DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF APPLES

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Abstract

The aim of this study was to determine the antioxidant activity of two varieties of apples: Reglindis variety and Rebella variety by the method using the reactivity to DPPH. Samples that were analyzed: crushed apple, apple without peel, apple juice, grated apple treated with pectin for different periods and filtered of the crushed apple. The other purpose of the analysis of these samples was to determine the part of the apple with the highest antioxidant activity. It has been found that greatest activity was obtained in the crushed apple with peel, demonstrating that the greatest amount of phytonutrients is found in the peel. We noted that the addition of pectin increases antioxidant activity in time. Analyzing the results on the antioxidant activity of samples of the REBLINDIS and REBELLA varieties, we found them to be almost equal, slightly higher for the Rebella variety.

Key words: flavonoids, apple, DPPH, quercetin.

INTRODUCTION

In recent years, there is an increasing interest in researching natural antioxidants. A growing number of plants, including fruits and vegetables are known as rich sources of antioxidants.

Because of the nature of Type I chemical antioxidants, also called chain interruption antioxidants, they can act as acceptors / neutralizers of free radicals.

They can delay or interrupt the chain of autoxidation reactions. Most primary antioxidants are polyhydroxylic phenols with various substituents namely carotenoids, flavonoids, phenolic acids, tocopherols and tocotrienols.

Apples (*Malus sp. Rosaeceae*) are an important source of phytonutrients that possess a variety of biological activities that contribute to the health of humans who consume them. Apples are a good source of vitamin C, potassium and dietary fibers and very low in saturated fat, cholesterol and sodium.

The important energy provided by apples is due to the sugars contained, especially fructose (about 60% of total carbohydrates), glucose (~ 20%) and sucrose (~ 20%). As a result the glycemic index (GI) of apples is GI = 28-44 and is part of the low glycemic index fruit. (Lu, 1999)

The apple is the most consumed fruit in the world; it is always available, regardless of the season, both in supermarkets and stalls in markets, in different colors and varied flavors.

Every day, we are advised by the media to eat fruit, but it is rarely specified what fruit we should eat. However, apples are the most available. Apples have curative and preventive qualities, certified in the most sophisticated laboratories worldwide.

Thus, it has been found that the apple juice reduces cholesterol, thus preventing cardiovascular diseases. It seems that quercetin, a substance found in apple, can reduce the risk of lung cancer, a reason why heavy smokers should eat apples.

Many phytonutrients found in apples are powerful antioxidants, especially polyphenols: quercetin, catechin, chlorogenic acid. There is a correlation between the amount of polyphenols and the antioxidant activity.

A definition of anti-oxidants has been given by Halliwell and Gutteridge (Packer, Valacchi, 2002). Antioxidants are substances that when present in small concentrations compared with the substrate significantly reduce or prevent its oxidation.

They later simplified the definition of antioxidants as a substance which delays, prevents or eliminates oxidative deterioration of target molecules.

The concentration of phenolic compounds and some antioxidant pigments in apples vary depending on the cultivation methods, season, the climatic region, the cultivation (conventional or organic), developmental stage of fruits, tissue type (peel or pulp), conditions of stress (pathogen attack) (Henriquez, et all, 2010)

The synthesis of antioxidant in plants occurs through the process of photosynthesis. Various agrochemicals used in apple cultivation have an influence in the synthesis of antioxidants. Fertilizers can significantly increase the speed of the photosynthesis process. (Perez, et all, 2008)

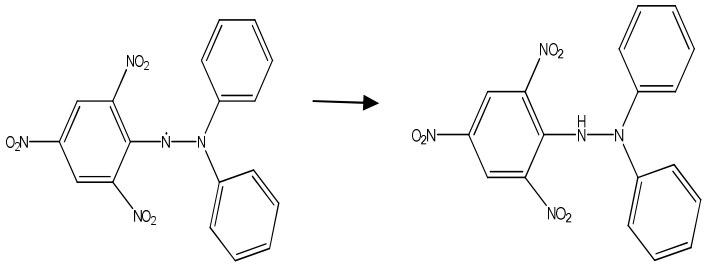
Common compounds found in the skins of apples are procyanidins, epicatechin and floridizin, but in much lower concentrations than in the peel of these fruits. Quercetin is found exclusively in the skins of apples. The compound found in the highest concentration in the pulp is chlorogenic acid. (Prakash, 2001)

