Action of Humic Acids, Boron, Calcium, Zinc and Galactomannans Extracted from the Seeds of *Gleditsia triacanthos* on the Content of Quercetin-3-O-rutinoside from *Lycopersicon esculentum*

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The consumption of plant nutrients is often very high, especially for vegetables, and especially those grown in protected systems. The use of fertilizers in order to assure the nutritional needs of plants is a very widely tool used in agriculture. This study is based on the effect of the fertilizer obtained from 0.1% humic acids, 1% calcium chloride, 1% zinc sulfate, 0.1% boron and 1% galactomannans extracted from the seeds of Gleditsia triacanthos on a culture of Prekos variety tomatoes which was nursed in a protected space. The fertilizer was applied on the leaves of the tomato culture in a concentration of 10 mL/L at 4 weeks after planting, in four stages and an interval of 10 days. The effect of the biofertilizer on the content of total polyphenols, flavonoids and rutin has been studied by measurements made on tomatoes fruits which were harvested at physiological maturity, and selected according to their firmness, without bumps or visible degradation. The content of total polyphenols from fertilized samples showed values of 350 mg/100 g fresh product and the unfertilized samples had the value of 110 mg/100 g fresh product. The content of total flavonoids was also different from 53.1 mg/100 g fresh product for the fertilized samples as compared to 22.5 mg/100 g fresh product for the unfertilized samples. The rutin concentration was 3.24 times higher in the fertlized samples as compared to the unfertilized samples.

*Keywords***:** *Lycopersicon esculentum, flavonoids, rutin, galactomannans*

Tomatoes (*Lycopersicon esculentum*) are vegetables grown in almost every country all over the world. They are one of the major sources of bioactive compounds such as folic acid, vitamin C, polyphenols and carotenoids, having special antioxidants in their composition [1]. These bioactive compounds are found in higher concentrations in skin, followed by seeds and pulp [2]. Tomatoes are the first source of lycopene (71.6%), secondary a source of vitamin C (12.0%), pro-vitamin A carotenoids (14.6%) and other carotenoids (17.2%) and third a source of vitamin E (6.0%) [3].

In addition to carotenoids (lycopene, β-carotene, and lutein), flavonoids have been confirmed as a group of polyphenols with great importance in the tomatoes antioxidant activity [4,5]. Flavonoids are polyphenols of vegetal origin, one of the most important components of human diet, due to their widespread distribution in food sources such as onion, grapes, buckwheat, beans, apples, tomatoes and beverages (black tea and red wine) [6-7]. They are both found under a free form (aglycones) and also as glycosides. Function of their structural characteristics, flavonoids are classified into the following groups: [8-9] flavones, flavonoles, isoflavone, anthocyanidins, flavanones and flavan-3-ol,(fig. 1.).

It is estimated that approximately 2% of all carbon fotosynthesized by plants is converted into different groups of flavonoids [10], representing one of the largest group of natural phenols from plants [11].

Therefore, in order to assess the nutritional quality of fresh tomatoes, it is important to study the main compounds having an antioxidant activity. The antioxidant content of tomatoes depends on the variety, maturity and agrochemical conditions [12,13].

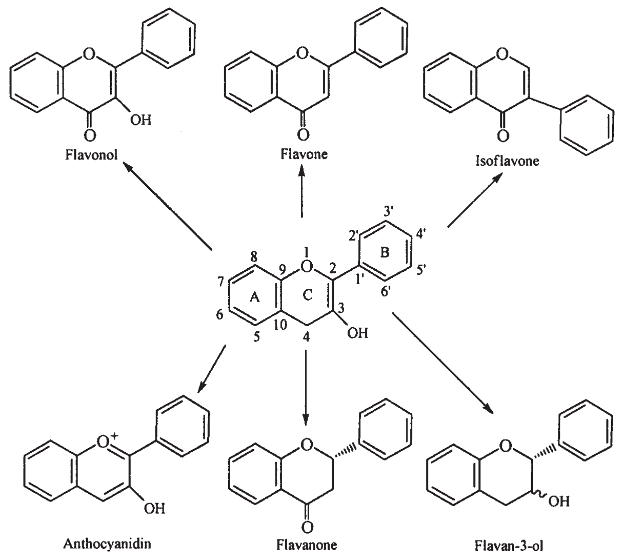

Fig. 1. Basic structure of different flavonoides

European countries consume remarkable quantities of fresh and processed tomatoes and they adapted various agricultural practices in order to increase the content of phenolic compounds in fruits. One of the most commonly used methods is the fertilization with components involved in the metabolism of plants. In this context, this study was following the effect of a fertilizer obtained from potassium humates mixed with galactomannans extracted from seeds of *Gleditsia triacanthos*, boron, calcium and zinc from tomatoes culture on the concentration of quercetin-3-rutinoside (rutin), the main flavonol from tomatoes (*Lycopersicon esculentum*) with a remarkable antioxidant activity [14,15] (fig. 2).

