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Total Phenolic Content and Antioxidant Capacity of Fruit Juices

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Abstract: The interest in polyphenolic antioxidants has increased remarkably in the last decades due to of their elevated capacity in scavenging free radicals associated with various human diseases. Previously, some fruits were shown to contain high antioxidant activities. Fifteen fruit juices were analyzed for total phenolic content and antioxidant capacity (oxygen radical absorbance capacity, ORAC). The total phenolic content (TPC) was measured by Folin-Ciocalteu assay and gallic acid used as standard. TPC varied from 7.3 mg GAE/100 mL for aloe vera juice to 71.8 mg GAE/100 mL for cranberry juice. The value of antioxidant capacity was determined by ORAC test, using 2,2'-azobis(2-amidino-propane) dihydrochloride as reactive species and Trolox as a standard. Obtained values were from 27.1 μmol TE/100 mL for aloe vera juice to 1271.8 μmol TE/100 mL for black currant juice. Results from the present study suggest further analysis on chemical composition of samples in order to identify compounds that might be responsible for antioxidant activity.

INTRODUCTION

Phenols are aromatic compounds containing one or several hydroxyl groups directly attached to the benzene ring. According to the number of hydroxyl groups, phenols are classified as dihydric, trihydric and polyhydric. By the year 2005, thousands of polyphenolic compounds have been isolated from plants (Prior, 1995). There are many spectrophotometric methods for the quantification of phenolic compounds in plant materials. Based on different principles, these methods are used to determine various structural groups present in the phenolic compounds. Spectrophotometric methods enable either the quantification of all extracted phenolics as a group (Swain, and Hillis, 1959; Price, and Butler, 1977; Earp et al., 1981), or the quantification of specific phenolic substances such as sinapine (Tzagoloff, 1963) or the sinapic acid (Naczk, et al., 1992). Spectrophotometric methods are also used in the quantification of a whole class of phenols such as phenolic acids (Price, et al, 1978; Mole, and Waterman 1987; Naczk, and Shahidi, 1989; Brune, et al, 1991).

Some of the most commonly used assay methods for phenolic compounds include the modified vanillin test (Price, et al., 1978), the Folin-Denis assay (Swain, and Hillis, 1959), the Prussian blue test (Price, and Butler, 1977) and the Folin-Ciocalteu assay (Maxson, and Rooney, 1972; Hoff, and Singleton, 1977; Earp et al, 1981; Deshpande, and Cheryan, 1987). The antioxidant capacity can be measured in pure substances as well as in mixtures of different samples of herbal and animal origin, such as plasma, blood, tissues homogenates of fruits and vegetables, juices and other foods. There are many methods

for measuring of total antioxidant capacity (AC), but in literature the most often cited are the following three: FRAP - Ferric Reducing Antioxidant Power (Benzie, and Strain, 1996), ORAC - Oxygen Radical Absorbance Capacity (Cao, and Prior, 1999), and TEAC - Trolox Equivalent Antioxidant Capacity (Rice-Evans, and Miller, 1994). Based on the reaction mechanism involved, major antioxidant capacity assays can be roughly divided into two categories (Huang, et al., 2005): hydrogen atom transfer (HAT) and single electron transfer (ET) reaction based assays. Most HAT-based assays monitor competitive reaction kinetics and the quantification is derived from the kinetic curves. Generally, these assays are composed of a synthetic free radical generator, an oxidizable molecular probe and an antioxidant. The aim of this study is to quantify the total phenolic content (TPC) and the antioxidant capacity (AC) in fresh juice of the following fruits: blueberry (*Vaccinum myrtillus* L.), cranberry (*Vaccinum macrocarpon* L.), black currant (*Ribes nigrum*), red currant (*Ribes rubrum*), red and white grapes (*Vitis vinifera* L .), red orange (*Citrus sinensis* L.), lemon (*Citrus limonia* L.), lime (*Citrus aurantifolia* L.), grapefruit (*Citrus paradisi* L.) kumquats (*Fortunella*), black chokeberry (*Aronia melanocarpa* L.), aloe vera (*Aloe vera* L.), apple (*Malus pumila*) and pomegranate (*Punica granatum*).

EXPERIMENTAL

Samples

Samples of blueberries, cranberry, black currant, red currant, red grape, white grape, red orange, lemon, lime, grapefruit, cumquat, commercial chokeberry, commercial aloe vera and pomegranate,were purchased from local markets.

Sample preparation

One mL of fresh juice from samples was diluted up to the volume of 25 mL. Part of solution was centrifuged at 15000 rpm for 20 minutes at 4°C. Supernatant solution was used for analysis. Also, non-centrifuged juices were analyzed.

Determination of total phenolic content

The total phenolic content (TPC) was determined by spectrophotometry, using gallic acid as a standard, according the method described by Singleton and Rossi (1965). Briefly, 0.2 mL of the diluted sample extract was transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 10 minutes, 0.8 mL of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 743 nm was measured. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 mL of fruit juice. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 0.2 to 4 mg/L.

