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CHARACTERISTICS OF BLACKBERRY AND RASPBERRY SEEDS AND OILS

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This study is concerned with the determination of technological quality characterristics of dried pomaces, i.e. blackberry and raspberry seeds, along with the quality parameters, content of total carotenoids and chlorophyl and transparency of crude extracted oil (using organic solvent). Blackberry seeds (Rubus fruticosus L.*) were obtained from a domestic variety Čačanska bestrna, while the raspberry seeds (Rubus idaeus* L.*) were of the variety Willamette. Oil content of the blackberry pomace was 13.97 and 14.34%, while the oil content of the raspberry pomace was 13.44 and 14.33% on dry basis (d.b.). In regard to technological characteristics of the pomaces, i.e. volumetric and specific weight, no considerably difference was found. However, a weight test for 1000 seeds showed a significant difference in weight: 3.5 g (d.b.) for the blackberry pomace and 1.5 g for the raspberry pomace (d.b.). Proximate analysis of blackberry seed oil showed that this oil had better quality since the FFA value was 3.43% (sample B1) and 3.53% (sample B2), while the peroxide value was 8.89 and 11.16 mmol/kg, respectively. Raspberry seed oil had higher FFA (8.59 and 8.83% for sample R1 and R2) and peroxide values (13.99 and 13.84 for sample R1 and R2) than the blackberry seed oil. Crude extracted blackberry seed oil had a brown-greenish color due to the high total chlorophyll content (around 3000 mg/kg dissolved in cyclohexane). Raspberry seed oil had a dark yellowish-orange color, due to lower chlorophyll content (around 200 mg/kg) compared to the blackberry seed oil, while the content of total carotenoids was slightly higher in this oil (around 40 mg/kg) compared to the blackberry seed oil (33 mg/kg).*

KEY WORDS: blackberry and raspberry seeds, seeds' technological quality characterristics, pigments

INTRODUCTION

Our country is known as a significant producer of berries, primarily blackberries and raspberries. Serbia exported 78460 tons of fresh raspberries and 28313 tons of blackberries in 2011. However, significant amounts of berries are processed in the country.

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During the processing, predominantly in the fruit and beverage industry for juice and jam production, a large amount of their by-product (pomace, consisting mostly of the seeds) is produced. Since blackberry and raspberry seeds contain lipids, these by-products are very interesting as a raw material for oil manufacturing in small facilities. Namely, berry oils are considered to be specialty oils and have been in demand on the market. These oils have a unique fatty acid profile and they possess interesting minor components (1).

Berry fruits are rich in phenolic compounds such as phenolic acids, flavonoids, and anthocyanins. The phenolic compounds in berries have been reported to have an antioxidant, anticancer, antiinflamatory, and antineurodegenerative biological properties (2). Tosun et al. (3) have shown that raspberries are a significant source of phenolic compounds and ascorbic acid, while antioxidant activity was high and varied greatly amongst different genotypes. Godjevac et al. (4) have investigated blackberry seed extracts and showed that three isolated polyphenolic compounds exhibited considerable protective effects on human lymphocytes DNA.

The study by Parry and Yu (5) confirmed that the presence of n-3 fatty acid and antioxidants suggests potential value-added utilization of black raspberry seeds for preparing functional foods or supplemental products. Reyes-Carmona et al. (6) have investigated a range of blackberries of different cultivars and determined high antioxidant capacity values that were highly correlated with total phenols and anthocyanin content.

The objective of this paper was to investigate the technological quality: moisture, volatile matter, oil and impurities content in seed, weight of 1000 seeds, specific weight and weight per liter of the blackberry and raspberry pomace, obtained as by-product of the industrial processing of berries, as well as the color quality parameters (acid and peroxide value), content of total carotenoids and chlorophyl, and transparency of the extracted oil.

EXPERIMENTAL

Materials and methods

Blackbery and raspberry seeds. Blackberry seeds (*Rubus fruticosus* L.) were of the domestic variety *Čačanska beztrna,* while raspberry seeds (*Rubus idaeus* L.) were of the *Willamette* variety. Fresh berries were frozen and stored in plastic freezer bags for 8 months, after which they were processed to extract the juice. After pressing the berries, the pomace (average water content 50.02% - blackberry and 48,38% - raspberry) was collected and dried under these conditions:

Blackberry seeds:

Sample B1, the pomace was dried in a thin layer (thickness of approx. 1 cm) on paper at ambient temperature (22 ºC) for 72 h, with occasional stirring.

