Journal of Agricultural, Food and Environmental Sciences Vol 77 No 2 (2023) 73-79
DOI: https://doi.org/10.55302/JAFES23772073j

Original scientific paper

SPECTROPHPTPMETRIC METHOD FOR DETERMINATION OF TOTAL POLYHENOLIC CONTENT IN SWEET CORN

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ABSTRACT

Polyphenolic compounds also known as polyhydroxyphenols, are a group of secondary plant metabolites that can be found as simple molecules but also as highly polymerized compounds such as tannins. Many food types are considered as a great source of polyphenolic compounds, such as fruit, herbs, vegetables, grains and so on. Therefore, the goal of the present study was the determination of total polyphenolic content (TPC) in two sweet corn cultivars Messenger F1 and Sentinel F1 using UV – Vis spectroscopy. TPC in sweet corn are evaluated by Folin – Ciocâlteu method in relation with gallic acid as a reference standard. The obtained results reveled that the TPC in the hybrid Messenger F1 is 16.81 ± 0.28 GAE (gallic acid equivalents), while the TPC in the hybrid Sentinel F1 is 22.79 ± 0.85.

Key words: sweet corn, polyphenols, gallic acid, UV – Vis specrophotometry, Folin – Ciocâlteu method.

INTRODUCTION

Sweet corn (*Zea mays saccharata Sturt*.) is a widely used crop which plays an important role as food, fodder and tehnical crop (Lertrat et al., 2007). The grain is the most important part of sweet corn because it has a high nutritional value as a result of the presence of carbohydrates, proteins, minerals and antioxidants (Jeftic, 1977). The chemical composition of corn can vary depending on the species, the place and the method of production (cultivation and agrotechnical measures). The antioxidant activity of sweet corn increases significantly during cooking despite the loss of certain amount of vitamin C. According to some researches, heat treatment of 115 °C for 25 minutes significantly increases the total antioxidant activity of sweet corn by 44% and 54%, although a loss of 25% of vitamin C is also observed (Dewanto, V., Wu, X., & Liu, R. H. (2002). The antioxidant properties of sweet corn are mainly due to the presence of polyphenols (Zhang et al., 2017; Acosta-Estrada et al., 2014). Polyphenols are secondary metabolites widespread in nature. So far, over 8,000 phenolic structures are known and are divided into four major groups: flavonoids, phenolic acids, polyphenol amides and other polyphenols (Musilova et al., 2013). The antioxidant properties of polyphenols are due to the fact that they bind with free radicals that damage cells and increase the risk of disease (Rice-Evans et al., 1996). Number of studies show that oxidative stress arising from oxidant/antioxidant imbalance plays a crucial role in the initiation and progress of chronic diseases. Some of the health benefits of fresh vegetables are attributed to the high abundance of phenolics and their potent antioxidant activity (Zhang et al., 2017). Other important properties that polyphenols have include anti-inflammatory, antiallergic, hypotensive, and hypoglycemic effects (Nichols & Katiyar, 2010;

Syedd-León et al., 2020). In the study of Xie et al., it has been indicated that the polyphenolic compounds are one of the largest groups of secondary metabolites that can exhibit antimicrobial activity (Xie et al., 2015; Turati et al., 2015).

As important elements in the composition of sweet corn and other vegetable crops, polyphenols can be quantitatively determined using different analytical methods, of which the most commonly used are chromatographic methods, spectroscopic methods and capillary electrophoresis (CE) (Khoddami et al., 2013). Chromatographic methods such as High Performance Liquis Chromatography (HPLC) and gas chromatography are used to quantify each of the polyphenols separately, while spectroscopic methods are used for determination the total polyphenol content (TPC). A frequently used approach for the evaluation of the total polyphenolic content in many different samples is to use a UV-Vis spectroscopy (Matić et al., 2017). Tus, qualitative and quantitative determination of TPC in sweet corn can be performed using spectrophotometric analysis, which are based on measuring the absorbed or emitted energy of radiation by atoms, molecules or ions of a substance (Harvey, 2000). During the reaction of polyphenols with a suitable redox reagent (the most commonly used is the Folin – Ciocâlteu reagent), a complex with a blue color is formed, the concentration of which can be determined with the help of visible spectroscopy. The intensity of the reaction of the phenolic compounds with Folin – Ciocâlteu reagent is in a direct dependence on the presence of hydroxyl groups on aromatic rings (Daneshfar et al., 2008). During the reaction between the Folin – Ciocâlteu reagent and the polyphenols, the color intensity of the complex is directly dependent on their concentration in the sample (Agbor et al., 2014). Gallic acid (3,5,7 threehydroxybenzoic acid) is often used as a reference standard, which due to its composition (presence of one aromatic ring and three free hydroxyl groups) has a higher specific absorption. The molecular formula of gallic acid is $C_7H_6O_5$ or $C_6H_2(OH)_3COOH$ (Zhao et al., 2011). While gallic acid is soluble in various solvents, literature results show that the solubility of gallic acid is highest in methanol, but it also dissolves in ethanol, acetone, and ethyl acetate (Daneshfar et al., 2008).

