

# THE EXTRACTION OF QUERCETIN FROM WASTE ONION (*Allium Cepa* L.) TUNIC BY THE AQUEOUS SOLUTIONS OF DIFFERENT DEEP EUTECTIC SOLVENTS

Biljana S. Đorđević\*, Zoran B. Todorović, Dragan Z. Troter, Ljiljana P. Stanojević, Vlada B. Veljković

University of Niš, Faculty of Technology, Leskovac, Serbia

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In order to find the most efficient natural solvent for the extraction of quercetin, its solubility was tested in the selected deep eutectic solvents (DESs) such as choline chloride:urea (ChCl:U), choline chloride:glycerol (ChCl:G), citric acid:D-glucose (CA:Glc), citric acid:D-fructose (CA:Frc), lecithin:urea (Lec:U) and lecithin:glycerol (Lec:G), as well as in their aqueous solutions, in a temperature range of 6-75 °C. Quercetin was most successfully dissolved in CA:Frc, followed by ChCl:U and ChCl:G, while CA:Glc was the least efficient. The lecithin-based DESs with urea and glycerol did not dissolve quercetin. The most optimal DES (ChCl:U) was employed in the extraction of quercetin from waste onion (*Allium cepa* L.) tunic and its efficiency was compared with those of methanol and ethanol. The largest amount of quercetin was extracted with ethanol, followed by methanol and ChCl:U, but quercetin extracted with this DES contained the least amount of impurities. Therefore, the ChCl:U DES was highly recommended for the selective extraction of quercetin.

**Keywords:** deep eutectic solvent, extraction, quercetin, choline chloride, citric acid, lecithin

## Introduction

Data on the solubility of various organic compounds in different solvents are very important for their use for separation and purification. Unfortunately, the conventional extraction methods are characterized by low selectivity and the presence of the employed extracting solvent in the final product. In most cases, the solvents used for this purpose are water, ethanol or aqueous ethanol solutions, but serious problems that need to be addressed are low efficiency of the extraction because of the variable nature and polarity of extractable bioactive compounds [1]. Various nutritional compounds and pharmaceuticals are obtained by solvent extraction from plant materials. Among these compounds, flavonoids, natural polyphenolic compounds, have been mainly studied. Quercetin (3,3',4',5,7 -pentahydroxyflavanone or 3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one) is a yellow flavonol widely present in plants. It is insoluble in cold water, poorly soluble in hot water, but quite soluble in alcohols and lipids. Some of its beneficial properties include antioxidant, Gastro-protective, antihypertension, *in vitro* anticancer, antiviral and antibacterial activities, as well as *in vivo* anti-inflammatory, anxiolytic and antidepressant effects [2]. The solubility of quercetin in aqueous alcohol solutions increased with either heating or increasing mole fractions of alcohols [3]. According to Dong et al. [4], the solubility of quercetin increases with the increase of the molality of cyclodextrins and heating. Chen and Jao [5] improved the solubility of quercetin in water by using phytoglycogen, a carbohydrate polymer extracted from sweet corn.

In recent years, deep eutectic solvents (DESs) have been used in different extraction processes. DESs are usually prepared by combining organic halide salts with a complexing organic agent (usually a hydrogen bond donor) in proper ratios, and are liquid at temperatures below 100 °C [6]. Beneficial properties of DESs are easy preparation, high purity, low cost, non-volatility at ambient condition, chemical and thermal stability, non-flammability, non-toxicity, biodegradability and good solubility of several organic compounds [7-8]. In addition, DESs produced from exclusively natural products, so-called natural deep eutectic solvents, show the potential to replace conventional organic solvents [9-11]. Choi et al. [12] hypothesized that DESs have a purpose of being a liquid phase for solubilizing, storing, and transporting non-water soluble metabolites in living cells and organisms. Some DESs were used extensively for the extraction of different compounds found in plants [13-17] and animals [18,19]. In order to propose a proper DES and to design an optimized production process, it is necessary to know the solubility of quercetin in different DESs.

