ALKYLRESORCINOLS EXTRACTED FROM RYE SEEDS BY SUPERCRITICAL CARBON DIOXIDE

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Abstract

The use of supercritical carbon dioxide (SC-CO_2) for extracting alkylresocinols from rye bran was investigated. Experiments were conducted at 35MPa and 55°C with pure SC-CO_2 and at four pressure levels; 8, 15, 30 and 35 MPa and two temperatures; 40 and 55°C with 10% ethanol as co-solvents for a constant SC-CO_2 flow of 5g/min for two hours. Acetone was also used in extraction for comparative purposes. Better extractions were achieved when SC-CO_2 was used with ethanol. In general, the extraction yield was higher the higher the pressure and the temperature above the crossover pressure. When compared to the acetone extract, the use of SC-CO_2 with ethanol has yielded 80% higher total extracts at 30 MPa and 55ºC. Non identified Alkylresorcinol homologues were extracted with pure SC-CO_2. From the results it is suggested that SC-CO_2 can be used for cleaner bran extracts by using pure SC-CO_2 followed by the addition of co-solvents.

1. Introduction

Supercritical fluid extraction (SFE) has received considerable attention is the recent years as an extraction or separation medium for plant material constituents [1]. In particular, supercritical carbon dioxide (SC-CO_2) advantages over organic solvent such as less time consuming in sample preparation, easy recovery of the extracts, low toxicity and cost are well documented [2;3]. The solvent capacity of supercritical carbon dioxide is mainly a function of density and this can easily be changed varying pressure and temperature conditions or adding small amounts of co-solvents [4-9].

Despite the vast work developed in the extraction of different plant materials, we could not find so far a single reference on the extraction of alkylresocinols from bran cereals. Alkylresocinols are amphiphilic 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring [10]. Derivatives with an odd number and length of 15 to 25 carbon atoms were shown to be common in plants from families Anacardiaceae, Ginkgoaceae or Proteaceae and Gramineae [11-13]. In food and feed materials, alkylresorcinols occur mostly in some members of the Gramineae family [10;14]. They are mainly concentrated in the bran of cereal grains [10;15;16], in high levels (>500µg/g) in wheat, rye and triticale and in low amounts in barley, millet and maize.

Alkylresorcinols are reported to possess wide range of biological activities like anticancer [10] antimicrobial[10;12;14], antiparasitic, antitumour and some antioxidant effect [10;14].

Rye alkylresorcinols are characterized by strait hydrocarbon chains with an odd number of carbon atoms (17-25) attached to position 5 of the phenolic ring [17]. Resorcinols with saturated aliphatic chains represent about 85% (alkylresorcinols), while other resorcinol analogues, including alkylresorcinols with mono- and di-unsaturated aliphatic chains and with keto groups, represent 15% [16].

The consumption of alkylresorcinols by humans, are currently achieved by the intake of whole grain cereals [18] or by incorporation of cereal bran in cereal products. For analytical or biosynthesis studies, these products require previous isolation to make them available. The isolation methods currently employed include extraction with organic solvent; acetone, chloroform [14;19-21] or ethyl acetate [10;16], especially for analytical purposes.

In the present work we employ supercritical carbon dioxide for extracting alkylresorcinols from raw rye rolled flakes. We present the yield and composition variation as a function of operation condition when ethanol is used as co-solvent.

2. Materials and Methods

2.1 Materials

Commercial raw rolled rye flakes were obtained in the local market. 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. Alkylresoscinol homologues used as standards were kindly provided by Prof. Arkadiusz Kozubek, Wroclaw University, Wroclaw, Poland. Water employed in the analysis was double distilled and de-ionized.

2.2 Methods

2.2.1 Sample preparation

Thirty grams of raw rolled rye flakes were milled in a KNIFETEC 1095 sample mill (Höganäs, Sweden) for 10 seconds under cooling.

2.2.2 Extraction with acetone

The extraction with acetone was carried out following the method described elsewhere [20].

