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# Antioxidant activity of phenolic compounds of selected cold-pressed and refined plant oils

## Aktywność antyoksydacyjna związków fenolowych wybranych olejów roślinnych tłoczonych na zimno i rafinowanych

**Key words**: phenolic compounds, free phenolic acids, SPE, HPLC, plant oils, DPPH

The increase of popularity of cold-pressed oils may indirectly influence the consumers' health by providing more native antioxidants such as polyphenolic compounds. In this paper the investigation of the content and properties of antioxidant polar substances extracted from non-polar oils is reported. Rapeseed oil, sunflower oil and soybean oil were investigated. The influence of the refining process on natural phenolic antioxidants content was also examined.

The antiradical activitiy of phenolic extracts from oils were determined using the free radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$). The percentage of radicals ($DPPH^{\bullet}$) scavenged by compounds contained in the extracts was determined. Concentration needed to scavenge 50% of radicals ($EC_{50}$) was also determined. Antiradical power (ARP) was calculated. Rapeseed oil was characterized by the highest quantity of phenolic acids, especially the sinapic acid (236.1 μg/100 g). This extract had the best antioxidant properties. In soybean and sunflower oils phenolic acids were not found after the refining. Correlation between the phenolic acids content and scavenging of free radicals ($DPPH^{\bullet}$) ($r = 0.94$) was found.

The results obtained confirmed significant influence of the refining process on native polyphenolic compounds content in plant oils.

**Słowa kluczowe**: związki fenolowe, wolne kwasy fenolowe, SPE, HPLC, oleje roślinne, DPPH

Wzrost popularności spożycia olejów roślinnych tłoczonych na zimno może pośrednio wpłynąć na zdrowie konsumentów dostarczając większą ilość natywnych antyoksydantów, jakimi są związki fenolowe. W pracy podjęto badania nad zawartością oraz właściwościami przeciwutleniającymi substancji polarnych wydobytych z niepolarnej matrycy, jakimi są oleje. Badaniu poddano olej rzepakowy, sojowy i słonecznikowy. Zbadano także wpływ procesu rafinacji na zawartość naturalnych przeciwutleniaczy fenolowych. Właściwości antyrodnikowe badano w stosunku do rodników 1,1-difenylo-2-pikrylohydrazylowych ($DPPH^{\bullet}$). Oznaczono procent zmiecionych rodników $DPPH^{\bullet}$ przez związki zawarte w ekstraktach, wyznaczono stężenie zmiatające 50% rodników ($EC_{50}$), obliczono także współczynnik antyrodnikowy (ARP — antiradical power). Otrzymane rezultaty potwierdzają znaczący wpływ procesu rafinacji olejów roślinnych na zawartość natywnych związków fenolowych. Olej rzepakowy charakteryzował się największą ilością kwasów fenolowych, szczególnie kwasu sinapowego (236,1 μg/100 g). Jednocześnie ekstrakt ten posiadał najlepsze właściwości przeciwutleniające. Nie stwierdzono obecności kwasów fenolowych w olejach sojowym i słonecznikowym poddanych procesowi rafinacji. Oleje tłoczone na zimno charakteryzują się wyższą

zawartością związków fenolowych ogółem oraz zawartością kwasów fenolowych w porównaniu do ich rafinowanych odpowiedników. Olej rzepakowy zawiera najwięcej związków o charakterze przeciwutleniaczy, kilkakrotnie więcej kwasów fenolowych w porównaniu do oleju sojowego i słonecznikowego tłoczonego na zimno. Stwierdzono istnienie korelacji pomiędzy zawartością kwasów fenolowych w badanych ekstraktach, a zdolnością wygaszania wolnych rodników $DPPH^{\bullet}$ ($r = 0,94$). Badania potwierdziły znaczący, zarazem niekorzystny wpływ procesu rafinacji na zawartość natywnych substancji o charakterze przeciwutleniającym w badanych olejach roślinnych.

