Identification and Quantification of Phenolic Compounds from Red Currant (*Ribes rubrum* L.) and Raspberries (*Rubus idaeus* L.)

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Abstract. The extracts obtained from two types of berries: red currant and raspberries, were evaluated for their phenolic content. They were identified and quantified by using an optimized HPLC method. During the analyze several phenolic compounds were found, like: gallic acid, (+)-catechin, syringic acid, cinnamic acid, chlorogenic acid, ferulic acid, rutin and quercetin. The total amount of phenolic compounds analyzed found in red currant was greater than the one found in raspberries, due to the low variety of phenolic compounds extracted. The greatest amount of gallic acid, (+)- catechin, syringic acid, cinnamic acid, chlorogenic acid, ferulic acid and rutin was determined from the extraction of red currant berries and the raspberries extract contained the greatest source of quercetin. This study shows that red currant can provide the highest and most varied content of phenolic compounds from the analyzed berries.

Introduction

Nowadays the exploitation of bioactive compounds from vegetable products is of great concern all over the world. These compounds have found many applications in different fields of interest.

Raspberries (*Rubus idaeus* L.) (Fig. 1) and red currant (*Ribes rubrum* L.) (Fig. 2) are berries widely spread all over Europe [1] and they are known for their food and flavoring qualities alongside with their involvement in preserving human health [2, 3], due to their important content of phenolic compounds [4].

Red currant has beneficial effects when consumed regularly. It has been shown that these berries reduce the incidence of several diseases as hypertension and other cardiovascular diseases, osteoporosis, diabetes, cancer and inflammatory diseases [5].

Raspberries are known for their antitumoral, antibacterial, antiinflammatory [6,7] and antioxidant activities [8].

It has been shown that the most health benefits of these berries are related to the content in phenolic compounds [4].

Phenolic compounds protect cells against damage due to their antioxidative, anti-inflammatory and anticarcinogenic activities. [9, 10].

The phenolic compounds are divided in different classes based on their chemical structures. Phenolic acids include hydroxybenzoic acids (gallic acid, syringic acid) and hydroxycinnamic acids (caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid). Flavonoids include flavonols (quercetin, rutin), flavanones, flavanols (catechin), flavonones, anthocyanins and isoflavones. The most representative stilbene is resveratrol. Plants rich in these active principles have antioxidant effects and are widely used nowadays for their health beneficial properties [11].

Scientists are most interested in the detailed chemical composition and the potential health benefits of these berries [5, 12].

Figure 1. Raspberries (*Rubus idaeus* L.) **Figure 2.** Red currant (*Ribes rubrum* L.)

The analyzed phenolic compounds have different applications concerning the preservation of the human health. For example, the gallic acid has antihyperglycaemic, anti-tumoral, anti-microbial, cardio protective and neuroprotective activities [13-17]. (+)- Catechin has implications in cardiovascular diseases, diabetes and it protects the skin against UVB radiation [18-20]. The syringic acid has antibacterial, anti-steatotic, anti-inflammatory and neuroprotective properties [21-23], the cinnamic acid has anti-tumoral properties and it has been proven that it improves the vascular function [24-25]. The chlorogenic acid has antihypertensive effects alongside with the improvement of the vessel function and the protection of the endothelial cells against oxidative damage and neuroprotective effects [26-28]. The ferulic acid prevents Alzheimer's disease, has antitumoral properties as well as antimicrobial and anti-inflammatory properties [29-31]. Quercetin has protective effects against retinal degeneration, anti-tumoral and antihyperglycaemic properties alongside with the neuroprotective and cardio protective effects [32-35] and rutin has the ability to decrease glucose concentrations in plasma, presents anti-inflammatory, anti-tumoral and neuroprotective effects [36-38].

All these phenolic compounds have great beneficial effect concerning human health. Their identification and quantification in raspberries and red currant is a matter of great interest due to the fact that these berries are widely consumed all around the world.

Materials and methods

Sample preparation

The berries were harvested from Sibiu County, Romania. They were frozen and stored at -20°C. Before the analysis they were defrosted, dried at 40°C and grounded on a domestic mill [7].

The extraction was performed by adding 10 mL of solvent methanol and purified water 70:30 (V/V) to 500 mg of berries. Covered and put in an ultrasound bath at 40°C for 30 minutes. The supernatant layer was centrifuged at 5000 rpm for 10 minutes, and the resulting supernatant layer was brought to dryness. The residue was dissolved in 10 mL of solvent, filtered and brought to volume to 10 mL using the same solvent [39].

