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Isolation of flavonoids from aspen knotwood by pressurized hot water extraction and comparison with other extraction techniques

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Abstract

Pressurized hot water extraction (PHWE) conditions (time, temperature, pressure) were optimized for the extraction of naringenin and other major flavonoids (dihydrokaempferol, naringin) from knotwood of aspen. Extracts were analysed by GC–FID, GC–MS, HPLC–UV and HPLC–MS. The results were compared with those obtained by Soxhlet, ultrasonic extraction and reflux in methanol. Flavonoids were most efficiently extracted with PHWE at 150 °C and 220 bar with 35 min extraction time. Soxhlet with methanol gave slightly higher recoveries, but an extraction time of 48 h was required. Naringenin concentration was highest in knotwood (1.15% dry weight) and much lower in the sapwood. PHWE proved to be cheap, fast and effective for the isolation of biofunctional flavonoids from aspen knotwood, producing higher recoveries than 24 h Soxhlet extraction, sonication or 24 h reflux.

Keywords: Pressurized hot water extraction; Soxhlet; Aspen (*Populus tremula*); Flavonoids; Naringenin; Dihydrokaempferol; Liquid chromatography; Knotwood; Isolation

1. Introduction

Naringenin (4′,5,7-trihydroxyflavanone) is a plant flavonoid (phenolic antioxidant), which is found in large amounts in citrus fruits and tomato. It exhibits anti-estrogenic activity [1], which may be responsible for the lower incidence of breast cancer in women consuming large amounts of phytoestrogens [2], and it could exert cholesterol-lowering properties by inhibiting cholesteryl ester synthesis [3]. Naringenin also seems to affect various oxidative processes associated with chronic degenerative diseases. It partially deactivates the Fenton reaction [4], restores glutathione-dependent protection against lipid peroxidation in α-tocopherol-deficient liver microsomes [5], and inhibits malonaldehyde production induced by ascorbic acid in rat brain mitochondria [6] or by autoxidation in rat brain homogenates [7]. Naringenin also may modulate cytochrome P450-dependent monooxygenase, the primary enzyme involved in the metabolism of drugs, carcinogens, environmental pollutants and other xenobiotics [8]. Furthermore, naringenin is reported to have inhibitory effects on microorganisms, in contrast to the corresponding glycoside (naringin), which appears to be inactive [9].

Heartwood, foliage, bark and cork of several species of trees have been identified as sources of natural phenolic antioxidants [10,11]. The extract yields obtained from these materials are low, however, and the extract usually contains a wide variety of phenolic and nonphenolic compounds, both as glycosides and as free aglycones. The degree of glycosylation affects the antioxidant properties of phenolic compounds. The major hydrophilic compounds in softwood knots are free aglycones of lignans, oligolignans, stilbenes and flavonoids which could be expected to have antioxidant properties [12–14]. The amount of extractable phenolic compounds, including flavonoids, tends to be much greater in knotwood than in other parts of the tree and in the best cases may account for up to 30% of the dry weight. The average is about 15% (w/w) [15].

Usually flavonoids are extracted from wood with a polar solvent, such as methanol, by Soxhlet-, ultrasonic- or accelerated

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solvent extraction [16]. Supercritical fluid extraction (CO_2) can be applied, but a modifier such as methanol is then required [17]. The use of pure water as an extraction solvent for phenolic compounds in wood material has been proposed by Holmbom et al. [18]. We have not, however, found any reports of the application of pressurized hot water extraction (PHWE) to knotwood.

The use of large amounts of organic hazardous solvents in sample preparation and the increasing cost of solvent waste disposal have created a growing demand for better and more environment-friendly extraction methods for analytical laboratories. Water is an interesting alternative to the usual solvents, particularly in view of its low cost, polarity and non-toxic character. The dissolving power (polarity) of water can be modified merely by adjusting the temperature. The dielectric constant is the key parameter involved in solvent–solute interactions and may be related to polarity. The dielectric constant of water is high at room temperature (78.5), but it decreases as the temperature increases. At the critical point (374 °C and 221 bar), dielectric constants and densities are the same for gaseous and liquid water. Exceedingly low values for the dielectric constant can be obtained for supercritical water, for example 1.5 at 500 °C and 225 bar. Although supercritical water is an excellent solvent for all kinds of organic compounds, its high critical temperature and corrosive nature hinder its wider use as an extraction medium. Fortunately, the solubility of many low polarity compounds in water is sufficient to allow their extraction at temperatures much below the critical temperature.

