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THE EXTRACTION OF HESPERIDIN FROM MANDARIN PEELS IN DEEP EUTECTIC SOLVENTS

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INTRODUCTION

Citrus peels make the largest amount of total produced citrus by-products and they can be utilized according to their bioactive compounds, especially flavonoids. Compounds found in mandarin peels, such as flavanone glycosides and polymethoxy flavones are recognized as major contributors to the biological activity of peels with hesperidin as the most abundant flavonoid and representing the main functional compound. The studies have shown that it has hypoglycemic, antioxidant and cytotoxic effect against human cancer cell lines, anti-inflammatory and antiproliferative activity.

The content of biologically active compounds can vary considering applied method and operating conditions. Therefore, it is very important to find the most efficient method to obtain selected bioactive compounds and then optimize the procedure to enable their application in the industry. In the last few years, deep eutectic solvents (DESs) showed as very efficient extraction media for obtaining different valuable compounds from various plant materials. They are characterized as green, nontoxic and cheap solvents formed of hydrogen bond acceptors (HBAs) in combination with hydrogen bond donors (HBDs).

The aim of this work was to obtain the extracts rich in hesperidin from mandarin peels of *Citrus reticulata* Blanco cultivars of four different variety including *Zorica rana, Chahara, Okitsu* and *Kuno.* The extraction was performed with 15 different DESs where HBA was choline chloride, while HBDs were compounds such as urea, acetamide, butane-1,4-diol, glycerol, citric acid, malic acid, sorbitol, xylitol, oxalic acid, levulinic acid, ethylene glycol, malonic acid, thiourea, N-methyl urea and lactic acid. The obtained extracts were analyzed by high performance liquid chromatography (HPLC) and the most suitable DES for the extraction of the highest amount of hesperidin was determined.

PLANT MATERIAL

The mandarin peels of *Citrus reticulata* Blanco cultivars of different variety (*Zorica rana, Chahara, Okitsu, Kuno*) were obtained in November 2017 from small family farm Dalibor Ujević (Opuzen, Croatia). Before extraction, the mandarin peels were dried and milled using laboratory mill (IKA M 20 Universal mill) and sieved applying a vertical vibratory sieve shaker (Retsch AS 200, Germany) for 20 min.


