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ORIGINAL PAPER

Shelf Life of Cold-Pressed Pumpkin (*Cucurbita pepo* L.) Seed Oil Obtained with a Screw Press

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Abstract The production of cold-pressed oil from non-roasted naked pumpkin seeds by pressing with screw presses started in the Republic of Serbia at the end of 1990s. This oil has the characteristic flavour of raw-dried seeds, and is a specific product of this region. The oil samples were prepared by pressing naked pumpkin seeds and pumpkin seeds with the hulls in a 3:2 ratio using a screw press. The changes of the oil sensory characteristics, basic chemical quality and oxidative stability were followed over a 2-year period. The results obtained showed that the oil sensory quality was stable during the first 12 months of storage, after which the flavour became musty. Although the chemical quality of the oil deteriorated to some extent, both the acid and peroxide values satisfied the legislative minimum during the whole investigation time. The oxidative stability of oil determined by a Rancimat apparatus was good, although the induction period after 2 years decreased by 25–40%. To ensure the use of only the highest quality oil, a 12-month shelf life is recommended.

Keywords Pumpkin seed oil · Sensory characteristics · Acid value · Peroxide value · Anisidine value · Oxidative stability

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Introduction

In recent years, there has been a growing interest in using natural and safe food, including oils and fats. Over the last few years, many nontraditional vegetable oils that are produced by mechanical extraction without use of any solvent have emerged and have become available to the consumers. These oils are obtained from different oilseeds or nutty fruits [1–3]. Pumpkin seed oil has an outstanding place in the group of the so-called seed oils [4].

Pumpkin (*Cucurbita pepo* L.) seed oil belongs to the group of oils of high nutritive value due to its favourable fatty acid composition and different minor components which have certain beneficial effects on the human organism [4, 5]. Besides the positive nutritive-pharmacological properties, pumpkin seed oil is characterised by specific sensory properties, such as colour [6, 7], odour, taste, and aroma. These are significantly different from other kinds of edible oils [4, 8, 9].

Pumpkin seed oil has been well known for a long time and appreciated as salad oil in a number of Southeastern European countries: Austria, Slovenia, Croatia, Hungary, with the consumption expanding in some other countries, too. In this particular market, pumpkin seed oil is known as *virgin oil***.** On an international scale, the Codex Alimentarius Standard for Named Vegetable Oils, Codex-Stan 210 [10], defines "virgin oils" as "obtained without altering the nature of the oil, by mechanical procedures, e.g. expelling or pressing and the application of heat only". According to traditional technology, virgin pumpkin seed oil is obtained from roasted (heat treated) seeds by pressing on hydraulic presses. Fresh water and table salt are added to the dried ground kernels to form a soft pulp. The pulp is roasted for up to 60 min at temperatures between 100 and 130 °C, which results in coagulation of the protein fraction and permits convenient separation of the oil by pressing. The roasting process of the seeds prior to pressing is responsible for the generation of the typical aroma of this oil. The pressing process is performed under isothermal conditions, at pressures between 300 and 600 bar [11, 12].

Cold-pressed pumpkin seed oil is a relatively new product in the Republic of Serbia and closer surroundings. Namely, the foundation of small and medium-sized facilities for production of cold-pressed oils started in this region at the end of the 1990s. Most of these small local oil mills use low-capacity screw presses (6–40 kg/h), ensuring simple production technology, a continuous pressing process and lower investment costs. Cold-pressed pumpkin seed oil is obtained by direct pressing of raw-dried, mostly hull-less pumpkin seeds, with continuous screw presses, with an outlet oil temperature below 50 °C [1]. In this process, neither the raw material nor the oil are exposed to higher temperatures in accordance with the Codex Alimentarius for cold-pressed oils. Namely, the Codex Alimentarius does not allow application of heat in the course of any production step of cold-pressed oils [10]. The technological process of cold-pressed pumpkin seed oil includes the following: the seed harvesting in the mid-autumn, immediate washing and drying to a residual water content of around 7%, and then storing. Prior to the processing, the seeds pass through the magnetic cleaner, followed by complete removal of organic impurities on the selector. The clean dry seeds are fed to the screw press, which grinds and presses the material, squeezing the oil, which is then collected in vessels. Turbid matter resulting from ruptured plant material of the seeds during pressing is removed by sedimentation or filtration at room temperature. Filtered oil is filled into dark glass bottles. Because of this simple process, the oil maintains the natural composition with regard to the constituents and flavour. Cold-pressed pumpkin seed oil produced using this process is specific to this Southeastern European region, and its consumption is currently increasing.

