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ORIGINAL PAPER

Supercritical CO_2 Extraction of Flax Lignans

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Abstract Lignans, such as flaxseed secoisolariciresinol diglucoside (SDG), have been implicated in the prevention of hormonally related cancers and other prevalent diseases. Lignans are typically extracted using organic solvents, which must then be removed from the extract. Supercritical carbon dioxide (SC-CO_2) is a non-toxic, inexpensive solvent, which, when combined with polar modifiers, can be used to extract polar phenolic compounds, such as SDG. The effects of processing conditions and pre-treatment on the extraction of SDG using SC-CO_2 were investigated. Extraction from defatted flaxseed was performed with SC-CO_2 modified with ethanol at levels of 0, 10 and 20 mol% at different temperature (40, 50 and 60 °C) and pressure (35, 40 and 45 MPa) conditions. Extracts were analyzed using RP-HPLC. The condition (7.8 mol% ethanol, 45 MPa and 60 °C) which produced the maximum CO_2 loading of SDG (0.49 μg/g CO_2) was used for the pre-treatment study, in which flaxseed defatted with petroleum ether and SC-CO_2, full-fat hulls, defatted hulls and pre-hydrolyzed flaxseed were used. Temperature, pressure and solvent modifier level had no significant effect ($p > 0.05$) on the SDG loading of CO_2. However, pre-hydrolyzed seed resulted in a significantly ($p \leq 0.05$) higher CO_2 loading of SDG (3.8 μg/g CO_2) compared to the other treatments studied. The yields obtained represented only a small fraction of the original lignan content (15 mg/g petroleum ether defatted flaxseed).

Keywords Flaxseed · Lignan · Secoisolariciresinol diglucoside (SDG) · Supercritical carbon dioxide (SC-CO_2)

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Introduction

Flaxseed, *Linum usitatissimum*, is an oilseed grown primarily in cool climates, such as the Canadian Prairie Provinces [1]. An important aspect of flax's increasing popularity is its high nutritional content. Health Canada, in its 2006 Flax Monograph, recognizes flaxseed as a source of essential fatty acids, especially α-linolenic acid and soluble and insoluble fibre [2].

Not as well known as its omega-3 fatty acid and fibre contents, is the flaxseed's lignan content. Lignans are phenolic compounds linked to many health benefits, including cancer prevention [3, 4]. In flax, the predominant lignan, secoisolariciresinol (SECO) is present as a glucoside, namely secoisolariciresinol diglucoside (SDG), which is linked to other SDG molecules by 3-hydroxy-3-methyl glutaric acid (HMGA) to form oligomers called lignan macromolecules [5, 6].

Once ingested, lignans are deglycosylated and converted by bacteria in the large intestine to produce enterolactone and enterodiol, the mammalian forms of plant lignans [4]. SECO, in the form of SDG, is the most prevalent mammalian lignan precursor in flaxseed, and thus attracts the most attention, although flax also contains several other lignans, including matairesinol and lariciresinol [7, 8]. SECO is also the lignan converted most efficiently into mammalian lignan forms. Once absorbed in the intestine, lignans, both SECO and its metabolites enter the circulation system and begin to exhibit their numerous physiological effects [4].

Since the discovery of their physiological value, lignans have been extracted from flax and other plants, in a variety of ways. Once extracted, lignans can be added to food or taken in a concentrated form, in an attempt to take advantage of their functionality and benefits. Traditionally, solvent

extraction has been used to recover lignans, typically with alcohols like methanol or ethanol, or acetone [9] due to the polar nature of these phenolic compounds. This extraction is either combined with, or followed by, hydrolysis of the lignan macromolecule using acid, base or enzyme. Use of large quantities of an organic solvent, such as methanol, requires its removal which usually involves high temperatures.

Recently, efforts have been made to use alternate protocols for the extraction of lignans from flax and other plant materials, including the use of pressurized low-polarity water [10], microwave-assisted extraction [11, 12] and supercritical fluid extraction [13, 14].

