

Effect of antioxidant butylated hydroxyl anisole on the thermal or oxidative stability of sunflower oil (*Helianthus Annuus*) by ultrasonic

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Abstract The aim of the current investigation was to evaluate the efficiency of butylated hydroxyl anisole (BHA) as an antioxidant in sunflower oil (*Helianthus Annuus*). The oxidation stability of sunflower oil have been investigated by the effects of varying amounts of BHA. The antioxidant incorporated sunflower oil system and control edible oil were subjected to heating at 180 ± 5 °C continuously for a period of 4 h per day for consecutive 4 days. The parameters used to assess the thermal degradation and oxidation properties of the oils include ultrasonic velocity, viscosity, density and peroxide value. The fatty acid compositions of the oils were measured by gas chromatography. Adiabatic compressibility, intermolecular free length, relaxation time and acoustic impedance have been calculated from experimental data. Viscosity, density and ultrasonic velocity change in control oil is from 3.72×10^{-2} to 13.2×10^{-2} Nsm⁻², 918 to 994 kg/m³ and 1412 to 1484 m/s respectively and in sunflower oil with 200 ppm BHA is from 3.88×10^{-2} to 7.52×10^{-2} Nsm⁻², 926 to 962 kg/m³ and 1418 to 1463 m/s respectively for 16 h of heat treated oil. The ultrasonic results obtained have shown reduction in thermal degradation and improvement in oxidation stability of antioxidant loaded oil in comparison to base oil. Hence, it can be recommended that sunflower oil with 200 ppm BHA can be used for frying without adverse effect on physical properties.

The ultrasonic velocity can be used for assessment of stability of frying oil.

Keywords Acoustical parameters · Gas chromatography · Sunflower oil · Peroxide value · BHA · Ultrasonic velocity

Introduction

Deep-fat frying is one of the oldest methods of food preparation. A series of complex reactions occur during the frying process due to the high temperature which include the hydrolysis, oxidation and polymerization of oils with hydroperoxides being important oxidation products formed during deep-fat frying (Rossell 2001). These decompose to give secondary products such as esters, aldehydes, alcohols, ketones, lactones and hydrocarbons. The change in quality of food and the loss of nutritional value is due to the secondary products formed, which also affect taste, flavor and aroma of the food. It has been also found that certain secondary products formed during the oxidation are toxic (Nawar 1996; Min and Boff 2001). Acceptable synthetic antioxidants are used to avoid these reactions. The important antioxidants used in the food industry are butylated hydroxyanisole (BHA), butyl-1-4-hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propylgallate (PG) of which, TBHQ has been found to be the most effective antioxidant (de Guzman et al. 2009; Pimpa et al. 2009).

Ultrasonic have been utilized for chemometrics for more than five decades as they are inherently well suited for characterization of composition, reacting system conditions, mixing and multiphase properties and provide real time images and help in characterization process. For example, the measurement of ultrasonic speed (Izbaim et al. 2009) enables the accurate determination of some useful acoustic and

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thermodynamic parameters and the variation in the acoustic parameters is due to the molecular interactions in liquid mixtures (Ali et al. 2004; Aralaguppi and Barragi 2006).

A few studies (Sarmento et al. 2006; Rehab 2010; Okoye et al. 2009; Valantina et al. 2010) have revealed that the measurement of viscosity and density can be used to determine the oxidative stability and ultrasonic velocity (Izbaim et al. 2009) to understand the molecular interactions. The molecular interaction (Kesavasamy et al. 2008; Priya et al. 2010) ultrasonic velocity (Izbaim et al. 2010) can be used to determine the stability of oil. However, the effect of a synthetic antioxidant on the ultrasonic velocity has not been fully elucidated.

Materials and methods

Reagents and chemicals

Sunflower oil and BHA was obtained from Sigma Aldrich and AR grade chemicals were used for the peroxide value determination.

Samples of sunflower oil (control) and sunflower oil with 50, 100, 150 and 200 ppm of BHA were heated in an oil bath to a temperature of 180 °C continuously for 4 h per day and for 4 days. Oil samples were exposed for 0, 4, 8, 12 and 16 h at 180 °C. Then, the samples were cooled to room temperature and characterized. All measurements were carried out in triplicate and average values reported.

