

PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF GREEN-SOLVENTS-BASED EXTRACTS OF RED ONION WASTES

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ABSTRACT

Onion represents one of the most important crop, based on its production, consumption and high pharmacological potential supported by its chemical composition. Phytochemicals of phenolic structure, in particular anthocyanins, are among the strongest antioxidant compounds with large biological and industrial applications. Because their conventional extraction involves the use of high amounts of polar organic solvents, there is a strong requirement for development of new strategies based on greener solvents. The aim of the present paper was to extract valuable compounds of polyphenolic structure from red onion wastes using deep eutectic solvents, as green strategy for their isolation. In addition, the total antioxidant activity as measured by ferric reducing antioxidant capacity assay was investigated. Extraction performed at 40°C in the selected solvent system was optimized for two parameters, solvent/sample ratio and extraction time. The obtained results showed the highest content of anthocyanins and antioxidant activity at a solvent/sample ratio of 30/1 and the extraction time of 90 min, at 40°C. Regarding the total phenolics and flavonoids, the optimal extraction parameters were 20/1 solvent/sample ratio and 90 min, at 40°C. Compared to conventional extraction in organic solvent, the values of the antioxidant activity were similar, while those of the content of bioactive compounds were higher in extracts obtained in ethanol solution. By further optimization of the extraction process, the obtained products may find useful application in obtaining natural ingredients intended for different purposes.

Keywords: *Red onion wastes, deep eutectic solvents, phenolics, anthocyanins, antioxidant activity.*

INTRODUCTION

Onion is an important crop, proved by its significant production, consumption and, not at least, potential valorization through extraction of biologically active compounds, such as fructo-oligosaccharides, minerals, fiber, and flavonoids. Apart the edible parts of onions, a lot of wastes, *e.g.* skins, outer fleshy scales and roots,

are produced mainly during industrial processing. As considerable non-edible parts of onions are generated, research has been focused on the potential valorization of its components for the development of bioactive ingredients. Phytochemicals of different chemical structures may provide human health benefits. The extraction of such molecules from various plant matrices is the first and most significant step for obtaining effective and safe final products. The most efficient extractive technology is that one which generates high amounts of targeted compounds, preserves as much as possible their biological activity and provides no negative environmental impact. Phytochemicals of phenolic structure, in particular anthocyanins, are among the strongest antioxidant compounds with great technological applicative potential (Kowalczyk *et al.*, 2003). Traditionally, they are extracted by using polar organic solvents or acidified solvent solutions which favor the stabilization of the red flavylum cation (Giusti and Wrolstad 2001; Revilla *et al.*, 1998). Several extraction parameters, such as solvent, solvent/sample ratio, time, temperature, and pH have been optimized such as to recover high amounts of anthocyanins, but also to avoid their degradation (Castañeda-Ovando *et al.*, 2009). The use of large amounts of organic solvents for the extraction of natural products may generate a negative environmental impact and may lead to small impurities in the final extract. Modern extraction techniques have been investigated and reported, such as ultrasound-assisted extraction (UAE) (Vinatoru, 2001), pressurized liquid extraction (PLE) (Ju and Howard, 2003; Feuereisen *et al.*, 2017) and supercritical fluid extraction (SPE) (Bleve *et al.*, 2005; Maran *et al.*, 2014). The current requirement for decreasing the impact of solvents moved the scientific research and the industry strategies toward new greener solvents, with low cost, reduced energy consumption, high solute solubility, selectivity and environmental compatibility. Deep eutectic solvents which are mixtures of compounds with lower melting points have been proposed as alternative solvents for green extraction being also biodegradable and having pharmaceutical accepted toxicity (Abbott *et al.*, 2007). Particular compositions of such solvents provide not only acceptable extractability but also the ability to stabilize some compounds with limited stability under various conditions, such as anthocyanins (in particular cyanidin) (Dai, 2013). The aim of the present paper was to get optimal extraction of phenolic compounds (anthocyanins, phenolics, flavonoids) under different parameters (solvent/solid ratio, extraction time) by using deep eutectic solvents. Solid red onion wastes (dry skins) were selected as potential chip source of valuable compounds, in particular red pigments (anthocyanins). In addition, the total antioxidant activity as measured by ferric reducing antioxidant capacity FRAP was determined.

MATERIALS AND METHODS

Plant material and chemical reagents: Commercially red onions (*Allium cepa* L.) were purchased from Romanian local market. Only dry skins were collected and grounded into powder before extraction processes (Grindomix GM 200, Retsch, Germany). The moisture content was determined at 105°C using the moisture

analyzer (MAC 210/NP Radwag, Poland). Chemical reagents of analytical grade were used.

