OPTIMIZATION OF THE EXTRACTION OF POLYPHENOLS FROM GRAPE SEEDS

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Abstract. *In the present work the extractability of polyphenols from grape seed has been studied. In winemaking, the degree of use of grapes as a material for the production of wine or juice reaches the maximum value of 80%, leading to the formation of considerable amounts of secondary products (marc, stalks, yeast, tartaric acid salts). Regarding the chemical composition, secondary winemaking products represent a valuable bonus material for the manufacture of a wide variety of products with an important role in the national economy. The determination of the quantity of the total amount of polyphenols with the help of Folin-Ciocalteu method in a stirred reactor at laboratory scale was made. Different values of temperature, time and concentration of the reagent were applied during the extractions to search for the optimal parameters where the extractability will be the most efficient in quantity and quality. A pulsed electric field was applied on the raw material to study its effect and check its fiability. The effect of the extractability was influenced by the parameters of the applied electric field, due to different values of intensity of the electric field, the number and the duration of impulse. The greater amount of extracted polyphenols was obtained at high temperatures, 30 minutes of extraction, with a concentration of ethanol of 40 %. The grape seed represents a valuable material which contains precious components and therefore the study of extractability is interesting from both scientific and economic point of view.*

Keywords: *Solid-liquid extraction, grape seed, polyphenols*

Introduction

A particular interest in the technology of waste treatment is the rational use of grape seed. Depending on variety, the bay contains up to 8.0% of seeds, which are not currently exploited[3]. Each year the primary wine making factories produce huge volumes of marc, for which is necessary to separate the seeds for drying and further processing. For several years, the wine tannins have diversified and are subject to many and varied uses. Besides the conventional tannin of galls, new tannins appeared from oak, chestnut trees, exotic fruits and, recently, grape seed. The chemical composition and properties of various tannins are not the same, especially the grape tannins that can a priori be considered "endogenous" to the wine compared to others, extracts of exotic fruits or trees. The constituents of grape are numbered by hundreds. This fruit is characterized by its richness in qualitative and quantitative phenol constituents localized mainly in the solid parts (seeds, skins, stalks), with the exception of grape varietals called dyers which also contain in the pulp. The phenol compounds of wine have many properties: • anthocyanins, red pigments, are responsible for wine color • tannins are responsible for the properties that give wines the characteristic taste of astringency[4] The phenol compounds are

able to polymerize and combine with many substances (polysaccharides, proteins, other polyphenols ...)[5]. These condensed forms to which they operate have a major role in the quality of wines: • the taste properties of tannins depend on their state of combination that provides them different taste characters); • the combinations Tannins-Anthocyanins (TA) is sought for the stability of the color of wine; From grape marc, distilleries recover, in addition to alcohol, seeds for the manufacture of edible oil, marc anthocyanin colorants for mainly for food industry, seeds of white grapes to extract oligomers proanthocyanidols for pharmaceutical industry (drugs against cardio-vascular diseases).

Materials and methods

As raw material we used dry seeds of red grapes from Moldova. The grape is a berry consisting of skins (7-11% by weight), seeds (2-6%) and juicy and fleshy pulp (80-85%). The quantity and types of polyphenols existing in grape seeds may change and are considerably influenced by the extraction process, the variety and storage of used seeds.

Solid-liquid extraction

Dry seeds of red grapes from Moldova were used for the experiments. The extracts were prepared using water-ethanol mixtures as solvents (ethanol concentration of 20, 40, 50, 60, 80 and 95% by volume). The experiments were performed in a water bath in a conventional batch, which eliminates the external resistance to mass transfer[6].Uncrushed seeds samples (3 g) were extracted by dissolving the appropriate volume (22.5 ml) at different temperatures up to 65 ± 2 ° C for different periods. After the extraction, the dry extract was obtained by evaporating the solvent on the water bath and the entire extraction yield was determined by weight differences. The results are expressed as mass fraction (total solid extract magnesium gram).