This study aims to determine the antioxidant activity in two types of apples:

Fig. 1. Apples-Reglindis variety Fig. 2. Apples-Rebella variety

Several methods have been developed for the determination of the antioxidant capacity of the different constituents of food compounds based on different principles. DPPH quantification is a simple, fast, accurate and cheap method to measure the ability of various compounds to neutralize free radicals or act as proton donors in order to evaluate the antioxidant activity of food. [6] DPPH is a stable radical: 1,1-diphenyl - 2 - picryl-hydrazyl. The method of analysis that is based on this compound is one of the most used.

DPPH is a stable, paramagnetic radical. The unpaired electron of the nitrogen atom is delocalized, belonging to the whole molecule. The solution is dark purple in color, exhibiting an absorption maximum at 520 nm. The DPPH radical can accept an electron or a hydrogen radical forming a diamagnetic molecule that is apale violet. If the test substance that has antioxidant activity is mixed with DPPH solution and it becomes a light violet, it suggests that this compound has antioxidant properties.



The concentration of the solution of DPPH in the working solution can range between 0.05 mM and 1.5 M [32]. The most often used solution is in a concentration of 0.1 mM DPPH. (Miller, et all, 2000)

Different concentrations of DPPH solution lead to different ratios between the volume of the sample and reagent. In the literature there are found ratios of 3: 1 through to 1: 600 (Gupta, et all, 2007; Lachman, et all, 2006)

Almost every developed method uses a certain amount of self volume / reagent.

The reaction time for the neutralization of the DPPH radical and antioxidants ranges from 1 minute (Sroka, et all, 2005) to 240 min (Miller, et all, 2000). Pavlov developed a method with a reaction time of 15 minutes. (Pavlov, et all, 2002)

Another specific feature of the method to determine the antioxidant activity with the DPPH is the wavelength at which measurement is made.

The absorbance of the sample was measured between 492 nm and 540 nm. Determinations at 515 nm wavelength are used by many researchers including Brand-Williams (Shikanga, et all, 2010)

The ability to neutralize the radicals by antioxidant substances can be calculated using various standard solutions. The literature discloses the use of ascorbic acid (vitamin C), trolox, Vitamin E (α-tocopherol), BHA.

Molyneux (Brad, et all, 1995) has studied this method of assessing the activity of antioxidants. He recommended, in order to increase the accuracy of the method, the use of tanks with a maximum volume of 4 mL, with 1 cm optical path.

In this method we use 2 ml DPPH solution, with concentration 50-100 μM, and 2 ml sample, reaction time 30 minutes, solvent methanol or ethanol. As standard, he proposes the use of ascorbic acid (vitamin C) or α-tocopherol (vitamin E). (Molyneux, et all, 2004)

MATERIAL AND METHOD

For the determination of polyphenol we used a PERKIN ELMER LAMBDA 35 UV / VIS spectrophotometer. There were used: Technical balance, analytical balance, laboratory centrifuge, beaker glasses, pipettes, mortar. The reagents used were: DPPH reagent, distilled water, methanol.

Preparation of the test samples

In this study we analysed two apple varieties, the following samples (Table1.)

Table 1

Tip of sample

No. sample	**REGLINDIS variety**	No. sample	**REBELLA variety**
1	Crushed apple	1A	Crushed apple
2	Raw apple, apple without peel	2A	Raw apple
3	Concentrated apple product	3A	Concentrated apple product
4	Apple treated with pectin 0 minutes	4A	Apple treated with pectin 0 minutes
5	Apple treated with pectin 45 minutes	5A	Apple treated with pectin 45 minutes
6	Apple treated with pectin 90 minutes	6A	Apple treated with pectin 90 minutes
7	Filtered crushed apple	7A	Filtered crushed apple
8	Crushed apple pulp after filtration	8A	Crushed apple pulp after filtration
9	Ultrafiltration product of crushed apple	9A	Ultrafiltration product of crushed apple

