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The effect of different berries in human nutrition

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Summary: The aims of this study were on the one hand to compare of examined compounds according to their importance in nutrition and human-health, and on the other hand we made preexperiments to investigate the relationships among antioxidant capacity and the endogenous substances which contribute the antioxidant status of the plants. The following species were involved in the experiment: raspberry, blackberry, black currant, elderberry and sour cherry. These fruits have potent health-promoting antioxidant power. Glucose, fructose, total phenol, formaldehyde and anthocyanin contents were determined in addition to ferric reducing ability. Our results reflected considerable differences in the measured parameters of the analysed species. In blackberries and elderberries the high antioxidant capacity is coupled with low carbohydrate content. Besides the formerly proven correlations between total phenol content, anthocyanin concentration and antioxidant capacity, these parameters also correlated with the measured formaldehyde concentrations, hereby we can follow the methylation /demethylation status of the plant.

Key words: antioxidant capacity, berries, carbohydrates, formaldehyde, trans-methylating processes

Introduction

Nowadays, increased interest in human health and nutrition has focused the prevention of degenerative processes caused by oxidative stress. Fruits contain many different dietary phytonutrients with strong antioxidant effect. Antioxidants of natural origin may be of major importance in preventing of oxidative stress, cancer and phytochemicals are inversely related to cardiovascular and coronary heart disease and have also genoprotective effects (*Barros* et al., 2006). Epidemiological studies have also been provided showing that dietary intake of fruits, their constituents, like vitamines, carotenoides, phenolic compounds are beneficial to human health and has antimicrobial, antifungal effects (*Uncini Manganelli* et al., 2005; *Rauha* et al., 2000). The aim of the present study was to determine carbohydrates, anthocyanin, HCHO and total phenol content and studying the biological properties of berries (raspberry – *Rubus ideaus* L., blackberry – *Rubus fruticosus* L., black currant – *Ribes nigrum* L., elderberry – *Sambucus nigra* L.) and sour cherry (*Prunus cerasus* L.). These plants are used in traditional human medicine and in recent years the use of plants as a way of treatment and their products is still very important for human beings. Many studies have been published in recent years the berries importance in the light of their phytonutrients content (*Marinova & Ribarova*, 2006; *Maisuthisakul* et al., 2007; *Landbo & Meyer*, 2004; *Hannum*, 2004; *Kikuzaki* et al., 1999).

Raspberry, blackberry and black-currant are among the berries that contain expressly high amounts of vitamine C and phenolic compounds. Fresh black currants are particularly rich in anthocyanins. Other principal phenolics present in black currants include flavonols, procyanidins and various phenolic acids (*Benvenuti* et al., 2004).

Dimitrios (2006) reviewed that the top antioxidant sources are fruits and vegetables. The dietary antioxidants as agents are capable to promote health. Dietary antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. The health benefits of fruits and vegetables are largely due to the antioxidant vitamins supported by the large number of phytochemicals, some with greater antioxidant properties.

A short-term effect of a phenolic-rich juice made from grape and berries on the antioxidant status of a group of healthy human subjects was evaluated (*Garcia-Alonso* et al., 2006). The data indicate that phenolics from the juice were bioavailable and able to bind with the lipid fraction of serum, therefore reducing lipid peroxidation. The increase in protein oxidation associated with the juice intake was observed. It has been also reported that natural carbohydrates from fruits, mainly soluble sugars, are capable of mediating oxidative stress in vivo, by increasing carbonyl proteins after intake.

Different plant varieties of black-currant berries were studied to investigate chemical and technological properties, to evaluate the amount of anthocyanins and their composition. The concentration of anthocyanins was from 14.54 to 11.19 mg g^{-1} cakes in different harvesting years. The same tendency was observed in juice: ranged between 1956 mg l^{-1} and 1199 mg l^{-1} (*Rubinskiene* et al., 2005).

