DETERMINING THE ANTIOXIDANT ACTIVITY OF SOME NUTRITIVE SUPPLEMENTS

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Abstract

Lately, the polyphenols have attracted scientists' interest due to their effects on health. The interest in analyzing the polyphenols, from different natural sources, has increased as polyphenols can be used as antioxidants in food industry and they are beneficial for human health. In this paper we studied the antioxidant activity of some nutritive supplements and the determination of the polyphenol content from these. We carried out studies on nutritive supplements with green tea extract content, curcuma, extract from grape kernel, detoxifying mixture. The experimental results obtained highlight a great content of polyphenols in the green tea extract (237,1 mg/g) and in the grape kernel extract (169 mg/g). It can be noted that in the case of the preparation which contains green tea extract (1), and grape kernel extract (3), the neutralizing activity of the free radical is quick, 81,15%, respectively 80% at the moment of introducing the extract în the DPPH solution.

Key words: Antioxidanti, Folin-Ciocalteau, DPPH

INTRODUCTION

A definition of anti-oxidants has been given by Halliwell and Gutteridge 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 (Thomas, 2017).They appear in a variety of fruits, vegetables, woody shell fruits, seeds, flowers, peel, drinks and even in some food products, like a component of the used natural ingredients. It was reported that the polyphenols would manifest anti-carcinogenic, anti-ATEROGEN, anti-ulcerative, anti-thrombotic, anti-inflammatory, immunomodulating, anti-microbial, vasodilator and analgesic effects. (Diaba et al, 2015; Şerban et al., 2008)

The interest in the polyphenol research, from different natural sources, increased due to the fact that polyphenols can be used as antioxidants in the food industry and they are beneficial for human health. The beneficial effects of the polyphenols on human health may be due to their cleaning properties of the free radicals, blocking the harmful actions of these molecules on cells. For determining the composition of total phenols

in extracts, the most frequent procedure is the F-C Test. (http://www.nal.ysda.gov/fnic/foodcomp;Spencer., Crozier, 2012)

The phytochimical composition of the apples varies extensively from one sort to the other. It is necessary the existence of a standard test for the purpose to standardize, compare and validate the results of the studies regarding the antioxidant activity of different compounds.

There have been developed several methods to determine the antioxidant capacity of varied constituent compounds of food, based on different principles (Şerban, Buhaş, 2014; Şerban, 2015): the neutralizing capacity of the peroxide radicals (the capacity of absorption of oxygen radicals; ORAC); the antioxidant power through radical neutralization (TRAP); the reducing capacity of metallic ions (the antioxidant capacity through ferric ion reduction, FRAP); the antioxidant capacity through cupric ion reduction (CUPRAC); the neutralization of organic radicals (acid 2,2 – azino-bic (3-etilbenz-tiazolin-6-sulfonic), ABTS; 2,2-difenil-1-picrilhidrazil, DPPH);the quantification of the formed product during the lipid peroxide activity (reagent sub layer acid tiobarbituric, TRAPS); lipoprotein oxidation with low density (LDLs). The most used methods to determine the antioxidant activities are FRAP, ABTS, TEAC and DPPH. (Pérez-Jiménez,, 2008; Prakash, 2001)

The DPPH quantification method to measure the neutralizing capacity of free radicals by different compounds or to function as proton donors, to evaluate the antioxidant activity of food, is simple, fast, exact and low-priced.

In this paper we have been concerned with the studying of the antioxidant activity of some nutritive supplement and with the determination of the polyphenols content from these nutritive supplements. We performed studies on: ***a. Nutritive supplement with green tea extracts*** (200mg green tea leaves/capsule).

The green tea is the most efficient antioxidant that is known, it contributes to the neutralization of the free radicals from the organism (accountable for the premature ageing of cells), immune system stimulation and it ensures the maintenance of a normal level of sugar in blood.(Lin 2006)

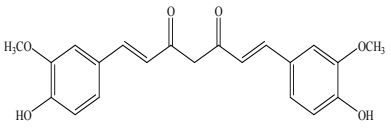

Fig. 1. Curcuma (1E, 6E) – 1,7-bis-(4-hidroxi -3-metoxifenil)-1,6-heptadien-3,5-diona