The aim of the present research was spectrophotometric determination of total polyphenolic content in the hybrid sweet corn Messenger F1 and Sentinel F1 according to the Folin – Ciocâlteu method in relation to gallic acid used as a reference standard.

MATERIALS AND METHODS

Reagents and equipment

Reagents: Folin – Ciocâlteu reagent (Merck), gallic acid (Alkaloid, 99.15%), methanol (Sigma Aldrich, 99.81%), sodium carbonate (Alkaloid, 99.9%) and deionized water.

Laboratory equipment: Micropipettes with different volume cups (50 mL and 100 mL), funnels, droppers, measuring flasks (10 mL and 20 mL), filter paper (plain and Whatman No.1), clock glass, graduated cylinders, erlenmeyers (50 mL), beakers, quartz and glass cuvette with a 1.0 cm optical path length.

Apparatus: UV – Vis spectrophotometer model Varian Cary 60, ultrasonic bath "Elma", shaker KIKA-WERKE KS 501 digital, kitchen blender, analytical scale (with less accuracy) Mettler, analytical scale Mettler P1200.

Materials

Two hybrids of sweet corn Messenger F1 and Sentinel F1 grown in the region of Kumanovo and Skopje, are analyzed. Messenger F1 is characterized with late maturing process. It has a remarkable resistance in the field. The plant has an average height of 210 cm, while the average length of the cob is 21 – 23 cm, with thickness of 5.1 cm and 18 – 20 rows of grains. The seed has a dark yellow color. This hybrid reaches maturity after 85 to 87

days. Messenger F1 is a durable plant resistant to strong winds in which it will not succumb due to the well – developed root system that provides support to the whole plant. In cold conditions, it germinates well and has excellent uniformity. Sentinel F1 is super sweet hybrid corn which is one of the leading varieties in Australia due to its excellent raste and appearance as well as good resistance against disease. The best growing climate for this hybrid is warm to hot. This plant is an early producing hybrid sweet corn which has large cobs, extremely sweet kernels with excellent taste and uniform appearance with 22 cm length. The germination is within 7 to 10 days and it reaches maturity after 12 to 15 weeks.

Preparation of standard solution

The standard stock solution of gallic acid (99.15%) is prepared by dissolution of around 4 mg in a 10 mL volumetric flask in pure methanol (CH_3OH, 99.8%) as a solvent. For completely dissolvation of the gallic acid, the solution is ultrasonified in an ultrasonic bath for 15 minutes. The concentration of gallic acid in the stock solution is 0.3966 mg/mL. Standard working solutions are prepared by diluting the stock solution in 10 mL measuring flasks with 80% methanol. For that purpose, different volume of the stock standard solution is put in each of the flasks (100 µL, 150 µL, 200 µL, 250 µL, 300 µL, 400 µL, 500 µL). All standard solutions are stored in the refrigerator at 4°C. They are stable during the period of analyses.

Preparation of sample solutions

Preparation of the sample solution started with homogenizing of a sweet corn hybrids separately in a blender, followed by measuring 5 g mass of the sample and transfering into an erlenmeyer to which 25 mL of solvent was added. For extraction of polyphenols 80% methanol is used as a solvent. Three samples of each variety were prepared paralelly. Furthermore, the samples are mixed for 2 hours, 15 minutes on a ultrasound and the remaining time on a shaker. After mixing the samples are filtered through plain filter paper and Whatman No.1 filter paper, as well. The obtained extracts before analysis are stored in a refrigerator at a temperature of 4 $^{\circ}$C.

