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Optical analysis, antioxidant activity and microbiological quality of cold-pressed pumpkin oil

Optička analiza, antioksidativna aktivnost i mikrobiološki kvalitet hladno-ceđenog tikvinog ulja

Vesna ĐUROVIĆ, Mirjana RADOVANOVIĆ (✉), Dalibor TOMIĆ, Miloš MARJANOVIĆ, Leka MANDIĆ

University of Kragujevac, Faculty of Agronomy in Čačak, Cara Dušana 34, Čačak, Serbia

✉ Corresponding author: mira.radovanovic@kg.ac.rs

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ABSTRACT

Consumer interest in minimally processed food, including cold-pressed oil, is growing year by year, along with evidence of the positive effects of various bioactive ingredients that promote health. This study aimed to characterize the cold-pressed oils obtained from non-intensively grown pumpkins. Some optical characteristics, content of total phenols and antioxidant activity were determined, as well as microbiological analysis of the obtained oils. Cold-pressed pumpkin oils differ in the ranges of specific absorbances: 3.40–7.04 at 232 nm and 1.96–3.33 at 270 nm. The refractive index at 23 °C varies from 1.4700 to 1.4730. The content of total phenols in pumpkin oil ranged from 1.83 to 4.56 mg GAE (gallic acid equivalent)/100 mL. The results of DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities, expressed as % inhibition in the lipophilic fraction from pumpkin oil ranged from 42.48% to 66.91%. The number of bacteria and molds determined was by the Regulations, and sulfite-reducing bacteria were not detected in the oils. Cold-pressed oils are a good source of antioxidants and their use is beneficial for human health.

Keywords: cold-pressed oil, *Cucurbita pepo*, refractive index, specific absorbance, antioxidants, microbiological quality

SAŽETAK

Interesovanje potrošača za minimalno prerađenom hranom, među kojima i za hladno ceđenim uljem raste iz godine u godinu uporedo sa dokazima o pozitivnim efektima različitih bioaktivnih sastojaka koji promovišu zdravlje. Cilj ovog rada bio je da se okarakterišu hladno ceđena ulja dobijena iz neintenzivno gajenih tikvi određivanjem nekih optičkih karakteristika, sadržaja ukupnih fenola i antioksidativne aktivnosti, kao i mikrobiološke ocene dobijenih ulja. Rezultati su pokazali da indeks refrakcije tikvinog ulja varira od 1,4700 do 1,4730. Hladno ceđena ulja su se razlikovala u opsegu specifičnih apsorbancija na 232 i 270 nm: 3,40–7,04 na 232 nm i od 1,96–3,33 na 270 nm. Sadržaj ukupnih fenola u ulju iznosio je od 1,83 do 4,56 mg GAE/100 mL, a antioksidativna aktivnost, izraženi kao % inhibicije u lipofilnoj frakciji od 42,48% do 66,91%. Ukupan broj bakterija i plesni je bio u skladu sa regulativom, a sulfitoredukujuće bakterije nisu detektovane u ispitivanim uzorcima ulja. Hladno ceđena ulja su dobar izvor antioksidanata i njihova upotreba je korisna za ljudsko zdravlje.

Ključne reči: hladno ceđena ulja, *Cucurbita pepo*, indeks refrakcije, specifične apsorbance, antioksidanti, mikrobiološki kvalitet

INTRODUCTION

Cold pressing is one of the oldest oil extraction techniques that excludes heat treatment, refining, solvent extraction or the use of chemicals. Cold pressing has the great advantage of reducing the breakdown of bioactive and nutritional components of the oil such as phytosterols, phenols, tocols, fatty acids, vitamins, and carotenoids. Such oils are more attractive to consumers due to their nutritional and functional properties. Therefore, in recent years, cold pressing has become an increasingly popular technique for the production of various seed oils (Akin et al., 2018; Radovanović et al., 2022).