Supercritical fluid extraction has been used previously for the extraction of oil and policosanols from flaxseed [15, 16]. Supercritical carbon dioxide (SC-CO_2) can be used as a solvent for plant components at temperatures and pressures above its critical point, 31 °C and 7.4 MPa [17]. SC-CO_2 is a favorable solvent for natural product applications because it is non-toxic, non-flammable, inexpensive, easily separated from the extract upon depressurization [15, 18] and it is ideal for nonpolar entities, such as lipids. However, co-solvents, such as water and ethanol, can be added at low levels to alter the polarity of CO_2.

The literature reveals limited information on the use of SC-CO_2 in regards to the extraction of lignans from flaxseed. It is primarily used as a preparatory step to remove the lipid fraction prior to solvent extraction and hydrolysis of lignans. Harris and Haggerty [19] investigated SC-CO_2 modified with tetrahydrofuran, water and acetic acid to extract lignans and phenolic acids from flaxseeds. This study [19], however, was not intended to extract the lignans for further use in food systems since tetrahydrofuran is not a food-grade solvent nor did it attempt process optimization.

Although SC-CO_2 has been used to extract lignans and related phenolics from plants such as *Schisandra chinensis*, with and without polar modifiers [13, 14], it has not been utilized, to any great extent, to extract lignans from flaxseed specifically. Therefore, the objectives of this investigation were (a) to determine the effect of temperature, pressure and level of ethanol addition on the CO_2 loading of SDG during SC-CO_2 extraction of flaxseed, and (b) to determine the effect of flaxseed pre-treatment on the CO_2 loading of SDG.

Materials and Methods

Materials

Industrial yellow flaxseed was generously donated by Agricore United (Winnipeg, MB, Canada) and stored at –20 °C until needed. All solvents and reagents used were of analytical grade.

Sample Preparation and Pre-treatments

Once removed from cold storage, seeds were ground in a coffee grinder (Philips Model HD5112, Markham, ON, Canada) for 30 s. Ground seed batches were then mixed to homogenize and to ensure uniformity in terms of particle size. Solvent defatting was performed over 7 h using a Goldfisch apparatus (Labconco Co., Kansas City, MO) with petroleum ether. Samples were placed in the fume hood overnight to ensure that all residual solvent was removed by evaporation. Where samples were defatted by SC-CO_2, the protocol of Bozan and Temelli [15] was followed at 70 °C and 55 MPa.

Dehulling was performed using a Buhler MLU 202 Flour Mill (Markham, ON, Canada) with the break rolls engaged (front and back break rolls set at a gap of 0.12 and 0.14 mm, respectively) and the reduction rolls blocked. The collected, crushed seed was sorted by a gravity table (Westrup, Slagelse, Denmark) in an attempt to collect a sample of hulls as pure as possible. Small pieces of residual meal were removed manually. Hulls were then ground as previously mentioned.

For ground, defatted seed, which was hydrolyzed prior to SC-CO_2 extraction, ground seed, defatted using the solvent method described above, and 0.5 N NaOH in 70% ethanol were combined in a 1:5 (w:v) ratio, and placed in a 60 °C water bath for 3.5 h with vortexing at 30-min intervals (personal communication with Dr. A. Muir, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada). Concentrated glacial acetic acid was used for neutralization. Samples were dried using a roto-evaporator, and then placed under a gentle flow of nitrogen to remove all remaining solvent. Hydrolyzed seed was then re-ground as previously mentioned.

