PRELIMINARY RESULTS ON ESTABLISMENT THE OPTIMAL EXTRACTION CONDITIONS STAGED PHENOLIC COMPOUNDS IN GRAPE SEED PROANTHOCYANIDINS

REZULTATE PRELIMINARII PRIVIND STABILIREA CONDIȚIILOR OPTIME DE EXTRACȚIE ETAPIZATĂ A COMPUŞILOR FENOLICI PROANTOCIANIDINICI

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Abstract. *Condensed tannins from grape seed the waste, also known as proanthocyanidins extracted with solvents, are soluble in water and therefore can not be used practically as the bioactive antifungal, antibacterial, cytostatic. In this context imposed, establish the optimal conditions for the method of extraction of phenolic compounds in stages: report solid / solvent extraction time, the number of extraction steps. They were tested five reports of plant material / solvent (1/4, 1/5, 1/6, 1/7, 1/8), following the dynamics of the extraction process of phenolic compounds in two steps, by monitoring the optical density at 280 nm. In the two-stage extraction, the maximum value of the optical density was recorded after eight hours, in the ratio 1/4, and the reports 1/5 - 1/8 after 10 hours. Cumulated extracts presented in terms of variations in the concentration of polyphenol, amounts being comprised between 2.81 to 2.88 g GAE / 100 g of delipidated grist.*
Key words: *seed, extraction, report, time*

Rezumat. *Taninurile condensate din deşeul de semințe de struguri, numite şi proantocianidine, extrase cu solvenți, nu sunt solubile în apă, motiv pentru care nu pot fi utilizate practic ca substanțe bioactive antifungice, antibacteriene, citostatice. În acest context s-a impus, stabilirea condițiilor optime pentru aplicarea metodei de extracție etapizată a compuşilor fenolici: raportul solid/solvent, timpul de extracție, numărul de etape de extracție. Au fost testate cinci rapoarte material vegetal/solvent (1/4, 1/5, 1/6, 1/7, 1/8), urmărindu-se dinamica procesului de extracție a compuşilor fenolici în două etape, prin monitorizarea densității optice la 280 nm. În cele două etape de extracție, valoarea maximă a densității optice s-a înregistrat după opt ore, la raportul 1/4, iar în cazul rapoartelor 1/5 – 1/8 după 10 ore. Extractele cumulate au prezentat variații nesemnificative în ceea ce priveşte concentrația de polifenoli totali, valorile fiind cuprinse între 2,81 – 2,88 g GAE/100 g şrot delipidat.*
Cuvinte cheie: *semințe, extracții, raport, timp*

INTRODUCTION

Grape seeds represent 38-52% of the dry pomace and are rich in oil, phenolic compounds, organic phosphorus compounds and minerals. Proanthocyanidins from

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the grape seed grits, resulting from extraction of the oil, are not soluble in water and therefore can not be used practically as bioactive antifungal, antibacterial, cytostatic substances . Their hydrosolubility represent the main prerequisite for the practical use.

The extractive quantitative methods used are continuously optimized thanks to deepen, on the one hand of the knowledge concerning the chemical nature of the phenolic compounds and on the other hand, the experience achievedregarding the physical factors involved in these extractive processes, namely: the size of the particles plant materials, the solvents that were used, the temperature of the process, the extraction time with the solvent plant material contact. In the literature there can be find numerous studies on these aspects, the authors using a variety of solvents, different temperature ranges and different reports solid / liquid extraction time (Spigno *et al.,* 2007; Huh *et al.,* 2004; Bensaruk *et al.,* 2012; Chew *et al.,* 2011; Khanal *et al.,* 2009; Amendola *et al., 2010*; Ross *et al.,* 2011; Lapornik *et al.,* 2005).

The study presents partial results obtained in the laboratory concerning the establishment of the optimal plant material or grape seed meal delipidated / solvent volume and the optimum extraction of phenolic compounds.

By applying a discontinuous process in the stationary and in the mining parameters ttaht t were seted, it was intended to achieve the best extraction yields of polyphenols from the seed oilcake delipidated with hexane, in a context of low

MATERIAL AND METHOD

The determination of the optimal raport solid / solvent (S / L), meal grape seed / alcohol 96 was conducted in 500 ml Erlenmeyer flasks closed with a glass stopper and wrapped in aluminum foil to avoid distortion of the phenolic compounds from the action of the light .