## Introduction

The increase of consumption of new and improved nutrient products, e.g. cold-pressed oils, may exert influence on improvement of our health and prevent us from civilization diseases. Free radicals may cause reversible or irreversible damage of biological molecules such as DNA, proteins or lipids (Goldberg 2003). These damages may cause cancer, heart disease, arthritis and accelerate aging of organisms (Cadenas and Davies 2000; Wang and Quinn 1999). Over the last few years the increase of interest in cold-pressed plant oils have been observed because these oils have better nutritive properties than those after refining process. Cold pressing is simple, ecological and does not require much energy. The disadvantage of this process is low productivity (high content of oil in pomace) and difficulties in obtaining the product with constant quality (Rotkiewicz et al. 1999). Such factors as geographical location, species and kind of processing may influence the final chemical composition of plant oils (Beardsell et al. 2002). Plant oils contain small amount of such compounds as free fatty acids, phenolic compounds, tocopherols, sterols, stanols, phospholipids, waxes, squalene and other hydrocarbons (Lecker and Rodriguez-Estrada 2000). In many products of plant origin substances having antioxidative properties were identified. Such substances are also present in oilseeds (Kalt et al. 1999; Yu et al. 2002a; Yu et al. 2002b; Yu et al. 2005). Natural polyphenols in oilseed crops stabilize oils by preventing lipids oxidation (Ruth et al. 2001; Quiles et al. 2002; Koski et al. 2003). The factors influencing antioxidation activity of phenolic compounds are position and quantity of hydroxide groups, polarity, solubility and stability of phenolic compounds during technological processes (Decker 1998). The content of non triacylglycerol fraction in cold-pressed oils is higher than in refined oils. These oils contain ~99% triacylglycerol and cold-pressed oils 94–98% (McGinley 1991). Cold-pressed oils contain more polar phenols whose concentration varies from 18 to 99 ppm caffeic acid equivalents (Koski et al. 2003). Olive oil is believed to be the most durable and stable because of high quantity of phenols (Maniak and Targoński 1996). The combination of phenolic compounds and α-tocopherol more effectively prevents lipids oxidation than combination of phenolic compounds with vitamin C (Paiva-Martins et al. 2003). Taking into consideration the fact that plant oils are mostly used in preparing food, health benefits for whole population could be significant,

especially considering prevention of heart diseases. Cold-pressed oils contain compounds with pro- and antioxidative properties of not univocally explained effect on the oxidation process because of multi-directional mechanism of free radical reaction. In the study, we compared phenolic compounds content in cold-pressed oils and in refined oils, and we also determined antioxidative potential of extracted phenolic compounds.

## Materials and methods

### Materials

Materials used in the study were cold-pressed soybean, sunflower and rapeseed oils and their refined equivalents. Examined oils were obtained from the local factory.

**Extraction of phenolic compounds** (Gomez-Alonso et al. 2003)

For extraction of phenolic acids fraction we used Chromabond® System (Macherey – Nagle, Germany) with SPE Supelco column filled with diol (500 mg). The process of extraction consisted of four stages.

I° Column conditioning (5 ml methanol and 5 ml n-hexane);
II° placing samples (2.5 g oil in 5 ml n-hexane + 5 ml n-hexane);
III° washing column (5 ml n-hexane/ethyl acetate 90 : 10v/v);
IV° elution of phenolic acids with methanol to 5 ml.

**Determination of total phenolic content** (Lampart-Szczapa et al. 2003)

The content of phenolic compounds in methanol extracts was determined by the Folin-Ciocalteu method. An aliquot (0.2 ml) of the methanolic extract was put in a flask (10 ml). Diluted Folin-Ciocalteu reagent (0.5 ml) was added. After 3 min. saturated sodium carbonate (1 ml) was added. And the flask was filled up to 10 ml with water. Absorbance was measured at $\lambda_{max}$ 725 nm exactly after 1 hour of preparing the sample. For determination, a standard curve was prepared and on that basis total phenolic compounds were measured as the caffeic acid equivalent

**Determination of extracts composition**

Separation and identification of free phenolic acids were carried out by high performance liquid chromatography (HPLC – Waters Milford, MA, USA). For separation a NovaPak®$C_{18}$ (3.9 × 150 mm; 5 μm) column was used. Solvent [A] was methanol; solvent [B] 2.5% acetic acid in water. The flow rate was 1 ml/min. The gradient profile was: 10% [A] (0–10 min.); 10–20% [A] (10–22 min.);

20–70% [A] (22–45 min.). The chromatograms were recorded at 250 and 320 nm (UV-VIS Waters). Identification and amount of phenolic acid was determined using external and internal standard of the individual phenolic compounds.