Traditional Lignan Extraction

As a comparison to SC-CO_2 extraction, the SDG content of the starting material was determined using the solvent extraction method of Muir (personal communication with Dr. A. Muir, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada). Two gram samples along with 10 mL of 0.5 N NaOH in 70% methanol were placed in a screw top test tube. Samples were then placed in a shaking water bath at 65 °C for 3.5 h, and neutralized as previously mentioned. Samples were then refrigerated until use (4 °C). Prior to HPLC injection, samples were centrifuged and the supernatant was passed through a 40 μm syringe filter tip (Millipore, Cork, Ireland). Solvent extractions were performed in duplicate. SC-CO_2 extraction residue samples were analyzed using the same methodology. Modifications to the extraction protocol were made for samples, both starting material and residue, which were

pre-hydrolyzed. These samples were not re-exposed to alkali conditions, but were instead just incubated in the 70% methanol solution.

Supercritical CO_2 Extraction

A laboratory scale supercritical fluid extraction system (Newport Scientific, Inc., Jessup, MD) used in the extraction of oil from flaxseed previously [15] was used for the determination of CO_2 loading of SDG. A four-gram sample was loaded into a 25-mL basket, which was placed in the 300-mL extraction cell. Carbon dioxide of 99.95% purity (Praxair, Edmonton, AB, Canada) was pressurized using a diaphragm compressor with a maximum rating of 69 MPa. Pressure was controlled (±1 MPa) by a back pressure regulator. The extraction vessel was heated with a heating jacket and the temperature controlled using a thermostat (±1 °C). Where ethanol was used as a modifier, liquid ethanol was pumped into the pressurized CO_2 stream using a separate co-solvent pump (Gibson 305, Middleton, WI). The flow rate of the CO_2 was 1 L/min, as previously determined to be appropriate for flax oil extraction using the same system [15], measured at ambient conditions by a dry gas meter and controlled by a heated micro-metering valve. Ethanol was removed upstream of the gas meter using a cold trap situated after the sample collection.

Glass collection vials were connected to the depressurization valve and held in a refrigerated bath (–20 °C). Sample collection tubing was washed using minimal (~5 mL) volumes of ethanol. Residual ethanol was removed from the samples by purging gently with nitrogen gas. Sample weights were determined gravimetrically (±0.0001 g). Collected samples were stored at –20 °C until ready to be analyzed by HPLC.

Experimental Design

Two sets of extractions were performed. Extract samples were collected as a function of time, and analyzed using HPLC to generate extraction curves. The effects of temperature, pressure and level of modifier addition were studied in the first set. Defatted, ground whole flaxseed was loaded into the extractor as described above. CO_2 was compressed to pressures of 35, 40 or 45 MPa and temperature was controlled at 40, 50 or 60 °C. Ethanol was introduced at 0, 10 or 20 mol%. Extractions were carried out for a total extraction time of 6 h and samples were collected at 20 min intervals for the first hour, 30 min intervals for the second hour and then once every hour after that, for a total of nine samples.

The effect of different pre-treatments was studied in the second set of extractions. The temperature, pressure and solvent composition found in the first set of extractions to result in the greatest CO_2 loading of SDG were utilized for the extraction of SDG from ground seeds defatted with petroleum ether (T DF WS), ground seeds defatted with SC-CO_2 (CO_2 DF WS), ground hydrolyzed seeds, defatted with petroleum ether (HY WS), ground hulls defatted with petroleum ether (DF H), and ground full-fat hulls (FF H). Extractions were performed in triplicate for 4 h. Samples were collected at intervals similar to those in the first set of extractions, and analyzed by HPLC. Different sample preparations used throughout the study are summarized in Fig. 1.

