THE LINEAR AND NON-LINEAR BI-DIMENSIONAL MATHEMATICAL MODELS, BETWEEN ANTIOXIDANT CAPACITY, ASCORBIC AND POLYPHENOLS CONTENTS IN SOME VEGETABLES

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A total of 17 vegetables: bell pepper (red and green); pepper (red and green), sallow thorn (Hyppophae rhammnoides) celery (leaves and root), cabbage (white, red, curly and Bruxelles), broccoli, cauliflower, radish, dill (leaves), parsley (leaves and roots); were analyzed for antioxidant activities using FRAP method, DPPH method, total polyphenols content by Folin Ciocalteu method and ascorbic acid content using 2,6-dichlorophenol indophenols. The highest TAC (Total Antioxidant Capacity) values was identified for sallow thorn (Hyppophae rhammnoides), red cabbage, red bell pepper and pepper. The smaller values were identified in parsley roots. The antioxidant capacity, Vitamine C and polyfphenols contents correlations on vegetables, have been studied using multiple regression mathematical elements. The obtained models, in accordance with the analysis of the dependence value between total antioxidant capacity, polyphenols and ascorbic acid contents. The nonlinear two-dimensional TAC = f(vit. C and polyphenols) model is indicating the increasing of the correlation coefficient comparative with the liniar model associated coefficient.

***Key words**: total antioxidant capacitity (TAC), FRAP and DPPH methods, polyphenols, ascorbic acid, vegetables*

From the oldest time, the fruits and vegetables were used as medicinal agents. In the last two decades, the scientific community recognized the "paramount" value of the fruits and vegetables, besides their nutritive contribution and their role in preventing vitamin deficiencies.

Having in mind all the means of protection that human body possesses, it's recommendable that humans should supply these means and use antioxidants, substances that have the power of preventing or even inhibiting per oxidation. An antioxidant is one of the many chemical substances which decrease and prevent the

oxidation and the destruction of cell and tissue towards free radicals from the body in which they are involved in a etiology of diseases.

Thus, the combination of phytochemical substances has an important role in antioxidant and anticancer activity and the real health benefits can come from the mixture of phytonutrients found in fruits and vegetables.

Vegetable polyphenols include a wide range of compounds with antioxidant activity, that is, hydroxycinnamates, flavan-3-ols (condensed tannins), Gallic acids derivatives (hydrolysable tannins), flavonols and anthocyanins. The phenol composition of vegetables varies greatly among cultivars and tissue. Peel tissues contain larger amounts of phenol, anthocyanins, and flavonols than flesh tissues.

The evaluation of vegetable antioxidants capacity is not an easy task, as much method can be used to determine this activity, and substrates, conditions, analytical methods, and concentrations can affect the estimated activity.

We used the iron-reducing capacity to evaluate the antioxidant capacity (FRAP method) of vegetables (Benzie and Strain, 1996), although we have understand that these simple method have limitation (Franke and Meyer, 2000) and the estimation of same parameter using the inhibition capacity of free radicals (DPPH method).

The aim of the present work was to determine the phenols, vitamin C contents as well as the total antioxidant capacity of some vegetables commonly consumed in Romania.

Several methods are know to measure the total antioxidant capacity (TAC) of biological samples, but we tried the FRAP assay, which depends upon the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant at low *p*H. This ferrous tripyridyltriazine complex has an intensive blue colour and can be monitored at 593 nm (Benzie and Strain, 1996). This method was elaborated for human plasma but many authors used these method for aqueous or hydroalcoholic extracts of medicinal plants (Szőllősi and Szőllősi Varga, 2002).

MATERIAL AND METHOD

1. Reagents and equipment

All chemicals and reagents were analytical grade or purest quality purchased from Sigma, Merck, Aldrich and Fluka; deionised water was used. Absorption determination for FRAP, DPPH and total phenol content was made using Specord 205 AnalitykJena.

2. Vegetables

In the present study, a total of 17 vegetables: bell pepper (red and green),, pepper (red and green), sallow thorn, celery (leaves and root), cabbage (white, red, curly and Bruxelles), broccoli, cauliflower, radish, dill (leaves), parsley (leaves and roots), were analyzed.

3. Evaluation of total antioxidant capacity (TAC)

a). Adaptation of FRAP method

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); 10mM/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM/L HCl; 20mM/L $FeCl_3 6H_2O$ in distilled water. FRAP working solution: 25mL acetate buffer, 2.5mL 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 0.1-1.0 mM/L.

For the preparation of calibration curve 1ml aliquot of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 μM/mL aqueous Fe(II) as Mohr salts solution were mixed with 5 mL FRAP working solution; FRAP reagent was used as blank. The absorption was read after 10 min. at 25 °C at 593 nm, 1cm lights path, and the calibration curve was drawn.