Phenolic profile

The quantitative and qualitative analysis of phenolic compounds was carried out on a Agilent Technologies 1200 series HPLC system, equipped with degasser, quaternary pump, diode array detector, thermostated autosampler and thermostated column compartment. The column used was Zorbax Eclipse Plus C18 (250 mm x 4,6 mm i.d. x 5µm), at controlled temperature of 25°C. The elution was performed using purified water (mobile phase A), methanol (mobile phase B) and purified water and glacial acetic acid 96:4 (V/V) (mobile phase C). The gradient program was used as follows: 0 min: 15% B and 85% C, 15 min: 75% A and 25% B, 20 min: 15% A and 85% B, 40 min: 40% A and 60% B, 45 min: 5% A and 95% B, 55 min: 5% A and 95% B, 60 min: 85% A and 15% B and 70 min: 85% A and 15% B. The flow rate program was used as follows: 0 min: 0.5 mL/min and 15 → 70 min: 0.8 mL/min. The injection volume was 5 µL and the detection was

performed at 280, 303, 330 and 360 nm [39]. The standards of gallic acid, ferulic acid, syringic acid, cinnamic acid, chlorogenic acid, caffeic acid, (+)-catechin, resveratrol, quercetin, rutin were purchased from Sigma Aldrich, at HPLC purity.

Results and discussions

The detection of the phenolic compounds was performed at different wavelengths because of the absorption maxima determined for each analyzed compound. Four wavelengths were determined to be suitable for the ten compounds analyzed: 280 nm for gallic acid, syringic acid, (+)-catechin and cinnamic acid, 303 nm for resveratrol, 330 nm for ferulic acid, caffeic acid and chlorogenic acid, and 360 nm for rutin and quercetin [39] (Fig. 3, 4).

The highest amount of phenolic compounds was found in red currant, the total amount reached was 94.43 mg / 100 g dry weight (d.w.) and for raspberries 5.79 mg / 100 g d.w. (Tables 1,2). The amount of phenolic compounds found in red currant shows a possible increase of effects in comparison with the raspberries due to the higher quantity of phenolic compounds [40].

Red currant had the highest amount of different compounds like rutin (8.50 mg/100g d.w.), cinnamic acid (0.32 mg/100g d.w.), ferulic acid (1.00 mg/100g d.w.), chlorogenic acid (3.63 mg/100g d.w) syringic acid (31.65 mg/100g d.w.), (+)-catechin (42.43 mg/100g d.w.) and gallic acid (6.90 mg/100g d.w.). The highest amount of quercetin (1.52 mg/100g d.w.) was found in raspberries (Tables 1, 2).

Table 1. Phenolic compounds from red currant

Standard	Sample area (mAU*s)	Standard area (mAU*s)	Sample mass (mg)	Standard mass (mg)	Standard concentration %	mg phenolic compounds/ 100 g d.w.
Quercetin	0	371.7557	521.47	5.19	95	0.00
Rutin	42.01485	225.8515		6.34	94	8.50
Gallic acid	73.69572	467.3984		5.79	98.5	6.90
Syringic acid	312.96799	392.7161		5.45	95	31.65
Caffeic acid	0	773.6238		5.21	98	0.00
Cinnamic acid	8.38993	1117.151		5.68	99	0.32
Ferulic acid	17.46582	673.0148		5.05	99	1.00
(+)-Catechin	109.55875	100.7379		5.19	98	42.43
Resveratrol	0	1058.129		5.04	99	0.00
Chlorogenic acid	34.52472	356.8267		5.15	95	3.63
Total						94.43

Figure 3. Chromatogram of red currant sample: A-280nm, B-303nm, C-330nm, D-360nm
1-gallic acid, 2-(+)-catechin, 3-syringic acid, 4-cinnamic acid,
5-chlorogenic acid, 6-ferulic acid, 7-rutin

Table 2. Phenolic compounds from raspberries

Standard	Sample area (mAU*s)	Standard area (mAU*s)	Sample mass (mg)	Standard mass (mg)	Standard concentration %	mg phenolic compounds/ 100 g d.w.
Quercetin	15.12688	371.7557	527.89	5.19	95	1.52
Rutin	0	225.8515		6.34	94	0.00
Gallic acid	13.32424	467.3984		5.79	98.5	1.23
Syringic acid	27.64087	392.7161		5.45	95	2.76
Caffeic acid	0	773.6238		5.21	98	0.00
Cinnamic acid	0	1117.151		5.68	99	0.00
Ferulic acid	4.88065	673.0148		5.05	99	0.27
(+)-Catechin	0	100.7379		5.19	98	0.00
Resveratrol	0	1058.129		5.04	99	0.00
Chlorogenic acid	0	356.8267		5.15	95	0.00
Total						5.79

Figure 4. Chromatogram of raspberry sample: A-280nm, B-303nm, C-330nm, D-360nm
1-gallic acid, 3-syringic acid, 6-ferulic acid, 8-quercetin

Resveratrol and caffeic acid were not detected neither in red currant or raspberries. Quercetin was not detected in red currant and rutin, cinnamic acid, (+)-catechin, and chlorogenic acid were not detected in raspberries.

