Solvent influence on antioxidant activity assay of selected cold-pressed plant oils

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**Abstract:** The objective of the study was to assess the antioxidant properties of extracts obtained from cold-pressed oils using different solvents. Commercially available: rapeseed, flax, pumpkin, walnut, evening-primrose and black cumin cold-pressed oils were analyzed. Samples were extracted using five solvents: acetone, methanol, acetone:water (50:50 V/V), methanol:water (50:50 V/V), methanol:water (70:30 V/V). The range of research performed in order to achieve the objective of the study covered what follows: the determination of antiradical properties (a test with the DPPH radical) and the determination of the Ferric Reducing Antioxidant Power (the FRAP method). Based on the analyses performed, it has been demonstrated that extracts of cold-pressed oils differ in antiradical activity and Ferric Reducing Antioxidant Power, which results from the use of different solvents for extraction. For all cold-pressed oils in the study, the highest antioxidant properties were shown by methanolic extracts. It has been confirmed that the antioxidant activity of oil extracts declines along with an increase in solvent polarity.

1. Introduction

Antioxidant activity is a parameter which recently has been often used to characterize the health-promoting properties of various products. It is connected with a common opinion on antioxidants’ crucial role in the prevention of oxidative stress-related diseases and on the reduction of total mortality, associated with diets rich in plant foods, particularly fruits and vegetables (Bazzano et al., 2002). Consequently, recent years have seen the development of many methods for the determination of the antioxidant capacity of various products. The most common of these methods are: the determination of the reducing power by using the FRAP (Ferric Reducing Antioxidant Power) assay, spectrophotometric methods using the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) or ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)), and the ORAC fluorometric method (Oxygen Radical Absorbance Capacity).

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(Huang et al., 2005; Rufino et al., 2010). It is advised to use at least two methods to obtain a reliable picture of the antioxidant activity of the sample, taking into account the strengths and weaknesses of each method and the possibility of their applications (Pérez-Jiménez et al., 2008). Therefore, there is a general need to develop a standardized methodology to measure total antioxidant capacity.

In the literature several ways of preparing the samples, choosing the end-point of assay, expressing the results and diverse antioxidants extraction conditions, such as solvent or temperature selection, even for the same method, are observed. For this reason, it is difficult to compare the results of studies carried out in different laboratories.

Antioxidant activity is measured in extracts obtained using diverse solvents including methanol, acetone, ethanol, chloroform or their mixtures with water in various proportions. The type of solvent and its polarity can affect the mechanisms of hydrogen atom transfer (HAT) and single electron transfer (SET), which are crucial for measuring antioxidant properties (Perez-Jimenez and Saura-Calixto, 2006). Hence, the critical point of research seems to be the choice of a suitable solvent.

Cold pressing is a technology that does not require high energy input, as it is based on the mechanical pressing of the product (Rotkiewicz et al., n.d.). Thanks to the simplicity of this method, the final product may contain many valuable health-promoting ingredients, including phenolic compounds, tocopherols, squalene, sterols and carotenoids – compared with refined oils which lose these bioactive compounds during various stages of production (Kania et al., 2004). Due to cold-pressed plant oils’ higher nutritive properties and consumers’ desire for natural and safe food products, these oils are recently gaining recognition. An attempt to measure their antioxidant capacity has been lately made (Małecka, 2002; Tuberoso et al., 2007; Siger et al., 2008).

Therefore, the objective of the present work is (1) to determine the antioxidant activity of selected cold-pressed plant oils and (2) to measure the influence of different extraction solvents applied.

2. Materials and methods

Cold-pressed, non-refined oils extracted from rape (Rp), flax (Fx), walnut (Wn), pumpkin seed (Ps), evening primrose (Ep) and black cumin (Bc) were purchased directly from a producer in the original packaging. All oils were within expiry date. Samples were freshly opened before analysis. The analyzed oil samples exhibited an overall good quality measured by standardized methods: peroxide value and acid value (data not shown).

Oil samples were extracted using five solvents: acetone (A), methanol (M), and mixtures of 50:50 V/V acetone:water (AW), 50:50 V/V methanol:water (MW) and 70:30 V/V methanol:water (MW7). The scope of the research undertaken to carry out the work included the determination of antiradical properties (a test with a DPPH radical) and the measurement of the reducing power (the FRAP method).

The samples were extracted in accordance with the methodology proposed by Szydlowska-Czerniak et al. (2006). Two grams of oil were shaken with 10 cm³ of each solvent for one hour in the dark. Afterwards, the flask was transferred to a freezer and freeze-dried for another hour. The extract layer was separated from the oil and
transferred quantitatively to glass flasks. The samples were stored in a freezer until analysis.

