Journal of Agricultural, Food and Environmental Sciences Vol 76 No 2 (2022) 12-19
DOI: https://doi.org/10.55302/JAFES22762012s

Original scientific paper

FOLIN–CIOCÂLTEU METHOD FOR DETERMINATION OF TOTAL POLYPHENOLS IN ONION

Viktorija Sokolovska*, Mirjana S. Jankulovska

Faculty of Agricultural Sciences and Food – Skopje, Ss. Cyril and Methodius University in Skopje, North Macedonia
*e-mail: vsokolovska@fznh.ukim.edu.mk

ABSTRACT

Onion is one of the most important crop in the world and the richest source of polyphenols. Polyphenols are organic compounds that are naturally present in onions and influence to its quality. The content of polyphenols depends on different factors. In this research determination of total polyphenols and flavonoids in two Macedonian local populations of onions was done by UV-Vis spectroscopy. The extraction of polyphenols from onions was performed with 60% methanol, while time for extraction was 120 minutes. Required time for completion of the reaction between polyphenols and Folin – Ciocâlteu reagent was examined. The total polyphenolic content was determined by the Folin – Ciocâlteu method with gallic acid as a reference standard. The obtained results were presented as mg GAE/100 g onion. Furthermore, the content of flavonoids in analyzed onions was determined with quercetin as a reference standard. The results were presented as mg QE/100 g onion. Polyphenols and flavonoids were determined in the onions' extracts immediately after extraction and in the extracts stored 18 hours in a refrigerator at 4 °C. The results showed that there is no significant difference in content of polyphenols in analyzed onions, while the flavonoid content was higher in a population Melnik.

Key words: Flavonoids, Folin–Ciocâlteu method, onion, polyphenols, UV-Vis spectroscopy.

INTRODUCTION

Polyphenols are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.). There can be easily oxidized, and oxidation reaction results in the formation of more or less polymerized substances, which lead to changes in the quality of food, particularly in color and organoleptic characteristics (Dai et al., 2010). Polyphenols are secondary metabolites, which are synthesized by plants and share a common structural feature (Spencer et al., 2008). They are classified into different groups as a function of the number of phenol rings that contain and on the basis of structural elements that bind these rings to one another. Polyphenols can be divided into four groups: flavonoids, phenolic acids, polyphenolic amides and other polyphenols (Williams et al., 2007). Previous studies showed that the main phenolics found in onion are quercetin, gallic acid, ferulic acid, and their glycosides (Singh et al. 2009; Pérez-Gregorio et al. 2010).

Allium cepa (Liliaceae) known as onion is a common vegetable widely cultivated and consumed all over the world presenting the second most produced vegetable after tomatoes (Griffiths et al., 2002). It contains different bioactive components such as antioxidants, which

possess various health benefits (Kim et al., 2004; Slimestad, 2007) and have aroused great interests for food industries (Benkeblia et al., 2005). Many reports have indicated that onions have a wide range of beneficial properties for human health such as anti-cholesterolaemic, anti-mutagenic and antioxidant capacity (Singh et al., 2009, Lu et al., 2011, Pérez Gregorio et al., 2011, Nile et al., 2013). The health benefits of onions beside other components are result of a presence of polyphenols. Consumption of foods rich in polyphenols is of great importance, because it contributes to the prevention of various diseases. Hence it is essential to study phenolic compounds as the major bioactive constituents in onions with important health-beneficial effects.

Researchers and food processors are increasingly interested in identifying and determining polyphenols in fresh and processed foods. The determination of phenolic compounds in natural products is a highly challenging task. The most commonly used method for determination of the total polyphenols content is UV-Vis spectroscopy (Lachman et al., 2003) using the Folin – Ciocâlteu reagent. This method is based on oxidation - reduction reactions in which polyphenols are oxidized and show maximum absorbance in the wavelength region between 725 and 765 nm (San et al., 2007; Yang et al., 2004). The aim of current research was determination of total polyphenolic content and flavonoids inside the onion, estimation the time required for completion of reaction between Folin – Ciocâlteu reagent and polyphenols from onions and determination of total polyphenolic content and flavonoids in onion extracts immediately after extraction and after 18 hours storage in a refrigerator at 4 ^{o}C.