In the present study, solubility of quercetin in different DESs, namely choline chloride:urea (ChCl:U), choline chloride:glycerol (ChCl:G), citric acid:D-glucose (CA:Glc), citric acid:D-fructose (CA:Frc), lecithin:urea (Lec:U) and lecithin:glycerol (Lec:G) and their aqueous solutions over the temperature range of 6-75 °C were determined. The most optimal DES was employed in the extraction of quercetin from waste onion (*Allium cepa* L.) tunic and its efficiency was

\*Author address: Biljana S. Đorđević, Faculty of Technology, University of Niš, Bulevar oslobođenja 124, 16000 Leskovac, Serbia  
E-mail: djbiljana89@gmail.com  
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compared with those of methanol and ethanol.

## Experimental

### Materials

Choline chloride (ChCl) (Sigma Aldrich,  $\geq 98\%$ ), urea (Zorka, Šabac, Serbia, 99.5%), glycerol (MeiLab Belgrade, Serbia, Ph Eur grade), citric acid monohydrate (Zorka, Šabac, Serbia, 99.5%), D-glucose (MosLab, Belgrade, Serbia, 99.0%), D-fructose (MosLab, Belgrade, Serbia, 99.0%), lecithin from soybean (Tokyo Chemical Industry, Japan, 99.0%), methanol (Zorka-Pharma, Šabac, Serbia, 99.5%) and absolute ethanol (Sigma Aldrich, St. Louis, USA, 99.5%) were used. Quercetin in a powder form was provided from Merck Chemicals Ltd. (Notingham, United Kingdom). Waste onion tunic was obtained from a local farm near Leskovac (Serbia).

### Preparation of DESs

The selected components for the preparation of DES were mixed in a round-bottomed flask. The flask was then placed on a rotary evaporator at 70 °C until a homogeneous liquid was formed. DESs were stored in well-closed glass bottles in a desiccator containing CaCl<sub>2</sub>. The properties of the prepared DESs at room temperature are given in Table 1. The physical and thermodynamic properties of the ChCl- and citric acid-based DESs can be found elsewhere [6,20].

**Table 1.** Prepared DESs and their visible properties at room temperature

Abbreviation	DES	Molar ratio (mol/mol)	Visible properties of DESs at room temperature
ChCl:U	Choline chloride:urea	1:2	Liquid, viscous, homogeneous, colorless
ChCl:G	Choline chloride:glycerol	1:2	Liquid, viscous, homogeneous, colorless
CA:Glc	Citric acid:D-glucose	1:1	Liquid, viscous, homogeneous, light yellow
CA:Frc	Citric acid:D-fructose	1:1	Liquid, viscous, homogeneous, dark brown
Lec:U	Lecithin:urea	1:2	Liquid, highly viscous, homogeneous, dark brown
Lec:G	Lecithin:glycerol	1:2	Liquid, highly viscous, homogeneous, dark brown

### Solubility of quercetin in different aqueous solutions of DESs

Pure DESs and their aqueous solutions at different concentrations of 25, 50 and 75 vol% were used. First, 10 mg of quercetin and 5 ml of the chosen solvent (water, DES or aqueous solution) were placed in a flask equipped with a magnetic stirrer, which was heated in a thermostated chamber at 25 °C for 1 h. After stirring, the mixture was kept in a refrigerator overnight at 6 °C in order to establish equilibrium, and the samples from the mixture were taken at 6, 25, 50 and 75 °C. Specified temperatures were achieved by heating in a water bath. The solubility of quercetin was determined by HPLC analysis and the concentration of quercetin (mg/ml) in

aqueous solutions DESs was read with a pre-prepared calibration curve for the quercetin standard.

Extraction of quercetin from waste onion (*Allium cepa* L.) tunic

Dried and powdered onion tunic (1 g) was combined in the flasks with 10 ml of methanol, ethanol or ChCl:U. After the initial shaking, a sample was taken from the solutions. Then, the flask was placed in a thermostated chamber at 30 °C and stirred with a magnetic stirrer. The samples were collected at different time intervals (5 min, 1 h, 7 h, 19 h, 25 h, 31 h and 69 h). The concentration of quercetin and quercetin-4'-O-monoglucoside were calculated from corresponding peak areas obtained by HPLC analysis by using calibration curves.

