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Short communication

Optimization of polyphenols extraction from grape residues in water medium

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ABSTRACT

The extraction in water of polyphenolics from grape red marc, grape white marc and grape pomace for the production of wood adhesives was optimized using microwave extraction in presence of Na_2CO_3. The reaction parameters studied were the temperature (60–120 °C), the residence time (5–20 min) and the sodium carbonate concentration (0–2.5%) using response surface methodology based on central composite design. The optimal values of the variables were as the followings: 100 °C, 8 min, without sodium carbonate from grape marcs and 100 °C, 8 min, 2.5% w/w of sodium carbonate for pomace. The microwave assisted extractions gave significantly higher yields as compared to traditional extraction and could be recommended as an alternative method for extraction of phenolic compounds from grape residues in water medium.

1. Introduction

Winemaking is one of the most important agricultural sectors in Europe. Thus, five European countries (France, Italy, Spain, Portugal and Greece) contribute to more than 60% of wine production worldwide. The winemaking process is known to generate large quantities of solid residue, namely the grape marc. This by-product consists of skins, and in certain cases of seeds and some stalks. After industrial extraction in distilleries of wide range of products (ethanol, oil, tartrate, etc.) only small amounts of these by-products are up-graded or recycled. The remaining pomace is used for composting, methanization and/or energy production. Nevertheless, the grape pomace retains high levels of phenolics because of low extraction during winemaking and ethanol extraction (Rondeau et al., 2013). The efficient utilization of these phenolic compounds is of great importance not only for minimizing environment impact but also for higher profitability. It was shown that polysaccharides (cellulose, pectins and glucomanan) were the most abundant components of grape skins and anthocyanidins and condensed tannins are among the principal phenolic constituents (Arnous and Meyer, 2008).

Phenolic-type compounds obtained from natural resources have been used for the production of wood adhesives since 1970s (Tondi and Pizzi, 2009). In previous works, we described the extraction at the lab scale of phenolic compounds from grape pomace in water medium (Lan et al., 2011a,b). The extracts were composed for a large part of condensed tannins (proanthocyanidins) (Lan et al., 2012a). Different adhesive formulations were prepared from these extracts and the first grape pomace extract-based particleboards were produced (Lan et al., 2011b,c, 2012a,b). However, the experimental conditions of the phenolics extraction from grape pomace were not fully optimized. This optimization is a key issue for future industrial developments. Moreover, utilization of marc as feedstock for the extraction of phenolic compounds has never been investigated for this application.

Microwave assisted extraction (MAE) has been described to be an alternative to conventional techniques due to its several advantages such as shorter extraction time, less amount of solvent usage, and higher extraction rate (Hyun-Ku et al., 2012; Liazid et al., 2011; Simsek et al., 2012). In literature, to the best of our knowledge, no studies have been presented on MAE for the extraction of total phenolic compounds from grape pomace in a water medium. As part of our ongoing works on the upgrading of grape industry by-products at an industrial scale, the aim of this study is to optimize the extraction of phenolics from solid residues from wine industry in water medium in presence of sodium carbonate using response surface methodology based on three variables central composite design. Three different feedstocks were investigated: a red marc, a white marc and a pomace. The objective was to determine the important experimental parameters for an industrial development and the optimal conditions for time, temperature and carbonate

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ions concentration. A systematic experimental approach was designed using microwave treatment. A comparison conventional and microwave extraction was also examined.

2. Material and methods

The samples correspond to mixtures of by-products of the winemaking process of red and white grape variety (*Vitis vinifera*), growing in south-west of France and were provided by a French distillery (Douence, Saint Genès de Lombaud, France). The air-dried grape residues were stored at room temperature during the course of this study.

2.1. Tannins extraction using parallel microwaves reactors

The device was composed of 8 quartz reactors of 100 mL each. These 8 reactors were disposed on carousel turning at constant speed in a microwave oven. The cooling at the end of each run was achieved by an accelerated circulation of cold air at room temperature inside the oven. The internal temperature and pressure were measured and recorded accurately with the help of the sensors present thereof.

2.2. Tannins extraction using conventional process

Twenty grams (oven-dry matter) of grape pomace were treated with an aqueous solution of sodium chemicals with a solid-to-liquid ratio of 1:8. The reaction mixture was heated at 100 °C during 8 min. The treatments were carried out in a round bottle flask connected by water condenser. After heating, the solid residue was cooled, washed and filtered through filter paper. The washed liquid was evaporated to a moderate concentration (rotary evaporator, temperature: 60 °C), then lyophilized.