Physical analysis

The density (ρ) of the pure oils and mixtures 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 (Advance Technocracy Inc.; Ambala; India) and it was calibrated with double distilled water. The Ostwald's viscometer (Saeed et al. 2012; Kimilu et al. 2011) was immersed in a temperature controlled water bath. The time of flow was measured using a Racer stopwatch with an accuracy of 0.1 s. Viscosity was determined using the relationship,

$$\eta_2 = \eta_1 \frac{t_2 \rho_2}{t_1 \rho_1} \quad (1)$$

Where, η_1 is the viscosity of water, t_1 is the time of flow of Water, ρ_1 is the density of water, η_2 is the viscosity of the experimental liquid, t_2 is the time of flow of the experimental liquid and ρ_2 is the density of the experimental liquid.

The velocity (U) of ultrasonic waves in the mixtures 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 high frequency generator excited a quartz crystal fixed at the bottom of the measuring cell, at its resonant frequency. A fine micrometer screw, with a least count of 0.01 mm at the top of the cell, was used to raise or lower the reflector plate in the liquid through a known distance. The measuring cell was connected to the output terminals of the high frequency generator through a shielded cable. Ultrasonic waves in the quartz crystal were reflected from the reflector plate, with stationary waves being formed in the region between the reflector plate and the quartz crystal. The micrometer was slowly moved till a number of maximum readings (n) of the anode current had passed. The total distance (d) moved by the micrometer was recorded. The wavelength and velocity of the ultrasonic waves in the liquid is given by, $\lambda = 2d/n$ and $U = f \lambda$ respectively, where, f is the frequency of the ultrasonic wave.

Secondary parameters

Adiabatic compressibility

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 using the thermodynamic relation as in

$$\beta = \frac{1}{V} \frac{\delta V}{\delta p} \quad (2)$$

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

$$\beta = \frac{1}{U^2 \rho} \quad (3)$$

Inter molecular free length

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

$$L_f = K_t \beta_{ad}^{1/2} \quad (4)$$

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

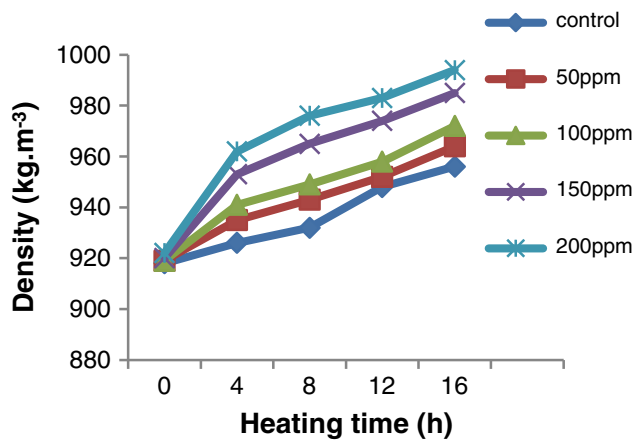


Fig. 1 Variation of density as a function of heating time for sunflower oil and sunflower oil with different concentrations of butylated hydroxy anisole

Relaxation time

The time taken for the excitation energy to appear as transitional energy is the relaxation time and it depends on temperature and impurities. The relaxation time (τ) can be calculated from Eq. (5) (Priya et al. 2010).

$$\tau = \frac{4}{3} \beta \eta \quad (5)$$

Acoustic impedance

The specific acoustic impedance (Z) is given by Eq. (6) (Ernest and Kavitha 2011)

$$Z = U \rho \quad (6)$$

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

The changes in ultrasonic velocity, density, viscosity, adiabatic compressibility, intermolecular free length, relaxation

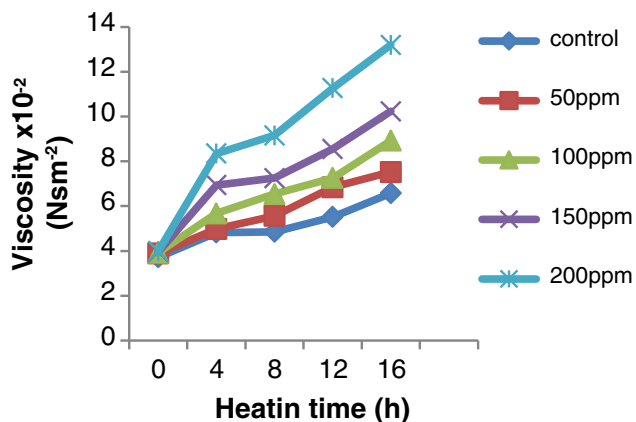


Fig. 2 The viscosity as a function of heating time for the control and sunflower oil with different concentrations of butylated hydroxy anisole