Pumpkin (*Cucurbita pepo*), belongs to the Cucurbitaceae family that grows widely in tropical regions and has a relatively high economic importance worldwide. The food industry uses pumpkin for the production of juices, purees, jams and alcoholic beverages. Pumpkin seeds contain some bioactive compounds that are often used as herbal medicines and functional foods. In addition, pumpkin seed oil has great importance in the fat and oil industry not only as an edible oil but also as a potential nutraceutical (Rezig et al., 2012). Because of their unique composition, seeds are considered the most important component of pumpkin. Apart from the high content of fats and proteins with essential amino acids, pumpkin seeds contain significant amounts of minerals such as potassium, magnesium, zinc, selenium, copper, molybdenum, and bioactive compounds such as tocopherols and carotenoids, pigments, squalene, sterols, phytosterols and phenolic compound (Akin et al., 2018; Özbek and Ergönül, 2020). However, the seed is also called an oilseed because of its very high oil content. Pumpkin seed oil contains about 98% triglycerides and 70% of all fatty acids are unsaturated. The main fatty acids are linoleic, oleic, stearic and palmitic acids. The importance of many of these compounds, especially tocopherols and phenolic compounds in pumpkin seed oil is reflected in their antioxidant effect and beneficial effect on human health (Andjelkovic et al., 2010).

The microbiological quality of oil is a very important issue because various pathogenic bacteria and yeasts can persist in these products (Zullo et al., 2018; Đurović et al., 2022a). It is recommended to consume unrefined oils without any thermal treatment, but there is the potential risk of introducing pathogenic bacteria and their toxins.

Therefore, the research aimed to determine the optical characteristics, antioxidant properties and microbiological quality of cold-pressed pumpkin oils.

MATERIALS AND METHODS

Collection and preparation of samples

Pumpkin samples were collected evenly from Central and Western Serbia during the 2021 harvest season. The pumpkin seeds were separated from the pumpkin fruit by hand, dried to storage humidity, and then stored in closed paper bags at ambient temperature. Whole seeds were ground in a blender before pressing. Twelve pumpkin oil samples (P1–P12) were analyzed.

Cold-pressed oils

The oil was obtained by pressing grounded whole pumpkin seeds on a screw press OP650W (Gorenje, Slovenia). The temperature during pressing did not exceed 50 °C. Collected oils were stored at 4 °C overnight to precipitate the sediment. Oils were separated by decantation. The procedure for obtaining pumpkin oil is shown in Figure 1.

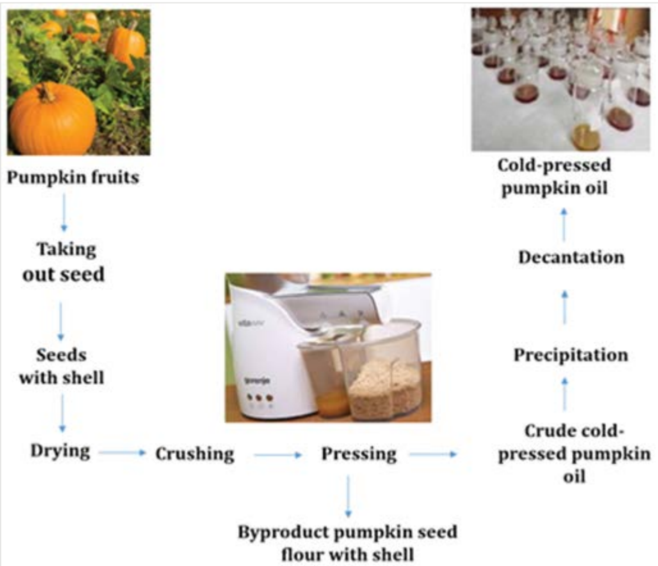

Figure 1. Obtaining cold-pressed oils of pumpkin seeds

Determination of moisture and volatile matter

Determination of the content of moisture and volatile matter was made according to the standard method (ISO 662:1980; Dimić and Turkulov, 2000). The result was expressed as the content of moisture and volatile matter in % (w/w).

Optical characteristics of pumpkin oils

Obtained oils were characterized by measuring the refractive index (RI), specific absorbances of the oil and recording characteristic spectra in the UV/Vis part of the spectrum as the most significant optical properties.

The RI of pumpkin oil was measured using a standard method (ISO 6320:1995; Dimić and Turkulov, 2000), with an Abbe refractometer (A. KRUSS, Germany), range of 1.3000–1.7000 ± 0.0001, and a monochromatic Na light (589.6 nm). Specific absorbances were determined on a UV-visible spectrophotometer (Cari 3000, Agilent, USA) using the standard method (ISO 3656:1989; Dimić and Turkulov, 2000). Absorbances were measured at 232 nm and 270 nm against n-hexane (Carlo Erba, France) as a blank. To measure the spectra, the oils were dissolved in n-hexane in a ratio of 1:9. Measurement of spectra was performed on a UV/Vis spectrophotometer from 200 to 800 nm with data interval 1 nm and scan rate 600 nm/min according to method described of Đurović et al. (2024) and using Cary WinUV software.