Oxygen Radical Absorbance Capacity (ORAC) Assay

The Oxygen Radical Absorbance Capacity (ORAC) assay measures the antioxidant scavenging function against peroxyl radical induced by 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH). Fluorescein is used as a fluorescent probe. The loss of fluorescence of fluorescein is an indication of the extent of damage from its reaction with the peroxyl radical (Cao, and Prior, 1999). The total reaction mixture of 100 μL of diluted supernatants of juices, 50μL solution of fluorescein (0.32 μM), and 1650 μL of water was incubated at 37 °C for 15 min. After the incubation, 200 μL of AAPH (320 mM) was added rapidly to start the reaction. The fluorescence was recorded every 5 min until relative fluorescence intensity of fluorescein was near to zero. Calibration of solutions of (±)-6-hydroxy 2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (0.1; 0.25; 0.5; 0.75, and 1 μM) were carried out. The final ORAC values were calculated using a linear equation from calibrated curve. ORAC values were expressed as μmol Trolox equivalents (TE) per 100 mL of fruit juice.

RESULTS AND DISCUSSION

Total phenol content (TPC) in fruit juices was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965) using gallic acid as the standard. Maximum wavelength for blue colored complex was at 743 nm. After determination of the λ_{max} of colored complex the absorbances of all standards were taken to construct a calibration curve (Fig. 1).

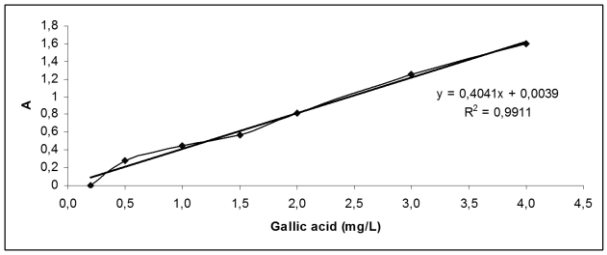

Figure 1: Calibration curve for gallic acid.

TPC was determined in 15 different fruit samples, first in non-centrifuged and then in centrifuged samples according to previously written procedure. As shown in Table 1 values for TPC varied from 6.16 to 71.76 mg GAE/100 mL. The highest TPC was in non-centrifuged (66.1 mg GAE/100 mL) and centrifuged sample (71.76 mg GAE/100 mL) of cranberry. The lowest TPC was in non-centrifuged (6.16 mg GAE/100 mL) and centrifuged (7.32 mg GAE/100 mL) sample of aloe vera.

Blueberries are a rich source of phenolic compounds such as phenolic acids and flavonoids. Literature value for TPC in blueberries ranges from 430 to 1990 mg GAE/kg of fresh fruit (Ehlenfeldt, and Prior, 2001). TPC values in blueberries measured in this work were 30.89 and 30.94 mg GAE/100 mL. For statistical analysis of the data t-test was used. The t-test showed statistically lower mean TPC values in non-centrifuged samples of nine fruits (cranberry, lemon, grapefruit, red orange, black chokeberry, black grapes, lime, apple and aloe vera) than in centrifuged samples ($p^{***}<0.001$). For the other six sorts of fruits (black and red currant, blueberry, kumquat, pomegranate and white grapes) the mean TPC values were higher in non-centrifuged than in centrifuged samples ($p^{*}<0.05$).

Measurement of antioxidant capacity (AC) was performed by manual ORAC method (Cao and Prior, 1999). Maximum of excitation (λ_{max}=485 nm) and emission (λ_{max}=520 nm) wavelengths were determined using trolox as a standard. After determination of wavelengths for excitation and

emission relative fluorescence intensity of all concentration of trolox was used to construct a calibration curve (Fig. 2).

Table 1: Total phenolic content in investigated samples.

Sample	***Non-centrifuged sample*** (mg GAE/100mL)	***Centrifuged sample*** (mg GAE/100mL)
Cranberry	**66.61**	**71.76**
Red currant	40.80	30.40
Black currant	37.20	36.72
Blueberry	32.89	30.94
Lemon	31.85	47.20
Grapefruit	30.60	45.12
Red orange	21.54	35.10
Black chokeberry	20.70	35.48
Black grapes	17.50	21.54
Kumquat	15.02	11.65
Lime	14.83	28.13
Pomegranate	12.23	10.22
White grapes	11.68	8.63
Apple	9.75	15.8
Aloe vera	**6.16**	**7.32**

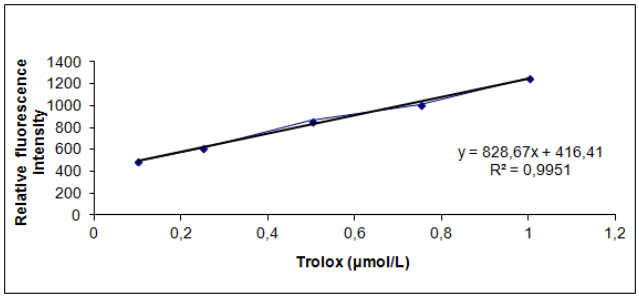

Figure 2: Calibration curve for trolox.