Sample B2, the pomace was dried in a thin layer in the oven in two stages: first at 63±2ºC for 20 h and then at 103±2ºC for 20 h.

Raspberry seeds:

Sample R1, the pomace was dried in a thin layer (thickness of approx. 1 cm) on paper at ambient temperature (22ºC) for 72 h, with occasional stirring.

Sample R2, the pomace was dried in a thin layer in the oven in two stages: first at 63±2ºC for 6 h and then at 103±2ºC for 4 h.

Blackberry and raspberry seed oil. Oil from dried blackberry and raspberry pomace was obtained using a standard laboratory method, extraction with hexane for 8 h. Upon completion of the extraction process the solvent was removed using a rotary vacuum evaporator at 70ºC.

Methods for seeds characterization. The quality of seeds was determined according to standard methods: moisture and volatile matter content in oilseeds (7), oil content in seeds (8) and impurities content (9). The weight of 1000 seeds was determined using a gravimetric method used for grains (10). Specific weight of seeds was determined by measuring the volume of the clean seed's weight. For volume determination, 60% ethanol solution was used. Specific seed volume (γ, g/ml) was calculated using the following equation: $\gamma = m/V_o - V$, where m was weight of seeds (g), V_o beaker's volume (ml), and V ethanol volume added to the beaker (ml). Weight per liter of seeds (kg/l) was determined using Shopper-s scale (10).

Methods for oil characterization. The peroxide value (PV), expressed in mmol/kg, was determined by the reaction of oil and 3:2 (chloroform to acetic acid) with potassium iodide in darkness. The free iodine was titrated with a thiosulfate solution (11). The acid value (AV), expressed in mg KOH/g, as well as free fatty acid content (FFA) expressed as % oleic acid, were determined by the titration of oil dissolved in a 1:1 ethanol to ether solution with an ethanolic solution of potassium hydroxide (12). Acidity was determined in the oil which was extracted by cold and hot extraction (13). Total carotenoid content was determined using a spectrophotometric method (14), by measuring the absorption of the oil dissolved in cyclohexane (1% solution) at 445 nm. Total chlorophyl content was determined by measuring the absorption of the oil solution in cyclohexane (1% solution) at 630 nm, 670 nm and 710 nm. In addition, oil absorption in chloroform (1% solution) was determined at 667 nm (15). In order to determine parameters for color characterization, transparency of oil solutions (1%) in chlorophorm and cyclohexane was measured at 455 nm. Spectrophotometric measurements were conducted using UV/VIS spectrophotometer, model T80+ (PG Instruments Limited, London).

Color determination. CIE *L*a*b** and CIE Y-xy graph color coordinates were determined using Minolta Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) in D-65 lighting, with a 2 angle and 8 mm opening. *L** - value is lightness, *a** - value represents red and green hue, while *b** - value represents yellow and blue hues. In CIE Y-xy tristimolous system, color characteristics are presented as a dominant wavelength - λ (nm) and color purity - P (%). Values: *L**, *a**and *b** were determined by reading, while the dominant wavelength was calculated based on the xy coordinates using a chromatic diagram.

All measurements were made in triplicates and the results were expressed as mean value ± standard deviation.

RESULTS AND DISCUSSION

Basic technological quality parameters of blackberry and raspberry seeds are shown in Table 1.

