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ANTIOXIDATIVE POTENTIAL OF SUBSTANCES CONTAINED IN COLD PRESSED SOYBEAN OIL AND AFTER EACH PHASE OF REFINING PROCESS

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Abstract. This study is an investigation of determining the changes of scavenger capacity of $DPPH^{\bullet}$ free radicals through substances contained in soybean cold pressed oil and in oils taken after individual phases of refinement. The content of tocopherols and phenolic compounds was determined. Scavenging of $DPPH^{\bullet}$ was examined and the coefficient of antiradical activity (AE) was counted in order to objectively determine the potential. Cold pressed oil had the highest ability of scavenging of $DPPH^{\bullet}$. This oil also possessed the largest coefficient of antiradical efficiency ($AE = 14.51 \cdot 10^{-3}$). The largest quantity of tocopherols was observed in crude oil and phenolic compounds in cold pressed oil. It was affirmed that every next stage of refinement causes the decreasing of tocopherols and phenolic compounds content.

Key words: Soybeans oil, tocopherols, free radical scavenging: $DPPH^{\bullet}$, antiradical efficiency, refining

INTRODUCTION

For several years there has been a growing interest in antioxidants as substances with antiradical properties. Many scientists support the theory that free radicals cause oxidative damage to DNA, proteins, phospholipids and other macromolecules. Free radicals contribute to the development of many illnesses and the aging of organisms. [Cutler 1991, Ames et al. 1993, Gey 1993]. Oil plants contain not only a great amount of lipids but also antioxidants that prevent auto oxidative processes.

Oil plants contain lipids consisting mostly of triacyloglycerols with a great amount of mono and polyunsaturated fatty acids, which are fundamental nutritious components and concentrated source of energy. In human organism lipids play many essential physiological functions; they are components of cellular membranes and white matter in

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brain. Oil plants are a good source of essential fatty acids, phospholipids, phytosterols and antioxidants, such as: tocopherols, carotenoids and polyphenolic compounds.

Soybeans (*Glycine hispida*) have in human organism the first place in the world as a source for oil production [Rejman 1997]. This oil is a compound of mayonnaise, margarine and it is also used for direct consumption and as a heating medium. American Food and Drug Department qualifies soybeans as the plants rich in izoflavones, phenolic acids, sterols, phospholipids and other phenolic compounds possessing antioxidative properties. Izofalvonoids possessing estrogenical activity were also extracted [Wang et al. 2000]. Soybean oil contains a relatively large amount of unsaturated fatty acids such as linolenic (48-58%) and linoleic acid (4-10%) essential to a proper growth and development of human organism [Niewiadomski 1993]. Soybean oil is a rich source of tocopherols and sterols mainly 91% beta-sitosterol, stigmasterol and campasterol [Hayes et al. 1977, Rhee et al. 1981, Kudou et al. 1991].

In this paper it is attempted to compare the ability of free radicals scavenging in cold pressed and refined soybean oils. It was also examined which phase of refining decreases the amount of free radicals scavenging substances.

MATERIALS AND METHODS

For the analysis the cold pressed soybean oil and the refined oil were used. Oils after individual phases of refining: extraction (crude oil), degumming and neutralization (degummed and neutralized oil), bleaching (bleached oil) and deodorization (refined oil) were sampled directly from the production line.

Quantitative and qualitative determination of tocochromanols homologues was performed using HPLC (Waters 600 Asc. Milford) system consisting of the gradient pump Waters Model 600, column, fluorimetric detector and Waters Millenium 32 data acquisition system. Samples dissolved in n-hexane were injected to the LiChrosorb Si 60 column (200 mm, 5 μm Merck), and the mixture of n-hexane and 1.4 dioxane (97:3 v/v) was used as a mobile phase. The flow rate was 1.5 ml/min. The fluorimetric detector (Water 474) worked by excitation $\lambda = 290$ nm and emission $\lambda = 330$ nm. The concentrations were calculated from the calibration curves made for individual tocopherols.

Peroxide value was determined according to the norm PN-ISO 3960 October 1996.

Different oils were mixed with methanol (1:1 v/v). Phenolic compounds were extracted by vigorously stirring for one hour and further centrifuged at 3000 g for 5 minutes to better separate methanolic phase (hydrophilic). The process was repeated threetimes.