Pumpkin seed oil belongs to the group of exclusive and very expensive edible oils, and its nutritive quality should justify the high price. The investigation of the stability of the oil is very important in order to determine the time during which the quality does not change significantly, i.e. to define the shelf life of the product. Although the stability is not defined by legislation, this quality parameter is requested by the market [13]. The determination of the stability, i.e. the shelf life of edible non-refined oils, is a very sensitive issue. Namely, due to the processing of this oil without refining, the oil may contain components (degradative oxidation products, metals, *etc.*) which decrease the stability, whereas higher contents of natural components, having antioxidative properties, contribute to better stability of the oil.

Since nowadays a variety of virgin oils are produced, the data about cold-pressed pumpkin seed oil are not often found in the literature. For this reason, the aim of this study was to investigate the sensory characteristics, quality and oxidative stability of cold-pressed oil obtained with a screw press from raw-dried, non-roasted pumpkin (*Cucurbita pepo* L.) seeds. To this end, the oil samples were kept at room temperature for 2 years, while monitoring the changes in their quality characteristics, to define the oil shelf life.

Experimental Procedures

Pumpkin Oil Samples

The basic chemical composition and quality characteristics of pumpkin seeds used for the preparation of oil samples is presented in Table 1.

The oil samples were obtained by pressing raw-dried and cleaned seeds with a screw press "Reinartz" TYPE AP 08 [Maschinenfabrik Reinartz GmbH, Neuss], run by a 4-kW electric motor, capacity of 30–40 kg/h, in a small-size facility for cold-pressed oils. The materials for pressing were prepared as follows: mixture of naked pumpkin seeds A (18 kg) and pumpkin seeds with hull B (12 kg); mixture of naked pumpkin seeds A (18 kg) and pumpkin seeds with hull C (12 kg) and naked pumpkin seeds A (30 kg). A double-step pressing process was applied, i.e. the cake obtained after the *first* (I) pressing was pressed again—*second* (II) pressing. The oils were collected in stainless steel vessels and decanted after 3 days at ambient temperature.

Altogether, nine cold-pressed oil samples were obtained, as presented in Table 2.

Table 1 The basic chemical composition and quality characteristics of raw-dried pumpkin seed

	Naked pumpkin seed[a]	Pumpkin seed with hull[b]	
	A	B	C
Moisture content, %	6.65	7.22	7.02
Oil content, %	43.94	37.83	35.65
Peroxide value[c], mmol/kg	2.95	5.04	4.87
Acid value[c], mg KOH/g	0.57	0.96	0.65
p-Anisidine value[c]	0.58	1.88	1.98
Share of hull, %	–	24	21

[a] Domestic sort olinka

[b] Domestic sort olivija

[c] Quality parameters related to oil obtained from seed by cold solvent extraction [14]

Table 2 Preparation and identification of cold-pressed pumpkin seed oil samples

Oil samples	Material for pressing	Extraction of oil	Temperature (°C)
1	Naked seed A and seed with hull B	*First* pressing and decantation	42*
2	Cake from seed A & B	*Second* pressing and decantation	46*
3	Mixture of oil samples 1 and 2	Filtration	22
4	Naked seed A and seed with hull C	*First* pressing and decantation	42
5	Cake from seed A & C	*Second* pressing and decantation	46
6	Mixture of oil samples 4 and 5	Filtration	22
7	Naked seed A	*First* pressing and decantation	45
8	Cake from seed A	*Second* pressing and decantation	49.5
9	Mixture of oil samples 7 and 8	Filtration	22

* Temperature of the outlet oil from the press

Oil samples 3, 6 and 9 were filtered on a chamber filter press Seitz/Schenk model 670/30, working volume 113 L, at pressures up to 11 bar.

