RADICAL SCAVENGING ACTIVITY AND TOTAL PHENOLIC CONTENT - COMPARISON BETWEEN APPLE AND RED GRAPE

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Abstract

The purpose of this study was to determine the antioxidant activity of organic apple and red grapes fruit (pulp, peel, seed and juice). The content of total phenolics in the extracts was determined spectrometrically according to the Folin -Ciocalteu method and calculated as gallic acid equivalents (mg GAE/100g). Antiradical activities of the extracts were evaluated by a microassay using 1,1¢-diphenyl-2-picrylhydrazyl, spectrophotometrically method. Juice was obtained by crushing fruit in a laboratory press.

The paper presents the possibility of improving the antioxidant activity of apple juice and grape seeds by extraction of polyphenolic compounds in the residue left after pressing fruit and added to apple juice.In terms of content in polyphenols, is very important to note that the value of grapes grow from 285.2 mg GAE/100g fruit to 385.2 mg GAE/100g in the juice. It was shown that the content of total antioxidant activity in juices varied up to 1.14 red grape juices up to 1.025 apple juice.

Key words: *organic apple, red grape, free radical scavenging activities.*

INTRODUCTION

Fresh fruits and fruit juices are rich in a ntioxidant compounds such as polyphenols, ascorbic acid and tocopherols which may protect us against chronic diseases. (Shui G., Lai Peng Leong, 2006, Lachman J., et al., 2006).

Total polyphenol content of apples peel is high compared to their pulp so it is possible to optimize the quality of apple juice on antioxidant activity. Vitamin content of fruit juices depends both on its production technology and storage conditions. Juices can have a significant role in child and convalescent patients with various diseases nutrition (Franke A. A., et al., 2003, Klimczak I et al., 2007, Giuseppe G., et al., 2012).

Consumption of fruits and vegetables has been shown to be effective in the prevention of chronic diseases. These benefits are often attributed to the high antioxidant content of some plant foods (Wolfe K. et al., 2003, Oszmian´ski J. et al., 2008, Giuseppe G. et al., 2012). Serra A.T. et al., 2012 evaluate the effect of diet supplementation with 20% of three Portuguese apple cultivators (Bravo de Esmolfe, Malápio Serra and Golden), containing distinct phenolic and fibre concentrations, on serum lipid profile and oxLDL of male Wistar rats fed a cholesterol-enriched diet (2%). After 30 days, only Bravo de Esmolfe apple was able to decrease significantly serum levels of triglycerides, total and LDL cholesterol concentrations.

Apples are commonly eaten and are large contributors in terms of phenolic compounds in European and North American diets. The peels of apples, in particular, are high in phenolics (Wolfe K. et al., 2003, Hasbay Adil I., et al., 2007). Apples significantly lowered lipid oxidation both in humans and rats and lowered cholesterol level in humans (Oszmian´ski J. et al., 2008). Due to the consumption of apple decreases the risk of cardiovascular disease.

Grapes are grown on all continents, especially for wine industry and for eating fresh. It is quite a popular and largely planted fruit in Romania and many other countries. Given the important role of antioxidants they are used in the treatment of many diseases, including inhibition of neoplasms. Recent research has shown that the juice of black grapes and also grape seeds and peel contains resveratrol, one of the most powerful antioxidants known**.**

Regarding the processing of grapes, most of the minerals is to be found in the final product but in terms of tartaric acid, it is found less in grape juice than in the fruit itself (Soyer Y., 2003, Nawaz H. Et al. 2006, Bozan B. et al., 2008). A study by Iacopini P. et al., 2008 showed that grape skin and seeds are a good and easily accessible source for nutraceutical.