One mL from diluted 1/10 vegetable extracts, was mixed with the same reagents as described above, and after 10 min. the absorption was measured. All determinations were performed in triplicate. Total antioxidant capacity in vegetable extracts in Fe(II) equivalents was calculated. Correlation coefficient for calibration curve was 0.959.

b) DPPH method

Hydrogen atom – or electron-donation ability of the corresponding vegetable extracts was measured from the bleaching of the purple-coloured ethanol solution of DPPH. This spectrophotometric assay uses stable 2.2'diphenylpicrylhydrazyl (DPPH) radical as reagent. 0.5 mL of various vegetable extracts were added to 2.5 mL of a 1 mM ethanol solution of DPPH. After 10 min. or 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):

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

4. The amount of phenolic compounds

The following reagents were used: 2.0 M Folin-Ciocalteu phenol reagent, gallic acid and anhydrous carbonate. The content of total phenolic compounds in methanolic extracts was determined by Folin-Ciocalteu method (1927).

For the preparation of calibration curve 0.5 mL aliquot of 0.16, 0.32, 0.60, 1.20 μM/mL aqueous gallic acid solution were mixed with 2,5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 2 mL (7.5%) sodium carbonate. The absorption was read after 2 h at 20 °C at 750 nm and the calibration curve was drawn.

0.5 mL from diluted 1/10 vegetable extracts was mixed with the same reagents as described above, and after 2 h the absorption was measured for the determination of plant phenols. All determinations were performed in triplicate. Total content of phenol compounds in methanol extracts in Gallic acid equivalents (GAE) was calculated. Correlation coefficient (r^2) for calibration curve was 0.975.

5. The amount of ascorbic acid (C vitamin)

Ascorbic acid contents were estimated titrimetrically by 2,6-dichlorophenol indophenol dye reactant. 5 mL of vegetable extracts was diluted with 10 mL water, ad 1mL HCl 1N, an titrate with 1mM solution of 2,6-dichlorophenol indophenols dye reactant to pink colour. For red coloured vegetables an adapted colorimetric method with the same dye was used.

6. Multiple linear and non-linear regression

In accordance with the multiple regression theories we presume that after "m" experimental measurements the final result "y" is linearly depending on the "n" factors $x_1, x_2,..., x_n$:

$$y = f(x_1, x_2, ..., x_n) \quad (1), \text{ where:}$$

$$y = \sum_{i=1}^{n} a_i x_i + a_{n+1} \qquad (2)$$

The main idea is to determinate the coefficients $a_1,a_2,...,a_n$ so that the expression of the function (2) is following as exactly as possible the points positions experimentally obtained. and for that the applied method is the one of minimum square roots

$$x_{11}, x_{12}, ..., x_{1m}, x_{21}, x_{22}, ..., x_{2m}, ..., x_{n1}, x_{n2}, ..., x_{nm}, y_1, y_1, ..., y_m$$

$$\varphi(a_i) = \sum_{j=1}^{m}\left[\left(\sum_{i=1}^{n} a_1 x_{ij} + a_{n+1}\right) - y_j\right]^2 \qquad (3)$$

where:

m - experimental measurements;

φ - sum of square differences between the value y_j , $j = \overline{1,m}$ experimentally obtained and

$$y = f(x_{1_j}, x_{2_j}, \ldots, x_{n_j}),\ j = \overline{1,m}$$

In such cases the function extremes are the points of minimum and by derivation of the application φ reported to a_i is constructing a mathematical coefficients system. The calculation of the coefficients consists in resolving the n+1 equations system with n+ 1 unknown factor (4):

$$\begin{cases} a_1\sum_{j=1}^{m} x_{1j}^2 + a_2\sum_{j=1}^{m} x_{2j}x_{1j} + \ldots + a_n\sum_{j=1}^{m} x_{nj}x_{1j} + a_{n+1}\sum_{j=1}^{m} x_{1j} = \sum_{j=1}^{m} y_j x_{1j} \\ a_1\sum_{j=1}^{m} x_{1j}x_{2j} + a_2\sum_{j=1}^{m} x_{2j}^2 + \ldots + a_n\sum_{j=1}^{m} x_{nj}x_{2j} + a_{n+1}\sum_{j=1}^{m} x_{2j} = \sum_{j=1}^{m} y_j x_{2j} \\ \ldots\ \ldots\ \ldots \\ a_1\sum_{j=1}^{m} x_{1j}x_{nj} + a_2\sum_{j=1}^{m} x_{2j}x_{nj} + \ldots + a_n\sum_{j=1}^{m} x_{nj}^2 + a_{n+1}\sum_{j=1}^{m} x_{nj} = \sum_{j=1}^{m} y_j x_{nj} \\ a_1\sum_{j=1}^{m} x_{1j} + a_2\sum_{j=1}^{m} x_{2j} + \ldots + a_n\sum_{j=1}^{m} x_{nj} + na_{n+1} = \sum_{j=1}^{m} y_j \end{cases} \qquad (4)$$

RESULTS AND DISCUSSION

1. The values of determined parameters. The total antioxidant capacity (TAC) by FRAP and DPPH methods, vitamin C and polyphenols content are presented in *table 1*.

