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RECOVERY OF BIOACTIVE COMPOUNDS FROM RED ONION SKINS USING CONVENTIONAL SOLVENT EXTRACTION AND MICROWAVE ASSISTED EXTRACTION

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Abstract

In this study two extraction methods, such as conventional solvent extraction (CSE) and microwave assisted extraction (MAE) were used for bioactive compounds extraction from red onion skins. The effects of several extraction parameters on the phytochemical content were investigated to discover which combination of parameters led to the highest concentration of phytochemicals. The results revealed that the optimal conditions for anthocyanin extraction (1.75±0.04 mg C3G/g DW) by CSE was achieved at 70% ethanol acidified with 0.1 N hydrochloric acid after 120 minutes of extraction at 25 °C. Also, the highest antioxidant activity (32.18±1.75 mM of Trolox/g DW) was obtained with 96% ethanol acidified with glacial acetic acid, after 3 hours of extraction at 25 °C. Meanwhile, for MAE the highest anthocyanin content of 1.60±0.05 mg C3G/g DW was found when using 50% ethanol acidified with 99.5% citric acid, at 735 W microwave power for 15 seconds of extraction. MAE with 96% ethanol and glacial acetic acid had the highest yield of antioxidant activity (33.41±0.59 mM of Trolox/g DW) at 315 W microwave power after 10 seconds of extraction. Therefore, the bioactive compounds from red onion skins might be available as a source of functional compounds useful in the pharmaceutical and food industry.

Keywords: extraction, red onion skins, antioxidant, anthocyanins, total phenolic content

Introduction

Allium cepa L. (*Liliaceae*), also known as bulb onion, is the most widely cultivated and consumed vegetable in the world, and its production is increasing every year

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due to increasing consumers demand (Kuete, 2017). It has a considerable economic importance, as it is one of the most valuable vegetable crops for flavouring various types of food, with important medical, nutritional and functional properties (Kuete, 2017).

Onion is the second most cultivated vegetable grown worldwide, after tomatoes, being cultivated in more than 170 countries for domestic use. About 8% of the global production is traded internationally. According to the UN Food and Agriculture Organisation (FAO) in 2017, the world production of dried onions was almost 98 million tones, led by China and India producing 25% and 23% of the total, respectively. Likewise, the onion production in 2017 was almost 10 million tons in Europe (FAOSTAT, 2016). Onion is an important vegetable for the human diet because it can be used raw, sliced for seasoning salads, fried with other vegetables and meat, or boiled with other vegetables, also being an essential ingredient in many sauces and dishes (Kuete, 2017). Aside from its use as a condiment and spice for flavouring and enriching diverse cuisines, onion has been recognized for its beneficial effects on health, for thousands of years (Lawande, 2012).

Onion is an important food because it supplies various active phytomolecules such as flavonoids, fructo-oligosaccharides (FOS), phenolic acids, thiosulfinates and other sulphur compounds (Slimestad *et al.*, 2007). However, red onion is rich in polyphenols, flavonoids with two major subgroups: flavonols and anthocyanins (Gorinstein *et al.*, 2008). Many compounds in onion (flavonoids and the alk(en)yl cysteine sulphoxides) have been reported to have a range of health benefits which include anticarcinogenic properties, antioxidant activities, antidiabetic, cardiovascular, antimicrobial and antiinflammatory effects (Kuete, 2017).

At industrial level, onion processing generates a large amount of waste which can lead to serious environmental problems. For instance, more than 500,000 tons of onion solid waste is produced annually only in Europe (Kiassos *et al.*, 2009). About 37% of fresh onions are discarded during processing as waste. Onion solid waste has a characteristic strong aroma and promotes the rapid growth of phytopathogenic agents like *Sclerotium cepivorum*. For this reason, they are not suitable as an organic fertilizer, animal feed or landfill suppression (Sharma *et al.*, 2016).

Consequently, urgent solutions must be found to allow the efficient use of these by-products, especially because of their high content of phenolic compounds, particularly anthocyanins such as glycosides of cyanidin, peonidin etc., flavonols such as glycoside forms of quercetin, kaempferol, and myricetin derivatives (Sharma *et al.*, 2016; Kuete, 2017).

Onion waste consists of the nonedible part of the onion bulb, that is the outer dry skin and semidry layers, as well as the apical and basal trimmings. The outer dry layers of the onion bulb, which constitute the main onion waste, is a source of valuable flavonoids, especially flavonols and anthocyanins (Slimestad *et al.*, 2007). The levels of flavonoids ranges between 2-10 g/kg in onions skins, and between 0.03 to 1 g/kg in edible portions of onions (Sharma *et al.*, 2016). Among the

flavonoids present in the onion skin, quercetin and its glycoside are the major compounds. Besides quercetin, onion skin contains lower concentrations of other flavonols, such as kaempferol, isorhamnetin, and myricetin with their derivatives (Sharma *et al.,* 2016). Flavonoids have antioxidant, anti-inflammatory, anti-allergic, antifungal, anti-platelet, anti-thrombotic, and anticancer properties (Horincar *et al.,* 2019a). Some researchers reported that the quercetin levels in red onions are 14-fold higher than in garlic. Red onion skins contain flavonoid levels that are 48-fold higher than its flesh (Gorinstein *et al.*, 2008).

Anthocyanins, mainly found in red onion skins are glycosides of cyanidin, peonidin, and pelargonidin (Sharma *et al.,* 2016). Slimestad *et al.* (2007) stated that there are at least 25 different anthocyanins in red onions. Moreover, Slimestad *et al.* (2007) added that in some red onions the content of anthocyanins represents about 10% of the total flavonoid content. Gennaro *et al.* (2002) reported the anthocyanin levels in red onion dry skins range from 4565 mg/kg to 7831 mg/kg, most of them being concentrated in the outer fleshy layer and the skin, whereas in the edible tissue they are limited to a single layer of cells in the epidermal tissue (Gennaro *et al.*,2002).

Therefore, an alternative way for the valorization of onion by-products could be the use of onion by-products as a natural source of high-value ingredients, such as anthocyanins, quercetin and other functional ingredients, by recovering the bioactive compounds from red onion by-products (Sharma *et al.,* 2016).

Extraction is an important step in the separation and purification of bioactive components from plant material. Various extraction techniques can be applied for polyphenol recovery from plants. These techniques can be divided into classical (solvent extraction or maceration, Soxhlet extraction and hydro-distillation) and modern (ultrasound assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, pulsed electric field assisted extraction, supercritical fluid extraction and pressurized liquid extraction) ones. Generally, the extraction efficiency is influenced by several factors, such as particle size, type and concentration of solvent, solid–solvent ratio, time, temperature, pH, etc. (Sagar *et al.*, 2018).

The conventional solvent extraction (CSE), or solid–liquid extraction, is one of the most commonly used, being a cheap and easy method of polyphenol extraction. It is based on a direct extraction of the dried plant material with an appropriate solvent using a homogenizer for a certain time. The disadvantages of CSE consist of long time of extraction and high solvent consumption (Sagar *et al.,* 2018).

Microwave assisted extraction (MAE) generates a higher extraction yield using a shorter extraction time and lower quantities of solvents. Also, the resulting extract is of a better quality and high selectivity. On the other hand, MAE is considered an expensive procedure and is not suitable for heat-labile molecules (Sagar *et al.,* 2018).

The objective of this study was to maximize the recovery of bioactive compounds from red onion skins using CSE and MAE. Thus, the effects of four parameters

(time, temperatures, ethanol concentration and acids) on the extraction efficiency of bioactive compounds were evaluated. Moreover, the quantitative analysis of individual phenolic compounds, *i.e*. anthocyanins, by high-performance liquid chromatography **(**HPLC), was also performed.

Materials and methods

Plant material

The red onions (*Allium cepa L.*) were purchased from the local market of Galati, Romania in august 2019. The onion samples were washed, their skins were manually separated, washed with distilled water and then blotted on paper towels. The separated skins were further dried for 2 hours at 40°C up to the equilibrium moisture content of 11.0% in an oven (Stericell 111MMM Medcenter, USA) with the air velocity of 0.7 m/s. Afterwards, the dried skins were grounded into a fine powder by using a grinder and stored in a hermetic container at a temperature of 4°C until performing the extractions.

Chemicals and reagents

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), sodium hydroxide, sodium carbonate, Folin-Ciocalteu reagent, sodium acetate, sodium nitrite, potassium chloride, aluminum chloride, formic acid, gallic acid, ethanol, and methanol (HPLC grade) were all obtained from Sigma Aldrich Steinheim, Germany.