Content of total polyphenolic compounds was determined by spectrophotometric assays using Folin-Ciocalteau reagent. Chlorogenic acid was used as a pattern according to Swain and Hillis [1959]. Antioxidative activity in oils was determined by monitoring changes of absorption band $DPPH^{\bullet}$ at 517 nm. [Brand-Wiliams et al. 1995, Espin et al. 2000]. Spectrophotometric measurement was carried out for 15 minutes at 30 s intervals from the time that the reagent was added.

Data were analyzed by an analysis of variance ($p < 0.05$) and Tuckey pos-hoc tests were used.

RESULTS AND DISCUSSION

One of main determinant of lipids quality is the peroxide value. Its level shows the advancement of lipids autoxidation process. Table 1 shows the changes of the peroxide value, in particular in oil samples. Crude oil, similarly to cold pressed oil, was characterized by the high peroxide value and there was no statistical difference between them (Table 1). Degumming and neutralization processes conducted in 80°C have not caused the decreasing of the peroxide value. Only further processes: bleaching and deodorizing have reduced peroxide value to the level consistent with the norm for refined oil.

Table 1. Peroxide value, milieq. of active oxygen/kg of examined product
Tabela 1. Liczba nadtlenkowa wyrażona w milirównoważnikach aktywnego tlenu na kilogram badanych produktów

Product Produkt	Peroxide value, milieq. of active oxygen/kg* Liczba nadtlenkowa milirównoważnika aktywnego tlenu/kg*				
	cold pressed oil olej tłoczony na zimno	crude oil olej surowy	acid degummed and neutralized oil olej odszlamiany i odkwaszany	bleached oil olej odbarwiony	fully refined oil olej rafinowany
Soybean oil Olej sojowy	4.21±0.25b**	5.21±0.2bc	6.02±1.01c	0.38±0.08a	0.49±0.19a

*Mean ±SD.
**Values followed by the same letter are not significantly different at α = 0.05.
*Średnia ±odchylenie standardowe.
**Wartości oznaczone tymi samymi literami nie różnią się istotnie na poziomie α = 0,05.

Figure 1 shows the sum of tocopherols contained in oils: cold pressed oil, crude oil and after degumming and neutralization, bleaching, deodorization. Jung et al. [1989] monitored the changes of tocopherols content in refining process in soybean oil and affirmed that 14-20% of tocopherols is removed during neutralization process, 10-15% – during degumming process and about 12% – during deodorization. In the sampled oils the amount of tocopherols decreased 15.55% – in neutralized oil, 9.87% – in bleached oil, 26.31% – in refined oil. Hexan extraction of oils causes statistically significant increase of tocopherols content in crude oil as compared to cold pressed oil.

The content of individual tocopherols homologues in the sampled oils is shown in Table 2. Composition of individual tocopherols homologues in refined soybean oil according to literature is: 4-10% alpha-T, 1-3% beta-T, 60-66% gamma-T, 24-29% delta-T [Evans et al. 2002]. Obtained results are consistent with the subject literature: 9.6% alpha-T, 2.8% beta-T, 62.3% gamma-T, 25% delta-T (Table 2). In the sampled cold pressed oil tocotrienols were also identified: alpha-T3 (0.44 mg/100 g) and beta-T3 (0.55 mg/100 g), that existence was reported by Bramley et al. [2000]. The presence of tocotrienols in other sampled oils has not been affirmed. Pressing and extraction resulted in a better extraction of alpha-T of 20.6%, gamma-T 10% than in a cold pressing. The greatest losses of alpha-T occurred in the first phase of refining (about 25%), beta-T (31%) and (25%) in the next 2 phases.