The determination of tannins

Preparation of samples for analysis: the grape seed extract (0.5 ml) was placed in the 50 ml volumetric flask and was dissolved in deionized water (approximately 25 ml), the Folin-Ciocalteu reagent (2.5 ml) and sodium carbonate solution (10 ml of 2% m/v) were added and then the sample was filled with water to 50 ml. The mixture was shaken thoroughly for 30 minutes and the formed blue color was measured at 750 nanometers using a spectrophotometer UNICAM. Due to relatively short linear part of absorbance values of standard curve obtained may extend between the absorbance of 0.210 at 0.340. If the absorbance is not in this field, the sample should be diluted or concentrated for it to be measured[7]. The measurement was performed using a blank reagent solution without extract. The results are calculated by the following analytic function: where m_t - total tannin mass, C_t (mol/liter) - the total concentration of tannins was reported on the calibration curve, M_t – tannins molar mass (gram/mol), V_{ext} - volume of the extract (ml). When the absorbance values fall without assigned support, the mixture was further diluted and then the elution volumes were taken into consideration [4]. Each sample was measured three times. The Folin-Ciocalteu phenol reagent and tannic acid (M = 1701.2 gram/mol) used as standard to determine the total phenol compounds were purchased from Sigma-Aldrich (Darmstadt, Germany). Ethanol and other chemicals were of analytical grade.

Identification and determination of phenol compounds

All tests have been carried out using the Shimadzu GC system coupled with a single quadrupole mass-spectrometer GCMS-QP2010 equipped with the three-dimensional automated system for the injection of samples AOC-5000 (GCMS-QP2010 PlusxAOC-5000).

For the identification, the general library of NIST-5 mass spectra was used. The accuracy of displacement has been verified according to the library of Covatz retention indices (MLRI). The analysis of the experimental data was carried out with the GC/MS Solution software (Shimadzu), which contains the SCAN/SIM options (Fast Automated Scan/SIM Type (FASST); creation of automatic SIM (Scan/SIM) table (COAST).

Research on the influence of extractions number on the extraction capacity of tannins

The use of grape seed tannins for extraction is very important because grape seeds are byproducts of winemaking that are not used practically. Numerous studies have shown that grape seeds are rich in polyphenols that can be extracted and used in different fields. The solid / liquid extraction is a transfer of material between a solid phase material to extract, and a liquid phase, the extraction solvent. Extraction is an operation used to remove old plants and certain organs of animals, food products, pharmaceutical or incense, for production of beverages, drugs or perfumes. The manufacturers use this particular separation process for extracting plant products by water, alcohols and organic solvents and chlorinated solvents. In thermodynamics, the solid is a homogeneous mixture at equilibrium is to say that in the absence of an external disturbance, it shall not be changed as opposed to treating a heterogeneous mixture can be divided by filtration, decantation or centrifugation, for example. In solid / liquid extraction, this disturbance is a heat exchange with the external and mechanical, coupled with the input of third body, the solvent. Following this disturbance, the solid will be more balanced and solid + solvent system will evolve towards a new equilibrium by mass transfer. The art of extraction is the knowledge of parameters influencing the nature and kinetics of mass transfer of solid to the solvent. To determine the maximum extractable was used 3 grams of basic equipment-whole seeds that were placed in an aqueous solution of 50% ethanol. The extraction was done for one hour at a temperature of 65 º C. After the first extraction the seeds were reused and placed in a newly prepared solution of 50% ethanol. This was done four times in the same way to achieve a solution with a minimal amount of tannins. The solutions with the extract were filtered in a Buchner and each filtrate was collected in a dark glass container.

The extract was analyzed by Folin-Ciocalteu method for determining the concentration and mass of tannins in each filtrate. So we conducted the analysis of the conductivity of materials using a circuit forms a galvanic cell and a digital multi-meter. From the results we can observe the variation of the total content of each polyphenol extraction. It is obvious that the content of polyphenols is extracted during the initial extraction and concentration of tannins decreased substantially after the second extraction. As a result we can say that it is reasonable to make two extractions for extracting the maximum amount.

