

RADICAL SCAVENGING ACTIVITY OF RASPBERRY (*RUBUS IDAEUS* L.) FRUIT EXTRACTS

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Thirty-one raspberry (*Rubus idaeus* L.) plants were collected in different natural habitats of Lithuania located in 17 districts and replanted in the experimental field. The fruits from different accessions were extracted with ethanol and the extracts were tested for their antioxidant activity (AA) by using ABTS^{•+} radical cation decolourisation and DPPH[•] radical scavenging methods. All tested raspberry fruit extracts were antioxidatively active; their radical scavenging capacity at the applied concentrations varied from 52.9 to 92.6% in DPPH[•] reaction system and from 52.5 to 97.8% in ABTS^{•+} system. The total amount of phenolic compounds in fruits varied from 5.6 to 13.7 mg of gallic acid equivalents in 1 g of plant extract. However, strong correlation between the radical scavenging capacity and the total amount of phenolic compounds in the fruit extracts was not observed, which indicates remarkable differences in the composition and antioxidant power of phenolic compounds present in the fruits harvested from raspberry accessions collected from different natural habitats. Most likely, environmental conditions and genetic factors should play an important role for the accumulation of active compounds in raspberry fruits.

Keywords: raspberry fruits, *Rubus idaeus*, radical scavenging, total phenolics

Antioxidatively active compounds are biosynthesised in various anatomical parts of plants. Plant-derived antioxidants have been shown to function as oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors and synergists (WANG & LIN, 2000). During last two decades, numerous studies have investigated various plant origin sources attempting to find new natural bioactive components for food and other applications. Some of these studies resulted in development of natural antioxidant formulations. However, taking into account the vast biodiversity in the plant kingdom, scientific information on antioxidant and other bioactive properties of a great number of plants is still insufficient. Therefore, the assessment of such properties remains interesting and useful task, particularly in searching for new sources of natural antioxidants and other bioactive compounds, which could be used for valuable and sustainable natural products, such as food additives, functional foods and nutraceutical ingredients (VENSKUTONIS, 2004).

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Raspberry (*Rubus idaeus* L.) is a perennial sub shrub growing in the deforestations, by the skirts and forests, by the roadsides and in the brushes. Raspberries are also cultivated in the gardens. Raspberry fruits contain high levels of vitamin C and phenolic compounds, and consequently they are rich source of dietary antioxidants. Eleven anthocyanins were reported in raspberries, the major being cyanidin derivatives, in addition, a number of ellagic acid derivatives were identified in raspberries together with trace levels of quercetin structure flavonol conjugates. Ellagic acid is of a particular interest from a dietary viewpoint, as it has been reported to exhibit antiviral and antioxidant properties and provides protection against colon, lung and oesophagus cancer (MULLEN et al., 2003). Most of fruit phenolics and flavonoids have beneficial effects on health as antioxidants and anticarcinogenic compounds (HÄKKINEN et al., 1999; WEBER et al., 2002).

Fruits and leaves from different cultivars of *Rubus idaeus* were analysed for total antioxidant capacity (oxygen radical absorbance capacity, ORAC) and total phenolic content. The results showed a linear correlation between total phenolic content and ORAC activity for fruits and leaves (WANG & LIN, 2000). ANTONEN and KARJALAINEN (2005) most recently analysed raspberry cultivars grown under northern European conditions in Finland, and determined that the content of total phenolics varied from 192 to 359 mg 100 g⁻¹ fresh weight. BEEKWILDER and co-workers (2005) have studied the antioxidant compounds in raspberry fruits by HPLC coupled to an on-line post-column antioxidant detection system in order to assess developmental and genetic factors by comparing fruits from a single cultivar of different ripening stages and by comparing ripe fruits of 14 raspberry cultivars, respectively. They found that dominant antioxidants could be classified as anthocyanins, ellagitannins, and proanthocyanidin-like tannins. Among the 14 cultivars, major differences (>20-fold) were observed in the levels of pelargonidin type anthocyanins and some proanthocyanidin type tannins.

Many plant flavonoids possess the ability to inactivate free radicals by donating hydrogen, consequently by using plant-derived products damaging effect of such radicals can be reduced both in foods and living cells. Therefore, the measurement of RSA of plant extracts is a useful method for the preliminary assessment of their antioxidant properties (BANDONIENĖ et al., 2002; MILIAUSKAS et al., 2004; GÜLCIN et al., 2005; THEN et al., 2005).