**Determination of antioxidative potential according** to Espin et al. (2000)

The method consisted in spectrophotometric measuring of changing intensity of the solution color depending on the amount of free radicals $DPPH^{\bullet}$. The reaction was started by adding ethyl acetate solution of $6.09 \times 10^{-5}$ mol/l $DPPH^{\bullet}$ (4.9 ml) to 100 μl of oil samples. The absorbance was measured at 517 nm at 0 min., 0.5 min., and every 0.5 min. until the reaction reached a steady state. This plateau was reached within 15 min.

In order to compare antiradical activity of an oil sample ARP was determined (Suja et al. 2005):

$$ARP = \frac{1}{(EC_{50})}$$

$EC_{50}$ — concentration needed to decrease initial content of $DPPH^{\bullet}$ by half.

Constant velocity of scavenging the free radicals $DPPH^{\bullet}$ ($k_{DPPH}$)

$$r = -\frac{\partial[DPPH]}{\partial t} = k \times [DPPH]$$

$$k = \frac{r}{[DPPH]}$$

**Statistical analysis**

The results were statistically analyzed using analysis of variance and Tukey's post-hoc tests for determining homogenous groups. All hypotheses were verified at the significance level $\alpha = 0.05$. Statistical analysis was carried out using the Statictica 6.0 (StatSoft) software.

## Results and discussion

It was found (Table 1) that cold-pressed oils contained higher total phenolic (TP) content than the corresponding refined oils. The highest TP content was found in the soybean oil (1.44 mg/100 g). Sunflower oil contained the least total phenolic content (1.19 mg/100 g). Refining brought about the biggest decrease in total phenolics of rapeseed oil (decrease 0.5 mg/100 g ) while in sunflower oil decrease was the least (0.04 mg/100 g). The decrease in total phenolic content was statistically significant in rapeseed and soybean oils. Kania et al. (2000) reported lower amounts of phenolic compounds — 5.38 mg/kg (cold-pressed oil) and

0.08 mg/kg (refined oil) in soybean oil than the amounts reported here. The differences may be caused by method of extraction of phenolic compounds and by phenolic standard used for quantification. Koski et al. (2003) reported higher level of TP content in the refined rapeseed oil 16 ppm caffeic acid equivalent than found here. On the other hand, Koski et al. (2002) also found that the cold-pressed olive and rapeseed oils contained the total phenolic content at 4 ppm.

Table 1

Total phenolic compounds content in plant oils extracts
*Zawartość związków fenolowych ogółem w ekstraktach z badanych olejów roślinnych*

| Oil<br>*Olej* | Total phenolic compounds content<br>*Zawartość związków fenolowych ogółem*<br>[mg/100 g]* | |
|---|---|---|
| | cold-pressed — *tłoczony na zimno* | refined — *rafinowany* |
| Rapeseed — *Rzepakowy* | 1.28 ± 0.02 (d)** | 0.78 ± 0.02 (a) |
| Soybean — *Sojowy* | 1.44 ± 0.01 (e) | 1.09 ± 0.02 (b) |
| Sunflower — *Słonecznikowy* | 1.19 ± 0.05 (c.d) | 1.15 ± 0.03 (c) |

* equivalent caffeic acid — *ekwiwalent kwasu kawowego*
** values followed by different letters are statistically significant at $\alpha = 0.05$
*wartości oznaczone różnymi literami różnią się statystycznie istotnie przy $\alpha = 0,05$*