1. *Sample preparation. Crushed apple*:
   Apples of every sort are washed. A quantity of 100 grams is cut into small pieces, with peel. The sample is milled in a glass jar. An amount of 10 grams of apple shredded pulp is placed in a 25 ml beaker and allowed to settle. The separated liquid at the surface, constitutes the sample.
2. *Sample preparation . Raw content:*
   Apples of every sort are washed and peeled. A quantity of 10 grams of pulp is cut into small pieces, with peel. The sample is milled in a glass jar. Apple crushed pulp is placed in a 25 ml beaker and allowed to settle. The separated liquid at the surface constitutes the sample.
3. *Sample preparation . Apple concentrate:*
   Apples of every sort are washed and peeled. A quantity of 100 grams of apple pulp is introduced into a fruit processor for obtaining juice, that is the apple concentratate.
4. *Sample preparation. Resistant apple treated with pectin:*
   An amount of 10 g of crushed apple prepared by the procedure described at point 1 is homogenized with 0.1 g pectin (1%). The sample is placed in a beaker and will be analyzed at the initial time, after 45 minutes and 90

minutes, in order to observe the influence of this addition over the antioxidant properties.

5. *Sample preparation. Filtered crushed apple:*
   A 10 g crushed apple obtained under pt. 1 was subjected to simple filtration. The filtrate constitutes the sample.
6. *Sample preparation. Crushed apple pulp after filtration:*
   After processing the crushed apple at pt. 5 a residue is obtained on the filter, that in this case represents the sample.

Procedure

In order to determine the antioxidant activity, namely the ability to neutralize free radicals we worked in the following manner:
We inserted 0.1ml of the sample and 2.9 ml reagent DPPH in the cuvette. It was kept at room temperature for 15 minutes to complete the reaction, after which we measured the absorbance at λ = 515 nm using methanol as a reference. Initially we measured the absorbance of the solution containing free radicals that will react with antioxidants.
The results obtained are shown in Table 2 and Figure 1.

Table 2

Result of analyses of apple sample

Sample no.	Sample	**REGLINDIS variety**	**REBELLA variety**
1	Crushed apple	0,4802	0,4899
2	Raw apple, apple without peel	0,1244	0,1356
3	Concentrated apple product	0,3066	0,322
4	Apple treated with pectin 0 minutes	0,4236	0,4628
5	Apple treated with pectin 45 minutes	0,4444	0,4707
6	Apple treated with pectin 90 minutes	0,4812	0,47
7	Filtered crushed apple	0,3491	0,3434
8	Crushed apple pulp after filtration	0,4906	0,4725
9	Ultrafiltration product of crushed apple	0,2277	0,2292

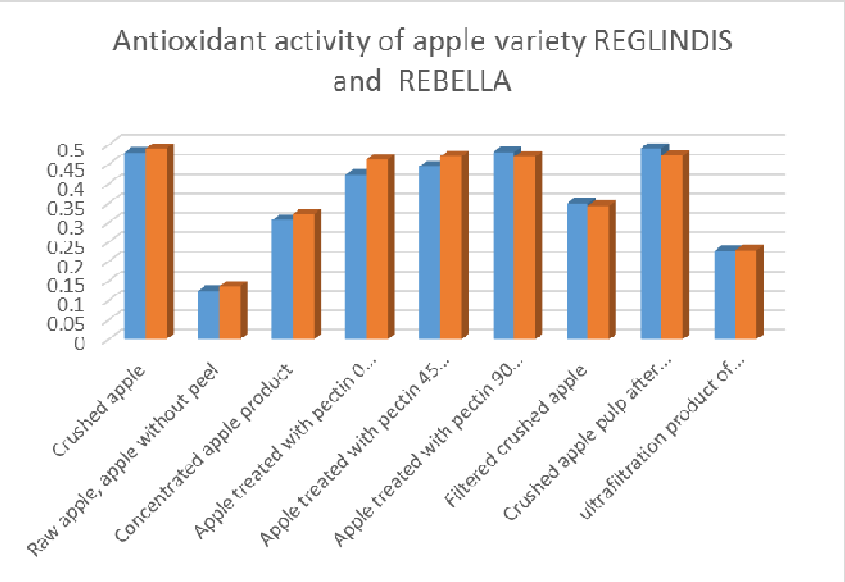


Fig. 3. Result of analyses of apple sample

It appears that higher antioxidant activity is achieved breaif the apple is crushed. This value is higher than that obtained for the crushed raw apple, sample containing pulp without peel. These results prove that the apple peel contains the highest amounts of antioxidant phytonutrients.

