Research Article

Secondary Anti-Oxidative Effect of Soya Lecithin in Bulk Soya Bean Oil

Pragasam A[1,2], Prithvi J[1], P. Majalikar[1], Preeti N. Tallur[1], and Vinayak M. Naik[1,2]*

[1]Department of Chemistry, Govt. Arts and Science College, Karwar- 581301, Karnataka, India
[2]Research & Development Centre, Bharathiar University, Coimbatore -641046, Tamilnadu, India

Abstract

Stability of oil is contributed by the chemical compositions of the oil. The degree of unsaturation plays a key role in contributing quality and hygienic for edible oils. Rancidity due to auto-oxidation is controlled by natural antioxidants like tocopherols, beta carotene etc present in the oil. Experiment was carried out by setting seven soya bean oil samples to study the pro-oxidative and anti-oxidative effect of soya lecithin. Extracted soya bean oil has lower peroxide and p- anisidine values (14.53 meq/kg and 0.15 meq/kg) with commercial soya bean oil (20.96 meq/kg and 0.16 meq/kg) after five hours heating at 60-65^{0}C. Commercial soya bean oil with 100 and 150 ppm lecithin gives a higher peroxide and p- anisidine values (21.47, 22.53 meq/kg and 0.18, 0.21 meq/kg) against commercial soya bean oil with 100 ppm α- tocopherol (10.82 meq/kg, 0.08 meq/kg). Commercial soya bean oil with 100 and 150 ppm lecithin having 100 ppm α- tocopherol gives a lower peroxide and p- anisidine values (8.54, 8.16 meq/kg and 0.06, 0.05 meq/kg) with commercial soya bean oil with 100ppm α- tocopherol (10.82 meq/kg,0.08 meq/kg). The decrease in peroxide value and p- anisidine value with of α- tocopherol and lecithin shows the synergic effect of lecithin. Increase in peroxide values with only lecithin is contributed to pro-oxidative effect of lecithin.

Keywords: pro-oxidant, anti-oxidant, soya lecithin, synergism, α- tocopherols

***Correspondence**
Author: Vinayak M. Naik
Email: nayakvinu06@rediffmail.com

Introduction

Quality of the oils is dependent on their chemical compositions, like the percentage of the degree of unsaturation. The peroxide value (PV), which depends on temperature, time and light, measures the extent of primary oxidation of oils (rancidification). Rancidity of oils can produce potentially toxic compounds associated with long-term health effects such as neurological disorders, heart and cancer [1]. Lipid oxidation is considered as main cause for deterioration in the quality of foodstuffs. It's not only imparts rancid and undesirable flavours to fat products, but also it generates reactive oxygen species, which are linked to carcinogenesis, inflammation, aging and cardiovascular disorders [2-4]. Lipid oxidation also influences the chemical, sensory, and nutritional properties of edible oils and fatty foods and thus plays an important role in determining their use and shelf-life [5, 6]. Assessment of oxidative deterioration of soybean oil determines its quality [7]. Lipid oxidation and the generation of secondary oxidation products have always been serious concerns of food quality and consumer health [8]. An extent of oxidative alterations in soybean oil (SBO), subjected to ambient and sunlight storage, over a long period gave a noted oxidative change [9-12]. Ascorbic acid and its derivatives, tocopherols, the esters of gallic acid, erythorbic acid and its sodium salt, BHA, BHT and other substances THBP and TBHQ are primary antioxidants. Sulphur dioxide and sulphites as well as lecithin are secondary antioxidants. Soya bean oil has unsaturated fatty acids more. So, it is more susceptible to auto-oxidation.

Pro-oxidation of phospholipids was observed in bulk oil. Bulk Oil is a heterogeneous system that contains triacylglycerides including traces of water, and different amphiphilic minor compounds like monoacylglycerides, diacylglycerides, free fatty acids, phospholipids, phytosterols and oxidation in the spontaneous formation of nano structures. For Instance, phospholipids in bulk oils form association colloids like reverse micelles. Native soya bean phospholipids could form reverse micelles in the mixture of hexane and soya bean oil containing less than 3% water [13]. Cryo-TEM gives direct visualization of phospholipids reverse micelles [14]. Many of the antioxidant properties of phospholipid reported in the literature are related to their ability to inhibit lipid oxidation synergistically with primary antioxidants, especially the tocopherols [15, 16]. Although many studies reported increased oxidative stability of food products when phospholipid and tocopherols were added together, the evidence of synergism is better demonstrated in studies showing that phospholipid alone does not inhibit lipid oxidation, but when they are in combination with tocopherols a strong antioxidant effect is observed. For instance, in Perilla oil that was depleted of mixed tocopherols and stored in dark at 37°C, neither 500 ppm PC, PE nor PS affected lipid oxidation. However, when 366 and 866 ppm mixed tocopherols were present, PE and PS prolonged the oxidation lag phase of the oil [17, 18]. Nevertheless, upon the addition of either 50 or 100 ppm α-tocopherol, synergistic activities were observed. PE

