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ORIGINAL PAPER

Cold-Pressed Pumpkin Seed Oil Antioxidant Activity as Determined by a DC Polarographic Assay Based on Hydrogen Peroxide Scavenge

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Abstract Antioxidant (AO) activity of cold pressed pumpkin (*Cucurbita pepo* L.) seed oil, produced from three naked and one hulled variety, was assessed using a DC polarographic assay based on a hydrogen peroxide scavenge (HPS). Results are expressed as the decrease of the anodic oxidation current of hydrogen peroxide obtained upon addition of methanolic extract of the investigated oils. Strict correlations of HPS and (1) radical scavenging capacity against the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (0.99), (2) the induction period estimated by a Rancimat test (0.99) and (3) total phenolic content estimated by Folin-Ciocalteu (FC) assay (0.99) were obtained. In addition, a significant correlation of HPS and the content of δ-tocopherol (0.87), squalene (0.67) and color CIE a* (−0.89) was found. Based on the results reported, the polarographic assay was found to be suitable for determination of AO activity as an indicator of the quality and oxidative stability of oil.

Keywords Antioxidant · Hydrogen peroxide · DC polarography · Oxidative stability · Phenolics · Pumpkin oil

Abbreviations

AO	Activity-antioxidant activity
HPS	Hydrogen peroxide scavenge or scavenging
SCE	Saturated calomel electrode
DC	Direct current
DME	Dropping mercury electrode
i_l	Limiting current
DPPH	1,1-Diphenyl-2-picrylhydrazyl free radical

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Introduction

Pumpkin (*Cucurbita pepo* L.) seed is used for oil production in some African countries, China and Southeastern Europe. Growing pumpkin as an oil crop has a long tradition in Southeastern European countries. Virgin pumpkin seed oil has been considered a regional specialty of Austria, Croatia, Hungary and Slovenia, while the cold-pressed oil is a product characteristic for Serbia. Production technology applied for obtaining virgin oil includes a roasting step at about 100 °C prior to seed pressing. Changes that occur during the roasting process, responsible for the development of the typical nutty aroma, have been studied [1]. Seeds of *Curcubita pepo* L. subsp. *pepo* var. Styrian, with stunted outer hulls, have been used in Austria's southeastern region, Styria, for the production of so-called

Styrian oil, whose production technology, regional significance and characteristics have been reported [2]. The production of cold-pressed oil from raw-dried naked or dehulled pumpkin seeds started in the 1990s in Serbia. Cold-pressed oil has been obtained from non-roasted pumpkin seeds by pressing on screw presses. In the course of pressing on screw presses, the temperature does not exceed 50 °C [3].

The total antioxidant (AO) activity plays an important role in the quality assignment as well as the identification and quantification of the antioxidants (AOs) present. An increased interest in oil AO activity has been led by the finding that edible oils rich in AOs provide health benefits, reducing the risk of some chronic diseases [4]. Also, edible oils with higher intrinsic AO contents may have higher resistance to oxidation and an extended shelf life. Antioxidants may protect oil from off-flavors, thus delaying the onset of rancidity.

Among numerous assays applied to test AO activity of edible oils are those based on various electrochemical techniques. Tests based on electrochemical properties, such as a flow injection analysis system (FIA) with electrochemical detection, have been used to measure the AO power of natural olive oil [5]. Hydrodynamic voltammetry performed in FIA has been applied to evaluate the contribution of total phenolics to the AO activity and oxidative stability of olive oil [6]. Also, an electrochemical method has been applied to evaluate the AO power of tocopherols and carotenoids [7]. The phenolic content of edible oils was evaluated using an array of voltammetric electrodes, chemically modified with electroactive materials [8], while a chronopotentiometric method was used to determine tocopherols [9]. In addition, electrochemical techniques were used to determine and quantify synthetic AOs added to improve oil stability and prevent rancidity. Synthetic AOs in vegetable oil solutions were directly measured by voltammetry [10] and differential-pulse polarography (DPP) [11].