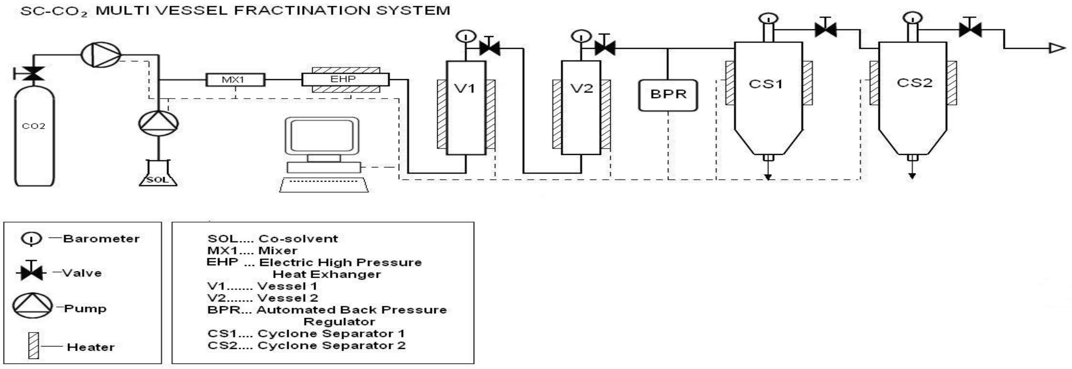

Figure 1. Schematic diagram of the continuous process of the SC-CO_2 extraction

2.2.3 Extraction with SC-CO_2

The extraction with supercritical carbon dioxide was performed in a system, SFE-2X100F, Thar Technology Inc., Pittsburgh, USA. A schematic draw of the experimental apparatus is shown in Figure 1. The extracting and separation vessels have nominal volumes of 100 and 200 mL each, respectively. Twenty five grams (25g) of milled material were placed in the extraction vessel 2 and carbon dioxide allowed to get through up to the desired pressure. The extractions were performed either with pure SC-CO_2 at 35 MPa and 55°C or with addition of ethanol as co-solvent. The co-solvent was admitted in the system as mass percentage of CO_2 flow. In all extractions the CO_2 flow was kept at 5g/min and the co-solvent (when used) as 10% of the CO_2 flow. In the extraction with use of co-solvent the pressures applied were 8, 15, 30 and 35 MPa and temperatures of 40 and 55°C for each pressure. The extraction time was set for two hours in all runs. The separators were kept at the same conditions (5 MPa, 20ºC) so that only total extract was collected. The CO_2 leaving the extraction process was vented to the hood atmosphere. The extracts obtained with co-solvent were evaporated in a BÜCHI Rotavapor R-200, Tamro MedLabA, Mölndal, Sweden.

2.2.4 Analytical method

All samples were analyzed with a HP 1050 HPLC equipped with a UV-detector and an auto sampler. A reversed phase column Kromasil 100-5c18 (250 × 4.6 mm) (Akzo Nobel, Sweden) was used. The UV wave length was kept at 280 nm. 20 µl of analyte dissolved in methanol were injected and the elution was conducted at ambient temperature. A 96:4 methanol/water mixture was used as the mobile phase at a flow rate of 1.5 mL/min. The alkylresorcinol homologues were identified by comparing the retention times of the extracted samples to the retention time of the standard homologues.

3. Results and Discussion

3.1 Extraction yield

The use of SC-CO_2 with ethanol has yielded 80% higher extract amounts at 30 MPa and 55ºC as compared with the extraction with acetone except for near critical point (8 MPa and 55ºC) where the yield is 23% lower (see Fig. 2)

The combination of a polar co-solvent with a non polar fluid (CO_2) has enhanced the extractability of the target compounds due to the hydrophilic moiety (phenolic ring) and the hydrophobic alkyl chain.

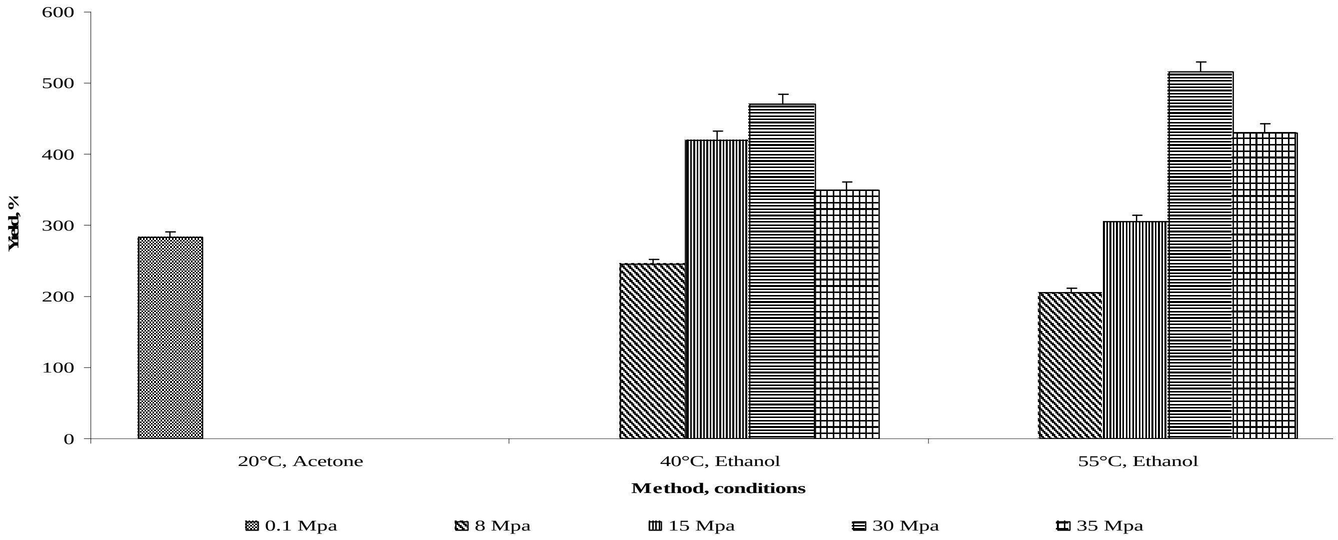

Figure 2. Extraction yield obtained by acetone and SC-CO in different conditions.

