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EVALUATION OF PHYSICAL PROPERTIES OF SELECTED VEGETABLE OILS OBTAINED BY COLD PRESSING

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A b s t r a c t. The aim of this work was to evaluate the physical properties of oils from winter rape, pumpkin and grape seeds in order to assess their potential as a source of functional oils. Seeds were analysed for water content, protein and fat. All the seeds met minimum requirements for technological quality. The effect of cold oil extraction on chemical compounds was studied in oils pressed from the above seeds. The properties of the oils were compared based on the lipid profiles, content of carotenoids, chlorophylls and tocopherols. The oxidation stability of the oils was estimated through the determination of the acid number, peroxide number and oxidation induction time.

K e y w o r d s: vegetable oil; fatty acids; oxidation stability; fat; protein

INTRODUCTION

The preparation of healthy and natural food depends strongly on the technology of production which is based on the selection of optimum temperature of processes as well as the reduction or elimination of unhealthy chemical compounds that are considered to increase productivity, commonly referred to as refiners. Furthermore, grape seed oil contains triglycerides which are rich in unsaturated fatty acids such as oleic (3-15% 18: 1n-9) and linoleic acid (58-78%, 18: 2n-6) and smaller amounts of saturated fatty acids (10%) (Kamel *et al*. 1985), poly-unsaturated fatty acids (90%), such as linoleic and linolenic acids (Baydar and Akkurt 2001, Martinello *et al*. 2007, Sosińska and Panasiewicz 2012).

Both oilseeds and their oils are characterised by different fatty acid composition. They contain monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs), particularly omega-3. These acids are the precursors for the synthesis of long chain fatty acids.

The unsaturated fatty acids comprise oleic and linoleic acids (Kamel *et al*. 1985) while polyunsaturated fatty acids comprise linoleic and linolenic acids (Baydar and Akkurt 2001). The fatty acids belonging to n-3 contribute to a reduction of the risk of cardiovascular diseases by lowering the levels of triglycerides and cholesterol in the blood and also by reducing the incidence of mental illness, including depression (Logan 2004). However, one has to deal with the presence of large amounts of saturated fatty acids (SFA). Their presence makes vegetable oils relatively unstable products, easily subject to oxidation processes which lead to adverse changes in the quality known as "fat rancidity".

The native substances known as the non-glyceride fraction, having antioxidant properties, include natural antioxidants such as tocopherols, phospholipids, carotenoids, sterols, phenolic compounds and squalene. In contrast, the compounds of the catalytic action of the oxidation process are heavy metals, particularly copper and iron, as well as chlorophyll pigments. They occur in small quantities (less than 2%), but they significantly affect the nutritional value and sustainability of oils (Mińkowski *et al*. 2011).

Tocopherols are a family of four derivatives (α, β, γ and δ-tocopherol; the alpha-derivative is also known as vitamin E) that are essential in preventing the formation of free radicals in biological systems. However, they are dependent on the processing conditions during oil extraction. Among them, α-tocopherol is the most biologically active (Nećeral *et al*. 2006). The tocopherol content of pumpkin seed oil is relatively high and varies from 400 to 700 mg kg^{-1}. The total content of tocopherols in cold pressed canola oil ranged between 430 and 2680 mg kg^{-1}, while refined oil had its content at the level of 51.2 mg 100 g^{-1} (512 mg kg^{-1}) (Goffman and Becker 2002, Kraljić *et al*. 2013). In the case of grape oil, the content of bioactive compounds such as tocopherols amounted to between 5 and 52 mg 100 g^{-1}.

The purpose of this study was to determine and compare the selected physicochemical parameters of seeds and oils from different varieties of winter rape, shell-less pumpkin and grape. The effect of cold oil extraction on chemical compounds of oils pressed from the above seeds was also studied. The properties of the examined oils were compared based on the lipid profiles, content of carotenoids, chlorophylls and tocopherols. The oxidation stability of these oils was estimated through the determination of the acid number, peroxide number and oxidation induction time.

