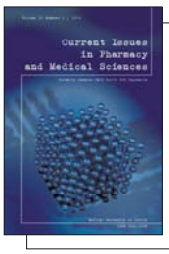

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Influence of extraction conditions on the recovery of the sum of phenolic compounds from daily food rations of students

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ABSTRACT

The aim of this study was to determine the influence of the applied extraction techniques in conjunction with the selection of an appropriate solvent system in the recovery of phenolic compounds from daily food rations of students (DFR). The study was performed on the averaged diets reconstructed on the basis of 24-dietary recall conducted among 162 randomly chosen people. It was shown that the most effective extraction technique was found to be the accelerated solvent extraction and the most optimal extrahents: mixture of acetone and water in a volume ratio of 7:3.

Keywords: extraction, polyphenols, students, diets

INTRODUCTION

Human diet should provide all necessary compounds including proteins, carbohydrates and fats which are essential for both rebuilding and energetic processes. Furthermore, supplementation with other nutrients, such as elements or vitamins, which participate in regulatory pathways is of high importance as well. In recent years many substances of plant origin which play an important role in major metabolic processes were discovered. Polyphenolic compounds belong to the components of this type, being present in the diet in the largest quantities. Numerous properties of these substances were described, including their anti-allergic action, antitumor and antioxidant properties. Many studies indicate their positive role in the prevention and treatment of cardiovascular and eye diseases, and even in the therapy of AIDS (2, 6, 7, 8). However, due to the large variety of flavonoid compounds, the quantitative analysis of phenolic compounds consumed in daily food rations (DFR) is problematic. The sum of all phenols presented as equivalents of gallic acid or (-) catechin is crucial in the estimation of daily consumption of these precious nutrients in contrast to chromatographic analysis, which enables to identify separate molecules, but does not show the total intake.

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Profound recovery of active components from food rations, although important, remains difficult. In view of the above, the choice of potent extraction technique may enable the assessment of total content of polyphenolic compounds in foods. Choice of efficient extraction techniques in combination with optimized selection of solvents systems increased the recovery of polyphenols from plant cells present in the human diet.

The aim of this study was to optimize the extraction conditions of polyphenols from daily food rations among students from Medical University in Lublin. A comparison of different extraction techniques together with chosen solvent systems in the process of total phenols amount estimation was performed. Folin-Ciocalteu method was chosen to determine the total content of polyphenols. Optimized parameters of the applied methodology allowed a detailed analysis of intake of this group of substances with student's diets.

MATERIAL AND METHODS

The study was conducted in 2012 and involved 162 randomly chosen students, 102 women and 60 men, from Medical University in Lublin. All students were volunteers; their lifestyles were characterized by moderate physical activity. The investigations were carried out using 24-hour dietary recall technique. On the basis of the information concerning qualitative and quantitative parameters of diets provided by students and using Dietetyk

2006 software average diets for both, women and men, were reconstructed. All products used to prepare food rations were from the retail market of the Lublin region. The daily rations included plate portions of main meals identical with the ones consumed by a particular individual and all other foodstuffs and beverages (including coffee, tea and juices) consumed daily. Diets duplicates were prepared according to generally accepted culinary techniques. Each average diet duplicate was homogenized and extracted.

Maceration

Three 75 g portions were weighted from each average daily diet into conical flasks with a volume of 300 mL and filled with a portion of 75 mL of methanol, methanol-water mixture (1:1 v/v) or acetone-water mixture (7:3 v/v), respectively. The mixture was extracted by 48-hour-long shaking maceration. Obtained solution was filtered and the residue poured with fresh portion of the extrahent. Action was repeated 3 times. The combined filtrates were evaporated under reduced pressure at a temperature not exceeding 40° C using rotary evaporator. Subsequently, their dry residue was dissolved in 100 mL of methanol-water (1:1 v/v). For prepared solutions total phenolic content was determined using Folin-Ciocalteau reagent.

