EXTRACTION OF BIOACTIVE COMPOUNDS IN GRAPE SEEDS

EXTRACȚIA COMPUŞILOR BIOACTIVI DIN SEMINȚELE DE STRUGURI

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Abstract. *The study of the bioactive compounds from the grape seed extracts represents an ever increasing preoccupation for the industry of food supplements and tonic drinks, as well as for the industry of pharmaceutical products and cosmetics. This scientific paper presents a practical method for extracting the proanthocyanidins from the grape seeds by using a hydroalcoholic solution. The method consists in autoclaving the mixture between the solid stuff and hydroalcoholic solution under temperature and pressure controlled conditions. The extraction itself is followed by the separation of the solids from the liquid fractions and thereafter the concentration of the extract rich in proanthocyanidins. The polyphenolic extracts are therefore easily obtained by applying this uncomplicated procedure, presenting the advantage that no toxic or polluting solvent is used during the stages of the technological process. Consequently, the product obtained is safe and secure for the human organism, complying also with the new regulations enforced for the food industry and for the pharmaceutical and cosmetic industries, too. The control concerning the quality of the extracts obtained was performed by assessing a range of analytical parameters (colorimetric measurement for DO 420 and 280 nm, evaluation of the amount of catechins, oligoproanthocyanidins and leucoanthocyanins, as well as the content in total polyphenols by practicing Folin Ciocalteu method).*

Rezumat. *Studiul compusilor bioactivi din extractele de seminte de struguri reprezinta o preocupare tot mai mare pentru industria suplimentelor alimentare, a bauturilor tonice, industria farmaceutica si a cosmeticelor. In lucrarea de fata este prezentata o metoda de extractie a proantocianidinelor din semintele de struguri utilizand o solutie hidroalcoolica. Metoda implica autoclavarea amestecului de material solid si solutie hidroalcoolica in conditii controlate de temperatura si presiune. Extractia propriu-zisa este urmata de o separare a fractiunilor solide de cele lichide si concentrarea extractului bogat in proantocianidine. Obtinerea extractelor polifenolice prin aceasta metoda este simpla si prezinta avantajul ca in nici o etapa a procesului tehnologic nu sunt utilizati solventi toxici sau poluanti, produsul fiind sigur pentru organismul uman si in conformitate cu noile reglementari impuse in industria alimentara, farmaceutica si cosmetica. Controlul calitatii extractelor obtinute s-a determinat prin evaluarea catorva parametrii analitici (masurarea colorimetrica la DO 420 si 280 nm, dozarea cantitatii de catechine si oligoproantocianidine si a cantitatii de leucoantociani, continutul de polifenoli totali prin metoda Folin Ciocalteu).*

Oligoproanthocyanidins (OPC) are molecules with pronounced anti-oxidizing properties, forming a family of flavanolic compounds with paler hues, included in a wide variety of plants.

Phenolic fraction grape seeds consists almost entirely of flavanols, mainly catechins (catechin and epicatechin, simple and galloilated) and their polymers also known as procyanidines, respectively proantocyanidines when the B nucleus includes monomer units differently substituted. By the HPLC technique (Fig. 1) there have been identified mainly monomers, dimers (B1,B2,B3,B4,B5) (Weinges and Piretti,1971; Piretti et al.,1976; Czochanska et al.,1979; Bourzeix et al.,1986) and trimers (C1,C2) (Lea et al.,1979; Romeyer et al.1986). The proantocyanidins with molecules including up to 10 monomer units each are designated as oligoproanthocyanidins (OPC).

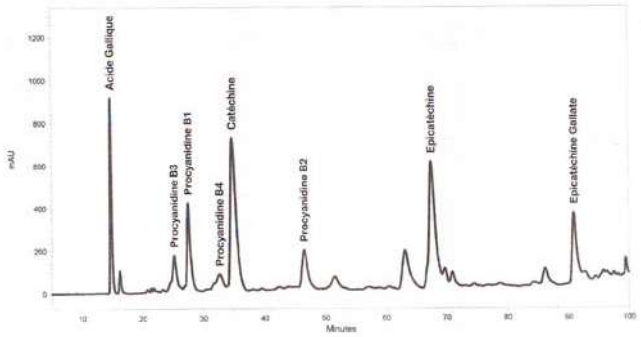


Fig.1 OPC in grape seeds, Nutrinov Laboratories, 2006

The studies revealed that 59% of the oligomeric proanthocyanidines (dimers, trimers, tetramers, pentamers) are found in seeds, 19-21% in stalks and skins and 1% in pulp.

The beneficial effects of the polyphenols included in grape seeds are related both to their anti-oxidizing properties and their affinity to proteins. The ability of this category of polyphenols to capture free radicals is higher than that of other well known anti-oxidizers, such as the C and E vitamins and beta-carotene.

The aims of this paper are the following:

1. to define the extraction mode by using non-toxic solvents and the raw material's characteristics
2. to appraise the bioactive potential of the extracts obtained from seeds of white and red grape varietees.

The results were obtained under the CEEX Project No. 33/2005.