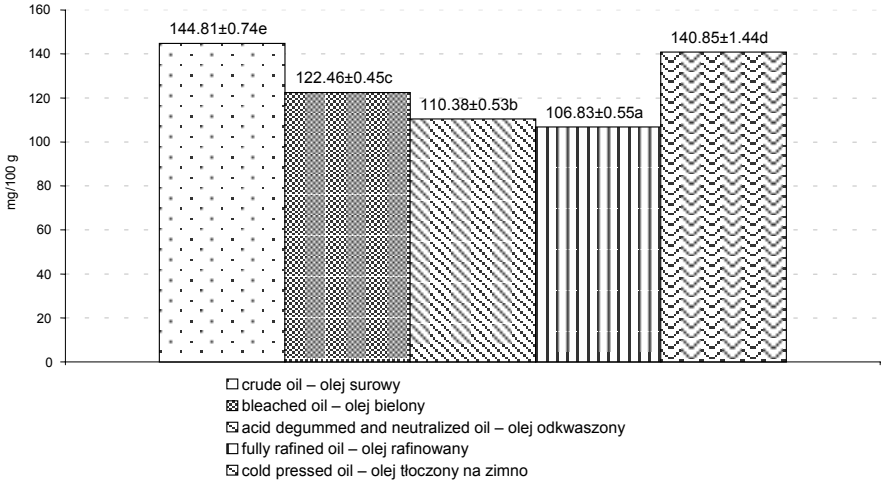

Fig. 1. Total tocopherols composition in oil samples. Mean ±SD, values followed by the same letter are not significantly different at α = 0.05
Rys. 1. Ogólna zawartość tokoferoli w badanych produktach. Średnia ±odchylenie standardowe, wartości oznaczone tymi samymi literami nie różnią się istotnie na poziomie α = 0,05

Table 2. Difference in the tocopherols homologues content in tested oils
Tabela 2. Zawartość homologów tokoferoli w badanych olejach

Product Produkt		Tocopherols content, mg/100 g product* Zawartość tokoferoli, mg/100 g produktu*				
		cold pressed oil olej tłoczony na zimno	crude oil olej surowy	acid degummed and neutralized oil olej odszlamiany i odkwaszany	bleached oil olej odbarwiony	fully refined oil olej rafinowany
Soybean oil Olej sojowy	α	12.03±0.24b**	16.36±0.36d	10.41±0.46a	13.86±0.25c	10.26±0.39a
	β	5.98±0.17e	6.96±0.31d	4.08±0.27b	5.93±0.22c	3.05±0.20a
	γ	72.40±1.42d	80.58±0.49e	68.31±0.42b	70.34±0.38c	66.53±0.29a
	δ	45.46±0.41d	40.91±0.57c	27.58±0.41a	33.78±0.51b	26.98±0.17a

*Mean ±SD.
**Values followed by the same letter are not significantly different at α = 0.05.
*Średnia ±odchylenie standardowe.
**Wartości oznaczone tymi samymi literami nie różnią się istotnie na poziomie α = 0,05.

Phenolic compounds occur mainly in hulls, leaves, flowers and stems [Herrmann 1993, White and Xing 1997]. Total phenols content is shown in Figure 2. In this paper we determined poliphenolic compounds content that passed to oil during the pressing and extraction processes. Phenolic compounds are sensitive to changes of temperature, radiation and oxygen. The greatest amount of poliphenolic compounds was extracted from

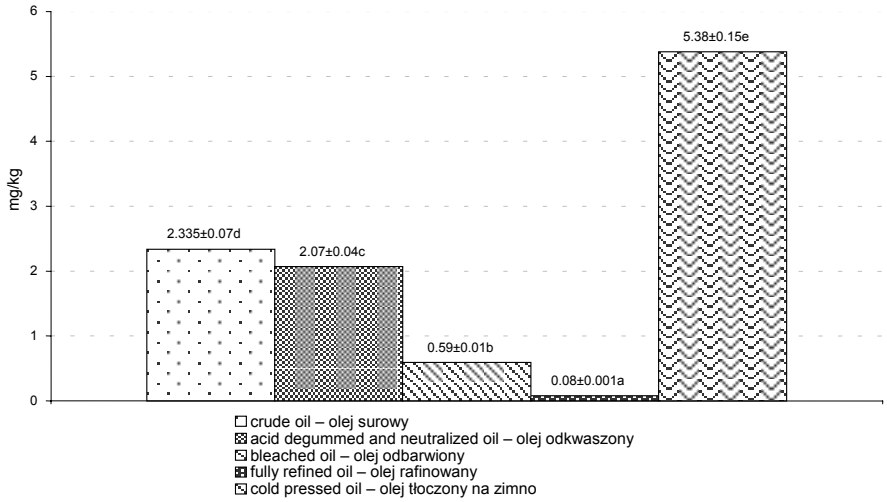

Fig. 2. The content of phenol compounds extracted from oil samples. Mean ±SD, values followed by the same letter are not significantly different at α = 0.05
Rys. 2. Zawartość związków fenolowych wyekstrahowanych z badanych olejów. Średnia ±odchylenie standardowe, wartości oznaczone tymi samymi literami nie różnią się istotnie na poziomie α = 0,05

