ASSESSMENT AND ADAPTATION OF METHODS OF EXTRACTION OF GRAPE SEED POLYPHENOL COMPOUNDS

EVALUAREA ŞI ADAPTAREA UNOR METODE DE EXTRACTIE A COMPUŞILOR POLIFENOLICI DIN SEMINȚELE DE STRUGURI

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Abstract. *The extraction of polyphenolic compounds in the plant material is influenced by the chemical and structural characteristics and the particle size of the plant material, as well as the presence of interfering substances. The solubility of the phenolic compounds depends on the solvent used, the degree of polymerization of the compounds, as well as interactions with other plant compounds. The paper presents several methods used for extracting polyphenolic compounds from grape seed: Soxhlet continuous extraction method; extraction with supercritical fluids (liquid CO_2); extraction under reduced pressure (3 bar) and high pressure (15 bar) with water and 75% alcohol concentration. Characterization of polyphenolic extracts carried out by HPLC alloweded the recommendation of two methods: with supercritical fluid and Soxhlet method. In the same time it was also observed a higher extraction yelds for the phenolic acids, hydrolysable tannins, flavones of stilbene using alcohol as a solvent.*
Key words: *seed, extraction, Soxhlet, supercritical fluids, presurre*

Rezumat. *Extracția compuşilor polifenolici din materialele vegetale este influențată din caracteristicile chimice structurale, metoda de extracție utilizată, dimensiunea particolelor materialului vegetal, precum şi de prezența substanțelor interferente. Solubilitatea compuşilor polifenolici depinde de solventul utilizat, gradul de polimerizare al compuşilor, precum şi de interacțiunile cu alți compuşi vegetali. În lucrare sunt prezentate rezultatele mai multor metode de extracție a compuşilor polifenolici din seminţele de struguri selectate din tescovină, respectiv: metoda extractivă continuă Soxhlet; extracția cu fluide supercritice (CO_2 lichid); extracția la presiune scăzută (3 bari) şi ridicată (15 bari) cu apă şi alcool etilic de concentrație 75%. Caracterizarea extractelor polifenolice realizată prin analiza HPLC a permis recomandarea metodelor de extracție cu fluide supercritice şi metoda Soxhlet. De asemenea, s-a constat o extracție superioară a acizilor fenolici, a taninurilor nehidrolizabile, a stilbenilor şi a flavonelor în cazul extractelor la care s-a utilizat ca solvent alcoolul etilic.*
Cuvinte cheie: *semințe, extracție, Soxhlet, fluide supercritice, presiune*

INTRODUCTION

Obtaining polyphenolic extracts raises several technological problems of which the most important one is to ensure maximum extraction efficiency, this

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being particularly dependent on the factors involved in physical and chemical extraction processes. The extraction procedures used, namely the extraction solid – liquid in continuous and dicontinuus system provides an advanced recovery of the crumbling degree, of the nature of the extraction solvent and a refresh of it, as well as adequate contact time or extraction (Maria Teresa Escribano – Bailon *et al.,* 2003).

In the case of grape seeds, the vegetable extracts obtained by Soxhlet continuous extraction method are richer in polyphenols, anthocyanins and tanoide materials, but the extraction time is higher, varying according to the plant material used (Jackson *et al.,* 1996; Luque de Castro *et al.,* 1998; Lin *et al.,* 1999; Lapornik *et al.,* 2005; Yilmaz *et al.,* 2005; Downey *et al.,* 2010; Schieber *et al.,* 2001).

Supercritical fluid extraction method is a technique for non-destructive separation, belonging to the processes occurring at high pressure (SPE) based on the power of leaching fluids at appropriate temperatures and pressures above the critical point. The solvent that is most commonly used is the carbon dioxide, due to its properties: low toxicity, non-polluting and the final extract does not contain other substances, allowing at the same time to achieve a selective extraction (Lin *et al.,* 1995, Palma *et al.,* 1999).

The extraction of the polyphenolic compounds at high / low pressure is a technique recently introduced among the methods used for the extraction of polyphenolic compounds. This method offers the posibility to conduct the extraction under an inert atmosphere and the stability of the polyphenolic compounds during this process is extremely high (Palma *et al.,* 2001).

At the moment an optimal procedure to ensure the total or fractional extraction of polyphenolic compounds isn`t available. Considering this aspect, in the present paper in order to obtain the polyphenolic extracts from the grape seeds selected from pomace, there were tested: the continuous Soxhlet method, the extraction with supercritical fluids method and the extraction at high / low pressure method.

MATERIAL AND METHOD

In order to remove the substances with lipophilic character it has been used a static method which consisted in keeping in contact for one hour the plant material with ethyl ether. Soxhlet extraction was carried out continuously with 96% ethyl alcohol, in relation to 1/10 (plant material (g) / solvent (ml), at a constant temperature of 78°C for 48 hours.

In the case of supercritical fluid method (liquid CO_2) there were performed five extractions with seven g of plant material each, using liquid CO_2 at a rate of 1 mL / minute and ethyl alcohol at a rate of 0.5 mL/min. The extraction time was 32 minutes for a total volume of 48 ml extraction fluid.

