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Study to Solve the Problem of Color Reversion in Refined Soybean Oil

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Abstract

This study carried out on the soybean oil samples taken from the processing lines during refining process (degumming, neutralization, bleaching and deodorization process) for crude soybean oil as well as on color reversed oil after deodorization step during storage for several hours to study the physical and chemical properties (color, refractive index (RI), free fatty acid (FFA), peroxide value (PV), p-Anisidine value (p-AV), total oxidative (TOTOX) value, oxidative stability (OS), saponification value (SV), iodine value (IV), unsaponifiable matter (unsap. %), soap content, minerals, waxes, total phenols content, K_{232} and k_{270} nm), the fatty acids composition, sterol compound, total tocopherols and their components (α, β, γ and δ tocopherol) and tocored compound for these oils and to find out the reason for the color reversion after a short period from storage for the deodorized soybean oil. Citric acid has been added (0.2%) to each color reversed and neutralized soybean oils, then procedure of bleaching and deodorization process on them, and studied its effect on the physical and chemical properties for them specially the color units (red and yellow), tocopherols contents and tocored compound. The results showed that refining process for soybean oil caused to a gradual decrease in values of AV, oxidative stability, IV, wax, % Unsapo., K_{232} and K_{270} nm, total polyphenols, minerals (P, Ca and Mg), total tocopherols and their compounds (α, β, γ and δ tocopherol) and also caused decrease in all sterol components, but they showed a few differences in percentages of fatty acids as result refining process. Color values (red and yellow unites) recorded gradually decreased during refining process, but these values were increased in deodorized soybean oil after storage (color reversed soybean oil). Reduction of α, β, γ and δ tocopherol contents was found to be linearly with the increase of red and yellow color units (color darking). The tocored is responsible for the color reversion phenomenon, where as maximum amount of tocored was in crude soybean oil 169.2 ppm, which gradually decreased during refining process 120.35, 99.82, 20.25, and 8.46 ppm, respectively, but it

was found to be 46.5 ppm in color reversed soybean oil. Addition of citric acid (0.2%) related to the removal of tocored from soybean oil during the bleaching and deodorization process of soybean oil before and after color reversion in parallel with the significant decrease in the color values.

Keywords

Soybean Oil, Color Reversion, Refining Process, Citric Acid

1. Introduction

Some vegetable oils are getting darker during storage. The phenomenon is called the color reversion of oil. Color reversion happens often in some refined vegetable oils, like soybean and rapeseed oils [1].

Soybean oil after deodorization is quite pale when freshly prepared but became darker during storage in the air. The degree of color change depends on the following condition: nature of raw soybean, refining and deodorization conditions duration of storage, temperature intensity of light and contact surface with air during storage. There are some oils that take only several hours to reach the maximum of color reversion, while others take several months. Phosphorus content of refined and deodorized soybean oil is closely related the color and the oxidation stability of oil [2].

The coloring of refined and deodorized soybean oil (this is called soybean salad oil) by oxidation has been presumed to be due to tocored in soybean oil. Tocopherol content in crude oil decreased when the soybeans were moistened and that the soybean salad oil obtained from them showed extremely distinct color reversion. Tocored in crude oil was completely removed by the refining process but 30% of it remained in the colorless from refined and deodorized oil and turned into tocored during storage or on heating the oil to a higher temperature. Tocopherols are relatively stable in refined oil and aren't oxidized to tocored under a milder condition such as the oxidation with air, tocored in colorless form. It is really a precursor to the color reversion in soybean oil [3].

Reduction of tocopherol was found to be linearly related to color darking, especially reduction of α-tocopherol which it was the most important cause of color reversion, but phytosterol had no effect on color reversion [1].

Oil extracted from soybean with high moisture content showed a corresponding increase in free fatty acids, Lovibond color and contents of oxidation deterioration products. As for color reversion, it can be prevented by reducing the enzyme activity of soy beans before cracking and flaking [4].