The content of phenolic acids in oils is shown in Table 2. Application of SPE columns with diol enable extraction of polar substances from non-polar matrix oils. Phenolic acids were not found in refined soybean and sunflower oils and rapeseed oil contained only sinapic (5.60 μg/100 g) and p-coumaric (0.28 μg/100 g) acids. In cold-pressed oils the content of these compounds varied from 6.50 μg/100 g for soybean oil to 256.8 μg/100 g for the rapeseed oil. Soybean oil contained the highest level of p-coumaric acid (1.58 μg/100 g), while sunflower oil contained vanillic (6.80 μg/100 g) and caffeic (4.84 μg/100 g) acids. Rapeseed oil contained mainly sinapic acid — 236.1 μg/100 g. Similar results were obtained by Koski et al. (2003) — 23 ppm. These authors did not find sinapic acid in the refined rapeseed oil. High phenolic acids level in rapeseed oil is caused by the fact that seeds of rape contain ten times more phenolic acids than other oilseed crops. In rapeseed phenolic acids composition consists mostly of the sinapic acid and its low-molecule derivative (Kozłowska et al. 1983; Zadernowski and Kozłowska 1983; Shahidi and Naczk 1992; Cai and Arntfield 2001; Siger et al. 2004). Percentage of free radicals $DPPH^{\bullet}$ scavenged by antioxidants contained in oils extracts is shown in Figure 1. All oil extracts scavenged free radicals $DPPH^{\bullet}$. Phenolic fraction of cold-pressed oils have better antioxidant properties than their corresponding fractions of refined oils. The best antioxidant properties were displayed by cold-pressed rapeseed oil (42.4%), the worst — by the refined soybean oil (7.8%),

Table 2

Phenolic acids content in plant oils extracts — *Zawartość kwasów fenolowych w ekstraktach z badanych olejów roślinnych*

| Oil<br>*Olej* | Phenolic acids content — *Zawartość kwasów fenolowych*<br>[μg/100 g] | | | | | |
|---|---|---|---|---|---|---|
| | p-hydroxybenzoic<br>*p-hydroksybenzoesowy* | vanillic<br>*wanilinowy* | caffeic<br>*kawowy* | p-coumaric<br>*p-kumarowy* | ferulic<br>*ferulowy* | sinapic<br>*sinapowy* |
| cold-pressed — *tłoczony na zimno* | | | | | | |
| Rapeseed — *Rzepakowy* | 1,62 ± 0,02 (b)* | – | 0,30 ± 0,01 (a) | 13,15 ± 0,05 (d) | 5,61 ± 0,08 (b) | 236,1 ± 0,32 (d) |
| Soybean — *Sojowy* | 0,85 ± 0,05 (a) | 1,14 ± 0,05 (a) | 0,80 ± 0,10 (b) | 1,58 ± 0,08 (b) | 1,26 ± 0,05 (a) | 0,91 ± 0,06 (a) |
| Sunflower — *Słonecznikowy* | 1,56 ± 0,08 (b) | 6,80 ± 0,10 (b) | 4,84 ± 0,06 (c) | 1,85 ± 0,05 (c) | 1,28 ± 0,08 (a) | 1,45 ± 0,05 (b) |
| refined — *rafinowany* | | | | | | |
| Rapeseed — *Rzepakowy* | – | – | – | 0,28 ± 0,03 (a) | – | 5,60 ± 0,06 (c) |
| Soybean — *Sojowy* | – | – | – | – | – | – |
| Sunflower — *Słonecznikowy* | – | – | – | – | – | – |

* values followed by different letters are statistically significant at α = 0.05
*wartości oznaczone różnymi literami różnią się statystycznie istotnie przy α = 0,05*