Table 1. Technological quality of blackberry and raspberry seeds

Parameter	Blackberry seeds		Raspberry seeds	
	B1	B2	R1	R2
Water content (%)	6.59 ± 0.07	5.24 ± 0.04	8.53 ± 0.02	6.08 ± 0.06
Oil content (%)				
- tel quel (as is)	13.05±0.05	13.59 ± 0.09	12.30 ± 0.13	13.46 ± 0.22
- on dry basis	13.97±0.05	14.34 ± 0.09	13.44 ± 0.13	14.33 ± 0.22
Impurities content (%)	4.68 ± 0.98	4.36 ± 1.11	10.06 ± 0.98	11.16 ±1.12
Pure seeds content (%)	95.32± 0.98	95.64 ± 1.11	89.94 ± 0.98	88.84 ± 1.12
Weight of 1000 seeds* (g)				
- tel quel (as is)	3.21 ± 0.05	3.32 ± 0.06	1.50 ± 0.07	1.45 ± 0.08
- on dry basis	3.45 ± 0.05	3.50 ± 0.06	1.64 ± 0.07	1.54 ± 0.08
Specific weight (g/ml)				
- pure seeds*	0.999±0.000	0.997 ± 0.010	0.968 ± 0.010	0.967 ± 0.000
- tel quel seeds (as is)	0.997±0.010	0.993 ± 0.010	0.964 ± 0.020	0.963 ± 0.010
Weight per 1liter (g/l):				
- pure seeds*	423.6 ± 0.4	429.2 ± 3.2	421.0 ± 1.0	419.2 ± 2.0
- tel quel seeds (as is)	384.8 ± 0.4	394.0 ± 1.6	375.8 ± 0.2	397.8 ± 2.2

*pure seeds were separated from the dry pomace by hand

Considering storage and oil extraction, water content is the most important characteristic of seeds as raw materials. Water content in dried seeds was pretty similar, ranging from 5.24 to 8.52%. It could be said that an equilibrium state of moisture was achieved using the mentioned drying conditions.

The most valuable macro component of the berry seeds is the oil. Results have demonstrated that investigated domestic blackberry and raspberry varieties were very similar in terms of oil content.

The oil content in the blackberry seed samples B1 and B2 was 13.97 and 14.34 % d.b., respectively, while in the raspberry seeds R1 and R2 it was 13.44 and 14.33 % d.b., respectively. Literature reviews have shown scarce data for oil content in blackberry seeds; the only value found was published by Kiss (16) and it was 14.7%, which is very similar to our results. Our results are in good agreement with published literature data for raspberry seed oil content too. According to Lampi and Heinonen (17) oil content was 10.7-23.2 %; Oomah et al. (18) reported 10.7% (d.b.), while Kiss (16) obtained 14%, and Šućurović et al. (19) 14.5% (at the water content of 9.7%).

The content of impurities in the blackberry seeds was low (4.5% of the total seeds weight), where 4% was dust, and the rest was organic plant material (dry fruit parts, thorns, membranes etc.), while the pure seeds content was about 95%. Raspberry seeds had higher impurity levels, 10.06 and 11.16% (with more than 10% dust), while the organic material quantity was negligible. The content of pure raspberry seeds was approximately 90 %.

The parameters such as the weight of 1000 seeds, specific weight and weight of 1 liter of seeds, are used to determine not only the seeds' quality, but also to help in the determination of volumetric seed weight for storage purposes. The weight of 1000 blackberry seeds was around 3.5 g, while for the raspberry samples the values were 1.54 and 1.64 g (d.b.), which means that blackberry seeds were bigger in size. The ratio between the weight of 1000 seeds of blackberries and raspberries was 2.29. Kiss (16) published similar results: 3.97 g for blackberry seeds and 1.42 g for raspberry seeds. According to Oomah et al. (18), the weight of 1000 blackberry seeds was 1.8 g. The specific volumetric (1 l) weight of pure seeds and tel quel (as is) seeds were pretty similar for both types of seeds. To the best of our knowledge, there are no published data on impurities content, or specific and volumetric weight of these seeds.

The most important quality parameters of berry seed oils are given in Table 2.

Table 2. Some quality parameters of blackberry and raspberry seed oils

Parameter	Blackberry seed oil		Raspberry seed oil	
	B1	B2	R1	R2
Acid value (mg KOH/g)				
- hot extraction	7.75 ± 0.22	8.23 ± 0.25	18.74 ± 0.26	17.65 ± 0.33
- cold extraction	6.85 ± 0.11	7.05 ± 0.30	17.18 ± 0.43	17.86 ± 0.51
FFA (% oleic acid) - cold extraction	3.43 ± 0.11	3.53 ± 0.30	8.59 ± 0.43	8.83 ± 0.51
Peroxide value (mmol/kg) - cold extraction	8.89 ± 0.12	11.16 ± 0.23	13.99 ± 0.44	13.84 ± 0.48