Table 1

Antioxidant capacity, vitamin C and polyphenols contents of vegetables

Crt Nr.	Vegetables	Vit. C, µM/100 g	Polyphenols, µM/100 g	TAC – FRAP, µM/100 g	TAC – DPPH, %	
					15 s	30s
1	red bell pepper	581,25	736	872	2,75	3,05
2	red pepper	468,75	744	824	1,89	3,06
3	sallow thorn	281,25	826	1160	38,05	43,91
4	celery leaves	37,50	462	426	5,31	6,11
5	celery roots	13,12	162	140	0	0,49
6	white cabbage	106,87	254	208	0,49	0
7	red cabbage	140,00	726	962	18,72	21,72
8	Bruxelles cabbage	166,87	488	434	3,13	3,80
9	curly cabbage	90,00	294	234	0,96	0
10	broccoli	183,75	284	260	0,47	1,40
11	cauliflower	144,37	240	192	0	0
12	green pepper	206,25	546	350	3,89	4,87
13	green bell pepper	150,00	492	242	0	0
14	red radish	170,62	200	234	1,42	1,40
15	dill leaves	59,47	270	330	3,88	2,25
16	parsley leaves	299,84	534	410	0,47	0
17	parsley roots	42,13	162	60	0,47	0

The highest vitamin C content can be observed in red bell pepper followed by red pepper and parsley leaves and is smaller in celery (leaves and roots) and parsley roots. Polyphenols content is high in sallow thorn (*Hyppophae rhammnoides)*, red pepper and bell pepper and smaller in celery roots and parsley roots. The highest TAC values by FRAP method were identified in sallow thorn (*Hyppophae rhammnoides)* extract followed by red cabbage. red pepper and bell pepper. The smaller values were identified in parsley roots. The values of TAC by DDPH method follow the same order as by FRAP method.

2. The correlations between experimental data

The aim of this paper is to present a nonlinear multifactor model simplified by using classical methods and reduced to the used linear model

The obtained models, in accordance with the analysis of the dependence value between total antioxidant capacity, polyphenols and ascorbic acid contents, separated by the obtained coefficient of multiple correlation, are the following:

a). The dependence TAC-FRAP (f) with ascorbic acid (C) and polyphenols (P) is presenting a multiple correlation coefficient r = 0,950, meaning a very high correlation.

$$f(C,P) = -1{,}067\ C + 1{,}219\ P + 1{,}431 \cdot 10^{-3}\ C \cdot P - 35{,}271$$

b). The dependence TAC-DPPH 30s (d_{30}) with ascorbic acid (C) and polyphenols (P) is presenting a multiple correlation coefficient r = 0,786, meaning a high correlation.

$$d_{30}(C,P) = -6{,}8 \cdot 10^{-2}\ C + 5{,}050 \cdot 10^{-2}\ P + 3{,}233 \cdot 10^{-5}\ C \cdot P - 6{,}792$$

c). The dependence TAC-DPPH 15s (d_{15}) with ascorbic acid (C) and polyphenols (P) is presenting a multiple correlation coefficient r = 0,780, meaning a high correlation.

$$d_{15}(C,P) = -6{,}19 \cdot 10^{-2}\ C + 4{,}821 \cdot 10^{-2}\ P + 3{,}128 \cdot 10^{-5}\ C \cdot P - 5{,}137$$

CONCLUSIONS

The highest TAC (Total Antioxidant Capacity) values was identified for sallow thorn (*Hyppophae rhammnoides)*, red cabbage, red bell pepper and pepper. The smaller values were identified in parsley roots.

The best correlation (r = 0,950) regarding the intensity of bounding and the level of association between two data series of values is obtained for TAC (FRAP) and the amount of reducing substances (vitamin C şi polyphenols).

A medium correlation (r = 0,786 and r = 0,780) obtained for TAC (DPPH) the amount of reducing substances (vitamin C şi polyphenols). That can be explained because the both methods estimate the total antioxidant capacity on the basis of two points of view: first, the reducing character of the vegetables substances with antioxidant properties and secondary, the scavenging property of free radicals.

The nonlinear two-dimensional TAC = f(vit. C and polyphenols) model is indicating the increasing of the correlation coefficient comparative with the liniar model associated coefficient.

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