Extraction procedures

Conventional solvent extraction

The extraction was performed using a quantity of 1g red onion skin powder and 15 mL of ethanol at three different concentrations (96 % (v/v), 70 % (v/v) and 50 % (v/v)). Then, the ethanol was mixed with one of the following acids: glacial acetic acid–CH_3COOH (99.8%), hydrochloric acid–HCl (0.1N), and citric acid (99.5%), with an acid–solvent ratio of 1:14. The extractions were performed at 25 °C and 50 °C for three different extraction periods (1, 2 and 3 hours) in an orbital shaker (SI-300R Medline Scientific, UK), at 150 rpm. The pH values of the extracts after acid addition varied between 2.12-2.67. Subsequently, the samples were centrifuged (Hettich Universal 320R, Germany) at 14000 rpm for 10 min at 4 °C, and the resulting supernatant was further used for characterization.

Microwave-assisted extraction

The extraction was performed using a microwave oven (Sharp Inverter, 1050 W, Germany). An accurate weight of ground red onion skins (1±0.001 g) was mixed with 15 ml of ethanol as described earlier. The extraction was performed at two different periods of time (10 s and 15 s) using two different microwave powers - 315 W (30%of the total power of the microwave oven) and 735 W (70 %of the total power of the microwave oven), respectively. Afterwards, the samples were centrifuged (Hettich Universal 320R, Germany) at 14000 rpm for 10 min at 4 °C and the resulting supernatant was further analysed.

Determination of total monomeric anthocyanins content (TMA)

The TMA was determined by using the pH differential method, as described by Horincar *et al.* (2019b) with minor modifications. Briefly, 200 µL of the extract were mixed with 800 µL of buffer solutions at pH 1.0 and pH 4.5. After 15 minutes the absorbance was measured at 520 nm and at 700 nm. The anthocyanins concentration was calculated based on equation 1:

$$\text{Anthocyanins (mg/g)} = \frac{A \cdot Mw \cdot DF}{\varepsilon \cdot L} \cdot \frac{Vt}{Wt} \qquad (1)$$

where A = [(A_{520}– A_{700}) pH1.0 − (A_{520}– A_{700}) pH4.5],

Mw–(molecular weight) of cyanidin-3-glucoside (449.2 g/mol), ε–is the molar extinction coefficient of cyanidin-3-glucoside (26.900 L/mol/cm), DF–the dilution factor, L–the length of the cuvette (1 cm), Vt–total volume (mL), Wt–sample weight (g). The TMAs were expressed in milligrams of cyanidin-3-glucoside (C3G) per gram of dry weight (mg C3G/g DW).

Determination of total flavonoid content (TFC)

The TFC was measured using the aluminium chloride spectrophotometric method as described by Horincar *et al.* (2019b) with slight modifications. Briefly, 0.5 mL of the extract was mixed with 2 mL of deionized water and, afterwards, a volume of 150 µL (5% $NaNO_2$solution) was added. After 5 minutes, 150 µL of 10% $AlCl_3$ solution was added into the reaction mixture. Then, after 6 minutes, 1 mL of 1 M NaOH was added. The sample absorbance was immediately measured at 510 nm against the prepared blank. The absorbance of the extract was compared with a quercetin standard curve to estimate the concentration of TFC in the sample. The TFCs were expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE/g DW).

Determination of total phenolic content (TPC)

The TPC was measured spectrophotometrically using the Folin–Ciocalteu (FC) method as described by Horincar *et al.* (2019b). In short, 200 µL of the extract was mixed completely with 15.8 mL deionized water and 1 mL of Folin–Ciocalteu reagent. After 10 minutes, 3 mL of 20% Na_2CO_3 was added to the mixture. The resulting mixture was kept for 60 minutes at room temperature in the dark, and then the absorbance was measured at 765 nm against the prepared blank. The absorbance of the extract was compared with a gallic acid standard curve to estimate the concentration of TPC in the sample. The TPCs were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

Antioxidant activity - DPPH scavenging method (AA)

The AA was determined using the DPPH free radical-scavenging activity as mentioned by Horincar *et al.* (2019b). The free radical scavenging capacity of the red onion skins extracts was tested by their ability to bleach the stable DPPH radical. Briefly, the absorbance of the prepared blank, 3.9 mL DPPH solution 0.1 M in methanol was measured at 515 nm. Then, over 100 µL extract, 3.9 mL DPPH

solution 0.1 M was added to the reaction mixture. Afterwards the mixture was kept for 1 hour and 30 minutes at room temperature in the dark, before the absorbance at 515 nm was recorded. A calibration curve using Trolox as standard was used. The AAs were expressed as millimoles of Trolox equivalents antioxidant activity per gram of dry weight (mM of Trolox/g DW).

High-performance liquid chromatography analysis of anthocyanins

The red onion skin extract contains a multitude of biologically active compounds such as anthocyanins. In order to assess the separation and identification of the anthocyanins from the red onion skins extracts, a ThermoFinnigan Surveyor HPLC system coupled with a Diode-Array Detector, controlled by Xcalibur software (Finnigan Surveyor LC, Thermo Scientific, USA), was used, following the method described by Horincar *et al.* (2019b) with minor modifications. To determine the anthocyanin profile and to achieve the anthocyanin separation from the rest of the compounds in the mixture, before injection, the samples were filtered through a C18 Sep-Pack cartridge (Cartridge-Waters, USA). The anthocyanins were analyzed at the specific wavelength of 520 nm. The column used was a Synergi 4u Fusion-RP 80A (150×4.6mm, 4μm), operated at the oven temperature of 25°C. The elution solvents were methanol 100% (A) and 10 % formic acid (B). The elution gradient employed to separate the targeted compounds was 0–20 min, 9–35% (A); 20–30 min, 35% (A); 30– 40 min, 35– 50% (A); and 40–55 min, 50–9% (A). A 10 μL injection volume was used, at a flow rate of 1 mL/min. The compounds were identified and quantified based on their retention time and by comparison to the available standards and the data reported in the literature.

Statistical analysis of data

Unless otherwise stated, the data reported in this study represented the average of triplicate analyses followed by the standard mean deviation. The statistical data analysis was performed using the data analysis tool pack of the Microsoft Excel software. The significant differences between samples were quantified using one-way ANOVA after checking the normality and variance equality conditions. Post-hoc analysis via the Tukey method was performed when the p-value resulting from the ANOVA analysis was $p<0.05$.

Results and discussion

Conventional solvent extraction

The average values for the content of TMA and TFC from the red onion skins extracts obtained by CSE under varying conditions of ethanol concentrations, acids, temperatures and extraction times are shown in Table 1.

Total monomeric anthocyanins contents

Table 1 revealed the average values of the TMA content from the red onion skins extracts. Regarding TMA there was significant difference ($p<0.05$) between the extraction with 50% ethanol and 96% ethanol, both extracts acidified with glacial acetic acid, at 25 °C after 1 hour of extraction.

Table 1. Total monomeric anthocyanins (TMA) and flavonoids content (TFC) of red onion skins extracts using conventional solvent extraction.