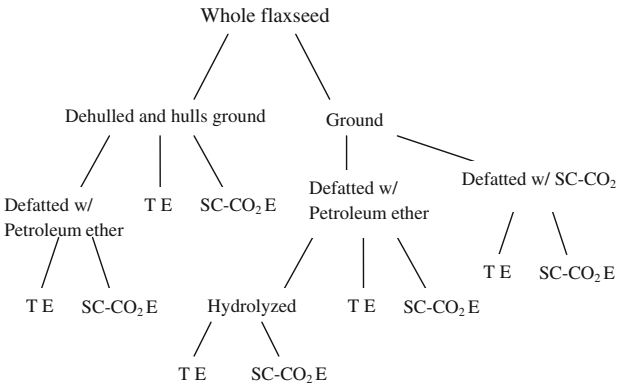

Fig. 1 Sample treatment steps for traditional and SC-CO_2 extractions (*T E* traditional lignan extraction, *SC-CO$_2$ E* SC-CO_2 lignan extraction)

HPLC Analysis

Starting material and SC-CO_2 extraction residue analysis were performed using a Varian Prostart 210 HPLC (Varian, Palo Alto, CA) equipped with a Waters 486 tunable absorbance detector (Waters, Milford, MA) set at 280 nm, as per the protocol developed by Muir (personal communication with Dr. A. Muir, Agriculture and Agri-Food Canada, Saskatoon, SK, Canada). The column used was a C18-5 μm, 4.6 mm × 150 mm reverse phase column (Supelco, Sigma–Aldrich, St. Louis, MO) and the mobile phase was a mixture of 0.05% trifluoroacetic acid in HPLC grade water (A) and 0.05% trifluoroacetic acid in HPLC grade acetonitrile (B). The gradient, $t = 0$ min, $A = 99\%$; $t = 0.5$ min, $A = 99\%$; $t = 5$ min, $A = 60\%$; $t = 6$ min, $A = 99\%$; was run at 1.5 mL/min for a total time of 15 min. Samples were compared to HPLC grade standards dissolved in 70% methanol. Quantification and SDG standard (ChromoDex, Irvine, CA) curves were established using Galaxie Version 1.9 software (Varian, Palo Alto, CA) in the range of 0.01–2 mg SDG/mL of 70% methanol.

For SC-CO_2 extracts from the first set of extractions focusing on process conditions, post-extraction hydrolysis

was necessary due to the potential existence of SDG polymers in the extract. Therefore, samples were solubilized in 4 mL of 0.5 N NaOH in 70% methanol directly in the extraction vials, and left in a shaking water bath for 3.5 h at 60 °C, as mentioned previously. They were then neutralized with concentrated glacial acetic acid, centrifuged and analysed by HPLC under conditions slightly modified from those above. The solvent gradient was stretched out to $t = 0$ min, $A = 99\%$; $t = 0.5$ min, $A = 99\%$; $t = 8$ min, $A = 75\%$; $t = 10$ min, $A = 0\%$; $t = 13$ min, $A = 0\%$; $t = 14$ min, $A = 99\%$; $t = 23$ min, $A = 99\%$; for a total run time of 23 min.

Extracts from the second set of extractions focusing on pre-treatments were prepared similarly to those from the first set with several modifications. First, samples were solubilized in only 2 mL of 0.5 N NaOH in 70% methanol in an attempt to improve quantification. Also, extracts from pre-hydrolyzed ground seeds were soaked in 70% methanol but were not re-hydrolyzed. The gradient time was slightly adjusted to reflect column usage over the time period in between the two sets of samples and the potential differences between samples to $t = 0$ min, $A = 99\%$; $t = 0.5$ min, $A = 99\%$; $t = 12$ min, $A = 75\%$; $t = 14$ min, $A = 0\%$; $t = 17$ min, $A = 0\%$; $t = 18$ min, $A = 99\%$; $t = 23$ min, $A = 99\%$. New standard curves were generated using this method, with concentrations in the range of 0.0001–0.01 mg SDG/mL of 70% methanol.

Statistical Analysis

A 3-variable Box–Behnken design was used for the investigation of pressure, temperature and co-solvent concentration effects with five replications at the center point. The order of all experiments was randomized. Design-Expert 7.1.6 software (Stat-Ease, Minneapolis, MN) was used to establish the Box–Behnken design, to conduct an analysis of variance (ANOVA) and to validate the model. Extraction curves based on the amount of SDG extracted (μg) versus g CO_2 were established for each extraction run. The slope of the initial linear portion of the extraction curves was determined, which represented the CO_2 loading of SDG and used as the response variable. The conditions (temperature, pressure and co-solvent concentration), which yielded the steepest initial slope (CO_2 loading) at $\alpha = 0.05$ were considered optimum.