and PC were pro-oxidative when added alone in stripped bonito oil which was stored in dark at 35-40°C [19]. But when combined with 500 ppm α-tocopherol, PE exhibited synergistic antioxidant activity, while PC still had no effect. The antioxidant activity of Phospholipid is absent when used alone but enhanced antioxidant activity when Present with α-tocopherol was also supported by the some researches [20]. In stripped soybean oil, it has also been reported PE alone promoted lipid oxidation but inhibited lipid oxidation upon the addition of α-tocopherol.

The present study was carried out to evaluate the physico-chemical characterisation of raw soya bean oil against commercial soya bean oil. Natural antioxidation and auto-oxidation of raw soya bean oil was related with commercial oil using α-tocopherol as a primary antioxidant and lecithin as secondary antioxidant. The pro-oxidation and antioxidation property of soya lecithin is evaluated and the possible mechanism is assigned.

Materials and Methods

Chemicals

The chemicals used are of analytical and HPLC grade. Glacial acetic acid (AR), Chloroform (AR), Potassium iodide (AR), Sodium thiosulfate (LR), Potassium dichromate (AR), Soluble starch (CP), Isooctane (HPLC) and Anisidine (AR). These chemicals are used to determine peroxide value and p- anisidine value.

Extraction of oil

50±0.5 g of seed powder is packed and stapled in a What Man No 42 filtered paper. The packet is inserted into the middle piece of soxhlet extractor. Oil was extracted using n-hexane for three hours. N-Hexane was recovered and the oil was dried 85^{o}C in a preheated oven for one hour. Oil obtained is cooled in a desiccator and weighed for constant weight. A total of about 200g of oil was extracted, flushed with N_2 gas and used for physical characterisation.

Sample preparation

Commercially available soy bean oil from local market was purchased. Different soy bean oil samples were prepared weighing with and without Lecithin. The oil samples under study were heated to 60-65^{0}C continuously for 8 hours. The oil samples were allowed to attain room temperature for determination of physico-chemical characteristics by keeping in the desiccator. The sample codes were given as;

Composition	Sample code
Extracted Soy Bean Oil	ESBO
Commercial Soy Bean Oil	CSBO
Commercial Soy Bean Oil with 100 mg Soy Lecithin	$CSBOL_{100}$
Commercial Soy Bean Oil with 150 mg Soy Lecithin	$CSBOL_{150}$
Commercial Soy Bean Oil with 1 ppm α- tocopherol	CSBOT
Commercial Soy Bean Oil with 100 mg Soy Lecithin and 10 ppm α- tocopherol	$CSBOL_{100}T$
Commercial Soy Bean Oil with 150 mg Soy Lecithin and 10 ppm α- tocopherol	$CSBOL_{150}T$

Physico-chemical Characteristics

Density

The densities (in g /ml) soya bean oil samples were determined by specific gravity bottle method. The experiment was conducted using thermostat maintaining the temperature at 28^{0}C and duplication of the determination was done for confirmation.

Peroxide Value (PV)

PV (in meq/kg) was determined iodometrically according to standard method for the oil analysis. PV of the soy bean oil samples was determined before and after heating at 60-65^{0}C.

P-Anisidine Value (p-AV)

The carbonyl content in oils was determined by standard method according to AOCS [21]. It measures the reactiveness of the aldehydes' carbonyl bond on the p-anisidine amine group forming a Schiff's base which absorbs at 350 nm. 2g (W) of soy bean oil was dissolved in 25 ml isooctane and absorbance A_1 was measured at 350 nm against a blank isooctane. An aliquot (5 ml) of this solution, respectively 5 ml of isooctane (as blank) was transferred to each

of two test tubes of 10 ml and 1ml anisidine solution (0.25% g/v glacial acetic acid) was added to each. After 10 minute the absorbance A_2 was measured at 350 nm against isooctane containing p-anisidine. The p-AV is determined as; p-AV = 25 x 1.2 x (A_2-A_1) / W [22].

FT- IR Spectra

Nicolet- 5700 FTIR spectrophotometer was used to record IR spectrum. A small drop of oil sample was rubbed on KBr (potassium bromide) pellet under the specification Thermospectra-Tech, KBr disc 32x3mm drilled 7000-467.