It is well known that the composition of secondary plant metabolites depends on numerous factors, particularly plant cultivar and its chemotype, cultivation conditions, and harvesting time. Remarkable variations in chemical composition can be observed in the plants grown in natural habitats, i.e. in a wild uncontrolled environment (LOŽIENĖ et al., 2002, 2003, 2005). Chemical and biological diversity of plants was an important impetus for studying raspberry accessions collected in different natural habitats of Lithuania. Collecting of raspberries in wild habitats is still popular in the country, and it is interesting to know the variations in the properties and chemical composition of fruits produced by different raspberry accessions. To the best of our knowledge such variations have not been a subject of studies in previously published articles. It is also

worth mentioning that raspberries have been used as natural medicines to treat various health disorders. The effects of such treatments would highly depend on the concentrations of bioactive components. The aim of this study was to assess radical scavenging capacity of raspberry fruit extracts harvested from 31 raspberry accessions collected in different locations of Lithuania (Table 1).

1. Materials and methods

1.1. Plant material and chemicals

Thirty-one accession of raspberry (*Rubus idaeus* L.) plants were collected in different locations of 17 districts in Lithuania. The samples were planted in the field collection of the Institute of Botany, Lithuania on a cultivated sandy loam soil without removal of canes and allowing 3 m² (1×3 m) of growth space for each accession. Common horticultural practices were applied for the plant management later on. The coordinates of the field collection were N 54°46'36" and E 25°17'38". Soil properties at the field collection were as follows: total nitrogen: 0.087%; available P₂O₅: 129.9 mg kg⁻¹; available K₂O: 183.6 mg kg⁻¹; humus: 2.83% and pH: 5.49.

Raspberry fruits were harvested on July 8, 2003, immediately frozen to minus 85 °C and freeze dried in a FD-5-130 apparatus (FTS systems, NY, USA) operating at 50 mTorr for 36 h; and dried berries were packed in glass containers and stored in a freezer before further analysis.

Food grade ethanol was obtained from the local distillery (AB Stumbras, Kaunas, Lithuania). 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH[•], 95%), anhydrous sodium carbonate and gallic acid were from Sigma–Aldrich Chemie (Steinheim, Germany), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), standard Folin–Ciocalteu's phenol reagent, KCl, NaCl, Na₂HPO₄ and K₂S₂O₈ were from Merck (Darmstadt, Germany); KH₂PO₄ from Jansen Chimica (Beerse, Belgium).

1.2. Preparation of extracts

Dried berries were ground in a mortar with a pestle and 1 g of ground sample was extracted with 25 ml of ethanol (96%) at room temperature for 2 h under constant shaking (Sklo Union LT, Teplice, Czech Republic). The extracts were filtered and the residue was re-extracted with 10 ml of ethanol for 2 h. The extracts were combined and dried in a rotary evaporator Büchi R-114 (Donau, Switzerland) at approximately 65 °C. The dry extracts were stored in a freezer; they were dissolved in 10 ml ethanol before further analysis.