Antioxidant properties

Sample preparation for the determination of total phenols and antioxidant activity was as follows: 1000 µL of oil was mixed with 1000 µL of methanol (80%), and centrifuged (5000 rpm, 10 min) to separate the hydrophilic (HF) and lipophilic fractions (LF). The total phenolic content (TPC) was determined in the hydrophilic fraction, and the antioxidant activity was determined in both the hydrophilic and lipophilic fractions (Đurović et al., 2024). The Folin-Ciocalteu colorimetric method was used to determine the total phenolic content (Singleton et al., 1965). The results were expressed as milligrams of gallic acid equivalents per 100 mL oil (mg GAE/100 mL). Spectrophotometric analysis with 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to determine the antioxidant activity of the oil. Results were expressed as a percentage of inhibition of the DPPH radical.

Microbiological evaluation of oils

Determination of the number of aerobic mesophilic bacteria and molds and the presence of sulfite-reducing bacteria (SRC) were determined using standard microbiological methods (ISO methods 4833 and 21527-1).

Statistical analysis

Experimental data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the least significant differences (LSD) test which was used to detect significant differences among the means. The level of significance was assigned at $P < 0.05$ for the content of total phenolics and antioxidative activity. The statistical analyses were performed using the program STATISTICA 12 (StatSoft, Inc. 2012).

RESULTS AND DISCUSSION

Moisture content and volatile compounds in the oil

The moisture and volatile compounds content of pumpkin oils was in the range of 0.08 to 0.22% (w/w) (Figure 2). Two pumpkin oil samples (P2 and P9) had a higher content than the maximum value (0.2%) defined for unrefined edible oils (Codex Alimentarius Commission, 1999).

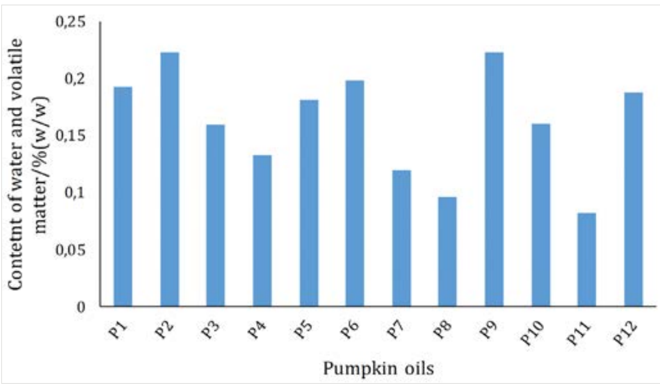

Figure 2. Content of moisture and volatile matter in cold-pressed pumpkin oils

Optical characterization

Pumpkin oils show significant color variations among samples: different shades of yellow, green and red (Figure 1). It is important to note that in addition to the botanical species, cultivar and geographical origin can also play a decisive role in the variability of the color of cold-pressed oils (Turrini et al., 2021).

According to the values of the RI the oil samples were grouped (Table 1). Values of RI values varied from 1.4700 to 1.4730, with a different distribution of the number of samples per group. Srbinoska et al. (2012) report an RI for extracted oils from whole pumpkin seeds, measured at 25 °C was 1.470. Similar values of RI for different cultivars of cold-pressed pumpkin seed oil were measured by Arslan et al. (2017) and values ranging from 1.475 to 1.480, at a temperature of 25 °C.

Table 1. Refractive index of cold-pressed oils from different pumpkin samples

Cold-pressed pumpkin oil	
Samples	RI (23 °C)
P8, P9, P10, P11	1.4700
P2, P4, P5	1.4705
P1, P3	1.4710
P7, P12	1.4715
P6	1.4730

* RI - Refractive Index

Cold-pressed pumpkin oils differ in the ranges of specific absorbances (Figure 3). There are significant variations in specific absorbances among pumpkin samples. The values of specific absorbances for pumpkin oils varied from 3.40–7.04 at 232 nm, and from 1.96–3.33 at 270 nm. Arslan et al. (2017) showed also differences of specific absorbances of pumpkin seed oil among varieties: 3.59–4.10 at 232 nm and 2.07–2.41 at 270 nm. Furthermore, Gohari et al. (2011) recorded specific absorbances of pumpkin oil at 4.8 and 3.52 at 232 and 270 nm, respectively.