Extraction procedure: Deep eutectic solvents, 1, 2-propanediol, choline chloride and water were prepared at a molar ratio of 1/1/1 as described by Dai (Dai *et al.*, 2013). The following extraction parameters were applied: solvent/sample ratio (30/1 and 20/1) and three predetermined extraction times (30, 60 and 90 min) at 40°C. Similar experiments were run with 70% (V/V) ethanol solution, for comparison. Further, samples were centrifuged at 4000 rpm for 10 min, filtered on 0.45 µm cellulose acetate membrane filters and diluted with water. The prepared crude extract was used for the determination of phenolics, anthocyanins, flavonoids, and total antioxidant activity.

Total phenolics: The content of total phenolics was determined spectrophotometrically according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). The Specord 200Plus UV-Vis spectrophotometer (Analytik Jena, Germany) was used. The results were expressed in milligram of gallic acid equivalents per 100 g dry mass (mg GAE 100g⁻¹ DM).

Total anthocyanins: The content of total anthocyanins was determined spectrophotometrically by the pH differential method (Giusti and Wrolstad, 2001). The content was expressed as milligram cyanidin-3-O-glucoside (Cyn-3-O-G) equivalents per 100 g dry mass (mg 100g⁻¹ DM).

Total flavonoids: The content of total flavonoids was determined using the aluminum chloride colorimetric method (Kumar *et al.*, 2008). The content was expressed as milligram quercetin equivalents per 100 g dry mass (mg 100g⁻¹ DM).

Antioxidant assay using Ferric Reducing Antioxidant Power (FRAP): The total antioxidant activity of crude extracts was determined by the ferric reducing ability assay described by Benzie (Benzie and Strain, 1996). The results were expressed as milligram ascorbic acid per 100 g dry mass (mg 100g⁻¹ DM).

Statistical analysis: Data presented are the average of two replicates, expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The choline chloride-based eutectic solvent (choline chloride/1,2-propanediol/water) was tested for extraction of the main antioxidant compounds of polyphenolic structure from red onion wastes. 1,2-propanediol is widely used as solvent in pharmaceutical formulations, cosmetics and food products. In the solvent system of organic salt, 1,2-propanediol acts as hydrogen donor while water is added for the decrease of viscosity. The mixture has a melting point lower than the individual components. The presence of many –OH groups, polarity and viscosity of deep eutectic solvents favors the extraction of bioactive compounds of phenolic structure due to the formation of H-bonds (Dai *et al.*, 2013). In order to further decrease the viscosity, extraction was conducted at 40°C. In our study, water was added to choline chloride and 1,2-propanediol (9% V/V) as it increases the extraction efficiency; it has been shown that higher water content (>50%) drastically decreased the yield of extraction probably due to the rupture/weakening

of the interactions between deep eutectic solvents and bioactive compounds (Bi *et al.*, 2013). The contents of the main antioxidant compounds extracted from red onion skins using choline chloride/1,2-propanediol/water are presented in Figures 1-3. As noticed, extractability of the investigated compounds increased with time of extraction, from 30 to 90 min., at 40°C. No significant difference was found between solvent/solid ratio regarding the content of total anthocyanins. However, mean values showed that 30/1 might be more efficient. Phenolic compounds were extracted with highest yield using a solvent/sample ratio of 20/1 and extraction time of 90 min. Similar results were obtained for flavonoids.

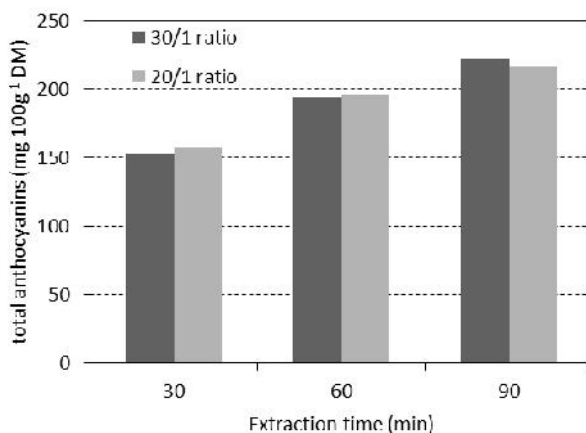


Figure 1. Total anthocyanins content of the crude extract of red onion skins in deep eutectic solvent according to different parameters.

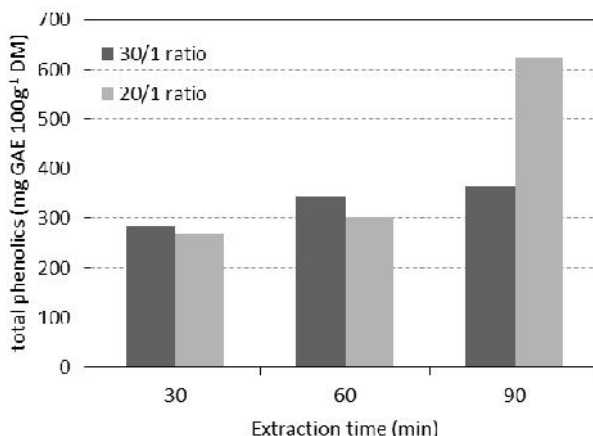


Figure 2. Total phenolics content of the crude extract of red onion skins in deep eutectic solvent according to different parameters.

