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EXTRACTION AND SPECTROPHOTOMETRIC QUANTIFICATION OF TOTAL POLYPHENOLIC CONTENT IN ONION

SUMMARY

Consumption of food rich in polyphenols such as fruits and vegetables is of great importance, because it contributes to the prevention of various diseases. Polyphenols are a group of bioactive compounds which can play significant role in preventing various health related problems. One of the richest sources of polyphenols in the human diet is the onion (*Allium cepa* L.). The main objective of the present investigation is quantification of total polyphenolic content (TPC) in the Macedonian local population of onion. A various organic solvents were tested (methanol, ethanol, ethyl acetate and acetone) and their different concentrations (20, 40, 60 and 80%) in order to establish optimum solvent concentration for extraction of polyphenols from onion. Time for extraction was another important parameter which was examined as well. TPC was determined by UV-Vis spectroscopy in accordance with the Folin–Ciocâlteu assay, using gallic acid as a reference standard. The results were expressed as mg gallic acid equivalents GAE/100 g fresh onion. The highest polyphenolic level was determined in 60% methanol (38.81±0.39 mg GAE/100 g), while lower total polyphenolic content was found in the ethyl acetate extracts (27.10±0.35 mg GAE/100 g). Regarding the time for extraction, the highest content of total polyphenols in onion was obtained during extraction of 120 minutes (37.95±0.63 mg GAE/100 g).

Keywords: UV-Vis spectroscopy, onion, extraction, gallic acid, total polyphenolic content (TPC).

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INTRODUCTION

In the recent years there has been great interest in the functional properties of fruits and vegetables, as well as their contribution to human health (Di Lorenzo *et al.* 2021; Chakraborty *et al*. 2022). Fruits and vegetables are source of many useful nutrients, among which polyphenols play an important role (Haminuk *et al.* 2012; Eseberri *et al.* 2022). Polyphenols are organic compounds naturally present in plant foods and characterized by the presence of one or more hydroxyl functional group attached to a single or to multiple aromatic rings (Kondratyuk *et al.* 2004). The relationship between polyphenol intake and human health was investigated with special merit to cardiovascular diseases, hypertension, diabetes, metabolic syndrome, obesity and cancer (Durazzo *et al.* 2019; Cory *et al.* 2018). Among all vegetables, onion (*Allium cepa* L.) is the second major important horticultural crop in the world. It is rich of organic compounds such as polyphenols, flavonoids, anthocyanins and vitamins which have potential beneficial properties for human health (Griffiths *et al.* 2002). The flavonoid compounds, especially quercetin and its derivatives are more common in onion (Slimestad *et al*. 2007; Rodriguez *et al*. 2008). Average total quercetin content in onion (347 mg/kg) is 5 – 10 times higher in comparison with other vegetables and depends on a variety of onion (Lee *et al.* 2014). Taking into consideration that the polyphenols influence on food quality it is essential to study them as major bioactive constituents in onions. Researchers and food processors are increasingly interested in identifying and determining polyphenols in fresh and processed foods (Khoddami *et al.* 2013; Sulaiman *at al.* 2013). The most commonly used methods for determination of total polyphenolic content in food are spectroscopic methods (Bezuneh *et al.* 2015). In the UV/Vis spectrophotometric method colorimetric reactions are widely used, which is easy to perform, rapid and applicable in routine laboratory use, and low-cost (Bueno *et al.* 2012). Folin–Ciocâlteu assay is frequently used for total polyphenolic content evaluation. Namely, polyphenols in plant extracts react with specific redox reagents (Folin–Ciocâlteu reagent) and form a blue complex that can be quantified by visible spectrophotometry (Andressa *et al.* 2013; (Stratil *et al*. 2007; Agbor *et al.*, 2014). Prior quantification of polyphenols first step is selecting a suitable solvent for the extraction of polyphenols from food matrix. Many factors affect the process of extraction of polyphenolic compounds from plant material. The literature data suggested that the recovery, yield and type of polyphenolics in an extracts are influenced among other factors by the type and polarity of extracting solvents and time for extraction (Sulaiman *et al.* 2011; Koffi *et al.* 2010). Selection of the appropriate solvent depends on the food sample among other factors (Lasano *et al.* 2019; Zhou *et al.* 2004).

