PRELIMINARY INVESTIGATIONS FOR THE VALORIZATION OF GRAPE SEEDS AFTER THE OIL EXTRACTION

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The wine industry produces a large amount of waste that must be valorized as imposed by ecologic as well as economic reasons. The grape seeds comprise around 17 % of the winery waste. A main valorization of seeds is the separation of oil, which makes 11-14 % from their content. After the extraction of oil there are still high quantities of residual materials left (over 80%). Possible solutions for their further valorization have been investigated in this paper. The experiments suggest several ways for obtaining new valuable products. Mixtures of polyhydroxyphenols (PPs,) which may be used as nutritional supplements, have been obtained by extraction with an appropriate polar solvent. They were analyzed by IR, NMR, and Folin Ciocalteu protocol. The new residue (over 70% from the grape seeds), obtained after the extraction of PPs, was valorized by two routes. A first attempt was to use it as dye adsorbent. The second one was a pyrolysis, when various organic compounds were identified by IR spectroscopy of the evolving gases. At over 800°C, char containing Ca, Mg, Na and K ions resulted, which may also be used as adsorbent for waste waters depollution.

Keywords: grape seeds, polyhydroxyphenols, dye adsorption, pyrolysis

1. Introduction

The *zero waste* policy imposes a new approach for all industrial processes [1]. The wine industry generates solid residues containing among others grape seeds. These components form around 17 % of the winery waste and may be used as an oil source. The oil content in the grape seeds is 11-14%, and this product is noteworthy for its high content (more than 70 %) in polyunsaturated fatty acids [2, 3].

The further valorization of the defatted seeds is compulsory due to their high amount (over 80%). A preliminary study in this respect is presented. Various solutions for the valorization of the residual grape seeds are proposed in this paper.

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2. Experimental

2.1 *Materials*

The defatted grape seeds resulted after a standardized Soxhlet extraction procedure [4] on grape seeds of **Pinot Noir** (**PN**) variety, supplied by the Research centre for viticulture and enology from Murfatlar, Romania.

Reagents and solvents were analytically pure being purchased from Sigma Aldrich.

2.2 *Apparatus*

NMR spectra were acquired on a Bruker Avance III of 500 MHz, and IR spectra were recorded with a JASCO 4200 spectrometer with Pike ATR unit in the 400–4000 cm^{-1} range.

STA Netzsch Jupiter 449 F3 apparatus coupled with a Bruker Tensor 27 FTIR spectrophotometer was used for pyrolysis. The samples were analyzed in Al_2O_3 crucibles, in 30-900^oC temperature range, with a heating rate of 10 K/min, and a nitrogen flow of 50 mL/min.

SEM images were obtained on a Quanta Inspect F 50 scanning electron microscope with field emission gun and energy dispersive X-ray spectrometer.

2.3 *Separation of PPs from the defatted seeds*

Defatted seeds were extracted with a polar solvent (ethanol/water – 2/1, v/v), in a ratio of 9/1 v/w with the solid, at room temperature, with magnetic stirring, for 2 h. After the solvent evaporation using a rotary evaporator, 0.08 g red solid (RS) was obtained per 1g of seeds. IR and ^{1}H-NMR spectra were recorded for this solid. After PPs extraction a biomass B, that accounts for about 90% of the defatted seeds, remains.

2.4 *Total PP content*

Folin-Ciocalteu method was applied [5] for the determination of polyhydroxyphenols (PPs) content. The red solid (0.02 g) was dissolved in 1 mL ethanol water mixture (2/1 v/v). A volume of 0.1 mL from this solution was mixed with: 0.5 mL Folin-Ciocalteu reagent, 1.5 mL 7.5% (w/v) sodium carbonate and 1.5 mL distilled water. The solution was kept for 2 h in the dark, then the absorbance at 750 nm was measured. The total content of PPs was expressed as gallic acid equivalents (GAL) based on a calibration curve previously drawn ($y = 0.0609 \cdot x$, where y = Absorbance and x = gallic acid concentration in μg/mL)

2.5 ***Dye adsorption on biomass B***

Various amounts of the biomass B (50, 75 and 100 g/L), resulted after the oil and PPs extraction, were introduced in *Acid Blue 62* dye water solutions (1 or 2 %). The discoloration in time of this textile dye solution was followed with UV-Vis spectroscopy.

3. Results and discussion

3.1 Study of the RS obtained by extraction

The aqueous ethanol was chosen for the extraction of RS because it is recommended as the best solvent for PP extraction, compared with hexane, chloroform or butanol [6].