Five reports have been tested: 1/4, 1/5, 1/6, 1/7, and 1/8, while maintaining a constant amount of 25 g of delipidated seed meal to which was added 100 mL, 125 mL, 150 mL, 175 mL and 200 mL of ethyl alcohol 96°C. The bottles that were prepared were placed on the mechanical stirrer programmed to 200 rpm, under a temperature of 25ºC. For each report two extractions were performed.

The extraction process monitoring was performed by determining the optical density (OD) at a 280 nm wavelength every two hours. At the timeframes referred it was also determined the concentration of the polyphenols, the samples being diluted 1/10. The amount of polyphenols was determined with Folin Cicâlteu reactif according to the Singleton and Rossi method, 1965. The concentration of polyphenols was determined on the basis of gallic acid standard curve, the amounts being expressed in g / l eq. gallic acid (EAG).

RESULTS AND DISCUSSIONS

Monitoring optical density shows a progressive increase during the extractive processes of total polyphenols compounds (Figures 1 and 2). The maximum optical density was recorded after eight hours in the two extractions at the ratio S / L 1/4.

For the reports 1/5 - 1/8 the maximum optical density was recorded after 10

hours but this is much more evident in the second extraction step.

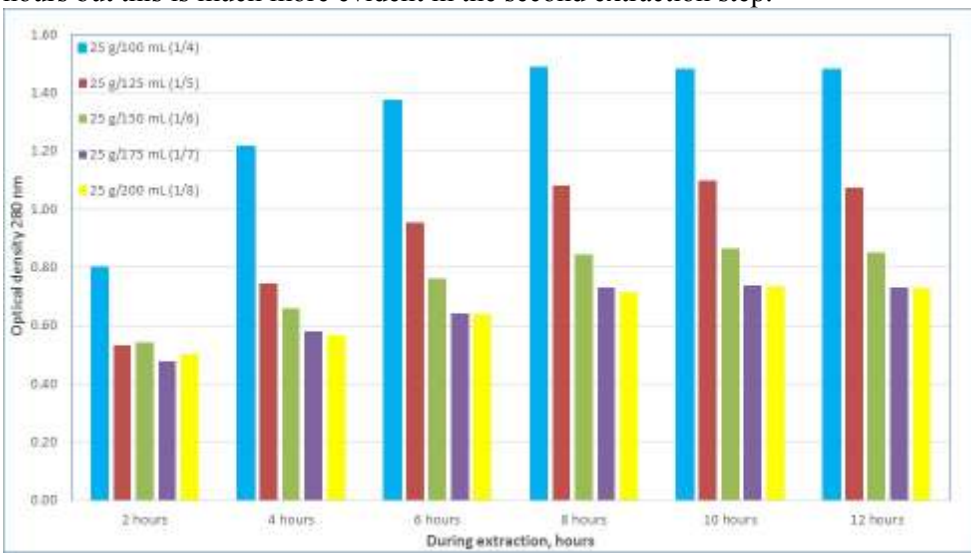

Fig. 1 – The dynamics of extraction processes phenolic compounds, during stage I

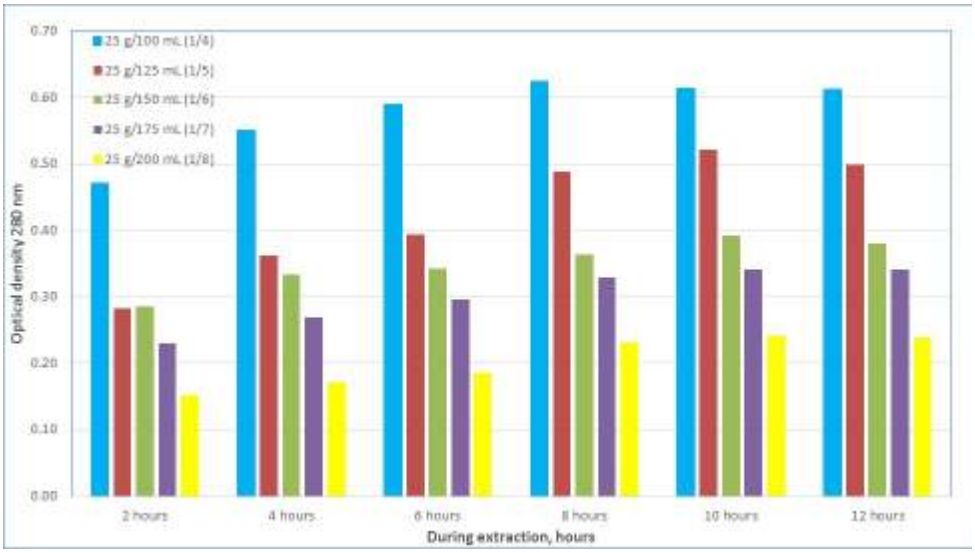

Fig. 2 – The dynamics of extraction processes phenolic compounds, during stage II