The main objectives of this study were (i) to evaluate the efficiency of four solvents with different concentration in extracting of polyphenols from fresh onion (ii) to estimate suitable time for extraction and (iii) spectroscopic determination of total polyphenolic content in onion by Folin–Ciocâlteu assay.

MATERIAL AND METHODS

Research material: Macedonian local population of summer yellow onion from village Vogani.

Chemicals and equipment: Folin–Ciocâlteu reagent (Merck), gallic acid (Alkaloid, 99.15%), methanol (Sigma Aldrich, 99.81%), ethanol (Alkaloid, 96%), acetone (Sigma Aldrich), ethyl acetate (Sigma Aldrich, 99.7%), distilled water. All spectrophotometric measurements were made on a Spectrophotometer Varian Cary 50 using a 1.0 cm optical path length glass cell.

Preparation of standard and sample solution: Standard stock solution of gallic acid was prepared when a known amount of the gallic acid was dissolved in methanol (99.81%) in a volumetric flask od 10 cm^3 (Daneshfar *et al.* 2008). Standard test solutions were prepared with dilution from stock solution. All standard solutions were stored at refrigerator at 4 °C. They were stable during the period of analyses. After harvesting the fresh onion samples were stored at room temperature, at a dark place. To prepare the onion samples, fresh onions was skinned, chopped, blended and homogenized.

Extraction of polyphenols from onion: Methanol, ethanol, acetone and ethyl acetate were tested for extraction of polyphenols from onion in different proportions of water (60% and 80%) resulting in different conditions of interactions with the matrices (Nguyen *et al.* 2015). Extraction procedures have been performed by mixing 5 g fresh onion and 25 mL of the desired solvent in a 50 mL conical flask, at room temperature. The conical flask with the mixture was kept under constant stirring using ultrasound bath 15 minutes followed by shaker to 2 hours' time. The onion extracts were filtered through (Whatman No. 1) filter paper and afterwards were stored at refrigerator (4°C) until further analysis.

Determination of total polyphenols: The procedure known from literature was used for quantification of total polyphenols in onion sample with the standard solution of gallic acid. One milliliter (1 mL) of onion's extract was mixed with 5 mL of 1:10 diluted Folin–Ciocâlteu reagent (Singh *et al.* 2017; Kamboj *et al.*, 2015; Agbor *et al*., 2014). The solutions were incubated at room temperature for 5 min. After that 5 mL of 7.5% Na_2CO_3 solution was added and solutions were stored at a dark place 120 minutes in order the reaction to be completed. The absorbance of the reaction mixture was measured at 760 nm. Every determination was made in triplicate. The total phenolic content (TPC) of the extracts was calculated from the regression equation of calibration curve and the results were expressed as mg of gallic acid equivalent (GAE) per 100 g onion.

RESULTS AND DISCUSSION

This study evaluated total polyphenolic content (TPC) after optimization of extraction of polyphenols from onion using different concentration of conventional organic solvents (methanol, ethanol, acetone and ethyl acetate) and different times. TPC in the onion extracts was determined by UV/Vis spectroscopy, applying Folin–Ciocâlteu assay with some modifications (Singh *et al.* 2017; Kamboj *et al.*, 2015; Agbor *et al.*, 2014). This assay is the simplest one

that is available for the measurements of phenolic compounds in different natural products. According to the literature the Folin–Ciocâlteu assay is used for TPC determination in different fruits and vegetables, as it is case in the work of Llupa and coworkers (2022). They used the Folin–Ciocâlteu assay for determination of TPC in quince and sweet cherry (Llupa *et al*., 2022). Colorimetric reactions are widely used in the UV/Vis spectrophotometric method, which is easy to perform, rapid and low cost (Bueno *et al.,* 2012). However colorimetric assay uses a reference substance and measures the total concentration of hydroxyl groups in the plant extracts (Kamboj *et al.,* 2015). The basic mechanism of this reaction is oxidation and reduction, with the phenolic group being oxidized and the metal ion from Folin–Ciocâlteu reagent reduced. In this research for TPC determination as reference standard gallic acid (3,4,5-trihydroxilbenzoic acid) was used (Daneshfar *et al.* 2008). The structural formula and UV spectrum of gallic acid is presented in the Fig. 1.

