

## Optimization of Bleaching Parameters for Soybean Oil

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### Summary

The final stage of edible soybean oil manufacture is refining, the most delicate phase of which is bleaching. At this step, undesirable substances are removed, such as pigments, traces of metals, phospholipids and certain degradation products. However, certain valuable compounds such as tocopherols and sterols may also be removed, significant loss of oxidative stability can occur, and fatty acid content may increase. To avoid these negative oil changes, bleaching parameters such as the concentration of bleaching clay, temperature and duration should be optimized. Since bleaching conditions depend on the properties of the bleaching clay as well as on the type of crude oil, bleaching parameters should be optimized with different types of clay for each vegetable oil. Since such optimization has not yet been reported for soybean oil treated with Pure-Flo® Supreme Pro-Active bleaching adsorbent, this study investigates the effect of bleaching parameters on bleaching efficiency, oxidative stability and the content and composition of bioactive compounds (tocopherols and sterols) using the above mentioned clay in this type of oil. Results show that the amount of clay had the greatest influence on bleaching efficiency, especially according to the Lovibond scale, on transparency, and on phosphorus content. Temperature and clay amount significantly affected oxidative stability, in particular the formation of secondary oxidation products. Increasing the amount of clay decreased tocopherol content of the bleached oil. Neutralized soybean oil bleached for 20 min at 95 °C with 1 % Pure-Flo® Supreme Pro-Active bleaching clay showed the highest oxidative stability, best bleaching efficiency, and most favourable sterol content, although tocopherol content was reduced.

**Key words:** bleaching, soybean oil, oxidative stability, sterols, tocopherols

### Introduction

Soybean is the dominant oilseed produced in the world (1) due to its favourable agronomic characteristics, high quality protein and valuable edible oil. Oil produced by mechanical pressing or, more often, by solvent extraction of soybean is termed crude soybean oil. The final stage of edible soybean oil manufacture is the complex refining process, of which the most delicate phase is bleaching (2,3). In this step, primarily undesirable substances are removed, including oxidation products,

colour bodies, phospholipids and glycolipids, soaps, contaminants and metal traces. Metal traces (particularly Cu and Fe) are naturally present in the seed. Their presence in the oil can also occur by transfer from the equipment containing Cu and Fe, although this is less common than before because of the modern use of stainless steel appliances in food processing plants. Nevertheless, metal traces should be removed from the oil during bleaching in order to produce oil with good oxidative stability (4). However, valuable compounds such as tocopherols and sterols may also be removed, significant

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loss of oxidative stability can occur, *trans* fatty acids can form, and free fatty acid (FFA) content may increase (5–9). Optimization of bleaching parameters, mainly temperature, time, and clay content, is necessary in order to minimize these undesirable oil changes.

If only the adsorbent properties of clay were important for bleaching, the most effective bleaching would occur at lower temperatures since at higher temperatures the adsorption equilibrium moves towards desorption and some of the adsorbed molecules dissolve back into the oil. However, decolouration is better at higher temperatures, which indicates that clay is much more than an adsorbent. Indeed, chemical reactions take place on the surface of the clay, and the kinetic constants of these reactions, both desirable and undesirable, increase 2- to 4-fold with a temperature increase of 10 °C (10). Therefore, there must be an optimum temperature for bleaching, and this optimum likely depends on the type of oil and levels of colour bodies, oxidation products and contaminants present. Most types of oil are treated in the temperature range of 90–100 °C (11), although bleaching may be carried out at up to 120 °C if problems are expected due to oil specificities (4).

The optimal bleaching time depends on the bleaching temperature and clay quality. Colour removal increases with time and temperature, although longer contact of oil and clay can cause colour reversion, which also increases with temperature. Bleaching for a long time at high temperatures seriously damages the oxidative stability of edible oil. Bleaching time for most types of oil is in the range of 20–30 min at 90–100 °C (4,12,13).

High clay content enhances the removal of undesirable substances in the oil. However, the minimum clay needed for effective bleaching is difficult to establish because different types of oil contain different amounts of substances and react differently with clay. Optimal clay content depends on the type of clay, oil pretreatments, and desired quality of the oil. It varies between 0.1 and 2.0 % (14), but it can be as high as 5 % in special cases (4).

These considerations make clear that bleaching parameters, *i.e.* temperature, duration and clay content, should be determined individually for each neutralized oil using different types of bleaching clay. The aim of this study is to optimize these bleaching conditions for neutralized soybean oil with Pure-Flo® Supreme Pro-Active bleaching clay on the laboratory scale, since optimal conditions have not yet been reported for this type of vegetable oil bleached with this clay. The effect of these parameters on bleaching efficiency, oxidative stability and content of bioactive compounds (tocopherols and sterols) has been investigated.

