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Original scientific paper

SPECTROPHOTOMETRIC DETERMINATION OF TOTAL POLYPHENOLIC AND FLAVONOID CONTENT IN TOMATO

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ABSTRACT

Polyphenolic compounds are a heterogeneous group of secondary plant metabolites and one of the most important classes of natural antioxidants. Polyphenols can be found in apples, onions, dark chocolate, red cabbage, tomatoes, and many other fruits and vegetables. The aim of the present investigation was the determination of total polyphenolic content (TPC) and total flavonoid content (TFC) in two tomato cultivars "Belle F1" and "Perugino F1" by UV-Vis spectroscopy. Determination of TPC was performed by Folin–Ciocâlteu assay with gallic acid as a reference standard. In order to determine TFC, quercetin was used as a reference standard. The obtained results showed that the TPC is higher in the tomato Perugino F1 (9.18 ± 0.16 mg / 100 g) compared to the hybrid "Belle F1" (6.23 ± 0.03 mg / 100 g).

Key words: polyphenols, flavonoids, gallic acid, quercetin, UV - Vis spectroscopy.

INTRODUCTION

Fruits and vegetables are the richest sources of polyphenols a group of bioactive compounds that play a significant role in preventing health-related problems (Haminiuk et al., 2012; Bezuneh and Kebede, 2015). The antioxidant and metal chelating capacity of polyphenols are responsible for reducing the risk of oxidative damage caused by free radicals to the cell (Sulaiman et al., 2013). Among vegetables, tomato is consumed worldwide due to its versatility and its status as an ingredient in a large variety of different receipts. Tomatoes and tomato products meet consumer demands in terms of cost, convenience, availability, and taste, while they also deliver beneficial health effects, being easily included in a large variety of culturally diverse dishes (Martí et al., 2016). Tomato is one of the most popular and the most produced vegetable in the world. It is a very important food in the human diet. Its nutritional value is due, among other things, to the presence of polyphenols that have antioxidant properties (George et al., 2004). Although tomato does not outstand for its high concentration in polyphenols, the high levels of tomato intake both fresh and processed, position tomato as one of the main sources of bioactive compounds (Chun et al., 2005).

Numerous studies have focused on the content of polyphenols due to their role in the nutritional properties of tomatoes. (Mimica et al., 2004; Urquiaga and Leighton, 2000). The determination of TPC and TFC is very important and common practice. Frequently used method for the determination of TPC and TFC is UV-Vis spectroscopy (Bezuneh and Kebede, 2015). This method is based on the absorption of electromagnetic radiation because of transitions of electrons from lower to higher energy state. The Folin–Ciocâlteu assay is the most commonly used procedure for the determination of TPC in different food extracts. This

assay is a colorimetric method based on electron transfer reactions between the Folin – Ciocâlteu reagent and polyphenols (Sánchez-Rangel et al., 2013). It is widely applied in the determination of the TPC in fruits and vegetables (Ainsworth and Gillespie, 2007). Gallic acid (3,5,7-trihydroxybenzoic acid) is a polyphenol with a higher specific absorption, therefore it is the best reference standard for determining the content of total polyphenols (Deneshfar et al., 2008). One major group of polyphenols is flavonoids. The flavonoid content of tomatoes can be determined in relation to quercetin as the reference standard (Slimestad et al., 2008). Quercetin (2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) forms coloured complex product in reaction with metal ions, due to its use as a reference standard solution for determining the total flavonoid content (Martí et al., 2016).

The main objectives of the present research were spectrophotometric determination of total polyphenolic content (TPC) and total flavonoid content (TFC) in the tomato hybrids "Belle F1" and "Perugino F1" according to the Folin–Ciocâlteu method. For determination of TPC gallic acid was used as a reference standard. In order to determine what percentage of total polyphenols are flavonoids, TFC was determined with quercetin as a reference standard.

MATERIALS AND METHODS

Chemicals and equipment

Folin – Ciocâlteu reagent (Merck), gallic acid (Alkaloid, 99.15%), quercetin (100%, Cayman), methanol (Sigma Aldrich, 99.81%), ethanol (Alkaloid, 96%), acetone (Sigma Aldrich), ethyl acetate (Sigma Aldrich, 99.7%), sodium carbonate (Alkaloid, 99.9%), aluminum nitrate (Carlo Erba, 99%), sodium acetate (Alkaloid) and deionized water. TPC and TFC determination was done on a Varian Cary 60 spectrophotometer in the wavelengths region from 400 to 800 nm. The spectra were recorded when the reaction was finished, at room temperature. The quartz and glass cells with a 1.0 cm optical path length were used for the ultraviolet and visible region, respectively.