Okitsu

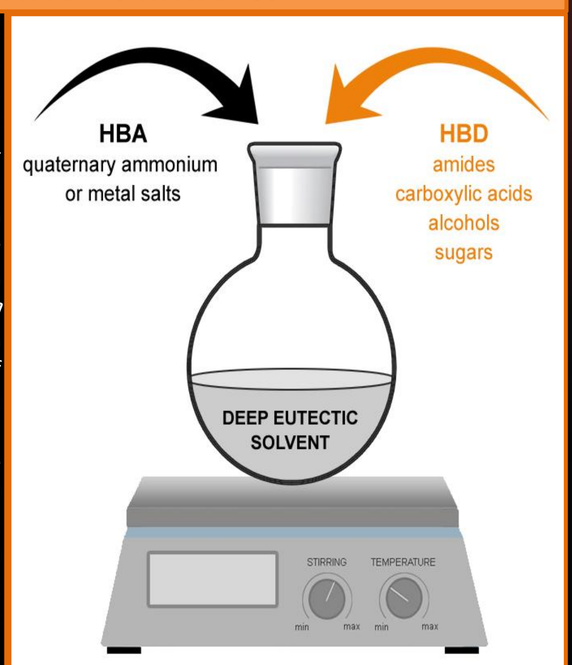

Kuno


Chahara


Zorica rana

EXTRACTION OF HESPERIDIN FROM MANDARIN PEELS WITH DESs

DESs were prepared by mixing choline chloride (ChCl) as HBA with 15 different HBDs (urea, acetamide, butane-1,4-diol, glycerol, citric acid, malic acid, sorbitol, xylitol, oxalic acid, levulinic acid, ethylene glycol, malonic acid, thiourea, N-methyl urea, lactic acid) in certain molar ratio as specified in Table 1. The each mixture was heated to 80°C under constant stirring until a stable homogeneous liquid was formed. Dried and milled mandarin peels of each variety (*Zorica rana, Chahara, Okitsu, Kuno*) were weightened (50 mg) and mixed with 1 mL of the mixture of DES with specific amount of distilled water. The mixture of DES and 20 % of added water (v/v) was then stirred at 50°C for 30 min. After the extraction, the mixture was centrifuged at 6000 rpm for 5 min and then decanted. The supernatant of 200 µL was then diluted with 800 µL methanol and filtered through a PTFE 0.45 µm filter before HPLC analysis. The extraction was performed in duplicate.



HPLC ANALYSIS

Hesperidin was determined using a RP-HPLC method on a Agilent 1260 Infinity II (Analytical Instruments, CA, USA) with chromatographic separation obtained on a ZORBAX Eclipse Plus C18 (Agilent, USA) column (100x4.6mm,5 µm) with isocratic elution of water as phase A and acetonitrile as phase B, at room temperature during 10 min. The flow rate was 1.0 mL/min, an injection volume of 20 µL was used and UV detection wavelength was 210 nm. Hesperidin standard stock solutions were prepared in the methanol and calibration was obtained at seven concentrations (20.0-200.0 mg/L). Linearity of hesperidin calibration curve was confirmed by R^2=0.99955 with the limit of detection (LOD) of 0.001062 mg/L, quantification limit (LOQ) of 0.00354 mg/L and hesperidin retention time was 4.153 minute. Results for obtained hesperidin content are given in Table 1.

RESULTS

Table 1. Hesperidin content (mg/g) in different varieties of mandarin peels (*Okitsu, Chahara, Kuno, Zorica rana*) obtained with 15 different DESs at 50°C for 30 min and 20 % (v/v) of added water

Deep eutectic solvents	Molar ratio	Hesperidin content (mg/g)			
		Okitsu	*Chahara*	*Kuno*	*Zorica rana*
Choline chloride:acetamide	1:2	102.01	68.31	88.73	112.14
Choline chloride:butane-1,4-diol	1:2	75.42	50.97	72.87	71.27
Choline chloride:glycerol	1:2	38.81	43.75	56.65	35.03
Choline chloride:citric acid	1:1	3.29	1.44	9.82	4.25
Choline chloride:malonic acid	1:1	62.68	51.22	70.74	59.19
Choline chloride:sorbitol	1:1	51.19	37.28	46.09	38.94
Choline chloride:xylitol	1:1	41.24	36.96	28.44	16.74
Choline chloride:oxalic acid	1:1	25.95	14.35	21.87	11.48
Choline chloride:levulinic acid	1:2	62.76	56.00	75.18	82.95
Choline chloride:ethylene glycol	1:1	58.84	50.66	83.73	59.00
Choline chloride:malic acid	1:1	48.08	16.42	47.34	32.07
Choline chloride:thiourea	1:1	38.76	26.84	7.32	17.62
Choline chloride:N-methyl urea	1:3	74.05	68.68	82.37	98.65
Choline chloride:lactic acid	1:2	38.76	26.84	7.32	17.62