Determination of TPC by Folin – Ciocâlteu method

The Folin – Ciocâlteu method is used for determination of TPC in both sweet corn hybrids using gallic acid as reference standard (Agbor et al., 2014). This method is based on a colorimetric reaction of the Folin - Ciocâlteu reagent with certain polyphenolic compounds. For TPC determination a diluted Folin - Ciocâlteu reagent is 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. 1 mL of the prepared sweet corn extract is transfer to a beaker in which 5 mL of freshly prepared Folin – Ciocâlteu reagent are added. After 5 minutes, 5 mL of sodium carbonate (Na_2CO_3) sre added. The mixture was kept 45 minutes in dark place, at a room temperature to complete the redox reaction. The absorbance of each of the solutions is then measured at 760 nm using UV – Vis spectrophotometer. At the same conditions with the samples, a blank sample was prepared in which destilled water was added instead of 1 mL of the sample. The final result is the average value of three measurements on each sample. The total polyphenolic content in the two sweet corn hybrids is expressed as mg gallic acid equivalents per 100 g sample (mg GAE / 100 g).

RESULTS AND DISCUSSION

The total polyphenolic content (TPC) in the two sweet corn hybrids Messenger F1 and Sentinel F1 is determined spectrophotometrically using the Folin – Ciocâlteu reagent. In order to determine TPC gallic acid was used as a reference standard. As a result of reaction

between Folin – Ciocâlteu reagent and the polyphenols from the samples the complex product with blue color is formed. UV – Vis spectra of the complex formed by gallic acid with polyphenols in standard working solutions are shown in Figure 1. From the presented UV-Vis spectra it can be seen that the obtained complex has a maximum absorption in the visible part of the spectrum, at a wavelength of 760 nm. Due to this the absorbance values determined at 760 are further used for TPC determination in the samples.

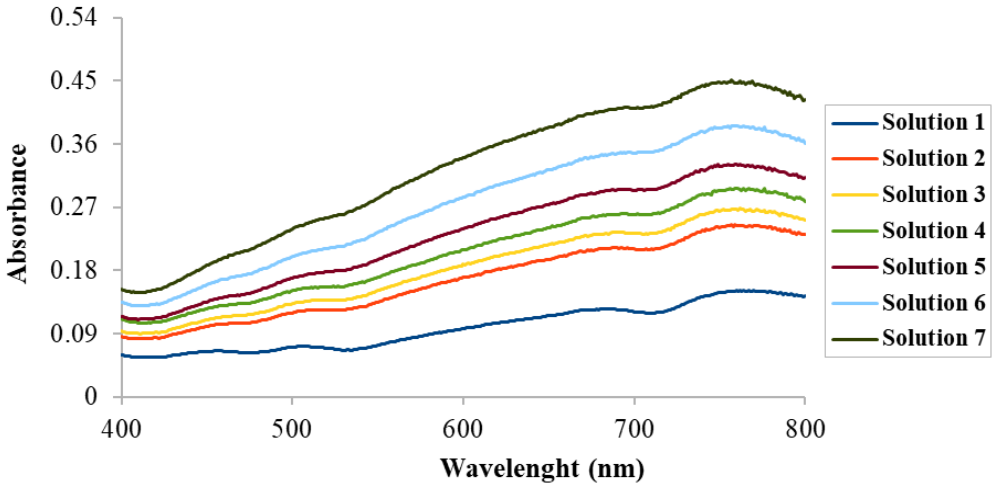

Figure 1. UV – Vis spectra of the complex of standard solution of gallic acid and Folin – Ciocâlteu reagent

The dependence between absorbance values determined at 760 nm on gallic acid concentration is linear (calibration curve). Table 1 gives the mass concentration of gallic acid and the absorbance value (measured at a wavelength of 760 nm) for each of the standard working solutions, while figure 2 shows the gallic acid calibration curve constructed according to the data shown in Table 1.

From the obtained calibration curve, it can be observed that there is a linear dependence between absorbance and concentration, which corresponds with various studies (Popova et al., 2004; Medina-Remón et al., 2009; Shaghaghi et al., 2008.). The concentration of polyphenols in the sample solutions is calculated using the absorbance values determined at a wavelength of 760 nm. For this purpose, the regression equitation y = 14.514x + 0.1674 is used.