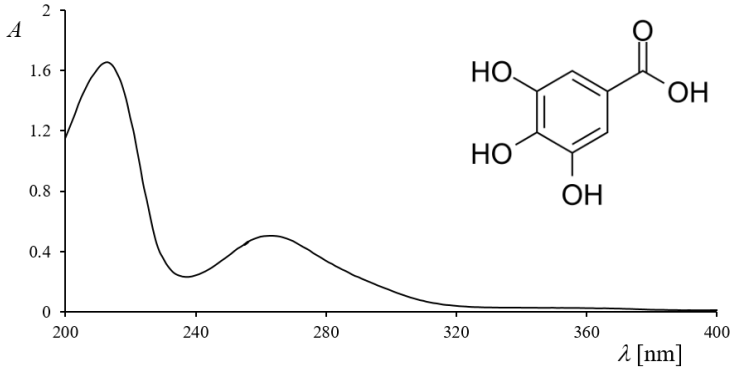

Figure 1. Structural formula and UV spectrum of gallic acid

In the UV spectrum of gallic acid two absorption maxima at around 215 and 265 nm can be observed. As a result of reaction of polyphenolic compounds with Folin–Ciocâlteu reagent blue complex was formed with absorption maximum in the visible region around 760 nm, and it can be quantified by visible spectrophotometry (Kamboj *et al.,* 2015). The intensity of a reaction of phenolic compounds with Folin–Ciocâlteu reagent is proportional to the availability of hydroxyl groups present on the aromatic ring and influences the relative potential of the molecule. Gallic acid has only one ring and three unsubstituted hydroxyl groups and is less influenced by electronic interactions and therefore has higher specific absorptivity and is the best reference substance for determination of TPC (Kamboj *et al.,* 2015).

In this research TPC was determined in the onion extracts obtained with different conventional organic solvents and different times for extraction. The absorbance of the extracts was compared with a gallic acid calibration curve for

estimating the concentration of TPC in the onion sample. The dependence of absorbance on concentration of gallic acid was linear as it is shown in the Fig. 2.

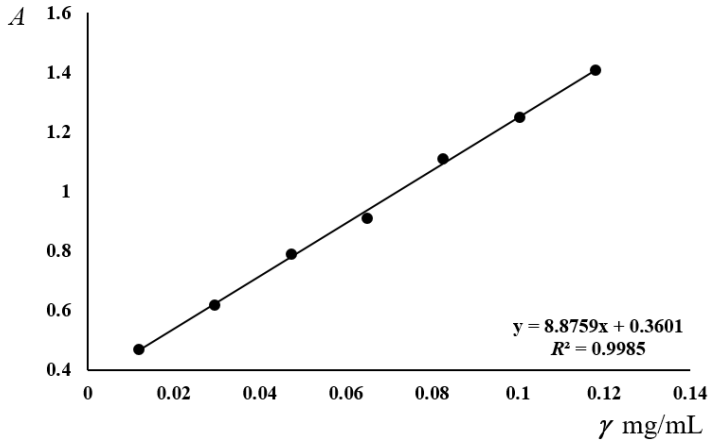

Figure 2. Calibration curve of gallic acid in the concentration range from 0.0118 mg/mL to 0.118 mg/mL

The calibration equation was y=8.8759x+0.3601, with correlation coefficient of 0.9985 which shows excellent linearity over tested concentration interval. TPC was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions, and expressed as mg gallic acid equivalent/100 g fresh weight of onion (mg GAE/100 g).

