Industrial Crops and Products 49 (2013) 286–291

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Integrated utilization of grape skins from white grape pomaces

Joana A.S. Mendes[a,b,c,d], Ana M.R.B. Xavier[a,b], Dmitry V. Evtuguin[a,b,*], Luísa P.C. Lopes[c,d]

[a] CICECO, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Environment Department, Polytechnic Institute of Viseu, 3504-510 Viseu, Portugal
[d] CI&DETS, Polytechnic Institute of Viseu, 3504-510 Viseu, Portugal

ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form 10 April 2013
Accepted 2 May 2013

Keywords:
Grape skins
Oleanolic acid
Low-density boards
Bioethanol

ABSTRACT

An approach for the integrated utilization of grape skins from white grape pomaces has been proposed. This consists of consecutive or simultaneous extraction of grape skins with neutral organic solvent and water under reflux. Organic extract is a valuable raw material for the isolation of oleanolic acid. The aqueous extract (ca 50%, w/w) is composed of essentially hexoses and suitable for the high yield (till 51%) bioethanol production at a maximum specific cell growth rate (μ_{max}) of 0.29 h^{-1}. The remained extracted grape skins are the complex of structural polysaccharides embedded into cutinous matrix. Extracted grape skins were shown to be a prospective raw material for the production of low-density boards ($d \leq 0.40$) for insulation needs. The boards produced from grape skins and bind of 8% urea–formaldehyde resin revealed reasonable tensile strength (0.4 MPa) and a low thermal conductivity (0.09–0.12 W (m K)$^{-1}$) over a wide range of temperatures (40–200 °C).

1. Introduction

The fluctuating prices, the reduction of world reserves and the adverse environmental effects of oil consumption have led to increasing demand and the development of alternative materials and energy sources that can reduce or even replace the use of fossil resources. These trends are coherent with necessities to utilize diverse agricultural residues both for economic and environmental reasons. In particular, winemaking sector produces high amounts of under-utilised by-products, which have significant economic potential being an urged challenge (Spigno et al., 2008; Ping et al., 2011; Prozil et al., 2012). This is especially important for Europe producing around 65% of wine worldwide (Commission E., 2013).

The conjunction of wastes generated in the winemaking process (grape pomaces) is constituted of skins, pulp, stalks and seeds, which account to 25–35 kg per 100 L of produced wine (Costa, 1983). Grape skins are the major component of grape pomaces contributing roughly to a half of grape pomace matter. Grape skins may be used to some extend in animal feeds, but only few other applications are known (Costa, 1983; Arvanitoyannis et al., 2006). For example, the extractives of grape skins represented by classes of organic compounds such as polyphenols and triterpenic acids can have interesting pharmaceutical and nutritional applications (Hichri et al., 2003; Ruberto et al., 2007).

Red grapes are entirely involved in fermentation and processed skins contain much less pulp and residual sugars than the skins from white grapes that are mechanically pressed to produce juice and are not subjected to ethanolic fermentation (Silva, 2003; Ruberto et al., 2007). This is an attractive feature for the eventual utilization of residual sugars of white grape skins as substrate for bioprocessing. Recently, aqueous extract of white grape skins was successfully used for the production of bacterial cellulose (Carreira et al., 2011). The eventual application of residual sugars in grape skins for the production of so-called second generation (2G) bioethanol would be another attractive application. Nowadays, bioethanol is the most recognized biofuel alternative to petrofuels and produced essentially from food stuff (so-called first generation bioethanol) (Bai et al., 2008). Last decade, 2G bioethanol has been the focus of studies using lignocellulosic materials as a carbon source (Mabee and Saddler, 2010; Naik et al., 2010; Quintero et al., 2011; Dias et al., 2012). Unlike to first generation bioethanol, 2G bioethanol derived from non-food raw materials do not compete with food production and has a higher potential for sustainable production (Naik et al., 2010; Dias et al., 2012).