Acids	Times	Ethanol concentration - temperature					
		TMA, mg C3G/g DW					
		50% - 25°C	50% - 50°C	70% - 25°C	70% - 50°C	96% - 25°C	96% - 50°C
Glacial acetic acid	1h	1.41±0.05^{aAB}	1.41±0.04^{aA}	1.27±0.06^{bD}	1.24±0.03^{bB}	0.31±0.03^{dC}	0.52±0.06^{cC}
	2h	1.28±0.09^{abC}	1.38±0.06^{aAB}	1.28±0.02^{abD}	1.23±0.04^{bB}	0.36±0.02^{dC}	0.53±0.02^{cC}
	3h	1.26±0.05^{bC}	1.42±0.06^{aA}	1.34±0.09^{abD}	1.23±0.03^{bB}	0.37±0.02^{dC}	0.55±0.02^{cC}
0.1N hydrochloric acid	1h	1.36±0.06^{bBC}	1.30±0.06^{bBC}	1.64±0.04^{aAB}	1.37±0.07^{bA}	1.39±0.05^{bA}	1.28±0.05^{bAB}
	2h	1.42±0.05^{bAB}	1.27±0.05^{dC}	1.75±0.04^{aA}	1.38±0.08^{bcA}	1.29±0.04^{cdA}	1.31±0.05^{cdAB}
	3h	1.43±0.05^{bAB}	1.28±0.05^{cBC}	1.63±0.05^{aAB}	1.38±0.06^{bcA}	1.37±0.08^{bcA}	1.38±0.06^{bcA}
99.5% citric acid	1h	1.47±0.06^{aAB}	1.44±0.05^{aA}	1.57±0.11^{aBC}	1.38±0.03^{abA}	1.13±0.16^{bcAB}	1.19±0.05^{cB}
	2h	1.49±0.05^{abA}	1.40±0.04^{bcA}	1.54±0.05^{aBC}	1.37±0.03^{cA}	1.16±0.15^{cdAB}	1.19±0.10^{dAB}
	3h	1.46±0.06^{aAB}	1.42±0.05^{aA}	1.47±0.09^{aC}	1.34±0.02^{abA}	0.94±0.07^{cB}	1.21±0.12^{bAB}
		TFC, mg QE/g DW					
Glacial acetic acid	1h	80.72±2.61^{bB}	95.42±3.16^{aAB}	80.05±3.74^{bC}	83.97±3.23^{bBC}	29.48±2.22^{dD}	52.54±6.65^{cC}
	2h	82.57±1.59^{bB}	94.93±3.77^{aAB}	83.44±1.86^{bC}	79.96±1.59^{bC}	31.36±2.83^{dD}	57.60±5.94^{cC}
	3h	83.86±5.49^{bB}	96.11±2.17^{aAB}	86.19±4.64^{bBC}	82.87±3.11^{bBC}	33.71±2.06^{dD}	59.35±7.67^{cC}
0.1N hydrochloric acid	1h	94.91±3.23^{cA}	100.25±1.96^{bA}	106.08±2.04^{aA}	107.71±3.76^{aA}	86.44±2.72^{dA}	97.32±1.86^{bcA}
	2h	98.79±2.65^{cA}	99.04±8.94^{abcAB}	109.41±5.26^{abA}	109.46±3.07^{aA}	82.63±6.10^{dA}	101.79±2.03^{bcA}
	3h	97.13±3.58^{bA}	100.19±6.17^{bAB}	104.33±3.06^{abA}	107.75±4.64^{aA}	83.76±3.11^{cA}	102.09±3.83^{abA}
99.5% citric acid	1h	77.40±4.77^{cB}	91.56±4.08^{aB}	91.14±3.49^{aB}	84.49±2.27^{bBC}	57.25±2.07^{dC}	77.79±4.12^{cB}
	2h	82.09±2.74^{bB}	91.61±3.04^{aB}	86.18±6.02^{abBC}	86.10±2.68^{abB}	68.58±5.52^{cB}	81.09±1.42^{bB}
	3h	81.32±3.14^{bB}	94.31±2.55^{aB}	85.55±3.67^{bBC}	84.90±1.80^{bBC}	65.85±6.10^{cBC}	81.71±2.65^{bB}

DW - dry weight. Results are expressed as mean±SD of six parallel measurements. Within a set of experiment, means that on the same row do not share a letter (a, b, c, d) are significantly different at $p<0.05$. Means that on the same column do not share an uppercase letter (A, B, C, D) are significantly different at $p<0.05$.

As shown in Table 1, the highest TMA content of 1.75±0.04 mg C3G/g DW was obtained with 70% ethanol acidified with 0.1N hydrochloric acid at pH=2.16 after 2 hours of extraction at a temperature of 25 °C. In the case of extraction with 50% ethanol, a high content of TMA (1.49±0.05 mg C3G/g DW) was found in the presence of 99.5% citric acid, at a temperature of 25 °C for 2 hours of extraction at pH=2.19. Additionally, in the case of the extraction with 96% ethanol, a slightly lower TMA content of 1.39±0.05 mg C3G/g DW, at a temperature of 25 °C, for 1 hour of extraction, at pH=2.17 in the presence of 0.1N hydrochloric acid was achieved. However, the lowest TMA content was found in the presence of 96% ethanol acidified with glacial acetic acid, at pH=2.67, after 1 hour of extraction at a temperature of 25 °C, which led to only 0.31±0.03 mg C3G/g DW. In terms of pH, the low pH value of the solvent helps the extraction efficiency and the low pH value of the extraction solvent can prevent the oxidation of anthocyanins.

Makris (2010) obtained by conventional extraction (25 °C, 3.7 hours, pH = 2.0) from onion skins extract using 60% ethanol a TMA content of 183.85 mg C3G/100 g DW. Our results are similar to those reported by Oancea and Draghici (2013), who found the highest TMA content (99.66 mg C3G/100 g fresh weight) for the extract derived from the dry outer skin of red onion (Red of Sibiu cultivar). In the above-mentioned study, the authors used CSE with a mixture of ethanol/acetic acid/water in a ratio of 50:8:42(v/v/v). The optimal conditions reported for the extraction were at pH=3.8, after 2 hours of extraction at 4 °C and solvent:sample ratio of 4:1(v/w). In another study, Viera *et al.* (2017) evaluated the content of TMA of the red onion skin extract obtained by conventional extraction at different periods of time (30, 60, 120 and 240 minutes) and using different concentrations of ethanol (20, 40, 60 and 80%). The highest content of TMA (470.2±16.2 mg C3G/100 g DW) was obtained with 60% ethanol, after 60 minutes of extraction. Moreover, Oancea *et al.* (2018) reported a higher content of TMA extracted from red onion skins (847.47±34.23 mg C3G/100 g DW), at 40 °C after 90 minutes of extraction by using 70% ethanol and a solvent/sample ratio of 30/1(v/w). In another study, Samir *et al.* (2019) reported that the highest yield of anthocyanins from red onion skins extract was 20 mg C3G/100 g DW using acidified ethanol with 1.5 N hydrochloric acid, 85:15 (v/v) by maceration at 4°C for 24 hours.

Total flavonoid contents

From Table 1, it can be observed that the maximum recovery of TFC (109.46±3.07 mg QE/g DW) from red onion skin extraction was obtained at 50 °C, after 2 hours of extraction, when using ethanol (70%) acidified with 0.1 N hydrochloric acid. However, as regards the TFC, a significant difference ($p<0.05$) between 50% ethanol extract and 96% ethanol extract, both extracts acidified with 0.1N hydrochloric acid at 25 °C after 2 hours of extraction, was observed. Furthermore, a high TFC of 102.09±3.83 mg QE/g DW was achieved for the extraction with 96% ethanol acidified with 0.1 N hydrochloric acid, after 3 hours of extraction at a temperature of 50 °C. On the other hand, the lowest TFC, 73% lower than previously mentioned, was noticed for the extraction with 96% ethanol acidified with glacial acetic acid after 60 minutes of extraction at a temperature of 25 °C.

Our findings are in agreement with a previous investigation conducted by Lee *et al.* (2014), who determined the TFC of 183.95±11.27 mg QE/g extract from onion skins extract (20 g skins were mixed with 200 mL70% ethanol(v/v)) by means of conventional extraction (60 °C, 3hours).

In another study conducted by Khiari *et al.* (2008) the TFC of onion solid waste extracts by conventional extraction (25 °C, 6 hours) using different ethanol concentrations (30%, 60% and 90%) and various acids (1% acetic acid, 1% citric acid and 0.1% hydrochloric acid) was determined. The highest TFC (3468±134 mg QE/100 g DW) was achieved with 60% ethanol acidified with 1% acetic acid. Viera *et al.* (2017) reported that the highest TFC of 40.9±0.9 mg QE/g DW was obtained for 60% ethanol, at 25 °C, after 60 minutes of extraction. Also, the same authors mentioned that increasing the extraction time led to a reduction in total flavonoid extraction, which can be associated to the exposure of the bioactive compounds to oxidative degradation. Likewise, Oancea *et al.* (2018) reported a high TFC of 121.49±27.59 mg QE/g DW for the red onion skin extract after 30 min extraction when using 70% ethanol as solvent and a solvent:sample ratio of 30:1 (v/w) at a temperature of 40 °C. Moreover, Ifesan (2017) reported a TFC of 177.33±1.56 μg QE/ml, for onion skins ethanolic extract, using conventional extraction (maceration 24h at room temperature) with 80% ethanol.

Figures 1 and 2 show the values of the TPC and AA of the red onion skins extracts obtained by CSE under varying conditions of solvent concentrations, acids, temperatures and extraction time.

