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Application of supercritical carbon dioxide for the extraction of alkylresorcinols from rye bran

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Abstract

The use of supercritical CO_2 (SC-CO_2) for extracting alkylresorcinols from rye bran was investigated. For pure SC-CO_2, 35 MPa and 55 °C were used. SC-CO_2 with 10% ethanol or methanol as co-solvents was tested at four pressure levels (8, 15, 30 and 35 MPa) and two temperatures (40 and 55 °C). Experiments were conducted at a constant SC-CO_2 flow of 5 g/min for 2 h. For comparative purposes acetone at ambient temperature and pressure was also used for extraction. The best extractions were achieved when SC-CO_2 was used with co-solvents. At 55 °C and 15–30 MPa, the use of SC-CO_2 with co-solvents has yielded from 8 to 80% (w/w) more extracted product than acetone. Yield of extracts was proportional to pressure (when above 30 MPa) and temperature. The results suggest that cleaner bran extracts can be obtained by using pure SC-CO_2 followed by the addition of co-solvents. There is no clear advantage of using either methanol or ethanol.

Keywords: Supercritical CO_2; Extraction; Rye; Cardol homologues; Alkylresorcinols

1. Introduction

Alkylresorcinols are defined as amphiphilic 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring [1,2]. The aliphatic chains of these compounds are predominantly saturated; however, the occurrence of double bonds has also been noted [3,4]. Derivatives with an odd (15–25) carbon atoms were shown to be common in plants from families Anacardiaceae, Ginkgoaceae or Proteaceae and Gramineae [4–6]. They are also found in a number of bacteria, fungi [1].

Alkylresorcinols with importance for food and feed occur mostly in numerous members of the Gramineae family [1,2]. Alkylresorcinols are mainly concentrated in the bran milling fraction of cereal grains [2,7,8]; namely, in wheat (>500 μg/g), also at high levels in rye and triticale, and in low amounts in barley, millet and maize. They are also found in rice and rye seedlings, mango latex and peel, and cashew nut shell liquid (CNSL), but are not present in the edible parts of these plants [1,9–12] except in minute amounts as in case of cashew nuts [2,13,14].

A wide range of biological activities, is attributed to the alkylresorcinols e.g. anticancer [2] antimicrobial [1,2,5], antiparasitic, antitumour, some antioxidant effect [1,2] and antifungal [5,6,15]. It was recently reported that 5-alkylresorcinols of type 1 (with a single acyl substitute on the OH group of the phenolic ring) can cleave DNA at high concentration in presence of copper(II) chloride and oxygen [5,16–18]. Additionally, the corresponding 5-alkenyl derivatives of this type of compounds, primarily those with a stilbene structure [5,19] show interesting antileukemic properties [5,20].

Rye, wheat and triticale alkylresorcinols are characterized by straight hydrocarbon chains with an odd number of

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carbon atoms (15–29) attached to position 5 of the phenolic ring [21]. These resorcinols with saturated aliphatic chains represent about 85% of total cereal grain resorcinol lipids, while other resorcinol analogues, including alkylresorcinols with mono- and di-unsaturated aliphatic chains and with keto groups, represent only 15% [8].

The consumption of alkylresorcinols by humans, are currently achieved by the intake of whole grain cereals [22] or by incorporation of cereal bran in cereal products however, for analytical or biosynthesis studies, these products require previous isolation to make them available for testing. The isolation methods currently employed include extraction with acetone, chloroform [1,15,23,24] or ethyl acetate [2,8].

Organic solvents have generally the drawback of requiring further purification of the extracts, especially if very pure isolates are required. Alternatively, supercritical carbon dioxide can be used. The solvent capacity of supercritical carbon dioxide is mainly a function of density and this in turn can easily be changed by varying pressure and temperature or by adding small amounts of co-solvents [25–30]. Supercritical carbon dioxide has a gas-like viscosity leading to favorable transport properties, so that for example extractions using supercritical fluid are faster than with traditional solvents. Furthermore, supercritical carbon dioxide is non-toxic, non-flammable, inexpensive and environmental friendly.