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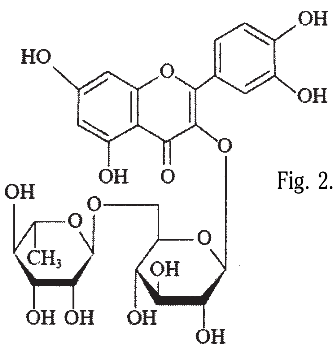

Quercitin-3-rutinoside (Rutina)

Fig. 2. Chemical structure of quercitin-3-rutinoside (rutin)

Rutin is a bioflavonoid, sometimes referred as vitamin P, which is essential for the assimilation of vitamin C. In fact, bioflavonoids like quercetin and hesperidin are almost identical with rutin composition and, therefore, most experts agree that rutin and quercetin actually work together through mutual harmonization. Therefore, it is suggested that rutin and quercetin are collectively considered. In fact, bioflavonoids are becoming increasingly popular due to their high nutritional value and their positive role for health. Therefore it is not surprising that the rutin is always present in highly nutritious foods, for example, red apples, broccoli, citrus fruits, onions and even in different teas. The rutin is normally available as a supplement and easily consumed. Since our bodies are unable to produce bioflavonoids, it is essential to be included in our diet.

Among the studied flavonoids, the rutin excelled due to its various pharmacological activities [16]. Among the therapeutic activities of rutin it is remarkable its action on the veins affected by some bleeding diseases or high presure by normalizing the resistance and permeability of the walls. It works on eyes function improving the field of vision and reducing visual loss.

These effects can be enhanced by combining the rutin with ascorbic acid which has an important role in the absorption of the rutin [17].

According to the studies made by Afanas'ev and co. [18] who have investigated the antioxidant activity of quercetin and rutin, there has been shown that these compounds have a therapeutic effect on diseases involving free radicals, and they are not toxic, in particular the rutin.

A number of other rutin activities on health highlighted its role in the treatment of arteritis, *Candida albicans* [19], suppression of cellular immunity [20], obesity [21], anticancer [22], anti-inflammatory diseases [23] .

Experimental part

The experiment was conducted on a tomato culture organized in a protected space (solar) consisting of a Prekos variety in Bechet city, Oltenia region in southwestern Romania (43° 47'N 23° 57'E) during the period March - August.

The fertilizer consisting of 0.1% humic acids, 1% calcium chloride, 1% zinc sulfate, 0.1% boron and 1% galactomannans extracted from the seeds of *Gleditsia triacanthos*, was obtained in the laboratories of the University of Craiova under a research contract [24] and was applied on the leaves of the tomatoes culture at a concentration of 10 mL/L at 4 weeks after planting during four stages, at an interval of 10 days.

Two sets of samples were made: a control (blank) sample and a sample treated with the fertilizer.

The effect of the biofertilizer on the content of total polyphenols, flavonoids and rutin was studied by measurements made on tomatoes fruits which were harvested at physiological maturity, and selected according to their firmness, without bumps or visible degradation.

Reactivi: Methanol HPLC; Folin-Ciocâlteu reagent, Sodium carbonate, Gallic acid; Sodium nitrite; Aluminium chloride, Sodium hydroxide; Rutin; Ammonium molibdate.

Obtaining the samples

A sample of 1 to 5 g was homogenised in a Braun MR 404 Plus vertical blender for 1 minute with 15 mL HPLC 80% methanol at room temperature. The homogenate was filtered and centrifuged at 2500 rpm for 5 min using a centrifugal type Hettich Universal 320/320R. The centrifuged, filtered samples were transferred in a 50 mL graduated flask obtaining the above mentioned volume by adding 80% methanol.

Determination of total polyphenol content of tomatoes

The total polyphenol content was measured using the Folin Ciocaletau colorimetric method. To 800µL of deionised water, 50 µL of Folin Ciocalteu's phenol reagent and a volume of sample ranging from 10 to 50µL were added and accurately mixed. After 1 min, 100 µL of 20% sodium carbonate solution was added and mixed. Deionised water was then added up to a volume of 1 mL. The solution was carefully mixed and total phenol content was spectrophotometrically estimated at 765 nm (Cary 50) after 2 h incubation Quantification was based on the standard curve generated with gallic acid. All determinations were carried out in triplicates [25,27].

Determination of total flavonoids

Briefly, a 0.25 mL of extract was diluted with 1.25 mL of distilled water. Then, 75µL of 5% $NaNO_2$ solution was added to the mixture. After 6 min. 150µL of 10% $AlCl_3 \cdot 6H_2O$ solution was added and the mixture was allowed to stand for another 5 min. Then, 0.5 mL of 1 M NaOH was added and, after mixing, the absorbance was measured immediately at 510 nm [28]. The final concentration of flavonoids was expressed as an equivalent of rutin (mg rutin / g FW). All determinations were carried out in triplicates.

