Health Promoting Compounds in Black Currants - the Start of a Study Concerning Ontogenetic and Genetic Effects

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Abstract

Many parts of black currant plants (buds, leaves and fruits) are rich sources of phenolic compounds with potential health promoting properties. For this reason we have started a project to investigate the ontogenetic, genetic and environmental effects on the content of specific phenolic compounds. Extraction, identification and analysis of phenolic compounds from different black currant plant organs have been optimized considering the extraction method and HPLC-DAD analysis. The compounds were identified and quantified using HPLC-ESI-MS characteristics and commercial standards. Here the variation in content of phenolic compounds in buds collected during one season is presented. The results show that swollen buds collected in March had highest content of phenolics with rutin, epicatechins and kaempferols being dominant, whereas the content of chlorogenic acid was very low through out the season.

INTRODUCTION

In recent years there has been an increased scientific interest toward the crops belonging to the genus *Ribes*, not only due to the desired taste of berries but also for the health benefits of different compounds associated with their consumption. The *Ribes* genus consists of approx. 150 diploid species of spiny and non-spiny shrubs, among which black currant (*Ribes nigrum* L.) is the most commercially important. Black currants are widely grown across temperate Europe, Russia, New Zealand, parts of Asia and to a lesser extent North America with an annual production of 160,000 tons in Europe and 185,000 tons worldwide (Jensen, 2009). In Sweden, black currant is cultivated on approximately 400–450 ha and is a popular plant in private gardens. The crop is primarily cultivated for juice and beverage production, processed for production of jams, purees, jellies, liqueurs, imparting colour and flavour in dairy products and to a lesser extent it is consumed fresh.

The reason for black currants being heavily marketed at the present time is due to its high content of bioactive compounds like vitamin C (ascorbic acid) and polyphenols including flavonoids such as anthocyanins, procyanidins, flavanols and phenolic acids with potential health promoting properties (Tabart et al., 2009; Brennan and Graham, 2010). A number of scientific studies performed using in vitro models have demonstrated that the mentioned bioactive compounds exhibit anti-inflammatory, vasomodulatory and anti-haemostatic activities (Hollands et al., 2008; Karjalainen et al., 2009). Industrially, fruits are considered to be of importance due to high content of tasty juice. Recent studies

show that other anatomical parts of black currant like buds and leaves also are good sources of total phenols that relate to the flavor, colour and health benefits which make them desirable extracts for functional foods (Brennan et al., 2008; Tabart et al., 2006). Black currants are thus very interesting to use in different products that could offer an added value with respect to health. In an unpublished pilot study at SLU Balsgård it was found that the total content of polyphenols is five times higher in the leaves compared to the fruits. Thus, the black currant leaves are a very interesting source of beneficial polyphenols that need to be further investigated.

Black currant cultivation is in different areas limited by a lack of adaptation in existing cultivars as well as their susceptibility to different pests and diseases. Also, the levels of bioactive compounds like ascorbic acid and polyphenols are influenced by genotype, environment and interactions between genotype and environment.

A breeding program is ongoing at the Department of plant breeding and biotechnology, Balsgård to develop new resistant cultivars that are well adapted to different climatic conditions with an increase in nutritional content and amenable to organic cultivation. The specific objectives of this research project, which is associated to the breeding program, are (1) to optimize the conditions for analysis of single polyphenols in buds, leaves and fruits by HPLC-DAD and HPLC-ESI-MS from black currant material, (2) to evaluate the performance and field resistance towards severe pests and diseases of black currant selections and cultivars in comparative organic field trials in the North (Öjebyn) and south (Balsgård) of Sweden, (3) to investigate the genetic effects, genotype environment interactions and reveal the role of ontogenetic stage of buds, leaves and fruits on the content of ascorbic acid and single polyphenols, and (4) to investigate the effects of year, latitude and growing seasons.

In this paper we present some preliminary results with the identification of the major phenolic compounds and their variation during different ontogenetic stages in black currant buds sampled during one season.

MATERIALS AND METHODS

Black currants represented by 3 advanced selections (SCRI 8872-1, SCRI 8944-13, BRi 9504-2-227) and 2 cultivars (‘Poesia’, ‘Titania’) consisting of 5 replicates each established in the South (Balsgård) and North (Öjebyn) of Sweden have been chosen for the whole study. For the part of the study reported here sampling of buds was done during one season (in the autumn 2010 and in the spring 2011). Buds, leaves and fruits will in the future be harvested at three occasions (Table 1) per location for 3 seasons (2010/11, 2011/12 and 2012/13).

The samples were freeze dried, ground and 50 mg extracted in triplicates with 1.5 ml of 50% ethanol (v/v) using ultrasonic bath for 15 minutes at room temperature. The extracts were centrifuged at 13000 x g for 10 min and the supernatant was transferred to HPLC vials. The extracts were analyzed on a Synergi hydro-RP 80A (250x4.60 mm, 4 micron) column using HPLC-DAD as well as ESI-MS detection for separation, identification and quantification of major phenolic compounds.