## Materials and Methods

### Standards and chemicals

All the standards used in this study were of HPLC grade. The following compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA):  $\beta$ -sitosterol (17-[5-ethyl-6-methyl-heptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol), campesterol (17-[5,6-dimethylheptan-2-yl]-10,13-

-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol),  $\alpha$ -cholestanol (10,13-dimethyl-17-[6-methylheptan-2-yl]-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-ol), and stigmasterol (17-[5-ethyl-6-methyl-hept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol). The compounds  $\alpha$ -tocopherol (2,5,7,8-tetramethyl-2-[4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol) and  $\gamma$ -tocopherol (2,7,8-trimethyl-2-[4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol) were obtained from Merck (Darmstadt, Germany). All other chemicals were of suitable purity grade.

### Materials

Industrially neutralized soybean oil (Zvijezda d.d., Zagreb, Croatia) was bleached using Pure-Flo® Supreme Pro-Active bleaching clay (Oil-Dri Corporation of America, Chicago, IL, USA). This is an adsorbent manufactured using a proprietary surface modification technology (SMT). Raw material of the adsorbent is an intergrowth of homite and smectite minerals. SMT processing, combined with the intrinsic qualities of the mineral, produces a highly active, fast-filtering product that removes colours from corn, canola and soybean oil, in addition to removing soaps, phospholipids and metals such as Ca, Fe, Mg and Ni. Manufacturer specifications of the bleaching clay (Oil-Dri Corporation of America) indicate a free moisture content of 14–18 % at 105 °C and pH=2.4 to 3.3 (5 % solids in deionized H<sub>2</sub>O).

### Bleaching of oil under laboratory conditions

Laboratory bleaching was performed in a round-bottom, three-necked flask of 500 mL equipped with a thermometer and attached to a vacuum pump and nitrogen source (Fisherbrand bleaching equipment, Fisher scientific Ltd, Loughborough, UK and Gast 0211 vacuum pump, Gast, Benton Harbor, MI, USA). Bleaching was conducted using an electromagnetic mixer with adjustable heater. All bleaching parameters are given in Table 1. Briefly, 200 g of industrially neutralized soybean oil was weighed into a round-bottom, three-necked flask

Table 1. Bleaching parameters of soybean oil samples

Sample	Bleaching parameters		
	w(clay) %	Time min	Temperature °C
1		20	105
2	0.5	30	95
3			115
4		40	105
5		20	95
6			115
7	1	30	105
8		40	95
9			115
10		20	105
11	1.5	30	95
12			115
13		40	105

and heated with mixing under a partial vacuum of 0.4 bar. Bleaching clay was added to a final concentration of 0.5, 1.0 or 1.5 % to the oil preheated to approx. 60 °C. The oil was then gradually heated to the desired bleaching temperature (95, 105 or 115 °C) with constant mixing under vacuum to disperse the clay completely. After reaching the desired temperature, the heating was temporarily turned off to allow the temperature to fall by approx. 2–3 °C. Then the heating was turned on again and kept constant for the desired bleaching time (20, 30 or 40 min). At the end of bleaching, heating and vacuum were turned off and nitrogen supply was turned on.

After bleaching, hot sludge from the flask was filtered through filter paper (Whatman no. 541) under vacuum. Samples of bleached oil were stored at –18 °C until further analysis.

#### Analytical method

Lovibond colour was determined according to the ISO method (15) using Lovibond Colourscan (Tintometer Ltd., Amesbury, UK). Transparency was measured at 455 nm on a Cary 100 Scan spectrophotometer (Varian, Palo Alto, CA, USA) following the method of Oštrić-Matijašević *et al.* (16). Transparency of samples was measured in undiluted oil using distilled water as a blank.

The elements P, Fe and Cu in oil were determined using an inductively coupled plasma atomic emission spectroscopy (ICP-AES) method (17,18) on a Vista-MPX (Varian). The three elements were analyzed by direct injection of a sample dissolved in PremiSolv ICP solvent (SCP Science, Conostan, Montreal, Quebec, Canada). Determination of the peroxide value (PV) was carried out following ISO 3960:2007 analytical method (19) and the anisidine value following ISO 6885:2006 method (20). These two values were used to calculate the Totox (total oxidation) value using formulae from Oštrić-Matijašević and Turkulov (21). Specific UV absorption at 232 and 270 nm ( $K_{232}$  and  $K_{270}$ , respectively) was measured following ISO 3656:2011 method (22). Free fatty acid (FFA) content was determined using ISO 660:2009 method (23).