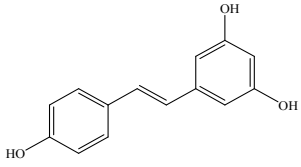

Fig. 2. Resveratrol

b. nutritive supplement with curcuma extract (100mg curcumin/ capsule) (image 1) In recent decades, curcurcumin has increased the scientists' interest from all around the world. It has been proved that it has several therapeutically effects such as: reducing the cholesterol, LDA prevention, inhibiting HIV replication, it helps at wound healing, prevents and treats cancer. It is a nonpoisonous compound even in high doses. Many of the therapeutically effects of curcuma are assigned to the high antioxidant properties. Most of the natural compounds with antioxidant activity are phenolic compounds or β-dicetonics. The curcumin has in its structure phenol groups but also β-dicetonics. Performed studies showed that the antioxidant effects are due to the phenolic groups. (Molyneux, 2004)

c. nutritive supplement with grape kernel extract(260 mg resveratrol/capsule) Resveratrol (image 2) from grapes, 3,5,4 - trihidroxi-trans-stilben, was identified as an ingredient in red wine. It is a poliphenol that is able to inhibit oxidation LDL cholesterol, reducing the coronary disease risk, it helps preventing, treating and relieving the symptoms of some disorders related to free radicals which include heart disease, diabetics, cancer and infections with Staphylococcus Aureus. Furthermore, it is efficient in reducing the symptoms of chronic venous insufficiency which can lead to pain, fatigue, visible veins, swelling of the limbs etc.

d. nutritive supplement with complex detoxifying extract (150 mg burdock root, 75 mg Viola Tricolor leaves, 75 mg Elderflower leaves, 25 mg Garden Angelica (Angelica archangelica) roots, 25 mg cardamom seeds, 2 mg Juniper essential oil/capsule)

MATERIAL AND METHOD

Reagents and equipment

For determining the polyphenol content and the antioxidant activity is is used ANALYTICJENASpecord 210Plusspectrophotometer..

The used reagents in determining the polyphenol content were: 2ethanol, natron / crystallized sodium carbonate with 10 molecules of water, ethanol Folin-Ciocalteau, gallic acid, distilled water and ethanol with antioxidants.

The used reagents in determining the antioxidant activity were: ethanol DPPH, distilled water.

The preparing of the solutions

a. *The preparing of the Folin-Ciocalteau reagent solution:* it is dissolved 1:10, 1 ml F-C reagent in 10 ml measuring flask, it is brought at the sign with distilled water. The solution is kept in the refrigerator.

b.The preparing of the reference solution of gallic acid. It is weight out an exact quantity of 0.05ggallic acid, it is passed in to a 25ml

measuring flask and it is brought to the sign with distilled water. From this solution the working ethanol is prepared. For this, pipette 0,5 ml, 0,75 ml, 1 ml, 1,5 ml, 2 ml in to a 10 ml measuring flask and it is brought to the sign with distilled water.

c. The preparing of the test solutions containing nutritive supplements. It is weight out, exact 5 tablets from each nutritive supplement proposed for analysis. The tablets are crushed until they are soft in a mortar, they are quantitatively transferred in Erlenmeyer flasks, 25 ml of methanol solution + distilled water is added and they are intensely shaken for an hour. The obtained suspension is filtrated. The filtrate is diluted 1:100 with the same solvent. The solutions are kept in the refrigerator.

Method of operation

A Method of operation for determining the polyphenol content

In Erlenmeyer flasks, are mixed 8 ml of distilled water, 1 ml from the working ethanol solutions and 1 ml from the Folin-Ciocalteau reagent. These are left to rest 1 minute, then 2 ml of sodium carbonate are added. The color of the solutions become blue. It is left for 1 hour in the oven at 40 ºC for the completion of the reaction and then the absorbance is measured ant the wave length of λ = 765 nm.

B. Method of operation for determining the antioxidant activity

In Erlenmeyer flasks are mixed 2 ml distilled water, 1 ml of testing (the 4 diluted tests and the gallic acid solution 0,15mg/ml) and 3 DPPH solution with the concentration of c = 0,003 M. the absorbance is read at the wave length λ = 517 nm. It is left for an hour at room temperature, in the dark, then the absorbance is read again.

The DPPH solution of 0,003M concentration presented the absorption A=1,0966.

The neutralizing percentage of the free radical DPPH is calculated with the relation>

$$\text{Activity (\%)} = (A0 - Ai) \cdot 100 / A_0$$

RESULTS AND DISCUSSION

In table 1 are presented the results obtained at the measurement of the gallic acid calibration solution absorbance. With these values the calibration curve is constructed according to the solution absorbency dependence with the gallic acid solution concentration (diagram 3).

Table 1

Variation of absorbance depending the concentration

Test number	Concentration mg/ml	Absorbance
1	0,05	0,3688
2	0,075	0,4876
3	0,1	0,6101
4	0,15	0,8661
5	0,2	1,0346

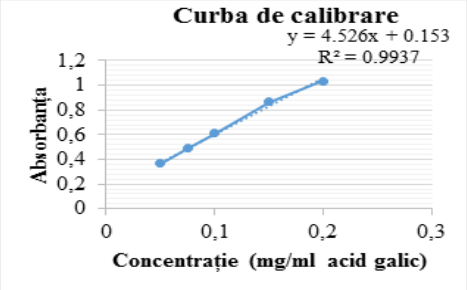

Fig.2. Calibration curve for salicylic acid content

In the concentration domain of 0,05 – 2,0 mg/ml (gallic acid) it is obtained a straight-line dependency of the concentration with the absorbance. The straight –line obtained has the equation y = 4,526x + 0,153, the correlation parameter R2 = 0,9937 showing a good correlation of the results. Based on the results it can be determined the calculation formula of the total phenols content.