Presence of nonhydratable phospholipid in the oil may reduce the efficiency of degumming and cause neutral oil loss. In addition, oil residual phospholipids will contribute to off flavors and colors in the refined oil. During the degumming process, phospholipids are eliminated by thermal treatment with water (hydratable phospholipids) and other degumming agents such as: phosphoric

acid, citric acid (nonhydratable phospholipids) [5].

This study aimed to know the reason of color reversion for deodorized soybean oil after several hours from storage and try to overcome this problem by study the effect of addition of 0.2% citric acid on the color reversion of soybean oil during packing and storage of it.

2. Materials & Methods

2.1. Materials

Samples of soybean oils were obtained from processing lines of Alexandria Oil and Soap Company, Alexandria governorate, Egypt) using chemical refining process. The obtained oils include crude soybean oil and soybean oil after each refining step *i.e.*, degumming, neutralization, bleaching and deodorizing, as well as on color reversed oil after deodorization step during packing and storage it for several hours. Representative samples were collected in dark glass bottles, purged with nitrogen gas after filling to prevent oxidation and stored in deep freezer, untill analysis.

Chemicals

All chemicals (hexane, cyclohexane, chloroform, acitic acid, ethanol, methanol, pottasiom hydroxide and sodium salphate anhydride) used in this study (analytical grades) were obtained from Oils and Fats Research Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Reagent and 2,2-diprydail were obtained from Gerbsaure Chemical Co. Ltd. Germany, caffeic acid and tocopherol standards were purchased from Sigma-Aldrich Co., St. Louis, USA.

2.2. Methods

2.2.1. Physical Properties of Soybean Oil Samples

Refractive index: The refractive index (RI) of oil samples were measured according to the methods described in A.O.A.C [6] using Abbe refractometer (NY RL-3-Poland), and the obtained results were standardized at 25˚C.

Color determination of soybean oil samples: The color of soybean oils was measured according to the method described in the AOCS [7] using Lovibond-Tintometer® Model F, and three colored scales (yellow, red and blue) in 5.25-inch quartz cell. The number representing the degree of oil color was read from the color scales.

2.2.2. Chemical Properties of Soybean Oil Samples

Acidity, peroxide, ansidine value (meq/kg oil), total oxidative (TOTOX) value, saponification value, soap content, wax content and unsaponifiable matter % values of oil samples were determined according to the A.O.A.C [6]. Conjugated dines (K232 nm) and conjugated trines (K270 nm) of oil samples were determined as described by Regulation sEEC/1989/2003 of the Commission of the

European Union [8] with a UV spectrophotometer (JENWAY 6405 UV/Vis. Spectrophotometer, England) using a 1% solution of oil in cyclohexane and a path length of 1 cm. Iodine value was calculated from fatty acids content according to [9].

2.3. Fatty Acids Composition of Soybean Oil Samples

2.3.1. Methylation of Fattyacids

An aliquot of fatty acids, about 10 mg, was dissolved in 2 ml hexane and then 0.4 ml of 2N KOH in anhydrous methanol was added [10], after 3 min, 3 ml water was added. The organic layer, separated by centrifugation, was dried over anhydrous sodium sulfate, and then concentrated, with a N_2 stream to around 0.5 ml for GC analysis of fatty acids methyl esters (FAMEs) as described below.

2.3.2. GC Analysis of FAME

Agilent 6890 series GC apparatus provided with a DB-23 column (60 m 0.32 mm 0.25 μm) was used. Oven temperatures were 150˚C ramped to 195˚C at 5˚C min^{-1}, ramped to 220˚C at 10˚C min^{-1} and flow rate was 1.5 min^{-1}. Fatty acids results after the previous procedures steps were transformed into methyl esters and directly injected into the GC.