Total phenolic contents

As it can be seen in Figure 1.a, the highest TPC was 81.48±2.69 mg GAE/g DW for the extract obtained with 70% ethanol and 99.5% citric acid, after 3 hours of extraction at a temperature of 25 °C. However, the values of TPC from red onion skin extracts varied significantly ($p<0.05$) between ethanol concentrations (50% ethanol and 96% ethanol both acidified with glacial acetic acid at 50 °C and 1 hour of extraction). Therefore, the values were 76.39±2.70 mg GAE/g DW in the case of 50% ethanol acidified with glacial acetic acid, after 60 minutes of extraction at 50 °C, and 45.06±5.31 mg GAE/g DW in the case of 50% ethanol acidified with glacial acetic acid after 60 minutes of extraction at 50 °C.

A key factor for the phenolics extraction from red onion skins was the solvent (ethanol) concentration. 70% ethanol with 99.5 % citric acid has been found to be more efficient for the TPC extraction due to disrupting the bonding between the solutes and the plant matrix, thus enabling a better mass transfer of the compounds. Temperature and time are important factors affecting extraction of bioactive compounds from red onion skins. By increasing the time of extraction (3 hours) and using a moderate temperature (25°C), the solubility of phenol compounds was enhanced.

The optimum recovery of phenolics is different from one sample to another, and relies on the type of plant and its active compounds and also on the combinations of solvents, time of extraction and temperature (Galanakis, 2018).

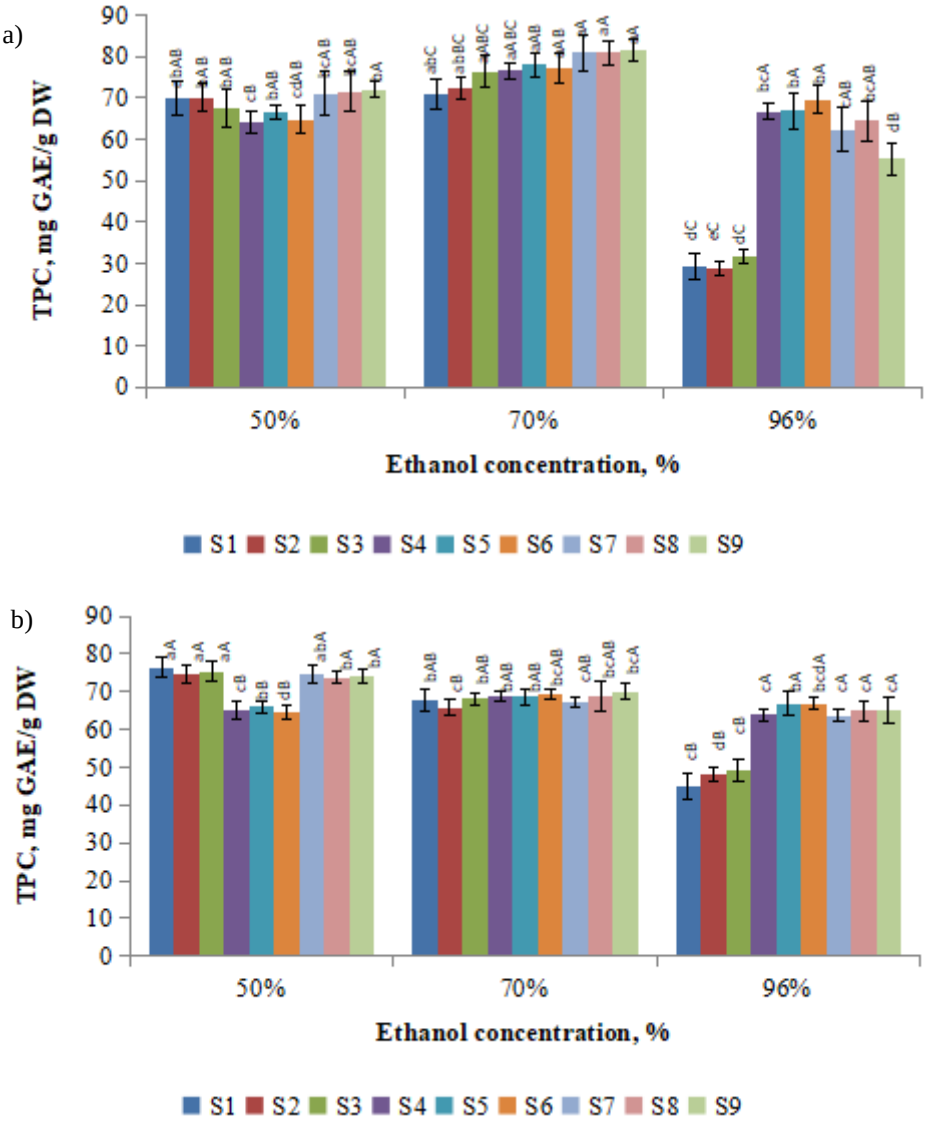

Figure 1. The content of total phenolics (TPC) of the red onion skins extract obtained by CSE under different conditions of solvent concentrations, acids, and extraction times at 25°C (a) and 50ºC (b). (S1-glacial acetic acid, 1h; S2- glacial acetic acid, 2h; S3- glacial acetic acid, 3h; S4- 0.1N hydrochloric acid, 1h; S5-0.1N hydrochloric acid, 2h; S6-0.1N hydrochloric acid, 3h; S7- 99.5% citric acid, 1h; S8-99.5% citric acid, 2h; S9-99.5% citric acid, 3h.). Bars of the same color with different lowercase letters from different groups with different ethanol concentration are significantly different at $p<0.05$. Bars of different color with different uppercase letters from the same group with the same ethanol concentration are significantly different at $p<0.05$.