The purpose of the present study was to explore the possibility of applying a DC polarography assay based on a hydrogen peroxide scavenge for evaluating the AO activity of cold-pressed pumpkin seed oils made on a small scale and obtained from three naked and one hulled variety of pumpkin. The hydrogen peroxide scavenge was monitored by DC polarography with a dropping mercury electrode (DME) [12]. This assay offers a rapid, direct and simple experimental procedure. In contrast to the artificial, non-physiological radical species used in common spectrophotometric assays, hydrogen peroxide has an advantage as a naturally present, powerful, physiologically relevant oxidant. The results, expressed as a percentage of the anodic current decrease of hydrogen peroxide upon addition of methanolic extracts of tested oils, have been compared with the radical scavenging capacity against the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). In addition, HPS and DPPH scavenges have been corroborated with the oxidative stability of oils evaluated through a Rancimat test, total phenolics, δ-tocopherols and squalene content, as well as with sensorial oils profiles (CIE L*a*b*). The possibility to use a polarographic assay to determine oil AO activity as a test of quality and oxidative stability has been discussed.

Experimental Procedures

Chemicals

Hydrogen peroxide, Folin-Ciocalteu reagent, caffeic acid, tocopherol standards, hexane, methanol and ethanol (95%, v/v) were purchased from Merck (Darmstadt, Germany). DPPH reagent was purchased from Aldrich (Milwaukee, WI). Hexamethyldisilazane, chlortrimethylsilane and pyridine were purchased from Supelco (Bellefonte, PA). All chemicals used in this study were of analytical grade.

Pumpkin Seed Material

Varieties of *Cucurbita pepo* L. grown in Serbia, naked "Olinka" (domestic), "SB" (domestic), "Gleisdorfer diamant" (Austrian) and hulled "Ljuska" (Hungarian), harvested in 2008, were obtained from the Institute for Crops and Vegetables, Novi Sad, Serbia.

Preparation of Cold-Pressed Pumpkin Seed Oil

The oil samples were obtained by pressing raw-dried naked or dehulled seeds with a screw press KK40/2-Standard (Kern Kraft, Germany), with a capacity of 40 kg/h. The temperature of oil from the press was not higher than 50 °C. The oils were collected in stainless steel vessels and decanted after 3 days at ambient temperature.

Chemical Analyses

The peroxide value (PV) [ISO 3960:2001], expressed in mmol/kg, was determined by the reaction of oil and chloroform:acetic acid (3:2) with potassium iodide in darkness. The free iodine was then titrated with thiosulfate solution. The acid value (AV) [ISO 660:2000], expressed in mg KOH/g, was determined by the titration of a solution of oil dissolved in ethanol:ether (1:1) with an ethanolic solution of potassium hydroxide. The unsaponifiable fraction was prepared according to the method for squalene determination.

Extraction and Sample Preparation

Crude oil (5.0 g) was dissolved in hexane (25.0 ml) and extracted with methanol (3 × 8.0 ml) by 2 min shaking for each extraction. Methanol extracts were left to stand overnight and then washed with hexane (25.0 ml). These extracts were carefully (without heating) evaporated on a rotatory vacuum evaporator and transferred to a volumetric flask, and the volume was adjusted to 10.0 ml with methanol.

Determination of AO Activity Using DC Polarographic Assay

The decrease of the anodic current of H_2O_2, i.e., the initial i_1 value (i_{10}), obtained by recording 5.0 mM H_2O_2 solution upon addition of methanolic extract of oil samples, was recorded. Samples were gradually added into an electrolytic cell with buffered H_2O_2 solution. Clarc Lubs. buffer (pH 9.8) was prepared from the mixture of 50.0 ml of 0.2 M H_3BO_3 and 0.2 M KCl to which 40.8 ml of 0.2 M NaOH was added. A starting H_2O_2 concentration of 5 mM was obtained by the addition of 10 µl of 30% H_2O_2 into 20.0 ml of buffer in an electrolytic cell. Before each *i-E* curve recording, a stream of pure nitrogen was passed through the cell solution. During the curve recording, the inert atmosphere was maintained by passing nitrogen above the cell solution. The initial potentials were 0.10 or 0.15 V, and the potential scan rate was 10 mVs^{-1}. Dropping mercury electrode current oscillations were filtered with a low pass filter positioned at 3 s. All experiments were done at room temperature.