2.3.3. Sterol Fractions of Soybean Oils

Five g oil sample was dissolved in 3 mL of hexane (purity 99.5%), and then 0.5 mL of 5 α cholestane (0.4 $mg{\cdot}mL^{-1}$) internal standard was added. The mixture was saponified with sodium Curr. Sci. Int., 9(4): 690-697, 2020 EISSN: 2706-7920 ISSN: 2077-4435 DOI: 10.36632/csi/2020.9.4.62 693 hydroxide solution in methanol (2N) at water bath for 1 - 2 h then, unsaponifiable matters were extracted. Then 1 μl of the sample was injected into Agilent 6890 series GC apparatus (setup: DB-5 capillary column, N_2 carrier gas at 0.9 mL 1 min and held for 10 min.), the internal standard method (5 α-cholestane) was used for quantification [11].

2.4. Mineral Analysis

The elements (P, Ca, Mg, Fe, Na and Cu) in soybean oil samples were determined using the PerkinElmer Model Optima 8×00 series ICP optical Emission Spectrometers (ICP-OES) (PerkinElmer, Inc., Shelton, CT, USA).

2.4.1. Determination of Total Tocopherols

Determination of total tocopherols of oil samples was determined according to the method described by [12]. Tocopherol compounds were determined by HPLC according to [13].

2.4.2. Determination of Total Polyphenols

Determination of total polyphenols of oil samples was determined according to the method of [14].

2.4.3. Oxidative Stability of Soybean Oilsamples

Oxidative stability of oils was evaluated by Rancimat method [15]. Stability was expressed as the induction time (hours), measured with the Rancimat 679 apparatus (Metrohm Herisou, Co., Switzerland), using an oil sample of 5 g heated to 100˚C with air flow rate of 20 L/h.

2.5. Statistical Analysis

Statistical analysis for all values are expressed as mean ± standard error for three independent samples for fresh and at the end of heating period (n = 3 ± SE). Analysis of variance (ANOVA) and the least significant difference (LSD) test at $P < 0.05$ were calculated to allow comparison between the mean values of the studied parameters using the COSTAT software package (Cohort Software, CA, USA). Differences between studied parameters were considered significant if $P < 0.05$.

3. Results and Discussion

3.1. Physical and Chemical Properties of Refined and Color Inverted Soybean Oils

Physicochemical characteristics (RI, color, FFA, PV, P-AV, IV, SV, K232 and K270 nm., stability, % UNS, soap content, wax, and total polyphenols) were determined in soybean oil during refining process (degumming, neutralization, bleaching and deodorization) and color inverted oil and compared them with those in crude soybean oil **Table 1**. The obtained results showed that there were observed difference in the previous mentioned parameters of soybean oils during refining process, values of acidity (FFA), stability, IV, soap content, wax, % unasp, K_{232} and K_{270} nm. And total polyphenols were reduced as a result all refining process compared them with crude soybean oil.

The decrease in value of acidity of soybean oil during all refining process may be due to elimination of fatty acids during the saponification process which used in neutralization process. On the other hand degumming and neutralization process caused increase in values of PV, p-AV, IV and color (red and yellow unites) but these values decreased as a results effect of bleaching and deodorization process. The high reduction in color unites (red and yellow) during bleaching and deodorization process may be due to that the pigments in soybean oil are absorbed on bleaching clay which used it in bleaching step. This results agreement with obtained by Nawar [16] Who mentioned that, the refining process remove some of impurities from the oil, such as; (FFA, phospholipids, pigments (chlorophyll) and carotenoids content and other minor impurities), after the refining, a pure oil with desirable properties for the consumers, such as (odor, taste, light color and stability).

As a result in **Table 1** it was found to be no clear changes in values of RI and SV of soybean oil during all refining process. With regarding the data in the **Table 1** it could be observed that, the value of color unites (red and yellow) of

Table 1. Physico-chemical properties of refined and color reverted soybean oils.