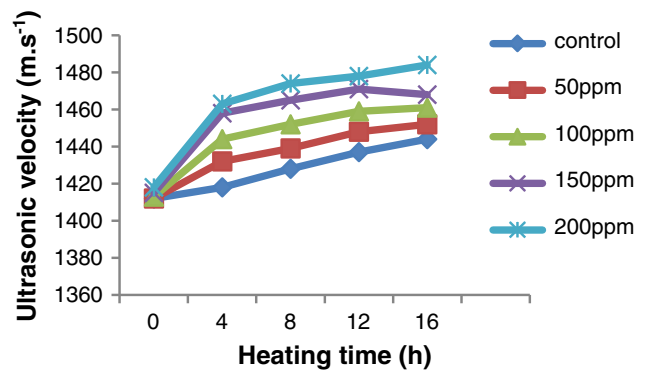


Fig. 3 Effect of heating time on ultrasonic velocity of the control and sunflower oil with different concentrations of Butylated hydroxy anisole

time and acoustic impedance of BHA in sunflower were calculated.

Chemical analysis

Fatty acid composition by gas chromatography

AOCS method No: Ce 1–62, 1998 (Firestone 1998). Fatty acid methyl esters (FAME) of the oil samples were prepared by trans esterification. 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 μ m SP2560 (Supelco Inc., Bellefonte, PA) as the stationary phase. The injector and FID (Flame ionization detector) were at 260 °C. The oven temperature was programmed from 140 to 240 °C at 4 °C/min with an initial hold at 140 °C for 5 min. A reference standard FAME mix (Supelco Inc.) was analyzed under the same operating conditions to determine the peak identity.

A comparison between the authentic standard mixture with those of retention times of the samples run on the same column under the same conditions, was made to facilitate

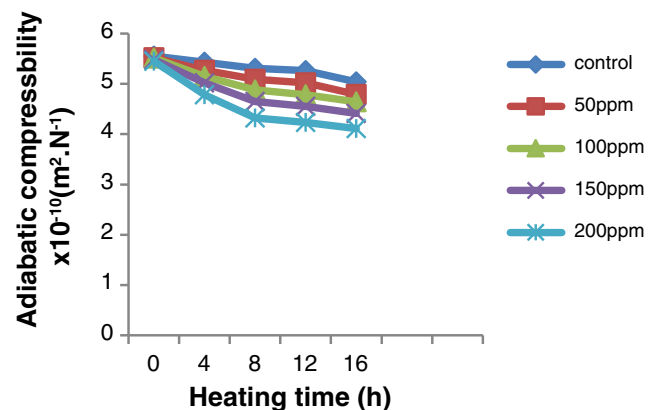


Fig. 4 Variation of adiabatic compressibility of the control and sunflower oil with different concentrations of butylated hydroxy anisole with heating time

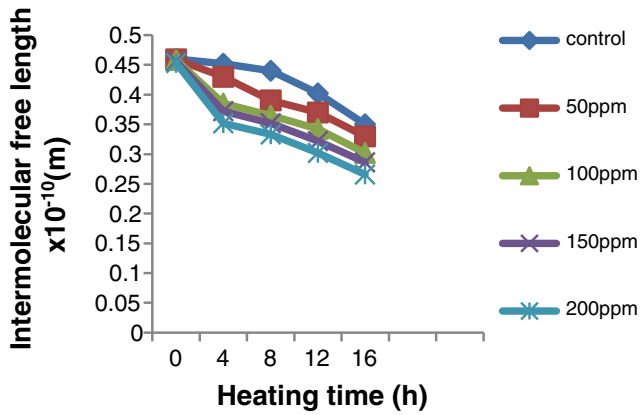


Fig. 5 Effect of heating time on intermolecular free length of the control and sunflower oil with different concentrations of butylated hydroxy anisole

identification. The FAMES results were expressed as relative area percentage.

Peroxide value determination

0.5–1 g of the oil was taken in a clean 250 mL iodine flask and dissolved in 30 mL glacial acetic acid and 20 mL chloroform. Then, 0.5 mL of saturated potassium iodide was added to the flask and kept in the dark for 15 min. Then, 50 mL distilled water was added and titrated against 0.02 N sodium thiosulphate solution using starch as an indicator. From the titer value, the peroxide value (PV) was calculated using the formula given in Eq. (7)

$$PV = \frac{V_{Na_2S_2O_3} \times N_{Na_2S_2O_3} \times 1000}{Weight\ of\ sample} \quad (7)$$

Where, V is the volume and N is the normality sodium thiosulphate solution.