The acidity of the oils was determined after their hot extraction, as well as after cold extraction. The acid value of blackberry oil after hot extraction was in the range from 7.75 to 8.23 mg KOH/g, while of the raspberry seed oil it was higher, ranging from 17.18 to 18.74 KOH/g. The increase of the acidity of both seed types can be due to longer storage time of the berries. Namely, the frozen berries were stored for 8 months before they were used, so that it was likely that the oil hydrolysis took place by the action of lipase. Van Hoed et al. (1, 20) reported that blackberry seed oils had FFA values of 0.96 and 0.80%, while raspberry seed oils had FFA values of 0.49 and 0.69% (expressed as oleic acid). However, the oils in their study were obtained from the seeds originating from fresh berries. Šućurović et al. (19) investigated freeze-dried raspberry seeds and found an FFA value of 1.32%. Peroxide values, as indicators of the primary oxidation process, were higher in the raspberry (13.99 and 13.84 mmol/kg) than in blackberry seed oils (8.89 to 11.16 mmol/kg). Although these values are high for crude plant oils, it could be said that they are in the range of PV reported in other studies for berry seed oils (1, 20, 21). Based on the PV, it is shown that raspberry seed oil is more prone to oxidation than blackberry seed oil.

The color perceptions of the extracted berry seed oils are presented in Table 3. Visual presentation of oils is very important for their potential application in food and cosmetic products.

Based on the visual color evaluation, blackberry seed oil had a dark brown-greenish color with an orange hue (viewed as a thin oil layer). Raspberry seed oil was dark yellowish-orange in color. Hence, both oils were pretty dark in color and not clear at room temperature.

Table 3. Color characteristics of blackberry and raspberry seed oils

Oil type	CIE system		CIE *L*a*b** system		
	λ (nm)	P (%)	L* (%)	a*	b*
Blackberry B1	589.0	8.19	16.94±0.03	2.32±0.04	0.99±0.09
B2	588.0	8.09	16.84±0.02	2.30±0.03	0.90±0.04
Raspberry R1	580.5	24.56	20.43±0.06	3.23±0.01	7.01±0.01
R2	580.0	25.87	20.23±0.03	3.63±0.01	6.99±0.02

λ – dominant wave-length; P – color purity; L^* – lightness; a^* – red hue; b* – yellow hue

Table 3 shows that the color purity (clarity) of the blackberry seed oil was very weak (around 8%), where the color determined using the CIE *L*a*b**system confirmed orange instead of green hue. The discrepancy between the visual and instrumental determination of the oil color was due to very weak clarity of the oil color. Color clarity of the raspberry seed oil was three times higher (around 25%), while the dominant wavelength was in the yellowish-orange hue range. With the rise in color clarity (purity), the hue became saturated, and the raspberry oil color determined by the CIE *L*a*b** system was in agreement with the color determined visually.

Visual color perception is mainly a function of the pigment quantity present in the oil. Total content of carotenoids, chlorophyl, as well as the transparency of blackberry and raspberry seed oil is shown in Table 4.

Table 4. Content of total carotenoids, chlorophyl and transparency of blackberry and raspberry seed oil

Oil	Total carotenoids[a] (mg/kg)	Total chlorophyl (mg/kg)		Transparency (%)	
		cyclohexane	chloroform	cyclohexane	chloroform
Blackberry B1	32.30±0.55	3049.52±17.79	1505.78±12.59	25.84±0.68	19.28±0.27
B2	33.92±0.67	3094.98±20.54	1583.62±11.66	24.33±0.34	18.87±0.23
Raspberry R1	39.06±0.25	208.50±1.44	129.97±4.06	12.46±0.49	9.83±0.65
R2	41.44±0.68	199.88±2.45	120.78±3.66	11.08±0.33	9.54±0.21

[a] expressed as β-caroten

Total carotenoids content, expressed as β-caroten, was pretty similar in all oil samples. Parry et al. (21) have used the HPLC method to determine total carotenoids content. They obtained the values of 23.4 μmol/kg for the blackberry seed oil and 12.5 μmol/kg for the raspberry seed oil. In the study of Oomah et al. (18), the total carotenoids content (expressed as β-caroten) of 23 mg/100g was reported. The values for total carotenoids content in this study are much lower compared to that found for the cold-pressed pumpkin seed oil, where the values were in the range from 138.67 to 218.67 mg/kg. Virgin cold-pressed pumpkin oil had these values even higher, ranging from 240.18 to 526.22 mg/kg (22).