Conc. (mg gallic acid/ml) = (A – 0,153) / 4,526

The obtained results at the polyphenol content determination from the nutritive supplements are presented in table 2 and figure 4.

Table 2

Results for nutritive supplements

Test number	Test	Absorbance	Polyphenol content in the solution to be analyzed (mg gallic acid)	Polyphenol content (mg/tablet)
1	Green tea extract	1,0172	477,35	95,47
2	Curcuma extract	0,7817	347,27	69,45
3	Grape kernel extract	0,5874	240,0	48,0
4	Detoxifying extract	0,1957	23,6	4,71

The obtained results at the determination of the antioxidant activity are presented in table 3 and figure 5:

Table 3

The results obtained for nutritive supplements and galic acid solution

Test number	test	Absorbance	Absorbance after 60 minutes	Activity at t = 0 (%)	Activity at t = 60 min. (%)
1	Green tea extract	0,2067	0,1394	81,1508	87,288
2	Curcuma extract	0,3826	0,2501	65,1103	77,1931
3	Grape kernel extract	0,2194	0,1822	79,9927	83,385
4	Detoxifying extract	0,5979	0,2858	45,4769	73,9376
5	Gallic acid solution 0,15 mg/ml	0,1598	0,1330	85,4277	87,8716

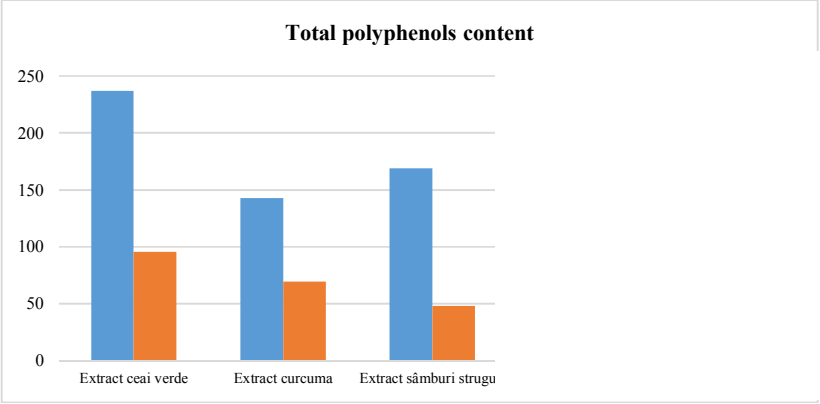

Fig. 4. Total content of polyphenols inactivity of free radicals

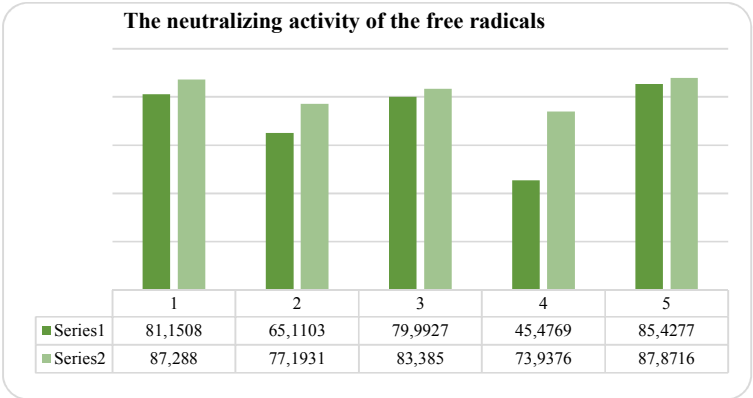

Fig 5. The neutralizing activity of free radicals nutritive supplements

The experimental results obtained highlight a great content of polyphenols in green tea extract (273,1 mg/g) and in the grape kernel extract (169 mg/g). The reason why the curcuma extract has in a tablet, a greater content of plyphelos, is due to the larger quantity of the tablet in comparison to that of the grape kernel. The detoxifying extract presents the smallest content of polyphenols, the pharmacological effect of this chemical being that of purification of the organism.

It is observed that in the case of the chemical containing green tea extract (1), and grape kernel extract (3), the neutralizing activity of the free radicals is fast, 81,15%, respectively 80% at the moment of introducing the DPPH solution extract. In the case of curcuma (2), initially the activity is of 65,11% reaching in 60 minutes to approximately 80%. The smallest antioxidant activity is presented by the detoxifying extract, first 45,47% and at 60 minutes 74%. These results are in congruence with the polyphenol content of these chemicals.

CONCLUSION

These experiments have shown that green tea extract and curcuma and grape extract have an intense antioxidant activity correlated with free radical neutralization activity.

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