3.2 Alkylresorcinol patterns in acetone and SC-CO_2 extracts

Figure 3a, shows an example of an HPLC chromatogram obtained from acetone extracts analysis. Acetone has been used earlier for extraction of alkylresorcinols from cereal bran [14;19-21] and the homologues C_{17}-C_{25} have been systematically reported in cereal bran to be the more abundant (up to 85%) [16]. In this study, these homologues account up to 77%. The small differences with the reported figure may be due to several factors, for example the difference on the sample source, extraction methods or protocols among others. The amount of C_{15} accounts for 5% while C_{29} appears in minor amounts. In our chromatograms we observe some minor compounds before the elution of the C_{15}. Since these compounds do not fall in the range of the target ones, they were not identified and no special attention will be addressed to them in this discussion. However, it is an indication of the occurrence of other lipid compounds in rye bran. Indeed these compounds were the only extracted when pure SC-CO_2 was used.

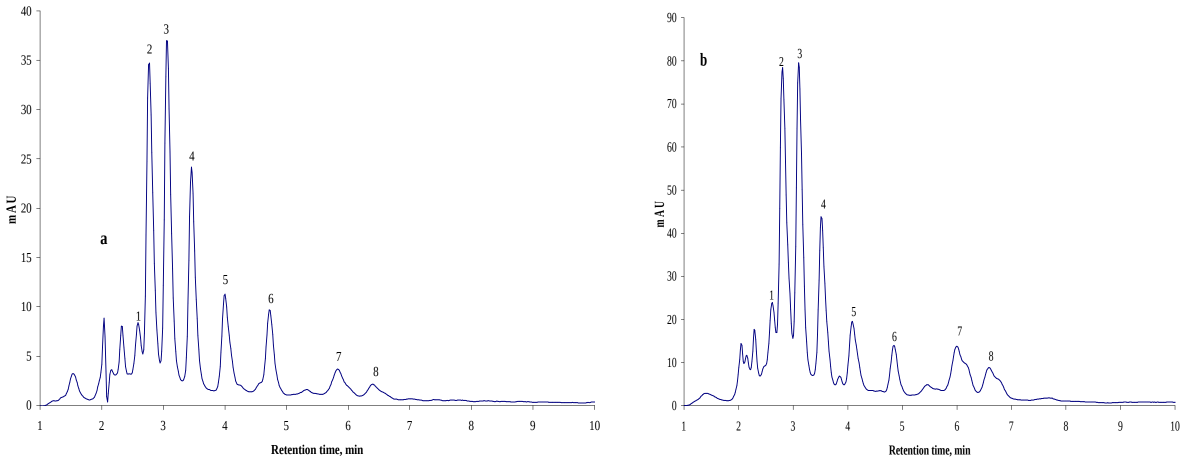

Figure 3. Examples of chromatograms obtained by (a) acetone and (b) SC-CO_2 at 350 bar and 55ºC with use of co-solvent. The pick numbers 1-8 stand for the alkylresorcinol homologues C_{15}, C_{17}, C_{19}, C_{21}, C_{23}, C_{25}, C_{27} and C_{29} respectively.

Figure 3b shows an example of HPLC chromatogram obtained for SC-CO_2 extracts with the use of ethanol as co-solvent. All picks present in the chromatogram fall in the range of the eluted in acetone extracts. The results obtained with pure SC-CO_2 at 35 MPa and 55°C are related to a number of factors. One factor is that alkylresocinols are phenolic lipids. Unlike neutral lipids and many terpenoid compounds, phenolic lipids posses some polarity that make their solubility in the non polar SC-CO_2 relatively lower when compared to acetone. Another factor is related to the Alkylresorcinol homologues chemical structure and their relatively high molecular weight. The effect of chemical structure on the solubility of substances in SC-CO_2 has been reported earlier [22].