Recently, both liquid water and steam at temperatures from 200 to 300 °C have been used to efficiently extract organics of different polarity from solid sample matrices. Satisfactory recoveries (>90%) have been reported, for example, for polar persistent organic pollutants such as phenols. Temperatures of about 200 °C have been used for quantitative extraction of more unpolar compounds such as pesticides and low-molecular-mass polycyclic aromatic hydrocarbons (PAHs) [19–21]. Temperatures of 250–300 °C were required for the extraction of polychlorinated biphenyls (PCBs) and high-molecular-mass PAHs from soil and sediments [22], and *n*-alkanes were extracted only at temperatures higher than 300 °C [19,22,23]. Steam conditions were necessary for the quantitative extraction of other highly hydrophobic pollutants such as polychlorinated dibenzofurans (PCDFs) and polychlorinated naphthalenes (PCNs) from naturally contaminated soils [23]. Isoflavones have been successfully extracted from defatted soybean flakes, and catechins with proanthocyanidins from winery by-products, at temperatures up to 150 °C and pressures up to 60 bar [24,25].

The primary aim of this investigation was to optimize pressurized hot water extraction conditions for the isolation of flavonoids and other phenolic compounds from aspen (*Populus tremula*) knotwood, and to compare the results with those obtained by other techniques (Soxhlet, reflux and ultrasonic extraction in organic solvent). A further aim was to efficiently isolate naringenin and dihydrokaempferol from the knotwood by PHWE and purify the extracts by solid phase extraction (SPE).

2. Experimental

2.1. Chemicals

(±)-Naringenin (95% purity) was purchased from Sigma–Aldrich Chemie Gmbh. (Steinheim, Germany), dihydrokaempferol (>95%) was from ArboNova (Turku, Finland), and taxifolin (≥85%) and naringin (≥95%) were from Fluka Chemie (Buchs, Switzerland). Silylation reagents N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) for GC analysis were purchased from Sigma (Steinheim, Germany) and Acros (Geel, Belgium), while internal standards heneicosanic acid, cholesterol, cholesteryl heptadecanoate and triglyceride standard (1,3-dipalmitoyl-2-oleyl-glycerol) were from Sigma. Milli-Q water (18 MΩ; Millipore, USA) was used for all solutions and PHWE. Methanol for standards and eluents was from VWR (Prolabo, West Chester, USA). Glacial acetic acid for eluents was 99–100% pure (J.T. Baker, Deventer, Holland).

2.2. Wood material

The wood material was aspen (*P. tremula*) from Närpes in Ostrobotnia, Finland. The tree was healthy and estimated to be 25 years old. A wood disc containing stemwood was cut out 1.5 m above the ground. Two other discs containing knotwood, one with a living branch and one with a dead branch, were sawn out from the same tree and transported directly to the laboratory. The outer branch was cut away and the knotwood discs were air dried before sampling of knotwood. The knotwood and ordinary wood were separated according to the fiber direction change in the boundary. Fibers perpendicular to fibers in the stemwood were considered as knotwood fibers. All reaction wood was removed and the samples did not contain any bark. The samples were splintered, freeze-dried, ground to 20 mesh size and then freeze-dried again.

2.3. Extraction of knotwood with PHWE

The instrument for pressurized hot water extraction is described in detail elsewhere [23,26]. The system consists of a Jasco PU-980 HPLC pump to pressurize the water and a Fractovap Series 2150 oven (Carlo Erba, Milan, Italy) to heat the laboratory-made, stainless steel 3 ml extraction vessel. The vessel has been described earlier [27]. A 30-15HF4-HT high temperature three-way valve (High Pressure Equipment, Erie, PA, USA), a manually adjustable pressure restrictor (model CC-A16A21APK, Tescom, Elk River, MN, USA) and a 10 or 5 ml measuring flask for the collection were employed. On/off valves were type 15-11AF1 from High Pressure Equipment. Approximately 3 m of 1/16 in. stainless steel tubing (i.d. 0.02 in.) was used for the preheating coil and about 1 m was used for the cooling coil. The same tubing was also used for all other connections.

Approximately 0.2 g of granular knotwood sample was placed in the extraction vessel. A sealing ring made of copper was inserted between the vessel body and the cover and the