All oil samples were packed into dark-green 250-mL glass bottles, closed with metal screw caps. The head-space above the oil was about 3% of the bottle volume. The bottles were kept at room temperature for 24 months. Oils were always from freshly opened bottles when used for periodic investigations.

Sensory Evaluation

Sensory evaluation of fresh oils. The samples were prepared according to the recommended practise for panel sensory evaluation of edible vegetable oils by AOCS official method [Cg 2–83]. Oil samples (10 mL) were kept in 50-mL closed beakers in an oven at 50 ± 1 °C for 30 min, after which a three-member panel of experienced assessors evaluated the oil sensory characteristics (colour, odour and taste) for this kind of oil, according to the standard [ISO 5492:1992]. The sensory analysis of the oil during storage (samples 3, 6 and 9) was performed in the identical way, but the emphasis was put on the change of the intensity of aroma and the development of an atypical flavour, which was characterised as musty. The aroma intensity evaluation was expressed on a 0–5 scale, 0—not perceivable and 5—strongly perceivable.

Chemical Analyses

The *peroxide value* (PV) [ISO 3960:2001], expressed in mmol/kg, was determined by the reaction of oil and 3:2 chloroform:acetic acid with potassium iodide in darkness. The free iodine was then titrated with a 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 1:1 ethanol:ether with ethanolic solution of potassium hydroxide.

The *p-anisidine value* (AnV) was determined following the AOCS official method [Cd 18–90], on a UV/VIS spectrophotometer, model T80+, PG Instruments Limited, London.

Each of these parameters was analysed in triplicate for each sample.

The Totox value was calculated as twice PV plus AnV.

Fatty Acid Composition

Fatty acid methyl esters (FAMEs) were prepared according to the standard method [ISO 5509: 2000]. The fatty acid profile was analysed by a gas chromatography method [ISO 5508:1990] on a Hewlett-Packard series II^{plus} GC analyzer equipped with an automatic sampler HP 7673, splitless injector, flame-ionisation detector and integrator. The gas chromatographic conditions were as follows: a capillary Supelco column SP-2560, 100 m length, injector and detector temperature 220 °C, oven temperature 175 °C, injection volume 1 μL. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. Identification of the individual FA was accomplished by comparing the GC retention time with that of the pure commercial standard. The fatty acid composition of oil samples 3, 6 and 9 was analysed in triplicate.

Oxidative Stability

The oxidative stability of oils was investigated by determining the induction period (IP) on a Rancimat 670 apparatus, at 100 °C and an air flow of 18 L/h [ISO 6886:1996]. Portions of oil (2.5 g) were carefully weighed into each of the six reaction vessels and analysed simultaneously. The IP was determined for all fresh oils. The changes of oxidative stability of samples 3, 6 and 9 were followed during the 24-month period, the IP being analysed every 6 months in triplicate.

All reagents used were of analytical grade (Merck, Germany).

Table 3 Sensory characteristics and quality parameters of fresh cold-pressed pumpkin seed oils