MATERIAL AND METHODS

Plant material

Two Macedonian local populations of onion: Dracevski onion, population of arpadzici (pungent onions) from Dracevo and Melnik, population of arpadzici (pungent onions) from Skopska Crna Gora.

Reagents and instrumentation

Folin – Ciocâlteu reagent (Merck), gallic acid (Alkaloid, 99.15%), methanol (Sigma Aldrich, 99.81%), quercetin (100%, Cayman), Destiled water, sodium carbonate (Alkaloid, 99.9%), aluminum nitrate (Carlo Erba, 99%), sodium acetate (Alkaloid), UV-Vis spectrophotometer Varian Cary 50, ultrasonic bath Elma, shaker (KIKA-WERKE KS 501) and analytical balance Mettler P1200.

Preparation of onion extracts

After harvesting onions were stored at room temperature at dark place. In order to prepare the onion samples, fresh onions were skinned, chopped, blended and homogenized. Five grams of the sample were placed in a 50 mL conical flask and 25 mL of 60% methanol were added. The conical flask with the mixture was kept under constant stirring using a ultrasonic bath and shaker. After extraction the samples were filtered through qualitative filter paper. The resulting extract were further used for determination of polyphenols and flavonoids. Every determination was made in triplicate.

Preparation of standard stock solution and standard working solution

Standard stock solutions were prepared in a volumetric flask од 10 cm^3. For that purpose, a known amount of gallic acid and quercetin was dissolved in methanol. Working standard solutions

were prepared with different concentration, taking the volume of 100, 250, 400, 550, 700, 850 and 1000 μL from standard stock solutions in a volumetric flask од 10 cm^3. All standard solutions were stored in a refrigerator at 4^oC. They were stable during the period of analyses.

Determination of total polyphenols

The content of total polyphenols was determined by the Folin – Ciocâlteu method using gallic acid (3,5,7-trihydroxybenzoic acid) as a reference standard (Yang et al., 2004; Kaur et al., 2010; Agbor et al., 2014). The polyphenols from onions were extracted with 60% methanol in 120 minutes. Further 1 mL of analyzed onion extracts was mixed with 5 mL of 1:10 diluted Folin – Ciocâlteu reagent. The solutions were incubated at room temperature for 5 min, then 5 mL of 7.5% Na_2CO_3 solution was added. The product of a reaction between phenolic compounds and Folin – Ciocâlteu reagent is a complex with blue color (Jozinovic, 2015). In order to examine whether the time required to complete the reaction affects the polyphenolic content, the prepared solutions were kept in a dark place for 45 and 120 minutes respectively. The absorbance of a reaction mixture was measured at 760 nm against the blank using the UV-Vis spectrophotometer. The calibration curve was prepared using gallic acid and the total phenolic content was expressed as mg GAE (gallic acid equivalents)/100 g onion.

Determination of flavonoids

The total flavonoids were determined using quercetin as a reference standard (3,3',4',5,7-pentahydroxy flavonoids). For that purpose, into 1 mL of onions' extracts following reagents were added: 1.5 mL of ethanol (96%), 0.1 mL of 10 % aluminium nitrate, 0.1 mL of 1 mol/L potassium acetate and 2.8 mL of distilled water. The reaction mixture was kept for 30 min at room temperature. During the reaction quercetin forms complex with Al^{3+} ions which have absorption maximum around 415 nm. The results were given as mg QE (quercetin equivalents)/100 g onion. The blank samples were prepared with all used reagents instead of onion extracts. All analyses were done in triplicate and final results were presented as average values.

RESULTS AND DISCUSSION

Spectroscopic determination of total polyphenolic contents by Folin – Ciocâlteu method

Polyphenols have become an intense focus of research interest because of their diverse and beneficial health effects. The scavenging ability of phenols is mainly due to the phenolic structure of hydroxyl substituent on the aromatic ring (Bahorun et al., 2004). Onion has been reported as one of the major sources of dietary polyphenols in many countries. In this research total polyphenolic content was determined in two onion populations according to the Folin – Ciocâlteu method using gallic acid as a reference standard. The structural formula of gallic acid is given in the Figure 1.