The analysis of chemical composition of grape skins shows that the main structural polysaccharides are embedded into the cuticle matter and remain inaccessible to acidic hydrolysis (Mendes et al., 2013). Hence the saccharification of grape skins either by enzymatic or by acidic hydrolysis is problematic. At the same time, the cuticle matter exhibits a chemical structure sound to cork material and could be proposed for the biocomposite applications. The use of grape skins for biocomposites was never reported yet though the economic and environmental benefits of the composites involving

* Corresponding author at: CICECO, University of Aveiro, 3810-193 Aveiro, Portugal.Tel.: +351 234 401 526; fax: +351 234 370 084.
E-mail address: dmitrye@ua.pt (D.V. Evtuguin).

0926-6690/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2013.05.003

natural fibres as fillers or reinforcing agents are of great interest (Fowler et al., 2006; Crespo et al., 2008). Natural fibres, like sugar cane bagasse, bamboo, jute, kenaf, cotton, rice husk have already been used as a biodegradable fillers (Avérous and Le Digabel, 2006; Satyanarayana et al., 2009; Faruk et al., 2012). Furthermore, the use of natural fibres derived from agro-based materials could develop and improve the economy of the specific agricultural sector.

Considering aforementioned peculiarities of chemical composition of skins from white grape, the comprehensive approach for their utilization has been proposed. This includes consecutive or simultaneous extraction of grape skins with neutral non-polar organic solvent and water under reflux. Both organic and aqueous extracts were exhaustively characterized and the aqueous extract was subjected to bioethanol production. The extractives free grape skins were examined for the production of low-density particle boards. The practical yields of bioethanol and biocomposite for insulation needs were estimated.

2. Materials and methods

2.1. Raw materials

The pomace of destemmed white grape (mixed varieties) was supplied by Quinta do Serrado (Penalva do Castelo, Portugal), a sub-holding of Tavfer group, during the 2008 vintage. The most abundant collected grape varieties were *Encruzado*, *Cerceal*, *Bical*, and *Fernão Pires*. The material was collected, dried at 40 °C and the grape skins were manually separated from the wine pomace. The grape skins were further extracted consecutively by hexane (4 h in Soxhlet) and water (1 h under reflux, solid-to-liquid ratio 1:10) or simultaneously with a mixture of hexane and water (1:1, v/v) for 4 h under reflux (solid-to-liquid ratio 1:10). After extraction by hexane–water mixture, the solid residue was separated by filtration and the liquid phase was separated to organic and aqueous phases. The solid residue had been used for preparation of biocomposites. The aqueous extract (AEX) was submitted to acid hydrolysis under reflux for 1 h (pH 1 adjusted by diluted aqueous solution of H_2SO_4). After cooling, the pH of the resulting solution was adjusted to 5.5 with NH_4OH aqueous solution. The AEX was aerated with compressed air and filtered using a 0.45 μm cellulose nitrate filter (Sartorius, sterilized). The purified AEX was used as a substrate for the production of ethanol.

2.2. Analyses

Grape skins were analyzed for ash, extractives and cellulose content. The ash content was determined by calcination of the material at 525 °C, according to the standard procedure Tappi T 211 om-93. Extractives in dichloromethane or in hexane were isolated by a Soxhlet extraction for 4 h. Cellulose content in dichloromethane extractive-free samples was determined by Kürschner–Hoffer method with relative error of 5% (Browning, 1967). Proteins in extractives-free samples were determined by treatment with 1% pepsin solution in 0.1 M HCl at 37 °C for 16 h (Pascoal Neto et al., 1996) and tannins were analyzed in extractives- and protein-free samples by the treatment with 0.1 M NaOH at 100 °C for 1 h in nitrogen atmosphere (liquid-to-solid ratio 100), followed by hot water washing until neutral reaction of filtrates as described elsewhere (Mendes et al., 2013).