### Analytical methods

The extracts were analyzed by an Agilent 1100 Series chromatograph equipped with a degasser, a binary pump, a thermostated column (Zorbax Eclipse XDB-C18, 4.6 × 150 mm, 5 μm) and a UV/VIS detector. The samples were dissolved in methanol. All solvents and samples were filtered by a 0.45 μm Millipore filter. The volume of the injected sample was 20 μl. The separation was performed at the 1 ml/min flow rate of a binary mixture of 1% aqueous formic acid solution (solvent A) and methanol (solvent B) with a linear gradient of 70% B of 8.5 minutes. The column temperature was 30 °C. The components (quercetin and quercetin-4'-O-monoglucoside) were detected at 370 nm.

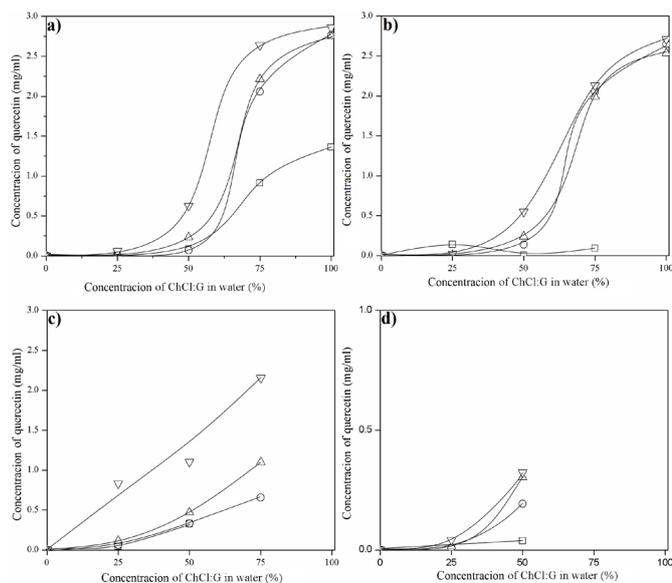
## Results and discussion

### Solubility of pure quercetin in aqueous solutions of different DESs

Figure 1a-d shows the solubility of quercetin in the choline chloride- and citric acid-based DESs and their aqueous solutions at different temperatures (6, 25, 50 and 75 °C). As expected, at all applied temperatures the solubility of quercetin in water was very sparing, close to zero. With ChCl:G, the highest solubility was achieved at the highest temperature (Figure 1a). The solubility of quercetin in the 25% and 50% aqueous solutions of ChCl:G at 6 and 25 °C was very low at all applied temperatures, but it gradually increased with increasing the amount of the DES, so the solubility of quercetin in the 75% aqueous solution was 1.0 mg/ml, while the solubility of quercetin in the pure DES was 1.3 mg/ml. However, the solubility of quercetin in the 75% aqueous solution and in pure ChCl:G at 25 °C increased abruptly, reaching the value of 2.0 and 2.7 mg/ml, respectively. The solubility of quercetin at 25 and 50 °C was similar. When the 50% and 75% aqueous solutions of ChCl:G were used, the solubility was only slightly higher at 50 °C, while the solubility of quercetin in the pure DES at 25 and 50 °C was the same. The solubility of quercetin in the 50% and 75% aqueous ChCl:G solutions at 75 °C was 0.6 and 2.6 mg/ml, respectively. In the pure ChCl:G, the solubility of quercetin had the maximum value of 2.8 mg/ml. When

the quercetin solubility in the aqueous ChCl:U solutions was tested (Figure 1b), the results differed from those achieved with ChCl:G, but the maximum solubility of quercetin was similar in both solutions. At 6 °C, ChCl:U was very viscous, so the measurement of solubility was impossible. At 25, 50 and 75 °C, the solubility of quercetin was approximately almost the same for all aqueous solutions of ChCl:U. However, only when the 50% solution of ChCl:U was employed, a difference in the solubility of quercetin was noticed. At 25 and 75 °C, the solubility of quercetin in all aqueous solutions of ChCl:U was 0.1 and 0.6 mg/ml, respectively. In the 75% aqueous solutions of ChCl:U, the solubility of quercetin was in the range from 1.9 to 2.1 mg/ml for all investigated temperatures.