Table 1. Detailed information about raspberry (*Rubus idaeus* L.) accessions

Site No.	Sample No. ^a	Collecting date of accessions	Habitat type	Municipality	Location ^b
1	JL01	25-04-2001	Lakeside hawthorn shrubs	Lazdijai dist.	Barčiai SE1
2	JL02	25-04-2001	Mixed forest regrowth	Alytus dist.	Pocelonys W2
3	JL07	17-05-2001	Margin of cultivated field	Vilnius city	Kairėnai S1
4	JL08	23-05-2001	Mixed forest regrowth	Ukmerge dist.	Uzulenis SW3
5	JL11	30-05-2001	Broadleaved forest glade	Vilnius city	Inst. Botany NE1
6	IZ01	03-06-2001	Mixed forest regrowth	Zarasai dist.	Rudakiskes E1
7	JL14	07-06-2001	Lakeside willow shrubs on peatland	Salcininkai dist.	Mikasiūnai SW2
8	JL15	09-06-2001	Pure middle-age pine crops	Vilnius city	Silas NE1
9	JL16	10-06-2001	Fen margin near conifer forest	Vilnius dist.	Pikeliskes SW3
10	JL18	25-06-2001	Curonian lagoon coast	Neringa city	Juodkrante N5
11	JL22	11-07-2001	Sprucewood cutting site	Vilnius dist.	Jociūnai NE1
12	JL27	17-07-2001	Highway embankment	Sirvintos dist.	Varanava
13	JL28	18-07-2001	Pinewood with broadleaves	Utena dist.	Labe lakeside, Tauragnai W1
14	JL29	18-07-2001	Conifer forest edge	Moletai dist.	Vysnialaukis
15	JL32	29-07-2001	Riverside shrubs and broadleaves	Taurage dist.	Liaudginal, W bank of Kertėnys river
16	JL35	26-08-2001	Lakeside pinewood with juniper	Moletai dist.	Island in Baltieji Lakajai lake
17	JL44	26-09-2001	Moist sprucewood cutting site	Vilkaviskis dist.	Gizai NW4
18	JL47	27-09-2001	Roadside shrubs	Svenčionys dist.	Mociške SE0.6
19	JL52	30-09-2001	Pinewood cutting site	Vilnius dist.	Galgiai E4, forest block 137
20	JL53	04-10-2001	Roadside shrubs	Sirvintos dist.	Sirvintos NW8

Table 1 (continued)

Site No.	Sample No. ^a	Collecting date of accessions	Habitat type	Municipality	Location ^b
21	JL55	04-10-2001	Young spruce crops	Sirvintos dist.	Ciobiskis ferry E0.5
22	JL56	04-10-2001	Premature pinewood at the roadside	Sirvintos dist.	Kernave NW1
23	JL59	16-10-2001	Sprucewood cutting	Vilnius dist.	Marijampolis S3
24	JL64	02-11-2001	Moist cutting site, shrubs	Kelme dist.	Kryžkalis NW8
25	BG02	17-05-2002	Field collection	Kaunas dist.	Babiai
26	JL72	05-06-2002	Young spruce crops	Biržai dist.	Latveliai N3.3
27	JL85	23-07-2002	Water-pool bank	Silalė dist.	Pepliskiu SE1
28	JL87	03-08-2002	Middle-age lakeside pinewood	Svenčionys dist.	Prudiskes
29	JL89	22-09-2002	Premature pinewood	Salcininkai dist.	Pamurinė E0.2
30	TK01	18-10-2001	Peatland shrubs	Zarasai dist.	Puscia
31	ST01	18-05-2003	Roadside shrubs	Salcininkai dist.	Salcininkeliai

^a All samples were collected in the wild except for the BG02, a cv. 'Meteor', which was cultivated in the experimental fields of Lithuanian Institute of Horticulture.

^b Location, e.g. Barčiai SE1, means that the colony is located 1 km south-east of the settlement Barčiai.

1.3. Evaluation of antioxidant activity

Radical scavenging activity (RSA) of raspberry extracts against stable DPPH• was determined by a slightly modified DPPH radical scavenging assay (BRAND-WILLIAMS et al., 1995). The solution of DPPH• (6×10^{-5} M) was prepared daily, before measurements on a UV/visible light spectrophotometer Spectronic Genesys 8 (Rochester, USA) at 515 nm. Two ml of this solution were mixed with 50 µl of extract solution in 1 cm path length disposable microcuvette (Greiner Labortechnik, Alphen a/d Rijn, The Netherlands). The decreasing absorbance was read during 16 min reaction time at 10 s intervals. Simultaneously, the absorption of a blank sample containing the same amount of methanol and DPPH• solution was measured daily. The measurements were performed in triplicates.

ABTS•⁺ radical cation was produced by reacting ABTS with potassium persulfate ($K_2S_2O_8$) (RE et al., 1999). The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the assessment of extracts, the ABTS•⁺ solution was diluted with phosphate buffered saline (PBS) to obtain the absorbance of 0.800 ± 0.030 at 734 nm. Three ml of ABTS•⁺ solution were mixed with 30 µl of ethanol solution of fruit extract in the 1 cm path length microcuvette. The absorbance was read at ambient temperature after 1, 4, 6 and 10 min. PBS solution was used as a blank sample. All determinations were performed in triplicate.