Physico-chemical properties		Oil samples of					
		Crude	Degummed	Neutralized	Bleached	deodorized	Color reversed oil
R.I at 25˚C		1.4715[a]	1.4715[a]	1.4715[a]	1.4715[a]	1.4717[a]	1.4715[a]
Color units	Red	6.2[c]	7.5[c]	8.6[d]	4.5[b]	1.3[a]	4.2[b]
	Yellow	35[d]	35[d]	35[d]	25[c]	8[a]	18[b]
FFA (% as oleic acid)		0.74[d]	0.36[c]	0.03[b]	0.03[b]	0.02[a]	0.03[b]
PV (meqO_2/kg oil)		1.79[c]	2.89[d]	2.89[d]	1.5[c]	0.42[a]	0.6[b]
p-Anisidine value (meq/kg oil)		4.46[b]	9.89[d]	9.89[d]	6.86[c]	2.98[a]	3.28[b]
Totox value		5.05[d]	6.49[de]	5.819[d]	3.059[c]	0.789[a]	1.259[b]
Oxidative stability (at 10˚C), air flow rate of 20 L/h		8.86[a]	8.46[a]	7.12[b]	6.42[c]	5.78[d]	5.54[d]
IV (I_2/100 g oil)		130.82[a]	128.96[a]	128.30[a]	129.60[a]	127.70[a]	127.95[a]
Saponification value (mgKOH/g oil)		194.67[a]	194.54[a]	194.89[a]	194.52[a]	194.62[a]	194.28[a]
Soap content (ppm)		55[d]	50[d]	43[c]	6[b]	0[a]	0[a]
Wax (ppm)		140[e]	86[d]	42[c]	30[b]	26[a]	26[a]
Unsap (%)		0.68[a]	0.68[a]	0.66[b]	0.64[b]	0.45[c]	0.40[d]
K232 (nm.)		2.313[c]	2.301[c]	2.297[c]	2.049[b]	1.661[a]	1.652[a]
K270 (nm.)		0.299[d]	0.280[c]	0.262[c]	0.244[ab]	0.237[a]	0.238[a]
Totalpolyphenols (ppm)		6.34[a]	4.81[b]	2.96[c]	2.44[c]	1.85[d]	1.64[d]

soybean oil recorded remarcable increase after several hours from deodorization process, that meaning the color of soybean oil getting darker during storage, this phenomena is called color reversion.

This increase in values of (red and yellow) color units of color reversed of soybean oil (deodorized soybean oil) during storage it for several hours may be related to the following conditions: moisture of raw soybean, refining and deodorization condition of soybean oil, temperature intensity of light and contact surface with the air during storage [4], and also may be related to increase of tocored content in deodorized oil during storage it (color reversed oil), that agreement with Mamoru [3] who found that the coloring of refined and deodorized soybean oil due to due to tocored in soybean oil.

3.2. Fatty Acids Composition of Refined and Color Reversed Soybean Oils

Fatty acids composition of soybean oil during refining steps and colored oil during storage were fractionated and determined by GC-capillary column. The obtained data are shown in Table 2, it is clear from the results in this table no clear change in the fatty acids composition in soybean oil as a results effect of refining process and in color inverted soybean oil (deodorized soybean oil) during

Table 2. Fatty acids composition of refined and color reversed soybean oil.

Fatty acids composition (%)	Oilsamplesof				
	Crude	Neutralized	Bleached	Deodorized	Color reversed oil
C14:0	0.07	0.07	0.07	0.08	0.08
C16:0	10.3	10.3	9.90	10.2	9.24
C16:1	0.11	0.11	0.09	0.10	0.10
C18:0	4.23	4.27	4.20	4.72	4.58
C18:1	21.4	21.5	23.2	22.0	23.0
C18:2	54.2	54.2	54.5	53.9	54.9
C18:3	8.79	8.79	8.74	7.86	7.95
C20:0	0.36	0.36	0.42	0.38	0.36
Others	0.55	0.42	0.37	0.53	0.53

storage compared them with crude oil. The unsaturated fatty acids C18:1 and C18:2 were increased, whilst the lenolenic acid (C18:3) recorded slight decrease in refined and color reversed soybean oil.