The solubility of quercetin in pure ChCl:U at 25, 50 and 75 °C was in the range from 2.5 to 2.7 mg/ml. Since the solubility was almost the same for the temperature range of 25-75 °C, it could be concluded that in the case of ChCl:U, the solubility of quercetin rose with heating up to 25 °C, but further heating had no impact on the solubility.



**Figure 1.** The concentration of quercetin extracted by aqueous solutions of ChCl:G (a), ChCl:U (b), CA:Frc (c) and CA:Glc (d) as a function of the concentration of DES in water (%) at different temperatures (6 °C - □, 25 °C - ○, 50 °C - △ and 75 °C - ▽)

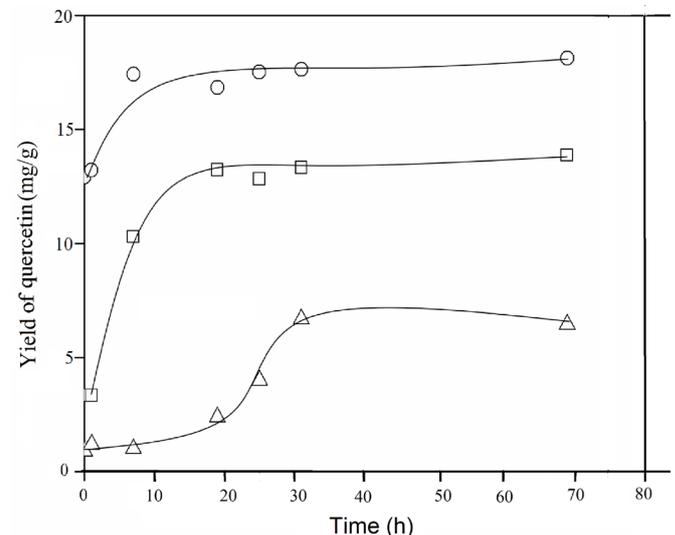
The determination of the solubility of quercetin in the pure citric-acid based DESs was impossible due to their high viscosity even at 75 °C, so the solubility of quercetin in CA:Frc and CA:Glc DESs was determined only in their aqueous solutions up to 75% and 50%, respectively (Figure 1c-d). The solubility of quercetin in the CA:Frc aqueous solutions was approximately the same for the temperature range of 6-25 °C. With increasing the amount of DES, the solubility of quercetin also increased. When the 75% solution was used, the solubility of quercetin in the citric-acid based DESs at 25 °C reached the value of 0.6 mg/ml. When the 25 and 50% solutions were used,

the solubility of quercetin in the citric-acid based DESs was almost the same at 50 °C. The solubility of quercetin in the aqueous solution of CA:Frc at 75 °C increased almost linearly with increasing the CA:Frc concentration.

The CA:Glc DES was highly viscous, so the solubility test of quercetin in those aqueous solutions could be performed only with the 25 and 50% aqueous solution. Fig. 1d shows the quercetin solubility in the CA:Glc aqueous solutions. At all applied temperatures, the quercetin solubility in all tested CA:Glc aqueous solutions was almost insignificant. The solubility of quercetin in the 75% CA:Glc aqueous solutions was 0.2, 0.3 and 0.3 mg/ml at 25, 50 and 75 °C, respectively. This eutectic mixture did not dissolve quercetin. Also, the quercetin solubility test with the lecithin-based DESs was impossible due to their viscosity.