A statistical analysis of variance (ANOVA) was carried out on the data (using Minitab software, version 16, State College, PA, USA) and Tukey’s *post hoc* test was performed to reveal any significant difference in content of specific polyphenols for the samples collected at different ontogenetic stages.

RESULTS AND DISCUSSION

All parts of the study have not yet been completed as we only have started sampling and analysis of buds and leaves this year and several papers presenting and discussing the results are expected in the years to come. However, we would like to present preliminary results with some of the phenols identified in buds and their variation among three different stages of ontogenetic development (Fig. 1, Table 2).

The HPLC-DAD analyses of black currant buds revealed chlorogenic acid (detected at 8.7 min on 280 nm), epicatechin (at 11.6 min on 280 nm), rutin (at 24.5 min), quercetin-3-β-D-glucoside (at 25.6 min), kaempferol-3-*O*-glucoside (at 33.4 min), isorhamnetin-3-*O*-glucoside (at 35.2 min) and quercetin (at 51.1 min) detected at 360 nm respectively. In addition, minor levels of chlorogenic acid and isorhamnetin-3-*O*-rutinoside were detected which was below the quantification limit in some of the samples. Also, 2 unknown peaks detected (peaks 3 and 6) at 360 nm are currently being investigated.

Here the variation in phenolic compounds in buds sampled from black currant selection SCRI 8872-1 is reported. On October 31, 2010, buds were dormant, on March 3, they were swollen and on April 4, the buds were in the onset of burst. In Fig. 2 and Table 2 it is shown that the content of epicatechin and rutin were higher than that of the other identified phenolic compounds during all three investigated bud stages, whereas chlorogenic acid (peak 8) and quercetin (peak 7) were the lowest. The content of the glycosides, quercetin-3-β-D-glucoside (peak 2), kaempferol-3-*O*-glucoside (peak 4) and isorhamnetin-3-*O*-glucoside (peak 5) were in between for all investigated bud stages. The concentration of epicatechin and rutin did not change significantly from the dormancy period to the time of bud swell. Later, at the onset of bud break the levels decreased to a level lower than the initial concentration found during the dormancy period. Quercetin-3-β-D-glucoside changed similarly as did epicatechin and rutin. The concentration of kaempferol-3-*O*-glucoside increased significantly at bud swell, and thereafter it decreased to a level similar to that of the dormancy stage.

The completion of this study will seek to provide valuable information on the phenolic compounds in different plant organs that could be used to enhance the nutritional content in the black currant material and support the development of resistant cultivars for sustainable growing. The information will thus (i) aid black currant breeders to develop new cultivars, adaptable and hardy enough for the changing climate, (ii) provide organic growers with information on cultivars resistant to severe pests and diseases, and with enhanced nutritional content, (iii) provide cultivars to North of Sweden with increased ascorbic acid and polyphenol content, (iv) support the health industry in preparation of functional food ingredients from different black currant parts with high antioxidant capacity, (v) support the processing sector with the introduction of fruits with superior quality and sensory attributes, (vi) support researchers in investigating the health properties of single polyphenols identified in this study and finally, (vii) benefit consumers and the nation on the whole due to health benefits associated from consumption of antioxidant and polyphenol rich black currant products.

CONCLUSIONS

This study shows that the content of different phenolic compounds in buds of black currant vary within the season and variations could mainly be found during the onset of the growth period. Among the studied phenolic compounds epicatechin and rutin occurred in highest amounts whereas the content of chlorogenic acid and quercetin were low.

ACKNOWLEDGEMENTS

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Tables

Table 1. Schedule for the planned sampling of buds, leaves and fruits in three seasons following their ontogenetic development.

Plant organ	Sampling 1	Sampling 2	Sampling 3
Buds	autumn	winter	spring
Leaves	spring	early summer	summer
Fruits	green	onset of ripening	fully ripened

Table 2. Concentration of identified phenolic compounds (µg/g DW) at different developmental stages. For each phenolic compound significant differences between developmental stages (Tukey, $P < 0.05$) are indicated by different letters.

Compound	Dormancy		Bud swell		Bud break	
Epicatechin	1734	b	1808	b	1136	a
Chlorogenic acid	37	a	39	a	40	a
Rutin	1795	bc	2043	c	1464	ab
Quercetin-3-β-D-glucoside	338	bc	434	c	283	ab
Kaempferol-3-*0*-glucoside	278	a	378	b	278	a
Isorhamnetin-3-*0*-glucoside	193	a	203	a	282	b
Quercetin	79	ab	96	c	66	a