The presence of primary oxidation products can be indicated by specific absorption at 232 nm, while absorption at 270 nm gives insight into possible secondary products of oil oxidation (Đurović et al., 2024).

Samples from P8 to P12 had a lower absorption value, so it can be assumed better oxidative stability of these oils. It is important to note that pumpkin oils are generally characterized by high values of specific absorption that are not caused by oxidative changes, which is confirmed by the literature data (Gohari et al., 2011; Arslan et al. 2017).

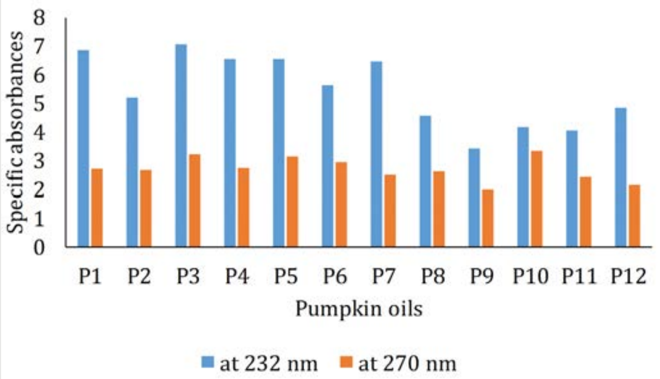

Figure 3. Specific absorbances of cold-pressed pumpkin

The spectra of pumpkin seed oil have pronounced peaks in the visible part of the spectrum, which are the result of the presence of the plant pigments (Figure 4 a, b, c). Fruhwirth and Hermetter (2008) noticed a similar spectrum for pumpkin seed oil. The spectra of pumpkin seed oils showed clear differences so all tested pumpkin oil samples were divided into three groups (Figure 4 a, b, c). For the grouping, the shape of the oil spectrum in the area from 420 to 440 nm was a discriminating parameter. Absorption maximum at 424 and 434 nm originate from carotenoids (Vorobyova et al., 2014).

For sample P7 (Figure 4a) the intensity of the peak at 440 nm is higher than the peak at 420 nm, while for the samples P1, P2, P9, P10, P11 and P12 (Figure 4 b) the order of peak maximum is reversed. The spectra for samples P3, P4, P5, P6 and P8 (Figure 4 c) are completely different, there are no clear absorption maximum characteristics of carotenoids. The shape of these spectra may indicate the carotenoid degradation in the oil samples.