The Ferric Reducing Antioxidant Power of extracts was evaluated according to the method of Benzie and Strain (Benzie and Strain, 1996) with modifications by Szydłowska-Czerniak et al. (2006). This method is based on an assessment of the ability of the test substance with antioxidant properties to reduce the Fe$^{3+}$-TPTZ complex (the ferric ion and 2,4,6-tripyridyl-s-triazine complex) to the corresponding ferrous ion complex, i.e. Fe$^{2+}$-TPTZ. The reduction is accompanied by a decrease in absorbance of the reaction system, which is measured spectrophotometrically at a wavelength of 593 nm.

For the determination of the reducing power, a reaction mixture was prepared containing 10 mmol/dm$^3$ TPTZ solution in 40 mmol/dm$^3$HCl, 20 mmol/dm$^3$ FeCl$_3$, 0.1 mol/dm$^3$ of acetate buffer (pH=3.6) at a ratio of 1:1:10. For the analysis, 0.3 cm$^3$ of the extract, 2 cm$^3$ of the reaction mixture was mixed with distilled water and filled up to 10 cm$^3$. Samples were shaken for 30 seconds and then allowed to stay for 6 minutes at room temperature. The samples were then centrifuged for 10 minutes and absorbance was measured. In parallel, a calibration curve was prepared using working solutions of Trolox. The antioxidant potential of extracts is expressed in µmolTrolox per 100g oil.

The spectrophotometric analysis of radical scavenging activity toward DPPH was performed by the method of Sanchez-Moreno et al. (1998) with the modification involving the use of DPPH methanolic solution with a concentration of 5.0 mg/100 cm$^3$ in the case of black cumin oil and 2.5 mg/100 cm$^3$ for other oil extracts. The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is stable under normal conditions. In the presence of an antioxidant it is reduced, which is accompanied by a change in the solution colour from purple to yellow and the decrease in absorbance at a wavelength of 515 nm. Antioxidant activity is expressed as a percentage reduction of the DPPH radical after sample incubation of 10 minutes in relation to the control.

The presented results are the arithmetic mean of at least three determinations of each two parallel extractions. Basic descriptive statistics were calculated for all parameters. In order to compare the mean values, the analysis of variance (ANOVA) was undertaken. To verify the significance of differences between mean values, the Tukey’s test was applied using the Statistica 9.0. program. In estimating, the statistical significance level was set at $p < 0.05$.

In order to compare and rank the effectiveness of solvents, the unitarization of variables was applied (Guzik et al., 2005). Thanks to this, uniform variables were obtained according to formula (1):

$$V_{ij} = \frac{x_{ij} - \min_i \{x_{ij}\}}{\max_i \{x_{ij}\} - \min_i \{x_{ij}\}} \quad (1)$$

where $i$ - type of oil; $j$ - type of solvent.

As a result of the implementation of the above formula, normalized variables with values in the range $[0, 1]$ were achieved. Then a synthetic variable was calculated on
the basis on formula (2):

\[ s_i = \frac{1}{p} \sum_{j=1}^{p} V_{ij} \]  

(2)

where p - quantity of variables.

3. Results and discussion

Extracts obtained from cold-pressed oils differed significantly in their Ferric Reducing Antioxidant Power (Fig. 1). The variability is visible between the oils and among extracts from different solvents within the same oil. Definitely the highest reducing power was revealed for black cumin oil, for which the values varied from 2102 µmol Trolox/100g oil for methanolic extracts to 678 µmol Trolox/100g oil for acetone:water (50:50 V/V) solvent. The lowest values were measured for flaxseed, walnut and pumpkin seed oils (5-199 µmol Trolox/100g oil).

![Fig. 1: Ferric Reducing Antioxidant Power of oil extracts obtained using different solvents (converted to Trolox).](image)

a-f – means with different letters indicate significant differences (p<0.05) among the samples


Taking into consideration the type of solvent used for extract preparation, it is clearly seen that extracts obtained with methanol exhibited a much higher reducing power compared to other solvent extracts of the same oil. In the case of methanol
as a solvent, the synthetic variable was equal to 1.000 which means that methanol extracts exhibited the highest reducing power across all oils. The average lowest synthetic value (0.035) achieved by 50:50 V/V methanol:watersolvent suggests that this type of solvent has the lowest antioxidant extraction abilities, measured by ferric reducing antioxidant power.

The evaluated extracts showed significantly different antiradical properties. Fig. 2 presents the reduction of DPPH, expressed in percentage, after 10 minutes of extract incubation with a DPPH solution. The DPPH assay showed that the strongest antiradical properties were exhibited by black cumin oil (the initial DPPH concentration in the solution was twice as high as for other oils and amounted to a concentration of 5 mg/100 cm$^3$). Moderately high quenching of DPPH radicals was observed in the case of evening primrose cold-pressed oil (5.6-84.3%). The weakest antiradical abilities were revealed for pumpkin seed oil regardless of the type of solvent used for extraction.