Characteristics of analyzed tomato hybrids

For the experiment, two hybrids of tomatoes were used "Belle F1" and "Perugino F1" which were grown in v. Vasilevo, Strumica with the conventional technology applied. Belle F1" is a high-yielding hybrid that matures early. It comes from the Netherlands and is a moderately branched bush with a height of up to 150 cm. The leaves are compound, medium in size, and dark green in color. 6-8 fruits are formed on each bunch. They have a very good yield, so, on average, each square meter yields up to 15 kg of tomatoes. The fruits are medium size, 120-200 g, and oval in shape. During the ripening process, the fruits change color from light green to dark red. The skin is thin and shiny while the flesh is juicy, with a large number of seed chambers. The taste is sweet, with a slight acidity.

The "Perugino F1" hybrid is an outstanding, early tomato hybrid, with strong growth and an excellent balance of vegetative and generative growth. It has excellent resistance to diseases, which is why it achieves high yields of up to 6 kg per plant. The fruits are of high quality, uniform in shape and size, usually weighing 220-250 g, intensely pink colored, with highly shiny skin, fleshy, with good firmness and taste, characteristics that are very rarely found collectively in one hybrid. "Perugino F1" is a hybrid that is recommended for cultivation in early spring, summer, and autumn in order to produce fruit of exceptional quality and taste, and is usually produced in greenhouses without additional heating.

Preparation of standard solutions

A known amount of gallic acid (99.15%) was measured for the preparation of the stock solution. This solution was prepared in a 10 mL volumetric flask in pure methanol (CH_3OH, 99.8%) as a solvent. In order to be completely dissolved, the solution of gallic acid was mixed with an ultrasonic bath for 15 minutes. The mass concentration of gallic acid in the stock solution was 0.9122 mg/mL. Standard working solutions were prepared with dilution from the stock solution. These solutions were prepared in 10 mL measuring flasks with 80% methanol. In each of the flasks a certain volume of the stock solution was placed (50 µm, 100 µm, 150 µm, 200 µm, 250 µm, 300 µm, 400 µm, 500 µm, 600 µm, 700 µm, 800 µm). All standard solutions were stored in the refrigerator (4 ^oC). They were stable during the period of analyses.

Preparation of sample solutions

TPC and TFC was performed in two tomato cultivars "Belle F1" and "Perugino F1". Preparation of the sample solution started with homogenization of tomato in a blender followed by measuring about 5 g of a sample and transferring to the Erlenmeyer. Then 25 mL of a solvent (80% methanol) was added to the sample. Three samples of each tomato cultivar were prepared paralely. All samples were mixed for 2 hours, 15 minutes on ultrasound, and the remaining time on a shaker. After mixing, the samples were filtered with Whatman No. 1 filter paper. The resulting extract was further used for the determination of TPC and TFC in tomatoes. Tomato extracts were stored in a refrigerator at a temperature of 4 °C.

Folin – Ciocâlteu method for TPC determination

The Folin – Ciocâlteu method is based on a colorimetric reaction of the Folin – Ciocâlteu reagent with certain polyphenolic compounds. To determine the content of total polyphenols, a diluted Folin – Ciocâlteu reagent was used at 1:10 (*V*/*V*), and a solution of Na_2CO_3 (7.5%). The reaction of polyphenols with the Folin – Ciocâlteu reagent is a redox reaction. Gallic acid is used as the reference standard. 1 mL of each tomato extract was transfer to a beaker and 5 mL of freshly prepared Folin – Ciocâlteu reagent was added. After 5 minutes, 5 mL of sodium carbonate (7.5%) was added. The mixture was kept for 45 minutes in the dark place at room temperature in order to complete the redox reaction. The absorbance of each solution was measured at 760 nm with UV-Vis spectrophotometer. At the same conditions with the samples, a blank sample was prepared. In the blank, distilled water was added instead of 1 mL of the sample. The mean value of the absorbances was calculated from the individual values of the three measurements. The TPC in the tomato samples was expressed as mg gallic acid equivalents per 100 g sample (mg GAE / 100 g).