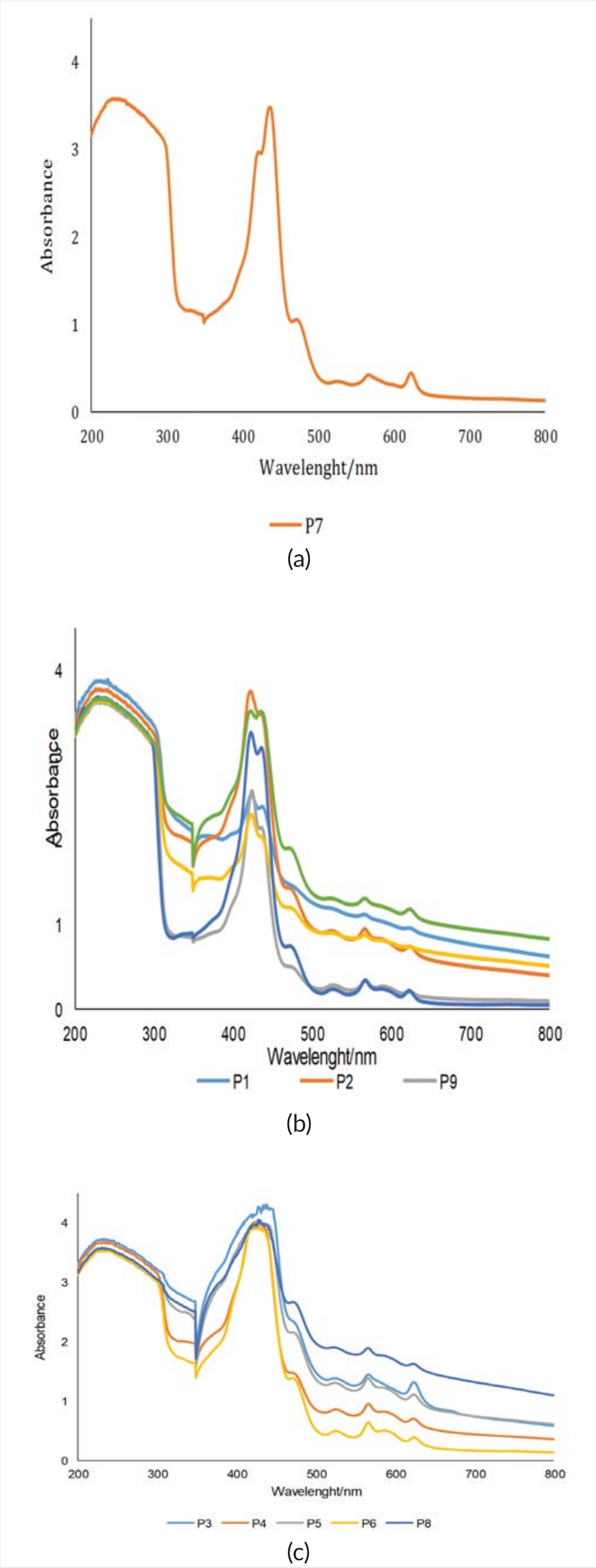

Figure 4. UV-Vis spectra of cold-pressed pumpkin oils

Lankmayr et al. (2004) have also grouped pumpkin oils based on the intensity of these specific peaks correlated with the sensory evaluation of the oil.

Total phenolic content and antioxidant activity of pumpkin oils

Cold-pressed pumpkin oils are characterized by a very diverse content of phenolic compounds. The content of total phenols in pumpkin oil in this study ranged from 1.83 (P5) to 4.56 mg (P9) GAE /100 mL (Table 2).

It can be seen that the pumpkin oil samples differed in total phenolic content. The obtained results are correlated with the results of other authors who state that the total phenolic content in the oil depends on many factors, the cultivar, the climatic and cultivation conditions, methods of obtaining oil, packaging or storage (Boskou et al., 2005; Kulaitienė et al., 2017). According to the literature, the content of total phenolics is mainly related to the oil from roasted pumpkin seeds and is reported to range from 0.28 to 2.46 mg GAE/100 g (Tuberoso et al., 2007; Siger et al., 2008). Akin et al. (2018) recorded values content of total phenolics (3.96–5.82 mg GAE/100 g), while Jiao et al. (2014) reported content from 7.33 to 128.84 mg GAE/kg.

The results of DPPH• radical scavenging activities, expressed as % inhibition, for pumpkin oil, are shown in Table 2. The antioxidant activity of the pumpkin oil varied among tested samples. The % inhibition in the lipophilic fraction ranged from 42.48% (P8) to 66.91% (P5), while in the hydrophilic fraction of pumpkin oil, the % inhibition ranged from 11.03% (P2) to 15.04% (P4). The ratio LF/HF in pumpkin oil in our study ranged from 3.38 (P7) up to 5.84 (P5). Similar results were recorded by Prescha et al. (2014) who measured that the antioxidant activity ratio LF/HF of pumpkin oil was from 2.88 to 3.38. It can be concluded that the tested cold-pressed oils had higher antioxidant activity in lipophilic fraction compared with hydrophilic. Espin et al. (2000) and Prescha et al. (2014) also reported that lipophilic antioxidants such as tocopherols are significantly more present in oils compared to hydrophilic antioxidants such as phenolic compounds.

Table 2. The content of total phenolics, antioxidative activity of the cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils