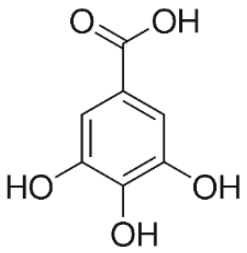

Figure 1. Structural formula of gallic acid

The reaction between polyphenols from onion and Folin – Ciocâlteu reagent which is described in materials and methods took place 45 and 120 minutes, respectively. Folin – Ciocâlteu reagent reacts with polyphenolic compounds and reducing substances with chromophores that can be detected spectrophotometrically are formed (Lorrain et al., 2013). The obtained results revealed that the absorbance values when the reaction mixture between polyphenols and Folin – Ciocâlteu reagent was kept 120 minutes at a dark place were higher compared to the absorbances measured after 45 minutes. Hence, further determination was performed at those experimental conditions. The dependence between absorbance values and concentration of standard working solution was linear with coefficient of determination 0.9984 (Figure 2).

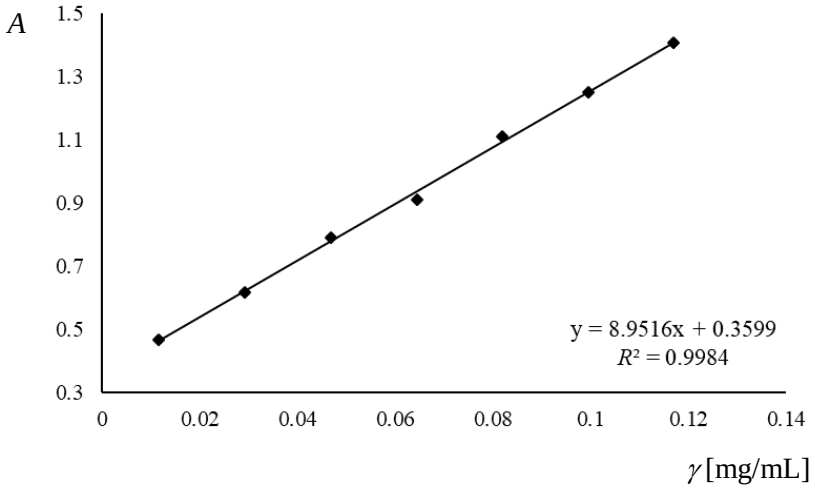

Figure 2. Calibration curve of gallic acid - absorbance values measured after 120 minutes

Using the calibration equation y = 8.9516x + 0.3599 total polyphenols were determined in in onion extracts immediately after extraction and in those stored 18 hours in a refrigerator at a temperature of 4 ºC. Determination was performed in analyzed populations of „arpadzi“ from Skopska Crna Gora and Dracevo. The obtained results are presented as mg GAE/100 g sample (Table 1).

Table 1. Total polyphenols determined in fresh onion extracts immediately after extraction and in the extracts stored in refrigerator 18 hours

onion		**A**	***GAE mg/100 g**	**average**	****SD**
Dracevski	immediately after extraction	1.4026	287.48	278.99 ± 9.64	8.52
		1.3412	270.45		
		1.3722	279.05		
Melnik		1.6989	281.48	277.24 ± 6.33	5.60
		1.6488	270.90		
		1.6888	279.35		
Dracevski	after storage of 18 hours	1.3858	282.83	279.51 ± 4.04	3.57
		1.3602	275.73		

	1.3755	279.96		
Melnik	1.6841	278.34	276.79 ± 1.52	1.35
	1.6736	276.14		
	1.6725	275.90		

*GAE (gallic acid equivalents), **SD - standard deviation

From the obtained results shown in the table 3 it can be seen that there is no significant difference in the content of total polyphenols determined immediately after extraction and after storage in the refrigerator until the next day, in 18 hours. Therefore, the extracts for further analysis can be stored in the refrigerator and analyzed afterwards. The analyzed onions have similar amount of polyphenols.

Determination of flavonoids by colorimetric method with $AlCl_3$

Total flavonoid content was determined by aluminum chloride colorimetric method with some modifications (Do et al., 2014; Duan et al., 2014). Flavonoids are the largest group of phenolic compounds in onions along with quercetin which is used as reference standard for determination of total flavonoid content in onion. Structural formula of quercetin is shown in the Figure 3.

