LWT - Food Science and Technology 61 (2015) 117–123

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Efficacy of olive oil mill extract in replacing sulfur dioxide in wine model

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ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form
6 November 2014
Accepted 9 November 2014
Available online 15 November 2014

Keywords:
Antioxidant
Anitimicrobial
Hydroxytyrosol
Olfactometry

ABSTRACT

Olive oil industry produces high amounts of olive mill solid waste. These have been described as natural source of hydroxytyrosol, which shows high antioxidant and antimicrobial properties joint to health-promoting effects. In the present work, an olive mill solid waste extract dissolved in model wine was evaluated to determine its potential capacity to replace and/or reduce sulfur dioxide in winemaking. Olive oil mill extract is a potent antioxidant of biological origin. The extract resulted in 4 and 40 mmol/L Trolox/mg extract by DPPH and ORAC respectively. Its antimicrobial activity was approaching to that of SO_2 for *Hanseniaspora uvarum*, *Candida stellata, Lactobacillus plantarum*, *Pediococcus damnosus and Acetobacter aceti*, higher for *Oenococcus oeni* and lower for *Dekkera bruxellensis* and *Botryotinia fuckeliana.*

Additionally, GC-olfactometry analysis showed that the most important odorants from the extract are naturally present in wines. However, some odorant zones might be significantly increased in wine after extract addition.

It can be concluded that olive mill solid waste extracts is a source of bioactive compounds of low cost, with high antioxidant activity and good antimicrobial properties.

1. Introduction

The olive oil industry is important in the Mediterranean region. Spain is the country with the largest area in the world dedicated to olive oil trees and the olive oil producing country (>40% world production) (FAO, 2012). Two process are able for producing olive oil: three-phase mill which generates two waste (orujo and aqueous liquor), and two phase centrifugation technology which produces a semisolid waste called olive cake or alperujo. In Spain 90%of olive oil is produced by two-phase system, which means between 4 and 6.5 million tons of alperujo by year. Olive oil byproducts are frequently detoxified prior to disposal for cattle feeding or composting. The occurrence of phenolic compounds in these residues obstructs its biological treatment. Greater environmental and economic benefits could result from the conversion of this byproduct to a product of higher added-value. Since olive mill solid wastes are a source of bioactive compounds, they may be used in nutraceutical, cosmetical, food and/or pharmaceutical industry (Fernández-Bolaños et al., 2004). However, it has not been effectively exploited yet.

Among the phenolic compounds present in olive, olive oil and olive byproducts, hydroxytyrosol (HT) is the major by quantity. Health-benefits such as anticancer, antimicrobial, antidiabetic and neuroprotective properties have been ascribed to HT (Fernández-Mar, Mateos, García-Parrilla, Puertas, & Cantos-Villar, 2012). The European Food Safety Authority (EFSA) has recently accepted the scientific evidence of health claims in relation to HT and protection from oxidative damage. In order to bear the claim, 5 mg of HT and its derivatives (e.g. oleuropein complex and tyrosol) in olive oil should be consumed daily (EFSA, 2011).

The application of natural antioxidants as preservatives in food industry is an emerging practice that is gaining importance lately. Phenolic extracts have already been proposed as preservatives in food industry. Hydroxytyrosol showed high efficiency in preventing

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http://dx.doi.org/10.1016/j.lwt.2014.11.024
0023-6438/

foodstuff rich in lipids from lipid oxidation (Pazos, Alonso, Sánchez, & Medina, 2008).

The most widely preservative used in food industry is sulphur dioxide (SO_2). This fact is extensible to wine industry. In wine, SO_2 exhibits an important antioxidant function that helps to reduce the effects of dissolved oxygen as well as to inhibit polyphenoloxidase, tyrosinase and peroxidase enzymes, which are endogenous in grape and also come from fungal infections. In addition, SO_2 inhibits the development of microorganisms, such as yeasts, lactic acid bacteria and, to a lesser extent, acetic acid bacteria (Santos, Nunes, Saraiva, & Coimbra, 2012). However, several human health risks, including dermatitis, urticarial, angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis, have been associated to SO_2 (Vally, Misso, & Madan, 2009). Consequently, the International Organization of Vine and Wine (OIV) has established limits for SO_2 content in wines (EC, 606/2009). Thus, there is great interest in research of other preservatives that can replace and/or reduce SO_2 content in wines.

Some chemicals have been tested as an alternative to SO_2: colloidal silver complex, dimethyl carbomate, ascorbic acid, hypophosphorous acid, thiodipropionic acid, Trolox C, stannous chloride, and Sporix, and even natural products (lysozyme and bacteriocins) (Santos et al., 2012). Among them, the use of phenolics has been proposed as a promising alternative. Enological tannins (Sonni, Cejudo Bastante, Chinnici, Natali, & Riponi, 2009), vegetal extract (Salaha, Kallithraka, Marmaras, Koussissi, & Tzourou, 2008), almond skin and eucalyptus leave extracts (González-Rompinelli et al., 2013) have resulted efficient in reducing SO_2 in wines.