After 8-10 hours of extraction, the values of the optimum density slightly increase, having decreasing trends after 12 hours, so the prolongation of extraction is no longer justified (Figure 3).

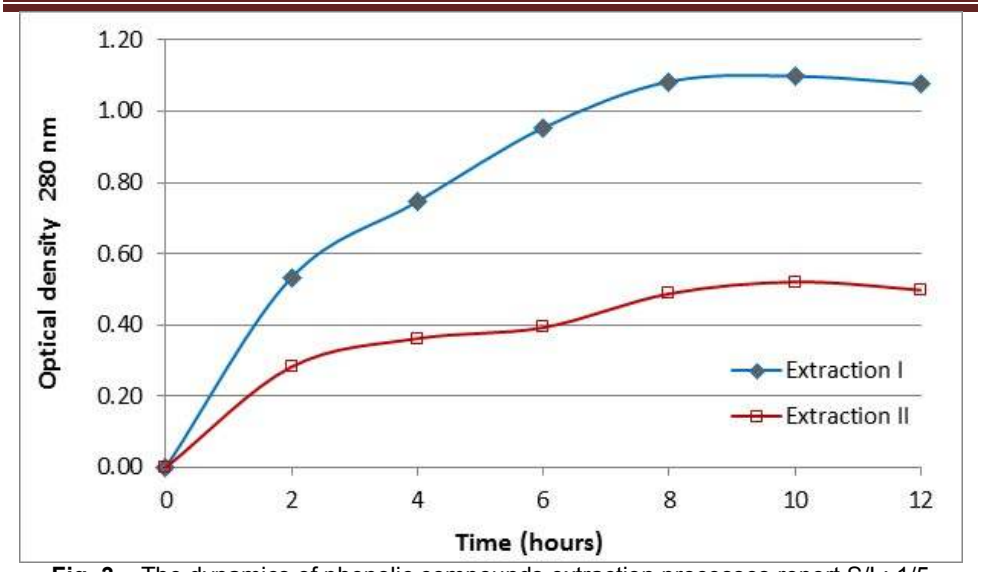

Fig. 3 – The dynamics of phenolic compounds extraction processes report S/L: 1/5

The results obtained by determining the concentration of total polyphenols compounds in the crude extracts obtained in stages I and II for each report solid / liquid are presented in Tables 1 and 2.

Table 1

The concentration of polyphenol compounds, g GAE/100 g delipidated grist - stage I extraction

The extraction time, hours	report solid/liquid, g/mL				
	1/4	1/5	1/6	1/7	1/8
2 hours	1,80	1,89	1,78	2,06	1,71
4 hours	2,09	2,15	1,94	2,25	2,23
6 hours	2,19	2,18	2,04	2,30	2,29
8 hours	2,26	2,22	2,15	2,31	2,35
10 hours	2,20	2,26	2,18	2,34	2,40
12 hours	2,19	2,26	2,17	2,33	2,34

Table 2

The concentration of polyphenol compounds, g GAE/100 g delipidated grist - stage II extraction

The extraction time, hours	report solid/liquid, g/mL				
	1/4	1/5	1/6	1/7	1/8
2 hours	0,40	0,49	0,53	0,39	0,34
4 hours	0,47	0,52	0,60	0,47	0,40
6 hours	0,54	0,53	0,61	0,47	0,42
8 hours	0,58	0,56	0,62	0,48	0,45
10 hours	0,59	0,57	0,63	0,49	0,48
12 hours	0,56	0,56	0,62	0,48	0,47

In the first extraction step, it is noted that the higher value for the total

polyphenols compounds was extracted after 10 hours at the report solid / liquid 1/8, namely 2.40 g / 100 g delipidated meal. In the case of the reports extracts 1/4, 1/5, and 1/7 16 similar values of total polyphenols concentrations were obtained, but lower than the extract obtained in 1/8 ratio (Table 1).