Extraction of polyphenols from onion with conventional solvents

There are several methods for extraction of polyphenols from fruits and vegetables. One of the most traditional methods uses organic solvents to improve efficiency, quality of extracts, extraction time and the consumption of a solvent. As it is known from literature, selection of solvent has significant effect on the extraction of polyphenols from different sample matrixes (Nguyen *et al.* 2015). Successful extraction of polyphenols from onions influence on the accuracy of the determination. Methanol, ethanol, or their mixture with water as well as ethyl acetate are usually used for the extraction of phenolic compounds (Dai and Mumper, 2010; Ignat *et al.*, 2011). Among organic solvents methanol and ethanol with different water content are the most frequently used, as it is presented in the paper of Krivokapić and coworkers. They determined TPC in two wild-growing plant species (*H. perforatum* L. and *M. officinalis* L) and found the highest values in 80% methanolic, while TPC values in 50% ethanolic extracts were lower (Krivokapić *et al.*, 2021). Furthermore, the Duan *et al.* (2015) found different TPC in onion extracts obtained with methanol and ethanol as solvents (Duan *et al.*, 2016).

The optimization of the extraction conditions in this work was performed by analyzing different organic solvents, and impact of solvent concentration was

investigated as well. The extraction of polyphenols from onion was performed with 80% methanol, 60% methanol, 80% ethanol, 60% ethanol, 80% acetone, 60% acetone, 80% ethyl acetate and 60% ethyl acetate. The average values of TPC expressed as GAE mg/100 g fresh onion ± the confidence interval with a 95% confidence level ($p<0.05$) are given in Table 1. The measurements were carried out in triplicate (n=3), and the standard deviation values are presented in Table 1, as well.

Table 1. TPC in onion extracts obtained using different organic solvents

No.	Solvent	GAE (mg/100 g)	SD (n=3)
1	80% methanol	37.53±0.38	0.33
2	60% methanol	38.81±0.39	0.35
3	80% ethanol	33.68±0.52	0.46
4	60% ethanol	32.38±0.31	0.27
5	80% acetone	37.85±0.60	0.53
6	60% acetone	38.24±0.59	0.52
7	80% ethyl acetate	27.73±0.32	0.28
8	60% ethyl acetate	27.10±0.35	0.31

GAE ± confidence level; SD - standard deviation

According to the results TPC determined in different onion extracts ranged from 27.10±0.31 mg GAE/100 g when ethyl acetate was used as a solvent to 38.81±0.35 mg GAE/100 g when polyphenols were extracted from onion with 60% methanol. As it is presented in Table 1, TPC in onion extracts decreased in following order: 60% methanol >60% acetone >80% methanol >80% acetone >80% ethanol >60% ethanol >80% ethyl acetate >60% ethyl acetate.

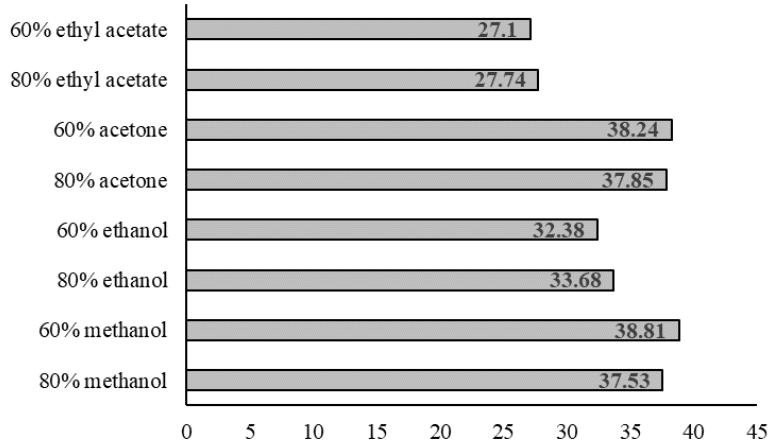

Figure 3. Extraction of polyphenols from onion using organic solvents with different concentration

The obtained results (Table 1, Figure 3) showed that 60% methanol as the extraction solvent gave the best yield of polyphenols compared to the other solvents used in this study. The obtained result is in accordance with the previous studies which reported that using methanol as a solvent for extracting polyphenols in onions is the best option i.e. gives the highest yield of polyphenols in onions' extracts (Singh *et al*. 2017; Hossain *et al.* 2018).