2.3. Analysis of the raw material and solid residue

Moisture content was determined using KERN MRS 120-3 infra-red moisture analyser. Carbohydrates were measured on extractive-free material (soxhlet extracted with dichloromethane overnight), ground to pass a 40-mesh screen, according to the laboratory analytical procedure (LAP). Samples were hydrolyzed with 72% sulfuric acid for 1 h and then autoclaved after being diluted to 3% sulfuric acid through the addition of distilled water (Rondeau et al., 2013). The dried residue was weighed to give the acid insoluble fraction (AIF). Monosaccharide contents in the filtrate were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The conditions of the analysis and an example of chromatogram are given in Supplementary materials. The ash contents are determined by incinerating the samples at 575 ± 25 °C during 180 min in a wet digestion system type Buchi B440. Total soluble polyphenols were extracted from the samples (4 g) with 100 mL of solvent (water/acetone/acetic acid, 29.5/70/0.5) during 2 h at 50 °C under stirring (Bozan et al., 2008). The method of Brownmiller et al. (2008) was used for the extraction of anthocyanins. 10 g of grape pomace or marc plus 100 mL of methanol/water/formic acid (60/37/3) was incubated for two hours at 50 °C. The total phenols were measured by the Folin–Ciocalteu method, based on a colorimetric oxidation/reduction reaction of phenols (Skerget et al., 2005). Condensed tannins were calculated from the absorbance at 550 nm of polyphenolic solutions obtained after 5% HCl–BuOH treatment (100 °C, 3 h) according Chamorro et al. (2012). Flavanols were estimated from the absorbance at 500 nm of polyphenolic solutions obtained after Vanillin treatment (30 °C, 20 min) (Nakamura et al., 2003). Results were given according to the calibration curve expressed in catechin equivalent.

2.4. Statistical approach

A systematic experimental method to optimize the polyphenolics extraction was designed using Central Composite Design (CCD) and Response Surface Methodology (RSM). Na_2CO_3 concentration, reaction temperature, residence time were studied as three independent variables. JMP software version 10 was used for regression analysis and analysis of variance (ANOVA).

3. Results and discussion

3.1. Composition

The samples studied correspond to by-products of the winemaking process (grape skins) of red and white grape variety (*V. vinifera*), growing in France and were provided by a French distillery. Table 1 gives the general composition of the two marcs (from red and white winemaking, recovered before extraction of ethanol in distillery) and of the pomace (residue after industrial extraction, see Supplementary materials). The monosaccharide contents were estimated after sulfuric acid hydrolysis of grape biomasses through analysis of the soluble fractions using HPAEC-PAD. The results were roughly similar in the three feedstocks and comparable to those reported by others for grape skins (Rondeau et al., 2013). Arnous and Meyer (2008, 2009) demonstrated that the method of hydrolysis of grape residues has a significant influence on the monosaccharide results. As a consequence, the compositional analyses given in the present study only provide a picture of the building blocks and enable a comparison between the samples compositions. As previously reported, the acid insoluble fractions (AIF) were relatively important (47.4–57.5%). This fraction is a consequence of the strong tendency for phenolics, largely present in pomace and others components (such as pectins, proteins, cutins) to become insoluble during this acid treatment (Llobera and Canellas, 2007). This can also suggests that pomace and marcs retain high content of polyphenolics not easily extractable (e.g. condensed tannins) and should explain the harsh conditions previously described for their extraction (Lan et al., 2011b).

The quantities of total phenolic substances contained in the grape marcs extracts (acetone extracts) were higher compared to those obtained from the pomace. The removal of water soluble phenolics during the process of extraction of alcohol and sugars in the distillery could explain this observation. As previously shown by Negro et al. (2003) on red grape marc, the data clearly show that an important part of the extracted phenolic substances had flavonoidic origin. The highest quantity of phenolics and flavanols were present in red marc. However, the analysis of the content in condensed tannins of the three fractions showed a different pattern with the highest content present in the red marc and in the pomace (∼1.5%), while the quantity in the white marc was lower (∼0.5%). The highest quantity of anthocyanidins was present in the red marc (1.46%). In grape pomace, the lower yield (0.56%) can be rationalized by the removal of a large proportion of anthocyanidins during the upstream processes. These compounds were not present in the white marc.

3.2. Response surface methodology

The response surface plots concerning the phenolic compounds extraction from the white marc, the red marc and the pomace, as a function of three variables studied are given in the Figs. 1 and 2. Fig. 1 depicts the interaction effect of temperature and residence time. It appears that (1) higher phenolics recovery is found at high