Electrochemical Instrumentation

A dropping mercury electrode as working electrode, with a programmed dropping time of 1 s, was used. A saturated calomel electrode (SCE) served as a reference and a Pt-foil as auxiliary electrode. The current-potential (*i-E*) curves were recorded using the polarographic analyzer PAR (Princeton Applied Research), model 174A, equipped with an X–Y recorder (Houston Omnigraphic 2000). The volume of the electrolytic cell was 30 ml.

Determination of AO Activity Using DPPH Assay

Volumes of 200 µl of methanol extracts were mixed with 1,800 µl of methanol solution of DPPH (0.04 mg/ml) and left to react in the dark for 30 min. After the reaction absorbance was measured on 517 nm, DPPH scavenging activity was determined from the equation:

$$\text{DPPH}\ (\%) = 100 \cdot \left[1 - \left(\frac{A_s - A_b}{A_c}\right)\right] \quad (1)$$

where A_s is the absorbance in the presence of the methanol extract in the DPPH solution, A_c is the absorbance of the control solution (containing only DPPH), and A_b is the absorbance of the sample extract solution without DPPH. All measurements were done in triplicate. Oil extracts were then compared by DPPH scavenging activity. A higher DPPH (%) value means a higher scavenging activity towards the DPPH radical.

The Content of Total Phenolics

The content of total phenolics was estimated according to the Folin-Ciocalteau spectrophotometric method [13]. The volume of 200 µl of oil extract was mixed with 1,000 µl of FC reagent previously diluted with distilled water in the proportion 1:10. After 6 min in the dark, 800 µl of 7.5% sodium carbonate solution was added, shaken and left in the dark for the next 2 h to react. Absorbance at 740 nm was measured after that. Deionized water was used as a blank. Appropriate sample dilutions were experimentally found for each extract. All measurements were done in triplicate. Each absorbance was adjusted for the value of the blank probe. The same procedure was done with caffeic acid standard (concentrations of 10, 25, 50, 100 mg/l), and the calibration curve was constructed. The total phenolic content is expressed in caffeic acid equivalents (CAEs)-concentration of caffeic acid (mg/l).

Determination of Tocopherols

Quantification of tocopherols was carried out using high-performance liquid chromatography (Waters M600E, USA) on a Nucleosil 50-5 C18 reversed phase column with fluorescence detection and external standard solutions of different tocopherols. The following procedures were applied: *n*-hexane extraction, extract vaporization and reconstitution in methanol using membrane filtration. Mobile phase was 95% ethanol with a flow rate of 1.2 ml/min. The fluorescence detector (Shimadzu RF-535, Japan) operated with the excitation wavelength at 290 nm and the emission wavelength at 330 nm.

Determination of Squalene

Squalene was determined by GC of the complete unsaponifiable fraction. A 7.5-ml volume of internal standard solution (0.1% of cholesterol in methylene chloride) was added to 4.5 g of pumpkin oil sample, and the methylene chloride was evaporated. The prepared sample was saponified with 20.0 ml of 6 M solution of KOH in water and

