STUDIA UBB CHEMIA, LXI, 3, Tom II, 2016 (p. 505-514)
(RECOMMENDED CITATION)

Dedicated to Professor Emil Cordoș
on the occasion of his 80th anniversary

EVALUATION OF THE ANTIOXIDANT CAPACITY AND TOTAL POLYPHENOLS IN DIFFERENT FRUIT TEAS

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ABSTRACT. A diet rich in antioxidants is a heath choice most people make when it comes to options in fighting free radicals, that are formed natural in the body, but that cause damage to DNA and are connected to aging. The study was done in order the evaluate fruit teas that are a sources of antioxidants that can be easily introduce in the diet and are found all year along. 20 tea samples were analyzed to evaluate the antioxidant capacity and total polyphenols content. For the antioxidant capacity a photochemiluminescence methods was used and the Photochem instrument from AnalytikJena and for the total polyphenols content the Folin-Ciocalteu method was used. The average results where compared to the results obtained from black tea. The antioxidant capacity values obtained where from 0,53 to 2,27 mg/l Ascorbic Acid equivalents. The total polyphenols content was between 54 to 96 mg/l Gallic Acid equivalents. A positive correlation of 0.9664 was obtained between the antioxidant capacity and the total polyphenols content. A negative correlation of -0.962031201 was obtained between the antioxidant capacity and the number of the ingredients found in the tea samples.

Key words*: antioxidants, tea, polyphenols, Gallic acid, Ascorbic acid, antioxidant capacity*

INTRODUCTION

One important aspect in our modern culture is to live longer and to age slower. Scientists have been searching for answers regarding the ageing

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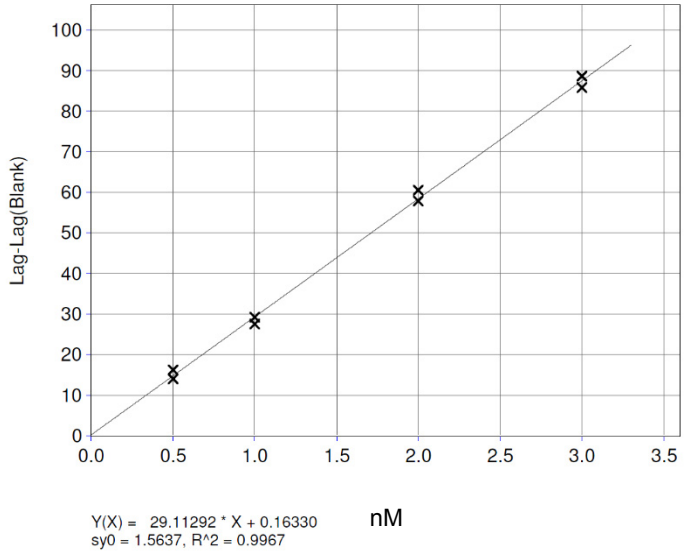
process. The result of these researches is the free radical theory of aging (FRTA) [1, 2] which states that organisms age because cells accumulate free radical damage over time [1, 3]. Free radicals are atoms with unpaired electrons that are the byproduct of normal cell function. The free radical theory of aging asserts that free radicals are linked to DNA damage, to protein cross-linking and other changes that accumulate over time and causes us to experience aging.[2 - 4] A chemical chain reaction of radical production occurs when a free radical pulls an electron off a neighboring molecule, causing the affected molecule to become a free radical itself, the new free radical can then pull an electron off the next molecule and so one. [2, 4] The free radicals produced in such reactions often terminate by removing an electron from a molecule which becomes changed or cannot function without it. Such an event causes damage to the molecule, and thus to the cell that contains it (since the molecule often becomes dysfunctional).[5] Antioxidants are a class of molecules that are capable of inhibiting the oxidation of another molecule. [2] They are electron donors that can break the free radical chain reaction by donating one electron, but without turning into free radicals themselves. [2] Studies have shown that increasing the amount of antioxidants in the diets of mice and other animals can slow the effects of aging. Since the creation of free radicals cannot be stop it is become more and more important to enrich our diets with higher quantities of antioxidants.[6, 7] A lot of food products have high concentrations of antioxidants like red wine, blueberries, red berries, dark green veggies, sweet potatoes, orange vegetables, tea, coffee, etc. [8 - 11] The problem with some food sources of antioxidants is that they are not available all year long like blueberries and raspberries and some are dietary restriction for people with different medical conditions like red wine, black tea and coffee.[12] The free radical theory of aging does not fully explain all the changes that occur during aging. It is likely that free radicals are only one part in the aging equation.

