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POLYPHENOLS CONTENT, TOTAL ANTIOXIDANT AND RADICAL SCAVENGING CAPACITIES FOR DIFFERENT TOMATOES AND APPLES JUICES

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Abstract

In the present study, a total of 8 fruit juices (6 apples and 2 tomatoes pure juices) from local markets were analyzed for total antioxidant capacity and polyphenols content. Total antioxidant capacity was analyzed using FRAP and DPPH methods and total polyphenols content by Folin Ciocalteu method. The results obtained for total antioxidant capacities varied between 1.93 – 5.78 mM Fe^{2+}/L (for FRAP method) and between 7.32 – 36.81 % (for DPPH method). The values for total polyphenols content are between 0.36 – 2.64 mM/L.

***Key words**: antioxidant capacities, radical scavenging capacity, FRAP method, DPPH method, total polyphenols and fruit juices.*

Introduction

Codex Alimentarius defines juice as unfermented but fermentable juice, intended for direct consumption, obtained by the mechanical process from sound, ripe fruits, preserved exclusively by physical means. The juice may be turbid or clear. The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factors of the juice. The addition of sugars or acids can be permitted but must be endorsed in the individual standard (FAO, 1992).

Over the past several decades there has been a growing trend toward adding value to raw agricultural products. As populations have become more urban, this trend has accelerated. Within the globalization of the food industry, the demand for quality juice and juice type beverages has markedly expanded. Juices such as orange,

grape, pineapple, apple, tomato and blends are well established in developed countries.

Juices can be consumed more conveniently than whole fruits. The very young, elderly and infirm may have problems eating, let alone peeling certain fruits. Drinking juice is an effective, nutritious alternative. The health-promoting properties of fruits are due to the presence of some vitamins (A, C, E and folates), dietary fiber and other bioactive compounds in these food products.

Free radicals are molecules capable of independent existence that contain unpaired electrons: hydroxyl (HO•), superoxide (O_2•), peroxyl (ROO•), alkoxyl (RO•), nitric oxide (NO•) and are responsible for oxidative stress, rip open of the cell membrane, oxidation of lipid, protein and DNA, etc. Among bioactive compounds, polyphenols deserve a special mention due their free radical scavenging activities and in vivo biological activities (Bravo, 1998). Fruit polyphenols include a wide range of compounds with antioxidant activity, that is, hydroxycinnamates, flavan-3-ols (condensed tannins), gallic acids derivatives (hydrolyzable tannins), flavonols and anthocyanins (Gil et al., 2002).

The aim of the present work was to determine the polyphenols contents and the total antioxidant capacity of apples and tomatoes pure juices commonly consume in Romania. Many methods are know to measure the total antioxidant capacity (TAC), but we tried the FRAP and DPPH assays.

Experimental

Reagents and equipment: All chemicals and reagents were analytical grade or purest quality purchased from Sigma, Merck, Aldrich and Fluka. Deionized water was used. Absorption determination for FRAP, DPPH and total polyphenols content was made using SPECORD 205 spectrophotometer by Analytik Jena.

Samples preparation: In the present study, a total of 8 fruit juices (6 apples and 2 tomatoes juices) were analyzed for total antioxidant capacity and polyphenols content. The apples samples used for determination were: Santal (apples clear juices), Bravo Alma (apples clear juices), Naturalis (apples clear juices), Raureni (apples clear juices), SunGold (apples clear juices) and Basarabia Nectar (apples turbid juices). The tomatoes juices were represented by Fruttia (tomatoes turbid juices) and Ciao (tomatoes turbid

juices). Fruit juices samples were purchased from local supermarkets. For polyphenols and other antioxidant compound extraction were prepared juices: ethanol (50%) extracts in ratio 10/20. All the extracts were diluted 1/10 with deionized water.