#### Extraction of quercetin from waste onion tunic

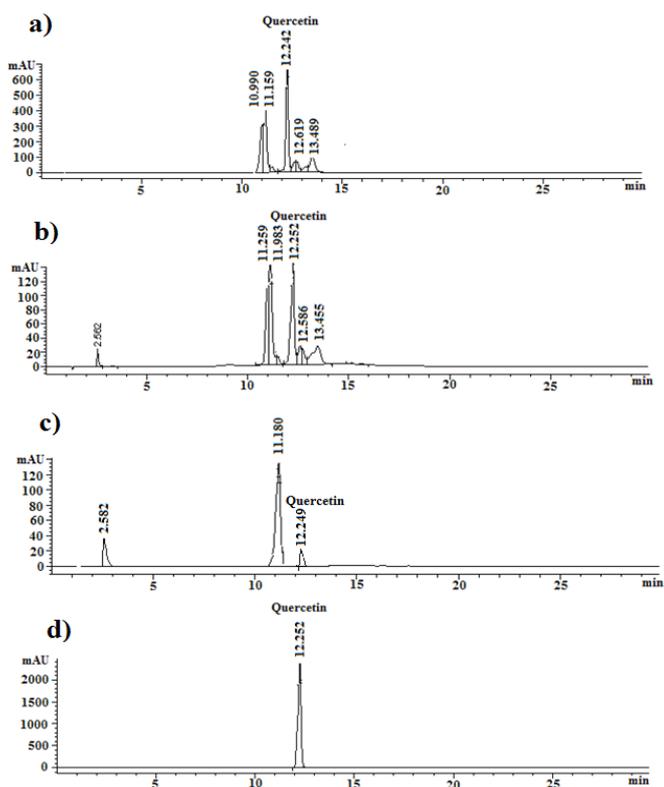
Solvents, such as methanol and ethanol, are commonly used for the extraction of biologically active substances from plant materials. In order to find the most suitable solvent for the selective extraction of quercetin from waste onion tunic, methanol, ethanol and ChCl:U were tested and their extraction efficiencies were compared (Figure 2). Among the tested DESs, ChCl:U was selected for the quercetin extraction because of its low viscosity and good dissolution capability.



**Figure 2.** Variations of the yield of quercetin extracted from waste onion tunic by ethanol, methanol and ChCl:U with the progress of extraction (methanol - □, ethanol - ○ and ChCl:U - △)

When ethanol was used for the flavonols extraction, the concentrations of quercetin and quercetin-4'-O-monoglucoside of 17 and 9 mg/g dry material, respectively, were reached after 8.3 h, and they remained constant afterwards. With methanol, the extracted quercetin and quercetin-4'-O-monoglucoside concentrations increased until they reached their maximum of 13 and 7 mg/g dry material, respectively, after about 16.7 h. The concentrations of extracted quercetin obtained

with ChCl:U was initially very low, but rose gradually after 12 h, reaching its maximum of 6 mg/g dry material, while the maximum concentration of quercetin-4'-O-monoglucoside of 12 mg/g dry material was achieved after 30 h and remained constant until the end of the experiment. Although these extractions were relatively long, these results have shown that the extraction with aqueous ChCl:U solutions can be successfully used to extract polyphenols under mild conditions. Many extractions of polyphenols (quercetin) were reported in the literature, but higher yields of phenolic compounds were obtained only by ultrasound-assisted and supercritical extractions. For instance, Nam et al. [21] applied ChCl:G (1:1) and CA:Glc (1:1) for the ultrasound-assisted extraction of quercetin, kaempferol, and isorhamnetin glycosides from dried *Flos sophorae*. Only de los Angeles Fernández et al. [22] used lactic acid:glucose (5:1), CA:Glc (1:1) and CA:Frc (1:1) for the ultrasound assisted extraction of phenolic compounds from by-products of the onion seed production besides by-products of olive oil, tomato and pear canning industries. The quercetin yield from onion seeds was  $2.06 \pm 0.23 \mu\text{g/g}$  dry by-product.