The extractions under reduced pressure (3 bar) and high pressure (15 bar) were carried out using as solvents water and ethyl alcohol with a concentration of 75% and an amount of 7.5 g vegetable material. The extraction time was 2 minutes and in the final stage it was collected a volume of 215 mL of extract.

Based on the nature of the solvent used, the concentration of the extracts was done differently. So, for the samples extracted with the ethyl alcohol, the concentration was carried out at a pressure of 120 mbar, at a temperature of 40 ° C

and a rotational speed of 32 rpm. Instead for the samples extracted with water, the conditions of concentration were: a pressure of 40 mbar, a temperature of 40°C and a rotational speed of 80 rpm. In order to characterize the polyphenolic extracts, there were determined the total polyphenols using the method of Folin-Ciocalteau, the index of tanoide materials (IMT) through the method established by Bourzex.

In addition, through HPLC analysis there were identified and quantified a series of phenolic acids, stilbenes, tannins that are not hydrolysable and several flavones using the method presented by M. Castellar (Castellar *et al.,* 2002).

RESULTS AND DISCUSSIONS

The data obtained during the characterization of the raw polyphenol extracts are presented in figures 1 and 2. Analysing the results, the vegetal extract from grape seeds obtained by supercritical fluid extraction, with a concentration in polyphenols 3.02 g GAE/L was noted (Figure 1).

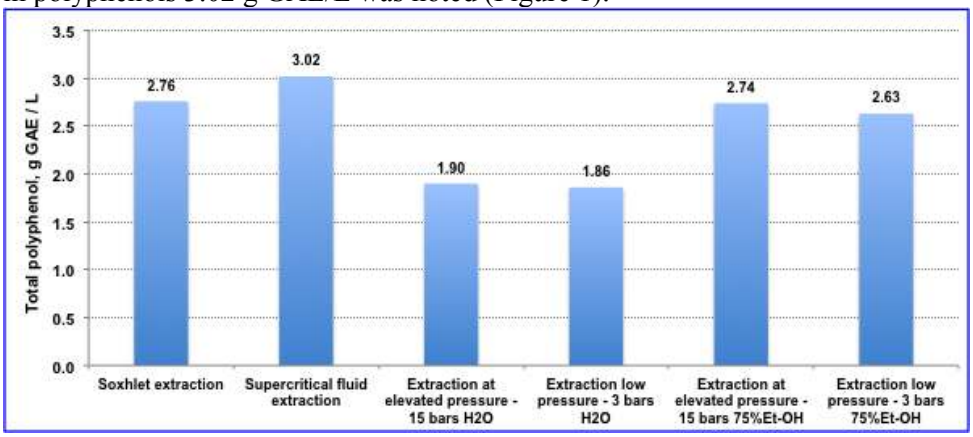

Fig. 1 - The total polyphenol content of the raw extracts obtained from the seeds of grapes

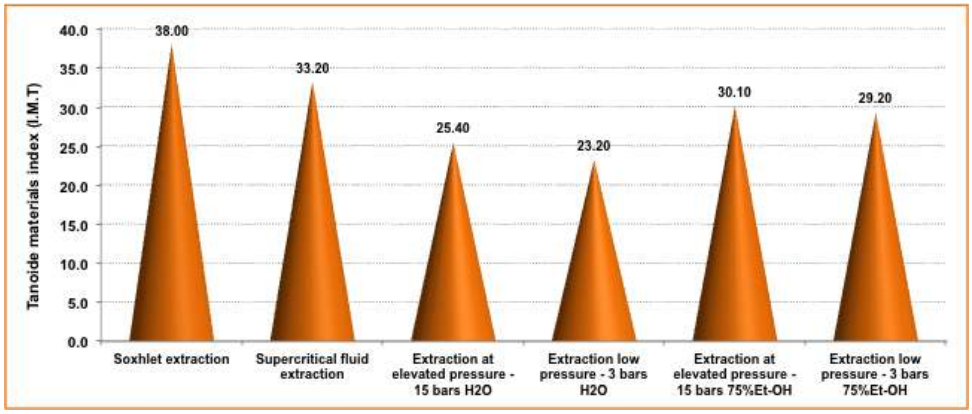

Fig. 2 – The tanoide materials index (IMT) of the raw polyphenolic extracts obtained by various methods of extraction

In what concerns the index of tanoide materials, the highest values were

determined at the extracts obtained using the Soxhlet method (38.0) and the supercritical fluid method (33.2). Lower values for both tanoide materials index (25.4 and 23.2) and for total polyphenol concentration (1.86 to 1.90 g GAE / L) are observed in the case of the extracts on which the water was used as a solvent (Figure 2).

Through the HPLC analysis of the concentrated extracts several phenolic acids were identified such as hydroxybenzoic acids and hydroxycinnamic acids (tables 1 and 2). Analyzing the data presented in Table 1 is particularly significant the extract obtained by supercritical fluid method, by showing the highest content of gallic acid, protocatehic acid, siringic acid, vanilic acid and m-hydroxybenzoic acid.