Due to the fact that raspberries have a lower quantity and diversity of phenolic compounds than the red currant, the beneficial effects of raspberries for human health are diminished in comparison to red currant.

Conclusions

This study was conducted in order to identify and quantify phenolic compounds extracted from red currant and raspberries using ultrasounds, raised temperature, and methanol and purified water as solvents.

The highest quantity of total phenolic compounds analyzed was determined from red currant. Raspberries had a low quantity of phenolic compounds due to the many compounds that were not detected.

Red currant can provide the highest and most varied content of phenolic compounds from the analyzed berries.

References

- [1] B. Djordjevic et al., Pomological and biochemical characterization of European currant Berry (*Ribes* sp.) cultivars, *Sci. Hort.* 165 (2014) 156-162.
- [2] S. Benvenuti et al., Polyphenols, anthocyanins, ascorbic acid and radical scavenging activity of *Rubus*, *Ribes* and *Aronia*, *J. Food Sci.* 69 (2004) 164-169.
- [3] H. Hajimehdipoor et al., Comparative study of the total phenol content and antioxidant activity of some medicinal herbal extracts, *J. Pharmacogn.* 1 (2014) 21–25.
- [4] M. Kula et al., Phenolic composition of fruits from different cultivars of red and black raspberries grown in Poland, *J. Food Comp. Anal.* 52 (2016) 74-82.
- [5] G. Zdunic et al., Black (*Ribes nigrum* L.) and red currant (*Ribes rubrum* L.) cultivars, in M. Simmonds, V.R. Preedy (Eds.), *Nutritional composition of fruit Cultivars*, Academic press, 2015, pp. 101-126.
- [6] D.D. Cetojevic-Simin et al., Bioactivity of Meeker and Willamette raspberry (*Rubus idaeus* L.) pomace extracts, *Food Chem.* 166 (2015) 407-413.
- [7] N. Shi, K.M. Riedl et al., Efficacy comparison of lyophilised black raspberries and combination of celecoxib and PBIT in prevention of carcinogen-induced oesophageal cancer in rats, *J. Funct. Foods.* 27 (2016) 84-94.
- [8] G. Bartosz, *Food oxidants and antioxidants. chemical, biological and functional properties*, CRC Press, Boca Raton, 2014.
- [9] A.M. Nderitu et al., Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated *in vitro* gastrointestinal digestion, *Food Chem.* 141 (2013) 1763–1771.
- [10] J.G. Xu et al., Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination, *J. Agric. Food Chem.* 57 (2009) 10392–10398.
- [11] K.B. Pandey, S.I. Rizivi, Biological activity and mechanism of action of plant polyphenols: relevance to human health and disease, in A.A. Farooqui, T. Farooqui (Eds.), *Phytochemicals and Human Health*, Nova Science Publishers, 2011, pp. 483-500.
- [12] R. Bobinate, P. Viskelis, P.R. Venskutonis, Chemical composition of raspberry (*Rubus* spp.) cultivars, in M. Simmonds, V.R. Preedy (Eds.), *Nutritional composition of fruit Cultivars*, Academic press, 2015, pp. 713-729.
- [13] V.R. Punithavathi et al., Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats, *Eur. J. Pharmacol.* 650 (2011) 465-471.
- [14] Y. Lu et al., Gallic acid suppresses cell viability, proliferation, invasion and angiogenesis in human glioma cells, *Eur. J. Pharmacol.* 641 (2010) 102-107.
- [15] X. Sun et al., The antimicrobial, mechanical, physical and structural properties of chitosan-gallic acid films, *Food Sci. Tech.* 57 (2014) 83-89.
- [16] J. Sun et al., Neuroprotective effects of gallic acid against hypoxia/reoxygenation-induced mitochondrial dysfunction in vitro and cerebral ischemia/reperfusion injury in vivo, *Brain Res.* 1589 (2014) 126-139.
- [17] H. Priscilla, P.S Maizen Prince, Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats, *Chem. Biol. Inter.* 179 (2009) 118-124.