**Figure 3.** HPLC chromatograms of methanol (a), ethanol (b) and ChCl:U (c) extracts of waste onion tunic and the standard solution of quercetin (d)

The application of subcritical water extraction of quercetin from onion tunic provided a maximum yield of quercetin ( $16.29 \pm 0.75 \text{ mg/g}$  onion tunic) at  $165^\circ\text{C}$  and 15 min [23]. Even though the application of subcritical water extraction provided a higher quercetin yield than the extraction with aqueous ChCl:U solutions, the overall

processing costs would be much higher. HPLC chromatograms of the methanol, ethanol and ChCl:U extracts from waste onion tunic are provided in Figure 3a-c. Although conventional solvents like ethanol and methanol extracted a large amount of quercetin, the quercetin extracted with ChCl:U was less impure, as shown by the HPLC analysis. Therefore, for the selective extraction of pure quercetin, the use of ChCl:U may be highly recommended.

## Conclusions

Various deep eutectic solvents were mixed with water to enhance the solubility of quercetin at the temperature range of  $25\text{--}75^\circ\text{C}$ . In pure ChCl:G at  $75^\circ\text{C}$ , the solubility of quercetin had a maximum value of  $2.8 \text{ mg/ml}$ . The solubility of quercetin in pure ChCl:U at the temperatures of  $25$ ,  $50$  and  $75^\circ\text{C}$  was in range from  $2.5$  to  $2.7 \text{ mg/ml}$ , so the temperature of  $25^\circ\text{C}$  was selected as the most optimal. Instead of the pure citric acid-based DESs, which were highly viscous and not suitable for the extraction of quercetin, their aqueous solutions were applied. With increasing the concentration of CA:Frc in the aqueous solution at  $75^\circ\text{C}$ , the solubility of quercetin increased almost linearly, reaching the maximum value of  $2.16 \text{ mg/ml}$  in the  $75\%$  solution, while CA:Glc was the least efficient. The lecithin-based DESs were also unsuitable for the extraction of quercetin. The quercetin extraction from waste onion tunic with ethanol, methanol and ChCl:U at  $30^\circ\text{C}$  showed that their efficiency decreased in the following order: ethanol > methanol > ChCl:U. However, quercetin extracted with ChCl:U was most pure, so the use of ChCl:U was highly recommended for the selective extraction of quercetin.

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## Izvod

**EKSTRAKCIJA KVERCETINA IZ OTPADNE LJUSPE CRNOG LUKA (*Allium Cepa* L.) VODENIM RASTVORIMA RAZLIČITIH EUTEKTIČKIH RASTVARAČA**

Biljana S. Đorđević, Zoran B. Todorović, Dragan Z. Troter, Ljiljana P. Stanojević, Vlada B. Veljković

Univerzitet u Nišu, Tehnološki fakultet, Leskovac, Srbija

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U cilju pronalaska najefikasnijeg prirodnog rastvarača za ekstrakciju kvercetina, njegova rastvorljivost je testirana u odabranim eutektičkim rastvaračima (DESs), kao što su holin hlorid:urea (ChCl:U), holin hlorid:glicerol (ChCl:G), limunska kiselina:D-glukoza (CA:Glc), limunska kiselina:D-fruktoza (CA:Frc), lecitin:urea (Lec:U) i lecitin:glicerol (Lec:G), kao i u njihovim vodenim rastvorima, u temperaturnom opsegu od 6-75 °C. Kvercetin se najbolje rastvorio u CA:Frc, a zatim u ChCl:U i ChCl:G, dok je CA:Glc bio najmanje efikasan. DES na bazi lecitina sa ureom i glicerolom nije rastvorio kvercetin. Najoptimalniji DES (ChCl:urea) primenjen je u ekstrakciji kvercetina iz otpadne ljuspe crnog luka (*Allium cepa* L.), a njegova efikasnost je upoređena sa metanolom i etanolom. Najveća količina kvercetina je ekstrahovana etanolom, pa metanolom a najmanja sa ChCl:U, ali kvercetin koji je ekstrahovan ovim DES-om sadržao je najmanju količinu nečistoća. Zbog toga se ChCl:U preporučuje za selektivnu ekstrakciju kvercetina.

**Ključne reči:** eutektički rastvarač, ekstrakcija, kvercetin, holin hlorid, limunska kiselina, lecitin