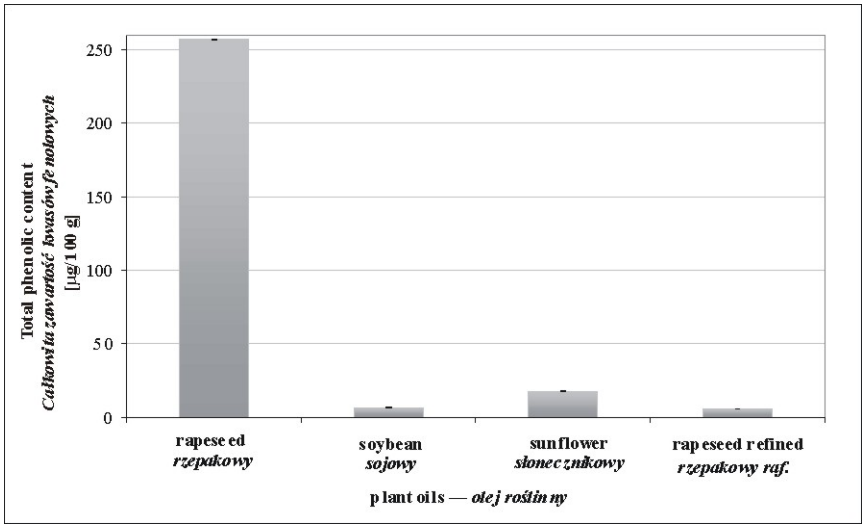


Fig. 1. Total free phenolic acids in plant oils extracts — *Sumaryczna zawartość kwasów fenolowych w ekstraktach z badanych olejów roślinnych*

cold-pressed soybean oil (10.8%) and refined sunflower oil (9.5%). In all these oils scavenging radicals DPPH$^{\bullet}$ were not significantly different ($p > 0{,}05$). The refined rapeseed oil was more efficient in scavenging radicals DPPH$^{\bullet}$ (19.8%) than cold-pressed sunflower oil (17.8%) but these values did not differ statistically $p > 0.05$.

Antioxidant components contained in rapeseed oil are characterized by the highest constant of scavenging free radicals (Table 3) which is $34.30\times10^{-3}$ for cold-pressed oil and $11.20\times10^{-3}$ for refined oil. Other extracts scavenge free radicals DPPH$^{\bullet}$ slower but still we see that cold-pressed oils have better properties than the refined one. ARP (Table 4) confirms higher antioxidants content in cold-pressed oils extracts than in refined oils extracts. Substances extracted from rapeseed oil were characterized by the best antioxidant properties with ARP several times greater than in other oils ($84.75\times10^{-2}$). Soybean oil antiradical power was four times lower and for sunflower oil it was two times lower than that found for rapeseed oil. The refining process influences antioxidative properties of oils extracts by decreasing the natural phenolic compounds content. ARP coefficients for the refined oils were two times lower. De Leonardis et al. (2003) examined antioxidant properties and oxidant stability of cold-pressed sunflower oil. Authors claim that phenols extracted from sunflower seeds more effectively protect sunflower oil against autoxidation process. Vuerola et al. (2004) confirm strong antioxidant properties of phenolic compounds extracted from rapeseed which scavenged over 60% radicals, inhibited formation of hexanal (over 90%) and hydroperoxides (over 80%). Matthaus (2002) confirms antiradical properties of rapeseed and sunflower extracts that inhibited conjugated dienes linoleic acid

Table 3

Constants of scavenging free radicals DPPH$^{\bullet}$ of phenolic compounds extracted from plant oils
*Stałe szybkości wygaszania wolnych rodników DPPH$^{\bullet}$ przez związki fenolowe ekstrahowane z badanych olejów roślinnych*

| Oil<br>*Olej* | Constant $k_{DPPH}$ — *Stała* $k_{DPPH}$<br>[% DPPH/min.$^{-1}$] | |
|---|---|---|
| | cold-pressed — *tłoczony na zimno* | refined — *rafinowany* |
| Rapeseed — *Rzepakowy* | $34.30 \times 10^{-3}$ | $11.20 \times 10^{-3}$ |
| Soybean — *Sojowy* | $4.90 \times 10^{-3}$ | $3.00 \times 10^{-3}$ |
| Sunflower — *Słonecznikowy* | $7.80 \times 10^{-3}$ | $2.90 \times 10^{-3}$ |