Determination of flavonoids

The TFC was determined with a quercetin as the reference standard. For this purpose, in a beaker 1.5 mL of 95% ethanol was added to 1 mL of tomato extracts. In the same beaker 0.1 mL of 10% $Al(NO_3)$, 0.1 mL of 1 M CH_3COONa and 2.8 mL of distilled water were added. After 30 minutes of storage at room temperature, the absorbance was measured at a wavelength of 415 nm. The obtained results were expressed as mg quercetin equivalents per 100 g sample (mg QE / 100 g). The measurements were repeated in triplicate and the absorbance values were used for further calculation.

RESULTS AND DISCUSSION

TPC and TFC were determined in two tomato cultivars "Belle F1" and "Perugino F1" by UV-Vis spectroscopy. TPC was determined by Folin – Ciocâlteu assay with gallic acid as the reference standard, while TFC was determined by using the values of absorbance of a yellow complex formed between quercetin and aluminum ions (Al^{3+}).

TPC determination

The product of the reaction between polyphenolic compounds with Folin – Ciocâlteu reagent is blue complex with an absorption maximum in the visible region around 760 nm (Craft et al., 2012). The absorbance of the extracts was compared with a gallic acid calibration curve for estimating the concentration of TPC in the tomato samples. The mass concentration of gallic acid and the absorbance value (760 nm) for each of the standard working solutions are given in Table 1.

The gallic acid calibration curve constructed according to the data shown in Table 1 is presented in Figure 1. The concentration of polyphenols in the samples was calculated from the absorbance values at a wavelength of 760 nm using the calibration curve.

Table 1. Absorbance values and concentration of working solutions of gallic acid

Solution	***γ* [mg/mL]**	***A***
1	0.0091	0.4224
2	0.0137	0.4823
3	0.0182	0.5781
4	0.0228	0.6375
5	0.0274	0.7027
6	0.0365	0.7965
7	0.0456	0.9022
8	0.0547	1.0040
9	0.0730	1.2245

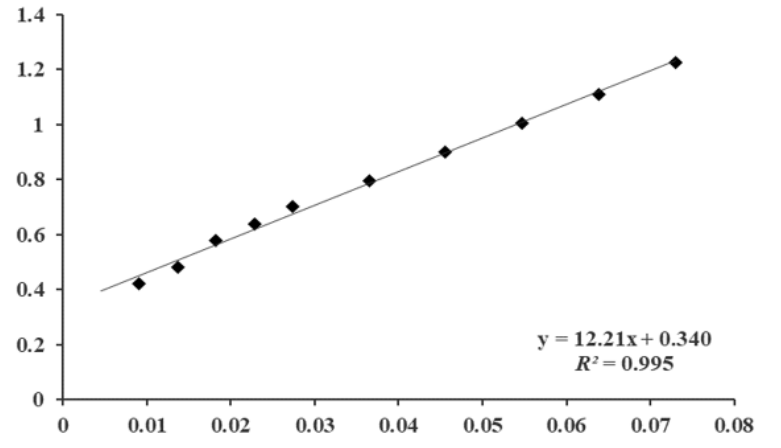

Figure 1. Calibration curve of gallic acid

The UV-Vis spectra of the blue complex formed between gallic acid and Folin – Ciocâlteu reagent are shown in Figure 2.

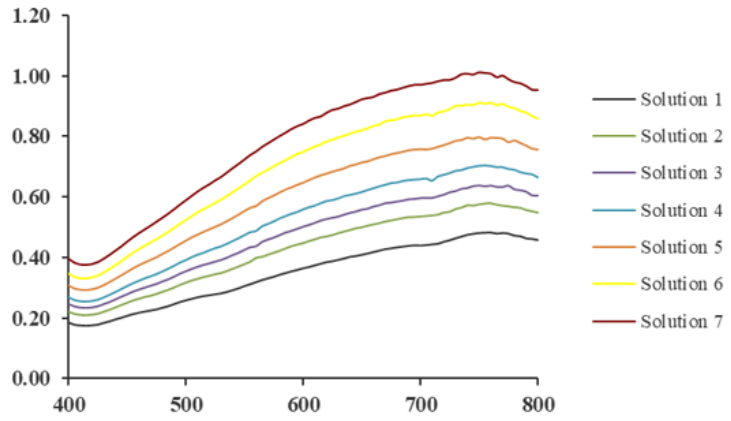

Figure 2. UV-Vis spectra of the complex formed in a reaction of gallic acid and Folin-Ciocalteu reagent

From the spectra presented in the Figure 2 it can be seen that the solutions showed a maximum absorption at a wavelength of 760 nm. In the Table 2 are given the obtained results for absorbance values, the TPC values of each measurement (GAE mg / 100 g), the mean values, standard deviation (SD) and the interval in which the obtained results are placed with confidence level of 0.05 (95%).