The objective of the present work is to test the possibility of using supercritical CO_2 for extracting Alkylresorcinols from rye flakes in set conditions such as pressure, temperature and co-solvent type. To our knowledge this technique has never been reported in the extraction of alkylresorcinols from cereal sources.

A full optimization process is a subject for another work in progress in which these and additional parameters are taken into account.

2. Materials and methods

2.1. Materials

Commercial raw rolled rye flakes were obtained in a local supermarket (Lund, Sweden). Acetone was purchased from Sigma, USA. Ethanol (99.5%) and methanol (HPLC grade) were obtained from Kemetyl AB, Sweden. The carbon dioxide (≥99.998%) used for extraction was from AGA Gas, Syndbyberg, Sweden. Alkylresorcinol homologues used as standards were isolated chromatographically from rye bran extracts according to Kozubek and Tyman [24]. Ultra-pure water (18 MΩ) was employed in the analysis.

2.2. Methods

2.2.1. Sample preparation

Thirty grams of raw rolled rye flakes were ground in a KNIFETEC 1095 sample mill (Foss, Sweden) for 10 s under cooling.

2.2.2. Extraction with acetone

The extraction with acetone was carried out at ambient temperature (20 °C) following the method described elsewhere [23]. Twenty-five grams of ground raw rolled flakes were place in an Erlenmeyer with 100 mL of acetone and kept overnight under continuous stirring. Then the slurry was filtered through a Buchner funnel filled with fiber glass filter. The sediment was additionally rinsed twice with 50 mL of fresh acetone. The filtrates were collected and centrifuged for 10 min at 5000 rpm in a Sorvall RT6000B, Dupont, France. The supernatant solution was separated from the precipitate and the solvent evaporated in a BÜCHI Rotavapor R-200, Tamro MedLabA, Mölndal, Sweden. The extracts were weighed and cooled for analysis. The extraction was performed in duplicates.

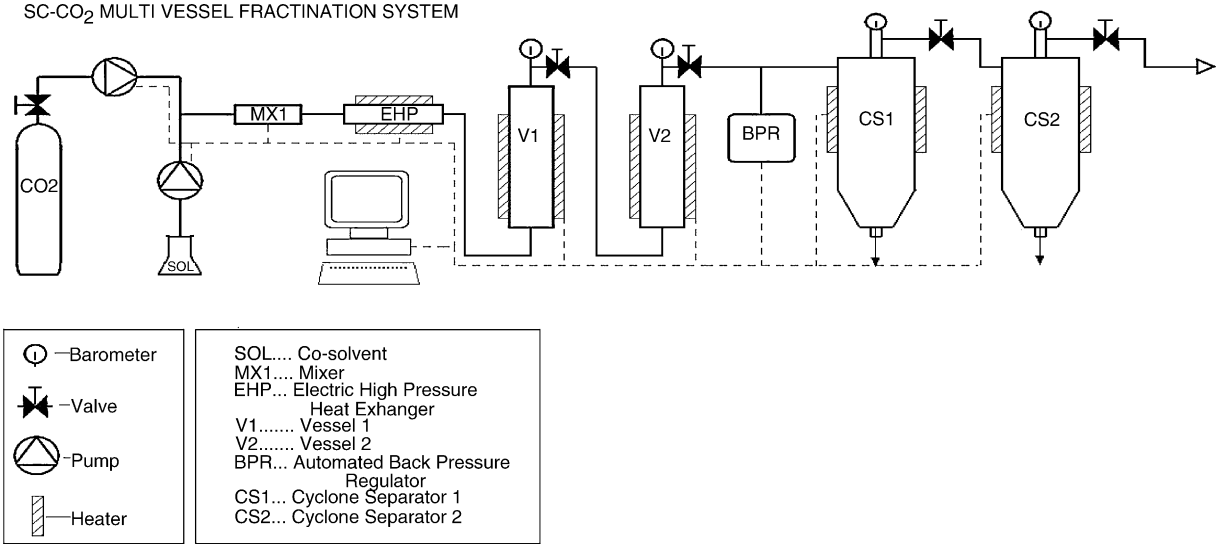

Fig. 1. Schematic diagram of the SC-CO_2 system.