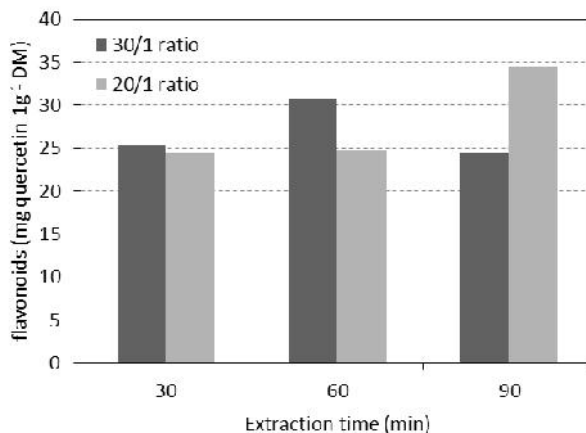


Figure 3. Total flavonoids content of the crude extract of red onion skins in deep eutectic solvent according to different parameters.

The contents of total anthocyanins and flavonoids were four time higher in extracts prepared under similar conditions but with conventional solvent (70% ethanol solution), while significant differences were found between total phenolics in extracts with deep eutectic solvents and ethanol solution. This might be due to the lower polarity of polyalcohol in the prepared deep eutectic solvent compared to other hydrogen donors, such as organic acids which may also improve extraction of some compounds (anthocyanins) due to the low pH.

To our knowledge there is no report on extraction of anthocyanins from red onion skins with the aid of deep eutectic solvents and on the evaluation of their antioxidant activity. However, literature reports the use of deep eutectic solvents based on other hydrogen donors (sugars, organic acids) which were tested for anthocyanins extraction from wine lees (Bosiljkov *et al.*, 2017). These authors found a promising combination of using natural deep eutectic solvents with ultrasonication for an efficient extraction of anthocyanins. Regarding phenolic compounds, the study of Fernández *et al.* showed that individual phenolics (caffeic acid, rutin, quercetin, tyrosol) were extracted from dry scapes and umbels from onion seed production using lactic acid/glucose/water assisted by ultrasonication (Fernández *et al.*, 2017).

The results regarding the total antioxidant activity of the crude extracts as measured by FRAP assay are presented in Figure 4.

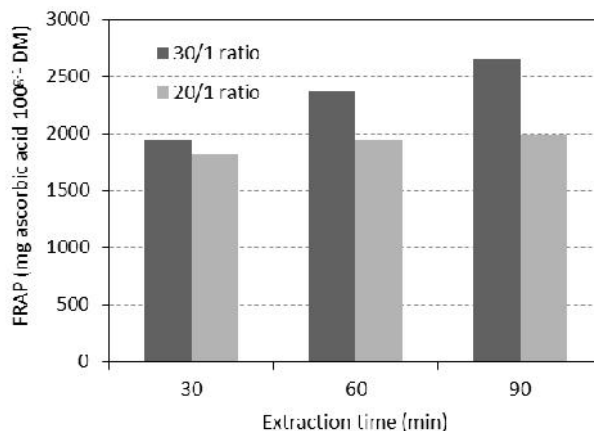


Figure 4. Ferric reducing antioxidant power (FRAP) of the crude extract of red onion skins according to different parameters.

The mean value of FRAP activity obtained using deep eutectic solvents (2120.09 mg ascorbic acid 100g⁻¹ DM) was similar to that of extracts under conventional extraction with 70% ethanol solution (2781.13 mg ascorbic acid 100g⁻¹ DM) highlighting the great potential of these solvents for eco-friendly extraction of plant antioxidants compounds. However, special attention has to be paid to practical aspects of deep eutectic solvents extraction and there is still room for further research in this area.

CONCLUSIONS

Horticultural and industrial wastes generated from red onion processing contain valuable compounds which may be exploited as ingredients for various purposes. Such approach of managing wastes was hereby combined with the testing of a green extraction technology of polyphenolic-based compounds.

The results of the study of the influence of extraction parameters showed that increased time (90 min) gave efficient extraction yield of bioactive compounds at 40°C. Promising results were obtained regarding the total antioxidant activity of the crude extracts, which were similar to those obtained under conventional extraction with ethanol solution (30/1 ratio, 90 min, 40°C).

Deep eutectic solvents emerged as new green solvents with promising results on recovery of important compounds from plant materials. Nevertheless, future research is required to evaluate different compositions of deep eutectic solvents such as to increase the extractability of anthocyanins, phenolics or flavonoids from red onion wastes compared to conventional organic solvents.

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