Table 2. TPC in the analyzed tomato varieties

Tomato cultivar	***A***	***GAE mg / 100 g**	**Average value**	****SD**
Belle F1	0.4992	6.27	6.23 ± 0.16	0.14
	0.4855	6.08		
	0.4953	6.36		
Perugino F1	0.555	9.17	9.18 ± 0.03	9.19
	0.5607	9.22		
	0.5595	9.17		

*GAE (gallic acid equivalents) - mg gallic acid per 100 g sample, **SD - standard deviation

From the obtained results it can be seen that the TPC is higher in the tomato hybrid "Perugino F1" (9.18. ± 0.03) compared to the hybrid "Belle F1" (6.23 ± 0.16).

TFC determination

The content of total flavonoids was determined spectrophotometrically, with quercetin used as a reference standard. Quercetin acts as a ligand and forms coloured complexes with metal ions. For example, with aluminum ions (Al^{3+}), quercetin forms a yellow coloured complex. The UV-Vis spectra of the complex formed between quercetin and aluminum ions are shown in the Figure 3.

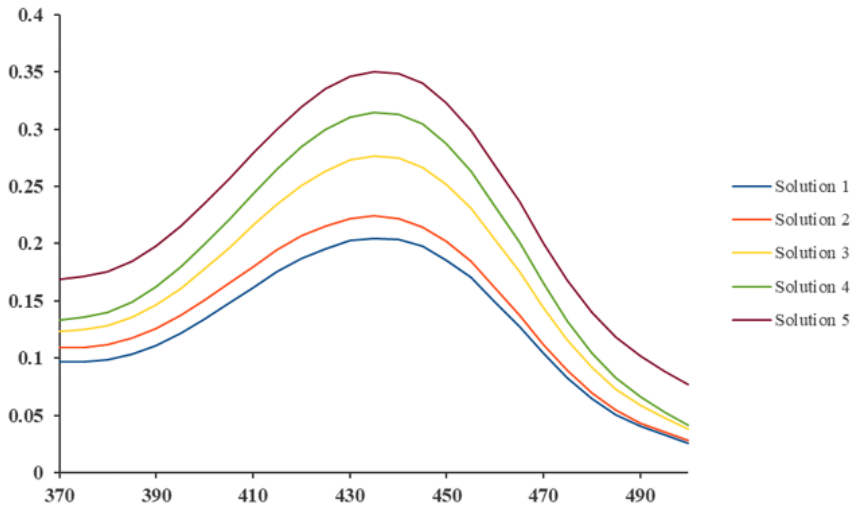

Figure 3. UV-Vis spectra of the yellow coloured complex between quercetin and Al^{3+} ions

From the spectra it can be seen that the formed complex showed a maximum absorption at around 415 nm wavelength. In the Table 3 are given the mass concentration of quercetin and the absorbance values (415 nm) for each of the standard working solutions.

Table 3. Concentration and absorbance values of standard working solutions of quercetin

Solution	***γ* [mg/mL]**	***A***
1	0.0042	0.1752
2	0.0063	0.1943
3	0.0084	0.2331
4	0.0105	0.2646
5	0.0126	0.3001

The calibration curve of quercetin, which is constructed based on the data shown in Table 3 is presented in the Figure 4.