Many bio-ingredients derived from plants are of high value chemicals with additional applications in food, cosmetics and pharmaceutical sectors. To satisfy a growing demand for a shorter time and

reduced cost in developing manufacturing processes, a systematic process is required to produce alternatives extraction process that transformed plants harvested in the desired products. Many factors affect the extraction and should be optimized for maximum recoveries - particle size of the sample, type of solvents, pH of the extraction time and extraction temperature, number of extraction and degradation of compounds during extraction. Among these factors, the type of solvent is most important because it can affect the quantity, biological activity and the types of compounds extracted. The thermodynamic principles on the solubility of structurally complex materials were not studied because of the complexity of molecular interactions involved. Hildebrand theory of regular solutions and concepts of the Hansen solubility parameter (HSP) provide a useful correlation for the solubility parameter of the solvent and solute. The Hildebrand solubility parameter is a numeric value that indicates the relative solvency behavior of a specific solvent. It is derived from the cohesive energy density of the solvent, which in turn is derived from the heat of vaporization:

$$\delta_H = \sqrt{\frac{E_{coh}}{V_m}} = \left[\frac{\Delta H^v - RT}{V_m}\right]^{1/2}$$

Where:

(1) E_{coh} *is* the molar cohesive energy

(2) V_m - molar volume

(3) ΔH^v – vaporization enthalpy

(4) R - ideal gas constant

(5) T- Temperature

The concept of HSP has brought all the cohesive energy (E_{coh}) by the amount of energy required to overcome the nuclear forces of dispersion forces between permanent dipoles of adjacent molecules (dipolar interaction) and to break the hydrogen bonds (exchange of electrons, proton donor / acceptor) between the molecules (δ_h)

$$\delta_t^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \qquad (2)$$

where δ_t (total solubility parameter) should be identical to δ_h. Defined the difference between the solubility parameters of solute i and solvent j indicates an affinity:

$$\Delta\delta_{i,j} = \sqrt{4(\delta_d^i - \delta_d^j)^2 + (\delta_p^i - \delta_p^j)^2 + (\delta_h^i - \delta_h^j)^2} \quad (3)$$

The parameters follow the rule that if the $\Delta\delta_{ij}$ is smaller, the affinity is high between the solute and the liquid. This method is often applied to pure chemicals with well-defined chemical structures. Recently, the concept of HSP has been extended to interpret the relationships involved in the miscibility of polymer-solvent, adsorption on solid surfaces, as well as for the solubility of components useful to plants in various solvents pure. This study is extracted from red grapes (*Vitis vinifera L.*), whose seeds are an important source of biologically active compounds such as polyphenols (also known as pro-anthocyanidins and condensed tannins) that act as strong antioxidants. The seeds are the parts richest in polyphenols, and a grape full beam is the part where polyphenols are very difficult to be extracted. The objective of this work was to experimentally determine the optimal operating conditions for a more complete recovery phenol grape seed. We determined the total mass of tannins extracted from four extractions is 124.2mg tannins from 3G base material (Figure 1). So we extracted 4.14% of the total mass.

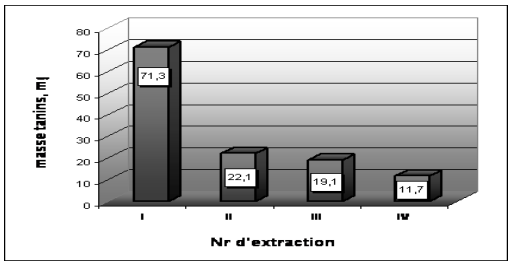

Figure 1. The effect of multiple extraction steps on the amount of tannin extracts

We also measured the total extracted mass by drying the filtrate which was obtained and the total mass of extract that can also contain mucilage. To see the change in total mass and the mass of extracted tannin the weights of the extracts are reported to 100 g and are shown in Figure 2.