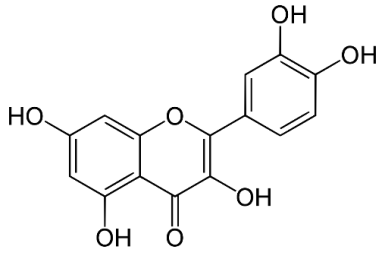

Figure 3. Structural formula of quercetin

The complex formed between flavonoids and Al^{3+} ions shows absorption maximum at around 415 nm. Hence, the absorbance of the standard working solutions was measured at that wavelength. The content of flavonoids in onion extracts were calculated in accordance with the calibration curve of quercetin (Figure 4). The obtained results are presented as mg QE/100 g sample as it is shown in the Table 2.

Table 2. Content of flavonoids determined in fresh onions immediately after extraction and after storage in the refrigerator 18 hours

onion	**Onion extracts**	**A**	***QE mg/100 g**	**average**	****SD**
Dracevski	immediately after extraction	0.4251	9.37	9.21 ± 0.21	0.19
		0.4155	9.00		
		0.4222	9.25		
Melnik		0.4560	10.53	10.86 ± 0.34	0.30
		0.4715	11.12		
		0.4666	10.93		
Dracevski	after storage of 18 hours	0.4312	9.60	9.55 ± 0.17	0.15
		0.4255	9.38		
		0.4333	9.67		

Melnik	0.4753	11.26	11.17 ± 0.27	0.24
	0.4657	10.90		
	0.4777	11.35		

*QE quercetin equivalents; **SD - standard deviation;

The obtained results showed that there is no significant difference in flavonoid content determined immediately after extraction and in extracts stored in a refrigerator at 4 °C for 18 hours. Hence, the extracts can be stored in a refrigerator at 4 °C until further analysis. Furthermore, it can be seen that the population Melnik has higher amount of flavonoids compared to Dracevski onion.

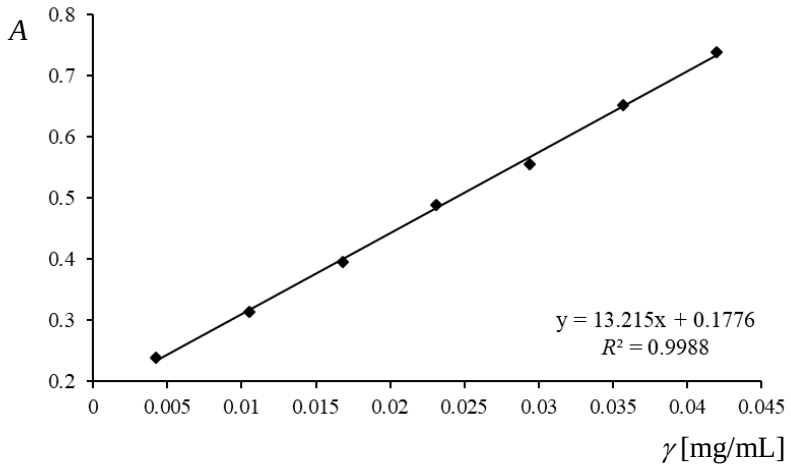

Figure 4. Calibration curve of quercetin

CONCLUSION

Onion is a rich source of polyphenols and flavonoids which influence on its quality. Determination of polyphenols and flavonoids in this research was performed by UV-Vis spectroscopy. Determination of total phenolic content was made using gallic acid as reference standard, while flavonoids were determined with quercetin as a reference standard. The reaction between polyphenols and Folin – Ciocâlteu reagent was completed after 120 minutes at a dark place. Hence this methodology was used for determination of total polyphenols. Furthermore, the obtained results showed no significant difference in total phenolic content and flavonoids determined immediately after extraction and in extracts stored 18 hours in a refrigerator at 4 °C. This suggest that onion extracts can be stored and analyzed afterwards. Furthermore, there is no difference in polyphenolic content in the analyzed onions, while population Melnik contains higher amount of flavonoids.

REFERENCES

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

Bahorun, T., Luximon-Ramma, A., Crozier, A., & Aruoma, O. I. (2004). Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. *Journal of the Science of Food and Agriculture, 84*(12), 1553-1561.