This piece of work joins the two current food industry research interests mentioned above, increasing added-value in olive mill waste and developing other preservatives that can replace and/or reduce SO_2 content in wine. Concretely, it aims at the evaluation of the antioxidant activity, antimicrobial activity and olfactometry profile of the olive mill waste extract as a potential alternative to SO_2 in winemaking. It is a preliminary study to test this extract in winemaking.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade methanol, acetic acid, diethyl ether, ethyl acetate and ethanol were supplied by Panreac (Barcelona, Spain). Chemical standards: 1-octen-3-one, Z-3-hexenol, *Z*-2-nonenal, terpinen-4-ol, 2-hydroxy-3-methyl-2-cyclopenten-1-one, guaiacol, 2-phenylethanol, eugenol, maltol, 2-acetylpyrrolle, 4-ethylguiacol, pantolactone, furaneol, *E,E*-farnesol, *E*-isoeugenol, γ-decalactone 4-ethylphenol, sotolon, γ-dodecalactone δ-dodecalactone, phenylacetic acid, vanillin, n-alkanes (C7-C40), Trolox (6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,20-azobis (2-amidinopropane) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescein (FL), potassium hydroxide solution and dichloromethane (LiChrosolv quality) were purchased from Sigma-Aldrich (Steinheim, Germany). Anhydrous sodium sulfate, tartaric acid and absolute ethanol (999 mL/L HPLC quality) were obtained from Panreac (Barcelona, Spain). Solfosol (water solution of SO_2) was supplied by Sepsa-Enartis (Penedès, Spain). Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used throughout this research.

2.2. Olive mill solid waste extract (HTE)

The olive hydroxytyrosol-enriched extract was obtained from olive mill waste through a patented process (HT80®, patent WO2007093659A1). Physico-chemical properties of the HT80® are shown in Table 1 (data supplied by Biomaslinic). HT80® was adjusted at 50 mg/L and 80 mg/L of hydroxytyrosol in model wine (120 mL/L ethanol, 4 g/L tartaric acid and adjusted to pH 3.4 with NaOH). These solutions are called hydroxytyrosol enriched extract (HTE) through the manuscript. The solutions without HT80® and with SO_2 at the same concentrations (50 mg/L and 80 mg/L) are called SO_2. Solutions without antioxidant are called control solutions (CT).

These concentrations were chosen as recommended for SO_2 in winemaking at warm climate (50 and 80 mg/L for red and 80 white wine respectively) (Puertas, Jiménez, Cantos-Villar, & Piñeiro, 2013).

2.3. HPLC analyses of phenolic compounds presented in the extract

Chromatographic analysis was carried out in a Jasco high-performance liquid chromatographic system equipped with a diode array detector (model MD-2010), a fluorescence detector (model FP-2020), an HPLC pump module (model PU-2089), a column oven module (model CO-2060) and an auto-sampler module (AS-2050), and controlled by Chrompass version 1.8 software. The method followed has been recently described in detail by Piñeiro, Cantos-Villar, Palma, and Puertas (2011). The fluorescence conditions were as follow: for hydroxytyrosol excitation 279 nm, emission at 631 nm. For tyrosol excitation 278 nm and emission at 598 nm.

2.4. Antioxidant activity

2.4.1. DPPH assay

Samples were analyzed according to the technique reported by Brand-Williams, Cuvelier, and Berset (1995). A volume of 10 μL of different HTE concentrations was added to 990 μL of 0.094 mmol/L DPPH in MeOH. To determine the reaction kinetics, the assays were continuously monitored at 515 nm over a 1 h period at 25 °C. Each sample was analysed in triplicate. The antioxidant activities were expressed as μmol/L Trolox equivalents/mg extract. Those data were also used for estimating scavenging effect (%) according He et al. (2012).

2.4.2. ORAC assay

The oxygen radical absorbance capacity was determined as previously described (Lucas-Abellán et al., 2008). Reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. The antioxidant activities were expressed as μmol/L Trolox equivalents/mg extract.

2.5. Antimicrobial assay

Pure cultures were obtained from the CECT (Spanish Collection of Type Cultures, Valencia, Spain). Yeasts: *Saccharomyces cerevisiae*

Table 1
Physicochemical properties of the olive mill solid waste (HT80®).

Description	Characteristic	Method
Physical state	Liquid syrup	
Color	Pale-brownish	
Odor	Processed olives	
Humidity (g/kg)	170.3	Gravimetry
Ash (g/kg)	3.4	Gravimetry
Viscosity (20 °C, mPa.s)	6190	Rheology
Density (20 °C, g/mL)	1.20	Gravimetry
Refraction index	1.340	Refractometry
Solubility (water, g/L)	73	
Solubility (ethanol, g/L)	700	