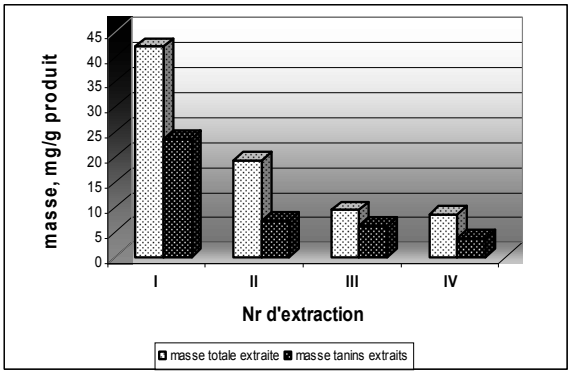

Figure 2. Representation of the total mass extracted from a mass of tannins extracted according to the number of extraction

So we can conclude that with the tannins extracted in essential quantities of other compounds. And the ratio of the total extract and extract tannins is 50%. The total extract obtained after 4 extractions is 0.08g per 100g of seeds and extract tannins is 0.04g per 100g of seeds.

Variation of parameters of tannin extraction

The solid / liquid extraction is influenced by many factors. The selection of raw materials, choice of solvent, use of the method, equipment performance is crucial. The implementation of a solid / liquid extraction is linked to the state of the solid and the solute, the nature of solvent, temperature and dispersion phases.

The nature and size of the particles have a direct influence on the rate of extraction. Indeed, if the solute is distributed throughout the grain, the first solvent will dissolve the solute at the surface will then have to progress within the grain to dissolve the solute particles.

Research on the influence of solvent on the extraction capacity of tannins

The most commonly used solvents are: water, ethanol, acetone, ethyl acetate, hexane and dichloromethane, regardless of the physical structure of plants and nature (flower, fruit), but they must be of suitable purity so that no remaining fraction of high boiling point.

To find the optimal concentration of solvent extractions were made extraction of tannins with different concentrations of alcohol. We prepare solvents with 0, 20, 40, 50, 60, 80.96 percent of ethanol. We prepared samples of seeds for each solvent extraction was placed at 65 º C for 30 minutes with the agitator, after each extraction being filtered and prepared for measurement.

By measuring all the samples we concluded that the solvent with the concentration 50% ethanol is the most optimal for the extraction.

Influence of solvent concentration on the degree of extraction of tannins

The best extraction of polyphenols in the compound solvent (50: 50 ethanol / water) is due to synergism between these two solvents. On the other hand, in pure ethanol (96%), extraction of total dry matter is more complete. But the rate of dietary fiber, insoluble in water, in this case is considerably higher.

In the compound solvent (50: 50-60: 40 ethanol / water) rate of polyphenol extracts ranged from 32-40 mg / 3 g of dry matter (Figure 3).

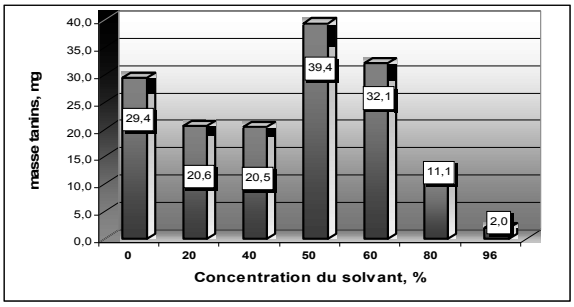

Figure 3. The influence of the concentration of solvent (ethanol) on the amount of tannin extracts

This degree of extraction after the first cycle, a total of 30 min is optimum as

allows extracting the most soluble fraction of tannins.

Research on the influence of temperature on the extraction capacity of tannins

The temperature is a factor that strongly influences the degree of extraction of tannins from grape seeds. The rise in temperature has a positive effect because it increases solubility and facilitates the diffusion of the solute and solvent viscosity decreases. However, an excessive rise in temperature can also facilitate the dissolution of harmful compounds or a deterioration of compounds extracted.

To have a temperature dependence with the mass of tannin, extractions were performed at different temperatures 25 º C, 45 º C and 65 º C. After measuring the samples we can conclude that the rise in temperature has a positive influence on increasing the amount of tannin extracts.