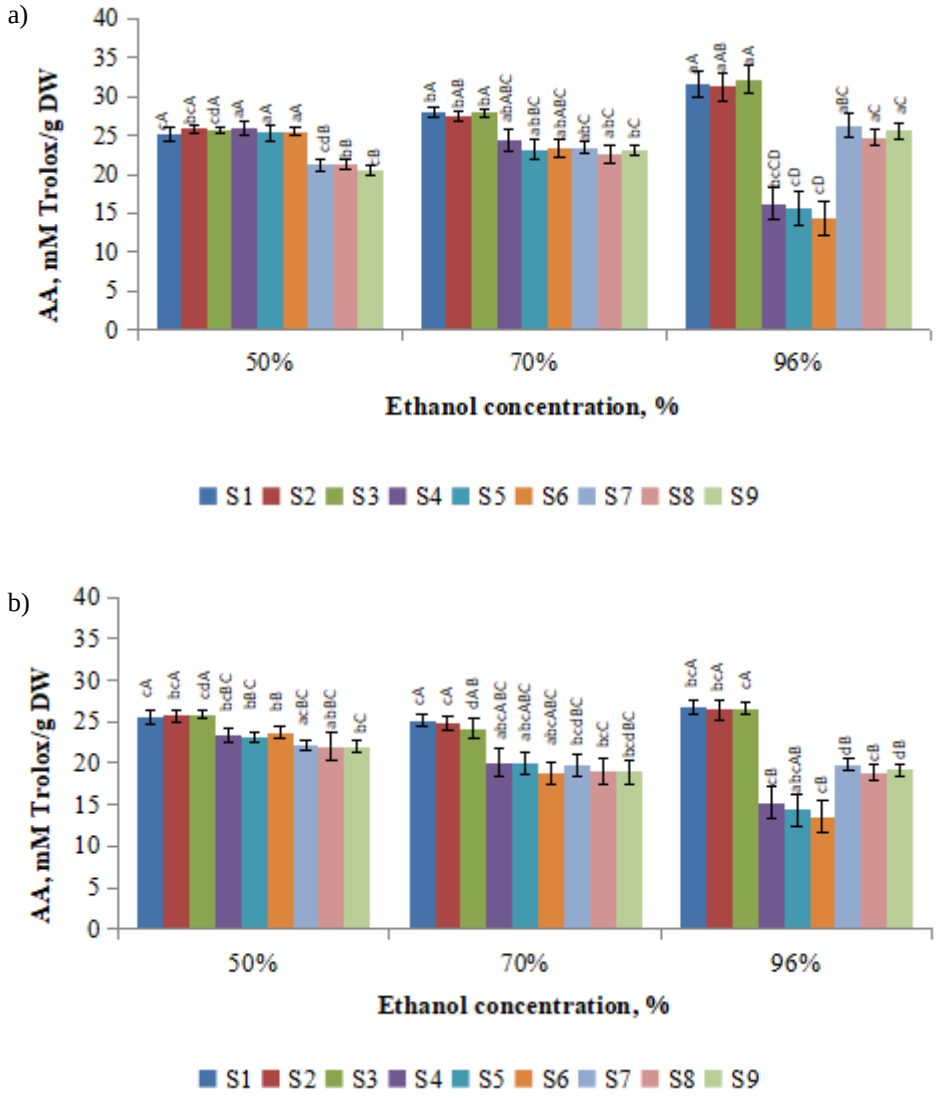

Figure 2. The DPPH radical-scavenging activity (AA) of the red onion skins extract obtained by CSE under different conditions of solvent concentrations, acids, and extraction times at 25°C (a) and 50°C (b). (S1-glacial acetic acid, 1h; S2- glacial acetic acid, 2h; S3-glacial acetic acid,3h; S4- 0.1N hydrochloric acid, 1h; S5-0.1N hydrochloric acid, 2h; S6-0.1N hydrochloric acid, 3h; S7- 99.5% citric acid, 1h; S8-99.5% citric acid, 2h; S9-99.5% citric acid, 3h). Bars of the same color with different lowercase letters from different groups with different ethanol concentration are significantly different at $p<0.05$. Bars of different color with different uppercase letters from the same group with the same ethanol concentration are significantly different at $p<0.05$.

Our results are in line with the findings of Kiassos *et al.* (2009), who determined the TPC from onion skins extract using conventional extraction, and the highest

total polyphenols yield was 9342±1435 mg GAE/100 g DW, under optimal conditions: 60% ethanol and citric acid 1g/L, pH=2.0, for 4.2 hours of extraction at 25 °C. In another study, Oancea and Draghici (2013) stated that the highest TPC (1345.74 mg GAE/100 g fresh matter), was found for the polyphenol extract resulting from the dry outer skin parts of red onion cultivar Red of Sibiu. The authors used conventional extraction with a mixture of ethanol:acetic acid:water in a 50:8:42 (v:v:v) ratio. The optimum parameters for the extraction were pH=3.8, extraction time 2 hours at a temperature of 40 °C and solvent:sample ratio 4:1. Later, Oancea *et al.* (2018) reported that the optimal conditions for TPC extraction (6183.85±268.96 mg GAE/100 g DW) from red onion skins were obtained during conventional extraction when using 70% ethanol (solvent:sample ratio of 30:1 (v/w)), at a temperature of 40 °C, after 90 minutes of extraction.

In another study, Khiari *et al.* (2007) found that the highest TPC of 1927±12 mg GAE/100 g DW was obtained for onion solid waste extracts using conventional extraction (40 °C, 6 hours) at optimum conditions with 60% ethanol acidified with 1% acetic acid. Additionally, Lee *et al.* (2014) determined a TPC of 372.50±6.85 mg GAE/g extract, from onion skins extract (20 g mixed with 200 mL ethanol) using conventional extraction (60 °C, 3hours) with 70% ethanol (v/v).

Antioxidant activity

AA in red onion skins extracts was measured by a well-known method, like DPPH. The highest AA of 32.18±1.75 mM of Trolox/g DW, was obtained for red onion skins extracts with 96% ethanol acidified with glacial acetic acid, after 3 hours of extraction at 25 °C (Figure 2a). Significant differences($p<0.05$) were observed among the samples extracted with 96% ethanol acidified with glacial acetic acid at 25°C and 3 hours of extraction, and the samples extracted with 96% ethanol acidified with 0.1 N hydrochloric acid for the same extraction time and temperature. However, for the same extraction variant and combination of parameters, if the glacial acetic acid was replaced with 0.1 N hydrochloric acid, lower values of AA (14.32±4.09 mM of Trolox/g DW) were determined. The red onion skin extracts exhibited a high AA even for the samples with low TPC when extraction was performed with 96% ethanol acidified with glacial acetic acid. The correlations between the scavenging activity and the total phenols are low, probably due to the fact that free radical scavenging activity depends on the presence of other plant compounds, such as alk(en)yl cysteine sulphoxides (ACSOs). The presence of bioactive compounds that possess antioxidant activity such as alk(en)yl cysteine sulphoxides affect DPPH radical scavenging more than polyphenols do (Rose *et al.*, 2005; Benitez *et al.*, 2011).

These results are higher than the values reported by Ifesan (2017) who stated that the highest AA was 27.76±0.91µg/ml, for the onion skins extract, using conventional extraction (maceration 24 hours at 25 °C) with 80% ethanol. In another report, Viera *et al.* (2017) found the highest DPPH radical scavenging activity of 100.1±4.9 µmol Trolox/g DW for the red onion skins extracts, obtained by conventional extraction with 60% ethanol after 240 minutes of extraction at 25 °C.

Lee *et al.* (2014) determined the AA of an onion skins extract (20 g onion skins were mixed with 200 mL of 70% ethanol) using conventional extraction (60 °C, 3 hours) with 70% ethanol (v/v). The ethanol extract exhibited the highest AA of about 72.25±2.74% inhibition of the DPPH radical. Oancea *et al.* (2018) reported a high antioxidant activity of about 87.36±0.36% inhibition of DPPH radical for an ethanolic extract from red onion skins. The optimal conventional extraction conditions were solvent/sample ratio of 20/1(v/w), after 60 minutes of extraction with 70% ethanol at 40 °C.

Furthermore, Khiari *et al.* (2007) determined the antiradical activity of onion solid waste extracts using conventional extraction (40°C, 6 hours). The highest antiradical activity (0.32±0.02 mM of Trolox/g DW) was obtained with 90% ethanol acidified with 0.1% hydrochloric acid.

Microwave-assisted extraction

Table 2 shows the average values for the content of TMA and flavonoid compounds from red onion skin extracts obtained by MAE under varying conditions of solvent concentrations, acids, temperatures and extraction times.

Total monomeric anthocyanins contents

According to the results in Table 2, the extraction with 50% ethanol gave the highest concentration of TMA (1.60±0.10 mg C3G/g DW) with 99.5% citric acid, at pH=2.19 after 15 seconds of treatment and at a microwave power of 735 W. As regards the TMA contents, a significant difference ($p<0.05$) was observed among the 70% ethanol extract and the 96% ethanol extract, both of them being acidified with 99.5% citric acid at 735 W and 15 seconds extraction time. The extraction with 70% ethanol resulted in a TMA concentration of 1.56±0.05 mg C3G/g DW, in combination with 0.1N hydrochloric acid at pH=2.16 after 15 seconds of microwave treatment, at a microwave power of 735 W. Moreover, in the case of 96% ethanol extraction, a slightly lower TMA concentration of 1.14±0.06 mg C3G/g DW with 0.1N hydrochloric acid at pH=2.17, after 15 seconds, at a microwave power of 735 W was achieved. In contrast, the lowest concentration of TMA recovered by the MAE was observed with 96% ethanol extraction and glacial acetic acid (0.35±0.03 mg C3G/g DW).

Authors such as Liu *et al.* (2019) developed an improved MAE of anthocyanins without dextrinization of starch, from purple sweet potato by-products. The optimal extraction conditions of purple sweet potato anthocyanins were as follows: solid:liquid ratio of 1:3 (g:mL), 30% ethanol acidified with 10% citric acid, 320 W microwave power, 500 seconds extraction time. Under these optimal parameters, the anthocyanins yield was 31.16±0.44 mg C3G/100 g fresh tissue. In another study, Piovesan *et al.* (2017) evaluated the content of TMA from blueberry extracts (*Vacciniumn ashei Reade*) of the Climax variety. The extracts were obtained by focused microwave extraction at different temperatures (30, 40, 50 and 60 °C) and different solvent concentrations (60% and 80% ethanol). The solvent was added to the sample in a ratio of 1:10 (w/v) which was then subjected to heating by microwave exposure for 20 minutes at 800 W microwave power. The highest

content of TMA of 21.20±0.7 mg C3G/100 g DW was obtained with 60% ethanol at a temperature of 60 °C.

Table 2. Total monomeric anthocyanins (TMA) and flavonoids content (TFC) of red onion skins extracts using MAE.