Extraction curves were again generated for the extractions of SDG from the flaxseed with different pre-treatments and an ANOVA of the results was performed using the general linear model procedure of SAS Statistical Software, version 9.1 (SAS Institute Inc., Cary, NC). Multiple comparison of the means was performed by a least significant difference (LSD) test at $\alpha = 0.05$ level.

Results and Discussion

Effects of Temperature, Pressure and Ethanol Concentration

Figure 2 shows typical HPLC chromatograms for the SDG standard, SC-CO_2 extract fraction, and the residue. Based on the quantification of SDG by HPLC, extraction curves were generated for each run, and a typical curve is presented in Fig. 3 as the amount of SDG extracted versus the amount of CO_2 used. In general, for dynamic extraction systems as the one described here, the slope of the initial linear portion of an extraction curve is reported as "solubility" or "thermodynamic solubility" when the starting material is a pure component or as "apparent solubility" for complex mixtures, like plant materials, as long as the equilibrium requirements are met. The requirements are to pump CO_2 at low enough flow rates to allow sufficient contact time and to have enough solute present to saturate the CO_2 [20]. In this study, the CO_2 flow rate was maintained at 1 L/min (measured at ambient conditions) and based on previous studies [21] should provide sufficient contact time to reach equilibrium. On the other hand, saturation level for SDG in CO_2 is not known. As well, SDG is mainly present as a macromolecule in the flaxseeds, so any free SDG would be available only at very low levels. Considering these limitations, the slope of the linear portion of extraction curves is reported as "CO_2 loading" rather than "apparent solubility". The linear portion or the constant rate period in Fig. 3 is followed by the transition and the diffusion-controlled periods where the extraction rate drops and the extraction curve asymptotically approaches complete recovery, as is typical in any extraction process.

The CO_2 loading of SDG in SC-CO_2 modified with ethanol varied from 0.087 to 0.55 μg/g CO_2 (Table 1). There were no significant differences ($p > 0.05$) between the CO_2 loading at each condition. As well, there were no significant ($p > 0.05$) interactions between temperature, pressure and co-solvent concentration. Choi et al. [13] investigated the effect of temperature and pressure on the yield of lignans schisandrol A and B and schisandrin A, B and C after 30 min of extraction. They found that there was no significant effect of temperature and pressure on the yield of these compounds. Their "optimum" condition was at 60 °C and the highest pressure investigated, 34 MPa.

In the case of the quantity of SDG in residue samples (Table 1), the interaction between co-solvent level and temperature was significant ($p \leq 0.05$), with the quantity of SDG in the residue decreasing with increasing ethanol level and temperature (Fig. 4). The residue which would have the least amount of SDG remaining after SC-CO_2 extraction, according to the model generated was obtained at 7.1 mol% ethanol, 37.9 MPa, and 60 °C with 1.8% SDG (w/w), and