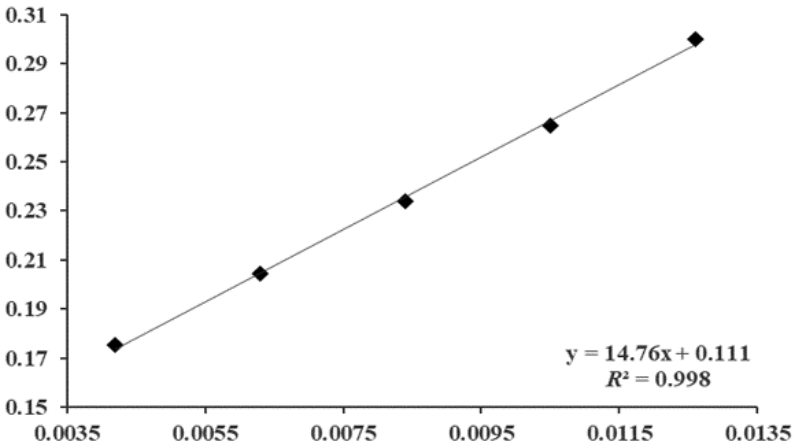

Figure 4. Calibration curve of quercetin

In the Table 4 are presented the absorbance values of the tomato samples. It can be seen that the absorbance values are very low. Therefore, it can be assumed that the content of flavonoids in tomato determined in relation to quercetin as a reference standard is very low. It is necessary to construct a calibration curve in an area with a lower concentration of quercetin or to apply another analytical method with higher sensitivity for the determination of flavonoids in tomato.

Table 4. The absorbance values in analyzed tomato cultivars

Tomato cultivar	***A*(mg / µL)**
Belle F1	0.0436
	0.0482
	0.0414
Perugino F1	0.0455
	0.0457
	0.0456

CONCLUSION

The UV-Vis spectrophotometric determination of TPC and TFC was performed in two tomato hybrids "Belle F1" and "Perugino F1". The TPC in tomatoes was determined by the Folin – Ciocâlteu method in relation to gallic acid as reference standard. From the obtained results it can be concluded that the TPC is higher in the tomato hybrid Perugino 0.16 mg / 100 g). An attempt was made for determination of TFC in analyzed tomatoes with quercetin as the reference standard. From the obtained results it can be concluded that the content of flavonoids in analyzed tomato sample is very low.

REFERENCES

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols, 2(4), 875-877.

Bezuneh, T. T., & Kebede, E. M. (2015). UV-Visible spectrophotometric quantification of total polyphenol in selected fruits. Int. J. Food Sci. Nutr, 4, 397-401.

Craft, B. D., Kerrihard, A. L., Amarowicz, R., & Pegg, R. B. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment. Comprehensive Reviews in Food Science and Food Safety, 11(2), 148-173.

Chun, O. K., Kim, D. O., Smith, N., Schroeder, D., Han, J. T., & Lee, C. Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. Journal of the Science of Food and Agriculture, 85(10), 1715-1724.

Daneshfar, A., Ghaziaskar, H. S., & Homayoun, N. (2008). Solubility of Gallic Acid in Methanol, Ethanol, Water, and Ethyl Acetate. Journal of Chemical & Engineering Data, 53(3), 776–778.

George, B., Kaur, C., Khurdiya, D. S., & Kapoor, H. C. (2004). Antioxidants in tomato (Lycopersium esculentum) as a function of genotype. Food chemistry, 84(1), 45-51.

Haminiuk, C. W., Maciel, G. M., Plata-Oviedo, M. S., & Peralta, R. M. (2012). Phenolic compounds in fruits–an overview. International Journal of Food Science & Technology, 47(10), 2023-2044.

Mimica-Dukic, N., Bozin, B., Sokovic, M., & Simin, N. (2004). Antimicrobial and antioxidant activities of Melissa officinalis L.(Lamiaceae) essential oil. Journal of agricultural and food chemistry, 52(9), 2485-2489.

Martí, R., Roselló, S., & Cebolla-Cornejo, J. (2016). Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. Cancers, 8(6), 58.

Sánchez-Rangel, J. C., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2013). The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Analytical Methods, 5(21), 5990-5999.

Slimestad, R., Fossen, T., & Verheul, M. J. (2008). The flavonoids of tomatoes. Journal of Agricultural and Food Chemistry, 56(7), 2436-2441.

Sulaiman, M., Tijani, H. I., Abubakar, B. M., Haruna, S., Hindatu, Y., Mohammed, J. N., & Idris, A. (2013). An overview of natural plant antioxidants: analysis and evaluation. Advances in Biochemistry, 1(4), 64-72.

Urquiaga, I. N. E. S., & Leighton, F. (2000). Plant polyphenol antioxidants and oxidative stress. Biological research, 33(2), 55-64.