Extraction time

The time required for the extraction plays an important role in efficient extraction of polyphenols from fresh onion and influence on the TPC (Viera *et al*., 2017). Some studies indicated that optimum time for the extraction of polyphenolic compounds fall in the region of 30 to 180 min, but it is dependent on the characteristics and the type of biomass used (Vergara-Salinas *et al.* 2012). In this investigation, the effect of the extraction time on the polyphenolic content in onion extracts was evaluated between 30 min and 180 min, at room temperature. For this purpose, 60% methanol was used as an extraction solvent. TPC obtained during different extraction time expressed as GAE mg/100 g fresh onion and the confidence interval with a 95% confidence level ($p<0.05$) are presented in the Table 2. The measurements were made in triplicate (n=3) and the calculated standard deviation values are given in Table 2, as well.

Table 2. TPC in onion extracts obtained at different extraction times

No.	Time (minutes)	GAE (mg/100 g DW)	SD (n=3)
1	30	36.08±0.32	0.28
2	60	36.37±0.73	0.64
3	120	37.95±0.63	0.56
4	180	36.55±0.54	0.48

GAE ± confidence level; SD - standard deviation

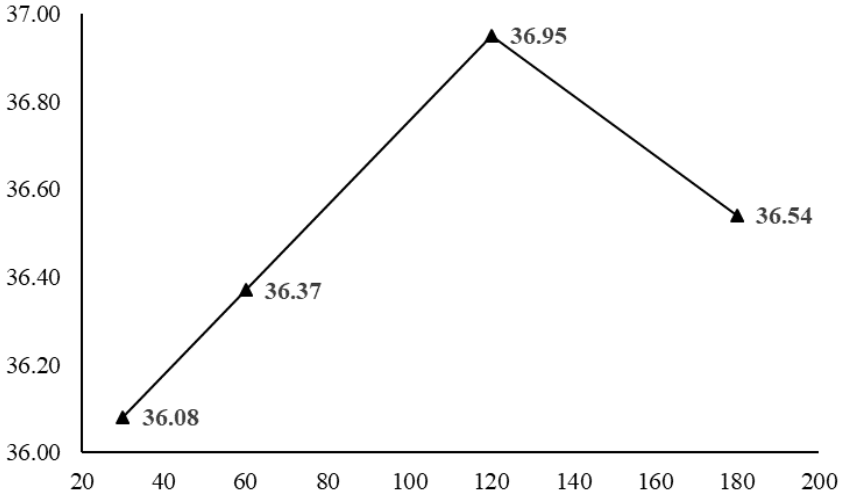

Figure 4. TPC depending on time for extraction

The results in Table 2 show that the TPC values increased gradually with an increase in extraction time from 30 to 120 min, reaching a limit after which they decreased. According to literature when final equilibrium is reached after certain time no more extraction is possible (Durling *et al*. 2007). Higher TPC values were obtained during extraction time of 120 minutes (37.95±0.63) as it can be seen from Table 2 and Figure 4.

The obtained results are in accordance with the previous studies obtained during the testing of extraction time (Pal *et al*. 2019; Souza *et al.* 2009).

CONCLUSIONS

Fruits can be listed as a principal source of polyphenols, among which onion is a great source of polyphenols, especially flavonoids. In the present study TPC was determined by UV-Vis spectroscopy in accordance with Folin–Ciocâlteu assay. This study was conducted in order to investigate which solvent is suitable for extraction of polyphenols from onion and to establish extraction time, as well. Different conventional solvents (methanol, ethanol, acetone and ethyl acetate) in different concentrations (60% and 80%) were evaluated. As it can be concluded from the result different solvent has different extraction efficiency in which the concentration of total polyphenol did not vary significantly with change in solvent. Time for extraction was evaluated, as well. Regarding time for extraction the best results for TPC were obtained during the extraction process of 120 minutes. This study showed that a higher concentration of TPC was found when the time for extraction was 120 minutes. Based on the results presented above, it can be concluded that the most suitable conditions to optimize the polyphenol extraction process from onion is 60% methanol as a solvent and 120 time for extraction.

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