The purpose of the study was to evaluate the antioxidant capacity of fruit tea found on the local market and to compeer those result with similar products that have high antioxidant content like black tea. Fruit teas are available all year long, are not part of dietary restrictions, can be easily incorporated in a day to day diet, come in many different flavors and can be enjoy by all age groups.[13] All these characteristics make fruit tea an easy and enjoyable source of antioxidants. The antioxidant capacity of the fruit tea was also correlated with the total polyphenols content and number of ingredients of the tea samples.

RESULTS AND DISCUSSIONS

The calibration curve in 4 points obtained using Photochem for vitamin C is presented in figure 1. The slope of the calibration curve was 0,9967.

The calibration curve in 5 points obtained using Lambda Spectrophotometer for Gallic acid is presented in image 2. The slope of the calibration curve obtained was 0,9993.



***** The interception point of the tangent with the x coordinate defines the Lag phase- Lag

Figure 1. Calibration curve of vitamin C

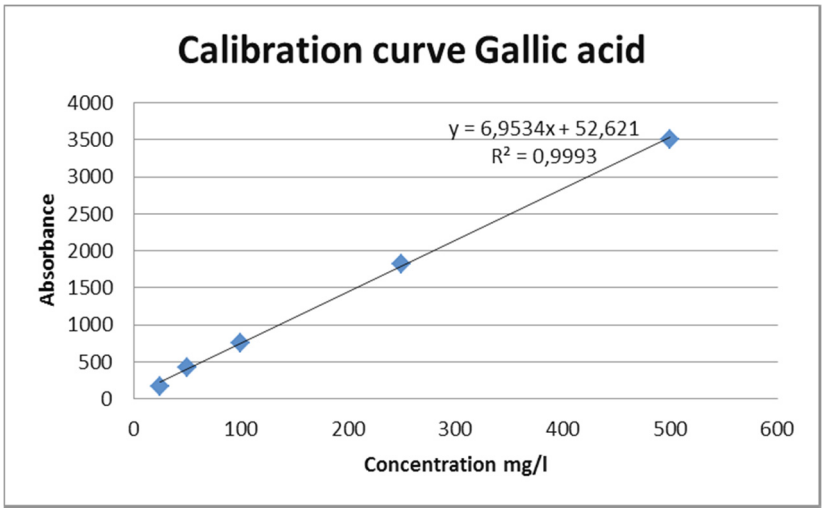


Figure 2. Calibration curve of Gallic acid

The average results obtained for the analysis of the 20 tea samples done in triplicates are presented in table 1.

Table 1. Antioxiodant capacity and polyphenol content of the 20 tea samples

Sample name	Antioxidant capacity mg/l Ascorbic Acid equivalents	Total polyphenols mg/l Gallic Acid equivalents	Total ingredients
Sample 1	1.57±0,007	93±0,57	7
Sample 2	2.27±0,009	96±0,55	6
Sample 3	2.23±0,09	96±0,53	6
Sample 4	2.19±0,009	96±0,53	6
Sample 5	1.25±0,006	76±0,44	7
Sample 6	0.53±0,003	66±0,41	9
Sample 7	0.56±0,003	67±0,39	9
Sample 8	0.58±0,003	68±0,39	9
Sample 9	0.67±0,004	68±0,40	9
Sample 10	1.07±0,005	90±0,53	7
Sample 11	1.26±0,006	81±0,51	7
Sample 12	1.32±0,005	71±0,48	8
Sample 13	0.75±0,004	70±0,48	9
Sample 14	0.74±0,004	72±0,48	9
Sample 15	1.56±0,007	95±0,56	7
Sample 16	0.68±0,004	54±0,35	11
Sample 17	0.98±0,005	63±0,39	9
Sample 18	1.17±0,005	75±0,48	8
Black tea 1	4.67±0,011	175±0,73	1
Black tea 2	3.96±0,009	149±0,69	1

The correlation between the results and between the number of ingredients and the results is presented in table 2, fig 3, fig 4 and fig. 5.