That may be related to the effect of different conditions of refining process *i.e.* temperature, pressure, time and steam on fatty acids composition of soybean oil. These results agree with those obtained by [17].

3.3. Mineral Contents of Refined and Color Reversed Soybean Oil

The changes in minerals composition of soybean oil during refining steps and in color inverted soybean oil during storage it for several hours were determined in Table 3. Results in Table 3 showed that refining process; degumming, neutralization, bleaching and deodorization steps reduced P, Ca, Mg and Cu elements from 146.12, 28.66, 18.09 and 0.04 ppm in crude soybean oil to (1.14, 1.20, 1.15 and 1.02 ppm), (0.15, 1, 0.08 and 0.002 ppm), (006, 0, 0 and 0 ppm) and (0.04, 0, 0 and 0 ppm) in degummed, neutralized, bleached and deodorized soybean oils respectively, and also color inverted soybean oil had trace metals i.e, P and Ca minerals were 1.05 and 0.002 ppm respectively, while Mg and Cu minerals were not detected.

As shown in the same table it could be noticed that Na element appearance after neutralization step (neutralized soybean oil), it was found to be 0.85 ppm and reduced to 0.43, 0.006 in samples taken from neutralization, bleaching and deodorization steps, and it was not determined to be zero in color inverted soybean oil. The higher decrease in phosphorous element must be lost refining process [18].

3.4. Tocopherol Compounds of Refined and Color Reversed Soybean Oils

Tocopherol contents oftentimes play an important role in the oil stability,

Table 3. Mineral contents of refined and color reversed soybean oils (mg/kg).

Oil samples	Minerals (ppm)					
	P	Ca	Mg	Fe	Na	Cu
Crude	146.12	28.66	18.09	0.00	0.00	0.04
Degummed-Neutralized	1.24	0.15	0.06	0.00	0.85	0.00
Bleached	1.15	0.08	0.00	0.00	0.006	0.00
Deodorized	1.02	0.002	0.00	0.00	0.00	0.00
Color reversed oil	1.05	0.002	0.00	0.00	0.00	0.00

therefore, the tocopherol compounds include alpha, beta, delta and gamma tocopherols in crude, industrially processed (degumming, neutralization, bleaching and deodorization process) and in color reversed soybean oils were determined and the obtained data are shown in Table 4. As clear from the data in this table it could be concluded that the crude soybean oil had the highest value from delta tocopherol (657.12 ppm) followed by alpha tocopherol (566.21 ppm) then gamma tocopherol (120.71 ppm) and beta tocopherol (61.70 ppm) and decreased to 628.4, 553.5, 115.5 and 58.12 ppm in degummed oil, to 598.67, 510.20, 108.32 and 55.12 ppm in neutralized oil, to 493.10, 411.79, 90.12 and 40.12 ppm in bleached oil and to 255.43, 253.86, 50.22 and 22.67 ppm in deodorized oil, respectively.

Additionally, the color reversed soybean oil had less amounts from the previous tocopherol compounds (α, β, γ and δ tocopherols) than those in others refined soybean oils, were 240.62, 22.94, 242.32 and 50.04 ppm respectively. Also, from the same table, it could be noticed that the crude soybean oil had the highest values from total tocopherols (1405.74 ppm), this value decreased to 1333.50, 1272.31, 1035.13, 582.2 and 555.92 ppm in degummed, neutralized, bleached, deodorized and color reverted soybean oils respectively.

With respect to tocored content of crude soybean oil, it was found to be 169.2 ppm, this value recorded a higher decreased as a result refining process; degumming, neutralization, bleaching and deodorization process 120.35, 99.82, 20.25 and 8.45 ppm, respectively, but it was increased to 46.5 ppm in deodorized soybean oil during storage it for several hours (color reversed soybean oil). This increase of tocored content in color reversed soybean oil may attributed to reduction of tocopherol compounds and turned into tocored during storage, that tocored in colorless form is really the precursor of color reversion in soybean oil [3].