Sterol content and composition were determined using gas chromatography and ISO 12228:1999 method (24). The prepared sterol fraction (1 µL) was injected into a gas chromatograph (series 610, ATI Unicam, Cambridge, UK) equipped with a DB-17 capillary column (30 m×0.32 mm×0.25 mm) containing 50 % phenyl-methylpolysiloxane stationary phase. Helium was used as carrier gas at a flow rate of 0.36 mL/min. The temperature of the injector was set to 280 °C and the temperature of the detector to 290 °C. The temperature of the column oven was programmed to increase at 6 °C/min from an initial value of 180 to 270 °C, and then to remain at this value for 30 min. Peaks were identified by comparing the retention times of sterols with those of the standards. Quantification of all sterols was based on an internal standard method using  $\alpha$ -cholestanol.

Tocopherol content and composition were determined using a standard ISO method (25). Analysis was performed by high-performance liquid chromatography (HPLC) using a ProStar 363 (Varian) equipped with a ProStar fluorescent detector and a Restek Pinnacle II silica column (15 cm×4.6 mm, 5 µm). Sample was prepared

by dissolving 0.1 g of soybean oil in 10 mL of *n*-hexane, and 20 µL of the solution was injected in the column. Detection of tocopherols was performed at an excitation wavelength of 295 nm and emission wavelength of 330 nm. Isocratic chromatography at room temperature was used with a mobile phase of 0.7 % propan-2-ol in *n*-hexane at a flow rate of 0.6 mL/min. Quantification of tocopherols was performed using standard calibration curves of  $\alpha$ - and  $\gamma$ -tocopherol covering the mass fraction range of 5–750 mg/kg.

#### Statistical analyses

A Box-Behnken design for three factors was used to design the samples (26). The Friedman-type statistics for ranking the data was calculated to rank the samples according to bleaching efficiency and oxidative stability. Nonparametric analogues to Fisher's least significant differences (LSD) for ranking the sums were calculated, and a multiple comparison procedure was performed to determine the significance of differences between the bleaching efficiency and oxidative deterioration parameters evaluated by the ranking test (27). Principal component analysis (PCA) was performed to reduce the number of correlated variables, and to determine which parameters significantly affected bleaching outcomes. All statistical analyses were performed using the software package STATISTICA v. 9 (28).

## Results and Discussion

Bleaching was performed on industrial neutralized soybean oil in the laboratory under vacuum and nitrogen atmosphere, using Pure-Flo® Supreme Pro-Active bleaching adsorbent. The values for temperature, duration of bleaching and clay content tested in this study were based on the literature (2,4,5,29) and on previous experience with bleaching at the refining plant of the Zvijezda oil factory, Zagreb, Croatia. Three bleaching temperatures (95, 105 and 115 °C), three clay amounts (0.5, 1 and 1.5 %) and three bleaching durations (20, 30 and 40 min) were tested. A Box-Behnken design for three factors was used to design the samples. The design consisted of 12 different cases, with the 13th central case representing an average valuation of variables (Table 1). All reported results are mean values of duplicate analytical determinations.

Bleaching efficiency was monitored by measuring the reduction in colour bodies, metal contaminants and oil retention by the bleaching clay. Colour bodies of oils and fats are pigments, primarily chlorophylls and carotenoids. Soybean oil contains small amounts of chlorophylls, and the main carotenoids are xanthophylls. Reduction of colour bodies in this work was measured according to the Lovibond method (Table 2). The greatest reduction in red (72 %) and yellow units (26 %) in neutralized oil was achieved using the highest clay content (1.5 %) at the highest temperature (115 °C) during 30 min. Substantial removal of colour substances makes the oil highly transparent. The highest transparency, 12-fold greater than that of neutralized oil, was obtained with the highest clay content, at higher temperature (105 and 115 °C) and through all time durations.