Results and Discussion

Density

Density of soya beanoil sample is a direct measure of the quality of soya bean oil. Densities of different sets of soya bean oils are measured against time duration of heating as shown in the **Table 1**. Samples were drawn after each hour at 60 -65^{o}C, allowed to cool to 28^{o}C by keeping in the desiccator and the density was determined.

Table 1 Densities of soy bean oils at temperature 60-65 ^{0}C

Duration of heating(minutes)	0	60	120	180	240	300
Density of ESBO (g/ml)	0.9081	0.9301	0.9558	0.9751	0.9980	1.0312
Density of CSBO (g/ml)	0.9071	0.9285	0.9452	0.9672	0.9901	1.0180
Density of $CSBOL_{100}$ (g/ml)	0.9104	0.9301	0.9479	0.9703	0.9998	1.1205
Density of $CSBOL_{150}$ (g/ml)	0.9157	0.9379	0.9504	0.9822	1.0087	1.1312
Density of CSBOT (g/ml- control)	0.9072	0.9074	0.9079	0.9086	0.9120	0.9180

Plot of density against time duration at 60-65 ^{0}C is given in the **Figure 1**, which explains the variation of density with time duration. The gradual increase in density of oils with time duration correlates the presence of some added mass such as water molecules, carbonyls and polymerized product of oils. These molecules are produced by absorption of moisture, oxidative cleavage of fatty acid double bonds and enzymatic hydrolysis of fats. Extracted soy bean oil and commercial soy bean oil shows a gradual increase in density with time duration whereas with lecithin the density is relatively higher with respect to control. The control is prepared with 100 ppm of α- tocopherol. $ESBOL_{100}$ and $CSBOL_{150}$ samples record relatively higher density compare to ESBO and CSBO. This variation is explained by the hydroscopic nature of lecithin.

The plot clearly explains gradual increase of peroxide value with time of heating as shown in **Figure 2**. This increase is due to the formation of peroxide at double bond which on simultaneous cleavage results in formation of aldehydes and ketones. Pro-oxidative and anti-oxidative ability of lecithin is conspicuous in this experiment. The synergic anti- oxidative effect of tocopherol with lecithin is well characterised by the peroxide values. Peroxide values of commercial soy bean oil with 100 and 150 mg lecithin reaches maximum after 6 hours heating at 60-65^{0}C whereas extracted and commercial soy bean oil without lecithin shows relatively less values. This indicates the pro-oxidative ability of lecithin.

Commercial soy bean oil with 100 and 150 mg lecithin having 100 ppm α- tocopherol has low peroxide values compare to soy bean oil with only 100ppm α- tocopherol after 6 hours heating at 60-65^{0}C. The decrease in peroxide values is the good indication of synergic anti- oxidative ability of α- tocopherol in the presence of lecithin.

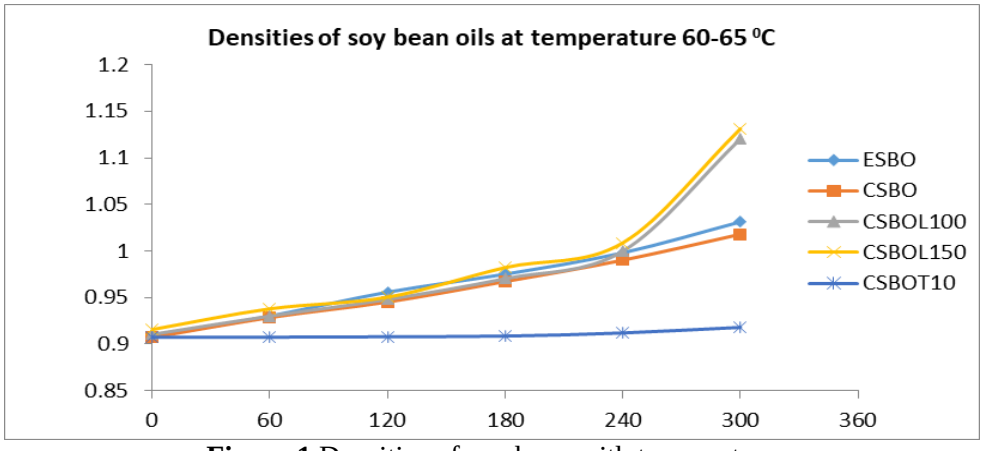


Figure 1 Densities of soy bean with temperature

Peroxide Value (PV)

The peroxide value of soy bean oil samples at temperature 60-65^{0}C was determined with time were recorded as shown in the **Table 2**. The variation in the peroxide value was plotted against time (**Figure 2**).