Ethanol concentration/ power		Acids/Times					
		Glacial acetic acid		0.1N hydrochloric acid		99.5% citric acid	
		10 s	15 s	10 s	15 s	10 s	15 s
		TMA, mg C3G/g DW					
50%	315W	1.49±0.06^{aAB}	1.41±0.07^{aBC}	1.32±0.07^{aC}	1.32±0.06^{bC}	1.58±0.05^{aAi}	1.55±0.07^{abA}
	735W	1.41±0.06^{aB}	1.44±0.10^{aB}	1.33±0.05^{aB}	1.40±0.07^{bB}	1.59±0.08^{aA}	1.60±0.05^{aA}
70%	315W	1.29±0.09^{bAB}	1.24±0.03^{bB}	1.38±0.06^{aA}	1.31±0.10^{bAB}	1.40±0.05^{bA}	1.43±0.11^{bA}
	735W	1.19±0.05^{bD}	1.14±0.04^{bD}	1.33±0.04^{aC}	1.56±0.05^{aA}	1.39±0.10^{bBC}	1.47±0.08^{abAB}
96%	315W	0.39±0.05^{cC}	0.44±0.04^{cC}	1.06±0.05^{bA}	1.05±0.07^{cA}	0.91±0.11^{cB}	0.93±0.07^{cAB}
	735W	0.35±0.03^{cD}	0.42±0.05^{cD}	1.02±0.09^{bAB}	1.14±0.06^{cA}	0.87±0.06^{cC}	0.98±0.12^{cBC}
		TFC, mg QE/g DW					
50%	315W	90.06±2.55^{aABC}	86.84±5.55^{aBC}	91.25±2.47^{bAB}	94.84±2.68^{bcA}	84.40±2.27^{aC}	84.56±3.84^{abC}
	735W	84.45±2.62^{bD}	90.47±3.34^{aBC}	93.60±2.24^{abB}	100.03±4.19^{bA}	82.76±2.81^{abD}	87.54±2.68^{aCD}
70%	315W	75.03±2.48^{cB}	77.66±3.32^{bB}	97.44±2.52^{aA}	93.43±2.74^{cA}	78.49±2.65^{bB}	77.59±2.53^{cB}
	735W	75.94±2.54^{cC}	77.93±3.53^{bC}	96.60±3.24^{aB}	106.29±4.06^{aA}	78.20±2.93^{bC}	79.16±3.49^{bcC}
96%	315W	29.41±1.96^{dD}	35.52±5.13^{cC}	74.20±1.84^{cA}	75.96±2.41^{dA}	49.83±4.14^{cB}	51.89±3.41^{dB}
	735W	28.89±2.05^{dD}	31.79±2.21^{cD}	74.07±2.88^{cA}	78.68±3.04^{dA}	47.52±2.36^{cC}	53.57±5.60^{dB}

DW - dry weight, Results are expressed as mean±SD of six parallel measurements. Within a set of experiment, means that on the same column do not share a letter(a, b, c, d) are significantly different at $p<0.05$.Means that on the same row do not share an uppercase letter(A, B, C, D) are significantly different at $p<0.05$.

Total flavonoid contents

The results in Table 2 indicated that the 70% ethanol combined with 0.1N hydrochloric acid recovered the highest TFC of 106.29±4.06 mg QE/g DW, at a microwave power of 735 W and after 15 seconds of treatment. In addition, for the same acid and the same time of extraction, a significant difference ($p<0.05$) was observed between the sample extracted with 50% ethanol at a microwave power of 735 W acid and the sample extracted with 96% ethanol at 735 W. The lowest concentration of flavonoids (73% lower than the highest TFC value) was found for the extraction with 96% ethanol in combination with glacial acetic acid, which can probably be explained by the fact that, after the microwave treatment, the solvent reached high temperatures (over 70 °C) that degraded the flavonoid compounds.

These results are higher than the ones reported by Shaileyee and Subhash (2015), who found the TFC of onion skins extracts at optimised MAE conditions to be 45.61±0.432 mg QE/g DW. The optimal MAE conditions were 210W microwave power, 15 minutes exposure time and 40 mL/g solvent to material ratio, using methanol as solvent. Jin *et al.* (2011), in a study on the optimization of the extraction methods for quercetin from onion skins, found a low content of quercetin (4.84±0.14 mg/g DW). This result was obtained using MAE at optimal parameters such as 700 W of microwave power after 117 seconds of treatment using 69.7% ethanol as solvent. Moreover, Kumar *et al.* (2014), in a study on the extraction of quercetin from red onion skins extract, determined the total quercetin content as being 209 mg/100 g fresh tissue. The authors used MAE and the optimal parameters were 1050 W of microwave power for 150 seconds at pH=6.25 using water as extraction solvent. In another study, Piovesan *et al.* (2017) reported the highest TFC of7.33±0.4 mg QE/g DW from blueberry extracts using MAE at optimum parameters such as 800 W microwave power, 20 minutes extraction time, at 60 °C and 60% ethanol.

Figures 3 and Figure 4 show the values of TPC and DPPH radical-scavenging activity for the red onion skins extracts obtained by MAE under varying conditions of solvent concentration, acids, temperature and extraction times.

Total phenolic contents

In the case of TPC obtained by MAE it was observed in Figure 3b that 96% ethanol with glacial acetic acid had the lowest extraction yield of TPC of 30.93±1.58 mg GAE/g DW. On the contrary, when the glacial acetic acid was replaced with 0.1 N hydrochloric acid the highest TPC of 75.56±2.33 mg GAE/g DW was achieved, with 50% ethanol, at a microwave power of 735 W, after 15 seconds of treatment. Similar values were also obtained when using 70% ethanol (71.88±2.60mg GAE/g DW) and 96% ethanol (65.46±4.23mg GAE/g DW) acidified with 0.1 N hydrochloric acid after 15 seconds of treatment at 735 W microwave power.

In the study conducted by Shaileyee and Subhash (2015), the maximum TPC of onion skins extracts in optimized MAE conditions was 94.34±0.225 mg GAE/g DW. The optimal MAE conditions were 210W microwave power, 15 minutes exposure time and 40 mL/g solvent to material ratio, using methanol as a solvent.

In anoother study, Kaderides *et al.* (2019) determined the TPC of pomegranate peel extract using microwave extraction. The optimum operating conditions of extraction were: solvent type - 50% ethanol; solvent:solid ratio of 60:1 (mL:g); microwave power600 W and 4 minutes extraction time. The extract had a maximum yield of the total polyphenols of 199.4 mg GAE/g DW.