Table 4

Antioxidant activity of phenolic compounds extracted from plant oils — *Właściwości przeciwutleniające ekstraktów związków fenolowych otrzymanych z badanych olejów roślinnych*

| Oil — *Olej* | $EC_{50}$ [ml] | ARP<br>antiradical power — *siła antyrodnikowa* |
|---|---|---|
| cold-pressed — *tłoczony na zimno* | | |
| Rapeseed — *Rzepakowy* | 1.18 | $84.75 \times 10^{-2}$ |
| Soybean — *Sojowy* | 4.61 | $21.69 \times 10^{-2}$ |
| Sunflower — *Słonecznikowy* | 2.83 | $35.34 \times 10^{-2}$ |
| refined — *rafinowany* | | |
| Rapeseed — *Rzepakowy* | 2.50 | $40.00 \times 10^{-2}$ |
| Soybean — *Sojowy* | 6.46 | $15.48 \times 10^{-2}$ |
| Sunflower — *Słonecznikowy* | 5.87 | $17.04 \times 10^{-2}$ |

formation. These results may be influenced by other substances contained in methanolic extracts which were not determined e.g. vinylsyringol (canolol) – decarboxylation product of sinapic acid which has strong ARP (Koski et al. 2003), and antibacterial properties, and protects DNA, fats and proteins from oxidation (Kuwahara and al. 2004). Kania et al. (2004) examined antiradical activity of soybean oil after each stage of refining concluding that this process leads to decreasing antioxidant compounds. Antiradical activity of cold-pressed soybean oil (both polar and non-polar fraction) decreased after refining from $14.51 \times 10^{-3}$ to $3.91 \times 10^{-3}$.

Espin et al. (2000) examined antiradical properties of plant oils such as soybean, sunflower and corn oil, in both lipid fraction and methanolic fractions, and reported that free radical scavenging capacity on DPPH$^{\bullet}$ in polar fraction was

not significant. Correlation between total phenolic acids content and scavenging free radicals DPPH$^{\bullet}$ (r = 0.94) was found. However, such correlation was not found between total phenolic compounds content and scavenging of free radicals DPPH$^{\bullet}$. Espin et al. (2000) give the following order of effectiveness: sinapic acid > oleuropein > hydroxtyrosol > sezamol > caffeic acid > protocatechuic acid > siringic acid. This may confirm better properties of rapeseed oil extract (the highest content of sinapic acid) than sunflower oil extract (caffeic acid – 4.84 μg/100 g; sinapic acid – 1.45 μg/100 g) and the worst properties of soybean oil extract.

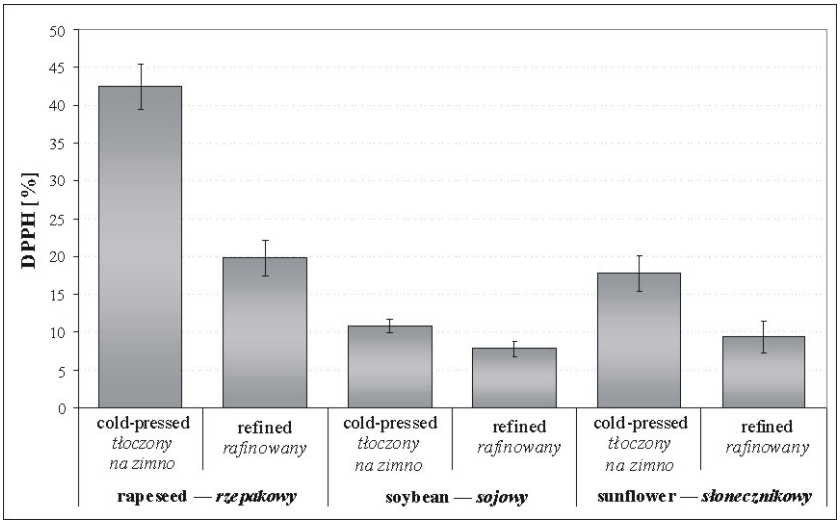


Fig. 2. Antiradical activity in plant oils extracts — *Właściwości antyrodnikowe ekstraktów z badanych olejów roślinnych*

## Conclusions

- Cold-pressed oils are characterized by higher total phenolic compounds content in comparison to their refined equivalents. In the refined soybean and sunflower oils phenolic acids were not found.
- Rapeseed oil contains the highest level of antioxidant compounds. It contains several phenolic acids more than cold-pressed soybean and sunflower oils.
- Correlation between total phenolic acids content and scavenging free radicals DPPH$^{\bullet}$ (r = 0.94) was found.
- Studies confirmed various effects of the refining process on the native antioxidant substances content in sampled plant oils.

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