The FTIR analysis of RS (see Figure 1) revealed the presence of OH (3254 cm^{-1}), C-O groups (1098.25 and 1035.59 cm^{-1}), aromatic CH (sh 3100 cm^{-1}) and of saturated carbon atoms CH (2924.52 cm^{-1}), as well as C=C (1604 cm^{-1}).

Several peaks suggested the presence of carbohydrates at 1200-1300 cm^{-1} (streaching of pyran ring),1098 cm^{-1} (glycosidic C-O-C), 817.67 cm^{-1} (α-glycosidic C-H), and 1519 and 1440 cm^{-1}(aromatic ring) [7, 8].

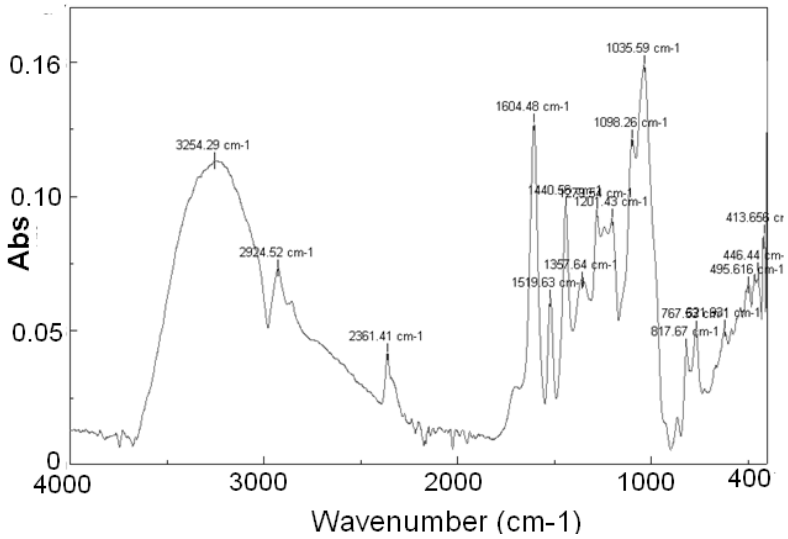

Fig.1. IR spectrum of ethanolic grape seed extract RS

The fact that PPs are present as glycosides, has been supported by the numerous peaks in the range 3.00-4.00 ppm [9] of the ^{1}H-NMR spectrum (see Figure 2a).

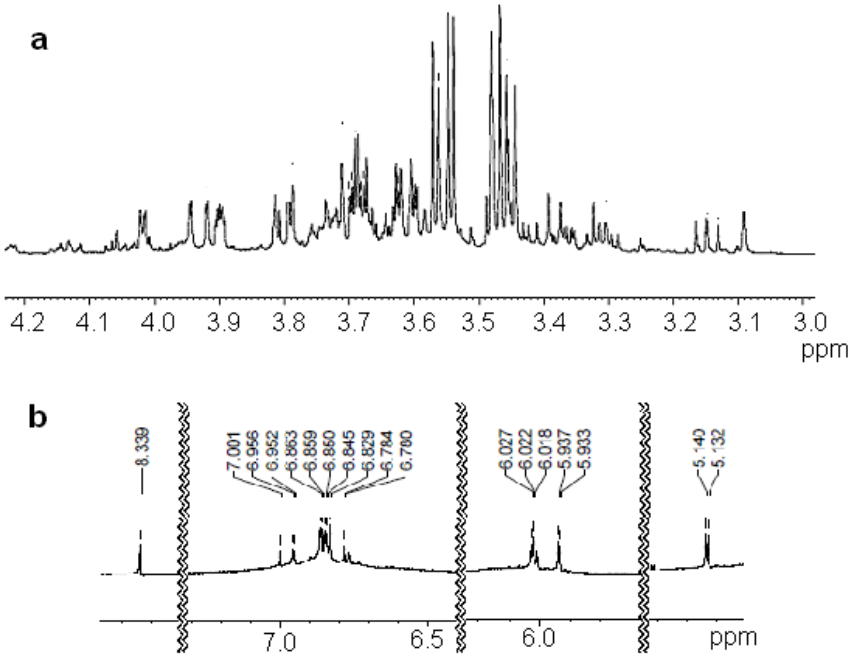

Fig. 2. ^{1}H-NMR spectrum of the red solid RS; chemical shifts for glycosides (a), and for the aromatic protons (b)

The red solid contains mainly anthocyanins, glycosides of anthocyanidins. The ^{1}H-NMR peaks suggesting this assertion are the following: 5.32, 5.40 and 3.10-4.10 ppm for the carbohydrate part, and 8.34, 6.78-7.00 ppm for anthocyanidins [9a]. The structure of anthocyanins is presented in Figure 3. Thus, the peak at 8.34 ppm was assigned to H4 from this structure (see Figure 3), according to literature [9, 10], the other aromatic protons resonating in the range 6.78-7.00 ppm (see Figure 2b). The presence of anthocyanins is also confirmed by the dark red color of RS.