The anthocyanin content, and more specifically the presence of cyanidin-3-glucoside, in blackberry, contributes a major part of the antioxidant ability to suppress both peroxyl radical-induced chemical and intracellular oxidation (*Elisia* et al., 2007). Antioxidant activities of both the crude and anthocyanin-enriched blackberry extracts were determined. It is of interest that, although the concentration of total anthocyanins in the anthocyanin-enriched extract was approximately 20 times greater than the crude extract, the antioxidant capacity of the anthocyanin enriched extract was increased relatively less (e.g., 7.3 times) than that measured in the original crude extract.

Buds and leaves were used for the extraction of total phenolics and antioxidant capacity from black currant. It has been measured remarkable antioxidant activities, the extent of which depends on the extraction conditions (*Tabart* et al., 2007).

Raspberry, blackberry, raspberry × blackberry hybrids, red-currant, gooseberry and Cornelian cherry cultivars was investigated for their antioxidant capacity, phenol, anthocyanin and ascorbic acid content. Considerable data suggest that FRAP (ferric reducing ability of plasma) values were highly correlated with phenol content, whereas a less linear correlation between total antioxidant capacity and anthocyanin content was recorded. Inversely, ascorbic acid content was negatively correlated with FRAP values (*Pantelidis* et al., 2007). This is in agreement with results of strawberries, where it has been shown that there is a positive correlation between antioxidant activity and total phenolic oranthocyan in content (*Wang & Lin*, 2000).

Other researchers demonstrated that there is no direct correlation between the level of flavonoids in the extracts and their antioxidant activity by analysed the flowers, berries and leaves of *Sambucus nigra* L. (*Dawidowicz* et al., 2006).

The influence of blackcurrant and elderberry concentrates was tested on the growth of microorganism too. Purified anthocyanins do not have significantly effect on the growth of microorganisms. Therefore the inhibitory effects may be attributed to other phytochemicals present in the concentrates and only 15–25% of the radical reducing activity of the blackcurrant concentrates was attributable to their anthocyanin content (*Werlein* et al., 2005). For in vivo studies, a special antioxidant juice (containing 30% white grape-, 25% blackcurrant-, 15% elderberry-, 10% sour cherry-, 10% blackberry- and 10% aronia-juice) was obtained in order to test the health protective potential. Consumption of a single dose of 400 ml antioxidant juice induces a significant rise in plasma antioxidant capacity and a significant decrease in plasma malondialdehyde in vivo. It could be demonstrated that after juice consumption valuable ingredients like ascorbic acid and anthocyanins are available for human volunteers and are active as antioxidants in vivo (*Netzel* et al., 2002).

Basic health-related constituents present in ten categories of fruit wines was determined and compared with traditional wines. Elderberry, blueberry and black-currant, wines were identified with the highest concentrations of phenolics comparable to red wine. Total antioxidant capacity of wines strongly correlated to total phenolic content (*Vasantha Rupasingh & Clegg*, 2007).

Healthy subjects receiving a single oral dose of elderberry juice, where the plasma antioxidant capacity and the total phenolics were significantly increased 1 h after ingestion, which may indicate that some of the absorbed phenolics have antioxidant function in vivo (*Netzel* et al., 2005).

Materials and methods

Beside the quantitative and qualitative identification of the different carbohydrate fraction's we analysed the total phenol content, the concentration of anthocyanin and capacity of antioxidant's in the bearing of raspberry, currant, cherry, blackberry and elderberry.

On the same homogenized samples we measured quantity of formaldehyde (HCHO) bondable by dimedon and made quantitative analysis of some quaternery ammonium compounds (choline, carnitine, N^{ε}-trimethyl-L-lysine, betaine, trigonelline).

The evaluated parameters show significant assortment dependency – based on other- and our published results – and this dependency was proved by several assortment vegetable and fruit.

As we aimed to make characterization the species between each others with our analysis, we didn't used exact cultivars for our measuring, but the available mixture of the grew cultivars from the given species.

