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ANTIOXIDANT CAPACITY EVALUATION IN RELATION WITH POLYPHENOLOS AND ASCORBIC ACID CONTENT FOR SOME NATURAL JUICES

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Abstracts

Twelve different commercial natural juices of fruits (six nectar and six clear juices) from Timisoara market were analyzed for some chemical characteristics (total acidity, soluble substance, total extract-determined on the basis of standardized method) and antioxidant activities (using FRAP method), total polyphenols amount (by Folin-Ciocalteu method) and ascorbic acid content (using 2,6-dichlorophenol indophenol method). The antioxidant capacity varied between 1.5-3 mM Fe^{2+}/L for clear natural juices and between 4-6.5 mM Fe^{2+}/L for fruit nectar. The polyphenols content it was situated between 0.8-1.1 mM acid gallic/L for clear natural juices and between 2-5 mM acid gallic/L for fruit nectar. The values obtained for ascorbic acid content was situated between 0.5-2.2 mM/L, different for clear juices and nectar.

***Key words**: antioxidant capacity, polyphenols, ascorbic acid, fruit nectar, clear natural juice*

Introduction

The natural juices are of great interest because of their potent antioxidant capacity and possible protective effects on human health. Flavonoids in fruits, vegetables and juices appear to play a significant role in cancer and heart disease health benefits, and proanthocyanidins account for a significant portion of the total flavonoids ingested by peoples. Antioxidants have been shown to prevent the oxidation of cholesterol, especially the bad cholesterol, low-density lipoprotein (LDL). High cholesterol is, of course, a scientifically documented risk factor for heart disease. Recently LDL oxidation has been hypothesized as the beginning of the atherosclerosis process that starts

in childhood. In short, your pipes begin to rust at an early age (Dragsted, 2003; Facino, 1999; Fitzpatrick, 2002).

In according to the many authors (Simonetti, 2002; Tosun, 2003), people drink fruit juice for many reasons: orange juice, for example, is an excellent source of Vitamin C, folate and potassium nutrients, many people may consume in sufficient quantity in their diets otherwise. But for people looking for dietary approaches to increase consumption of natural antioxidants that may help reduce the risk of heart disease, purple grape juice appears unique in its potential ability to provide a beneficial effect to a variety of cardiovascular functions. By drinking this juice, you are going to give yourself the antioxidant profile you need to protect your arteries, your brain cells, and literally every part of your body from oxidation. You will also maximize and optimize your health and energy levels with something that actually tastes delicious.

The purpose of this paper was to determine the total antioxidant capacity and to found some correlations between total polyphenos amount, ascorbic acid and antioxidant capacity for natural juices from Romanian market. Fruit polyphenols include a wide range of compounds with antioxidant activity, that is: anthocyanins, flavonols, flavan-3-ols, hydroxycinnamates (Harmanescu, 2005).

The measurement of total antioxidant capacity (TAC) can be making using different methods (photometric, chemiluminescence's and spectrophotometric methods). On the basis of the mentioned data in literature and on the experience of the authors regarding the determination of antioxidant capacity, we chose the modified method FRAP. This method it was based of the ability of antioxidant substances present in analyzed juices to reduce the ferric ions (Fe^{3+}) from tripyridyltriazine complex to the ferrous ions (Fe^{2+}). The ferrous tripyridyltriazine complex has an intensive blue color and can be monitored at 593 nm (Benzie & Strain, 1996).

This method is properly in the case of total antioxidant capacity determination for different natural products (Tosun, 2003; Gergen, 2004, Gergen, 2005).

For polyphenols determination (expressed such as mM/L acid galic) will be use the spectroscopic method with the Folin Ciocalteu reactiv (Folin, 1927; Gergen, 2004; Gergen, 2005).

Experimental

Reagent and equipment: All chemicals and reagents were analytical grade or purest quality purchased from Merck, Fluka, Sigma. Was used distilled water. Absorption determination for FRAP and total phenol content was made using Spectrophotometer Specord 205 by Analitik Jena.

Nectar and clear drinks: In the present research, a total six fruit clear natural juices (red orange, orange, tutti frutti, strawberry&banana, grapefruit, pine-apple) and six nectars (apricot, pear, orange, peach, cherry, tutti frutti) were investigated. These juices were processed of S.C. EUROPEAN DRINKS S.A., Bihor, Romania (FRUTTIA mark) and purchased from Timisoara supermarkets.