Oil sample	Sensory characteristics	PV (mmol/kg)	AV (mg KOH/g)	AnV (100 $A^{1\%}_{350nm}$)	IP (h)
Oil obtained from naked pumpkin seed A and seed with hull B					
1	Flavour: specific[1], very pleasant	$2.22 \pm 0.03^{\pounds,\S}$	$0.83 \pm 0.04^{\pounds,a}$	$3.00 \pm 0.05^{\pounds}$	$17.5 \pm 0.2^{\pounds,a}$
I pressing	Colour: light green ochre-reddish[2]				
2	Flavour: typical, very pleasant	$2.61 \pm 0.04^{\pounds,\S}$	$0.87 \pm 0.03^{\pounds,b}$	$3.25 \pm 0.15^{\pounds}$	$17.0 \pm 0.1^{\pounds,b}$
II pressing	Colour: dark green-reddish[2]				
3	Flavour typical, very pleasant	$1.94 \pm 0.06^{\pounds,\S}$	$1.13 \pm 0.01^{\pounds,b}$	$3.15 \pm 0.13^{\pounds}$	$17.1 \pm 0.1^{\pounds,b}$
Filtered	Colour: dark green-reddish				
Oil obtained from naked pumpkin seed A and seed with hull C					
4	Flavour: specific, very pleasant	$2.29 \pm 0.02^{\pounds}$	$0.90 \pm 0.01^{\pounds,a}$	$3.22 \pm 0.02^{\pounds}$	$17.3 \pm 0.2^{\pounds,a}$
I pressing	Colour: light green ochre-reddish				
5	Flavour: typical, very pleasant	$2.99 \pm 0.05^{\pounds}$	$1.05 \pm 0.03^{\pounds,b}$	$3.55 \pm 0.05^{\pounds}$	$16.9 \pm 0.3^{\pounds,b}$
II	Pressing Colour: dark green-reddish				
6	Flavour: typical, very pleasant	$2.99 \pm 0.03^{\pounds}$	$1.15 \pm 0.02^{\pounds,b}$	$3.46 \pm 0.03^{\pounds}$	$17.0 \pm 0.1^{\pounds,b}$
Filtered	Colour: dark green-reddish				
Oil from naked pumpkin seed A					
7	Flavour: specific, very pleasant	$1.13 \pm 0.02^{\S}$	$0.50 \pm 0.05^{\S,a}$	$1.02 \pm 0.15^{\S}$	$18.4 \pm 0.3^{\S,a}$
I pressing	Colour: light green ochre-reddish				
8	Flavour: typical, very pleasant	$1.96 \pm 0.04^{\S}$	$0.78 \pm 0.05^{\S,b}$	$1.00 \pm 0.13^{\S}$	$18.2 \pm 0.2^{\S,b}$
II pressing	Colour: dark green-reddish				
9	Flavour: typical, very pleasant	$1.58 \pm 0.01^{\S}$	$0.85 \pm 0.02^{\S,b}$	$0.99 \pm 0.01^{\S}$	$18.2 \pm 0.1^{\S,b}$
Filtered	Colour: dark green-reddish				

[1] Specific = mild fruity, raw (nonroasted) pumpkin seed-like

[2] Concomitantly with a red fluorescence

[£,§] Superscript letters within columns indicate significant differences ($p < 0.05$) among the pressed materials

[a,b] Superscript letters within columns indicate significant differences ($p < 0.05$) among the oil samples

Statistical Analysis

The experimental values were expressed as the means of three determinations. Statistical analysis was performed using the Statistica 8 software package. Statistical differences between the oil samples were estimated by applying two-way ANOVA and using the Tukey test at a significance level of 5% (*$p < 0.05$*). The multiple regression method was used for model determination.

Results and Discussion

Sensory and Chemical Characteristics and Oxidative Stability of Fresh Cold-Pressed Pumpkin Seed Oils

The results of sensory evaluation, basic chemical characteristics, and induction periods of nine freshly pressed oil samples of pumpkin seeds are presented in Table 3.

The sensory characteristics of cold-pressed pumpkin seed oil, both flavour and colour, are specific and "unique". The oil's flavour and taste was mild and fruity (as raw pumpkin seeds), without any "roasted" taste. The odour of all samples was also specific, but rather mild and poorly expressed. Literature data on odour components of cold-pressed pumpkin seed oil are lacking, so that we cannot compare our data with the findings that have been reported for virgin oils. However, oils obtained from roasted seeds have been analysed by a number of authors. Matsui et al. [15] identified 24 active odoriferous components, attributing the characteristic flavour of virgin pumpkin seed oil to pyrazines. The furans—2-pentylfuran—generally contribute to a "roasted" smell (caramel like) [16]. Siegmund and Murkovic [17], and Haiyan et al. [18] also analysed the volatile compounds profile of roasted pumpkin seed oil, and obtained different results. Namely, the latter authors found significantly fewer volatiles than the former ones. This is not surprising, given the importance of roasting temperature to the production of volatiles, and particularly those derived from pyrazine. In view of the fact that aroma (odour and taste) of our oils is different and much less expressed compared to those studied by these authors, it can be concluded that the mentioned compounds are absent, or present to a much smaller extent in our oil samples.