Table 2 Peroxide Value of soy bean at temperature 60-65 ^{0}C.

Duration of heating(hrs)	0	1	2	3	4	5
ESBO (meq / kg)	3.50	4.38	6.12	8.74	11.45	14.53
CSBO (meq / kg)	1.72	4.52	8.23	13.42	16.12	20.96
$CSBOL_{100}$ (meq / kg)	1.72	7.88	13.67	14.75	15.62	21.47
$CSBOL_{150}$ (meq / kg)	1.72	8.06	13.97	15.43	16.54	22.53
CSBO T (meq/kg) control	1.72	3.24	5.34	7.12	8.47	10.82
$CSBOL_{100}$T (meq / kg)	1.72	2.16	4.67	5.97	6.72	8.54
$CSBOL_{150}$T (meq / kg)	1.72	2.06	4.31	5.23	6.14	8.16

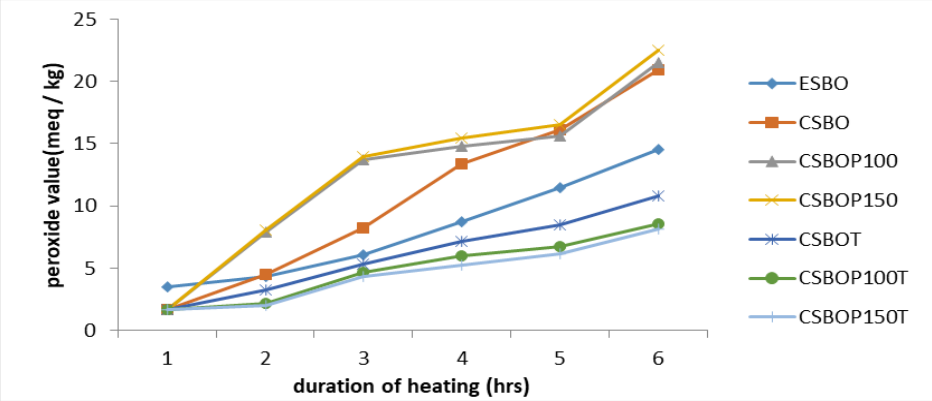


Figure 2 Peroxide Values soya bean oil samples with heating

P-Anisidine Value (p-AV)

Secondary oxidation of soy bean oil is determined by p-anisidine value. It measures high molecular weight saturated and unsaturated carbonyl compounds in triacylglycerols [4]. The range of p-anisidine values with time of heating at 60-65^{0}Cwere listed in **Table 3**.

Table 3 p-Anisidine Value of soy bean at temperature 60-65 ^{0}C

Duration of heating(hrs)	0	1	2	3	4	5
p-AV of ESBO (meq / kg)	0.01	0.03	0.06	0.09	0.12	0.15
p-AV of CSBO(meq / kg)	0.01	0.03	0.05	0.10	0.13	0.16
p-AV of $CSBOL_{100}$ (meq / kg)	0.01	0.04	0.07	0.11	0.14	0.18
p-AV of $CSBOL_{150}$ (meq / kg)	0.02	0.05	0.07	0.13	0.15	0.21
p-AV of CSBO T(meq/kg) control	0.01	0.02	0.04	0.05	0.07	0.08
p-AV of $CSBOL_{100}$T (meq / kg)	0.01	0.01	0.02	0.03	0.05	0.06
p-AV of $CSBOL_{150}$T (meq / kg)	0.01	0.01	0.02	0.02	0.04	0.05

The plot of p-anisidine value against time of heating shows gradual increase as indicated in the **Figure 3**. The increase in the p-anisidine value gives total mass of the carbonyls formed by secondary oxidation in triacylglycerol. The increase p-anisidine value due to the formation of carbonyls is well explained by the pro-oxidation and anti-oxidation of lecithin. The graph correlates the pro-oxidant effect of lecithin on soy bean oil by the increase of p-anisidine value. The synergic anti-oxidant effect of α- tocopherol is enhanced by the lecithin indicating lower p-anisidine value.