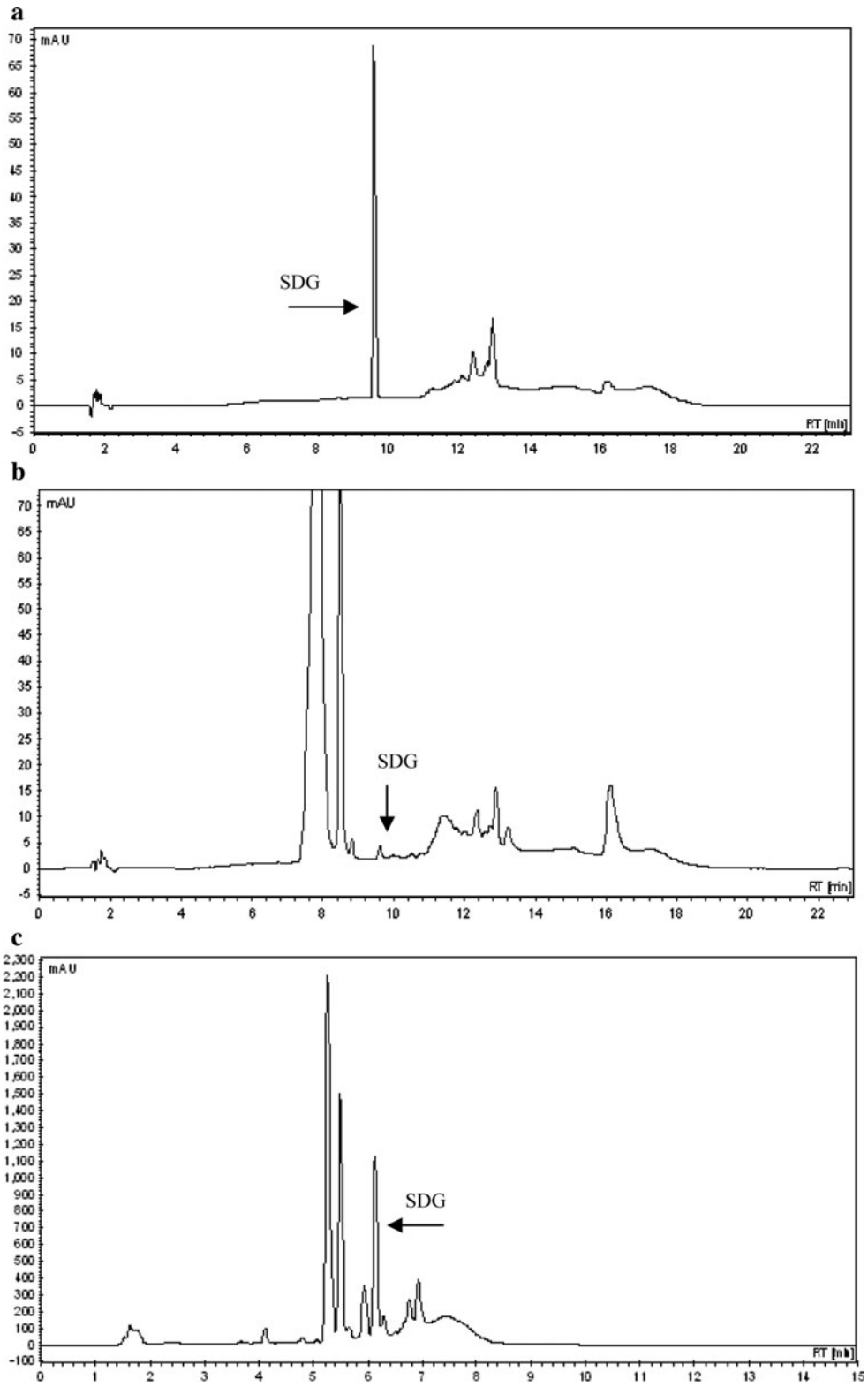

Fig. 2 Sample HPLC chromatogram: **a** SDG Standard (0.09 mg/mL of 70% methanol); **b** SC-CO_2 extract fraction collected between 40 and 60 min at 45 MPa, 50 °C and 0 mol% ethanol; **c** residue from SC-CO_2 extraction at 45 MPa, 50 °C and 0 mol% ethanol

the residue which would have the most SDG remaining was at 0 mol% ethanol, 45 MPa, and 50 °C with 2.7% SDG (w/w). Because SDG is a polar compound, it was expected that an increase in ethanol concentration would increase the CO_2 loading of SDG; however, this effect was not found to be significant ($p > 0.05$). At high ethanol concentrations, such as 20 mol% ethanol, it is likely that not all ethanol was in the supercritical state and dissolved in CO_2. There are