MATERIALS AND METHODS

Experimental material consisted of 2 kg samples of seeds from oilseed plants such as winter rape of mixed variety Abakus, Seed-less pumpkin Olga variety, as well as Grape of Fredonia variety. All the plants were grown in South-East Poland in the Lublin Province. All the crops were collected in 2014. Prior to the experiments, the seeds were dried under natural conditions and stored under laboratory conditions at 20°C and relative humidity of 60-70%.

Determinations of fat (CLA/GC/3b/2011) and protein (CLA/PSO/13/2013) were carried out at the Central Agro-Ecological Laboratory of the University of Life Sciences in Lublin.

Seed moisture content was determined using a moisture analyser Radwag max 50/1/WH (oven-dry test). Analysis of moisture was carried out during drying at sample load of 4 g at 120°C .

Fatty acids were determined by the use of gas chromatography to determine the qualitative and quantitative composition of a mixture of fatty acid methyl esters prepared in accordance with PN-EN ISO 12966-2:2011.

The oil was pressed using a screw press with variable nozzle with a diameter of 8 mm and a set of microscopic strainers from Farmet DUO (Czech Republic), in the continuous operation mode. The press was started and after the stabilisation of the working temperature the oil pressing process was commenced. The stabilisation was achieved after the pressing of oil from a mass of about 1 kg of seeds when the temperature was about 70°C. The temperature during pressing was measured with the use of an ama-digit thermometer. The oil after extraction was stored in dark glass bottles at a temperature of 5°C, in order to obtain natural decantation for 6 days, and then subjected to analyses.

The contents of chlorophyll and carotenoids were determined by spectrophotometry with fresh oil, using a double-beam UV – VIS Jasco V-630 spectrophotometer. Oil samples were diluted 20-fold with acetone and the spectrum was measured between wavelengths of 350 and 700 nm. Calculations of concentrations of chlorophyll a and b and total carotenoids content (in mg ml) were made in accordance with the procedure of Lichtenthaler and Buschmann (2001). All the measurements were done in three independent replicates.

Evaluation of the quality of oils included determination of the acid number (AN) by titration, in accordance with DIN EN ISO 660:2005, and of the peroxide number (LOO) by titration in accordance with DIN EN ISO 3960:2005.

The oxidative stability was measured using a Rancimat 670 apparatus (Metrohm AG, Herisau, Switzerland). Oil samples (2.5 g) were weighed into reaction vessels and heated at 120°C under a dry air flow of 20 l h^{-1}. The volatile compounds released during the oxidation were collected into a cell containing distilled

water, and the increasing water conductivity was continually measured. The time taken to reach the conductivity inflection point was recorded as the induction period (IP) expressed in hours. All determinations were carried out in triplicate.

Tocopherols (α, β, γ, δ) in oils were determined with the use of high performance liquid chromatography, after saponification of the contents in the presence of potassium hydroxide (60%, w/v) and ethanol containing the antioxidant (pyrogallol in ethanol 6%, w/v). After the addition of NaCl (1% , w/v), tocopherols and tocotrienols were extracted twice with the mixture of ethyl acetate and hexane (1/9, v/v). Solvents were removed by evaporation and the residue was dissolved in isopropanol (1% v/v) in n-hexane. Determinations of tocopherols and tocotrienols were made based on the work of Panfili *et al*. (2003). Tocopherols and tocotrienols were determined using the Agilent 1100 method, using a fluorescence detector (Ex290 nm EM330 nm) on a column packed with silica. Hexane, ethyl acetate and acetic acid (97.3/1.8/0.9 v/v/v) mixture was used as eluent.

Results are presented as means ± standard deviation from three replicates of each experiment. A significance level $p < 0.05$ was used to denote significant differences between mean values determined by the analysis of variance, Pearson correlation, post-hock Tukey tests (ANOVA) with the assistance of Statistica 10 software.