Analyzing the results obtained for samples 3-6, respectively 3A - 6A, it is found that the addition of pectin increases the antioxidant activityin time. Pectin is extracted from the apple peel, so it has significant antioxidant activity.

The determinations made for samples 7-9, respectively 7A - 9A show that the largest amount of antioxidants is found in apple peel and pulp. The juice obtained by simple filtering with a certain content of pulp presents ahigher antioxidant activity than apple juice obtained by ultracentrifugation.

Analyzing the results on the antioxidant activity of different samples of the two apple varieties Reblindis and Rebella, we found them to be almost equal, with a slightly higher activity in the Rebella variety.

REFERENCES

1. Brand-Williams, W., M. Cuvelier and C. Berset, 1995, Use of free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28 (1): 25-30
2. Gupta, M., U. Mazumder and P. Gomathi, 2007, Antioxidant and antimicrobial properties of Galega purpurea root. Asian Journal of Plant Sciences, 6 (3): 533-537
3. Henriquez C., Almonacid S., Chiffelle I., Valenzuela T., Araya M., 2010, Determination of antioxidant capacity, total phnolic content and mineral

composition of different druit tissue of five apple cultivars grown in Chile, Chilean J. Agricultural Research 70(4), 523-526
4. Kim., J. K., J. H. Noh, S. Lee, J. S. Choi, H. Suh, H. Y. Chung, Y.O. Song and C. Lee, 2002, The First Total Synthesis of 2,3,6-Tribromo-4,5-dihydroxybenzyl Methyl Ether (TDB) and Its Antioxidant Activity. Bull. Korean Chem. Soc., 23 (5): 661-662
5. Lachman, J., M. Šulc and O. Pavlíková, 2006, Polyphenol content and antiradical activity in different apple varieties. Hort. Science (Prague), 33 (3): 95-102
6. Liebenberg, L., 2004. Evaluation of biologically active compounds in Coleonema album. Ph.D. Thesis, University of Johannesburg, South Africa
7. Lu Y., Foo LY., 1998, Constitution of some chemical components apple seed. Food Chem, 61, 29-33
8. Miller, H., F. Rigelhof, L. Marquart, A. Prakash and M. Kanter, 2000, Antioxidant Content of Whole Grain Breakfast Cereals, Fruits and Vegetables. Journal of the American College of Nutrition, 19 (3): 312S-319S
9. Miller, H., F. Rigelhof, L. Marquart, A. Prakash and M. Kanter, 2000, Antioxidant Content of Whole Grain Breakfast Cereals, Fruits and Vegetables. Journal of the American College of Nutrition, 19 (3): 312S-319S
10. Molyneux, Ph., 2004, The use of stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science Technology, 26 (2): 211-219
11. Packer L., Valacchi G.,2002, Antioxidants and the response of skin to oxidative stress: vitamine E as a key indicator. Skin Pharmacol Appl Skin Physiol,15, 282-90
12. Pavlov, A., P. Kovatcheva, V. Georgiev and I. Koleva, 2002. Biosynthesis and Radical Scavenging Activity of Betalains during the Cultivation of Red Beet (Beta vulgaris) Hairy Root Cultures. Zeitschrift für Naturforschung, 57:640-644
13. Pérez-Jiménez, J., S. Arranz., M. Tabernero, M. E. DiazRubio, J. Serrano., I. Goñi and F. Saura-Calixto, 2008, Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. Food Research International, 41 (3): 274-285
14. Prakash, A., 2001, Antioxidant Activity. Medallion Laboratories Analytical Progress, 19 (2)
15. Shikanga, E. A., S. Combrinck and T. Regnier. 2010, South African Lippia herbal infusions: Total phenolic content, antioxidant and antibacterial activities. South African Journal of Botany, 76: 567-571
16. Sroka, Z. and W. Cisowski, 2005, The anti-ROS Activity of Various Plant Extracts, Adv. Clin. Exp. Med., 14 (3): 423-433