IR Spectra

The IR- spectra clearly gives the frequency and percentage transmission of relevant functional groups oil samples. The spectra obtained have strong C-H absorption between 3010 and 2850 cm^{-1}. The **Figure 4** clearly showed separate band with asymmetrical CH stretching (CH_2) at 2925 cm^{-1} and symmetrical C-H stretching (CH_2) at 2855 cm^{-1} with

weak peak at 3008 cm^{-1} due to methylene asymmetrical stretching band. Experimental oil samples gave strong bands at 1746, 1464 and 1163 cm^{-1} that corresponds to C=O (ester) stretching, C-H bending (Scissoring) and CO, CH_2 stretching, bending respectively

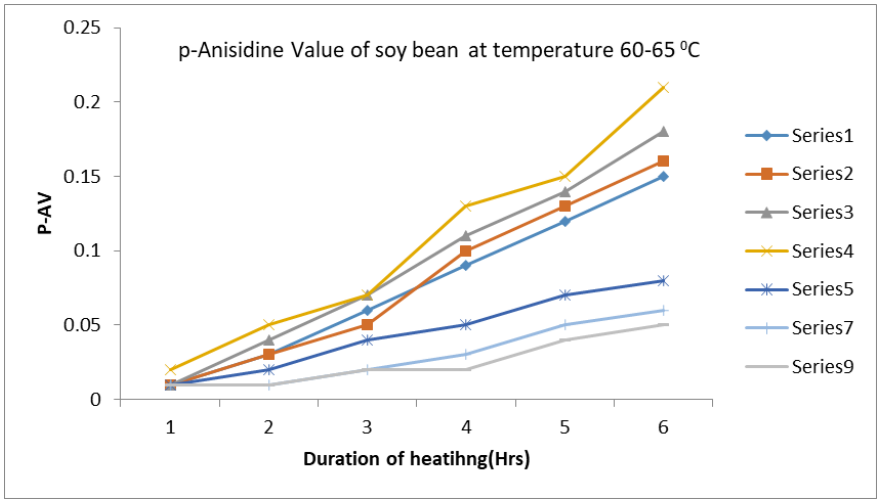


Figure 3 P-Anisidine Value of soya bean oil samples with heating.

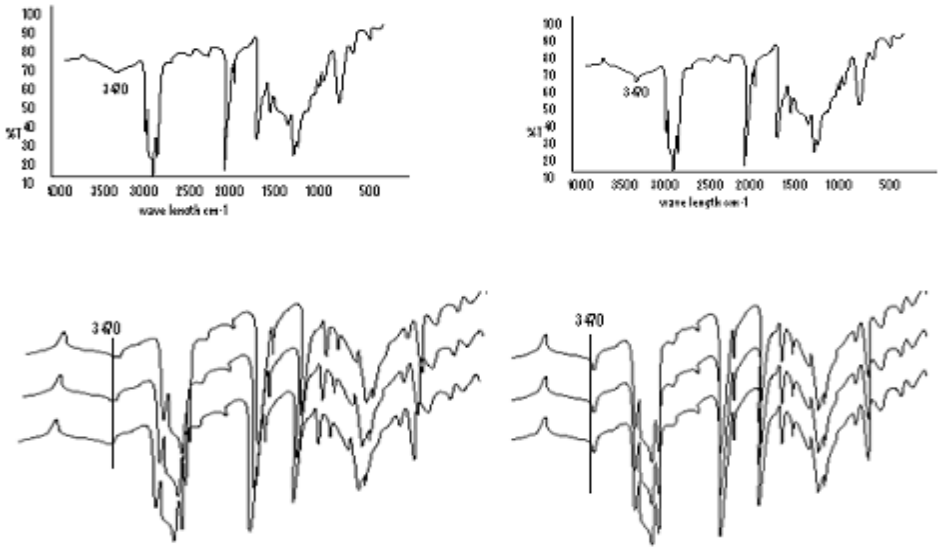


Figure 4 FT-IR Spectra of soya bean oil samples

Pro-oxidation of Lecithin

Radar plot compares the p-anisidine and peroxide value of soya bean oil samples for pro-oxidation and anti-oxidation effect of lecithin. ESBO and CSBO samples have maximum p-anisidine and peroxide value as a radar values (0.15, 0.16 meq/kg and 14.53, 20.96 meq/kg) at end of continuous heating for six hrs at 60-65^{0}C.The gradual increase of radar value is contributed by the natural antioxidant (α-tocopherol) present in ESBO and added antioxidant in CSBO. The increased P-anisidine and peroxide value of CSBOP100, CSBOP150 (0.18, 0.21 meq/kg and 21. 47, 22.53 meq/kg) indicates the faster oxidation of oil samples. The increased trend of radar values along fifth radar coordinate axis correlates the pro-oxidation ability of lecithin.