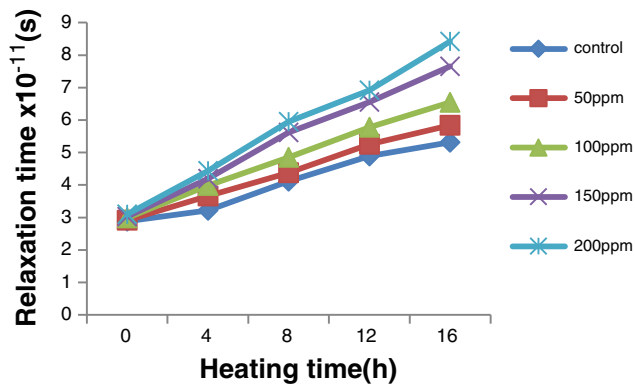


Fig. 6 Variation of relaxation time of the control and sunflower oil with different concentrations of butylated hydroxy anisole as a function of heating time

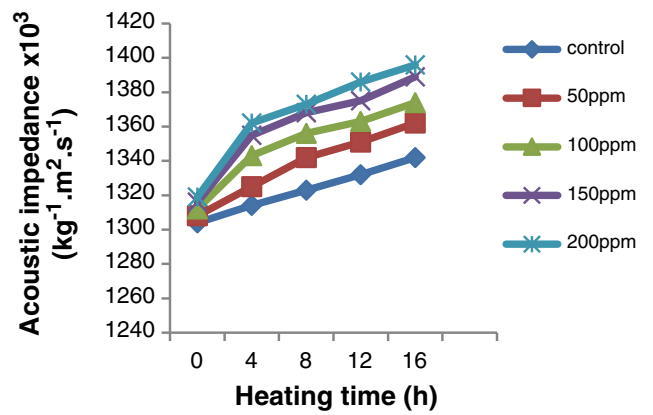


Fig. 7 Variation of acoustic impedance of the control and sunflower oil with different concentrations of butylated hydroxy anisole as a function of heating time

Free fatty acids

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

Polar compounds

The contents of total polar compounds were determined using the method proposed by IUPAC, 1992 (Izbaim et al. 2010).

Results and discussion

The study of thermal degradation and antioxidant stability of the sunflower oil was carried out by heating the oil at a frying temperature of 180 °C. After heating for the desired time, the viscosity, density and ultrasonic velocity of the control and the

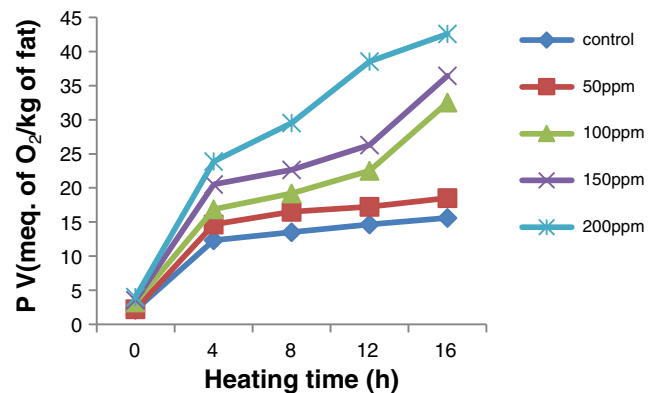


Fig. 8 Variation of peroxide value (PV) of the control and sunflower oil with different concentrations of butylated hydroxy anisole with heating time

Table 1 Changes in the fatty acid composition (%) of sunflower oil during heating process

| % Fatty acid composition | Duration of heating (h) | | | | |
|--------------------------|-------------------------|------------|------------|------------|------------|
| | 0 | 4 | 8 | 12 | 16 |
| C16:0 | 13.4±0.11 | 16.43±0.31 | 18.71±0.47 | 19.4±0.54 | 21.2±0.62 |
| C16:1 | 0.54±0.03 | 0.23±0.02 | 0.0 | 0.0 | 0.0 |
| C18:0 | 4.31±0.05 | 6.35±0.34 | 8.12±0.24 | 11.14±0.43 | 13.68±0.46 |
| C18:1 | 38.4±1.3 | 40.32±1.27 | 43.62±1.38 | 40.64±1.24 | 38.53±1.21 |
| C18:2 | 46.3±1.6 | 41.53±1.42 | 38.83±1.28 | 36.12±1.19 | 32.43±1.14 |
| C18:3 | 0.32±0.01 | 0.29±0.01 | 0.14±0.01 | 0.0 | 0.0 |

C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; α -C18:3, α -linolenic acid

oil with BHA at different concentrations were measured at 30 °C.