As far as the flavour is concerned, no difference was observed between oil samples obtained after the first and second pressing, i.e. filtration. Also, the addition of seeds with hulls (as a drainage material) had no influence on the oil flavour. However, some differences in the oil colour were observed. Namely, the colour of oil samples obtained after the first pressing of prepared seed material (samples 1, 4 and 7) was light green and orange-reddish, while the samples after the second pressing, i.e. the filtered oils, were darker green and concomitantly showed red fluorescence. The viscous oils were dark red in colour, depending on the thickness of the sample. They appeared red in a thick layer, but green in a thin one.

The peroxide value of oil samples 1–6, obtained from the mixture of naked seeds and seeds with hull, was between 2 and 3 mmol/kg. This value for the oil samples 7–9, obtained from naked pumpkin seeds, was the lowest (1.13 mmol/kg) after the first pressing, significantly higher (1.96 mmol/kg) after the second pressing, while for the filtered oil it was somewhere in between (1.58 mmol/kg). The fact that the peroxide value was always lower in oil samples obtained by the first pressing than in the oils after the second pressing is most likely due to somewhat higher cake pressing temperatures (46 and 49.5 °C). Oil filtration resulted in a certain decrease in the peroxide value.

The acid value ranged from 0.50 to 1.1 mg KOH/g, and the lowest value was always measured after the first pressing. After the second pressing, and after filtration, the acid value was slightly higher. This increase might be a result of the activity of lipolytic enzymes, bearing in mind the pressing (42–49.5 °C) and filtration (around 22 °C) temperatures.

The *p*-anisidine values ranged from 0.99 to 1.2 for the oil samples from naked pumpkin seeds (7–9), and from 3 to 3.55 in oil samples from mixed pumpkin seeds (1–6). Higher values were most probably due to the lipoxygenase activity. According to the results obtained by Al-Khalifa [19], lipoxygenase activity of crude pumpkin seed was 6,000 units per gram, while the residual activity of roasted seeds was 60%.

The oxidative stability of fresh cold-pressed oil samples, expressed by the induction period, was in the range of 16.9–18.4 h, and this classifies pumpkin oil in the group of stable edible oils. However, the induction period of pumpkin seed oil obtained from roasted seeds is significantly longer, 31.2 h [20].

Summarising the results presented in Table 3, it can be concluded that the highest quality cold-pressed oil is obtained by the first pressing of pumpkin seeds on a screw press, as the acidity and peroxide values of the oil are the lowest, and the induction period is the longest. This has been proven for both the pressing of naked seeds and pressing of naked seeds with addition of seeds with hull. A similar observation holds also for the production of virgin oil by pressing roasted seeds on a hydraulic press. In Austria, for example, only the highest quality oil from the first pressing is allowed to be sold as the brand "Styrian virgin pumpkin seed oil", and is classified as a product protected in its geographical origin by European law [4, 21].

It was noticed too, that the highest quality oil was obtained by pressing only naked pumpkin seeds. This has been proven by statistical analysis of the results presented in Table 3. To our knowledge, no data is available on the influence of hull on the quality of pumpkin seed oil. However, the negative influence of the presence of hull during the processing of virgin sunflower oil was confirmed [3].

Evaluation of Sensory and Chemical Characteristics and Oxidative Stability of Cold-Pressed Pumpkin Seed Oils During Storage

Considering the high price of raw pumpkin seeds, most facilities which use screw presses at lower temperatures apply "double pressing", aiming to increase the yield of oil extraction. The mixture of oils after the first and second pressing is sold to the market. This was the reason why the change of quality and stability was investigated in samples 3, 6 and 9, i.e. in the filtered oils. The fatty acid composition was also determined in these samples, since the shelf life of oil depends on this characteristic (Table 4).

The results of the flavour evaluation of oil samples during storage are presented in Fig. 1.

The changes in the sensory quality of edible oils during storage are unavoidable. However, as presented in Fig. 1, no change of flavour was observed during the first 6 months. After that period, a certain loss of aroma was noticed as it became very mild. The freshness of all samples changed after 18 months, and the flavour became musty. No differences in colour, assessed visually, were observed.