Determination of AC in 15 different fruit samples was carried out first for non-centrifuged and then for centrifuged samples with peroxyl radicals generated from AAPH and result are shown in Table 2. As shown in Table 2, there were big differences in antioxidant capacity between selected samples. The AC values varied from 27.07 to 1271.8 μmol TE/100 mL.

The highest value for AC was in black currant (1271.8 μmol TE/100 mL) and lowest in aloe vera (27.07 μmol TE/100 mL). For non-centrifuged sample the highest value for AC was in black chokeberry (1086.6 μmol TE/100 mL) and lowest in white grapes (30.82 μmol TE/100 mL). The t-test showed statistically significant higher mean AC values in non-centrifuged than in centrifuged samples of nine sorts of fruits (black chokeberry, apple, cranberry, pomegranate, blueberry, lime, lemon, aloe vera and red orange ($p^{**}<0.01$). For other six sorts of fruits (red and black currant, black and white grapes, kumquat, and grapefruit) the mean AC values were higher in centrifuged than in non-centrifuged samples, but no stastically significant difference was evident ($p>0.05$).

In a living system, phenolic compounds, some enzymes, peptides and vitamins serve as protection agents against oxidative damage caused by free radicals and are called antioxidants. Consumption of foods rich in this type of compounds have resulted in an increase in total antioxidant capacity (AC) in the blood plasma of people (Cao, & Prior, 1999; Sofić et al., 2005). As one potential source, plant phenols have primary antioxidant activity (Shahidi & Wanasundara 1992).

Table 2: Antioxydant capacity in investigated samples

Sample	***Non-centrifuged samples*** ORAC (μmol TE/100 mL)	***Centrifuged samples*** ORAC (μmol TE/100 mL)
Black chokeberry	**1086.60**	666.60
Black currant	500.80	**1271.80**
Red currant	422.60	540.50
Apple	389.40	196.40
Cranberry	379.70	336.60
Pomegranate	340.50	151.40
Blueberry	297.80	206.80
Lime	285.10	111.90
Lemon	223.10	125.90
Grapefruit	105.04	200.30
Aloe vera	81.35	**27.07**
Red orange	71.72	35.68
Black grapes	58.60	94.30
Kumquat	38.16	133.50
White grapes	**30.82**	31.10

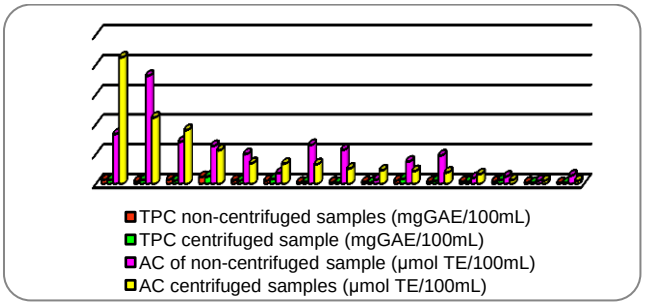

Figure 3: Total phenolic content and antioxidant capacity of fruit juices.

In summary, TPC and AC of 15 samples of fruit juices that is non-centrifuged and centrifuged was determined by the Folin-Ciocalteu method and ORAC assay with peroxyl radical generator. Black currant and black chokeberry showed the highest value of an antioxidant capacity but they have lower content of TPC than cranberry. Among all of this samples aloe vera showed lowest content of TPC and lowest value for AC.

CONCLUSIONS

Proteins residing in solutions of non-centrifuged samples increased the antioxidant capacity of those fruits. The influence of the preparation procedure on the total phenolic content and antioxidant capacity for each sample remains a subject for further research.

There is no linear corelation between the total phenol content and antioxidant capacity in neither the centrifuged nor in non-centrifuged samples. Our findings suggest that further studies should be conducted with non-centrifuged samples as such produce higher AC values.

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Summary/Sažetak

Interes za antioksidante polifenolske prirode se povećao zadnjih decenija zbog njihove sposobnosti hvatanja slobodnih radikala, povezanih sa različitim bolestima. Od ranije je već poznato da voće posjeduje visok sadržaj antioksidanata. Petnaest različitih voćnih sokova je analizirano na sadržaj ukupnih fenola i antioksidativnu aktivnost. Ukupni fenolski sadržaj određen je Folin-Ciocalteu metodom, uz upotrebu galne kiseline kao standarda. Vrijednosti variraju od 7.3 mg GAE/100 ml za sok od aloe vera do 71.8 mg GAE/100 ml za sok brusnice. Vrijednost antioksidativnog kapaciteta određena je ORAC testom, koristeći 2,2 '-azobis (2-amidino-propan) dihidrohlorid kao reaktivnu vrstu i troloks kao standard. Dobivene vrijednosti su od 27.1 μmol TE/100 ml za sok od aloe vera do 1271.8 μmol TE/100 ml za sok crne ribizle. Rezultati ovog istraživanja ukazuju na daljnju analizu hemijskog sastava uzoraka kako bi se identificirali spojevi koji bi mogli biti odgovorni za antioksidativnu aktivnost.