Mechanism pro-oxidation of soya lecithin

The possible mechanism of pro-oxidant effect of lecithin is based on the accumulation of hydrophilic end of lecithin trapping moist oxygen from air phase lead to form reverse micelle nanostructures in the bulk oil phase. Lipid besides the reverse micelles can entrap moisture or molecular oxygen forms lipid peroxide adjacent to double bond (**Figure 5**). In the absence of lecithin, the micelle nanostructures are not so conspicuous. Lecithin can enhance the formation of reverse micelles and increase the rate of oxidation of lipids.

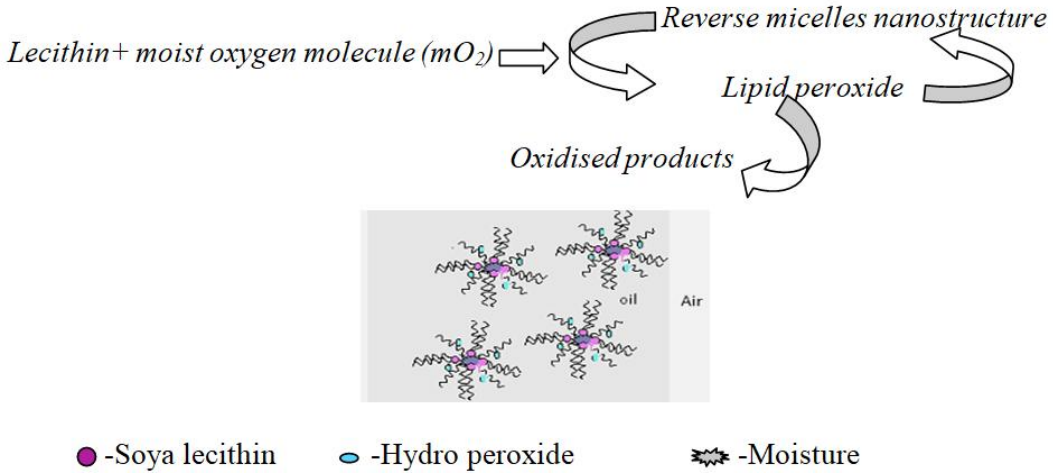


Figure 5 Bulk soya bean oil with reverse micelle nanostructures

Synergic antioxidant effect of Lecithin

A radar plot of peroxide values and p-anisidine values (**Figure 6**) were versus time duration at 60-65^{0}C explains the pro-oxidative and anti-oxidative property of lecithin

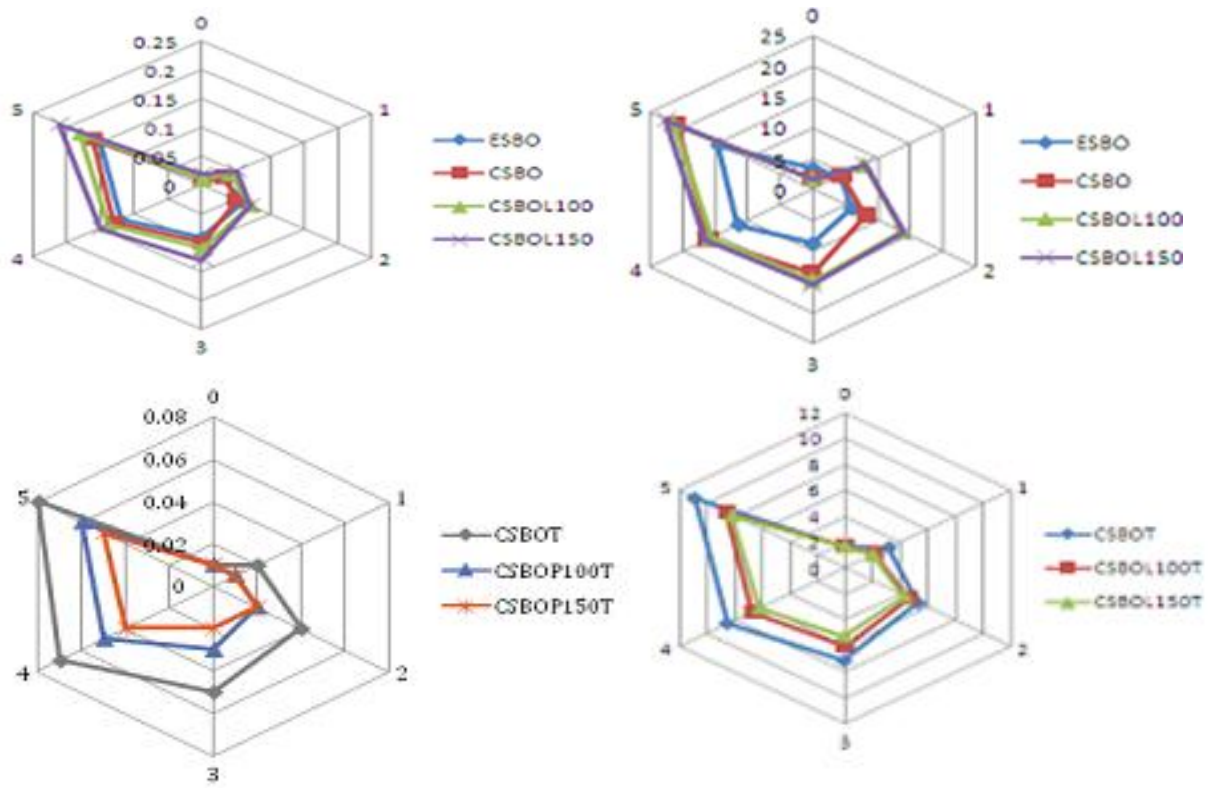
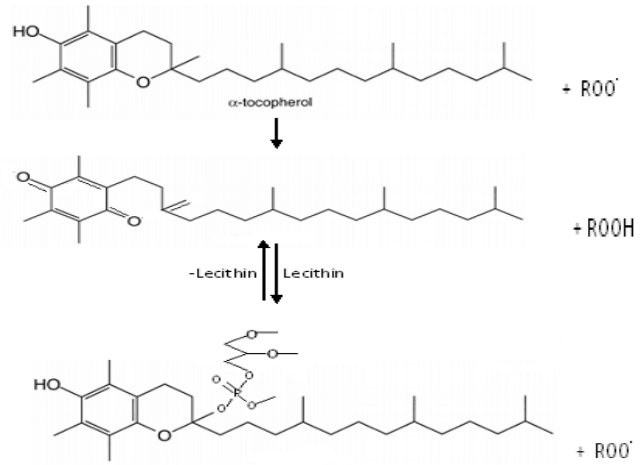