The neutral monosaccharides of the grape skins, obtained after the Saeman hydrolysis were analyzed as alditol acetate derivatives (Blakeney et al., 1983; Selvendran et al., 1979) by gas chromatography (GC), using a Varian 3350 chromatograph equipped with a capillary column DB-225 J&W (30 m × 0.25 mm internal diameter; 0.15 μm film thickness). The chromatographic conditions were the following: column temperature: 220 °C; injector temperature: 220 °C; flame ionization detector (FID) temperature: 230 °C. Before injection of the samples, calibration curves for rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose were obtained using high purity (99.9%) commercial products (Sigma–Aldrich Chem. Co., Madrid). The sugars analysis in AEX was carried out by HPLC according to previously published procedure (Fernandes et al., 2012). The total amounts of reducing sugars in AEX were quantified by dinitrosalicylic acid method according to a known procedure (Miller, 1959).

SEC analyses of AEX, dissolved in ultra pure water with 0.1 M $NaNO_3$ and 0.02% NaN_3 to a concentration of 0.4–0.5% (4–5 mg mL^{-1}), were performed on a PL-GPC 110 system (Polymer Laboratories Ldt., UK) equipped with refraction index (RI) detector using two Plaquagel-OH MIXED 8 μm columns (300 mm × 7.5 mm). The temperature of the injector and the columns was kept constant at 36 °C. The eluent, ultra pure water containing of 0.1 M $NaNO_3$ and 0.02% NaN_3, was pumped at a flow rate of 0.9 mL min^{-1}. The SEC columns were calibrated using pullulan standards (Polymer Laboratories, UK).

About 10 mg of extractives soluble in dichloromethane or in hexane were trimethylsilylated (TMS) and analyzed by gas chromatography coupled with mass detector (GC–MS). Briefly, the sample was dissolved in 0.5 mL of pyridine containing tetracosane as internal standard and silylated by adding 250 μL of bis(trimethylsilyl)trifluoroacetamide and 50 μL of trimethylchlorosilane. The mixture was heated to 70 °C for 40 min and the derivatized products analyzed by GC–MS. Each sample was injected twice and the results were averaged. GC–MS analysis was carried out on a Trace Gas Chromatograph 2000 series coupled with a Finnigan Trace MS mass detector, employing a DB-1J & W capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness), using helium as carrier gas (35 cm/s). The chromatographic conditions were as follows: initial temperature – 80 °C for 5 min; temperature rate – 4 °C/min; final temperature – 285 °C for 10 min; injector temperature – 290 °C; transfer line temperature – 290 °C; split ratio – 1:75. Compounds were identified as TMS derivatives by comparing their mass spectra with those in GC–MS spectral library or with data from the literature and in some cases, by injection of standards.

2.3. Fermentation assays

Saccharomyces cerevisiae 4072 was provided by the Portuguese Yeast Culture Collection on agar tubes. The culture was transferred to yeast medium (YM) agar plates, containing 20 g of agar, 10 g of glucose, 3 g of yeast extract and 3 g of malt extract per litre of distilled water and maintained at 4 °C, after grown at 28 °C ± 0.5 °C during 3 days. The inocula for the fermentations were prepared with one colony of the yeast strain reactivated in YM liquid medium and incubated at 28 °C during 15 h.

Fermentations of 250 ml working volume were carried out with 70% (v/v) of ennobled AEX, 10% (v/v) of supplementary medium (SM1 or SM2) and 20% of inoculum in Erlenmeyer flasks of 500 mL incubated at 180 rpm and 28 °C in an orbital incubator.

SM1 was used for *fermentation 1*, contained 2.5 g L^{-1} yeast extract, 2.0 g L^{-1} $(NH_4)_2HPO_4$ and 1.0 g L^{-1} $(NH_4)_2SO_4$ and $MgSO_4 \cdot 7H_2O$ and SM2 for *fermentation 2*, containing the same supplement except yeast extract that was not added. Sterile samples were taken during time for analysing biomass sugars and ethanol concentrations.

Biomass was determined by monitoring of optical density at 620 nm (OD^{620}) and using a calibration curve of OD^{620} versus biomass dry weight. Sugars and ethanol concentrations were analyzed by high-pressure liquid chromatography (HPLC) as described previously (Xavier et al., 2010).