The stability of sunflower oil at different times of heating and at different amounts of BHA has been investigated using the parameters of viscosity (Sarmiento et al. 2006; Valantina et al. 2010), density and ultrasonic velocity (Kesavasamy et al. 2008; Priya et al. 2010). These parameters were evaluated after different times of heating. It was found that the antioxidant stability of BHA with sunflower oil was better even at high temperature and after prolonged heating (Okoye et al. 2009; Rehab 2010).

Density

The period of heating gradually and substantially increased density of sunflower oil. Incorporation of 200 ppm of BHA in to oil causes little increase in the density values as compared to the control oil. The density of the oil containing 200 ppm of BHA hardly changed as there are no molecular changes due to the antioxidant activity of the oil. The variation in density is due to severe damage in chemical composition as there is an increase in the saturation composition of the oil (Valantina et al. 2010).

Table 2 Changes in the fatty acid composition (%) of sunflower oil with 200 ppm BHA during heating process

| % Fatty acid composition | Duration of heating (h) | | | | |
|--------------------------|-------------------------|------------|------------|------------|------------|
| | 0 | 4 | 8 | 12 | 16 |
| C16:0 | 13.4±0.9 | 14.43±0.29 | 16.12±0.42 | 18.3±0.39 | 19.43±0.41 |
| C16:1 | 0.56±0.03 | 0.32±0.02 | 0.21±0.01 | 0.0 | 0.0 |
| C18:0 | 4.33±0.05 | 4.92±0.41 | 6.73±0.11 | 9.87±0.94 | 11.72±0.91 |
| C18:1 | 38.2±1.3 | 39.43±1.29 | 41.62±1.25 | 39.73±1.13 | 36.85±1.11 |
| C18:2 | 46.8±1.6 | 43.54±1.46 | 40.35±1.32 | 38.12±1.12 | 37.32±1.18 |
| C18:3 | 0.32±0.01 | 0.31±0.01 | 0.21±0.014 | 0.0 | 0.0 |

C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; α -C18:3, α -linolenic acid

Viscosity

The period of heating gradually and substantially increased the viscosity (η) of sunflower oil. Adding 50, 100, 150 and 200 ppm of BHA led to different increments in the viscosity values during the heating period. Oil with 200 ppm of BHA had the lowest viscosity shown in Figs. 1, 2, 3, 4, 5, 6 and 7. The obtained results were in good agreement with the results (Rehab 2010; Farag et al. 2003; Shaker 2006; Anany 2007). The fried oil with various dosages of phenolic compounds didn't alter the viscosity.

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 (Valantina et al. 2010).

Ultrasonic velocity and acoustical parameters

The ultrasonic velocity and attenuation depends on the physico-chemical properties of the oil (McClements 1997). The ultrasonic evaluation of food properties use ultrasonic velocity, as it is more reliable than attenuation and related to the physical and chemical properties of the medium (Benedito

Table 3 Variation of % free fatty acids (as oleic acid) with different heating periods of sunflower oil with and without BHA

| Sample | 0 h | 4 h | 8 h | 12 h | 16 h |
|-----------------------|------|------|------|------|------|
| Sunflower oil | 0.12 | 0.15 | 0.19 | 0.24 | 0.32 |
| Sunflower+200 ppm BHA | 0.11 | 0.13 | 0.16 | 0.18 | 0.20 |

et al. 2007) After each frying period the ultrasonic velocity increases and thus it is possible to distinguish the two oils (Izbaim et al. 2010). The ultrasonic velocity increased linearly as the density and the viscosity was increased with the heating time. The adiabatic compressibility and free length are deciding factors of the ultrasonic velocity in liquid systems (Priya et al. 2010).

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 (Kinsler and Rray 1989) and in such a situation, the molecules get rearranged due to a cooperative process (Ali et al. 2000).