Figure 6 Radar plot for p-anisidine and peroxide values

Hexane extracted oil ESBO has 0.15 as radar value. The samples CSBO, CSBOL100 and CSBOL150 are shown increased radar value and a maximum of 0.21 radar value is given by CSBOL150. Lesser radar value of ESBO is due antioxidant ability of tocopherol present in the raw extracted oil. The increased radar values are the indication of pro-oxidative property of soya lecithin. CSBOT was used as control containing zero ppm lecithin. P-anisidine value of CSBOT is marked 0.08 as radar value after six hrs heating continuously at 60-65^{0}C. SBOP100T and SBOP150T samples have lesser P-anisidine values as radar values of 0.06 and 0.05 respectively. The decrease in radar value correlates the enhancement of antioxidant ability of α- tocopherol by lecithin. Here α- tocopherol acts as primary antioxidant whereas lecithin is secondary antioxidant.



Possible mechanism of synergic antioxidant effect of lecithin
(ROO^0 = Lipid peroxy radical)

The above mechanism concludes enhancement of antioxidant activity of tocopherol by reversing the reaction to regenerate the tocopherol.

Conclusions

The study shows that the peroxide value and p-nisidine value of samples with only lecithin are increased. This proves that lecithin has prooxidative effect on soyabean oil.The samples with lecithin and α-tocopherol have decreased peroxide value and p-nisidine compare to the that contain only α-tocopherol without lecithin as control. Conspicuous FT-IR spectrum at 3470 cm^{-1} is the indication for formation of peroxide. This concludes that lecithin could act as an antioxidant in the presence of α-tocopherol and as pro-oxidant without α-tocopherol. Thus the study clarifies that lecithin can act as secondary antioxidant to the α-tocopherol whereas α-tocopherol is a primary antioxidant. Synergism between phospholipid and tocopherols could be due to ability of phospholipids to 1) altering the physical location of tocopherols and 2) regeneration of tocopherols.

Acknowledgement

I would like to acknowledge the support of our beloved Principal and group members for their invaluable cooperation to work on this topic.