Regarding the variety of apples used in our study were preferred a juicy and succulent one called *Patule*. Fruits for juice are harvested when fully ripe and healthy. In this state of maturity they contain maximum amounts of sugars, flavors, are pleasant to the taste, balanced in terms of composition, with specific characteristics of the variety**.** It is considered the best local variety. They are harvested in October and consumed until April-May. Grape juice must be of the right wine. For high quality juice, grapes have to be used as fresh as possible preferably the ones harvested on the same day**.** The high content of phenolic compounds, antioxidant activity and anti proliferative activity of apple peels indicate that they may impart health benefits when consumed and should be regarded as a valuable source of antioxidants (Wolfe K. et al., 2003, Rosnah S., et al., 2012).

Therefore the purpose of this study is to test the antioxidant activity of an extract obtained from organic apple and red grapes fruit (pulp, peel, seed and juice).

MATERIALS AND METHODS

PLANT MATERIAL:

Mature fruit samples of apple (*Mallus pumilla)* and red grapes *(Vitis Vinifera)* (cultivars Suceava) were freshly harvested from the field in September, 2012 and transported to Laboratory of Faculty of Food Engineering, Suceava. A selection of the fruits was washed and apples sliced prior to juicing in a press. After drying, all seeds were separated from peels and pulps and ground into fine powder. Each type of juice sample was stored in previously labeled plastic containers at 4°C, after sterilizer.

CHEMICALS

All chemicals used for experiments were of analytical grade and procured from Sigma Merck, Aldrich and Fluka. Deionizer water was used. Absorption determination for total polyphenols content was made using UV–VIS spectrophotometer.

CHEMICAL ANALYSES

The determination of moisture in fruits was effectuated according to the European Standard EN ISO 665/2000 by the drying process in a drying chamber at the temperature of 103 °C.

Total polyphenol content was determined using the Folin Ciocalteu method. After preparing, grape and apple seeds, peel, and pulp were extracted with 80% ethanol (0.1 g sample/10 mL of 80% ethanol), and total phenol contents (TPC) of the extracts were determined. The fruit samples aliquot (0.2 mL) was added to 1.5 mL of freshly prepared Folin Ciocalteu reagent (1:10, v/v, with water). The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 mL of 60 g/L sodium carbonate solution. After incubation at room temperature for 120 min, the absorbance of the mixture was read at 760 nm using the respective solvent as blank. The results were expressed as mg of gallic acid equivalents (mg GAE).The correlation coefficient (r^2) for the calibration curve was 0.9954.

2,2-Di (4-tert-octylphenyl)-1-pycrilhydra-zyl (DPPH) scavenging capacity assay

The method used for determining the antioxidant activity of juice, pulp, peel and seeds extracts is based on scavenging 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radicals. The fruit samples aliquot (0.5 mL) was added to freshly prepared DPPH reagent. After incubating for 5 min, the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer. The control was conducted in the same manner, except that distilled water was used instead of sample.

Using a calibration curve with different amounts of DPPH, the IC_{50} was calculated. The IC_{50} is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the experimental conditions given.

Vitamin C in sample fruits and juices was determined using 2, 6-dichlorophenolindophenol titration. The vitamin C contents of fruit juices were reported as mg/100 ml.

STATISTICAL ANALYSIS

Statistical analysis was conducted using Excel Data Analysis for 4 different samples (pulp, peel, seed and juice, respectively) extract from red grape *(Vitis vinifera)* and apple *(Mallus pumilla)*. The PCA was performed using Unscrambler X 10.1 (CAMO Process AS, Oslo, Norway), all the physicochemical parameters were weighted and normalized for performing the PCA. The experiments were made in three replicates and the results are expressed as mean ± standard deviation.

RESULTS AND DISCUSIONS

Table 1 plays the acid ascorbic values, TP and The IC_{50} of red grape and apples samples.

Moisture presented the lower values from seeds. Moisture content was higher in S1 red grape pulp (85.3 %) and lower in S3 red grape seed (21.5%). Thus grape pulp contains 85.3% and apple pulp contains 78.1%, to as expected, get more grape juice than apple juice**.**

Table 1.