Table 2. Parameters of bleaching efficiency for soybean oil samples

Sample	Lovibond colour		Transparency	$w(\text{Cu})$ mg/kg	$w(\text{Fe})$ mg/kg	$w(\text{P})$ mg/kg
	red units	yellow units				
NS	10.6	68.0	1.30	0.009	0.020	16.77
1 <sup>bd</sup>	7.3	68.0	5.64	0.002	0.001	13.44
2 <sup>b</sup>	8.2	69.0	5.08	0.000	0.000	12.65
3 <sup>bd</sup>	7.0	67.0	7.27	0.000	0.002	13.23
4 <sup>b</sup>	7.1	68.0	6.72	0.000	0.005	13.00
5 <sup>a</sup>	5.4	68.0	12.76	0.005	0.006	9.84
6 <sup>ab</sup>	4.3	69.0	13.73	0.000	0.000	10.72
7 <sup>bcd</sup>	8.2	69.0	12.44	0.000	0.000	11.00
8 <sup>ab</sup>	5.0	68.0	11.60	0.006	0.003	10.54
9 <sup>ab</sup>	4.3	69.0	12.77	0.000	0.003	11.55
10 <sup>ac</sup>	3.4	62.0	16.13	0.000	0.006	10.19
11 <sup>a</sup>	3.4	69.0	14.91	0.002	0.000	9.79
12 <sup>ac</sup>	3.0	50.0	15.95	0.002	0.000	10.33
13 <sup>ad</sup>	3.3	70.0	15.17	0.013	0.005	7.22

NS=neutralized soybean oil

Bleaching parameters for each sample are given in Table 1

Samples with different letters in superscript differ significantly ( $p \leq 0.05$ )

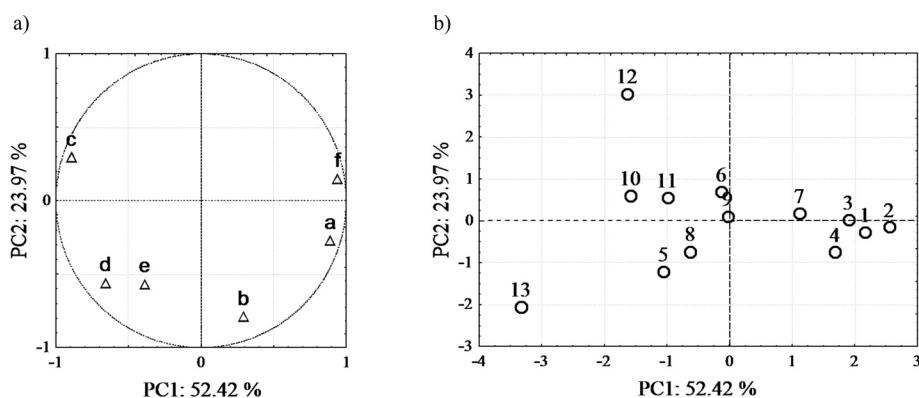
Maximum mass fraction of Cu or Fe permitted in bleached oil is 0.1 mg/kg (4,8,30). All samples in this work had metal traces far below 0.1 mg/kg (Table 2).

Phospholipids have good emulsifying properties and can cause losses of neutral oil during refining. Therefore, they have to be removed, mainly by degumming or to a lesser extent by bleaching. Phospholipid elimination is checked indirectly by analyzing phosphorus content. Maximum bleaching should decrease the level of phosphorus in oil to 10 mg/kg (2). Table 2 shows that higher clay amounts and lower temperatures reduced phosphorus content. The best result (7.22 mg/kg) was achieved by bleaching for 40 min at 105 °C using 1.5 % clay.

The results in Table 2 were analyzed by the Friedman-type statistics for ranking the data (27), which showed that bleaching of neutralized soybean oil was the most efficient when performed for 30 min at the lowest temperature (95 °C) and using the highest clay amount (1.5 %). Fisher's LSD for ranking the sums of the examined samples were calculated and a multiple

comparison procedure was performed to identify significant differences in bleaching efficiency among the samples ( $p \leq 0.05$ ). Clay content was the most important factor causing differences between samples. The same conclusion was obtained when the results were analyzed using PCA (Fig. 1).

PCA conducted on the results from Table 2 showed that only the first two of six principal components (PCs) have eigenvalues  $> 1$  and together they explain more than 76 % of the total variance. Fig. 1a shows a plot of the original variables for the first two PCs obtained from the PCA analysis of the data on bleaching efficiency. PC1 has a high positive correlation with red units and amount of phosphorus, and high negative correlation with transparency. Fig. 1b shows a loading plot for the first two PCs. Samples fell into three groups along the PC1 based on the amount of clay content used for bleaching. Samples bleached with a higher amount of clay showed higher transparency and lower levels of red units and phosphorus than the samples bleached with lower amounts of clay.



a: red units, b: yellow units, c: transparency, d: Cu / (mg/kg), e: Fe / (mg/kg), f: P / (mg/kg)