1.4. Total amount of phenolic compounds

The concentration of phenolic compounds in ethanol extracts of raspberry fruits was determined by the FOLIN and CIOCALTEU (1927) method. For the preparation of calibration curve 1 ml reference gallic acid solutions in ethanol (aliquots of 0.025, 0.075, 0.100, 0.175 and 0.350 mg ml^{-1}) were mixed with 5 ml of standard Folin–Ciocalteu reagent and diluted with distilled water (1:10) and 4 ml of 7.5% sodium carbonate solution in water. The absorption was read after 30 min at 765 nm and the calibration curve was drawn, which showed very good linear dependence between absorption and gallic acid concentration ($y = 9.699x + 0.104$; $R^2 = 0.997$). For determination of phenolics, 1 ml of raspberry fruit extract solution in ethanol was used. All determinations were performed in triplicates. Total concentration of phenolic compounds (C) was expressed in mg of gallic acid equivalents (GAE) per g of plant ethanol extracts and recalculated in 1 g of dried raspberry fruits, taking into account the yield of the extract.

1.5. Statistical analysis

In most cases, the results are provided as a mean of three measurements. Standard deviations (SD) did not exceed 3% for all measurements. Analyses of variance were performed at a level of $P < 0.05$ to evaluate the significance of differences between mean values. Correlation coefficients (R) to determine the relationship between antioxidant

activities obtained in two different RSA reaction systems and between RSA and the concentration of total phenolic compounds in the extracts were calculated using MS Excel software (CORREL statistical function).

2. Results and discussion

It was found that all extracts isolated from raspberry fruit samples with ethanol were antioxidatively active. However, the RSA of extracts from the fruits harvested from the plant accessions collected in different locations (Table 2) varied in a wide range, from 52.9 ± 2.6 to $92.6 \pm 0.7\%$ in DPPH• reaction system and from 52.5 ± 1.4 to $97.8 \pm 0.5\%$ in ABTS•+ reaction system. It indicates remarkable variations in the composition of radical scavengers in the analysed raspberry fruits.

Table 2. Radical scavenging activity of ethanolic raspberry fruit extracts in DPPH• and ABTS•+ reaction systems

Sample number	Inhibition, %		Sample number	Inhibition, %	
	DPPH•	ABTS•+		DPPH•	ABTS•+
JL01	65.7±0.40	56.6±0.81	JL47	63.5±2.63	87.0±2.90
JL02	67.9±0.04	61.1±0.37	JL52	86.8±1.39	62.7±2.53
JL07	66.1±1.10	79.4±1.68	JL53	65.0±2.92	86.8±0.76
JL08	91.1±1.99	95.6±2.74	JL55	92.1±0.45	82.6±2.48
JL11	64.9±1.32	79.9±1.47	JL56	72.7±2.83	54.0±1.12
JL14	70.1±0.90	52.5±1.44	JL59	86.0±0.77	78.2±1.45
JL15	77.9±0.78	73.9±1.50	JL64	89.2±1.30	82.3±0.75
JL16	73.9±2.05	73.7±2.42	JL72	82.5±0.51	60.3±2.80
JL18	65.4±1.63	53.0±2.42	JL85	76.1±0.84	56.3±1.70
JL22	90.1±2.61	66.1±2.69	JL87	84.0±1.76	76.5±2.01
JL27	61.4±1.67	76.6±1.38	JL89	92.6±0.66	80.4±1.31
JL28	52.9±2.60	60.6±2.64	BG02	67.3±1.34	96.3±0.94
JL29	73.8±0.96	63.8±0.85	IZ01	81.6±0.63	70.9±1.47
JL32	90.6±0.64	97.8±0.50	ST01	73.6±1.34	64.8±0.36
JL35	69.5±2.43	96.3±2.25	TK01	84.2±0.54	81.1±2.77
JL44	71.9±2.72	70.5±2.13			

Raspberry samples JL08, JL22, JL32, JL55, JL64 and JL89 had the strongest antioxidant activity; the RSA of their extracts was approximately 90% in DPPH• radical scavenging reaction, while the extracts isolated from the samples JL27, JL28 and JL47 exhibited the lowest RSA in DPPH• reaction, approximately 55%.