RESULTS AND DISCUSSION

Table 1 shows the water, fat and protein content in the examined seeds. Statistical analysis showed significant differences between the species of seeds. The highest mean fat content was determined at pumpkin seeds (47%), followed by rapeseeds (45.5%) and grape seeds (12.6%). The values for fat contents obtained in this study were comparable or higher than those published previously (Misiura, 2010; Rezig 2012). The same was observed for proteins, although according to Hanganu (2012) protein content in grape seeds ranges at 10-20%. Such rather minor differences may result from the effect of variety or cultivation conditions.

Table 1. Profile of seeds

Specification	Unit	Winter rape	Pumpkin	Grape
Water content in seeds	(%)	6.50 ±0.10	10.40 ±1.61	7.17 ±0.21
Fat	(% d.m.)	45.50± 0.23	47.23 ±0.20	12.6 ±0.04
Protein	(% d.m.)	20.40±0.30	35.30 ±0.21	9.4 ±0.12

Statistical analysis of correlation coefficient showed a strong correlation $r = 0.875$ (at the significance level of $p < 0.05$) between fat and protein contents for all the examined seeds

Table 2 shows the results of determination of the fatty acids profiles. Rapeseeds were characterised by the lowest content of saturated fatty acids (SFA) – 7.62%,

polyunsaturated fatty acids **(**PUFA) – 25.61%, as well as Omega-6 acids which consists of linoleic, γ-linolenic and arachidonic acids – 24.59%. On the other hand, rapeseeds had the highest content of mono unsaturated fatty acids (MUFA), amounting to 66.68%. The fatty acid content in pumpkin and grape seeds was similar in the case of SFA, and amounted to 18.80 and 18.18%, respectively. They also had similar levels of PUFA acids which amounted to 62.02 and 66.46% for pumpkin and grape seeds.

All the examined oils contained similar levels of Omega-3 acids, that is α-linoleic and eicosapentaenoic (EPA) acids, whose concentrations amounted to 0.37% for rapeseeds and 0.36% for pumpkin seeds. The obtained values were comparable with the results obtained by other authors (Krygier *et al*. 1998, Murkovic *et al*. 2004, Nyam *et al*. 2009).

Table 3 shows the results of analysis of carotenoids and chlorophylls a and b content in the oils. The highest amount of chlorophyll a (4.41 μg ml^{-1}) was found at the oil pressed from grape seed, while the lowest concentration (1.26 μg ml^{-1}) was found in the pumpkin seeds. According to literature, the amount of chlorophyll a in the seeds should not exceed the value of 25 μg kg^{-1} (Daun 1987) as it has a negative effect on the quality and stability of oils, as well as supports oxidation, which results in the darkening of oils (Mag 1993).

Table 2. Fatty acid content of the seeds (%)

Specification	Winter rape	Pumpkin	Grape
C14:0	0.07±0.03	0.11±0.01	0.11±0.01
C16:0	5.37±0.32	12.77±0.35	12.20±0.34
C16:1	0.32	0.13±0	0.08±0
C17:0	0.06	0.07±0.01	0.07±0.01
C17:1	0.09±0.01	0	0
C18:0	1.59±0.17	5.24±0.20	5.18±0.20
C18:1n9c +C18:1n9t	66.28±2.15	18.89±0.97	15.08±0.77
C108:2n6c+C18:2n6t	24.59±0.30	61.65±1.63	66.15±1.74
C18:3n6 (gamma)	0.05	0	0
C18:3n3 (alpha)	0.07±0.01	0	0.32±0
C20:0	0.54±0.03	0.36±0	0.37±0
C20:5	0.30±0	0	0
C20:1	0	0.37±0	0.11±0
C22:0	0	0.18±0	0.19±0
C22:2	0.66±0	0	0
C24:1	0	0.07±0	0.07±0
SFA	7.62	18.8	18.18
MUFA	66.68	19.11	15.26
PUFA	25.61	62.01	66.46
OMEGA 3	0.37	0.36	0.32
OMEGA 6	24.59	61.65	66.15

The highest carotenoid content was determined at pumpkin seed – 22.46 μg ml^{-1} while the lowest content of this pigments was found at grape seed – 0.90 μg ml^{-1}. Statistical analysis of the correlation coefficient showed a strong correlation (r = 0.918) between carotenoid content in the examined oils and the fat oxidation induction time (at the significance level of $p < 0.05$).