Ultrasound-Assisted Maceration (UAE)

Three 10 g portions of daily diet were transferred into three 300 mL conical flasks and suspended in 20 mL of methanol, methanol-water mixture (1:1 v/v) and acetone-water mixture (7:3 v/v), respectively. Ultrasonic Assisted Extraction was performed for each sample at 40°C within 45 minutes. Then the extracts were evaporated and their dry residues were dissolved in 50 mL of methanol-water (1:1 v/v) solution. The obtained extracts were subjected to further analysis.

Accelerated Solvent Extraction (ASE)

Dionex ASE 100 apparatus (Sunnyvale, CA, USA) was used for the pressurized liquid extraction. Homogenized daily food rations were evaporated under reduced pressure using a rotary evaporator. From the obtained dry residue of average diets three 1.0 g samples were collected, placed in an extraction cell, placed into ASE carousel for extraction proces with three extraction systems: methanol, methanol: water (1:1 v/v) and a mixture of acetone: water (7:3 v/v). The extraction was carried out at 75°C. The elution volume was set at 60% of the cell's volume. Following conditions were employed: static time – 5 min, purge time – 100 sec, while the pressure was set at 90 bar. The extracts were evaporated to dryness using a rotary evaporator at 40°C.

Determination of total polyphenols content using Folin-Ciocalteu assay

The total content of polyphenols in the diet sample extracts was determined using a modified method of Folin-Ciocalteau (5). For this purpose, 0.5 mL of the extract solution was mixed with 30 mL of distilled water and 2.5 mL of Folin-Ciocalteau reagent. After 1 minute (but no longer than 8 minutes) 7.5 mL of 20% Na_2CO_3 solution was added and the mixture was filled to volume of 50 mL with distilled water. The absorbance was measured at a wavelength of λ = 760 nm in a 1-cm cuvettes by Thermo Fisher Scientific Evolution UV-Visible Spectrophotometer after 2h. The assay was performed for a series of standard solutions as well. The same procedure was repeated to all standard gallic acid solutions (50-500 mg/L) and standard curve was obtained. The total phenolic content was calculated in gallic acid equivalents (GAE) based on the plotted calibration curve.

RESULTS

The extraction techniques employed in conjunction with the selection of solvents systems allowed to determine the total phenolic content in the reconstructed daily food rations (DFR) of students. The average amount of polyphenols contained was within the range of 389-1316 mg and 484-1276 mg in women's and men's diets, respectively. Obtained results were presented in Table 1 and Table 2.

Table 1. Total polyphenols content in men's diets depending on conducted method of extraction and solvent system

Extraction method	Parameters	Solvent system		
		Methanol	Methanol:Water (1:1 v/v)	Acetone:Water (7:3 v/v)
Maceration	Polyphenols content [mg]	**553**	**484**	**670**
	SD	42.3	45.4	25.0
	Range	507-581	412-531	627-712
UAE	Polyphenols content [mg]	**1015**	**714**	**1266**
	SD	184	111	98.8
	Range	793-1331	483-899	1168-1433
ASE	Polyphenols content [mg]	**809**	**1266**	**1275**
	SD	172	219	177
	Range	611-920	1028-1313	1097-1452

Table 2. Total polyphenols content in women's diets depending on conducted method of extraction and solvent system

Extraction method	Parameters	Solvent system		
		Methanol	Methanol:Water (1:1 v/v)	Acetone:Water (7:3 v/v)
Maceration	Polyphenols content [mg]	**467**	**389**	**599**
	SD	12.4	39.6	29.8
	Range	445-486	336-440	550-645
UAE	Polyphenols content [mg]	**791**	**560**	**1042**
	SD	75.7	92.6	209
	Range	692-945	401-681	823-1556
ASE	Polyphenols content [mg]	**834**	**1316**	**965**
	SD	116	118	184
	Range	775-967	1212-1444	798-1163

DISCUSSION

The highest recovery of investigated compounds, both in women's and men's diets, was achieved using Accelerated Solvent Extraction (ASE) technique. The least efficient method of extraction constituted the repeated maceration. It is worth noticing that very good recovery of phenolic substances was obtained using ultrasound-assisted maceration. This fact remains of high importance as UAE is a simple and cheap extraction technique. It does not require the use of expensive equipment, or large amounts of solvents.