Separation and determination of carbohydrates

The fruit mass were suspended in methanol:distilled water (80:20, V/V) solution. This suspension was centrifuged at 13000 *g* for 10 minutes at 4 °C. The clear supernatants were used for overpressured layer chromatographic separations (OPLC chromatograph developed by OPLC-NIT Ltd., Budapest, Hungary). OPLC separations were carried out on TLC and HPTLC silica gel 60 F_{254} precoated chromatoplates (Merck Co., Darmstadt, Germany) using acetonitrile: distilled water (85:15, V/V). Staining was performed by aniline – diphenyl amine – phosphoric acid reagent. For densitometric determination a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), λ = 540 nm was used (*Sárdi* et al., 1996, 1999).

Separation and determination of HCHO and fully N-methylated compounds

The fruit mass were suspended in dimedone solution (0.05% dimedone in methanol) (e.g. 0.30 g plant powder/ 0.7 ml of 0.05% dimedone solution). This suspension was centrifuged at 1500 g for 10 minutes at 4 °C. The clear supernatants were used to OPLC separations.

The separations were carried out on OPLC silica gel 80 F_{254} precoated chromatoplates using a chloroform – methylenechloride mixture (35:65,V/V) for formaldemethone determination and an i-propanol – methanol – 0.1M sodium acetate mixture (20:3:30,V/V) for quaternary ammonium compounds. Calibration curves were made by means of authentic substances (at λ = 265 nm for formaldemethone and at λ = 525 nm for quaternary ammonium compounds which were detected by Dragendorff reagent) (*Gersbeck*, et al., 1989). Densitograms were taken with a Shimadzu CS-930 scanner. Samples were applied with a NANOMAT sample applicator.

Determination of total phenol content

Total phenol content was determined by spectrophotometer (λ = 760 nm) using Folin-Ciocalteu reagent (*Singleton & Rossi*, 1965).

Determination of antioxidant capacity

Antioxidant power was measured by the FRAP (Ferric Reducing Ability of Plasma) method at λ = 593 nm using tripyridyl-triazine (*Benzie & Strain*, 1996).

Determination of anthocyanin content

Anthocyanin content was evaluated spectrophotometerically in absorption maximum according to the method of *Füleki & Francis* (1968).

Results

From the nutritional point of view in case of fruits the carbohydrates first of all have role to determine level of enjoyment. Furthermore in case of some diseases, for example serious diabetes, the quality and quantity of the carbohydrate consumed up by eating fruits is highly meaningful.

By analysing the homogenised samples of raspberry, black currant, cherry, blackberry and elderberry glucose and fructose were determined in well detectable amount. In the glucose content of the fruits significant differences were detected. (*Figure 1*) Glucose level was the highest in black currant and sour cherry and lowest was in raspberry and blackberry.

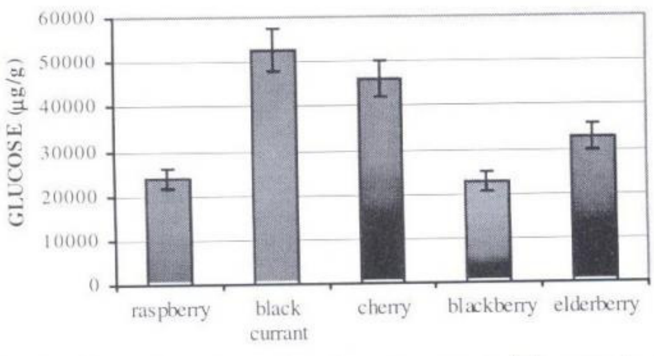

Figure 1. Comparison of genera based on measurement of glucose content.

Based on fructose content similar differences were detected between the species (*Figure 2*). Fructose content was about two times higher than glucose content in raspberry and black currant, about 1.5 times higher in sour cherry and nearly the same was in blackberry and elderberry.