References

[1] Afshan Kaleem, Sana Aziz and et al 2015 Investigating changes and effect of peroxide values in cooking oils subject to light and heat, Fuuast J. Biol., 5(2): 191-196.
[2] Pezzuto JM, Park EJ. 2002. Autoxidation and antioxidants, in Swarbrick J, Boylan JC, (Eds.), Encyclopedia of Pharmaceuticals Technology, (vol. 1,2nd ed.) 97-113, Marcel Dekker Inc., New York.
[3] Siddhuraju P, Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (M. Oleifera Lam.). J. Agric. Food Chem., 51, 2144-2155.
[4] Min DB and Lee HO. 1998. Lipid Oxidation of Edible Oil,Food Lipid: Chemistry, Nutrition and Biochemistry, Akoh CC and and Min DB (Eds.), 283-296, Marcel Dekker, New York.
[5] Dobarganes MC, Márquez-Ruiz G. 2003.Oxidized fats in foods. Curr. Opin. Clin. Nutr. Metabolic Care.6, 157-163. International Union of Pure and Applied Chemistry (IUPAC). 1987. Standard Methods for the Analysis of Oils, Fats and, 7th revised and enlarged ed., edited by C. Paquot and A. Hautfenne, Blackwell Scientific, London.
[6] Anwar F, Bhanger MI, Yasmeen S. 2003. Antioxidant activity of some natural extracts in corn oil. in Murata N, Yamada M, Nishida I, Okuyama H, Sekiya J & Hajime W (Eds.) Advanced Research of Plant Lipids,24-27, Kluwer Publishers, Netherlands
[7] Farooq Anwar, Shahzad Ali ShahidChatha and Abdullah IjazHussain 2007 Assessment of oxidative deterioration of soybean oil at ambient and sunlight storage Grasasy Aceites, 58 (4), 390-395

[8] Berliner, J. A.; Subbanagounder, G.; Leitinger, N.; Watson, A. D.; Vora, D. 2001 Evidence for a role of phospholipid oxidation products in atherogenesis. Trends Cardio Vasc. Med, 11, 142–147.
[9] F. Anwar, S. Ali Shahid Chatha and A. Ijaz Hussain, 2007 Assessment of oxidative deterioration of soybean oil at ambient and sunlight storage. Grasasy Aceites, 58 (4), Octubre-diciembre, 390-395, issn: 0017-3495).
[10] J. Velasco and C. Dobarganes., 2002 Oxidative Stability of virgin olive Oil. European Journal of Lipid Science and Technology, 104, 661-676
[11] Chaiyasit W, Elias RJ, Mc Clements DJ, EA 2007 Role of Physical Structures in Bulk Oils on Lipid Oxidation. Crit Rev Food Sci 47:299-317
[12] E. Choe and D. B. Min, 2006, Mechanisms and Factors for Edible Oil Oxidation. Food Science and Food Safety, 5 (4) 169-186
[13] Gupta R, Muralidhara HS, Davis HT 2001 Structure and Phase Behavior of Phospholipid --- Based Micelles in Non-aqueous Media. Langmuir 17:5176-5183
[14] Danino D, Gupta R, Satyavolu J, Talmon Y 2002 Direct Cryogenic-Temperature Transmission Electron Microscopy Imaging of Phospholipid Aggregates in soya bean oil. J Colloid InterfSci 249:180-186
[15] H. J. Kim and D. B. Min. 2008 Tocopherol stability and prooxidant mechanisms of oxidized tocopherols in lipids. In Food lipids, chemistry, nutrition and biotechnology. Edition C. Press. 435- 447
[16] E.N. Frankel. Antioxidants in food & biology Dundee. The oily press Ltd. 2007.
[17] Kashima M, Cha GS, Isoda Y, Hirano J, Miyazawa T 1991. The antioxidant effects of phospholipids on perilla oil. J Am Oil Chem Soc 68:119-122
[18] Diana Moigradean, Mariana, et al., 2012 Quality characteristics & oxidative stability of coconut oil during storage, J. Agro alimentary process and Technologies, 18(4), 272- 276
[19] Lee J, Choe E 2011 Effects of phospholipids on the antioxidant activity of α-tocopherol in the singlet oxygen oxidation of canola oil. N Biotechnol 28:691-697
[20] Shimajiri J, Shiota M, Hosokawa M, Miyashita K 2013 Synergistic Antioxidant Activity of Milk Sphingomyeline and Its Sphingoid Base with α-Tocopherol on fish oil Triacylglycerol. J Agr Food Chem 61:7969-7975
[21] AOCS Official and recommended practices of the American oil chemists, Official methods and recommended practices 5th edition, edited by Fire Stone D. Publisher, AOAC Press; ChamppaignIllinose, USA, 1998
[22] Pragasam A, Vinayak M. Naik and Preeti N. Tallur., 2015. Assessment of physio- chemical characteristics deterioration of lipids by FTIR spectra for successively used for soy bean and sunflower oils in frying spice food stuffs, IOSR J. Of Applied Chemistry, 8(3), 47-51.

Publication History
Received 08th Aug 2018
Revised 18th Sep 2018
Accepted 04th Oct 2018
Online 30th Oct 2018