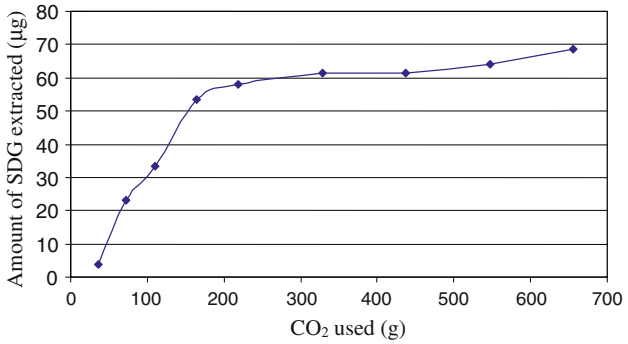

Fig. 3 Cumulative amount of SDG extracted as a function of mass of CO_2 at 35 MPa, 60 °C and 10 mol% ethanol

several reasons why there may be no evident trends in CO_2 loading and that significant optimization conditions could not be established through these experiments. First, SDG typically occurs in a polymer form in flaxseeds, and is linked to other SDG molecules via 3-hydroxy-3-methyl glutaric acid (HMGA) to form a lignan macromolecule, with most commonly five SDG molecules and four HMGA molecules [5, 6]. Recent work has revealed that the macromolecule may also contain *p*-coumaric acid glucoside, ferulic acid glucoside, caffeic acid glucoside and herbacetin diglucoside [22]. Because the macromolecule has a much greater molecular mass than SDG alone, it would not be solubilized to the same degree that molecular SDG could be. The values reported reflect the SDG released from all soluble forms, including the larger macromolecules. Choi et al. [13] and Lojkova et al. [23] found much higher extraction efficiencies in their studies with lignans from *Schisandra chinensis* with a recovery of 80% after 30 min of SC-CO_2 extraction compared to methanol extraction for fruits, and 96% from seeds and 26% from fruits after 60 min of SC-CO_2 extraction, compared to petroleum ether and methanol extraction, respectively. This is most likely due to differences in the structures and associations of these lignans compared to those in flax. The lignans in *S. chinensis* contain free lignans to a greater extent with much lower polarities compared to flax lignans and therefore they are likely to have much higher affinities to SC-CO_2 than do flax lignans.

Another contributing factor to error was the difficulty in maintaining constant pressure and flow rate when ethanol was incorporated into the system, especially at the maximum level of 20 mol%. Manual control of the micrometering valve becomes difficult when relatively large quantities of ethanol have to pass through the small opening around the stem upon pressure drop down to atmospheric level. The flow meter used was a dry gas flow meter; if the ethanol in the exhaust stream was not completely condensed in the cold trap between the outlet and the flow meter, it may have caused problems with the flow meter reading. Lojkova et al. [23] also found it difficult to control the flow, especially during the beginning of the extraction, due to the high concentration of easily extractable lipophilic compounds.

The next set of extractions, investigating the effect of pre-treatments, required an "optimum" set of conditions to be selected, regardless of statistical significance. The

Table 1 SDG extraction from 4 g of flaxseed using SC-CO_2 modified with ethanol

Ethanol addition (mol%)	Pressure (MPa)	Temperature (°C)	CO_2 loading of SDG (µg/g CO_2)[a]	Amount of SDG in SC-CO_2 extract at 6 h (µg SDG)[a]	SDG in residue (% w/w)[a, b]
10	40	50	0.38	70.4	1.6
20	40	40	0.12	46.0	2.2
0	40	60	0.18	54.3	2.7
10	40	50	0.39	44.7	2.1
10	35	60	0.34	68.6	1.6
0	40	40	0.13	27.4	2.4
10	40	50	0.35	46.9	1.6
10	40	50	0.25	107.2	2.1
10	35	40	0.24	28.9	2.3
10	45	60	0.55	84.7	1.9
20	40	60	0.11	33.6	1.4
0	35	50	0.087	10.9	2.4
10	40	50	0.11	21.9	1.4
20	35	50	0.23	42.2	2.3
0	45	50	0.22	56.7	2.5
10	45	40	0.25	44.3	2.1
20	45	50	0.13	44.9	2.1