Table 2. Value of correlation factors

Crt. nr.	Antioxidant capacity	Polyphenols	Total ingredients
1	1		
2	0.9664	1	
3	-0.9620	-0.9702	1

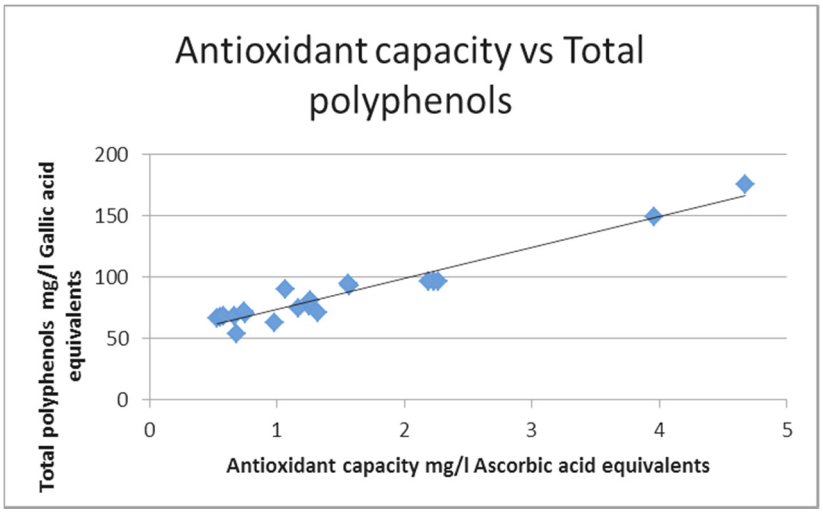


Figure 3. Trend line correlation between antioxidant capacity and total polyphenols

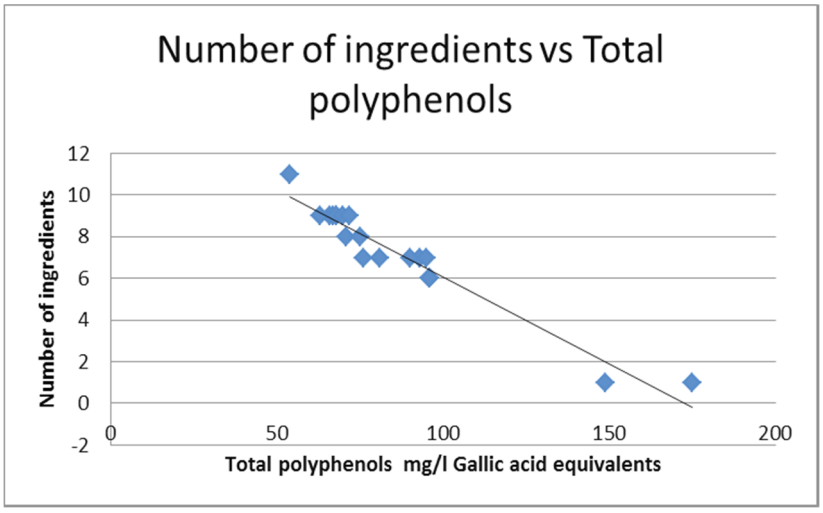


Figure 4. Trend line correlation between number of ingredients and total polyphenols

The values obtained correlating the results are significantly different from 0 which prove that the antioxidant capacity of the fruit tea is proportional with the total polyphenols content. The data also shows that there is an indirect correlation between ingredients and antioxidant capacity/ total polyphenols content. The more ingredients a tea has the less antioxidant capacity and total polyphenols content values where obtained.