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- [18] H.M. Eid et al., A combination of (+)-catechin and (-)-epicatechin underlines the in vitro adipogenic action of Labrador tea (*Rhododendron groenlandicum*), an antidiabetic medicinal plant of the Eastern James Bay Cree pharmacopoeia, *J. Ethnopharmacol.* 178 (2016) 251-257.
- [19] F.S. Jung et al., A metabolomics approach shows that catechin-enriched green tea attenuates ultraviolet B-induced skin metabolite alterations in mice, *Metabolomics.* 11 (2015) 861-871.
- [20] M. Murray et al., Green tea catechins and cardiovascular disease risk factors: Should a health claim be made by the United States Food and Drug Administration?, *Trends Food Sci. Technol.* 41 (2015) 188-197.
- [21] C. Shi et al., Antimicrobial activity of syringic acid against *Cronobacter sakazakii* and its effect on cell membrane, *Food Chem.* 197 (2016) 100-106.
- [22] J.R. Ham et al., Anti-steatotic and anti-inflammatory roles of syringic acid in high-fat diet-induced obese mice, *Food Funct.* 7 (2016) 689-697.
- [23] M. Tokmak et al., The axon protective effects of syringic acid on ischemia/reperfusion injury in a rat sciatic nerve model, *J. Turk. Neurosurg.* 27(1) (2017).
- [24] A. Rodrigues-Mateos et al., Cranberry (poly)phenol metabolites correlate with improvements in vascular function: A double-blind, randomized, controlled, dose-response, crossover study, *Mol. Nutr. Food Res.* 60 (2016) 2130-2140.
- [25] M. Marcin, L. Bogdan, Cinnamic acid derivatives and inhibitors of oncogenic protein kinases structure, mechanism and biomedical effects, *Curr. Med. Chem.* 10 (2016) 954-982.
- [26] R. Rodrigo, Beneficial effects of chlorogenic acids on essential hypertension, *J. Food Nutr. Sci.* 3 (2016) 1-5.
- [27] R. Jiang et al., Chlorogenic acid improves ex vivo vessel function and protects endothelial cells against HOCl-induced oxidative damage, via increased production of nitric oxide and induction of Hmox-1, *J. Nutr. Biochem.* 27 (2016) 53-60.
- [28] F. Taram, A.N. Witer, D.A. Linseman, Neuroprotection comparison of chlorogenic acid and its metabolites against mechanistically distinct cell death-inducing agents in cultured cerebellar granule neurons, *Brain Res.* 1648 (2016) 69-80.
- [29] E. Park, H. Lee, Ferrulic acid as a natural bioactive compound enhances PARP inhibitor sensitivity in breast cancer, *Cancer Res.* 76 (2016) 1338.
- [30] J.S. Jung et al., Protective effects of a dimeric derivative of ferulic acid in animal models of Alzheimer's disease, *J. Pharmacol.* 782 (2016) 30-34.
- [31] S. Chao et al., Antimicrobial activity of ferulic acid against *Cronobacter sakazakii* and possible mechanism of action, *Food. Path. Dis.* 13 (2016) 196-204.
- [32] M. Lee, E.G. McGeer, P.L. McGeer, Quercetin, not caffeine, is a major neuroprotective component in coffee, *Neurobiol. Aging.* 46 (2016) 113-123.
- [33] Y. Wang et al., Protective effect of quercetin against visible light-induced retinal degeneration in vivo, *FASEB J.* 30 (2016).
- [34] M.C. Serban et al., The effect of quercetin on blood pressure: a systematic review and meta-analysis of randomized controlled trials, *J. Am. Coll. Cardiol.* 67 (2016) 2003.
- [35] S.M. Snyder et al., Consumption of quercetin-containing apple and cherry extracts affects blood glucose concentration, hepatic metabolism and gene expression patterns in obese C57BL/6J high fat-fed mice, *J. Nutr.* 146 (2016) 1001-1007.

- [36] R. Tian et al., Rutin ameliorates diabetic neuropathy by lowering plasma glucose and decreasing oxidative stress via Nrf2 signaling pathway in rats, *J. Pharmacol.* 771 (2016) 84-92.
- [37] F. Nasiri et al., Rutin enhances the antiproliferative effect of 5-FU and oxaliplatin in colon cancer cells, *Cancer Res.* 76 (2016).
- [38] R. Gautam et al., Rutin attenuates intestinal toxicity induced by methotrexate linked with anti-oxidative and anti-inflammatory effects, *Bio. Med. Central.* 16 (2016) 1-6.
- [39] A. Frum et al., The quantitative and qualitative analysis of several phenolic compounds and polyphenols from blueberries (*Vaccinium myrtillus* L.), *Research result, Bus. Serv. Tech.* (in press)
- [40] N. Balasundram, K. Sundram, S. Samman, Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses, *Food Chem.* 99 (2006) 191-203.