Finally, reduction of total tocopherols, alpha, beta, delta and gamma tocopherol compounds and tocored of soybean oil during refining process were found linearly with darking color oil (color reversion) as a shown in Table 1 and Table 4. That may be due to the decrease in delta tocopherol compound was accompanied by an increase in dimeric oxidation products. Formation of dimeric oxidation produced from delta γ-tocopherol was proposed to be the reason of color

Table 4. Tocopherol compounds of refined and color reversed soybean oils (mg/kg).

Oil samples	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total-tocopherol	Toco-red
Crude	566.21[a]	61.70[a]	657.12[a]	120.71[a]	1405.74[a]	169.2[a]
Degummed	533.5[b]	58.12[b]	628.40[a]	115.5[b]	1335.52[b]	120.35[b]
Neutralized	510.20[b]	55.12[b]	598.67[b]	108.32[b]	1272.31[b]	99.82[c]
Bleached	411.79[c]	40.12[c]	493.10[c]	90.12[c]	1035.13[c]	20.25[d]
Deodorized	253.86[d]	22.67[d]	255.43[d]	50.22[d]	582.2[d]	8.45[e]
Color Reversed Oil	240.62[e]	22.94[d]	242.32[d]	50.04[d]	555.92[d]	46.5[f]

reversion [1].

3.5. Sterol Fractions of Refined and Color Reversed Soybean Oils

The sterol fractions of unsaponifable matters in soybean oil during refining steps (degumming, neutralization, bleaching and deodorization process) and in color reversed soybean oil (deodorized soybean oil during storage it for several hours) and compared them with those in crude soybean oil were presented in Table 5. Ten sterol fractions were identified (cholesterol, brassicaesterol, egrosterol, compestrol, compestanol, stigmasterol, B-sitosterol, Δ5 Avenasterol, Δ7 stigmasterol and Δ7 Avenasterol compounds), whereas the B-sitosterol fraction was the major sterol fraction in all previous samples, it was found to be 178.27 ppm in crud soybean oil and decreased to 172.57, 167.84, 165.57, 162.03 and 161.87 ppm in degummed, neutralized, bleached, deodorized and color reversed soybean oil, respectively.

Also, campesterol and stigmasterol fractions were found to be 82.24 and 68.18 ppm, respectively in crude soybean oil which they decreased to (78.15, 72.47, 65.7, 62.68 and 61.99 ppm) and (65.24, 62.47, 54.34, 50.23 and 49.98 ppm) in degummed, neutralized, bleached, deodorized and color reversed soybean oils, respectively. Also, others determined sterol fractions in degummed, bleached, neutralized, deodorized and color reversed soybean oils were decreased compared with those in crude soybean oil. This decrease in all sterol fractions during refining steps may be due to degradation of these fractions as a result temperature which used during process made of soybean oil and storage it.

3.6. Effect of Addition 0.2% Citric Acid to Color Reversed Oil on the Physical and Chemical Properties of Bleached and Deodorized Soybean Oil

Table 6 and Table 7 show the changes in the physco-chemical characteristics of bleached and deodorized soybean oil as a result addition of citric acid (0.2%) to color reversed and neutralized soybean oils. Results in these tables indicated that addition of citric acid to color reversed and neutralized soybean oils and after that procedure bleaching and deodorization process on previous oils caused a higher decrease in values of color (red and yellow unites) of bleached and

Table 5. Sterol fractions of refined and color reversed soybean oils (mg/kg).