Fig. 1. PCA loading (a) and score (b) of the plots of the data on bleaching efficiency. Samples in b are described in Table 1

Bleaching clay accelerates oxidation of oil because of its high specific area and high catalytic activity. As a result, bleached oil has the lowest oxidative stability among the oils obtained during the various stages of refining (8). Thus, it is strongly recommended that bleaching be conducted under vacuum, which significantly reduces, but does not eliminate, oxidation. Table 3 shows oxidative deterioration of the soybean oil samples bleached under vacuum (0.4 bar) and different conditions. The peroxide value (PV) indexes the amount of oxygen that has reacted with the oil, resulting in the formation of hydroperoxides. The reduction in PV during bleaching was 14–36 %, and the lowest PV was achieved with 1 and 1.5 % clay at 95 and 115 °C, respectively. The best PV obtained here (0.9 mmol of O<sub>2</sub> per kg) was higher than the 0.5 mmol of O<sub>2</sub> per kg reported by Doleschall *et al.* (31) and Ortega-García *et al.* (32). Our higher PV may reflect the relatively high phosphorus content of 16.77 mg/kg in the neutralized soybean oil that was bleached. Wang (2) has suggested that low levels of phosphorus (5–10 mg/kg) in neutralized oil are required to maximize the bleaching effect. These hydroperoxides can be removed during the final refining stage of deodorization in the form of low molecular mass carbonyl compounds that arise from hydroperoxide breakdown during bleaching. These secondary oxidation products can be measured using the anisidine value (AV). Table 3 shows that samples with higher PV had lower AV, and *vice versa*. Higher AVs occurred at higher clay amounts, higher bleaching temperatures, and longer bleaching times. The lowest AV (3.3) was obtained by bleaching for 30 min at 95 °C using 0.5 % clay. This value is slightly higher than the previously published values for edible soybean oil, which range from 1.5 to 2.5 (31,33). Although our value was higher, our method may still be adequate for industrial production, since deodorization usually removes such secondary oxidation products.

Since PV and AV index only part of oxidative deterioration, we determined Totox values of our samples. Totox combines PV and AV to act as a marker of oil oxidative history and current oxidation status. High quality oil should have a Totox value under 10 (34). According to this indicator, bleaching at 95 °C gave the highest quality oil (Table 3).

Decomposition of hydroperoxides and isomerization of unsaturated fatty acids lead to the formation of conjugated dienes and trienes, which show absorption maxima at 232 and 270 nm, respectively. These compounds are very unstable to oxidation and are much more sensitive than oleic and linoleic acids, so bleaching parameters should be chosen to avoid formation of these compounds. In our study, oil treated with higher clay content had lower levels of conjugated dienes than the neutralized oil (Table 3). On the other hand, all samples had more conjugated trienes than the neutralized oil. Oil bleached using 0.5 % clay showed the lowest amounts of these compounds. For crude soybean oil intended for margarine production, Kondal Reddy *et al.* (35) recommended a maximum absorption of 3.3 at 232 nm, and they suggested that soybean oil with absorption above 4 at 270 nm would give a final product with bad sensory characteristics. All of our samples gave absorbances below these values (Table 3).

Although the oil bleaching process normally does not increase the content of FFA, this can occur after hydrolysis of triglycerides. It can be suppressed by properly drying the oil before bleaching and choosing clay that contains enough water necessary to become fully active but not more (5). Pure-Flo® Supreme Pro-Active clay, used for bleaching in this work, has a water content of 14–18 % and a pH of 2.4–3.3 (Oil-Dri Corporation of America). The increase in FFA in our samples during bleaching was minimal (Table 3), with a relative increase of 1 % over