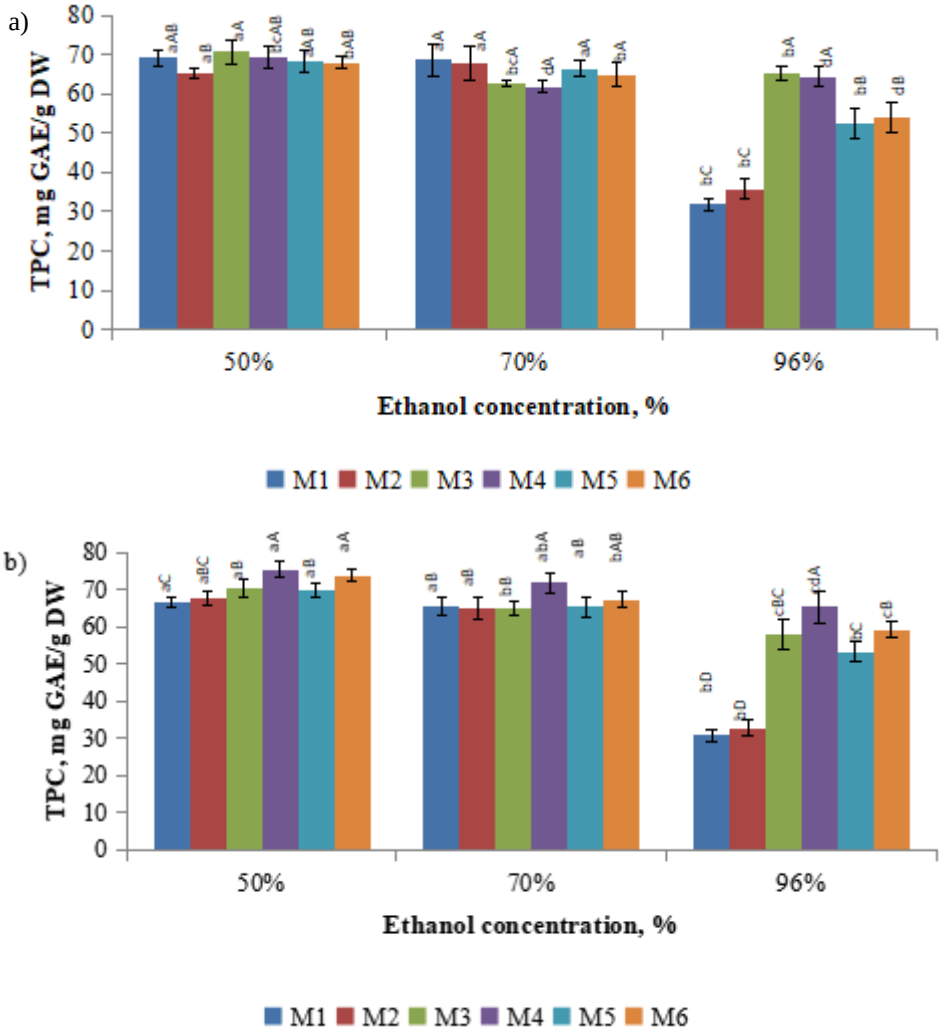

Figure 3. The content of total phenolics (TPC) of the red onion skins extract obtained by MAE under different conditions of solvent concentrations, acids, and extraction times at 315 W (a) and 735 W (b). (M1-glacial acetic acid, 10 s; M2- glacial acetic acid, 15 s; M3- 0.1N hydrochloric acid, 10 s; M4- 0.1N hydrochloric acid, 15 s; M5-99.5% citric acid,10 s; M6- 99.5% citric acid,15 s). Bars of the same color with different lowercase letters from different groups with different ethanol concentration are significantly different at $p<0.05$. Bars of different color with different uppercase letters from the same group with the same ethanol concentration are significantly different at $p<0.05$.

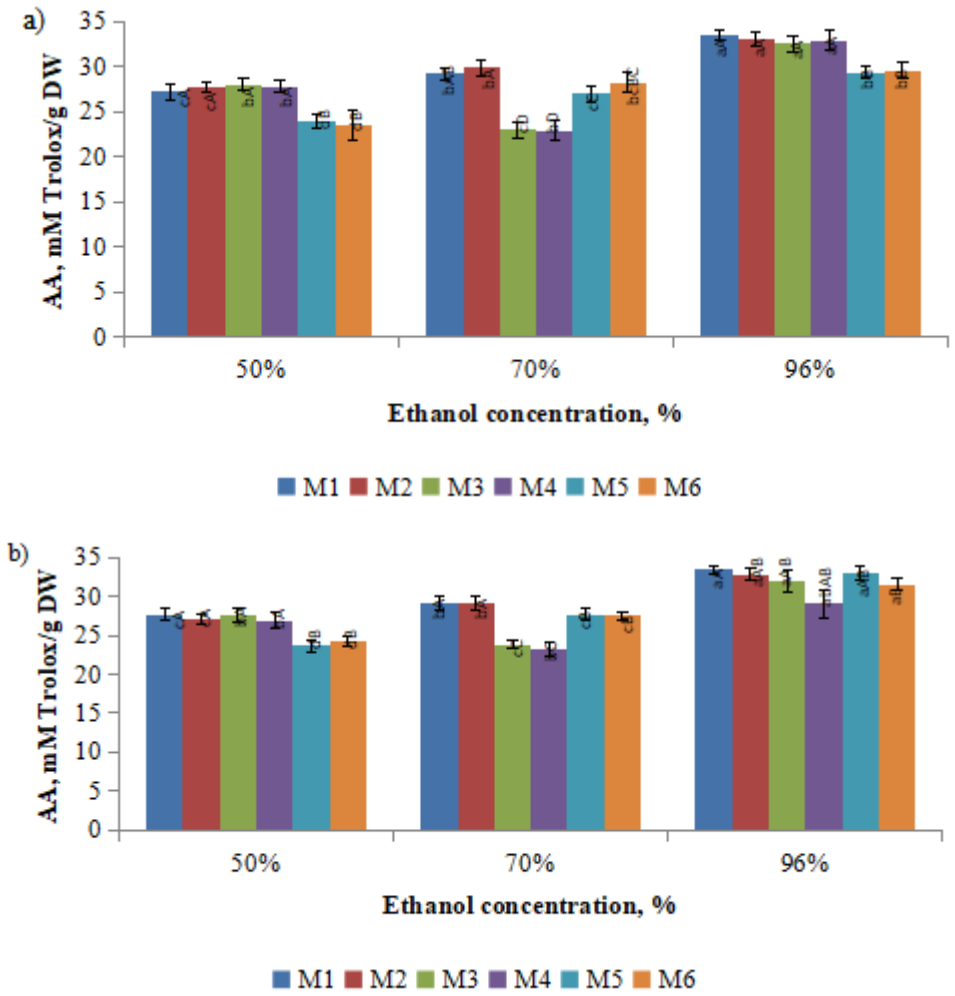

Figure 4. The DPPH radical-scavenging activity (AA) of the red onion skins extract obtained by MAE under different conditions of solvent concentrations, acids, and extraction times at 315 W (a) and 735W (b). (M1- glacial acetic acid, 10 s; M2- glacial acetic acid,15 s; M3- 0.1N hydrochloric acid, 10 s; M4 - 0.1N hydrochloric acid, 15 s; M5- 99.5% citric acid, 10 s; M6- 99.5% citric acid,15 s). Bars of the same color with different lowercase letters from different groups with different ethanol concentration are significantly different at $p<0.05$. Bars of different color with different uppercase letters from the same group with the same ethanol concentration are significantly different at $p<0.05$.

Additionally, Pal and Jadeja (2019) determined the TPC of 80.45 mg GAE/g DW, for red onion skins extracts using an optimized MAE. For this extraction a green solvent (choline chloride:urea:H_2O in the molar ratio of 1:2:4) was used. The

optimum parameters of the extraction were 100 W microwave power, 15 minutes of exposure time, and liquid to solid ratio of 54.97 ml/g. In anoother study, Piovesan *et al.* (2017) stated that the highest TPC of 113.38±4.3 mg GAE/g dried fruit from blueberry extracts was obtained with 80% ethanol at 60 °C using microwave extraction (20 minutes microwave exposure and 800 W microwave power).

Antioxidant activity

The AA of red onion skins extract was determined using the DPPH• radical scavenging assay. Overall, it can be observed in Figure 4a that MAE with 96% ethanol and glacial acetic acid had the optimum yield of AA of 33.41±0.59 mM of Trolox/g DW after 10 seconds of exposure at315 W. However, for this type of extraction the lowest values of AA (22.89±1.14 mM of Trolox/g DW) were obtained for the extraction with 70% ethanol acidified with 0.1 N hydrochloric acid and 315 W microwave power for 15 seconds exposure time.

In another study, Shaileyee and Subhash (2015) reported a MAE method for extraction of DPPH scavenging activity from onion skins extract. The AA of the extract was 92.25 % expressed as inhibition rate. The optimal MAE conditions were 210W microwave power, 15 minutes exposure time and 40 mL/g solvent to material ratio, using methanol as solvent. Kaderides *et al.* (2019) reported an AA of pomegranate peel extract under the following optimum conditions: solvent type-50% ethanol; solvent:solid ratio-60:1 mL/g; microwave power-600 W, 4 minutes extraction time. The extract showed a high AA of 94.91%, expressed as inhibition rate. Moreover, Piovesan *et al.* (2017) evaluated the AA from blueberry extracts, and the highest AA of 8.46±0.4mM of Trolox/g DW was obtained using 60% ethanol at 60 °C and 800W, 20 minutes exposure time.

The anthocyanins profile determination by high performance liquid chromatography analysis

Among the polyphenolic compounds, anthocyanins represent the most used antioxidants class due to their various applications in the food and pharmaceutical industry and their well-known effects on human health. In order to determine the anthocyanins' chromatographic profile for the red onion skins extract, a HPLC analysis was performed and the results are shown in Figure 5.

The anthocyanin profile from red onion skins extract obtained by CSE was determined by HPLC analysis. The red onion skins extract obtained by CSE was chromatographically analyzed because it had shown the highest anthocyanin content of 1.75±0.04 mg C3G/g DW, achieved with the optimal parameters of 70% ethanol and 0.1N hydrochloric acid, after 2 hours of extraction at 25 °C. The identification of the anthocyanin compounds was achieved at the wavelength of 520 nm based on the retention time (RT) and by comparison to the available standards (cyanidin-3-rutinoside), as well as the existing data in the specialized literature.