Determination of Total Antioxidant Capacity (Adaptation of FRAP method): Reagents: acetate buffer, 300 mM/L, pH = 3.6 (3.1 g sodium acetate $3H_2O$ and 16 mL conc. Acetic acid per 1L off buffer solution); TPTZ (2,4,6-tripyridyl-s-triazine) solution 10 mM/L (0.31 g TPTZ in 100 mL HCl). Prepared freshly always utilization; $FeCl_3$ solution 20mM/L (0.54 g $FeCl_3 \cdot 6H_2O$ in 100 mL distilled water). Prepared freshly always utilization; FRAP working solution (25 mL acetate buffer, 2.5 mL TPTZ solution, 2.5 mL $FeCl_3$ solution). Prepared freshly always utilization; Standard solution - Mohr salt 1mM/L.

Aqueous solution of known Fe concentration was used for calibration, in a range of 0.1-0.8 mM/L. For the preparation of calibration curve 0.5 mL aliquot of 0.1, 0.2, 0.4, 0.6, 0.8 μM Fe^{2+}/mL aqueous as Mohr salt solution were mixed with 2.5 mL FRAP working solution. FRAP reagent was used as blank. One mL from diluted 1/10 juices and nectars was mixed with the same reagents as described above, and after 10 min. absorption was read after at λ= 593 nm.

The Total antioxidant capacity in fruit juices and fruit nectar in Fe (II) equivalents was calculated. Correlation coefficient (r^2) for calibration curve was 0.998.

Determination of phenolic compounds: The content of total polyphenolic compounds was determined by Folin-Ciocalteu method. Reagents: Folin-Ciocalteu′s phenol reagent solution 1:10; Na_2CO_3 solution 7.5%; Standard solution - Gallic acid 10mM/L. For the preparation of calibration curve 0.5 mL aliquot of 0.2, 0.3, 0.4, 0.8 and 1.2 μM/mL aqueous gallic acid solution were mixed with 2.5 mL

Folin-Ciocalteu reagent and 2.0 mL sodium carbonate. One mL from diluted 1/10 juices and nectars was mixed with the same reagents as described above, and absorption was read after 2 h at λ= 750 nm. Total content of polyphenols in fruit juices and fruit nectar in gallic acid equivalents (GAE) was calculated. Correlation coefficient (r^2) for calibration curve was 0.995.

Determination of ascorbic acid: Ascorbic acid content was estimated titrimetrically with 2,6-dichlorphenolindophenol natrium dye reactant. 10 mL of clear natural juice (diluted 1/10) or nectar (diluted 1/20) with 10 mL distilled water and 1 mL HCl 1N it was titrated with 2,6-dichlorphenolindophenol natrium to pink color. On the basis of 2,6-dichlorphenolindophenol natrium volum it was determined the ascorbic acid amount. [Szeto, 2002; Gergen, 2004]

Results and Discussions

The commune chemical characteristics (total acidity, soluble substance, total extract) of natural juices are presented in Table 1.

Table 1. Total acidity, soluble substance and total extract of some clear juices

Nr. crt.	Natural juice	Total acidity (g malic acid /100 mL)	Soluble substance (°Bx)	Extract (g/L)
Clear juice				
1.	Red Orange	0.43	11.2	11.5
2.	Orange	0.36	10.2	10.4
3.	Tutti Frutti	0.45	11.2	11.5
4.	Strawberry& banana	0.17	11.4	11.7
5.	Grapefruit	0.25	10.2	10.5
6.	pine-apple	0.25	10.6	11.0
Nectars				
1	Apricot	0.88	15.4	18.2
2	Pear	0.47	15.8	17.4
3	Orange	0.86	13.0	13.8
4	Peach	0.70	15.9	19.7
5	Cherry	0.60	14.1	16.4
6	Tutti Frutti	0.61	13.1	14.9

The values for characteristics showed in table 1 are in according to the natural juices type (clear juices or nectars). The fruit percent in

clear juices is min. 5-10% while in nectar min. 35-50%. This fruit percent is proceeded to concentrate from natural fruit juice.