Figures

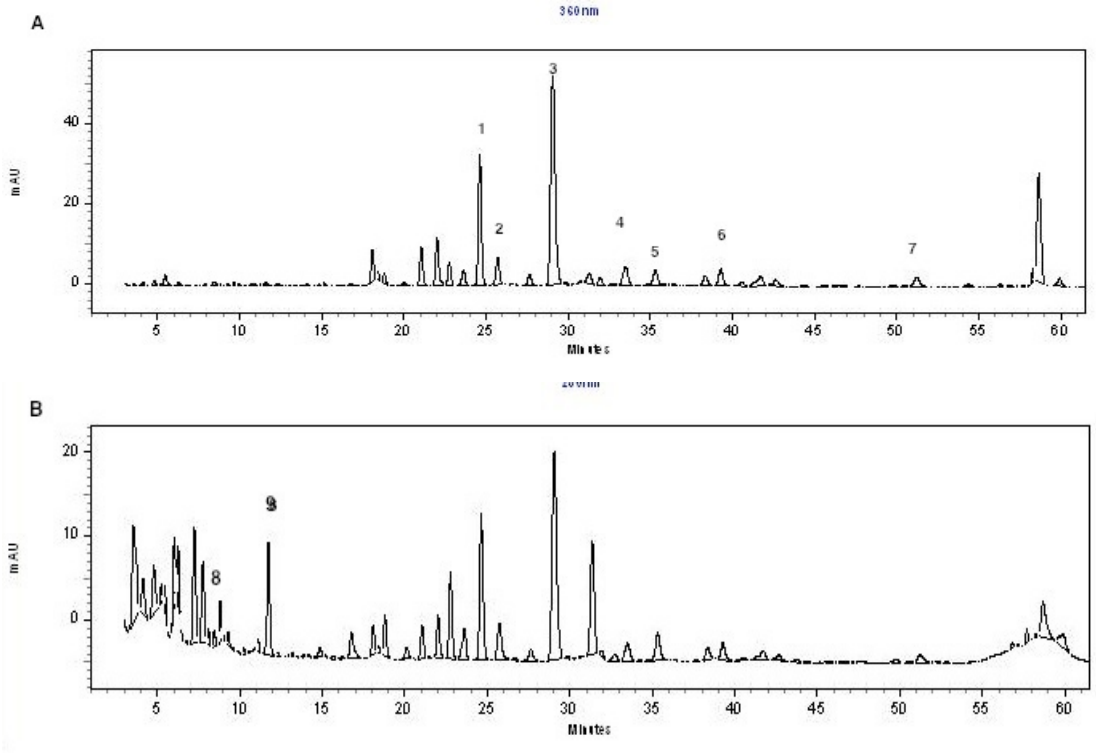

Fig. 1. HPLC-DAD traces of phenolic compounds identified in buds recorded at (a) 360 nm and (b) 280 nm. 1. Rutin, 2. Quercetin-3- β-D glucoside, 3. Unknown, 4. Kaempferol-3-*O*-glucoside, 5. Isorhamnetin-3-*O*-glucoside, 6. Unknown, 7. Quercetin, 8. Chlorogenic acid, and 9. Epicatechin.

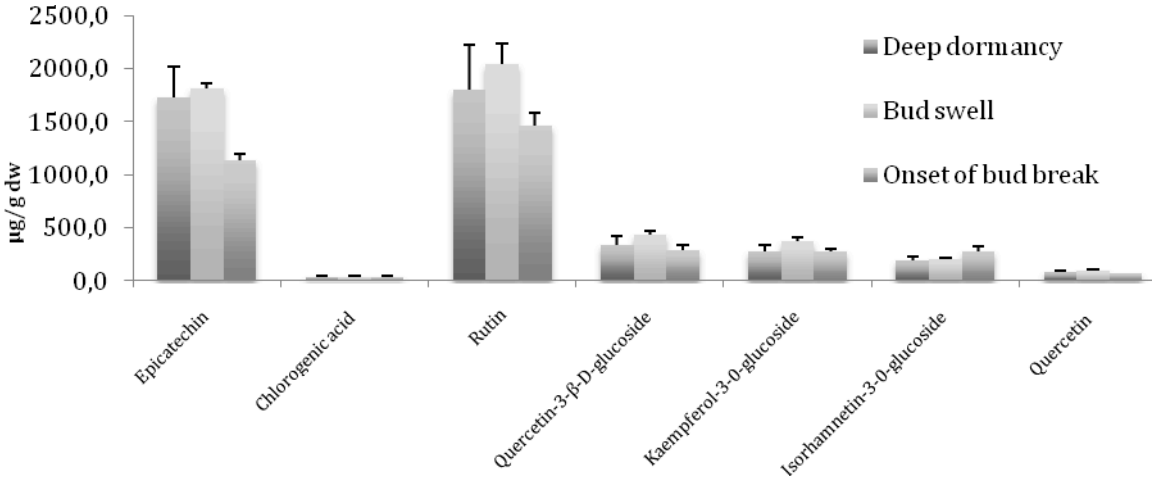

Fig. 2. Differences in the concentrations of specific polyphenols in buds collected from selection SCRI 8872-1 over three ontogenetic stages. It could be observed that concentrations of different phenols tended to increase at the time of bud swell followed by a decrease to a level lower than that of the dormancy period at the time of bud burst.