MATERIAL AND METHOD

1. Defining the extraction mode and raw material characteristics

The extraction of bioactive compounds from grape seeds was performed in accordance with the pre-established schedule, during the 4th stage of the Project. This operation includes the following steps: extraction of C+OPC in 20% alcoholic solution

by autoclaving at 1 atmosphere for 1 hour, separation of the liquid fraction rich in C+OPC, clarifying the liquid fraction by centrifugal action and concentration (fig.2).

The seeds used as raw material were taken from red and white marc, resulted after the pressing of the destemmed mash resulted during the harvest years in 2005, 2006 and 2007. The seeds were further separated, sorted and dried for storage. Preparation of the seeds before extraction included the separation of organic or mineral foreign materials and washing in warm water to eliminate the pectic and protein substances and sugars.

The physical and chemical parameters of the raw seeds were examined in accordance with STAS 1069-77 and STAS 6124/1-73. Information was processed by statistical mathematical methods and by graphs.

The method for determining the catechine and oligoproanthocyanidines proportion in the seeds was based on the vanillin reaction (FV 829/1623-220589- as modified by ICDVV).

2. Appraisal of the bioactive potential for extracts taken from white and dark grapes

The bioactive potential of extracts was evaluated by the following parameters: total dry extract, DO 420 nm, DO 280 nm, Catech + OPC, tannins.

The method for determining the catechine and oligoproanthocyanidines proportion in the extracts was based on the vanillin reaction and colour measurement at 510 nm (FV 829/1623-220589- as modified by ICDVV). The total proportion of polyphenols was determined by the Folin Ciocalteu method (Singleton and Rossi, 1965), tannin by the MP-VT-PF-02.01-TAN spectrophotometric method and color structure by the MP-VC-07.03-CUL spectrophotometer method.

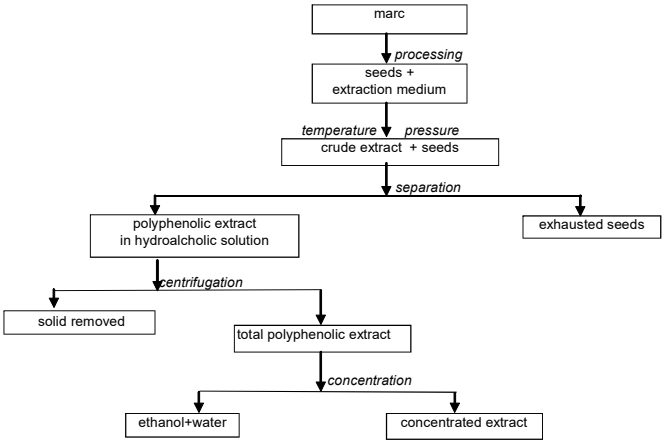


Fig.2 Flow diagram for obtaining polyphenolic extracts

RESULTS AND DISCUSSIONS

The organoleptic characteristics of the seeds were in compliance with the acceptance requirements defined in the professional standard 40-92.

The seeds were characterized by: aspect, color and odor.

- *Aspect*- full grown, mature, healthy
- *Color* – reddish brown, free of mould stains
- *Odor*- specific, without traces of scalding, molding or souring.

Table 1

Quality Parameters for Seeds

Analyzed material	Foreign material (%)			Humidity (%)	Hectoliter Weight (kg)
	mineral	organic	total		
White seeds	0,52	3,54	4,06	9,5	60,1
Red seeds	0,55	2,66	3,21	8,7	59,8

Seed quality was evaluated by determining the proportion of catechins and oligoproanthocyanidins, as well as by the absorption of polyphenolic compounds at 280 nm after 1:100 dilution (table 2).

Table 2

Catechin and OPC Quantities in White and Red Seeds

Analyzed Parameters	Seeds	
	White Grapes	Red Grapes
Catechins and oligoproanthocyanidins g/kg	12,19	24,99
DO 280 nm	25,81	51,89

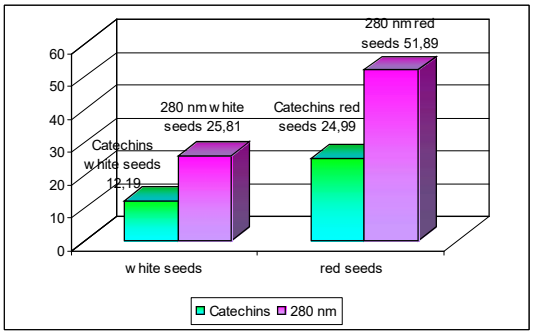


Fig.3 Comparison of analyzed parameters for white and red grape seeds

The seeds of black grapes are approximately 2 times richer in monomer and oligomer proanthocyanidins as those from white varieties. The same applies to the absorption of total phenolic compounds at 280 nm, where higher values were recorded for red grape seeds (fig.3). Quality control for extracts was carried out by determining the analytical parameters and by organoleptic examination (table 3).