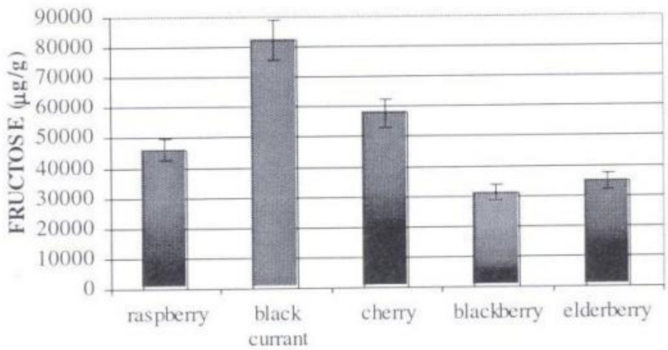

Figure 2. Comparison of genera based on measurement of fructose content.

The parameters, what were analysed in connection with the ability of bounding free radicals were the total phenol content, the concentration of anthocyanin and antioxidant capacity (the ferric reducing ability). On the same samples the amount of formaldehyde bondable by dimedone was also measured.

The quantity of quaternary ammonium compounds was also determined. These compounds can be realised initial materials of HCHO, but because of methodological causes this measurement did not gave valuable results in this experiment.

On the basis of measured parameters correlating to free-radical scavenging activity all results prove the advantage of blackberry and chiefly elderberry compared to raspberry, black currant and sour cherry (*Figures 3, 4, 5*).

The HCHO content of fruit samples, correlating to trans-methylating process of fruits, can be seen on *Figure 6*. The blackberry and elderberry show the highest amount of HCHO content, the elderberry has twofold more formaldehyde than raspberry or black currant.

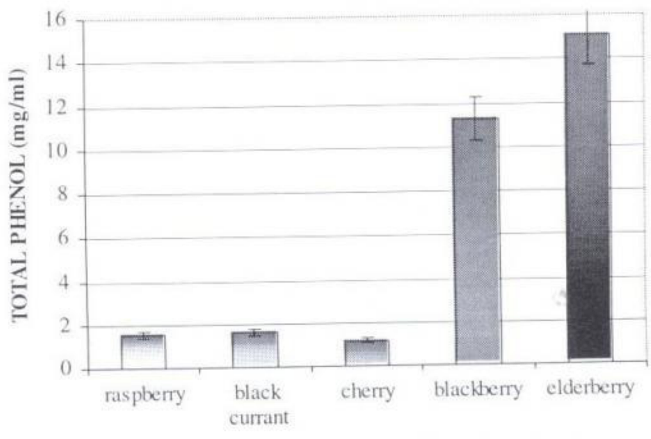

Figure 3. Comparison based on measurement of total phenol content.

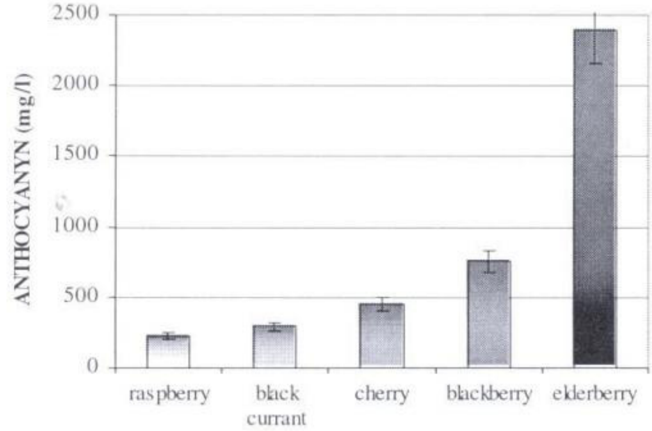

Figure 4. Comparison based on measurement of anthocyanin content.