In regard to the anthocyanin profile of the extract obtained through the CSE technique, the chromatographic profile revealed the presence of several

compounds, out of which 8 compounds were identified as follows: Peak (1) – cyanidin 3-glucoside; Peak (2) –cyanidin 3-laminaribioside; Peak (3) –cyanidin 3-(3"-malonylglucoside); Peak (4)–peonidin 3-glucoside; Peak (5) –cyanidin 3-(6"-malonylglucoside); Peak (6)–cyanidin 3-(6"-malonyl-laminaribioside); Peak (7)–peonidin 3-malonylglucoside and Peak (8)–cyanidin 3-dimalonylaminaribioside, as previously reported by many authors (Donner *et al.*, 1997; Downes *et al.*, 2009; Perez-Gregorio *et al.*, 2010).

The two major compounds from red onion skins extract were cyanidin 3-laminaribioside and cyanidin 3-(6"-malonyl-laminaribioside). For the red onion skins extract obtained by CSE, the two compounds displayed a content of 27.25% and 38.65% of the total anthocyanin content. Peonidin 3-malonylglucoside, as the third major compound, showed a content of 9.13% of the total anthocyanin content, in the case of the red onion skins extract obtained by CSE. Furthermore, all the other identified compounds exhibited contents lower than 8% of the total anthocyanin content.

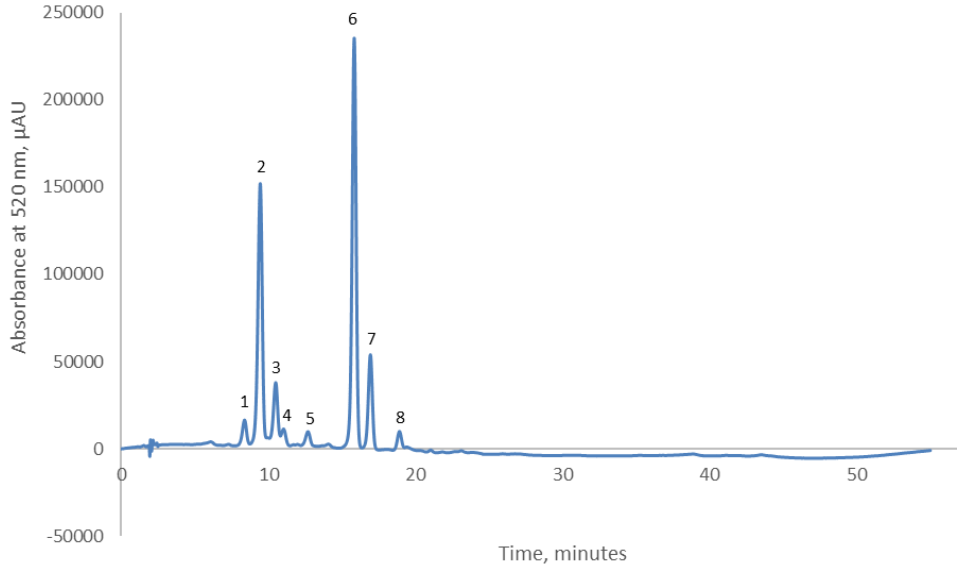

Figure 5. Chromatographic profile of the anthocyanins from the red onion skins extract obtained by a conventional technique: Peak (1) cyanidin 3-glucoside; Peak (2) cyanidin 3-laminaribioside; Peak (3) cyanidin 3-(3"-malonylglucoside); Peak (4) peonidin 3-glucoside; Peak (5) cyanidin 3-(6"-malonylglucoside); Peak (6) cyanidin 3-(6"-malonyl-laminaribioside); Peak (7) peonidin 3-malonylglucoside and Peak (8) cyanidin 3-dimalonylaminaribioside.

This results are in agreement with Perez-Gregorio *et al.* (2010), who used HPLC-DAD detection for the analysis of anthocyanins from red onion and the extraction solvent was methanol:formic acid:water (50:5:45 v/v).The authors identified cyanidin 3-(6"-malonylglucoside) and cyanidin 3-glucoside as the major anthocyanins, the same as the two major compounds found in our red onion skins

extract. Moreover, Perez-Gregorio *et al.* (2010) identified 8 peaks that were isolated in red onions with the spectral properties of anthocyanins. Based on the results and the scientific literature, the identities of these 8 compounds were determined as cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-(3"-malonylglucoside), peonidin 3-glucoside, cyanidin 3-(6"-malonylglucoside), cyanidin 3-(6"-malonyl-laminaribioside), peonidin 3-malonylglucoside and cyanidin 3-dimalonylaminaribioside. The identities of these 8 compounds were in agreement with those found by Donner *et al.* (1997). Other authors identified delphinidin or petunidin derivatives, although they were found at very low levels as regards cyaniding (Gennaro *et al.*, 2002). More than 20 derivatives of anthocyanins were identified in red onions (Slimestad *et al.*, 2007). Cyanidin glucosides and acylated glucosides of cyanidins are the main anthocyanins in red onions (Donner *et al.*, 1997).

In another study carried out by Downes *et al.* (2009), they used methanol:water:hydrochloric acid in a ratio 70:29.5:0.5 (v/v/v) to extract anthocyanins from red onion skins. The authors reported that the major anthocyanins found in the skins of red onion (*Red Baron* cultivar) were cyanidin 3-(6"-malonylglucoside) and cyanidin 3-glucoside. The mobile phase consisted of HPLC grade water with 2.5% acetonitrile and 5% formic acid. These two anthocyanins were also dominant in the dry outer skins of red onion (*Red Jumbo* cultivar) and the flesh of red onion (*Red Baron* cultivar) (Donner *et al.*, 1997).

In a study conducted by Wu and Prior (2005), the HPLC chromatogram of the major anthocyanins found in the skins of red onion (*Red Baron* cultivar) contained ten anthocyanins such as (1) cyanidin 3-(malonoyl)-glucose-5-glucose; (2) cyaniding 3-glucose; (3) cyanidin 3- laminariboside; (4) cyanidin 3-(3"-malonoylglucoside); (5) peonidin 3-glucose; (6) cyanidin 3-(3"-acetoyl)glucoside; (7) cyanidin 3-(6"-malonoylglucoside); (8) cyanidin 3-(6"-malonoyl-laminariboside); (9) peonidin 3-(malonoyl)glucoside; (10) cyanidin 3-(malonoyl)(acetoyl)glucoside. The major anthocyanin compound found was (7) cyanidin 3-(6"-malonylglucoside), which is consistent with our findings.

The anthocyanins extracted from a red onion (*Tropea* cultivar) were analysed by HPLC-MS (Gennaro *et al.*, 2002). The authors found the following compounds: cyanidinglucoside; cyanidin (glucosylglucoside); delphinidin (glucosylglucoside); petunidin (glucosylglucoside); cyanidin (malonylglucoside); cyanidin (malonylglucosylglucoside); cyanidinaglycon; delphinidin 3-glucoside; petunidinglucoside and delphinidinaglycon. Gennaro *et al.* (2002), also stated that the major anthocyanins compound found was cyanidin 3-(6"-malonylglucoside), the same as in our study, and that the derivatives of mono- and diglucosylatedcyanidin, with or without a malonyl moiety, account for >50% of the total anthocyanin content.

Conclusions

Onion (*Allium cepa L.*) is a common vegetable crop, being cultivated and largely consumed all over the world. It is one of the major sources of various biologically

active compounds such as phenolic acids, flavonoids, organosulfur compounds, dietary fibres that have many health benefits. The results reported in this paper are focused on two different methods (CSE and MAE) used for the extraction of biologically active compounds found in red onion skins. The extracts were evaluated in terms of TPC, TFC and TMA contents and their AA.

The results showed that for the CSE method 70% ethanol acidified with 0.1N hydrochloric acid was the best combination for the extraction of biologically active compounds. In terms of the anthocyanin profile, in the red onion skins extract obtained by CSE, a number of eight anthocyanins were separated, with cyanidin 3-(6"-malonyl-laminaribioside) being the most abundant.

Our results revealed that CSE under optimum conditions can be considered the most efficient method for the extraction of bioactive compounds from red onion skins. Therefore, the skin of red onion may be considered as a new source of biologically active compounds useful in the pharmaceutical and the food sector.

Acknowledgments

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