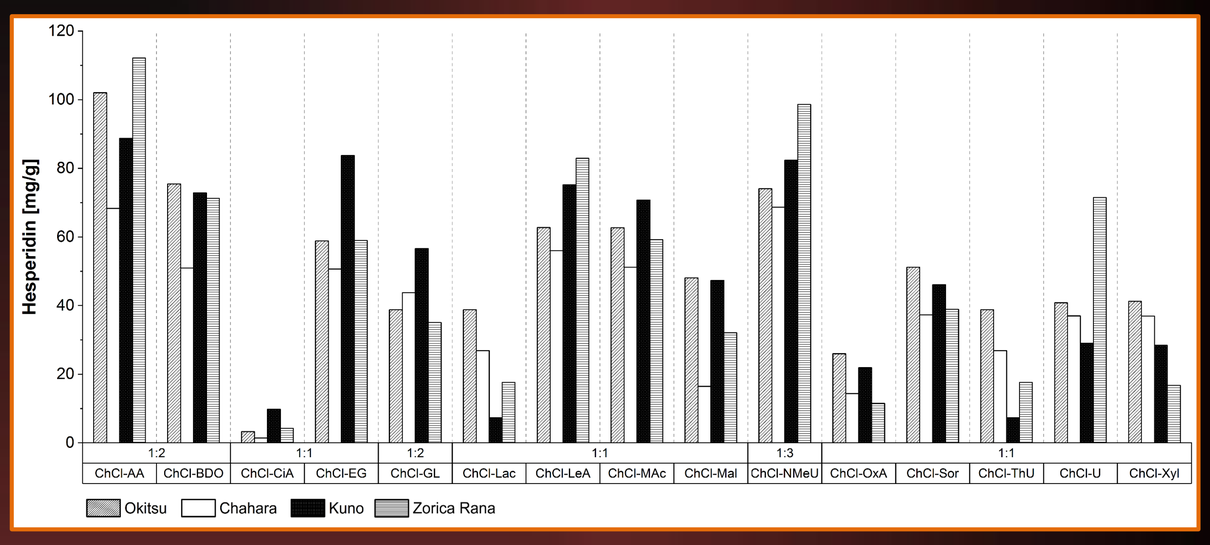


Figure 1. Comparative study of obtained hesperidin yields using different combinations of deep-eutectic solvents (DESs) comprising choline chloride (ChCl) and acetamide (AA), butane-1,4-diol (BDO), citric acid (CiA), ethylene glycol (EG), glycerol (GL), lactic acid (Lac), levulinic acid (LeA), malonic acid (MAc), malic acid (Mal), N-methyl urea (NMeU), oxalic acid (OxA), sorbitol (Sor), thiourea (ThU), urea (U), and xylitol (Xyl).

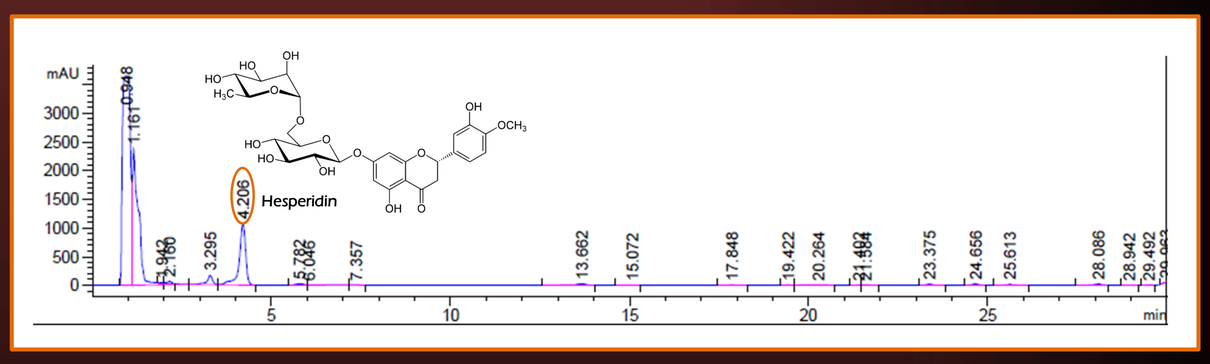


Figure 2. Chromatographic separation of hesperidin (RT 4.206) from mandarin peel variety *Zorica rana* extracted with choline chloride:acetamide by RP-HPLC

CONCLUSION

Screening showed that the best solvent was choline chloride:acetamide (1:2), followed by N-methyl urea (1:3). On the other hand, the solvent with choline chloride:citric acid (1:1) showed as the most unsuitable solvent for the extraction of hesperidin. The highest amount of hesperidin was found in variety *Zorica rana*, followed by the amount found in variety *Okitsu*. The results showed that the amounts of extracted hesperidin were completely different according to the used mandarin variety. Furthermore, application of deep eutectic solvents for the extraction exhibited a strong potential for the production of the extracts rich in bioactive compounds.

ACKNOWLEDGMENT

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