ABTS•+ radical cation decolourisation assay is another widely used method for the screening of antioxidant activity, which is applicable for both lipophilic and hydrophilic antioxidants. It was found (Table 2) that raspberry samples JL08, JL32, JL35 and BG02 were the most effective in ABTS•+ reaction (their RSA was approximately 95%), while the samples JL01, JL14, JL18, JL56 and JL85 showed the lowest inhibition of ABTS•+ (their RSA was approximately 55%).

The results obtained suggest that the kinetics of radical scavenging reaction in the two systems differ. However, in many cases the total antioxidant activities of extracts in DPPH• reaction were similar to those in ABTS•+ reaction system. However, data comparison of two radical scavenging tests did not result in a strong correlation between the two reactions systems ($R=0.242$).

Table 3. Yield of raspberry fruit extracts (%) and total amount of phenolic compounds in raspberry fruit extracts and dry fruits, mg g⁻¹ (in GAE)

Sample number	Extract yield	Phenolic compounds in extracts	Phenolic compounds in dry fruits	Sample number	Extract yield	Phenolic compounds in extracts	Phenolic compounds in dry fruits
JL01	26.9	6.4±0.21	1.5±0.05	JL47	30.0	12.9±0.34	3.1±0.08
JL02	25.2	6.2±0.18	1.5±0.04	JL52	27.5	12.7±0.28	3.1±0.07
JL07	26.9	9.6±0.17	2.3±0.04	JL53	33.6	12.9±0.30	2.3±0.04
JL08	25.1	8.5±0.26	2.0±0.06	JL55	33.0	10.5±0.24	2.5±0.06
JL11	27.6	12.7±0.19	3.0±0.05	JL56	29.3	8.3±0.27	2.0±0.06
JL14	29.7	11.6±0.30	2.8±0.07	JL59	25.3	13.7±0.19	3.3±0.05
JL15	32.2	8.4±0.15	2.0±0.04	JL64	38.0	7.7±0.11	1.8±0.03
JL16	24.9	12.4±0.27	3.0±0.07	JL72	23.7	9.8±0.31	2.4±0.07
JL18	29.4	8.7±0.25	2.1±0.06	JL85	37.1	6.6±0.20	1.6±0.05
JL22	24.8	13.2±0.37	3.2±0.09	JL87	31.4	11.7±0.22	2.8±0.05
JL27	33.4	13.7±0.18	3.3±0.04	JL89	31.5	8.2±0.15	2.0±0.04
JL28	31.0	5.6±0.12	1.3±0.03	BG02	30.7	7.8±0.25	1.9±0.06
JL29	30.6	6.3±0.20	1.5±0.05	IZ01	29.98	13.1±0.38	3.1±0.09
JL32	26.2	12.4±0.21	3.0±0.05	ST01	29.80	7.9±0.19	1.9±0.05
JL35	33.3	11.8±0.34	2.8±0.08	TK01	25.21	9.4±0.17	2.3±0.04
JL44	26.8	12.9±0.27	3.1±0.07				

Published reports show that RSA of plant extracts, as a rule, is in good correlation with the concentration of phenolic compounds determined by the Folin–Ciocalteu method (MILIAUSKAS et al., 2004). It is well established that various phenolic compounds can scavenge free radicals; therefore, they act as primary antioxidants or free radical reaction terminators. The RSA of phenolic compounds depends on their structure, particularly the number of hydroxyl substitutes and their position in the molecule.

The concentrations of phenolic compounds in the analysed ethanolic extracts from raspberry fruits are presented in Table 3. It varied in different samples from less than 5 to almost 14.0 mg of GAE in 1 g of extract or from 1.3 to 3.3 mg of GAE in 1 g of dried fruits. The amount of phenolics in extracts exceeded 12 mg g⁻¹ in 35% of all studied samples, e.g. JL11, JL27, JL32, JL59 and IZ01, while 16% of the samples contained less than 7 mg g⁻¹ of phenolics, among them JL01, JL02, JL28, JL29 and JL85. It was reported previously that the total amount of phenolics in the four investigated raspberry varieties varied from 359.2 to 512.7 mg in 100 g of berries and the antioxidant activity of the raspberry fruits was directly related to the total amount of phenolics and flavonoids (LIU et al., 2002). However, in our study there was no clear correlation