30.0 ml of EtOH (ethanol with up to 5% diethyl ether). The solution was refluxed for 90 min at 70 °C (water bath 85–90 °C). After saponification, 30.0 ml of water was added, and non-saponifiable compounds were extracted first with 45.0 ml of petroleum ether and then with 45.0 ml of diethyl ether. The two extracts were combined and washed twice with 20.0 ml of 0.5 M solution of KOH in water and with 20.0 ml of 5% NaCl solution in water until the pH of the washing water was neutral. The organic fraction was dried with Na_2SO_4 and filtrated over folded filter paper into a conical flask. The residue obtained after evaporation was derivatized by adding 1.5 ml of dry pyridine, 0.2 ml hexamethyldisilazane and 0.1 ml chlortrimethylsilane as silylation agent. The flask was placed in an oven at 80 °C for 30 min for completion of the silylation. The derivatized sample was transferred into a vial and was ready for injection in GC. All samples were analyzed within 6 h after derivatization. GC and GC/MS analyses were performed on an Agilent 7890A GC equipped with inert 5975C, inert XL EI/CI MSD and a FID detector connected by a capillary flow technology two-way splitter with make-up. An HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The temperature for the GC oven was programmed from 60 to 300 °C at 3 °C/min and held for 10 min. Helium was used as carrier gas at 16.255 psi (constant pressure mode). The sample was analyzed in the splitless mode. The injection volume was 1 μl. The GC detector temperature was 300 °C. MS data were acquired in EI mode, with a scan range of 30–550 *m/z*; the source temperature was 230 °C and quadrupole temperature 150 °C. Solvent delay was 3 min. Identification was confirmed by the retention time lock (RTL) method and RTL Adams database.

Sensorial Profiles of Pumpkin Seed Oils Determined by the CieLab Colour-CIE L*a*b* Coordinates

Pumpkin seed oil has a specific shade, and therefore its color was assessed using a manual colorimeter-spectrophotometer MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Japan). The analyzed samples (10.0 g) were weighed into Petri dishes. The color was measured using the manual colorimeter-spectrophotometer with direct reading, at random, at ten different points on their surface, taking the average value. The L* value is the "lightness" of a sample from 0 to 100, with 100 being pure white; the a* value describes red (+) to green (−); the b* value represents yellow (+) to blue (−); and zero values for a* and b* represent gray.

Rancimat Test

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. The volatile compounds released during 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), and it is expressed in hours. All determinations were carried out in triplicate.

Statistical Analysis

The experimental values were expressed as the means of three determinations and the standard deviation. Correlations were studied by regression line and coefficient of correlation (r) using StatSoft STATISTICA 7.

Results and Discussion

Chemical Characteristics

The common chemical properties of investigated cold-pressed pumpkin seed oils, such as PV and AV as well as the content of unsaponifiable matter, were determined (Table 1). PV varied from 1.77 to 2.27 mmol/kg, which are values lower than those described in the EU regulations (10 mmol/kg). The acid values, ranging from 0.92 to 1.75 mg KOH/g, indicated that seed samples were fresh and stored under good conditions prior to cold pressing. The content of the unsaponifiable fraction ranged from 0.60 to 0.80%. According to the literature, this content can be higher and often is affected by the production process [14].

Determination of AO Activity Using HPS

The antioxidant activity of methanolic extract of tested oils was studied. Examined samples were added in 200-μl aliquots into the initial peroxide solution. Polarograms of H_2O_2 before and after addition of oil samples were recorded (Fig. 1). The height of the initial peroxide-limiting current (i_{l0}) was compared with the residual peroxide-limiting current (i_{lr}) obtained upon gradual addition of tested samples. The percentage of i_l decrease was calculated upon each addition of tested oil according to Eq:

$$\% \text{ scavenged } [H_2O_2] = \left(\frac{i_{lr}}{i_{l0}} - 1\right) \times 100 \quad (2)$$

The percentage of i_l decrease was plotted against the volume of added samples. Dose response curves (percentage of i_l decrease *vs* volume of added sample) are shown in Fig. 2.