Fat oxidation induction time obtained during the analysis allows to estimate the oils' resistance to oxidation under accelerated conditions. Induction time for all the oils stored for 6-8 days was relatively low (Table 4). For the oil obtained from grape seed, the analysis showed the lowest oxidation stability with mean oxidation time of 2.59 h. This value can be explained in terms of a relatively high MUFA and PUFA content, although other authors report a relatively higher induction time as compared to this study (Besbes *et al*. 2004, Rezig *et al*. 2012). For the same conditions, the highest oxidative stability was determined for pumpkin seeds for which it was 4.75 hours. These results are comparable to those for rapeseed oil reported in the literature (Krygier *et al*. 1998).

Table 3. Chlorophyll and carotenoid content of the oils (μg ml^{-1})

Specification	Cold pressed oils		
	Winter rape	Pumpkin	Grape
Chlorophyll a	1.76±0.47	1.26 ±0.11	4.41±0.03
Chlorophyll b	2.02±0.79	7.83±0.84	3.16±0.27
Carotenoids	8.41±0.76	22.46±2.51	0.90±0.09

Table 4. Acid number, peroxide number and the induction time of oils

Specification		Cold pressed oils		
		Winter rape	Pumpkin	Grape
AN (mg KOH g^{-1})		1.4 ±0.01	1.4±0.01	2.8±0.01
LOO (mmol O kg^{-1})		1.12±0.01	2.0±0.02	6.72±0.02
Oxidative stability (h)	Induction time	4.05±0.07	4.75±0.18	2.59±0.02
	Normal time	21.56±0.45	25.33±0.95	13.85±0.11

The factors negatively affecting oil durability can be a high content of chlorophyll pigments as well as free fatty acid content determined by the acid (AN) and peroxide (LOO) numbers. They are indicatory values that describe the hydrolytic changes in triglyceride and oxidation in both the seeds and the oil. The best parameters were obtained for cold-pressed oils derived from rape seeds and pumpkin seeds, for which the acid number and peroxide number were 1.4 mg KOH g^{-1} (both oils) and 1.12 mmol O kg^{-1} and 2.0 mmol O kg^{-1}. The highest parameters were found for grape seed oil, in which the acid number was 2.8 mg KOH g^{-1} which falls within a range of standards for the content of AN and LOO. In the case of LOO its value was significantly higher and amounted to 6.72 mmol O kg^{-1}. The

results of statistical analysis of the correlation coefficient showed a strong negative correlation between the acid value and peroxide value of oils and induction time ($r = -0937$ and $r = -0873$, respectively; at the level of $p < 0.05$). This can be explained in terms of a change of one of the analysed factors which have a significant impact on the level of the other one in oil. The performed post-hoc tests on the dependencies of the oxidation induction time and the oxidative stability showed statistically significant differences between all oils.

A very strong negative correlation ($r = -0.967$) was found between the chlorophyll a content and fat oxidation induction time. Post-hoc tests showed statistically relevant differences between chlorophyll a content in oils pressed from grape seed and oils from rapeseeds and pumpkin seeds. In the case of chlorophyll b, the statistical differences were found between pumpkin seed oil and other examined oils. Relatively high correlation coefficient was also found for fat content and the fat oxidation induction time ($r = 0.931$).

The antioxidant potential of tocopherols in the examined oils is presented in Table 5. All the examined oils were relatively rich in γ-Tocopherol. The results showed that rapeseed oil had the largest content of γ-tocopherol, averaging 434.17 mg kg^{-1}, and α-tocopherol – averaging 141.8 mg kg^{-1}. β- and δ-tocopherols had incomparably low concentrations, which was consistent with the findings of other authors (Kraljić 2013).