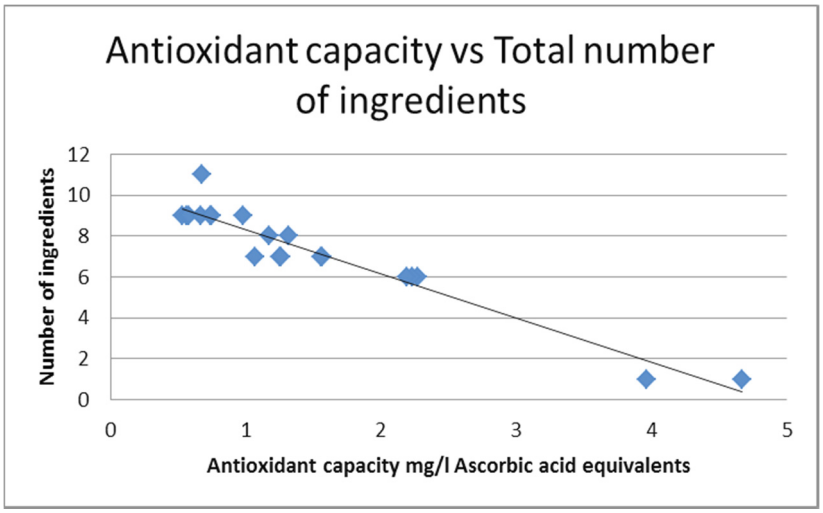


Figure 5. Trend line correlation between number of ingredients and antioxidant capacity

The antioxidant capacity of the black tea was significantly higher than that of fruit tea also the total polyphenols content. (figure 6, 7)

The antioxidant capacity was more them 3,5 higher than that of the average values obtained for fruit tea, but the total polyphenols was only 2 times higher.

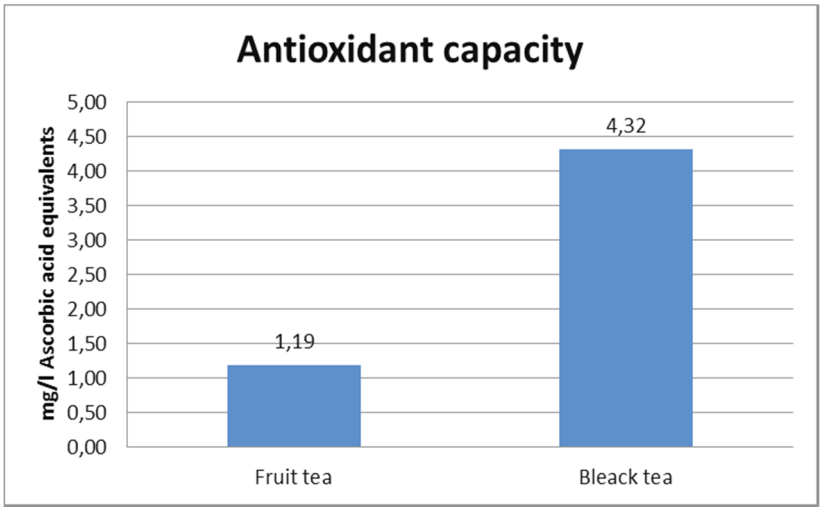


Figure 6. Average antioxidant capacity values for fruit tea and black tea

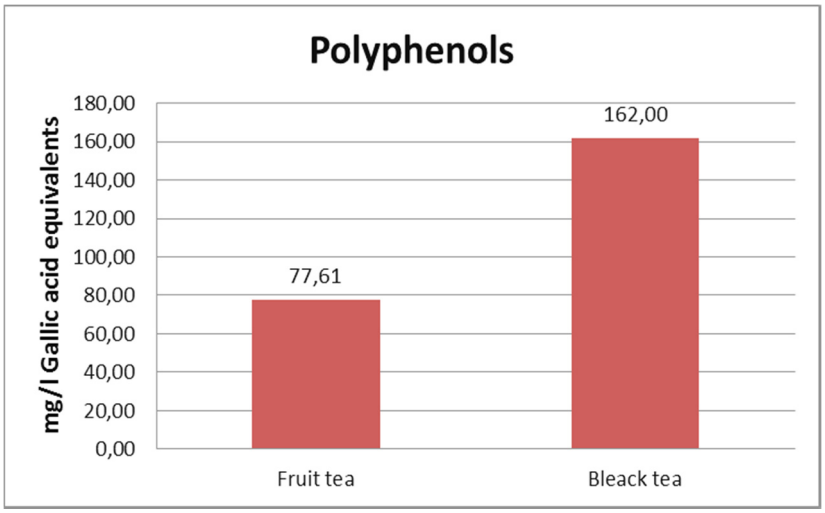


Figure 7. Average total polyphenols content for fruit tea and black tea

CONCLUSIONS

The antioxidant capacity values obtained where from 0,53 to 2,27 mg/l Ascorbic Acid equivalents and the total polyphenols content was between 54 to 96 mg/l Gallic Acid equivalents.

The highest antioxidant capacity values obtained was 2,27 mg/l Ascorbic Acid equivalents the tea had only 6 ingredients and it had the heights total polyphenols content of 96 mg/l Gallic Acid equivalents.

