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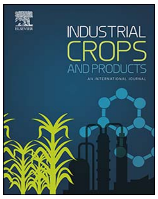

Extraction of natural flavorings with antioxidant capacity from cooperage by-products by green extraction procedure with subcritical fluids

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ABSTRACT

A green extraction procedure with different subcritical GRAS solvents has been developed to produce flavoring extracts from cooperage by-products. Results showed that, in comparison with ethyl lactate, both ethanol:water (80:20) and pure water are excellent solvents to obtained flavoring extracts. Components such as furanic compounds, β-methyl-γ-octalactones, terpenes, norisoprenoids and benzenic compounds were detected. Pressurized pure water seemed to be more effective for furanic compounds, terpenes and norisoprenoids, while lactones and benzenic compounds were found in higher concentration in hydroalcoholic extracts. The temperature seemed not to be such a relevant factor like the nature of solvent, although major amounts of identified compounds were observed for certain volatile compounds at higher temperatures tested. Furthermore, flavoring extracts also exhibited antioxidant capacities, especially in those hydroalcoholic extracts attained at 120 °C. Consequently, valuable compounds from oak wood residues can be extracted as excellent natural flavoring and preservative agents to be used in food and cosmetic industry among others.

1. Introduction

Concerned about rising agricultural food processing wastes and residues generated by the industrial sector, new strategies and industrial policies have to be developed to manage the rise of agricultural food processing wastes and residues generated by the industrial sector. In most cases, agri-food residues are important sources of compounds with commercial interest. Therefore, the most sustainable and profitable challenge could be the recovery of target compounds both to decrease the volume of residues generation, and also to improve the economic feasibility of the main process, by producing secondary streams of value-added compounds (Vardanega et al., 2015). However, the recovery of valuable compounds from natural sources is not an easily accomplished task. In the agri-food residues, target compounds usually coexist with a multitude of other components and process used for their extraction must be, as much as possible, environmentally benign to fulfil the requirements for a sustainable framework in which process can fit more efficiently.

In this sense, in the last years, sustainable green technologies have emerged to meet these requirements (Chemat et al., 2012; Mendiola et al., 2007; Heng et al., 2013). Green technologies such as supercritical fluid extraction (SFE) or pressurized liquid extraction (PLE) often entail the use of GRAS (Generally Recognized As Safe) solvents as CO_2, ethanol or even water in the case of pressurized liquid extraction. Furthermore, in order to improve the solvation power and reactivity of these solvents, extractions are performed under different and controlled combinations of pressure and temperature. These facts point out to green extraction techniques as an excellent tool to extract target compounds of interest for food, cosmetic and pharmaceutical industries preserving their functional properties (Barba et al., 2016; Chemat et al., 2012; Heng et al., 2013; Vardanega et al., 2015).

Green extraction techniques have been recently applied to the oenological field (Barba et al., 2016). Wine production represents one of the major agricultural activities worldwide which entails the generation of large amounts of wastes and by-products, rich in interesting compounds, but which threaten the environment (Lempereur and Penavayre, 2014). Grape stalks, grape marc, exhausted yeast, wine lee and high loaded wastewater have been used for alternative purposes due to their richness on high value-added compounds. Grape pomace can be used such as soil conditioner, as a source of fibers and energy (Kamel et al., 1985), to stabilize color, to increase the complexity of flavor and modulate taste of beverages (De Torres et al., 2015; Villegas et al., 2016), as source of protein or antioxidant bioactive compounds with health-promoting activities (Teixeira et al.,

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2014). Vitamin E, tocopherols and tocotrienols have been recovered from grape seeds (Gornas et al., 2015) and enzyme production has been enhanced by the use of grape stalks (Masutti et al., 2015).

Winemaking also involves the manufacturing of oak wooden barrels by cooperages where wines elaborated undergo the chemical modifications of ageing stage with the aim of improving sensorial properties. Cooperage woods are regarded as high quality woods due to their richness in volatile and phenolic compounds. However, during the barrels manufacturing, a large amount of potentially valuable residues, around 80%, are generated (Tonnellerie Vicard, 2012). Due to the pleasant aromas of oak woods (coconut, vanilla, spice, clove, smoky, bitter almond, toasty, caramel, sweet, cinnamon, floral) their main profitable industrial use is flavoring wines and distillates by means of chips as an alternative ageing technique, faster and cheaper than barrels (Guchu et al., 2006).

Recent trends in food industry have focused on adding oak extracts to different products in order to flavor them and improve their organoleptic properties. In this sense, oak extracts have been applied to brandy products to induce ageing character (Van Jaarsveld et al., 2009) and more recently, they have been applied to grapevines with the aim of achieving wooden aromas in wines as if has been aged in oak barrels (Martínez-Gil et al., 2011).

Consequently, oak wood is postulated as a natural source of valuable volatile compounds whose recovery can be used for imparting pleasant aromas and developing new products with new sensorial properties. With this aim, our main goal has been to establish a green and sustainable extraction procedure using different pressurized liquids to produce pleasant flavoring extracts from cooperage by-products capable to be used mainly in the food industry.