The total antioxidant capacity–TAC by FRAP method, Ascorbic Acid and polyphenols are presented in Table 2 for soft drinks and in Table 3 for analyzed nectars.

Table 2. Antioxidant capacity, polyphenols and Vitamin C content of some clear drinks

Clear juice	Antioxidant capacity (mM Fe^{2+}/L)	Poliphenols (mM acid gallic/L)	Ascorbic acid (mM/L)
Red Orange	2.57	1.13	0.88
Orange	2.48	0.91	0.96
Tutti Frutti	2.87	1.12	1.04
Strawberry & banana	1.40	0.58	0.48
Grapefruit	2.02	0.83	0.72
pine-apple	2.36	1.00	0.96

Table 3. Antioxidant capacity, polyphenols and Vitamin C content of some nectars

Nectars	Antioxidant capacity (mM Fe^{2+}/L)	Poliphenols (mM acid gallic/L)	Ascorbic acid (mM/L)
Apricot	4.64	3.9	1.28
Pear	5.27	3.56	1.60
Orange	4.90	2.26	2.24
Peach	4.56	3.86	1.76
Cherry	6.52	5.20	1.28
Tutti Frutti	4.30	3.18	2.08

The nectars present TAC more then clear juices. The same variation was observed for total polyphenols and ascorbic acid. For nectar, the highest polyphenols amount was found in cherry nectar and the smaller in orange. For clear juices, the highest polyphenol content is in tutti frutti juice and the smaller value was identified in strawberry &banana juices. For nectar, the ascorbic acid content is highest in orange and minim in apricot and cherry. For clear juices, the smaller values were identified in strawberry&banana juice and the maxim values in tutti frutti. We explained the different values for these characteristics on the basis of applied processing technology (for clear juices the phenolic compound were removed by clearing operation).

Conclusions

The fruit nectars were presented more total antioxidant activity, polyphenols and ascorbic acid content than clear natural juices. For clear natural juices, TAC is very good correlated with the sum of ascorbic acid and polyphenols content. For analyzed nectars, the correlation is not was observed. The results of this research show that, the natural juices have an excellent source of natural antioxidants being consumed with pleasure because are more readily assimilated than fruit tissues.

References

Benzie I.F.F., Strain L. (1996). Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem.*, 239, 70-76.

Dragsted L.O. (2003). Antioxidant actions of polyphenols in humans. *Int J Vitam Nutr Res.* 73(2),112-119.

Facino R.M., Carini M., Aldini G., Berti F., Rossoni G., Bombardelli E., Morazzoni P. (1999). Diet enriched with procyanidins enhances antioxidant activity and reduces myocardial post-ischaemic damage in rats. *Life Sci*., 64(8), 627-642.

Fitzpatrick D.F, Bing B., Maggi D.A., Fleming R.C, O'Malley R.M. (2002). Vasoɛdilating procyanidins derived from grape seeds. *Ann N Y Acad Sci*., 957, 78-89.

Folin O., Ciocalteu V. (1927). On tyrosine and tryptofane determination in protein. *Journal of Biological Chemistry*, 24, 627-650.

Gergen I., Poiana M.A., Parvulescu L., Moigradean D., Harmanescu M. (2005). Antioxidant Capacities, Poliphenols and Ascorbic Acid Contents of some Fruit Juices and Soft Drinks. *Papers of Int.Symp.Euro-aliment 2005, Galati,*, 139-142.

Gergen I. (2004). *Analiza produselor Agroalimentare*, Editura Eurostampa, Timisoara

Hărmănescu M., Vargias L., Poiană M.A., Gergen I., (2005). Antioxidant capacity and compozitional of different beverages, fruits nectars and syntehetic fruit juices. *Scientifical researches Agroalimentary Processes and Technologies*, XI(1), 193-198.

Simonetti P., Ciappellano S., Gardana C., Bramati L., Pietta P. (2002). Procyanidins from Vitis vinifera seeds: in vivo effects on oxidative stress. *J Agric Food Chem.* 50(21), 6217-6221.

Szeto Y. T., Tomlinson B., Benzie I.F.F. (2002). Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implication for dietary planning and food preservation. *Br. J. Nutr.* 87, 55-59.

Tosun I., Ustun N.S. (2003). An investigation about antioxidant capacity of fruit nectar. *Pakistan Journal of Nutrition*, 2, 167-169.