Evaluation of total antioxidant capacity (TAC) by FRAP method: FRAP method depend upon the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant at low pH. This ferrous tripyridyltriazine complex has an intensive blue color and can be monitored at 593 nm (Benzie & Strain, 1996). Reagents: acetate buffer, 300mM/L, pH 3.6 (3.1g sodium acetate $3H_2O$ and 16 mL conc.; Acetic acid per 1L of buffer solution); 10 mM/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM/L HCl; 20 mM/L $FeCl_36H_2O$ in distilled water. FRAP working solution: 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3$ solution. The working solution must be always freshly prepared. Aqueous solution of known Fe (II) 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/mL aqueous Fe(II) as Mohr salts solution (1mM) were mixed with 2.5 mL FRAP working solution; FRAP reagent was used as blank. The absorption was read after 10 min. at 25°C and 593 nm. All determinations were repeated for three times. Total antioxidant capacity in apples and tomatoes juices in Fe (II) equivalents was calculated. Correlation coefficient (r^2) for calibration curve was 0.9994.

Evaluation of total antioxidant capacity (TAC) by DPPH method: Hydrogen atom – or electron-donation ability of the corresponding apples and tomatoes juices was measured from the bleaching of the purple-colored ethanol solution of DPPH. This spectrophotometric assay uses stable 2.2'diphenylpicrylhydrazyl (DPPH) radical as reagent. 0.5 mL of various ethanol juices extracts diluted 1/10 were added to 2.5 mL of a 1 mM ethanol solution of DPPH. After 40 min. incubation at room temperature the absorbance was read against a blank at 517 nm. TAC as inhibition of DPPH free radical in percent was calculated in following way (Burits & Bucar, 2000; Cuendet et all, 1997):

$$TAC_{DPPH}\ (\%) = (A_{blank} - A_{sample}/A_{blank}) \cdot 100$$

The amount of phenolic compounds: It was used the following reagents: 2.0 M Folin-Ciocalteu phenol reagent, gallic acid and anhydrous carbonate. The content of total polyphenolic compounds in apples and tomatoes pure juices ethanol extracts diluted 1/10 was determined by Folin-Ciocalteu method (1927). 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 (diluted ten-fold) and 2.0 mL sodium carbonate (7.5%). The absorption was read after 2 h at 20°C, at 750 nm. All determinations were performed in triplicate. Total content of polyphenols in apples and tomatoes juices in gallic acid equivalents (GAE) was calculated. Correlation coefficient (r^2) for calibration curve was 0.9618.

Results and Discussions

The results for total polyphenols contents and total antioxidant capacity (TAC) by FRAP and DPPH methods are presented in Table 1.

Table 1. Total polyphenols contents and total antioxidant capacity (TAC) by FRAP and DPPH methods for apples and tomatoes juices

Samples	Polyphenols (mM/L)	TAC_{FRAP} (mM/L)	TAC_{DPPH} (%)
Santal, apples clear juices	1.87	3.36	23.43
Bravo Alma, apples clear juices	0.36	0.75	7.32
Naturalis, apples clear juices	1.95	3.24	21.87
Raureni, apples clear juices	1.96	3.5	22.93
SunGold, apples clear juices	1.12	1.84	11.17
Basarabia Nectar, apples turbid juices	2.64	5.69	36.81
Fruttia, tomatoes turbid juices	2.48	5.68	18.22
Ciao, tomatoes turbid juices	2.33	5.19	16.24

The highest total polyphenols content was identified for turbid apples juice (Basarabia Nectar) followed by tomatoes juices. The lower content in polyphenols was identified for Bravo Alma apples clear juice.

The total antioxidant capacity (TAC) by FRAP method is in good correlation with total polyphenols content ($r^2 = 0.9856$) both for apples and tomatoes juices, Figure 1.