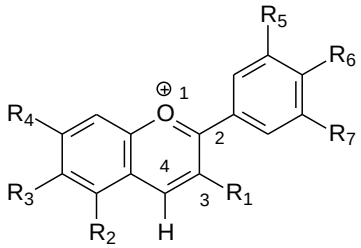

Fig. 3 Chemical structure of anthocyanins ($R_{1\text{-}7}$ = H, OH, OCH_3, O-glycone)

RS containig anthocyanins is a good antioxidant. The Folin-Ciocalteu analysis evidenced a quantity of 1.41 mg GAL per 1 g of extracted seeds.

Consequently, it can be recommended as food supplement for prevention of neuronal and cardiovascular diseases, as well as for diabetes or cancer [11].

3.2. The biomass B as dye adsorbent

The textile industry is very polluting due to the number of chemicals necessary for the preparation and/or finishing of textile materials [12]. Textile dyes are highly polluting agents, mostly because of the colored waste waters resulted. By coloration of the surface waters the vegetation is destroyed due to the lack of transparency and consequently, the blockage of the photosynthetic process results [13]. One of the less expensive procedures for the elimination of the textile dyes from the waste waters is the adsorption on solid supports [14]. Several experiments have been performed using the biomass B as adsorbent. Solutions of 1 and 2 % 1-amino-4-cyclohexylamino-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid, sodium salt *(Acid Blue 62*, **AB 62**), having a blue color (λ_{max}=585 nm) were prepared and tested for discoloration. Results are presented in Table 1 for a quantity of 50g biomass B per 1L dye solution.

Table 1

Adsorption of AB 62 on biomass B (50g/L)

Time (min)	Dye concentration (%)	
0	1	2
30	0.42	1.42
60	0.37	1.37
90	0.36	1.13
120	0.35	1.01
180	0.34	0.95
1440	0.31	0.72

The dye adsorption was established by measuring the solution absorbance (Abs) in time; the concentration of **AB2** was calculated based on a previously drawn calibration curve (Abs *vs* conc. %). The discoloration process is visible in the first 30 minutes for the solution of 1% and in 120 min for the solution of 2 %.

This result may be explained by the fact that the process of adsorption is influenced by the adsorbent surface so it is important to find the optimum amount of biomass B as adsorbent in this process. Figure 4 shows the evolution in time of the discoloration process for 1% aqueous solutions of the dye, using different amounts of biomass B (50, 75 and 100 g/L).

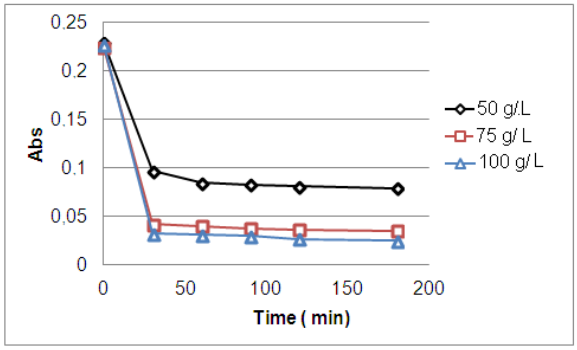

Fig. 4. Influence of the biomass B quantity on the adsorption process

Based on these experimental results, an amount of B per L of 1% dye solution is 75g, and a contact time of 50 minutes seem to be the appropriate conditions for the elimination of most of the dye from the solution.

3.3. Thermal degradation of the biomass B

Another way to valorize the biomass B is the thermochemical conversion by pyrolysis, combustion, etc. [15]. The combination of thermal analysis (TA) with the Fourier transform infrared (FTIR) study of the evolved gases is a resourceful method for the characterization of the biomass B material decomposition [16]. The thermal investigations were performed in nitrogen flow, after drying the biomass B to constant weight at room temperature (25^{o}C). The thermogravimetry (TG) and differential scanning calorimetry (DSC) plots are presented in Figure 5. A small mass loss (~ 6 %) was detected above 200^{o}C showing an endothermic effect. It is most probably due to the elimination of volatile compounds. In the range 200-370^{o}C a process of degradation leads to a mass loss of ~34 %, DSC plot showing two exothermic peaks, at 285 and 362^{o}C. The degradation continues slowly in the range 370-800^{o}C, a residual mass of char (~ 37 %) being finally obtained.