The total chlorophyl content was determined by dissolving oil samples in cyclohexane and chloroform. It has to be noted that there was a difference in the values obtained by using these two solvents. Namely, when the total chlorophyl content was determined by dissolving oil in cyclohexane, the results were two times higher compared to the values obtained by dissolving oil in chloroform. Thus, the values obtained for the blackberry seed oil were 3000 mg/kg (in cyclohexane) and 1500 mg/kg (in chloroform). The raspberry seed oil had much lower values: the total chlorophyl content of 200 mg/kg (in cyclohexane) and 120 mg/kg in chloroform solution. The total chlorophyl content of blackberry seed oil was around fifteen times higher than of the raspberry seed oil.

Transparency was also determined using both cyclohexane and chloroform solutions. Regardless of the method used, the values for oil transparency of the blackberry seed oil were approximately twice than those for the raspberry seed oil. This can be explained by the fact that the raspberry seed oil has a much higher content of total carotenoids, while the oil transparency was measured at 455 nm, i.e. at the wavelength where carotenoids exhibit maximum absorption.

CONCLUSION

Results obtained in this study have shown that pomace, a by-product from berry processing, specifically blackberry and raspberry juice, can be used as a potential raw material for oil extraction. This is supported by the fact that dried pomace has around 14% of oil (d.b.). Although both seeds were very small in size, blackberry seeds were twice the weight of the raspberry seeds. Conversely, due to the small seed size, there was no considerable difference in the volumetric weight, which was around 420 g/l. The FFA and PV values for the oils extracted from dried seeds are much higher than those found in other plant oils. Further studies should encompass investigations of storage time on the primary oil characteristics of frozen berry seed oil.

The crude blackberry seed oil extracted using organic solvent was dark brown-greenish in color, primarily due to the high total chlorophyl content (around 3000 mg/kg in cyclohexane). The raspberry seed oil was dark yellowish-orange in color, due to lower chlorophyl content (around 200 mg/kg) compared to the blackberry seed oils, while the total carotenoides content was slightly higher (40 mg/kg) compared to the blackberry seed oil (33 mg/kg).

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КАРАКТЕРИСТИКЕ СЕМЕНА И УЉА КУПИНЕ И МАЛИНЕ

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У овом раду су одређене технолошке карактеристике осушеног тропа, односно, семена купине и малине, као и параметри квалитета, садржај укупних каротеноида и хлорофила, транспаренција и боја сировог уља издвојеног из семена помоћу органског растварача. Семе купине (*Rubus fruticosus* L.) је добијено из плода домаће сорте Чачанска безтрна, а семе малине (*Rubus idaeus* L.) из плода сорте *Willamette*. Садржај уља у семену осушеног тропа купине износио је 13,97 и 14,34%, а код малине 13,44 и 14,33% на суву материју (с.м.). По технолошким карактеристикама, литарској и специфичној маси семена, нису нађене разлике, међутим маса 1000 семена је код купине износила око 3,5г, а код малине око 1,5 г на с.м. Основни хемијски квалитет уља семена купине је био знатно бољи у односу на уље малине, будући да је садржај слободних масних киселина (СМК) био 3,43 и 3,53%, а пероксидни број (Пбр) 8,89 и 11,16 ммол/кг, док су код малине вредности СМК биле 8,59 і 8,83%, а Пбр 13,99 и 13,84 ммол/кг. Сирово екстраховано уље семена купине било је тамне мрко-зелене боје, пре свега због високог садржаја укупних хлорофила, око 3000 мг/кг (у циклохексану). Уље малине је било тамне жућкасто-наранџасте боје, будући да је садржај хлорофила био знатно мањи, око 200 мг/кг, у односу на уље купине, док је садржај укупних каротеноида био нешто већи, око 40 мг/кг (код купине око 33 мг/кг).

Кључне речи: семе купине и малине, технолошке карактеристике семена, пигменти

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