Extraction of Wood Compounds by Use of Subcritical Fluids

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Key Words
Gas chromatography - mass spectrometry
Solid-liquid extraction
Subcritical fluids
Oak wood

Summary
A study of the extraction of oak wood compounds with subcritical water-ethanol mixtures as extractants, with an ethanol content between 0 and 60%, is reported. Identification and characterization of the extracted compounds were achieved by spectrophotometry and by gas chromatography with either flame ionization or mass spectrometric detection. Extraction was performed statically by use of a single cycle or repeated cycles. All variables affecting the extraction process were studied and optimized. Extraction time and temperature were 60 min and 200 °C, respectively. Comparison of the extract thus obtained and commercial extracts showed the former to be rich in compounds characteristic of the commercial extracts. The method also enables manipulation of extract composition by changing the temperature and the water/ethanol ratio used. It is faster than traditional procedures for obtaining wood extracts.

Introduction
The ageing of alcoholic beverages is traditionally performed in oak wood barrels, because extraction of key compounds from the wood produces desirable changes in the composition of wines and spirits. Oak wood is mainly composed of three large insoluble polymers, cellulose, hemicellulose, and lignin but also contains different compounds with lower molecular weights, for example volatile and non-volatile acids, sugars, steroids, terpenes, volatile phenols, and lactones, etc. [1–3], which can be extracted by ethanol-water mixtures in very different ratios, depending on the alcohol content of the extractant. The heating and charring operations performed during the manufacture of oak wood barrels modify the macromolecular structure of the wood, resulting in degradation of polysaccharides and polyphenols and the appearance of new classes of volatile substances, for example pyrazines, furans, and phenols, etc. Several studies have revealed changes in the composition of oak when it is toasted over a wood fire [4, 5]. Small amounts of hundreds of volatile compounds can be found in untoasted oak wood [6]; the relatively small number of volatile compounds present in significant amounts include vanillin, eugenol, and cis- and trans-β-methyl-γ-octalactones [2, 7, 8]. Other volatile compounds are formed by thermal degradation of the main wood polymers during the toasting process, and the amounts of the compounds found in untoasted oak wood are also increased by toasting. Although phenol and furan aldehydes are the most abundant aromatic compounds identified in toasted wood, only vanillin contributes substantially to the aroma. Other volatile phenols responsible for the ‘spicy’ and ‘smoky’ characteristics of toasted wood [5] are formed during this step.

Extracts obtained from toasted or untoasted wood containing different amounts of these wood compounds have traditionally been used to modify the colour and flavour of alcohol beverages. The composition of these wood extracts is subject to European legislation [9] and the extraction procedure uses exclusively alcohol-water mixtures. Extracts of fragrance and flavour substances obtained from plants are known as pomades, concentrates, absolutes, resinoids, or tinctures, depending on the method used for preparation. Industrial manufacture of wood extracts is performed by treating natural raw material with ethanol or water-ethanol mixtures, thus obtaining a tincture [10]. Commercial wood extracts are usually obtained by re-circulating water-ethanol mixtures through a bed of chips at ambient temperature and pres-
Subcritical fluid extractions were performed with a Knauer (Berlin, Germany) 64 high pressure pump which was used to propel the extractant liquid through the system. The extractor, depicted in Figure 1, consisted of a stainless steel cylindrical extraction chamber (150 mm × 11 mm i.d., 14 mL internal volume) which was closed with screws at both ends to enable filling with extractant and emptying of the extract. The screw caps also contained stainless steel filter plates (2 mm thick, 6.35 mm i.d.) to ensure the wood chips remained in the extraction chamber. This chamber, with a stainless steel preheater, was located in a gas chromatograph oven (HP 5720A; Agilent Technologies, Wilmington, DE, USA) used as heating source and designed to work up to 400 °C. A loop made from a 1-m length of stainless-steel tubing and cooled with water at room temperature was used to cool the extract from the oven to a temperature close to 25 °C. A pressure needle valve coupled to the outlet of the cooler, and a selecting valve located between the high-pressure pump and the oven enabled flushing of the extract with N₂ after extraction.

Analysis of the extracts was performed by means of a Varian 3900 gas chromatograph equipped with a Chrompack CP-Wax 57CB fused-silica capillary column (50 m × 0.25 mm i.d., 0.2 μm film thickness) and a flame ionization detector (FID). A Saturn 2200 mass spectrometer (Varian) equipped with a Chrompack CP-Sil 8CB fused-silica capillary column (50 m × 0.25 mm i.d.) was used to characterize the compounds. A Cary 50 Conc spectrophotometer from Varian (Mulgrave, Australia), connected to a computer running Cary WinUV v.2.0 (Varian) software for data collection and treatment was used for measurement of the total polyphenol index (t.p.i.) of the extracts.

**Experimental**

**Instruments and Apparatus**

Table I. Results from the optimization study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range tested</th>
<th>Optimum value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EtOH (% v/v)</td>
<td>0 – 60</td>
<td>60</td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraction time (min)</td>
<td>10 – 75</td>
<td>60</td>
</tr>
<tr>
<td>Extraction temperature (°C)</td>
<td>80 – 300</td>
<td>200</td>
</tr>
<tr>
<td>Sample mass (g)</td>
<td>–</td>
<td>1.5</td>
</tr>
<tr>
<td>Pressure (atm)</td>
<td>–</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure 1. The extraction system used. hpp = high-pressure pump, er = extractant reservoir, ph = preheater, ec = extraction cell, o = oven, c = cooler, v₁ = selection valve, v₂ = restriction valve.

Figure 2. GC-FID chromatograms obtained from extracts from wood. Study of degradation at (a) 200 °C and (b) 300 °C.

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