In the case of DPPH reduction, methanolic extracts (with the synthetic variable at 0.900) proved to be the most effective ones in all oil samples except black cumin oil (for which methanol:water (70:30 V/V) solvent was more efficient). The weakest radical reduction was recorded for methanol:water (50:50 V/V) extracts, with the synthetic value at a level of 0.003.

The present study found a strong positive correlation (0.81) between antioxidant activity expressed as a percentage of reduced DPPH and reducing power (FRAP), which allows to rank the analyzed cold-pressed oils for their antioxidant properties: black cumin > evening primrose > rapeseed > flaxseed > walnut > pumpkin seed. Significant antioxidant properties of black cumin oil were recently evaluated (Lutterodt et al., 2010). It is believed that black cumin oil might enhance the oxidative stability of food products and provide potential health benefits to consumers. Because of that, an attempt was made to prepare oil blends with the use of sunflower oil and cold-pressed black cumin oil (10% and 20%, w/w), and the enrichment caused a decrease in peroxide levels during incubation (Ramadan, 2013).

Moreover, the research showed that the selection of extraction solvent strongly determines the value of measured parameters. The results are consistent with the literature. Perez-Jimenez and Saura-Calixto (2006) measured the antioxidant properties of catechin and gallic acid mixture extracted with solvents such as methanol, water and a mixture of acetone/water and methanol/water. The biggest impact of solvent was visible in the ORAC method, then in the ABTS, FRAP and DPPH methods. The authors concluded that the observed differences may be even greater if the food products which are analyzed are a complex matrix where different components can interact with each other or with the solvent.

A factor with a key influence on the determination of antioxidant activity seems to be the level of solvent polarity. In the study with quercetin it has been reported that with increasing polarity of the solvent a decrease of antiradical properties was observed (Pinelo et al., 2004). This relation was confirmed by Valavanidis et al. (2004) who demonstrated that hydrogen bonding can have large impact on phenolic antioxidants functioning as hydrogen donors. According to the polarity paradox, the hydrophilic antioxidants are effective in non-polar solvents, while the lipophilic compounds are better antioxidants in polar solutions. The presence of water, also during extraction, may affect the distribution of antioxidants between the polar and a polar fraction.
Fig. 2: The reduction of the DPPH radical after 10 minutes of incubation with oil extracts obtained using different solvents [%].

In the present study solvents of different polarity were chosen. The polarity paradox was confirmed, as extracts obtained with a lower polarity solvent exhibited greater antioxidant activity. Among the solvents, methanol had the lowest polarity and the antioxidant activity of its extracts was the highest. The mixture of 50:50 V/V methanol:water was characterized by the highest polarity, which resulted in the lowest antioxidant activity and reducing power in most of the oil samples investigated.

Another important aspect of the comparison of the antioxidant properties is a type of fraction in which the determination is made. The study discussed measuring the activity only in the polar fraction. In the literature, different reports can be found on which phase (polar, a polar or whole oil without prior extraction of antioxidants) has the highest antioxidant activity (Arranz et al., 2008; Espin et al., 2000; Valavanidis et al., 2004). In the next stage of the research, antioxidant activity of the non-polar fraction and oil without prior extraction will be measured.
4. Conclusions

The presented study showed a strong positive correlation (0.81) between antioxidant activity expressed as percentage of reduced DPPH and reducing power (FRAP) which allows to rank the analyzed oils for their antioxidant properties: black cumin > evening primrose > rapeseed > flaxseed > walnut > pumpkin seed cold-pressed oil.

Extracts obtained from cold-pressed oils showed diverse antiradical activity and reducing power resulting from the use of different solvents for extraction. The highest antioxidant activity was measured for methanolic extracts, while the lowest was obtained for methanol:water(50:50 V/V) extracts.

An important parameter in determining the antioxidant properties is the polarity of the solvent. It was confirmed that the higher the solvent polarity, the stronger the antioxidant properties of extracts decrease.

The presented study found a strong positive correlation (0.81) between antioxidant activity expressed as percentage of reduced DPPH and reducing power (FRAP), which allows to rank the analyzed cold-pressed oils for their antioxidant properties: black cumin > evening primrose > rapeseed > flaxseed > walnut > pumpkin seed.

Comparison of the antioxidant properties of different samples is appropriate when the analysis is carried out under the same conditions, using the same method, solvent and unified presentation of the final results.

References