Table 1

The hydroxybenzoic acids identified in the polyphenolic extracts concentrated

Hydroxybenzoic acids, mg	Soxhlet extraction	Supercritical fluid extraction	Extraction at elevated pressure		Extraction low pressure	
			H_2O	75%Et-OH	H_2O	75%Et-OH
gallic acid	10,08	317,42	40,12	54,73	20,16	67,07
protocatehic acid	-	366,87	35,81	41,78	11,00	51,56
p-hydroxybenzoic acid	0,95	2,79	1,40	1,18	0,96	1,79
gentisic acid	-	0,31	0,91	2,27	0,14	0,68
syringic acid	114,05	126,51	1,53	13,92	6,31	0,30
vanillic acid	0,45	34,77	5,51	3,83	1,07	4,61
m-hydroxybenzoicacid	0,40	29,86	5,32	2,50	0,06	0,25
salicilic acid	92,86	2,26	0,53	6,17	-	4,83

The most significant hydroxybenzoic acid, namely the galic acid showed values between 10.08 mg / L of the extract obtained by Soxhlet extraction method and 317.42 mg / L of the extract obtained by method of supercritical fluids.

Although in the literature is remarked that salicylic acid is formed only during the alcoholic fermentation process, it was identified in the largest quantity in the extract obtained by Soxhlet method (96.64 mg/L) and in amounts more small, between 0.53 and 6.17 mg / L in the extracts obtained by other methods.

It has also been identified syringic acid, with very large variation limits between 0.30 mg/L (the extract obtained at low pressure with 75% alcohol) and 126.51 mg/L (the extract obtained by extraction with supercritical fluids). In lower amounts other hydroxybenzoic acids were identified, such as: protocatechinic acid, p-hydroxybenzoic acid, gentisic acid, m-hydroxybenzoic acid and vanillic acid.

In the concentrated polyphenol extracts there were determined a number of hydroxycinnamic acids respectively, caffeic acid, , chlorogenic acid, p-coumaric, ferulic and synapic acids (table 2). It can be observed, that in the case of extracts obtained at low pressure using water the amount of the hydroxycinnamic acids was below the detection limit.

Table 2

The hydroxycinnamic acids identified in the concentrated polyphenol extracts

Hydroxycinnamic acids, mg/L	Soxhlet extraction	Supercritical fluid extraction	Extraction at elevated pressure		Extraction low pressure	
			H_2O	75%Et-OH	H_2O	75%Et-OH
cafeic acid	0,53	1,27	1,54	2,32	-	0,15
clorogenic acid	2.29	15,06	2,62	1,90	-	2,78
p-cumaric acid	4,58	0,25	0,31	0,68	-	0,71
ferulic acid	0,98	0,38	0,33	1,94	-	3,44
sinapic acid	0,36	0,20	-	-	-	0,21

Through HPLC analysis of the polyphenols extracts it was revealed the presence of some non- hydrolysable tannins (condensed), namely, the catechin and epicatechin (table 3). In what concerns the content of catechin, it can be observed a high variation limit, larger amounts being extracted with the method that used ethyl alcohol as a solvent. In the case of epicatechin, significantly higher amounts were found in the extracts obtained by extraction with supercritical fluids (157.91 mg/L).

Table 3

The non-hydrolysable tannins identified in the concentrated polyphenolic extracts

Method of extraction	catechin, mg/L	epicatechin, mg/L
Soxhlet extraction	6,05	4,58
Supercritical fluid extraction	4,18	157,91
Extraction at elevated pressure - 15 bars H_2O	2,33	0,14
Extraction low pressure - 3 bars H_2O	0,97	-
Extraction at elevated pressure - 15 bars 75%Et-OH	3,76	1,64
Extraction low pressure - 3 bars 75%Et-OH	0,40	3,97

In addition to phenolic acids, in the extract samples it was also identified the trans-resveratrol (table 4). As for the phenolic acids, the higher amount was found in the extracts obtained by extraction with the Soxhlet method and the supercritical fluid method, respectively, 2.40 and 1.38 mg/L.

Table 4

The content in stilbene and flavones from the concentrated polyphenol extracts

Method of extraction	trans-resveratrol, mg/L	rutine mg/L	quercitine, mg/L
Soxhlet extraction	2,40	0,55	1,54
Supercritical fluid extraction	1,38	0,75	0,37
Extraction at elevated pressure - 15 bars H_2O	-	0,72	-
Extraction low pressure - 3 bars H_2O	-	-	-
Extraction at elevated pressure - 15 bars 75%Et-OH	0,02	0,29	0,11
Extraction low pressure - 3 bars 75%Et-OH	0,21	20,60	1,15

Among the flavones were identified routine and quercetin, standing out especially the extract obtained by Soxhlet extraction method with 1.54 mg/L

quercetin and the extract obtained at low pressure using water 20.6 mg/L rutine.

CONCLUSIONS

1. The characterization of polyphenolic extracts carried out by HPLC (high performance liquid chromatography) allows the recomandation of the supercritical fluid extracting method for and the Soxhlet method.

2. In the case of the extracts that used ethyl alcohol as a solvent, it could be remarked a superior extraction of the phenolic acids, of non- hydrolyzed tannins, of the stilbene and the flavonols.

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