Rutin assay

The analyses of rutin content were performed according to the AOAC official method [29]. This method has been modified using HPLC 80% methanol [30]. 0.5 g of the sample is dissolved into 50 mL HPLC 80% methanol. The 2 mL of the extract thus obtained is transfered into 50 mL volumetric flask. 2 mL ddH_2O and 5 mL ammonium molybdate are added. Then the mixture is diluted with ddH_2O. The standard solution is prepared through disolving of 0.02 rutin in 50 mL HPLC 80% methanol. Then 1 mL of this solution is used following the same procedure as for the sample analysis. The absorbance of the sample against ddH_2O as a blank sample was determined at 360 nm with an UV-Vis spectrophotometer Cary -50. All determinations were carried out in triplicates.

The content of rutin (R), % in the sample is calculated as follows:

$$R\ (\%) = A_{sample} \cdot C \cdot 50 \cdot 100 / A_{standard} \cdot W \cdot 2$$

Where: A_{sample} – absorbance of the sample at 360 nm

$A_{standard}$ - absorbance of the standard solution at 360 nm

C – concentration of standard solution of rutin, g.mL^{-1}

W – weight of the sample , g

2 – volume of the sample, mL.

Sample	Total polyphenols, mg GAE/100g fresh product	Total flavonids, mg RE/100g fresh product	Rutin, mg/100g fresh product	Total flavonoids / Total polyphenols
Blank sample	110	22.5	2.9	0.20
Fertlized sample	350	53.1	9.4	0.15

Table 1
RESULTS OBTAINED FROM MEASUREMENTS OF TOMATO FRUITS

Statistical analysis - Three replicates of each sample were used for statistical analysis. Analysis of the data was performed on the original data by one-way analysis of variance (ANOVA) or regression analysis. Differences at $P < 0.05$ were considered significant.

Results and discussions

Among the studied flavonoides, the rutin excelled in the analysis due to its various pharmacological activities [16]. So, it has been studied the possibility of increasing the concentration of rutin in tomatoes fruits by applying a fertilizer of 0.1% humic acids, 1% calcium chloride, 1% zinc sulfate, 0.1% boron and 1% galactomannans extracted from the seeds of *Gleditsia triacanthos* obtained in the laboratory of the University of Craiova, Department of Chemistry.

The content of total polyphenols, total flavonoids, rutin and the ratio of total flavonoids and total polyphenols from the polyphenolic extract obtained from the fruit of tomatoes is shown in table 1. The content of total polyphenols was determined from the regression equation of gallic acid from the calibration curve. Similarly, the total flavonoids concentration from the regression equation of the calibration curve of the rutin was determined.

By corroborating these results obtained after the treatment with a fertilizer that contain the extract of galactomannans from the seeds of *Gleditsia triacanthos* mixed with humic acids, boron, calcium and zinc, their positive effect on the concentration of total polyphenols, total flavonoids and of rutins is shown. Thus, the boron, by its action on the enzymathic system, contributes to increase the activity of the enzyme chalcone isomerase which leads to an increase in the content of rutin [31-33].

Humic acids, beside their effect on the growth of metabolism, also determine an increase of enzymes activity and carbohydrate content [34-35]. By increasing the enzymathic activity and especially of the enzyme chalcone isomerase, enzyme that catalyzes the conversion of the naringerin chalcone to naringerin quercetin, so that an increase of the content of rutin of 3.24 times higher in fertilized sample compared to the blank sample is obtained [32,33,36]. The increase of the carbohydrate content accelerates the biosynthesis of flavonoids by shikimic acid and acetyl-CoA route. The values obtained are consistent with other results in the literature [37].

The content of total polyphenols presents an increase from 110 mg/100 g fresh product in blank samples to 350 mg/100 g fresh product in fertilized samples and the content of total flavonoid content increases from 22.5 mg/100 g fresh product in blank samples to 53.1 mg/100 g fresh product in fertilized samples. These values are similar to other results obtained by some authors [38-39].

The difference in the obtained results is due to the stimulatory effect of fertilizer components. Thus, the zinc controls the generation of toxic oxygen free radicals by interferring with NAPDH oxidation and oxygen radical capture. According to this effect it helps to increase cell membrane permeability [40]; it maintains the structural orientation of the macromolecules while maintaining the ion transport [41-43].

Boron is involved, with calcium, in the cell wall structure and the movement of calcium in plants. It acts, directly or indirectly, in many physiological and biochemical processes during the plant growth acting on the nutritional status of plants, on the enzymathic system and contributes to the integrity of the cell membrane [44-47].

Galactomannans, by their property to form films on the applied surface prevent the fertilizes leakage. After application on the leaves, by water evaporation, galactomannans form a semi-solid film on the surface of the leaf incorporating the entire mass of the aqueous solution. As water is lost by evaporation, the mass transfer from the surface of the leaf is reduced and the daily natural rewet of the leaves causes the resumption of daily food intake until the complete foliar absorption of the fertilizer components.

Conclusions

The obtained results highlight the role of endogenous substances contained by the fertilizer on the metabolism of phenolic compounds from tomatoes. These substances act competitively in controling the tomatoes plants metabolism through the formation of phenolic compounds in increased concentrations and from their balance it results their control on plant metabolism.

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