Sterol fractions (%)	Oil samples					
	Crud	Degummed	Neutralized	Bleached	Deodorized	Color Reversed Oil
Cholesterol	1.45	1.34	1.28	0.76	ND	ND
Brassicaesterol	5.80	5.12	4.89	2.95	1.65	1.60
Egrosterol	3.15	2.95	2.20	1.98	1.04	1.04
Campesterol	82.24	78.15	72.47	65.20	62.68	61.99
Campestanol	5.98	4.84	3.78	2.79	1.98	1.92
Stigmasterol	68.18	65.24	62.47	54.34	50.28	49.98
B-Sitosterol	178.27	172.57	167.84	165.57	162.03	161.87
Δ5 Avenasterol	12.88	12.45	11.79	10.46	7.95	7.89
Δ7-stigma sterol	16.92	16.27	15.89	14.21	13.58	12.98
Δ-7 Avenasterol	14.79	14.07	13.48	12.82	10.83	10.57

Table 6. Effect of addition 0.2% citric acid to color reversed oil on physiochemical properties of bleached and deodorized soybean oils.

Physico-chemical properties of oils		Color reversed oil	Bleached	Deodorized
R.I at 25˚C		1.4715^{a}	1.4718^{a}	1.4717^{a}
Color units	Red	4.2^{c}	1.8^{b}	0.5^{a}
	Yellow	18^{c}	12^{b}	5^{a}
FFA (%) as Oleic acid		0.03^{a}	0.03^{a}	0.02^{a}
PV (meqO_2/kg oil)		0.6^{b}	0.35^{a}	0.23^{a}
p-Anisidine value (meq/kg oil)		3.28^{b}	2.05^{ab}	1.85^{a}
Totox value		1.259^{b}	0.759^{a}	0.485^{a}
Oxidative stability at 100˚C		5.54^{b}	5.84^{b}	6.25^{a}
IV (I_2/100 g oil)		127.95^{a}	127.85^{a}	127.64^{a}
Saponification value (mgKOH/g oil)		194.28^{a}	194.52^{a}	194.68^{a}
Soap content (ppm)		0	0	0
Wax (ppm)		26^{b}	24^{b}	20^{a}
Unsap (%)		0.44^{a}	0.42^{a}	0.40^{a}
K232 (nm.)		1.652^{a}	1.604^{a}	1.562^{a}
K270 (nm.)		0.238^{a}	0.224^{a}	0.219^{a}
Totalpolyphenols (ppm)		1.64^{a}	1.60^{a}	1.58^{a}

Table 7. Effect of addition 0.2% citric acid to neutralized soybean oil on physiochemical properties of bleached and deodorized soybean oils.

Physico-chemicalproperties of oils		Neutralized	Bleached	Deodorized
R.I at 25˚C		1.4715[a]	1.4715[a]	1.4717[a]
Color units	Red	8.6[c]	2[b]	0.7[a]
	Yellow	35[c]	20[b]	6[a]
FFA (%) as Oleic acid		0.02[a]	0.03[a]	0.02[a]
PV (meqO_2/kg oil)		2.89[c]	1.5[b]	0.42[a]
p-Anisidine value (meq/kg oil)		9.89[c]	6.86[b]	2.98[a]
Totox value		5.82[c]	3.059[b]	0.88[a]
Oxidative stability at 100˚C		7.12[a]	6.98[b]	6.68[b]
IV (I2/100 g oil)		128.3[a]	129.6[a]	127.7[a]
Saponification value (mgKOH/g oil)		194.89[a]	194.52[a]	194.62[a]
Soap content (ppm)		43[c]	6[b]	0[a]
Wax (ppm)		42[b]	30[a]	26[a]
Unsaponifable matters (%)		0.66[a]	0.64[a]	0.45[b]
K232 (nm.)		2.601[b]	2.049[b]	1.661[a]
K270 (nm.)		0.546[b]	0.244[a]	0.237[a]
Totalpolyphenols (ppm)		2.96[a]	2.35[a]	1.76[b]

deodorized soybean oils, and also values of PV, p-AV, TV, wax content, (%) Unsap, soap content, K_{232} and K_{270} nm recorded slight decrease by adding citric acid. With regarding the data in Table 6, stability of bleached and deodorized soybean oils after added citric acid to color reversed soybean oil was increased compared with that color reversed oil (without citric acid), while it was recorded a slight decrease in bleached and deodorized soybean oils after adding of citric acid (0.2%) to neutralized soybean oil Table 7.