Table 3. Scavenging of DPPH• radicals in oil samples, %
Tabela 3. Zdolność wygaszania rodników DPPH• przez badane oleje, %

Product Produkt	% remains of the DPPH• radical* % pozostawionych rodników DPPH•*				
	cold pressed oil olej tłoczony na zimno	crude oil olej surowy	acid degummed and neutralized oil olej odszlamiany i odkwaszany	bleached oil olej odbarwiony	fully refined oil olej rafinowany
Soybean oil Olej sojowy	31.95±1.32a**	49.69±3.37d	41.56±1.54 b	44.03±2.65bc	46.1±2.12 bc

*Mean ±SD.
**Values followed by the same letter are not significantly different at α = 0.05.
*Średnia ±odchylenie standardowe.
**Wartości oznaczone tymi samymi literami nie różnią się istotnie na poziomie α = 0,05.

cold pressed oil. The greatest loss of poliphenolic compounds took place during degumming and neutralization. According to Dąbrowski and Sosulski [1984] phenolic acid content in soybean is about 69 mg/100 g and 93% of it exists in estrification form (p-hydroxbenzoic, p-cumaric, caffeic trans, ferulic trans).

Free radical DPPH• scavenging ability in oil samples is shown in Table 3. The best ability for scavenging free radicals is shown in cold pressed oil. This ability is correlated with the high value of tocopherols and phenolic content (Table 3).

Statistical analysis has shown that there is a correlation between individual tocopherols homologues content, total tocopherols content and the ability for scavenging free radicals. There is no correlation between the ability of scavenging DPPH$^{\bullet}$ and phenolic content.

Figure 3 illustrates the kinetics of free radicals DPPH$^{\bullet}$ scavenging reaction. There is no statistically significant difference in free radicals DPPH$^{\bullet}$ scavenging between degummed and neutralized oil, refined oil and bleached oil. According to Evans et al. [2002] antioxidative potential of tocopherol homologues is in following order: alpha-T > gamma-T > delta-T. Despite of its lower alpha-T content the cold pressed oil reduced free radicals better than the crude oil (Fig. 3). This phenomenon may be connected with greater phenolic compounds content and shortage of substances which may act as generators of radicals and penetrate crude oil during pressing and extraction processes.

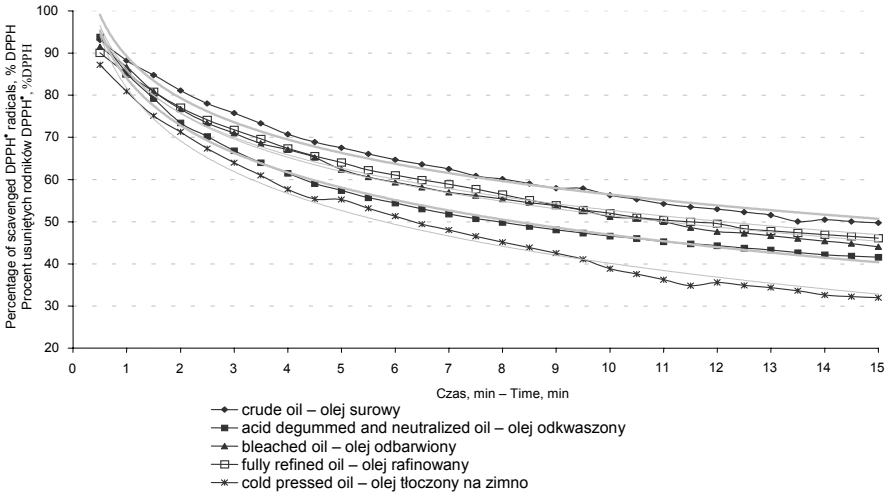

Fig. 3. Kinetic behavior of DPPH$^{\bullet}$ radicals scavenging in oil samples during the time
Rys. 3. Kinetyka wygaszania rodników DPPH$^{\bullet}$ przez badane oleje w czasie