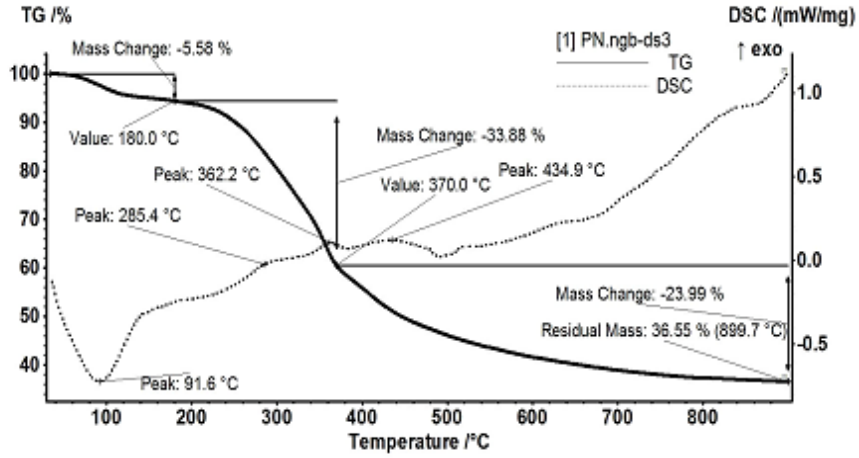

Fig. 5. Thermal behavior (TG-full line and DSC-dotted line) of biomass B and mass loss values during the thermal degradation

The 3D FTIR data (see Figure 6) of the evolving gases evidenced carboxylic acids in the temperature range 180-370ºC, assumption based on the large peak at 3000-2700 cm^{-1} together with the high peaks in the region 1800-1650 cm^{-1} which suggest the presence of C=O groups (from COOH) and the peaks at 3800-3600 cm^{-1} characteristic for OH [17]. These peaks decrease with temperature in time, and char is obtained in the end.

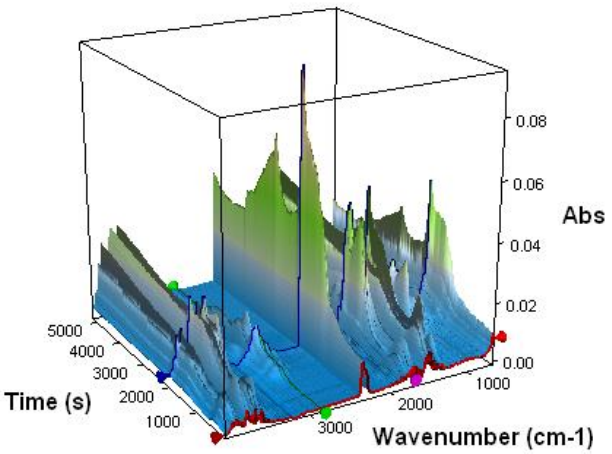

Fig. 6. 3D FTIR spectra of the gases evolved during the pyrolysis process

The SEM analysis of the char shows a granular morphology, as previously described [14] for other biomasses, while the Energy Dispersive X-Ray Analysis (EDAX) indicates carbon as major element, accompanied by traces of Ca, Mg, K, Na (see Figure 7).

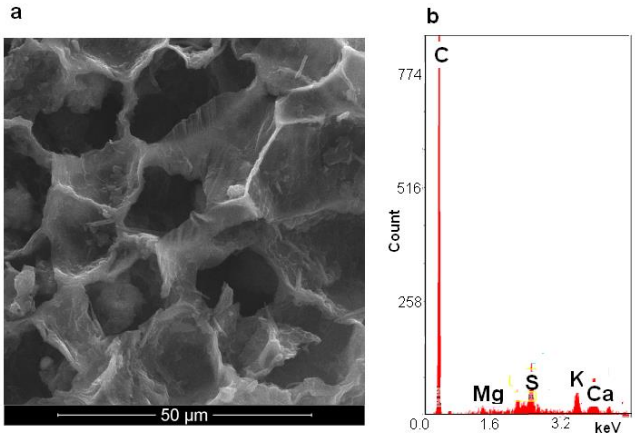

Fig. 7. **a)** Texture of carbonized inner tissue of the char; **b)** Char chemical elements analysis (EDAX)

The char texture and content suggest a direction for the valorization of this product, namely its use as adsorbent. The presence of alkaline elements is an asset for the elimination by adsorption of acid compounds from polluted waste waters.

4. Conclusions

The high quantity of wastes (over 80 %) resulted after the oil extraction from grapes seeds makes compulsory their further valorization. Preliminary investigations suggest the following possibilities in this respect:

- extraction with a polar solvent of the residual polyhydroxyphenols (PPs), which may be valorized as food supplement or as drug component;
- use of the biomass B (90%), obtained after the PPs extraction, as adsorbent for depollution of textile waste waters from acid dyes;
- pyrolysis of the biomass B in order to produce energy and valuable compounds (carboxylic acids, char, etc.).

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