2. Materials and methods

2.1. Samples and reagents

American naturally seasoned oak chips (*Quercus alba*), sized 2 × 1 × 0.1 cm, coming from the manufacturing barrels were supplied by the cooperage Magreñán S.L. (La Rioja, Spain). Prior to the extraction process, oak chips were ground with a mechanical mill and sieved (size < 1 mm) to obtain a homogenous sawdust.

As GRAS solvents, deionized water (Milipore), absolute ethanol, obtained from Panreac and ethyl lactate food grade, acquired from Sigma–Aldrich were tested. Standards used for identification and quantification process were obtained from Sigma–Aldrich: γ-caprolactone, α-terpineol, benzaldehyde, cinnamaldehyde, guaiacol, furfural, 2-phenylethanol, eugenol, isoeugenols, *trans*-β-methyl-γ-octalactone, *cis*-β-methyl-γ-octalactone, syringol, syringaldehyde, sinapaldehyde, vanillin and α-ionone.

2.2. Pressurized liquid extraction

Isolation of volatile compounds from oak wood was performed using of commercial pressurized liquid extraction with an accelerated solvent extractor ASE 200 (Dionex Corp. Sunnyvale, CA). Three GRAS solvents water, ethanol/water (80:20) and ethyl lactate were tested. Extractions were performed at different temperatures: 60, 80, 100 and 120 °C with the aim to point out the best condition to carry out the extraction of volatile compounds. Samples consisting of 2 g of wood sawdust, mixed with 500 mg of diatomaceous earth as a dispersing agent, were placed in a 11 mL inox extraction cell. Two extraction cycles of 10 min each one were performed under 10.34 MPa of pressure with a flush volume of 60% and a purging time of 80 s (Alañón et al., 2009). Between extractions, a rinse of the complete system was performed to avoid any carry-over. All extractions were done in duplicate.

2.3. Analysis of volatile compounds

For the analysis of volatile compounds, 100 µL of γ-caprolactone as internal standard (0.45 g/L) were added to 25 mL of pressurized extracts. The samples were submitted to a solid phase extraction (SPE). The SPE were carried out using 500 mg styrene divinyl benzene cartridges (Lichrolut EN Merck, KGaA, Darmstadt, Germany). The cartridges were previously conditioned by passing first 10 mL of dichloromethane, then 5 mL of methanol, and finally 10 mL of 10% (v/v) aqueous ethanol at a flow rate of 2 mL/min. Non-volatile hydrophilic compounds were washed out of the cartridges using 50 mL of bidistilled Milli Q Plus water. Subsequently, volatile compounds were eluted with 10 mL of dichloromethane. The extracts were concentrated to 200 µL under a gentle stream of nitrogen and stored in a freezer (−20 °C) prior to their chromatographic analysis.

2.4. Gas chromatography analysis

One microliter of extracts was injected in splitless mode (0.73 min) for analysis into an Agilent Technology 7820A GC System coupled to an Agilent Technology 5977E Mass Selective Detector equipped with an autosampler. The column used was a HP-5MS UI capillary column (30 m × 250 µm × 0.25 µm). The injector temperature was 250 °C and the oven temperature was programmed as follows: 70 °C during 3 min ramped at 3 °C/min to 250 °C. Then, the temperature was ramped at 8 °C/min to 290 °C which was kept during 30 min. The carrier gas was Helium with a flow rate of 1 mL/min. The total chromatographic time was 98 min per each analysis. The MS operated in the electron impact mode with electron energy of 70 eV, the ion source temperature was 230 °C and the scanning was made from 40 to 450 a.m.u.

The chemical data were dealt with the software Mass Hunter Qualitative Analysis B.07.00 version. The identification was based on comparison of the GC linear retention times and mass spectra with those provided for authentic standards, by the NIST library and by those reported in bibliography (Vichi et al., 2007). The linear retention index was calculated using n-alkanes mixture from C_{10} to C_{28} as external references obtained from Sigma–Aldrich (Madrid, Spain). Calibration curves of standards were made to carry out the quantification process (Table 1). For that compounds whose standards were not available, those calibration curves of compounds with similar chemical structures were employed. The total amount of identified compounds from pressurized extracts after each extraction process was calculated as a sum of the all volatile compounds identified.

2.5. Total phenolic content

The total phenol content of extracts was determined according to

Table 1
Calibration curves of standards made to carry out the quantification process.

Standards	Slope	Intercept	R^2
α-Terpineol	2.760	−0.000	0.997
Benzaldehyde	0.453	−0.001	0.991
Cinnamaldehyde	1.877	+0.005	0.991
Guaiacol	2.320	−0.007	0.994
Furfural	0.391	+0.000	0.990
2-Phenylethanol	2.416	−0.005	0.997
Eugenol	3.445	+0.003	0.992
Isoeugenol	2.304	−0.005	0.997
Trans-β-methyl-γ-octalactone	1.598	−0.000	0.990
Cis-β-methyl-γ-octalactone	0.968	+0.000	0.995
Syringol	2.647	−0.005	0.996
Syringaldehyde	0.023	−0.000	0.997
Sinalpaldehyde	0.219	−0.002	0.990
Vanillin	0.651	+0.003	0.999
α-Ionone	0.304	−0.002	0.994