Physico-chemical indicators of samples

Samples	**Botanical name**	**Samples**	**Moisture (g/100 g)**	**TP[a] (mg GAE/100g)**	**IC_{50}**
S1	Red Grape *(Vitisvinifera)*	pulp	85.3	285.2 ±29.3	1.60
S2		peel	84.2	352.7±32.5	0.70
S3		seed	21.5	62.1±2.15	1.82
S4		juice	-	385.2±35.6	1.14
S5	Apple *(Malluspumilla)*	pulp	78.1	428.8±45.2	1.205
S6		peel	75.2	482.3±40.1	0.95
S7		seed	22.1	75.2±5.21	1.61
S8		juice	-	338.8±32.1	1.025

[a]Values are mean ± SD (*n* = 2).TP = content of conținut de Total Polifenoli (mg GAE/100 g)
IC_{50} = Free Radical Scavenging Activities

Total phenols. The contents of total phenolics, calculated as gallic acid equivalent (GAE), of 80% methanolic extracts of samples are presented in Table 1.Phenols are compounds predominant in fruit metabolism have antioxidant properties and contribute to the color and taste of fruit and fruit juice.

The results showed that red grape peel extracts contained highest content of poliphenols 352.7 mg GAE/100 g. The juice all had significantly higher total poliphenols 385.2 mg GAE/100 g than the peel and pulp of the red grape examined.

Kim S.Y. et al., 2006 in their study evaluate the effect of heating and physical conditions of grape seeds on the antioxidant activity of their extracts. According to her the maximum total phenol contents (TPC) and radical scavenging activity (RSA) of whole grape seed extract (WGSE) were achieved when the seeds were heat-treated at 150 ^{0}C for 40 min, while that of powdered grape seed extract (PGSE) were at 100 ^{0}C for 10 min.

The values of the peels were compared to those of the seeds, pulp and juice of the organic apples. Within each samples, the total phenolic were highest in the peels, followed by the pulp and the juice. Apple peels had the highest total phenolic contents 482.3 ± 40.1 mg of gallic acid equivalents/100 g of peels, respectively).

As expected, the peel of all the samples analyzed had significantly higher concentrations of total phenolic than those determined for pulp. The results are in agreement with those reported by Mamzoor M. et al., 2012, who found that peel of the tested apple cultivars had superior antioxidant capacity.

According of Pavlina D. Drogoudi et al., 2008, the greatest antioxidant activity and total phenolic content were found in peel tissue of Starkrimson whereas the lowest values were found in Golden Delicious and Granny Smith. Shui G., Lai Peng Leong, 2006, investigate antioxidant capacities and total phenolic contribution of the residue from star fruit used to produce fruit drinks. According this researcher were found that the residue accounted for over 70% of the total polyphenolic content in whole fruits.

DPPH scavenging capacity assay

The lowest IC_{50} value indicates the highest free radical scavenging activities. Concerning samples red grape extracts, the peel extract was the one that had the strongest antiradical activity (IC_{50} of 0.7), followed by pulp and seed extracts with very similar activities (IC_{50} of 1.6 and 1.82, respectively).Among the apple samples, the peel extract was the one that showed the strongest antioxidant activity (IC_{50} of 0.95), followed by the pulp extract with an IC_{50} of 1.205 and the seed extract with an IC_{50} of 1.61.

All the rest correlation is negligible.

Larrauri J.A. et al., 1998 study the effect of processing temperature (80, 100, and 120 °C) on the free radical scavenging capacity of extracts from red and white grape pomace peels. The heating of the sample extracts up to 120°C influenced the free radical scavenging capacity, being the amount of the remaining DPPH more affected than the time at the steady state. The results showed a lower reduction in the free radical scavenging capacity of white grape pomace peel extract with processing temperature as compared to that from red grape pomace peel.

The ascorbic acid content in red grape has recorded different discounts, depending the samples analyzed. Thus, the greatest values of acid ascorbic concentration were registered in the peel of red grape 28.9±4.2 mg/100g. The greatest reduction in the content of ascorbic acid was in the juice red grape samples (29.06%); followed by pulp (22.14 %). As seen in the figure 1, the ascorbic acid content in apple was higher in peel 30.2±9.3 mg/100g.

