO. At the same time there is increase in SFA; in SO is 16.9 to 32.66%, in SFO is 9.8 to 27.6% and in PO is 50.8 to 66.2% (Akhtar 2012).

These changes in fatty acid profiles are responsible for major changes in physical properties of untreated oil mainly. The fatty acid profile of all the three oils with TBHQ showed insignificant changes in the fatty acids composition even after heating, which reflected that antioxidant addition in the unsaturated oils control the degradation of oils. The addition of TBHQ enhanced the oxidative stability of the heated oil samples. The FA composition of oil has marked effects on its frying performance as well as on its physico-chemical behaviour. The FA profile of the heated oils changed as a result of cyclization, polymerization and hydrolytic, oxidative and other chemical reactions promoted by frying conditions (Nawar, 1996).

Polar compounds and free fatty acids

Generally, degradation of oil during frying is accompanied by increasing the polar compounds of

Table 3. Variation in % of polar compounds and free fatty acids in oils upon heating

Heating time (h)	Total polar compounds (%)					
	SO	SOQ	SFO	SFOQ	PO	POQ
0	4.1±0.21	4.15±0.17	3.8±0.25	3.8±0.24	5.0±0.26	5.0±0.26
4	7.6±0.34	6.2±0.31	7.4±0.23	5.5±0.24	8.1± 0.21	7.2±0.34
8	11.5±0.26	7.9±0.24	12.5±0.05	7.4±0.18	12.1±0.34	8.9±0.05
12	15.8±0.21	9.1±0.19	16.6±0.42	8.5±0.24	18.1±0.45	10.1±0.51
14	17.2±0.41	9.8±0.24	17.8±0.21	9.0±0.34	20.6±0.41	10.8±0.48
16	18.5±0.52	10.5±0.32	19.4±0.21	9.5±0.52	22.3±0.43	11.5±0.47
Heating time (h)	Free fatty acid (%)					
0	0.14 ±0.01	0.11± 0.02	0.15± 0.01	0.15± 0.02	0.18± 0.01	0.16± 0.01
4	0.27± 0.01	0.19± 0.01	0.27± 0.03	0.21± 0.02	0.25± 0.02	0.20± 0.01
8	0.38± 0.03	0.27± 0.02	0.39± 0.03	0.27± 0.02	0.33± 0.03	0.24± 0.02
12	0.49± 0.01	0.33± 0.02	0.51± 0.01	0.31± 0.02	0.42± 0.02	0.28± 0.03
16	0.61± 0.02	0.38± 0.03	0.63± 0.02	0.37± 0.03	0.51± 0.02	0.32± 0.02

Values are mean ± S.D (n= 3) at $p < 0.05$

oil (Innawong, 2004). Many researchers reported that the total polar components (TPC) to be the most reliable indicator of oil degradation (Fritch, 1981; Gere, 1982). Polar compounds include all oxidized triglycerides, dimerized triglycerides, FFA's, monoglycerides, diglycerides, sterols, antioxidants, antifoamers, hydrogenation catalyst residues and soaps (Blumenthal, 1996) formed during repeated heating of oils. Table 3 shows the percentage of TPC formed during heating process of oils with and without TBHQ. Initially, the TPC contents of base oils and formulated oils were similar. The increase in the rate of TPC formation in the base oils were different from formulated oils. After 16 h of heating, the final TPC values were 18.5% and 19.4% in SO and SFO respectively, and in case of PO, it is 22.3% which is more compare to SO and SFO. TPC indicates the state of oil deterioration and in all of them it was noticed that oils with TBHQ were more stable than virgin oils. Furthermore, the amounts of polar compounds can be a good indicator of quality of fried oils. Polar compounds are sum of non triglycerides including fatty acids, sterols, tocoferols, mono and di triglycerides, alcohols, aldehydes, ketones and other soluble compounds in oils (Melten *et al*., 1994). According to few researchers (Billek *et al*., 1978; Nawar, 1996) the degradation of oils can be measured by polar compounds, which indicate the breakdown of triglycerides. During frying process the polar compounds accumulate on the surface of frying pan and foods. It can be imagined that the most poisonous components are exist in the polar compounds of oil (Frankel and Huang, 1994). If the TPC exceed more than 25%, the oil should be disposed according to FDA regulations. It is observed from Table 3 that the polar compound values are not exceeding 25% when the edible oils formulated with 200 ppm of TBHQ and heated repeatedly i.e., polar compounds are found to be 10.5% for SO, 9.5% for SFO and 11.5% for PO with TBHQ. This result clearly indicates that 200 ppm TBHQ is the optimized composition for these edible oils.

Formation of free fatty acids (FFA) during frying process is considered to be a measure of rancidity of oils. The obtained percentage of FFA formed during repeated heating of oils is addressed in Table 3. Initially, the base oils and formulated oils with TBHQ had a similar FFA content, as the heating period is continued, all the three untreated oils showed a higher values of FFA content (Izbaim *et al*., 2010) than the TBHQ loaded oils. FFA is formed due to hydrolysis of triglycerides and may be promoted by the reaction of oil with moisture. Addition of antioxidant caused significant reduction in FFA values of heat processed oils. FFA content is frequently used data to probe the shelf life of frying oils, but it is not recommended to be the only indicator. Because of the cleavage of triglycerides during oxidation and hydrolysis, FFAs are formed. Previous studies have shown that FFA and other volatile substances increases during deep-frying of oil and affect the smoke point (Kalapathy and Proctor, 2000). Oils with high FFA are known to have a lower smoke point (Augustin *et al*., 1987) and the surfactant effect of FFA contributes to foaming, which leads to further oxidation of the oil. From Table 3, it is observed that FFA increased to almost same extent in SO (0.61) and SFO (0.63) but in case of PO, FFA level is increased to lesser extent (0.51). This result indicates that, in addition to FFA, there are other compounds also may be formed during frying of oils.

As long as the ultrasonic velocity is related to the physical properties of the medium like viscosity and density, velocity should also be related to these chemical indicators. Relationship between the ultrasonic velocity with both TPC and FFA during 16 h of heating for SO, SFO and PO at 30°C is shown in Figures 2[A] and [B] respectively. The changes in polar compounds with ultrasonic velocity are found to be almost linear for all the three oils (Izbaim *et al*., 2010). These findings are in accordance with literature data (Benedito *et al*., 2007). These physico-chemical properties, widely considered as oil quality indicators and significant polynomial fits were found when relating the velocity. Therefore, measurement of ultrasonic velocity can be used as a simple technique that relates to changes in percentages of polar compounds and FFA in the oil.

Conclusions

In the current study a simple ultrasonic method has been used for the measurement of ultrasonic velocity and calculation of secondary parameters like adiabatic compressibility, intermolecular free length, relaxation time and acoustic impedance to probe the thermal stability of SO, SFO and PO. Addition of 200 ppm of TBHQ was found to be an effective in all the oils irrespective of degree of unsaturation or different types of chemical changes occurring during repeated heating of oils and its addition resulted in the retardation of oxidative deterioration. It is found that the fatty acid composition of the oil became saturated to a very little extent due to the presence of 200 ppm of TBHQ. The changes occurred in SO, SFO and PO determined by ultrasonic velocity is in good agreement with other physico-chemical results found elsewhere. SO, SFO and PO with TBHQ have better thermal stability than oil without antioxidant and can be used at frying temperatures. Finally it can be concluded that ultrasonic velocity method is a simple and fast approach to evaluate the oxidative stability of edible oils.

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