Finally addition of citric acid to each neutralized and color reversed soybean oils caused a high reduction in color values of bleached and deodorized soybean oils. The reduction of color maybe due to eliminate the nonhydratable phospholipid in soybean oil (the presence of these nonhydratable phospholipid in oil colors the refined oil) by citric acid as chelating agents [5].

3.7. Effect of Addition of Citric Acid (0.2%) on the Tocopherol Contents of Soybean Oil

Table 8 and Table 9 illustrated the changes in the total tocopherols and their contents α, β, γ and δ tocopherols and tocored compound of bleached and deodorized soybean oils as a result addition of citric acid (0.2%) to neutralized and color reversed soybean oils, then procedure of bleaching and deodorization process on these oils. From the result in the same previous Table 8 and Table 9

Table 8. Effect of addition 0.2% citric acid to color reversed oil on the tocopherol contents (mg/kg) of bleached and deodorized soybean oils.

Oil samples	*α*-tocopherol	*β*-tocopherol	*γ*-tocopherol	*δ*-tocopherol	Total-tocopherol	Toco-red
Color Reversed Oil	240.62[b]	22.94[a]	242.32[b]	50.04[b]	555.92[b]	46.5[b]
Bleached	235.54[b]	21.57[a]	240.38[b]	40.74[a]	538.23[b]	Nil[a]
Deodorized	220.85[a]	20.49[a]	225.46[a]	39.52[a]	506.32[a]	Nil[a]

Table 9. Effect of addition 0.2% citric acid to neutralized soybean oil on the tocopherol contents (mg/kg) of bleached and deodorized soybean oils.

Oil samples	*α*-tocopherol	*β*-tocopherol	*γ*-tocopherol	*δ*-tocopherol	Total-tocopherol	Toco-red
Neutralized	510.20[c]	55.12[c]	598.67[b]	108.32[b]	1272.31[c]	99.82[b]
Bleached	322.42[b]	34.57[b]	282.14[a]	51.48[a]	609.61[b]	Nil[a]
Deodorized	232.41[a]	22.48[a]	231.72[a]	40.07[a]	526.68[a]	Nil[a]

it could be noticed that, adding citric acid to neutralized and color reversed soybean oils caused a higher decrease in total tocopherols and their contents (*α*, *β*, *γ* and *δ*), whilst tocored compound was disappeared (not detected) in bleached and deodorized soybean oils. The disappearance of tocored content was linearly related to color lighting (reduction values of red and yellow unites) by adding citric acid (0.2%) as a shown in Table 6 and Table 7. This strongly indicated that tocored content may contribute more to the color darkening (color reversion) of soybean oil during packaging and storage it.

Finally, addition of citric acid (as chelating agents to deactivated trace metals and to increase the efficiency bleaching earth on the color reversion of soybean oil during packing and storage it) to bleaching earth to both color reversed and neutrtlized soy bean oils then procedure bleaching and deodorization process on these oils caused disappearance the tocored content, which responsible for the phenomenam of color reversion of soybean oil after deodorization step during oil storage.

4. Conclusion

Considering comprehensively, the neutralization stage and the deodorization stage have the greatest influence on the color units, AV, PV, K_{232}, K_{270}, oxidative stability and micronutrients (*T. polyphenols*, *T. tocopherols*, tocopherol contents [*α*, *β*, *γ* and *δ* tocopherols], tocored compound and sterol fractions) of soybean oil, followed by the bleaching stage, and the least affected is the degumming stage. A similar trend was observed for the saponification value, iodine number and fatty acids composition changes of soybean oil during refining process, which indicates that there was no significant difference between these parameters of RBD soybean oil. Addition of citric acid before addition of bleaching earth leads to increase in bleaching efficiency therefore, increasing the

amount of tocored adsorptions on the surface of bleaching earth and then protecting soybean oil from the phenomenon color reversion after deodorization step.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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