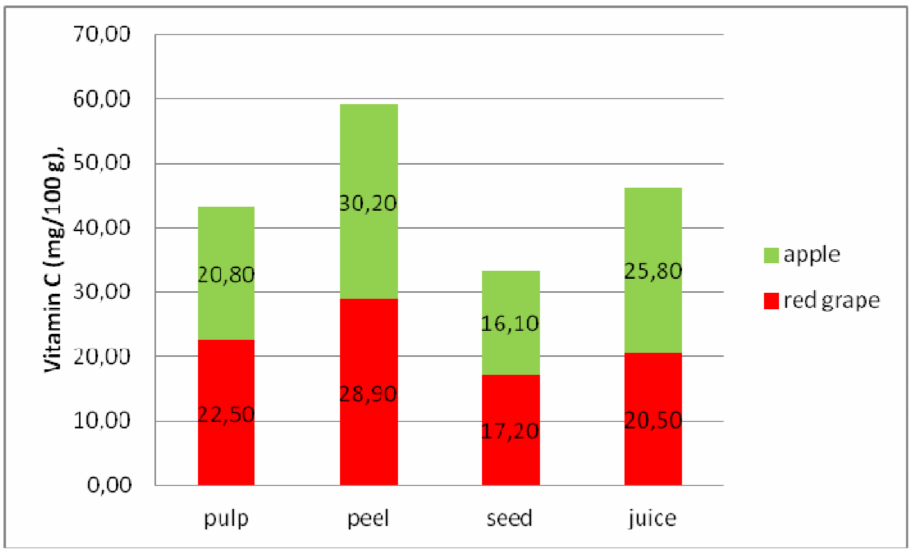

Figure 1. Content of ascorbic acid mg /100g of analyzed samples

Ming-Jun Li et al., 2008, evaluate the ascorbic acid (AsA) distribution, biosynthesis and recycling in different tissues of young and mature fruit of cv. Gala apple (*Malus domestica* Borkh). The results showed that the peel of 'Gala' apple had the highest AsA levels among all the tissue types.

Principal component analysis (PCA) was used to test for the correlation between the variables: moisture (g/100 g), vitamin C (mg/100 g), TP (mg GAE/100g) and IC_{50}.

A stronger positive correlation between vitamin C and TP measured in red grape and apple, of the value 0.744, and a negative correlation between IC_{50} and vitamin C, of the value -0.601 can be observed in table 2. All the rest correlation is negligible.

Table 2

Correlation matrix for the variablesmeasured in red grape and apple: Moisture (g/100 g), Vitamin C (mg/100 g), TP (mg GAE/100g) and IC $_{50}$

	Moisture(g/100 g)	Vitamin C mg/100 g	TP (mg GAE/100g)	IC_{50}
Moisture(g/100 g)	1			
Vitamin C mg/100 g	0.461	1		
TP (mg GAE/100g)	0.393	**0.744**	1	
IC_{50}	-0.006	-0.601	-0.336	1

The biplot provides a useful tool of data analysis.In PCA the biplot can show inter-unit distances among units as well as display variances and correlations of the variables (Gabriel, 1971). The biplot graphic based on principal component analysis (PCA) for the most important factors (F1 and F2) is given in figure 2.

The variation of the parameters studied implies the existents of 4 factors. The principal components that have eigenvalue more than 1 are, as follows: F1 (2.349), F2 (1.009). The eigenvalue for F3 and F4 are less than 1, that implies that these factors are not important. The percentage of variability represented by the first two factors is high (83.88 %).