Another important aspect of the study was the selection of appropriate elution system, allowing the most efficient extraction of designated substances. In most cases the best solvent system was a mixture of acetone: water in the ratio 7:3 v / v. The only exception was the extraction of the female diet by the ASE extraction, whether the best recovery was obtained using a mixture of methanol and water in the ratio of 1:1. However, it should be stressed, that in case of DFR of students, the use of this particular mixture of solvents in ASE extraction also resulted in high recovery, which was comparable to the one obtained by the mixture of acetone and water.

In the methods of multiple and ultrasound-assisted maceration pure methanol proved to be a better extrahent compared with 50% aqueous solution of the solvent. However, in the method of ASE, the situation was reversed: the least efficient extraction solvent was 100% methanol.

The total intake of phenolic compounds determined in the Folin-Ciocalteu assay in the extracts obtained by multiple maceration was comparable to the results obtained in other similar studies (3). Moreover, these results were similar to the ones obtained by other authors evaluating the intake of polyphenolic compounds with national daily food rations (1, 4). However, it should be emphasized, that these two scientific papers refer only to the consumption of flavonoid compounds and are based on foreign theoretical data bases, rather than on analytical assays. What's more, the total content of phenols in the samples extracted by UAE and ASE techniques is significantly higher, than in the scientific papers cited. On the basis of the obtained data, it can be concluded that these two listed extraction techniques have much greater efficiency compared to simple maceration. The total phenolic intake with DFR's studies need to be developed and confirmed in future research.

CONCLUSIONS

It was shown that Accelerated Solvent Extraction (ASE) was found to be the most effective technique for the extraction of polyphenols from the daily food rations. Performed optimization of the extraction systems revealed that the highest recovery of marked compounds was obtained using a mixture of acetone and water at volume ratio of 7:3. It should be also emphasized that very high degree of recovery of investigated compounds was obtained using ultrasound-assisted extraction. For traditional extraction techniques such as maceration, 100% methanol seems to be more effective than its water dilutions. The estimation of phenolic intake with daily food rations was directly associated with the extraction methods used, allowing higher recovery of these substances from investigated material.

REFERENCES

1. Ilow R. et al.: Evolution of bioflavonoid intake in the diets of 50-year-old inhabitans of Wrocław. *Adv. Clin. Exp. Med.*, 17, 3, 327, 2008.
2. Malińska D., Kiersztan A.: Flawonoidy-charakterystyka i znaczenie w terapii. *Post. Biochem.*, 50, 2, 182, 2004.
3. Marzec Z. et al.: Total intake of zinc, manganese, copper, vitamin C and phenols in students' daily food rations. *Ann. UMCS Sect. DDD.,* 23, 4, 67, 2010.
4. Regulska-Ilow R. et al.: Ocena pobrania bioflawonoidów z dietą przez studentów z Wrocławia. *Bromat. Chem. Toksykol.,* 3, 41, 675, 2008.
5. Singleton V. L. et al.: Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. *Methods Enzymol.,* 299, 152, 1999.
6. Vejkovic V.: Simple criterion for selection of flavonoid compounds with anti-HIV activity. Bioorg. *Med. Chem. Lett.*, 17, 5, 1226, 2007.
7. Woodman O. L., Chan E. Ch.: Vascular anti-oxidant actions of flavonols and flavones. *Clin. Exp. Pharmacol. Physiol.*, 31, 11, 786, 2004.
8. Yao L. H. et al.: Flavonoids in food and their health benefits. *Plant Foods Hum. Nutr.*, 59, 3, 113, 2004.