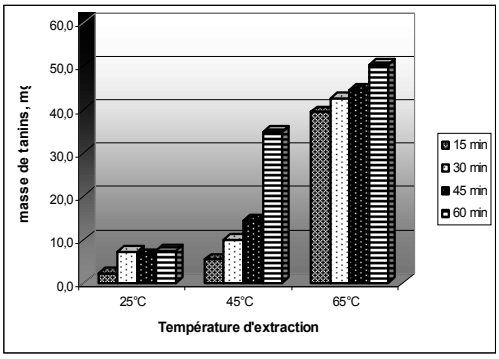

Figure 4. The effect of temperature on the amount of tannin extracts

It is obvious that increasing the temperature of the solvent and extraction time have a positive influence on the rate of polyphenol extracts.

The duration of the extraction has a great influence on the amount of tannin extracts. We observe that increasing the extraction time has a direct influence on the proportional amount of tannin extracts.

It is very difficult to evaluate separately the influence of temperature and extraction time. This gives a higher concentration at 65 ° C for one hour of extraction.

But it is important to take into account the fact that you can not get the same results for a longer duration and temperature decreased because we observed that at 65 º C for 15 minutes you get twice as 45C and 45 min or so for a period of 15 min at 45 ° C extracted twice for 60 minutes at 25 º C. So the temperature plays a key role. In order not to destroy the antioxidant tannins may be considered maximum duration of 60 minutes at 45 º C when the mass reached 11.57 g of tannins extracted from 100 g of seeds. This result is equivalent with the result obtained at 65 ° C for 15 minutes when extracted 12.19% of the tannins.

Identification and quantification of extracted phenol compounds

The identification and quantification of phenol extracts was performed by gas chromatography coupled with mass spectrometer (GC / MS). The results are presented in Table 14.

The experimental data obtained show the presence of 10 phenol compounds, which have been identified, including: vanillin, cinnamic acid, p-hydroxybenzoic acid, floretic acid , vannilic acid, gallic acid, p-coumaric acid, caffeic acid, resveratrol, quercetin. The other seven peaks were not identified, and their contribution was taken into account. The presence of catechin and epicatechin, as the presence of polyphenols and their derived conjugates esters have not been highlighted by this study.

Also, a sizeable rate of resveratrol (0.14 0.01) was documented in the extracts of grape seed. The predominant compounds, in descending order, are: vannilic acid (1.24 0.25), caffeic acid (0.55 0.08), gallic acid (0.52 0.15) .

Conclusions

1. A study concerning the influence of the number of fractional extraction on the extraction rate of wine-tannins was performed. It was established that two extraction steps are used to extract 80-85% of the total the quantity of tannins, which make ineffective the following steps. With the extracted tannins an important quantity of other compounds are extracted. The ratio of the total extract and tannin extract is 50%.

2. The study on the composition influence of the extracting on the extraction rate of wine-tannins showed that the extraction is more complete in the case of ethanol / water mixture (50:50) due the synergism between these two solvents. In pure ethanol (96%), extraction of total dry material is more complete. But the rate of wine-tannins extracts in this case is considerably lower.**3.** The extraction time greatly influences the rate of extraction of wine-tannins: 2.2 to 7.5 mg tannins / g 3 seed (25 º C) of 5.4 to 34.8 mg tannins / g 3 seed (45 º C) from 39.4 to 50.4 mg tannins / g 3 seed (65 º C). Another important factor is the temperature of extraction. Under the same conditions, the extraction is 7-8 times higher at a temperature of 65 º C as compared to room temperature. **4.** The identification and quantification of the extracted phenol compounds helped to highlight the presence of 10 phenol compounds, including predominant compounds (mg / 3g seeds): vannilic acid (1.24± 0.25); caffeic acid (0.55± 0.08), gallic acid (0.52± 0.15). Also, a sizeable rate of resveratrol (0.14± 0.01) was documented. This composition reflects the high biological activity of wine-tannins from grape seed.

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