Table 1. Concentration (mg/mL) and absorbance of standard working solutions of gallic acid

Solution	γ [mg/mL]	A
1	0.005	0.2432
2	0.007	0.2669
3	0.009	0.2964
4	0.011	0.3306
5	0.015	0.3801
6	0.019	0.4471

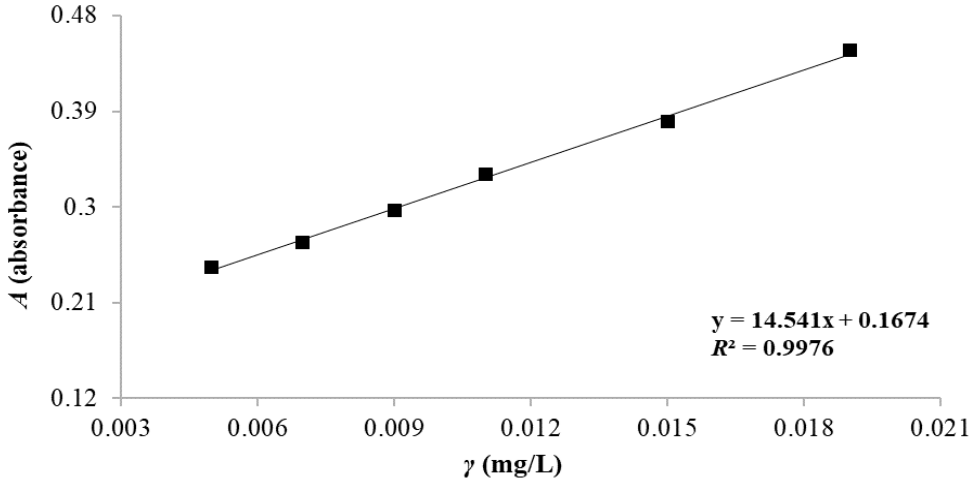

Figure 2. Calibration curve of gallic acid in concertation region from 0.005 to 0.019 mol/L

In order to determine if there is a statistically significant difference between the obtained results for the TPC in the hybrids of sweet corn, an analysis of variance (ANOVA) was performed with a confidence level of 0.05 (95%). The results from the analysis of variances showed that there is a statistically significant difference between them, i.e. the r parameter value (0.00019) is lower compared to the confidence level of 0.05.

In the Table 2 are shown the absorbance values (obtained from three measurements), the values of the TPC (GAE mg/100 g) of each measurement, the average values, the values of standard deviation, as well as the interval in which the results are placed with a confidence level of 0.05 (95%).

Table 2. The total polyphenolic content in the analyzed samples of sweet corn hybrids

Hybrid sweet corn	***A***	***GAE mg/100 g**	**Average value**	*****SD**
Messenger F_1	0.6416	16.56	16.81 ± 0.28**	0.25
	0.6490	16.81		
	0.6563	17.06		
Sentinel F_1	0.8479	23.65	22.79 ± 0.85	0.75
	0.8142	22.49		
	0.8069	22.24		

*GAE (gallic acid equivalents) – mg gallic acid per 100 g sample, ** interval in which the results obtained from the performed measurements with a confidence level of 0.05 (95%), ***SD – standard deviation

The obtained results (Table 2) showed that the TPC in the hybrid Messenger F1 is 16.81 ± 0.28, while in the hybrid Sentinel F1 is 22.79 ± 0.85. Therefore, the hybrid Sentinel F1 has a higher polyphenolic content compared to the hybrid Messenger F1.

The UV-Vis spectra of the complexes formed between Folin – Ciocâlteu reagent and polyphenols in the analyzed samples are presented in the Figure 3.

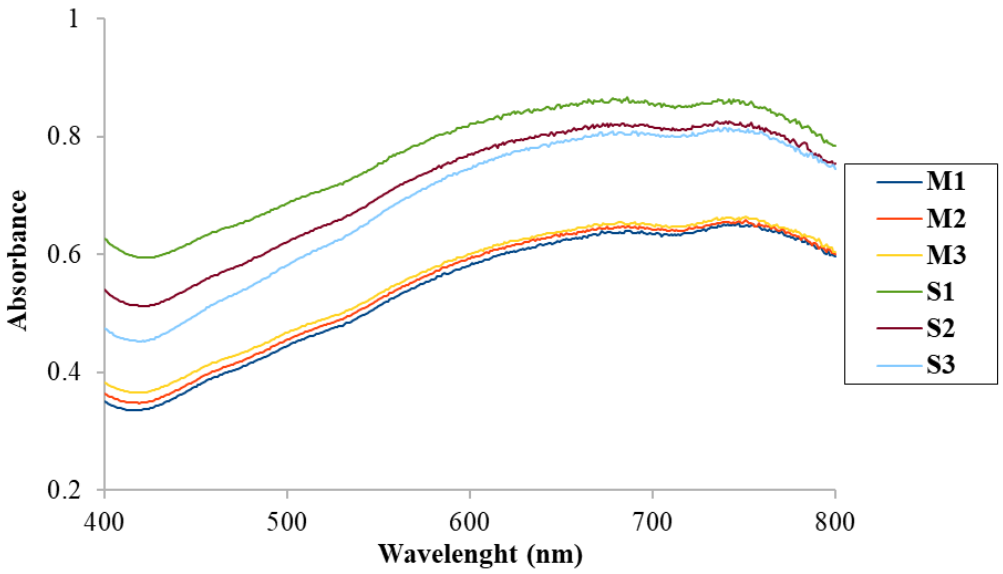

Figure 3. UV-Vis spectra of the analyzed sweet corn samples: Messenger F1 (M1, M2 and M3) and Sentinel F1 (S1, S2 and S3)

From the Figure 3 it is clear the difference in absorbances and TPC as well between two analyzed sweet corns.