The lowest antioxidant capacity values was obtained for a tea with 9 ingredients, 0,58 mg/l Ascorbic Acid equivalents, that had total polyphenols content of 68 mg/l Gallic Acid equivalents.

The lowest total polyphenols content was obtained for a tea with 11 ingredients, 54 mg/l Gallic Acid equivalents that had antioxidant capacity value of 0.68 mg/l Ascorbic Acid equivalents.

The extra ingredients added to the fruit tea did not give a higher antioxidant capacity or a higher total polyphenols content which mean that the 3 base ingredients hibiscus, blackberry leaves (*Rubus suavissimus*) and apple are the ones that give the antioxidant capacity, all the other ingredients add lower the content of the basic tea and thus lower the antioxidant capacity. The extra ingredients have no antioxidant capacity because either they don't have it as raw material, the compounds that are antioxidants are no extracted in water or the hot water temperature denatures the compounds.

The types of polyphones present in the teas have different antioxidant capacity of that is way the antioxidant capacity was more them 3,5 higher than that of the fruit tea and the total polyphenols content was only 2 times higher.

EXPERIMENTAL SECTION

Tea samples

18 fruit tea samples where purchase from different local shops they had different flavors but the same base notes (hibiscus and apple) and 2 black tea samples. Samples consisted of two batches of each commercial tea.

Reagents

The fallowing reagents where used in the analysis: Folin-Ciocalteu solution (Supelco), Gallicacid (Supelco), sodium carbonate (Merck), ultra-pure water (EVOQVA), Ethanol (Supelco), ACW kit from AnalytikJena, HPLC grade Methanol (Merck).

Preparation of tea infusion

The infusion was prepared using a bag of tea or 2 g of tea in 200 ml of ultra-pure water at 97°C. After 5 minutes of infusion the tea was filter and left to cool for 1h in an Erlenmeyer flask with lid to prevent any water evaporation. Every sample was done in triplicate.

Antioxidant capacity

For the determination of the antioxidant capacity expressed in equivalents of vitamin C the Photochem instrument from AnalytikJena was used and the specially design ACW kits. ACW kit contains a standard solution of vitamin C, stock solution (Photo sensitizer and detection reagent), a buffer solution and a diluting solution. The instrument uses the fast photochemical excitation of radical formation combined with sensitive luminometric detection. The measurment is done in 2 steps:

1. Optical excitation of a photosensitizer substance S and subsequent generation of the superoxide anion radicals

$$S + hv + O2 \rightarrow [S^*O2] \rightarrow S^{\bullet +} + O2^{\bullet -}$$

2. Detection of the radicals (left after the reaction with antioxidants) by means of a chemiluminogenic substance.

A calibration curve of vitamin C was done in 4 points 0,5nM, 1 nM, 2 nM and 3 nM. The samples were diluted 1:20-1:50 to be in the range of the calibration curve.

The formula used for calculation is:

$$Concentration\ \left[\frac{\mu g}{ml}\right] = \frac{Quantity \cdot Dilution \cdot M}{Pipetted\ Volume}$$

Where:
Quantity - nmol Ascorbic acid
M - Molar mass Ascorbic acid = 176,13 ng/nmol
Pipetted volume: used volume in the vial in µl
Dilution - at 1:10 dilution factor = 10.

Total polyphenols analysis

For the determination of polyphenols a modified version of the Folin-Ciocalteu method was used. Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry [14].

0,5 ml of sample, was pipette in a 10 ml volumetric flask, which contained 0.5 ml Folin-Ciocalteu solution, 5 ml ultra-pure water and 1,5 ml sodium carbonate solution (20%), the flask was filled up to the mark with ultrapure water. The volumetric flasks samples were left 90 de minutes and where then measure at 765 nm using the Spectrophotometer UV/VIS Lambda 25 from Perkin Elmer. Measurements were compared to a calibration curve of Gallic acid (25, 50, 100, 250, 500 ppm), and the results were expressed as Gallic acid equivalents. All samples where done in triplicate.

ACKNOWLEDGMENTS

This work was funded by Core Program, under the support of ANCSI, project no. PN 16.40.02.01, Sectoral Operational Programme “Increase of Economic Competitiveness”, Priority Axis II, Project Number 1887, INOVAOPTIMA, code SMIS-CSNR 49164

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