The data presented in Table 5 demonstrate a significant change in the oil acidity during storage. The greatest changes took place during the first 3 months, when the acid values increased more than twice in all three oil samples. Afterwards, until the end of the investigations, the changes in acidity were slower, and after 6 months they were not statistically significant. At the end of the storage period, the acid values of filtered oils were 3.05–3.64 mg KOH/g, and these values were lower than the maximum value permitted for cold-pressed and virgin oils prescribed by the Codex standards for the named vegetable oils [10].

The content of primary oxidation products, expressed by the peroxide value, is an important quality characteristic susceptible to changes during storage and directly affecting

Table 4 Fatty acid composition of pumpkin seed oil obtained with a screw press

Fatty acids*	Sample 3£	Sample 6£	Sample 9£
14:0	0.08 ± 0.01d	0.09 ± 0.01d	0.1 ± 0.01d
16:0	11.88 ± 0.78c	11.15 ± 0.65c	10.21 ± 0.55c
18:0	4.45 ± 0.65cd	5.15 ± 0.48cd	4.54 ± 0.31cd
18:1	30.35 ± 3.69a	33.49 ± 2.08a	42.07 ± 2.32a
18:2	52.15 ± 4.31b	49.77 ± 3.37b	43.68 ± 4.63b
18:3	0.26 ± 0.10d	0.21 ± 0.05d	0.16 ± 0.01d
20:0	0.33 ± 0.01d	0.30 ± 0.00d	0.28 ± 0.01d
MUFA	30.35	33.49	43.68
PUFA	52.41	49.98	42.23
PUFA/MUFA	1.73	1.49	1.04

* Wt% of total FA, mean ± SD ($n = 3$)

Different superscript letters in the first row (£) and columns (a, b, c, d) indicate significant differences ($p < 0.05$)

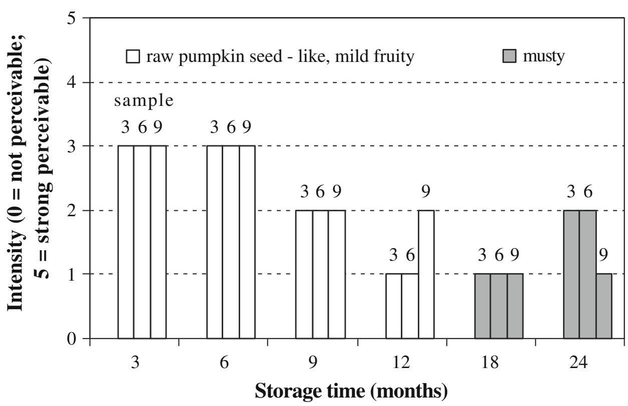

Fig. 1 Results of the flavour evaluation of cold-pressed pumpkin seed oil during storage

the stability of the oil. During storage, the PV constantly increased in all three samples (Table 5); the most significant changes became noticeable after 18 months. However, a statistically significant difference was observed between samples 6 and 9. The lowest PV values were detected in oil processed by pressing solely naked pumpkin seeds (sample 9), whereas the highest values (over 5) were recorded for sample 6, in the 12–24 month period.

The *p*-anisidine value, in combination with the peroxide value, is often used to determine total oxidative value (Totox), which is considered a very suitable indicator of the quality of oil. The Totox values of oil samples for the whole investigation period are also presented in Table 5. During the storage period, the Totox values also changed to a certain extent, and sample 9 exhibited a significantly different behaviour from samples 3 and 6.

As expected, the oxidative stability of the oil samples decreased with time (Table 6; Fig. 2). The smallest changes were noticed in the first 6 months: the induction periods decreased by 4, 5 and 8% for sample 9, 3 and 6, respectively. The most stable appeared to be the oil from naked seeds (sample 9), whose induction period decreased by 25% in the course of 2 years, while the respective values for samples 3 and 6 decreased by 40 and 30%. The statistical analysis of data in Table 6 by two-way ANOVA, where the categorical predictors were sample number and storage time (month) and the dependant value the induction period, showed a strong correlation between the storage time and the decrease in the induction period ($R^2 > 0.98$ for samples 3 and 9, $R^2 > 0.86$ for sample 6).