CONCLUSION

Sweet corn, as a cereal, is an important source of polyphenolic compounds. The most important characteristic of polyphenolic compounds is their antioxidant activity and positive health benefits. Spectroscopic determination of total polyphenolic content in two hybrids sweet corn Messenger F1 and Sentinel F1 is performed using Folin – Ciocâlteu method in relation to gallic acid as a reference standard. From the obtained results, it can be concluded that the better sweet corn hybrid in terms of polyphenolic content is Sentinel F1.

REFERENCES

Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. *Food chemistry*, *152*, 46-55.

Agbor, G. A., Vinson, J. A., & Donnelly, P. E. (2014). Folin – Ciocâlteu reagent for polyphenolic assay. *International Journal of Food Science, Nutrition and Dietetics (IJFS)*, *3*(8), 147-156.

Ainsworth, E. A., and 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.

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.

Dewanto, V., Wu, X., & Liu, R. H. (2002). Processed sweet corn has higher antioxidant activity. *Journal of Agricultural and food Chemistry*, *50*(17), 4959-4964.

Jeftic. S. L., (1977). Kukuruz. „Nolit". Beograd.

Zhao, J., Khan, I. A., & Fronczek, F. R. (2011). Gallic acid. *Acta Crystallographica Section E: Structure Reports Online*, *67*(2), o316-o317.

Harvey, D. (2000). *Modern analytical chemistry*. McGraw Hill.

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328-2375.

Lertrat, K., & Pulam, T. (2007). Breeding for increased sweetness in sweet corn. *International Journal of Plant Breeding*, *1*(1), 27-30.

Matić, P., Sabljić, M., & Jakobek, L. (2017). Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. *Journal of AOAC International*, *100*(6), 1795-1803.

Medina-Remón, A., Barrionuevo-González, A., Zamora-Ros, R., Andres-Lacueva, C., Estruch, R., Martínez-González, M. Á., ... & Lamuela-Raventos, R. M. (2009). Rapid Folin–Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Analytica Chimica Acta*, *634*(1), 54-60.

Musilova, J., Lachman, J., Bystricka, J., Polakova, Z., Kovacik, P., Hrabovska, D.(2013). The changes of the polyphenol content and antioxidant activity in potato tubers (Solanum tuberosum l.): Due to nitrogen fertilization. Vol.7.Potravinarstvo. No. 1, pp. 167-170.

Nichols, J. A., & Katiyar, S. K. (2010). Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. *Archives of dermatological research*, *302*, 71-83.

Popova, M., Bankova, V., Butovska, D., Petkov, V., Nikolova-Damyanova, B., Sabatini, A. G., & Bogdanov, S. (2004). Validated methods for the quantification of biologically active constituents of poplar-type propolis. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *15*(4), 235-240.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.

Shaghaghi, M., Manzoori, J. L., & Jouyban, A. (2008). Determination of total phenols in tea infusions, tomato and apple juice by terbium sensitized fluorescence method as an alternative approach to the Folin–Ciocalteu spectrophotometric method. *Food chemistry*, *108*(2), 695-701.

Syedd-León, R., Orozco, R., Álvarez, V., Carvajal, Y., & Rodríguez, G. (2020). Chemical and antioxidant charaterization of native corn germplasm from two regions of Costa Rica: A conservation approach. *International journal of food science*, *2020*.

Turati, F., Rossi, M., Pelucchi, C., Levi, F., & La Vecchia, C. (2015). Fruit and vegetables and cancer risk: a review of southern European studies. *British Journal of Nutrition*, *113*(S2), S102-S110.

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current medicinal chemistry*, *22*(1), 132-149.

Zhang, R., Huang, L., Deng, Y., Chi, J., Zhang, Y., Wei, Z., & Zhang, M. (2017). Phenolic content and antioxidant activity of eight representative sweet corn varieties grown in South China. *International journal of food properties*, *20*(12), 3043-3055.