Since the oil samples, with an exception of oil obtained from "Gleisdorfer diamant," did not reach 50% of the i_l

Table 1 Chemical properties of cold-pressed pumpkin seed oil

	Naked seed oils			Husk seed oil
	Olinka	SB	Gleisdorfer Diamant	Ljuska
Acid value (mg KOH/g of oil)	0.95 ± 0.14	0.93 ± 0.05	1.75 ± 0.01	0.92 ± 0.09
Peroxide value (mmol/kg of oil)	1.91 ± 0.08	1.77 ± 0.09	1.87 ± 0.17	2.27 ± 0.15
Unsaponifiable (%)	0.80 ± 0.04	0.60 ± 0.13	0.79 ± 0.03	0.78 ± 0.10

Data are the means ± standard deviation values ($n = 3$)

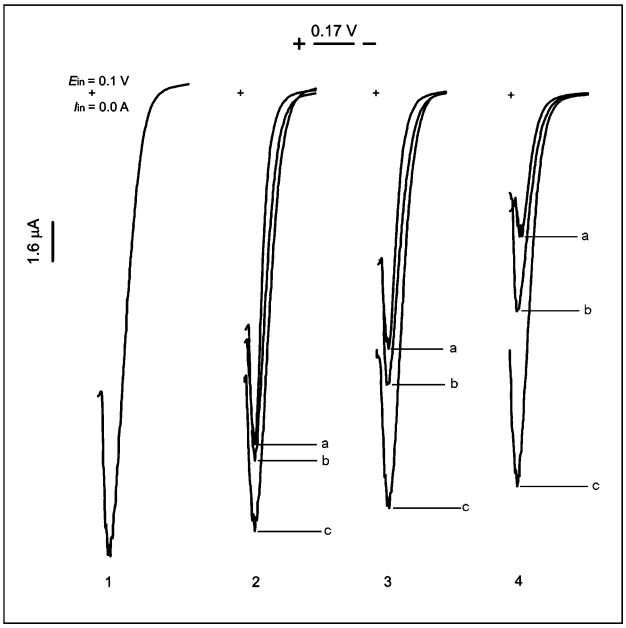

Fig. 1 Anodic polarographic curves of H_2O_2 before *1* and after addition of *2* 400, *3* 800 and *4* 1,200 µl of methanolic extract of cold-pressed pumpkin seed oil from variety "Gleisdorfer diamant" *a*, "Ljuska" *b* and "Olinka" *c*

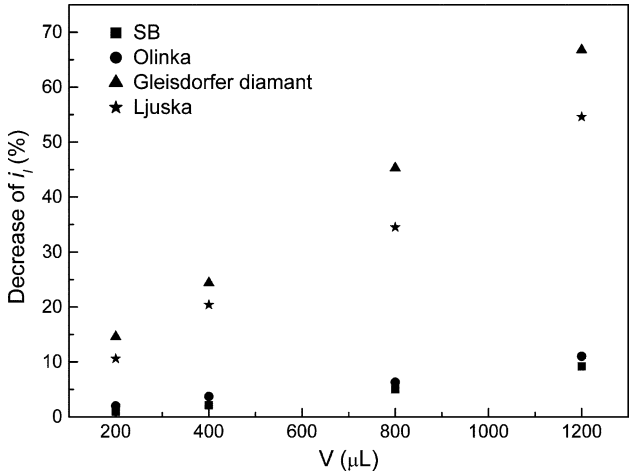

Fig. 2 Dose dependence of cold-pressed pumpkin seed oil obtained from "Gleisdorfer diamant," "Ljuska," "SB" and "Olinka" varieties and their effects on the anodic-limiting current of H_2O_2, i_l, decrease, i.e., scavenged H_2O_2 (%) versus volume of methanolic extracts (µl)

decrease, they could not be used as a criterion for AO activity, as previously [12, 15]. The value of i_l decrease upon addition of 1,200 µl was established as a measure of AO activity. Oil samples were compared according to this value. The following rank of order was obtained: "Gleisdorfer diamant" > "Ljuska" > "SB" > "Olinka" (Table 2). The decrease of i_l upon addition of 500 µl was used as the measure of strong alcoholics [16] and raspberry's extract AO activity [17].