Parry et al. [22] reported the oxidative stability index of roasted pumpkin seed oil at 80 °C to be 61.7 ± 2.1 h (at an air flow of 7 L/h). According to Murkovic and Pfannhauser [13], the mean value of the induction period at 120 °C was 6.83 h. The differences between the pumpkin oil quality and shelf life may have been due to the production process or the quality of the raw material. Thus, Fruhwirth and Hermetter [4] suggested the antioxidant capacity of Styrian pumpkin seed oil as a novel and important criterion for quality control with respect to the stability and shelf life of this oil.

Sample 3: $y = -0.3067x + 17.42$; $R^2 = 0.9838$
Sample 6: $y = -0.2217x + 16.56$; $R^2 = 0.8663$
Sample 9: $y = -0.2017x + 18.32$; $R^2 = 0.9893$

Besides, the oxidative stability of oil depends on the fatty-acid profile. According to the fatty acid composition, the pumpkin seed oil belongs to the group of oleic-linoleic type of oils, as can be seen from Table 4.

The content of these two dominant fatty acids in the final oil samples was 30.35–42.07% for oleic and 43.68–52.15% for linoleic acid. The content of linolenic acid was 0.16–0.26%. Generally, it is accepted that the higher the degree of unsaturation of an oil, the more susceptible it is to oxidative deterioration. The highest PUFA/MUFA ratio (1.73) was found for sample 3, characterised by the greatest decrease in the induction period during the storage. At the same time, the quality of the raw material probably had a high impact on the oil stability, since the seeds with hull B had the highest PV and a high AnV value (Table 1). The ratio PUFA/MUFA for the oil obtained using only naked seeds was 1.04. This oil had a similar ratio of the contents of oleic and linoleic acids.

It is obvious from the data analysis (Tables 4, 6) that the PUFA/MUFA ratio of each sample is not correlated with the storage times of 6 ($R^2 < 0.13$) and 12 ($R^2 < 0.5$) months. However, there is a very strong correlation for the storage times of 18 ($R^2 > 0.99$) and 24 ($R^2 > 0.9$) months. When we applied multiple linear regression, the dependant value—decrease of IP for 18 and 24 months, where the independent values are storage time and PUFA/MUFA

Table 5 Change of AV, PV and Totox value of cold-pressed pumpkin seed oil during storage

Storage time (months)	AV (mg KOH/g)			PV (mmol/kg)			Totox value		
	Sample			Sample			Sample		
	3§,*	6§	9§	3§,†	6†	9§	3†	6†	9§
0	1.13a	1.15a	0.85a	1.94a	2.99a	1.58a	7.03a	9.44a	4.15a
3	3.01ab	2.55ab	2.12ab	3.23ab	4.01ab	2.17ab	9.56ab	11.47ab	5.23ab
6	3.27b	3.02b	2.75b	4.44ab	4.57ab	3.25ab	12.05ab	12.64ab	7.52ab
9	3.37b	3.13b	2.90b	4.25ab	4.76ab	3.77ab	11.64ab	13.07ab	8.84ab
12	3.32b	3.08b	2.95b	4.32ab	5.11ab	3.41ab	11.84ab	13.80ab	8.29ab
18	3.76b	3.56b	2.90b	4.76b	5.56b	3.51b	12.76b	14.68b	8.51b
24	3.64b	3.38b	3.05b	4.64b	5.38b	3.84b	13.13b	14.31b	9.37b

* Different superscript letters in this row indicate significant differences ($p < 0.05$) among the oil samples

Different superscript letters within columns indicate significant differences ($p < 0.05$) in oils during storage

Table 6 Decrease of IP (h) of cold-pressed pumpkin seed oil during storage

Storage time (months)	Sample 3£		Sample 6£		Sample 9£	
	IP	Share[1]	IP	Share	IP	Share
0	17.1 ± 0.2a	100	17.0 ± 0.0a	100	18.2 ± 0.1a	100
6	16.2 ± 0.1a	94.73	15.6 ± 0.3a	91.76	17.4 ± 0.1a	95.6
12	13.5 ± 0.3b	78.94	12.5 ± 0.2b	73.53	15.8 ± 0.2b	86.81
18	11.8 ± 0.1b	69.01	12.5 ± 0.3b	73.53	14.5 ± 0.0b	79.67
24	10.1 ± 0.4b	59.06	11.9 ± 0.1b	70.00	13.6 ± 0.5b	75.72