Table 3. Parameters of oxidative deterioration in bleached soybean oil samples

Sample	<i>b</i> mmol of O <sub>2</sub> per kg	Anisidine value	Totox value	Absorbance		w(free fatty acids)* %
				A <sub>232 nm</sub>	A <sub>270 nm</sub>	
NS	1.4	1.5	4.3	2.96	0.38	0.10
1 <sup>ac</sup>	2.1	3.6	7.8	3.05	1.20	0.07
2 <sup>a</sup>	1.7	3.3	6.7	3.04	1.09	0.09
3 <sup>bc</sup>	3.7	6.0	13.4	3.43	1.64	0.10
4 <sup>ab</sup>	3.5	4.2	11.2	3.12	1.39	0.11
5 <sup>a</sup>	1.1	4.1	6.3	2.81	1.73	0.09
6 <sup>ab</sup>	1.2	9.8	12.2	2.81	2.58	0.09
7 <sup>ab</sup>	1.9	6.7	10.5	2.83	2.19	0.11
8 <sup>ac</sup>	2.3	3.7	8.3	2.82	2.00	0.11
9 <sup>b</sup>	2.5	10.5	15.5	3.21	2.67	0.11
10 <sup>ab</sup>	1.5	9.0	12.0	2.58	2.51	0.07
11 <sup>ab</sup>	1.9	6.8	10.6	2.61	2.27	0.09
12 <sup>bc</sup>	0.9	14.5	16.2	2.95	3.08	0.10
13 <sup>bc</sup>	1.8	10.4	14.0	2.93	2.74	0.06

*b*=peroxide value, NS=neutralized soybean oil

Bleaching parameters for each sample are given in Table 1

Samples with different letters in superscript differ significantly (*p*≤0.05)

\*in % of oleic acid

neutralized oil occurring in only 4 samples. This indicates that the clay used is suitable for soybean oil bleaching.

The Friedman-type rank test showed that soybean oil bleached for 20 min at 95 °C using 1 % clay had the best oxidative stability. Fisher's LSD for ranking the sums showed statistically significant differences among the samples ( $p \leq 0.05$ ), due primarily to the influence of temperature and secondarily to the influence of clay content.

PCA was also performed on parameters of oxidative deterioration. The first two PCs had an eigenvalue  $>1$  and together they explained 80 % of the total variance. Score plot (parameters of oxidative deterioration and FFA) and loading plot (samples of bleached oil) as a function of PC1 and PC2 are shown in Figs. 2a and 2b, respectively. Fig. 2a shows a high negative correlation of PC1 with the indicators of secondary oxidation products, while PC2 has a good positive correlation with primary oxidation products. Fig. 2b shows that although the samples do not fall into non-overlapping groups based on the oxidative deterioration, soybean oil samples bleached with 1 or 1.5 % clay at 115 °C fall to the left side along the PC1. These samples have higher values of AV and  $K_{270}$ . No clustering of samples is evident along PC2. These findings suggest that clay amount and bleaching temperature had a strong influence on the secondary oxidation products, but no effect on the primary oxidation products.

The Friedman-type rank test was applied to all results in Tables 2 and 3. It indicated that sample 5 showed the most efficient bleaching with minimum oxidative deterioration of the oil; this sample was bleached for 20 min at 95 °C using 1 % clay.

The influence of bleaching conditions on the content and composition of bioactive components of soybean oil was also examined. These analyses were carried out on a subset of samples bleached at different temperatures using different clay amounts, since statistical tests of the results showed that the duration of bleaching had no significant influence on bleaching efficiency or oxidative deterioration of oil. Of course, the sample bleached using optimal parameters of 1 % clay, 95 °C and 20 min was also included.

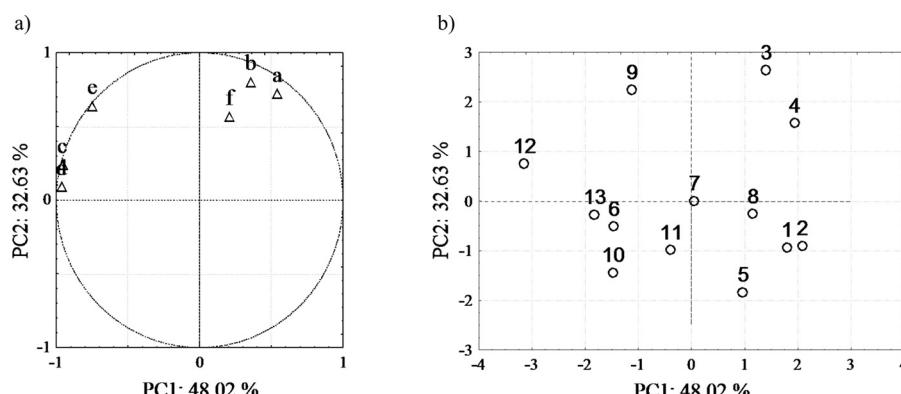
Bioactive compounds in vegetable oil, including soybean oil, contribute to their oxidative stability and nutritional value. The most important bioactive components are tocopherols, strong antioxidants with a significant biological effect. Levels of total and individual tocopherols decrease during neutralization, bleaching and deodorization (6,36). Table 4 presents the content and composition of tocopherols of selected soybean oil samples. The dominant tocopherol is  $\gamma$ -tocopherol, as reported in previous studies (8,36,37). It is obvious that the levels of  $\alpha$ - and  $\gamma$ -tocopherol in the neutralized oil decreased during bleaching. This reduction was greater when increasing clay content and bleaching temperature. The levels of  $\delta$ -tocopherol and total tocopherols also decreased, but there was no significant association with clay content or temperature. The Friedman-type rank test showed that the sample bleached at 105 °C using 1 % clay had the highest levels of individual and total tocopherols. The Fisher's LSD for ranking the sums confirmed that the samples with different clay content and bleaching temperature showed significant differences ( $p \leq 0.05$ ). In fact, the bleaching parameters found to be optimal based on the bleaching efficiency and oxidative deterioration of soybean oil (1 % clay, 95 °C and 20 min) did not give opti-