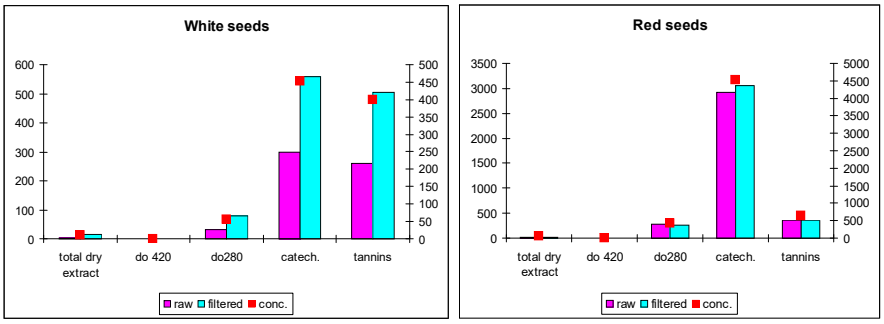
Table 3

Comparison Between White and Red Grape Seed Extracts during Technological Flow

Analyzed Parameters	White Seed Extracts			Red Seed Extracts		
	raw	filtered	conc.	raw	filtered	conc.
Total dry extract g/l	4,04	16,35	9,67	22,77	21,63	41,04
DO 420 nm	0,686	1,448	0,976	3,44	3,32	4,49
DO 280 nm	32,42	79,68	56,61	274,25	259,75	424,02
Catech + OPC mg/l	299	558	452	2930	3058	4523
Tannins mg/l	260	505	400	360	360	640

The results of the comparative examination of parameters during the technological flow indicated that the red grape seed extracts are richer than those obtained from white grape seeds. The parameters examined for white seed extracts indicated maximum values for the extracts taken after centrifuging, during the filtering operation, as compared with the raw and concentrated extracts (fig.4.a). Color intensity also diminishes after concentration, thus confirming the occurrence of certain condensation and insolubilization reactions.

The parameters examined for red seed extracts indicated a phenol content enrichment during the technological flow, from the raw extracts up to the concentrated ones (fig.4.b).



a. **b.**

Fig.4 Variation of Analyzed Parameters for White and Red Grape Seed Extracts

Red seed extracts are about 10 richer in catechins-OPC after autoclaving than white seed extracts. The same can be noticed for concentrated extracts from red seeds (4523 mg/l against 452 mg/l).

In the red seed extracts the catechins and OPC's are predominant in comparison with the tannin content of the same extract.

Determination of the total polyhpenol proportion in white seed extracts of the Feteasca Regala variety by the Folin Ciocalteu method, in comparison with the red seeds of the Merlot variety indicated that the latter are richer in polyphenolic compounds (fig.5).

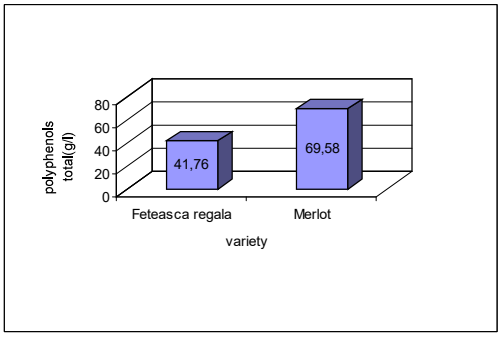


Fig.5 Comparison of Concentrated Extracts

CONCLUSIONS

- Hydro alcoholic extraction by autoclaving is a simple method for obtaining catechin and OPC rich extracts.
- Extraction was achieved by the diffusion of polyphenolic compounds in hydro alcoholic solution, without the destruction of the vacuolar seed walls.
- Extraction was performed under controlled conditions, without any modification of the resulted phenolic compounds.
- The extraction method uses non-polluting organic solvents which render the obtained products safety for use in cosmetics, pharmaceutical and food industry.
- Seeds taken from black grape varieties remain an important source for bioactive compounds extraction even after wine production.

REFERENCES

1. **Ariga T, Hosoyama H, Yuasa K**. - *Process for the preparation of proanthocyanidins. United States Patent 5773262;1998-06-30;*
2. **Campeanu R, Florescu C, Kontek A**. - *Method for Obtaining Natural Coloring Concentrate from Spent Grains. OSIM license no. 99206/1989;*
3. **Frangi E, Bertani M, Mustich G,Tuccini G. -** *Process for preparing grape seeds extracts enriched in procyanindol oligomers. United States Patent 5484594,1996-01-16;*
4. **Nafisi-Movaghar K, Svanoe T T, Seroy W A. -** *Method for extraction of proanthocyanidins from plant material United States Patent 5912363,1999-06-15;*
5 **Ricardo-DA-SILVA J.M, BELCHIOR A.P., SPRANGER MARIA ISABEL, BOURZEIX M. -** *Procyanidines oligomeres de trois cepages et vins du Portugal,Scienes des Aliments, p.213-221.*
6. **Shrikhande A J, Race E J, Wightman J D, Sambueso R D**. - *Process for extraction, purification and enrichment of polyphenolic substances from whole grapes, grape seeds and grape pomace Patent 6544581, 2003-04-08.*