Data are reported as means ± SD ($n = 3$)

Different superscript letters in the first row (£) and columns (a, b) indicate significant differences ($p < 0.05$)

[1] Share (%) compared to the initial value

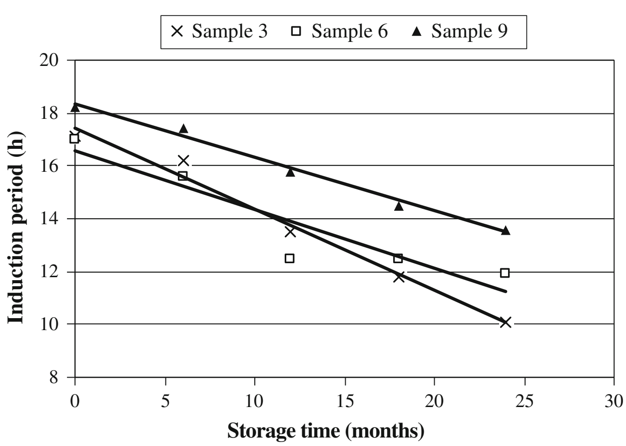

Fig. 2 Change of the induction period of cold-pressed pumpkin seed oils during the 24 month period

ratio, we obtained a very strong ($R^2 > 0.9$) model: IP = 118.72 ST–19.30 PUFA/MUFA (ST—storage time in months). This model can be slightly improved by applying an exponential transformation to the PUFA/MUFA values ($R^2 = 0.945$), which becomes: IP = 101.45 ST—0.32 exp(2.485 PUFA/MUFA).

According to data of Fruhwirth and Hermetter [4], the mean value of oleic acid content of 100 different breeding lines of Styrian oil pumpkin plants was 33.3%, and of linoleic acid 48.6%. Haiyan et al. [18] found 37.7% of oleic acid and 44.0% of linoleic acid in cold-pressed pumpkin seed oil, while Younis et al. [23] found that the content of oleic acid ranged from 28.3 to 34.0%, and that of linoleic acid from 43.0 do 50.3%. According to Neđeral Nakić et al. [24], the oleic acid content in oil samples of six different pumpkin seeds from factories in West Slovenia and North Croatia was 35.12 ± 4.31%, and of linoleic acid 46.58 ± 4.41%. The contents of the same fatty acids in the oil from seeds with hull were 30.46 ± 2.08 and 51.51 ± 2.19%.

In considering the oxidative stability of oil, the presence of some natural compounds with antioxidative capacity is a very important factor. Tocopherols act as antioxidants by trapping the hydroperoxide intermediates and stopping the autoxidation chain reaction. According to the chemical structure, γ- and δ-tocopherols are more effective than α-tocopherol [25]. Tuberoso et al. [26] determined the antioxidant activity and antioxidant compounds in commercial oilseeds for food use and concluded that the variability of the correlation between the antioxidant activity and the composition of the oilseeds could be attributed to the differences in the contents of squalene, chlorophylls, carotenoids, and phenolics of the oils and their mutual interactions. They found that pumpkin seed oil showed a preponderance of γ-tocopherol (more than 78% of total tocopherols). Fruhwirth et al. [27] suggested that 59% of the antioxidant capacity of pumpkin seed oil is due to the polar phenolics, and only 41% to tocopherols. According to Andjelkovic et al. [28], there is no strong correlation between the phenolic content and the OSI values in pumpkin seed oil. In view of these facts, the presence and interaction of some relevant minor compounds in cold-pressed pumpkin seed oil should be further investigated, to get an insight into its oxidative stability.

Conclusion

Cold-pressed pumpkin seed oil obtained by pressing the seeds with a screw press is characterised by a specific and pleasant flavour of raw, non-roasted seeds. It is dark green-reddish in colour and of a very good chemical quality. The oil samples fulfill the quality demands prescribed by legal regulations for a 2-year period. However, for the highest quality of oil, a 12-month shelf life is recommended.

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