Table 4. Tocopherol content and composition of bleached soybean oil samples

Sample	w(tocopherol) mg/kg			
	$\alpha$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol	Total
NS	87.1	431.7	202.8	721.6
2 <sup>c</sup>	62.6	405.1	167.9	635.6
3 <sup>b</sup>	54.0	403.6	199.5	657.1
5 <sup>d</sup>	47.6	388.2	171.3	607.1
7 <sup>a</sup>	51.9	410.6	210.2	672.6
11 <sup>c</sup>	42.4	399.2	201.5	643.0
12 <sup>d</sup>	41.3	376.1	198.3	615.7

NS=neutralized soybean oil

Bleaching parameters for each sample are given in Table 1  
Samples with different letters in superscript differ significantly ( $p \leq 0.05$ )



a: PV/(mmol of O<sub>2</sub> per kg), b:  $K_{232}$ , c: AV, d:  $K_{270}$ , e: Totox, f: FFA/%

**Fig. 2.** PCA loading (a) and score (b) of the plots of the data on oil deterioration during the process of bleaching. Samples in b are described in Table 1

mal yields of tocopherols. Under these conditions, the levels of total tocopherols and  $\delta$ -tocopherol fell by 16 %, of  $\alpha$ -tocopherol by 45 % and  $\gamma$ -tocopherol by 10 % (Table 4).

Sterols are a very important nonglyceride fraction of vegetable oil. They can reduce blood cholesterol (38) and protect the oil from polymerization during thermal processing (39). Sterols are also very important for monitoring vegetable oil authenticity. Table 5 shows the content and composition of sterols in soybean oil samples after bleaching. All samples showed levels of individual and total sterols in accordance with international and Croatian regulations (40,41). Bleaching led to decrease in total sterol content ranging from 2 to 11 %, but this could not be associated with the changes in the three bleaching parameters studied here. Other studies have reported decreases in total sterols of 2.6 % (36) and 8 % (37), while Verleyen *et al.* (7) reported an increase in total sterols of 0.7 % during bleaching. This last study further found an increase in the levels of campesterol, stigmasterol and sitosterol, which was also reported by Costa *et al.* (36). The Friedman-type rank test showed that the sample bleached at 95 °C using 1.5 % clay had the highest levels of individual and total sterols. The Fisher's LSD for ranking the sums confirmed that the samples with different

clay content and bleaching temperature showed significant differences ( $p \leq 0.05$ ). During bleaching, increase in  $\Delta 7$ -stigmastanol (1 %) and  $\Delta 7$ -avenasterol (6 %) over neutral oil was observed. We assume that this increase was due to the formation of steradienes.

PCA of bioactive compounds showed that the first three PCs of the total of five had an eigenvalue  $> 1$ . Together, these three PCs explained nearly 93 % of total variance. PC1 and PC2 together accounted for almost 74 % of the variance. For simplicity, only plots of PC1 and PC2 were shown in this study, because PC2 and PC3 had similar factors of correlation with the original variables. Fig. 3a shows correlation of the amounts of bioactive compounds with PC1 and PC2. PC1 shows a high negative correlation with the amounts of all identified sterols in soybean oil. Tocopherols, except for  $\delta$ -tocopherol, show a very high positive correlation with PC2. Fig. 3b shows loading plot (selected samples) in the same coordinate system. Although there was no defined group forming of the samples along PC1, samples 3 and 12 bleached at higher temperature (115 °C) were placed on the positive side of PC1. Those samples had lower sterol content. Along the PC2, samples were separated based on the amount of clay. Samples bleached with 1.5 % of bleaching clay together with sample 5 (bleached with 1.0 % of