Benkeblia, N. (2005). Free-radical scavenging capacity and antioxidant properties of some selected onions (Allium cepa L.) and garlic (Allium sativum L.) extracts. *Brazilian archives of biology and technology*, *48*, 753-759.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of food and drug analysis*, *22*(3), 296-302.

Duan, Y., Kim, M. A., Seong, J. H., Chung, H. S., & Kim, H. S. (2014). Antioxidative activities of various solvent extracts from haw (Crataegus pinnatifida Bunge). *Korean Journal of Food Preservation*, *21*(2), 246-253.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onions—a global benefit to health. *Phytotherapy research*, *16*(7), 603-615.

Jozinović, A. (2015). *Svojstva kukuruznih snack proizvoda obogaćenih s nusproizvodima prehrambene industrije*, Doctoral dissertation, Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology, Department of Food Technologies, Sub-department of Carbohydrates Technology.

Kaur, C., Singh, M., Walia, S., Joshi, S., & Munshi, A. D. (2010). Variations in phenolics and antioxidants in Indian onions (Allium cepa L.): Genotype selection for breeding. *Nutrition & Food Science*, *40*(1), 6-19.

Kim, J. W., Kim, Y. S., & Kyung, K. H. (2004). Inhibitory activity of essential oils of garlic and onion against bacteria and yeasts. *Journal of food protection*, *67*(3), 499-504.

Lorrain, B., Ky, I., Pechamat, L., & Teissedre, P. L. (2013). Evolution of analysis of polyhenols from grapes, wines, and extracts. Molecules, 18(1), 1076-1100.

Lachman, J., Pronek, D., Hejtmánková, A., Dudjak, J., Pivec, V., & Faitová, K. (2003). Total polyphenol and main flavonoid antioxidants in different onion (Allium cepa L.) varieties. *Horticultural science*, *30*(4), 142-147.

Lu, M., Yuan, B., Zeng, M., & Chen, J. (2011). Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. *Food Research International*, *44*(2), 530-536.

Nile, S. H., & Park, S. W. (2013). Total phenolics, antioxidant and xanthine oxidase inhibitory activity of three colored onions (Allium cepa L.). Frontiers in Life Science, 7(3-4), 224-228.

Pérez-Gregorio, R. M., García-Falcón, M. S., Simal-Gándara, J., Rodrigues, A. S., & Almeida, D. P. (2010). Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. *Journal of Food Composition and Analysis*, *23*(6), 592-598.

Pérez-Gregorio, M. R., García-Falcón, M. S., & Simal-Gándara, J. (2011). Flavonoids changes in fresh-cut onions during storage in different packaging systems. *Food Chemistry*, *124*(2), 652-658.

Sellappan, S., & Akoh, C. C. (2002). Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. *Journal of agricultural and food chemistry*, *50*(19), 5338-5342.

Slimestad, R., Fossen, T., & Vågen, I. M. (2007). Onions: a source of unique dietary flavonoids. *Journal of agricultural and food chemistry*, *55*(25), 10067-10080.

Singh, B. N., Singh, B. R., Singh, R. L., Prakash, D., Singh, D. P., Sarma, B. K., ... & Singh, H. B. (2009). Polyphenolics from various extracts/fractions of red onion (Allium cepa) peel with potent antioxidant and antimutagenic activities. *Food and Chemical Toxicology*, *47*(6), 1161-1167.

Spencer, J. P., Abd El Mohsen, M. M., Minihane, A. M., & Mathers, J. C. (2008). Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *British Journal of Nutrition*, *99*(1), 12-22.

Sun, T., Powers, J. R., & Tang, J. (2007). Evaluation of the antioxidant activity of asparagus, broccoli and their juices. *Food chemistry*, *105*(1), 101-106.

Williams, G. J., Zhang, C., & Thorson, J. S. (2007). Expanding the promiscuity of a natural-product glycosyltransferase by directed evolution. *Nature chemical biology*, *3*(10), 657-662.

Yang, J., Meyers, K. J., van der Heide, J., & Liu, R. H. (2004). Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *Journal of agricultural and food chemistry*, *52*(22), 6787-6793.