Determination of DPPH Scavenge and Oxidative Stability by Rancimat Test

For validation reasons, the obtained results were corroborated with a DPPH scavenge. The DPPH assay was chosen as one of the widespread methods. It has been used to determine the AO activity of virgin [18] as well as cold-pressed pumpkin seed [19] oil. The Rancimat assay is a method commonly used for the determination of oil oxidative stability; the results are expressed as induction period (IP) (h). The rank of order of the DPPH scavenge of tested oils corroborates the rank of HPS well, while IP corroborates both the HPS and DPPH scavenges well (Table 2). High correlation was found between DPPH and HPS ($r = 0.99$), as seen in Table 3. A strong correlation ($r = 0.99$) was obtained between DPPH and IP. Correlation between HPS and IP was high as well ($r = 0.99$). This is an important aspect since most results concerning the antioxidant capacity of oils have been reported using the Rancimat assay.

Determination of Antioxidant Compounds Content in Pumpkin Seed Oils

Total AO activity of the oil reflects the integrated action of the complex mixture of different classes of polar as well as lipophilic AOs able to neutralize reactive oxygen species, delaying quality deterioration because of the autooxidation during processing and storage. Pumpkin seed oils from naked and hulled pumpkin seeds were evaluated for various compounds known to contribute to AO activity and

Table 2 Antioxidant activity of cold-pressed pumpkin seed oils determined by HPS and DPPH assays, and oxidative stability determined by Rancimat test

	Naked seed oils			Husk seed oil
	Olinka	SB	Gleisdorfer Diamant	Ljuska
HPS (%)	9.2 ± 0.53	11.0 ± 0.64	66.8 ± 1.44	54.6 ± 1.73
DPPH (%)	38.25 ± 0.00	38.49 ± 0.01	57.39 ± 0.01	52.25 ± 0.00
Rancimat IP (h)	4.0 ± 0.09	4.1 ± 0.01	4.5 ± 0.10	4.4 ± 0.00

Data are the means ± standard deviation values ($n = 3$)

Table 3 Matrix of correlation of selected parameters of cold-pressed pumpkin oils from different varieties

Correlation matrix	HPS	DPPH	IP	Phenols	Delta toc	a*	Squalene
HPS	1						
DPPH	0.99	1					
IP	0.99	0.99	1				
Phenols	0.99	0.98	0.97	1			
Delta tocopherol	0.87	0.89	0.88	0.78	1		
a*	−0.89	−0.91	−0.91	−0.81	−0.99	1	
Squalene	0.67	0.70	0.60	0.58	0.80	−0.77	1

oxidative stability. The amount of total phenolics, tocopherols and squalene in cold pressed oil is given in Table 4. As a quite natural product obtained without using thermal treatment before pressing or subsequent refinement, unrefined cold-pressed oil keeps the original amount of AOs.

The content of total phenolics in cold-pressed pumpkin seed oil determined previously (2.4 mg/100 g as CAEs) [19] was within the range found in this study (1.39–3.16 mg/100 g as CAEs). The total phenolic content in virgin pumpkin oil was found to be high, from 24.71 to 50.93 mg CAE/kg [18]. The main individual phenolics identified were tyrosol, vanillic acid, vanillin, luteolin and sinapic acid. The phenolic content of some edible oils correlated with different radical scavenging tests [20]. The analogy between AO activity of analyzed oils and total phenolics was found in this study as well. Significant correlations were found between FC CAE and both AO assays, HPS ($r = 0.99$) and DPPH ($r = 0.98$) (Table 3).