In the second stage of extraction close values of total polyphenols concentration were obtained after 10 hours of extraction area for the reports 1/5, 1/8, this value being reached with the report 1/4 after eight hours of extraction (Table 2).

In the two extraction steps five crude polyphenol extracts resulted with insignificant variations in the concentration of total polyphenols, the values measured being situated between 2,05- 2,88 g GAE / 100 g of delipidated meal (Table 3).

Table 3

The total polyphenol compounds (I + II extraction), g GAE/100 g delipidated grist

The extraction time, hours	report solid/liquid, g/mL				
	1/4	1/5	1/6	1/7	1/8
2 hours	2,20	2,38	2,31	2,45	2,05
4 hours	2,56	2,67	2,54	2,72	2,63
6 hours	2,72	2,71	2,65	2,77	2,71
8 hours	2,84	2,78	2,77	2,79	2,80
10 hours	2,79	2,83	2,81	2,83	2,88
12 hours	2,75	2,82	2,79	2,81	2,81

In the case of the report 1/4 the highest quantity of phenolic compounds was extracted after eight hours, namely 2.84 g GAE / 100 g, and in the case of other reports (1/5, 1/6, 1/7 and 1/8) after 10 hours of extraction, 2.81 - 2.88 g / GAE / 100 g.

It can be also noted that regardless the solid / liquid and the time of extraction, the highest amount of phenolic compounds, over 80% was extracted in the first stage (Figure 3).

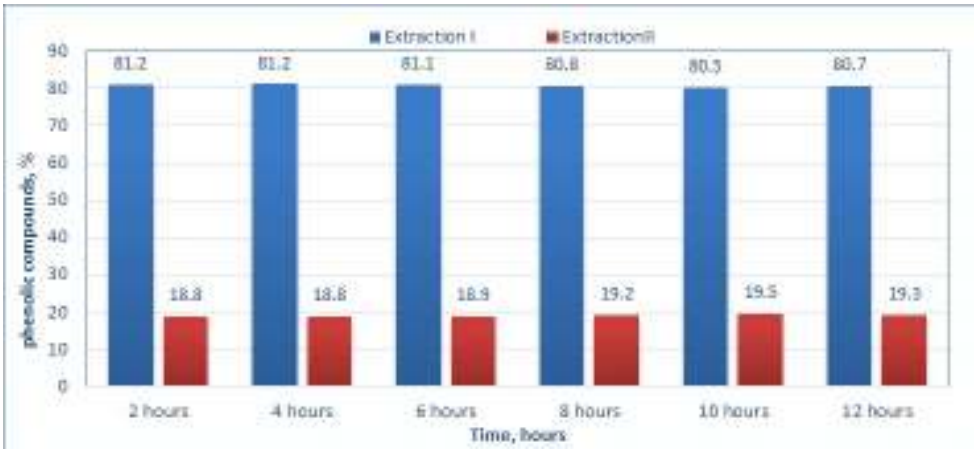

Fig. 3 - The percentage concentration of total phenolic compounds, during the two-stage extraction

The results obtained confirm the data reported in the literature and justifies further research or the study of the influence of the type of solvent and the source (variety) of plant material.

CONCLUSIONS

1. In the two-stage extraction, the maximum value of the optical density was recorded after eight hours at the report solid / liquid 1/4 and in the case of the reports 1/5 - 1/8 after 10 hours. After 10 hours of extraction, the values of the optical density increased insignificantly having decreasing trends after 12 hours.

2. In the first extraction stage, the highest amount of total polyphenolic compounds was extracted after 10 hours at the S / L 1/8, and 2.4 g / 100 g delipidated meal. In the second extraction stage, the data shows close values of total polyphenols concentration after 10 hours of extraction area for the reports 1/5, 1/8, this value being reached also in the case of 1/4 ratio after eight hours of extraction.

3. The crude polyphenol extracts obtained in the two phases presented insignificant variations in terms of the concentration of total polyphenols (2.81 - 2.88 g GAE / 100 g of delipidated meal).

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