between the concentration of phenolic components and RSA, although some tendencies could be observed. For instance, the sample with the lowest concentration of phenolics, JL28 possessed the weakest RSA in DPPH• reaction. Other extracts containing less than 7 mg g⁻¹ phenolics were also among the weakest radical scavengers. Samples with high concentration of phenolics, e.g. the sample JL32 (12.44 mg g⁻¹), were able to scavenge almost all free radicals at the applied reaction conditions. However, some extracts, e.g. JL11, JL14, with high content of phenolics showed only medium RSA. In general, slightly better correlation was found between the total content of phenolics and RSA in ABTS•+ reaction system (R=0.323) as compared with DPPH• (R=0.153). Most likely, in this case the difference in the concentration of the individual phenolic components in the analysed raspberries is the most important factor in defining antioxidant properties of their extracts. Remarkable differences in antioxidant power of individual phenolic constituents were reported in many literature sources. It is also worth noting that the accessions of raspberry plants in our study were collected from the natural habitats with different environmental conditions, while previously performed studies used mainly cultivated berries (WANG & LIN, 2000). Consequently, genetic factors should play an important role for the accumulation of active compounds in raspberry fruits, which were transferred from wild locations to the controlled growing environment. The information on the properties and the composition of raspberry fruits collected in wild environment is rather scarce. Recently, it has been reported that environment had a significant effect on the content of quercetin in red raspberries grown in Finland. It is most interesting to note that the concentration of quercetin, which is known to be a powerful antioxidant (MAKRIS & ROSSITER, 2001), in some samples with the highest content of the total phenolics was remarkably lower than in the samples containing lower amounts of total phenolics. The authors concluded that breeding material should be evaluated for their potential health benefits over several regions in northern raspberry breeding (ANTTONEN & KARJALAINEN, 2005). Earlier, HÄKKINEN and co-workers (1999) reported a high proportion of ellagic acid relative to other phenolic components in red raspberry extracts, however, later GONZÁLEZ and co-workers (2003) in their study of 4 raspberry cultivars found no significant correlation between ellagic acid content and antioxidant activity in fruit extracts, as determined by antiradical efficiency assay. BEEKWILDER and co-workers (2005) detected in ripe raspberries from 3 cultivars sanguin H6 as one of the main component; it possessed 40% of the total antioxidant activity of the all raspberry components assessed by the on-line HPLC-PDA-ABTS method. Moreover, the ratio of the highest and the lowest content of this component during development of raspberries was 7.5:1.1. Based on the analysis of 14 raspberry cultivars, the same authors suggest that the content of individual health-promoting compounds varies significantly in raspberry due to both developmental and genetic factors. CONNOR and co-workers (2005) reported that antioxidant activity and total phenolic content in red raspberries were highly phenotypically correlated (r=0.93), while genetic correlation between these characteristics was much lower.

The results obtained in our study as well as the most recently reported data clearly suggest that further studies should be focused on the quantitative and qualitative composition and RSA of individual antioxidatively active components in the raspberry fruits harvested from the selected plant accessions. Most likely, such data would explain rather weak correlation between the total concentration of phenolics in the berries and antioxidant power of their extracts which was determined in our study.

3. Conclusions

Measurements of radical scavenging activity of raspberry fruit extracts in model DPPH• and ABTS•+ reaction systems indicate that the concentration of antioxidatively active compounds in the analysed berries varies in a wide range: the extracts with the highest RSA were able to inhibit approximately twice larger amount of DPPH• and ABTS•+ radicals compared to the extracts with the lowest RSA. Total concentration of phenolic compounds in raspberry fruits varied from 5 to 14 mg g⁻¹ extract or from 1.3 to 3.3 mg g⁻¹ dried fruits as expressed in gallic acid equivalents. However, strong correlation between the radical scavenging capacity and the total amount of phenolic compounds in the fruit extracts was not determined. It indicates remarkable differences in the composition and antioxidant power of phenolic compounds present in the fruits harvested from raspberry accessions collected from different natural habitats. Some of the most recent literature sources indicate that these differences might depend on phenotypic, developmental and genetic factors. Further research should be focussed on the quantification of individual raspberry extract components and assessment of their radical scavenging properties.

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