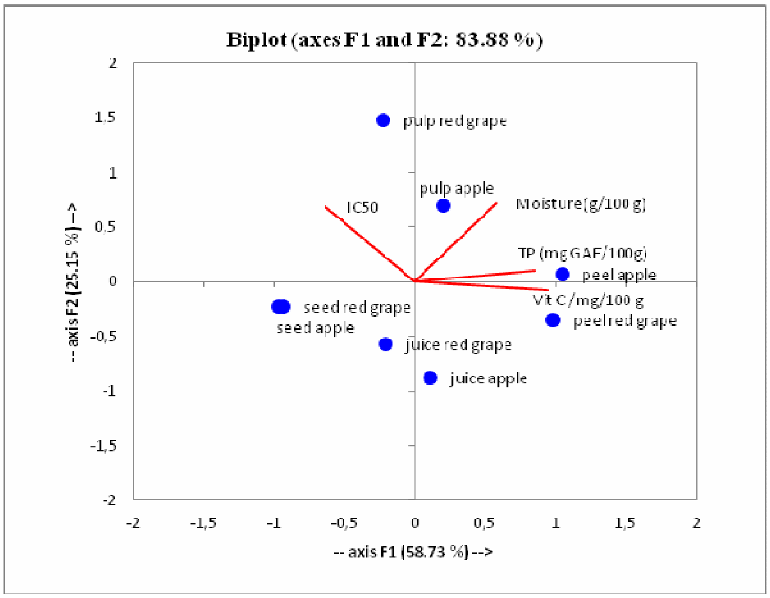

Figure 2. Biplot graphic for the parameters studied in analyzed samples

The first factor (F1) explains 58.73 % of the total variance with a significant parameters vitamin C and TP. The values of vitamin C show a strong positive loading, having a factor loading 0.947. Also, Tp values imply a factor loading 0.848. An important contributions of the observations (%) for the factor F1 have peel aple (36.79 %), peel red grape (23.965 %), seed apple (23.312 %) and seed red grape (21.834 %).

The second factor (F2) explains 25.15 % of the total variance with a significant parameters Moisture and IC_{50}. The values of moisture and IC_{50} show a strong positive loading, having a factor loading 0.723. Also, IC_{50} values imply a factor loading 0.682. An important contributions of the observations (%) for the factor F2 have pulp red grape (54.91%) and juice apple (19.08 %).

CONCLUSIONS

Ascorbic acid as one of the main vitamins is important in assessing the nutritional value of fresh and processed fruit.

Following the analysis of samples, ascorbic acid content was 22.5±2.3mg/100 g in the red grape pulp but the value decreased to 20.5±3.6mg/100 g. in the juice.

Both remarkably high phenolic content and radical scavenging activties were found in the apple peel. In general, the methanolic extracts of organic fruits was found to exhibit a higher phenolic content as well as antioxidant and radical scavening activities compared to seed extracts.

In conclusion, this study suggests that phenolic compounds are the main antioxidants in grape fruits and apple. This fruits can be used as good sources of antioxidants in our diet and may have relevance in the prevention of diseases in which free radicals are implicated. Natural juice, freshly squeezed, has the advantage of no preservatives added and, as long as it is to be consumed fresh, will keep its properties and nutrients. In addition, grape juice and apple byproducts (peels and seeds) are a good and cheap source of antioxidants, which could be industrially exploited.

REFERENCES

1. Bozan B. , Göksel Tosun, Derya Özcan, 2008, Study of polyphenol content in the seeds of red grape (Vitis vinifera L.) varieties cultivated in Turkey and their antiradical activity, *Food Chemistry*, Volume 109, Issue 2, Pages 426-430.
2. 17. Franke A. A., Laurie J. Custer, Christi Arakaki, Suzanne P. Murphy, 2003. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii, Journal of Food Composition and Analysis, volume 17, issue 4, Pages 17 -35.
3. Gabriel K.R, 1971*,* The biplot graphic display of matrices with application to principal component analysis, *Biometrika, 58 (3): 453-467*
4. Giuseppe G., Patrizia Iacopini, Massimiliano Baldi, Annamaria Ranieri, Paolo Storchi & Luca Sebastiani, 2012, Temperature and storage effects on antioxidant activity of juice from red and white grapes, International Journal of Food Science and Technology, 47, 13–23.
5. Hasbay Adil I., H.˙I. Cetin, M.E. Yener, A. Bayındırl, 2007, Subcritical (carbon dioxide + ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts, J. of Supercritical Fluids 43, 55–63.
6. Iacopini, P., Baldi, M., Storchi, P., Sebastiani, L., 2008, Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, *in vitro* antioxidant activity and interactions, Original Research Article *Journal of Food Composition and Analysis*, 21, 589– 598.
7. Klimczak I., Maria Małecka, Mirosława Szlachta, Anna Gliszczyńska-Świgło, 2007. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. Journal of Food Composition Analysis, volume 20, issue 3-4, pages 313-322.
8. Lachman J., Šulc M., Sus J., Pavlíková O., 2006, Polyphenol content and antiradical activity in different apple varieties. Hort. Sci. (Prague), *33*, (3): 95–102.