3.3 Extraction of rye bran with SC-CO_2 with addition of ethanol. The effect of pressure and temperature

A co-solvent is another solvent that is added the SC-CO_2 to confer to it some polar properties. Alkylresorcinol homologues are amphiphilic compounds. For this reason pure CO_2 was not efficient in extracting them. Due to this unsatisfactory result, the extraction with SC-CO_2 was performed with the addition of ethanol. Ethanol is commonly used co-solvent in SC-CO_2 extraction due to its tolerable presence in food, medical or cosmetic sample applications, and its intermediate polar character (polarity 4.3).

When the extraction was performed with the presence of ethanol, the SC-CO_2 extraction yielded all the homologues obtained by pure acetone. The chromatograms are similar to these obtained for acetone extracts (Fig. 3a) and the quantity of the homologues have increased, especially these with high molecular weight. Figure 4 summarize the extraction composition of rye bran with SC-CO_2 with the addition of a ethanol.

When ethanol was used as co-solvent, the effect of pressure followed the following pattern? An increase of pressure favored the extractability of the target compounds. The extractability of a substance in SC-CO_2 is a function of the density and this in turn a function of the pressure. The higher the pressure, the higher the density and so the solvent power of the SC-CO_2 [22].

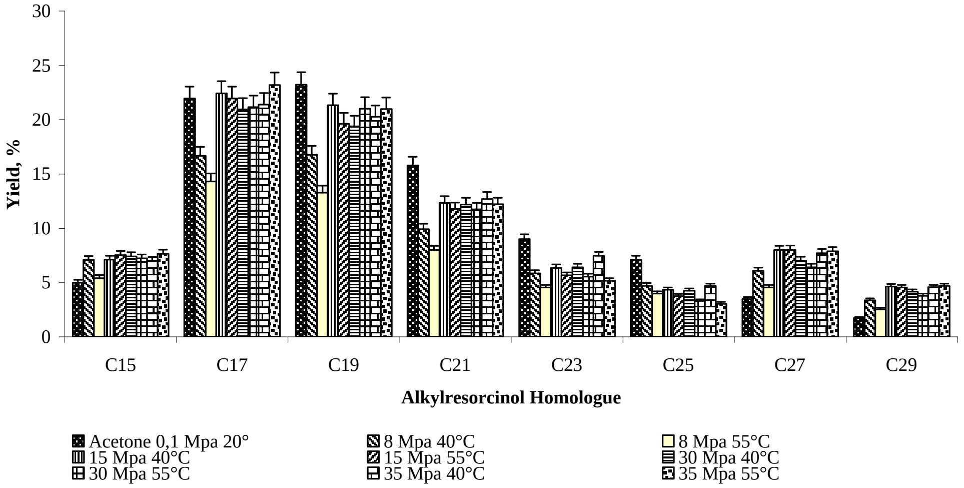

Figure 4. Alkylresocinol homologues composition of the SC-CO_2 extracts with the addition of ethanol expressed as percentage of the relative pick area in the HPLC chromatogram at different pressures and temperatures compared to acetone.

Temperature, however, had a dual effect. At 8 and 15 MPa, the lower the temperature, the higher was the extractability of the target compounds. At this pressure range, an increase in temperature decreases the density. At higher pressures, both the increase of the pressure and temperature increased the extractability of the target compounds, as it is illustrated for 30 and 35 MPa, at 55ºC the yield is greater than at 40ºC. This phenomenon is observed above the crossover region (Pressure =182 bar)[23]. At higher pressure the density of the SC-CO_2 is less dependent on temperature. Thus the negative effect of the decrease of the density by the increase of the temperature is compensated by the increase on the vapor pressure of the compounds to be extracted. In this way the increase on the extractability at higher pressures and temperature may be explained by other reasons like the increase on the vapor pressure of the target compounds.

It is observed (Fig. 4) that at 40ºC, the composition of the SC-CO_2 extracts obtained at 8 MPa contains lower concentration of the Alkylresorcinol homologues when ethanol was used as co-solvent as compared to the acetone extracts. For the remaining pressure used the yields are comparable. At 55ºC the same pattern as described above for 40ºC is observed, however at 30 and 35 MPa the yields of the Alkylresorcinol homologues obtained in the extracts when ethanol is used as co-solvent is almost similar to the reference acetone extract.

An overall observation is that the use of SC-CO_2 with co-solvents brings higher yields that the use of the traditional organic solvent at pressure above the critical point with the help of a co-solvent. At 30 MPa and 55°C the highest yield was obtained corroborating the pressure and temperature effect above the crossover region. A higher pressure (35 MPa) and temperature (55°) however has shown better performance for the yield in terms of the compositions of the alkylresorcinol homologues. The pure SC-CO_2 couldn't isolate the alkylresorcinol homologues intended at the operating conditions but it was possible to obtain unwished compounds before the C_{15}. This suggest that it is possible to use pure SC-CO_2 in a pre-stage of the extraction for removing unwished compounds and further add co-solvent for recovering cleaner alkylresorcinol homologues.

Acknowledgements

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