For objective comparison of antiradical activity in sampled oils AE parameter was calculated. This parameter takes into consideration both the concentration of antioxidant and the time needed to reach a steady state of the concentration, corresponding to EC_{50} (Table 4).

$$AE = \frac{1}{T_{EC50} \cdot EC_{50}}$$

EC_{50} – the amount of antioxidant necessary to decrease the initial DPPH$^{\bullet}$ concentration by 50%,

T_{CE50} – the time needed to reach a steady state at the concentration DPPH$^{\bullet}$ corresponding to EC_{50}.

Table 4. Antiradical efficiency of oil samples
Tabela 4. Aktywność przeciwrodnikowa badanych olejów

Product Produkt		Cold pressed oil Olej tłoczony na zimno	Crude oil Olej surowy	Acid degummed and neutralized oil Olej odszlamiany i odkwaszany	Bleached oil Olej odbarwiony	Fully refined oil Olej rafinowany
Soybean oil Olej sojowy	EC_{50} (g/kg $DPPH^{\bullet}$)	15.25	33.04	45.05	39.72	39.19
	T_{EC50} (min)	4.52	18.75	7.21	10.48	7.27
	AE ($\times 10^3$)	14.51	1.61	3.08	2.40	3.91

According to Sanchez-Moreno et al. [1989] examined oil can be classified to the group of medium radical activity except for cold pressed oil in which radical activity is very high. Very high value of AE may be caused by large amount of phenolic compounds content (Fig. 2), tocopherols content (Fig. 1) and by the presence of tocotrienols.

CONCLUSIONS

1. The best free radicals $DPPH^{\bullet}$ scavenging ability was found in the substances contained in cold pressed oil. Statistical analysis has shown that other oil samples form a homogeneous group.

2. In all analyzed oil samples AE varied from 1×10^{-3} to 5×10^{-3} except for the cold pressed oil where $AE \geq 10\cdot10^{-3}$.

3. The greatest amount of tocopherols was found in crude oil. Furthermore it has been shown that each next phase of refining causes a decrease of total tocopherols content. It has been affirmed that the greatest losses of alpha-T occurred in degumming and neutralization phase of refining (about 25%), beta-T (31%) in the losses of bleaching phase and (25%) in deodorizing phase.

4. The greatest amount of phenolic compounds was determined in the cold pressed oil. Crude oil had the lowest amount of phenolic compounds and every next phase of refining caused further decreasing of its content.

5. It has been shown that there is a correlation between the ability of scavenging free radicals $DPPH^{\bullet}$ and the amount of tocopheros. There is no correlation between the content of phenolic compounds and the ability of scavenging free radicals $DPPH^{\bullet}$.

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POTENCJAŁ ANTYOKSYDACYJNY SUBSTANCJI ZAWARTYCH W OLEJU SOJOWYM TŁOCZONYM NA ZIMNO ORAZ PO JEDNOSTKOWYCH PROCESACH RAFINACJI

Streszczenie: Podjęto badania nad określeniem zmian zdolności wygaszania wolnych rodników DPPH przez substancje zawarte w oleju sojowym tłoczonym na zimno oraz w olejach pobranych po poszczególnych etapach rafinacji. Oznaczono zawartość tokoferoli oraz ogólną zawartość związków fenolowych. Zbadano zdolność wygaszania wolnych rodników $DPPH^{\bullet}$ oraz dla obiektywnego wyznaczenia potencjału przeciwutleniającego obliczono współczynnik aktywności antyrodnikowej (AE). Najwyższą zdolność wygaszania $DPPH^{\bullet}$ miały substancje zawarte w oleju tłoczonym na zimno. Olej ten miał także

największy współczynnik aktywności antyrodnikowej (AE = $14{,}51 \cdot 10^{-3}$). Największą ilość tokoferoli oznaczono w oleju surowym, a związków fenolowych w oleju tłoczonym na zimno. Stwierdzono, że każdy kolejny etap rafinacji powoduje obniżenie zawartości tokoferoli i związków fenolowych.

Słowa kluczowe: olej sojowy, tokoferole, wygaszanie rodników DPPH, aktywność antyrodnikowa, rafinacja

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