The acoustic impedance was low in the oil with BHA. The intermolecular free length and acoustical impedance depend upon the intermolecular attractive and repulsive forces (Ernest and Kavitha 2011). Excess acoustical impedance may be due to the geometrical effect allowing the fitting of molecules of different sizes after collapsing the triglyceride structure.

The acoustical parameters such as β , τ , Lf and Z were correspondingly found to change to a large extent in the control is measured. The antioxidant BHA, did not allow any breaking up of the molecular clustering interaction between the molecules of oil and hence very small structural changes occurred during heating in the presence of an antioxidant. The break up in the molecular clustering, releasing several dipoles for the interaction in the control, the antioxidant activity is much less.

However, 150 ppm of BHA produced nearly the same acoustical parameter values as shown for 200 ppm of BHA loaded oil. In India, 150 ppm of BHA is allowed in vegetable oils whereas the general standard for food additives (Codex 1995) allows the usage of antioxidant upto 200 ppm. Hence, the maximum stability of oil can be maintained with 200 ppm of BHA.

Table 4 Variation of % polar compounds with different heating periods of sunflower oil with and without BHA

| Sample | 0 h | 4 h | 8 h | 12 h | 16 h |
|-----------------------|------|-----|------|------|------|
| Sunflower oil | 4.3 | 8.4 | 12.8 | 17.2 | 21.3 |
| Sunflower+200 ppm BHA | 4.36 | 7.5 | 8.3 | 10.5 | 12.4 |

Fatty acid composition

The effect of different conditions on the fatty acid composition of fats can be studied by useful analytical technique for Gas chromatography (GC). The oxidative stability of the heated oil samples is enhanced the incorporation of BHA. The concentration of the unsaturated fatty acids by relative GC peak areas showed that sunflower oil samples treated with 200 ppm BHA had significantly lower values in comparison with oil.

The physical and chemical behaviors of the FA composition of oil has marked effects on its frying performance. The FA profile of the frying oils changed as a result of cyclization, polymerization and hydrolytic, oxidative and other chemical reactions promoted by frying conditions (Nawar 1996). The frying stability of highly unsaturated vegetable oils such as sunflower oil be improved by incorporation of BHA.

Peroxide value

Peroxides can be used as an oxidation index (OI) for the early stages of lipid oxidation (Barthel and Grosch 1974). Figure 8 shows changes in the peroxide values of sunflower oil during the heating process. At the end of the frying period, the peroxide value (PV) of sunflower oil without antioxidant was about 42.2 meq. of oxygen/kg of fat and the sunflower oil mixed with 50, 100, 150 and 200 ppm of BHA was about 21.3, 16.8, 11.80 and 7.48 meq. of oxygen/kg of fat.

These results were consistent with the data published elsewhere (Azuma et al. 1999; Rehab 2010; Farag et al. 2003; Shaker 2006; Anany 2007).

Many scientist (Clark and Serbia (1991), White (1991), Tyagi and Vasistha (1996) and Pimpa et al. (2009) have reported that frying oils used continuously or repeatedly at high temperature in the presence of oxygen and water from the food being fried, were subject to thermal oxidation, polymerization and hydrolysis. Hence, the resulting decomposition products adversely affected the flavor and color.

Free fatty acid value

Free fatty acids (FFA) may get promoted by the reaction of oil with moisture (Frega et al. 1999). FFA values of sunflower oil were caused significant reduction in addition of antioxidant. FFA content is the frequently used data to probe the life of frying oil, but it is not recommended to be the only indicator. FFA and other volatile substances 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.

Polar compounds in oil

Generally, degradation of oil during frying is accompanied by increasing the polar compounds of oil (Innawong et al. 2004). 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, FFAs, monoglycerides, diglycerides, sterols, carotenoids, antioxidants, antifoamers, hydrogenation catalyst residues and soaps (Blumenthal 1996).

After 16 h of frying, the final TPC values were 18.5 % in base oil and 10.5 % in formulated oil. If the total polar compounds exceeds 25 %, the oil should be disposed (Romero et al. 2000) according to FDA regulations (Tables 1, 2, 3 and 4).

Conclusion

Mixing different concentration of BHA (50, 100, 150 and 200 ppm) with sunflower oil improve antioxidative potency. Subsequently, the oil would have longer shelf-life, stability and more nutritional value. BHA was found to be the most effective at the concentration of 200 ppm. The variation in the parameters η , ρ , U , β , τ , L_f and Z of sunflower oil with 200 ppm of BHA compared with the control on heating. Sunflower oil with BHA has better thermal stability.

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