Table 5. Tocopherol content of the oils (mg kg^{-1})

Specyfication	Cold pressed oils		
	Winter rape	Pumpkin	Grape
α-Tocopherol	141.8±2.69	20.83±0.76	18.20±0.36
β-Tocopherol	37.83±0.80	51.03±0.50	4.80±0.40
δ-Tocopherol	9.10±0.26	12.07±0.50	4.20±0.26
γ-Tocopherol	434.17±3.63	551.43±1.44	69.73±0.76
Total	624.90*	635.37*	96.93*

*Sum of the means

In the case of pumpkin seed oil, the mean amount of γ-tocopherol was 551.43 mg kg^{-1}, while the mean for α-tocopherol amounted to 20.83 mg kg^{-1} and it was relatively higher than reported for other pumpkin cultivars (Stevenson *et al*. 2007). Other results are comparable with those published previously. The lowest tocopherol content was observed at the oil pressed from grape seeds, although γ-and α-tockopherols (69.73 and 18.20 mg kg^{-1}, respectively) exceeded other tocopherols in this oil. Statistical analysis showed significant differences between the contents of the various types of tocopherols among all oils, apart from δ-tocopherol, whose concentration level was similar for all the oils. The tests performed on the

correlation coefficient revealed no correlations between the time of induction of oils and the content of α-tocopherol. A strong correlation exists between the other tocopherols and the induction time (β-tocopherol: r = 0.993; γ-tocopherol: r = 0.989; δ-tocopherol: r = 0.987).

CONCLUSIONS

The presented study demonstrated that the seeds of winter rape, pumpkin and grapes can be a rich and valuable source of many important nutrients, including fat and protein. Oil production by using the technique of cold pressing results in the preparation of raw material with a high content of polyunsaturated acids, both MUFA and PUFA, in the range of 15.26 to 66.68% and 25.61 to 66.46%, respectively.

A low content of γ-linoleic acid (0.05%) and α-linolenic acid (0.07%) at rapeseeds or α-linolenic acid (0.32%) at grapes as well as α-linoleic acid content at the detection level at pumpkin seeds can provide their relatively high oxidative stability.

The high tocopherol content and quality of tocopherols provide protection against oxidative stress. The oil which contained the highest average level of tocopherols was pumpkin seed oil (635.37 mg kg^{-1}), followed by rapeseed oil (624.90 mg kg^{-1}) and grape seed oil (96.93 mg kg^{-1}). Shelf-life and other desirable characteristics may indicate potentially a wide range of applications of the examined oils.

The presented study proves that the examined oils can serve as excellent sources of vitamin E (tocopherols). Preparation of the oils by cold-pressing ensures favourable fat and antioxidant content.

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WPŁYW TŁOCZENIA NA ZIMNO ROŚLIN OLEISTYCH NA JAKOŚĆ WYTŁOCZONEGO OLEJU

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S t r e s z c z e n i e. Zamierzeniem pracy było określenie parametrów fizycznych olejów z rzepaku ozimego, dyni i winogron w celu oceny ich potencjału jako źródła olejów funkcjonalnych. Nasiona analizowano na zawartość wody, białka i tłuszczu. Parametry nasion spełniały minimalne wymagania technologiczne. Badano zawartość związków chemicznych uzyskanych z powyższych nasion w olejach tłoczonych na zimno. Właściwości olejów porównano na podstawie zawartości lipidów, karotenoidów, chlorofili i tokoferoli. Stabilność oksydacyjną tych olejów oszacowano przez oznaczenie liczby kwasowej, liczby nadtlenkowej oraz czasu indukcji utleniania.

S ł o w a k l u c z o w e: olej roślinny, kwasy tłuszczowe, stabilność oksydacyjna, tłuszcz, białko