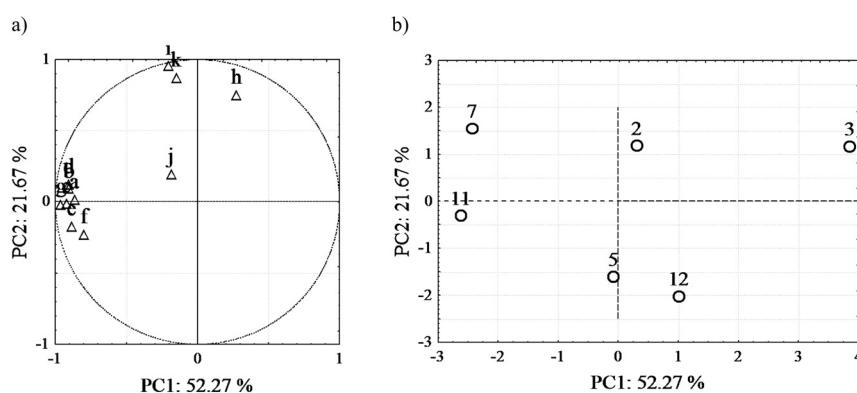
Table 5. Sterol content and composition of bleached soybean oil samples

Sample	<i>w(sterol)</i> mg/kg						
	campesterol	stigmasterol	$\beta$ -sitosterol	$\Delta 5$ -avenasterol	$\Delta 7$ -stigmastanol	$\Delta 7$ -avenasterol	Total
NS	529	543	1612	55	94	47	2880
2 <sup>bcd</sup>	510	527	1557	47	82	34	2757
3 <sup>bd</sup>	466	491	1433	45	78	36	2549
5 <sup>bcd</sup>	494	511	1515	51	93	47	2712
7 <sup>ac</sup>	507	526	1561	55	95	50	2794
11 <sup>a</sup>	508	536	1582	53	93	50	2822
12 <sup>ac</sup>	494	511	1520	46	84	41	2697

NS=neutralized soybean oil

Bleaching parameters for each sample are given in Table 1

Samples with different letters in superscript differ significantly ( $p \leq 0.05$ )



a: campesterol/(mg/kg), b: stigmasterol/(mg/kg), c:  $\beta$ -sitosterol/(mg/kg), d:  $\Delta 5$ -avenasterol/(mg/kg), e:  $\Delta 7$ -stigmastanol/(mg/kg), f:  $\Delta 7$ -avenasterol/(mg/kg), g: total sterols/(mg/kg), h:  $\alpha$ -tocopherol/(mg/kg), i:  $\gamma$ -tocopherol/(mg/kg), j:  $\delta$ -tocopherol/(mg/kg), k: total tocopherols/(mg/kg)

Fig. 3. PCA loading (a) and score (b) plots of data on bioactive compounds of selected samples. Samples in b are described in Table 1

clay at 95 °C) fell on the negative side of PC2. Those samples contain lower amounts of all tocopherols. We can conclude that increasing the bleaching temperature can decrease the amount of total and individual sterols, and that higher amounts of clay can lead to lower levels of tocopherols.

## Conclusion

Indicators of the efficiency of soybean oil bleaching were strongly affected by the amount of Pure-Flo® Supreme Pro-Active bleaching adsorbent used. Bleaching with 1.5 % clay gave significantly greater transparency and lower levels of red units and phosphorus. Bleaching with higher amounts of clay at higher temperatures resulted in significantly greater levels of secondary oxidation products, based on the AV values and absorbance at 270 nm. In contrast, the formation of primary oxidation products was not significantly influenced by the bleaching parameters analyzed.

Soybean oil bioactive compounds were influenced by the investigated bleaching conditions. Oil samples bleached with high amount of clay had a lower content of  $\alpha$ -tocopherol,  $\gamma$ -tocopherols and total tocopherols. Sterols decreased with higher bleaching temperature.

The most suitable parameters for bleaching soybean oil with Pure-Flo® Supreme Pro-Active bleaching clay were 1 % adsorbent at 95 °C for 20 min, although these parameters were not the best choice for tocopherol preservation. Bleaching is only part of oil refining process and certain negative effects can be corrected during deodorization, such as reducing secondary products of oxidation, or during stabilization of edible soybean oil, such as adding tocopherols in accordance with international and Croatian legislation (40,42). We believe that these optimized parameters for bleaching soybean oil using Pure-Flo® Supreme Pro-Active clay can be applied to industrial conditions. We expect substantial cost savings and the production of contaminant-free, edible soybean oil with high oxidative stability.

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