The antioxidant activity of tocopherols has been studied extensively. Tocopherols trap the hydroperoxide intermediates stopping the autoxidation chain reaction. Differences in the chemical composition of virgin pumpkin seed oil from naked and hulled seeds were studied, and higher amounts of total tocopherols in hulled seed than in naked were observed [14]. Variability within each seed oil variety was explained by genetic factors [21]. Differences in relative amounts of tocopherols regarding the influence on human health (α-tocopherol) and protection of seed compounds such as fatty acids (γ-tocopherols) are important. Although there were certain differences in the levels of separated individual tocopherols, γ-tocopherol was identified as the major

Table 4 Content of total phenolics [FC CAE (mg/100 g)], tocopherols (mg/100 g) and squalene (mg/100 g) in cold-pressed pumpkin seed oil

	Naked seed oils			Husk seed oil
	Olinka	SB	Gleisdorfer Diamant	Ljuska
Total phenolics FC CAE (mg/100 g)	1.39 ± 0.06	1.39 ± 0.07	3.16 ± 0.10	3.14 ± 0.16
α-Tocopherol (mg/100 g)	5.39 ± 0.37	4.57 ± 0.15	2.98 ± 0.10	4.72 ± 0.11
γ-Tocopherol (mg/100 g)	44.59 ± 0.15	40.09 ± 0.04	34.65 ± 0.10	46.99 ± 0.30
δ-Tocopherol (mg/100 g)	2.99 ± 0.15	4.26 ± 0.40	10.54 ± 0.13	5.55 ± 0.10
Squalene (mg/100 g)	655 ± 2	549 ± 2	788 ± 1	615 ± 2

Data are the means ± standard deviation values ($n = 3$)

Table 5 Sensorial profiles of pumpkin seed oils. L*a*b* color space measurements

	Naked seed oils			Husk seed oil
	Olinka	SB	Gleisdorfer Diamant	Ljuska
L*	20.49 ± 0.02	18.70 ± 0.21	19.76 ± 0.06	20.39 ± 0.09
a*	5.51 ± 0.09	4.50 ± 0.31	0.68 ± 0.30	3.54 ± 0.11
b*	0.60 ± 0.14	0.40 ± 0.09	−0.16 ± 0.10	0.33 ± 0.08

Data are the means ± standard deviation values ($n = 3$)

tocopherol in all oil samples. γ- and δ-Forms possess much higher AO activity than the α- and β-forms. Prevalent γ-tocopherol together with δ-tocopherol is considered to be the main contributor to pumpkin oil AO activity [22]. In accordance with previous findings, good correlation with γ- and especially with δ-tocopherols and AO activity was observed. The correlation between δ-tocopherol, and DPPH and HPS was $r = 0.89$ and $r = 0.87$, respectively (Table 3).

Squalene was detected in oil seeds. The presence of this triterpene, a key intermediate in the steroid biosynthetic pathway (precursor of cholesterol, steroid hormones and vitamin D) was considered to be important because of its AO activity. Antioxidant activity of squalene was demonstrated against PUFA [23]. Although AO activity of squalene was found to be lower than the activity of phenols and tocopherols, its role in olive oil stability was verified [24]. The squalene amount varied from 170.5 to 3,529.9 mg/kg in sunflower and pumpkin seed oils [21]. The content of squalene in four investigated samples of cold-pressed oil was 2–3 times higher (5,490–7,880 mg/kg) than values reported for virgin pumpkin seed oil (2,259–3,513 mg/kg) [14]. The well-known fact that squalene has weak AO activity explains the positive but not strong correlation between squalene and HPS ($r = 0.67$) (Table 3).

Correlation Between Color and AO Activity

Color analysis of oils is usually performed in order to evaluate the processing grade. Less refined oils have the darkest color (Table 5). Obtained results for both HPS and DPPH confirm that the darkest oil has the highest level of AO activity. Significant correlations between color CIE a* and AO activity were found ($r = 0.91$ for DPPH and a*; $r = 0.89$ for HPS and a*) (Table 3).

Conclusion

Based on the correlations obtained, it was concluded that DC polarography is suitable for the determination of AO activity of pumpkin seed oil as a test of the quality and oxidative stability. This study indicates that a DC polarographic assay could be a practical and simple way to evaluate the AO activity of pumpkin oil. The oxidative stability of the tested oils as well as the total phenolic content highly correlated with AO activity as estimated by both DC polarographic and DPPH assay. Also, significant correlation was found between an HPS and DPPH scavenge and the tocopherol and squalene contents.

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