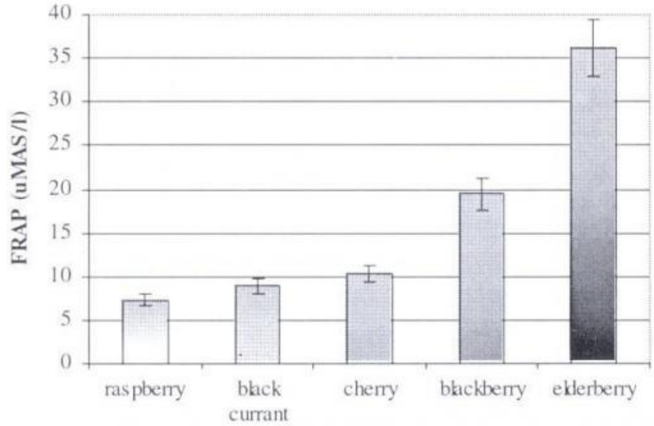

Figure 5. Comparison based on measurement of total antioxidant capacity by FRAP-assay.

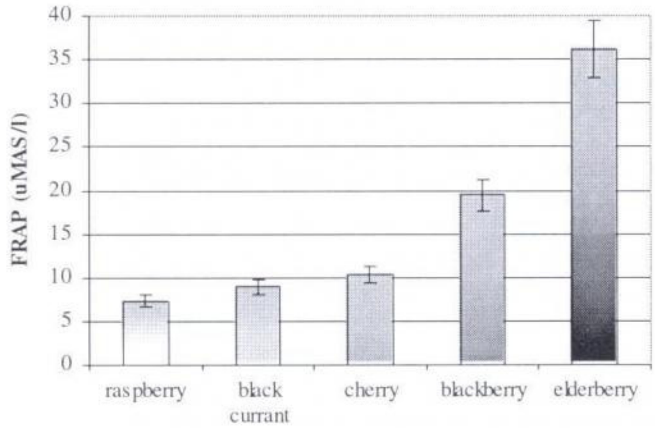

Figure 6. Comparison based on measurement of formaldehyde content.

Discussion

Besides quantitative and qualitative analysis of the different carbohydrate fractions the parameters correlating to free radical scavenging activities, as total phenol and anthocyanin content and antioxidant capacity were measured. Amounts of removable methyl-groups (dimedone bound formaldehyde) and quaternary ammonium compounds were also measured in raspberries, red currants, sour cherries, blackberries and elderberries.

These measured parameters highly depend on cultivars, but by the degree of differences between the observed species our results confirm that the effects of biologically active compounds of blackberry and elderberry fruits compared to raspberries, red currants and sour cherries are more significant.

According to former experiments fruit with lower antioxidant capacity also contain substantial compounds and have also an important role in maintaining the antioxidant status of the body. As *Szamosi* et al. (2006) described the antioxidant capacity of apples consumed in winter was ca. tenth of the measured value of raspberry.

In blackberries and elderberries the high antioxidant capacity is coupled with low carbohydrate content, which can be very important in connection with carbohydrate intake with fruits, especially for patients having diabetes or diabetes-connected diseases.

Besides the formerly proven correlations between total phenol content, anthocyanin concentration and antioxidant capacity, these parameters also correlated with the measured formaldehyde concentrations corresponding also to our former observations (*Sárdi* et al., 2004, *Sárdi & Tordai*, 2005, *Tordai* et al, 2006), which is indicative of importance of endogen trans-methylating processes. With the quantitative determination of the quaternary ammonium compounds (which are considered to be the pre-forms of HCHO) no detectable amounts were measured at the applied measuring conditions. The reason of this was the high pigment content of fruits, which hindered the fractionation and detection of these compounds. For this reason it can be stated that the methods of analytical determination of these compounds need to be further developed.

The species- and cultivar-comparing methods based on quantitative determination of the measured components could be – with further method developments – appropriate for developing faster testing methods and therefore could be helpful in breeding and cultivation. Our former and planned experiments could also be important in discovering new correlations in antioxidant protection of organisms.

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