Radical scavenging capacity determined by DPPH methods (read after 40 min.) for analyzed juices samples are presented in Table1. The highest TAC radical scavenging capacity values (DPPH) was identified for Basarabia Nectar (apples turbid juices) followed by clear apple juices (Santal, Naturalis, Raureni). The medium values were identified for tomatoes juices. Bravo Alma and SunGold apples clear juices present the lower values. The correlation between polyphenols content and TAC_{DPPH} values ($r^2 = 0.9331$) are lower than the correlation between polyphenols and TAC_{FRAP} values, Figure 2.

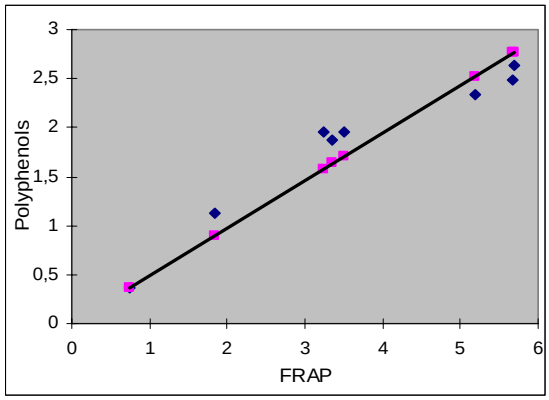

Fig. 1. Correlation between TAC_{FRAP} and polyphenols content for apples and tomatoes juices

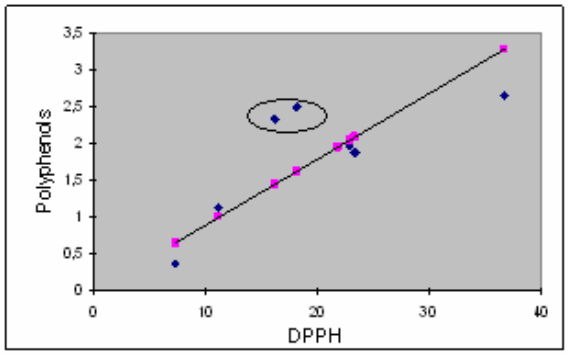

Fig. 2. Correlation between TAC_{DPPH} and polyphenols content for apples and tomatoes juices

The TAC_{DPPH} values for tomatoes juices are outlier for this correlation. Without these values, the correlation coefficient (r^2) is better (0.9872). That means that the radical scavenging activity of tomatoes juices is lower then the radical scavenging activity of apples juices.

The correlation between TAC_{FRAP} and TAC_{DPPH} for all values is present in Figure 3 with correlation coefficient $r^2 = 0.8941$. From this figure we observed also that the values for tomatoes juices are outlier. That confirms the previous observation that the radical scavenging activity of tomatoes juices is lower then the radical scavenging activity of apples juices.

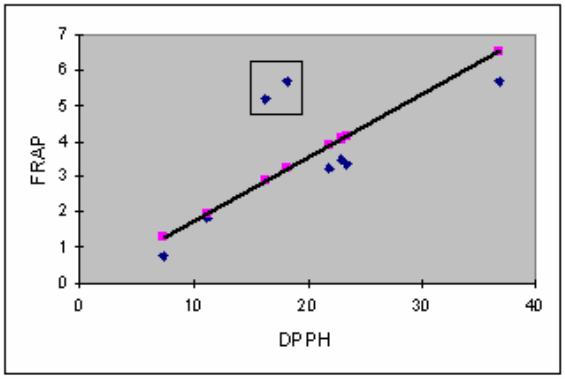

Fig. 3. Correlation between TAC_{DPPH} and TAC_{FRAP} for apples and tomatoes juices

Conclusions

This study shows that the polyphenols content in some clear apples juices are poor than the turbid apples juices and the radical scavenging activity for tomatoes juices are lower than some apples juices.

The correlation between polyphenols content and TAC_{DPPH} values are lower than the correlation between polyphenols and TAC_{FRAP} values. This observation shows that the compounds with antioxidant activity from apples juices present a better radical scavenging activity than the tomatoes juices compounds.

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