9. Larrauri J. A., Concepcio´n Sa´nchez-Moreno, and Fulgencio Saura-Calixto, 1998, Effect of Temperature on the Free Radical Scavenging Capacity of Extracts from Red and White Grape Pomace Peels, J. Agric. Food Chem., 46, 2694−2697.
10. Ming-Jun Li, Feng-Wang Ma, Min Zhang, Fei Pu, 2008, Distribution and metabolism of ascorbic acid in apple fruits (*Malus domestica* Borkh cv. Gala), *Plant Science*, Volume 174, Issue 6, Pages 606–61.
11. Oszmian´ ski J., Michał Wolniak, AnetaWojdyło, IwonaWawer, 2008, Influence of apple pure´e preparation and storage on polyphenol contents and antioxidant activity, Food Chemistry 107, 1473–1484.
12. Pavlina D. Drogoudi, Zisis Michailidis, George Pantelidis, 2008, Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. *Scientia Horticulturae*, Volume 115, Issue 2, Pages 149–153.
13. Rosnah S., Wong, W. K., Noraziah, M. and Osman, H., 2012, Chemical composition changes of two water apple (*Syzygium samaragense*). International Food Research Journal 19(1): 167-174.
14. Maleeha M., Farooq Anwar, Nazamid Saari and Muhammad Ashraf, 2012, Variations of Antioxidant Characteristics and Mineral Contents in Pulp and Peel of Different Apple (*Malusdomestica*Borkh.) Cultivars from Pakistan, *Molecules*, *17*, 390-407;
15. Nawaz H., Shi J., Gauri S. Mittal, Yukio Kakuda, 2006, Extraction of polyphenols from grape seeds and concentration by ultrafiltration, Separation and Purification in the Food Industry, volume 48, issue 2, pages 176–181.
16. Serra A.T., J. Rocha, B. Sepodes, Ana A. Matias, Rodrigo P. Feliciano, Agostinho de Carvalho, Maria R. Bronze, Catarina M.M. Duarte, M.E. Figueira, 2012, Evaluation of cardiovascular protective effect of different apple varieties – Correlation of response with composition, *Food Chemistry*, Volume 135, Issue 4, Pages 2378–2386.
17. Soyer, Y., Koca N., F Karadeniz, 2003, Organic acid profile of Turkish white grapes and grape juices, *Journal of Food Composition and Analysis*, 16, Issue 5, Pages 629-636.
18. So-Young Kim, Seok-Moon Jeong, Woo-Po Park, K.C. Nam, D.U. Ahn, Seung-Cheol Lee, 2006, Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts, *Food Chemistry*, Volume 97, Issue 3, Pages 472–479.
19. Vieira, F.G.K.; Borges, G.D.S.C.; Copetti, C.; Amboni, R.D.D.M.C.; Denardi, F.; Fett, R. 2009, Physico-chemical and antioxidant properties of six apple cultivars (*Malus domestica* Borkh) grown in southern Brazil. *Sci. Hortic*., *122*, 421–425.
20. Shui G., Lai Peng Leong, 2006, Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals, Food Chemistry 97, 277–284.
21. Wolfe K., Xianzhong Wu and Rui Hai Liu, 2003, Antioxidant Activity of Apple Peels. *J. Agric. Food Chem.*, 51 (3), pp 609–614