Oil sample	TPC mg GAE 100 mL^{-1}	DPPH % Inhibition LF	DPPH % inhibition HF	Ratio LF/HF
P1	2.68 ± 0.26^{d}	57.37 ± 6.04^{b}	13.33 ± 1.40^{abc}	4.30
P2	3.99 ± 0.10^{ad}	45.67 ± 6.74^{cde}	11.03 ± 0.32^{c}	4.14
P3	3.07 ± 0.31^{cd}	46.71 ± 3.34^{cde}	13.11 ± 1.44^{abc}	3.56
P4	2.75 ± 0.07^{d}	61.36 ± 4.40^{ab}	15.04 ± 4.18^{a}	4.08
P5	3.54 ± 0.37^{bc}	66.91 ± 3.72^{a}	11.45 ± 0.04^{c}	5.84
P6	2.58 ± 0.50^{de}	57.72 ± 0.61^{b}	11.30 ± 0.19^{c}	5.11
P7	2.61 ± 0.30^{d}	48.82 ± 5.56^{cde}	14.43 ± 1.25^{ab}	3.38
P8	1.83 ± 0.25^{e}	43.48 ± 2.59^{e}	12.65 ± 1.15^{abc}	3.44
P9	4.56 ± 1.86^{a}	46.96 ± 3.03^{cde}	13.04 ± 1.62^{abc}	3.60
P10	2.86 ± 0.16^{cd}	51.55 ± 0.06c	12.21 ± 0.78^{bc}	4.22
P11	2.95 ± 0.17^{cd}	50.78 ± 0.85^{cd}	12.51 ± 0.58^{abc}	4.06
P12	2.98 ± 0.19^{cd}	45.10 ± 0.73^{de}	13.58 ± 0.56^{abc}	3.74

Hydrophilic (HF) and lipophilic fractions (LF)
Results are reported as mean value ± standard deviation of three replicates (n = 3)
$^{a\text{-}d}$ Means with the different superscripts within each column are significantly different ($P < 0.05$)

The antioxidant potential of vegetable oils is directly related to the content of phenolic compounds, tocols and carotenoids (Latif and Anwar, 2011). Literature data show that there is a correlation between the content of total phenols and antioxidant activity (Đurović et al., 2021; Đurović et al., 2022b). The presence of natural bioactive compounds with antioxidant activity is a vital factor for the oxidative stability of oils. Important factors that determine the content of antioxidant compounds are variety, genetic factors, extraction method and solvent, production and storage conditions of seed oil (Nawirska-Olszanska et al., 2013).

Microbiological evaluation of pumpkin oils

In all tested pumpkin oil samples, the total number of bacteria and molds was lower than the limit values prescribed in the regulation "Guide to the application of microbiological criteria for food" (Ministry of Agriculture, Forestry and Water Supply Trade, 2011). The presence of sulfite-reducing bacteria was not detected in pumpkin oil (Table 3).

Table 3. Microbial evaluation of pumpkin oils

Oil	Bacteria	Molds	SRC
P1	10–100	<10	nd
P2	>100	10–100	nd
P3	10–100	<10	nd
P4	10–100	<10	nd
P5	10–100	10–100	nd
P6	>100	<10	nd
P7	>100	<10	nd
P8	10–100	<10	nd
P9	>100	<10	nd
P10	>100	15	nd
P11	>100	10	nd
P12	>100	<10	nd

n.d.- not detected
SRC - sulfite-reducing bacteria

In products and raw materials, there can be microbial contamination from various sources, especially soil and irrigation water (Mapanda et al., 2005; Doyle and Erickson, 2008). In addition, microbial contamination of fruits and vegetables is directly dependent on hygienic practices during their production, harvesting, handling, processing, storage and distribution of products (Heaton and Jones, 2008). Good agricultural and production practices are important in order for the final product to meet chemical and microbiological criteria.

CONCLUSIONS

All samples of oil, except for two samples (P2 and P9), had moisture and volatile matter content, of less than 0.2% which follows regulations for edible unrefined oils. The refractive index at 23 °C varied from 1.4700 to 1.4730 for pumpkin seed oils. Large variations were noted among pumpkin oils in specific absorbance ranges from 3.40 to 7.04 at 232 nm, and from 1.96–3.33 at 270 nm. Twelve cold-pressed pumpkin oil samples were categorized into three different groups based on the shape of the spectrum. Discriminating differences were in the visible part of the spectrum from 420 to 440 nm. The content of total phenols in pumpkin oil ranged from 1.83 to 4.56 mg GAE/ 100 mL. The lipophilic fraction of pumpkin oils showed a significantly higher antioxidant potential compared to the hydrophilic fraction. The total number of bacteria and molds was lower than the limit values prescribed in the “Guide to the application of microbiological criteria for food” and the presence of sulfite-reducing bacteria was not detected in pumpkin oil. Based on the obtained results, it is not possible to determine which oil has the best characteristics within all the tested properties, because the oils P2 and P9 had the highest content of total phenols, while the lipophilic fractions of the oils P4 and P5 showed the strongest antioxidant potential. On the other hand, based on the appearance of the spectra, it can be assumed that carotenoid degradation occurred in samples P3, P4, P5, P6 and P8.

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