[a] Mean ± standard deviation for CO_2 loading, amount of SDG in extract and in residue are 0.30 ± 0.12 µg/g CO_2, 58.2 ± 32.3 µg and 1.8 ± 0.31%, respectively, for the center point, based on five replications

[b] SDG content of starting material = 2.2% (w/w)

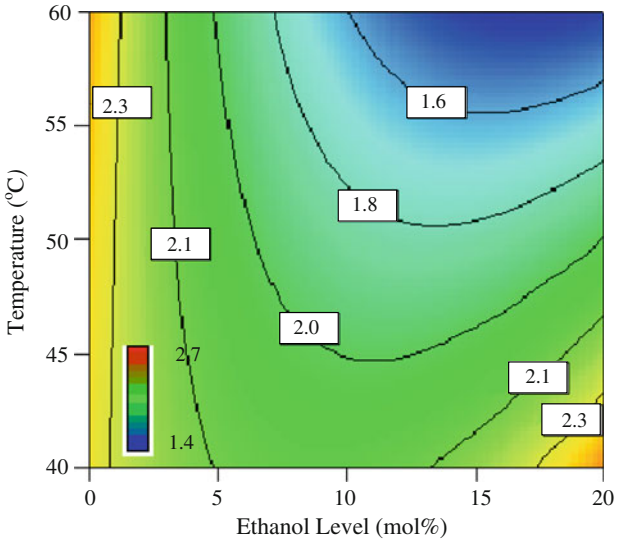

Fig. 4 Quantity of SDG in residue (% w/w) at 37.9 MPa (Design-Expert 7.1.6, Stat-Ease, Minneapolis, MN)

statistical software generated an optimum CO_2 loading of 0.49 μg/g CO_2 at 7.8 mol% ethanol, 45 MPa and 60 °C. Figure 5 illustrates that, although not statistically significant, when co-solvent concentration is fixed at 7.8 mol% ethanol, the CO_2 loading of SDG increases with both temperature and pressure. Statistically, however, any of the conditions tested could have been chosen.

Effect of Sample Pre-treatment

Based on the findings from the investigation into the effects of process conditions, it was predicted that the maximum CO_2 loading of SDG from whole, ground flaxseed defatted by petroleum ether, 0.49 μg/g CO_2, could be achieved at

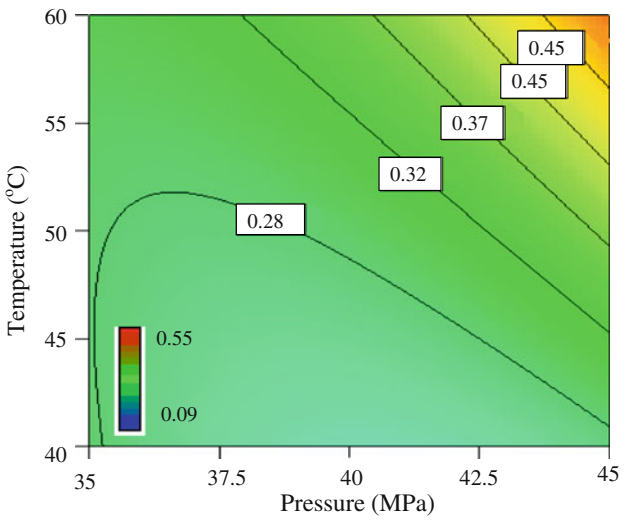

Fig. 5 CO_2 loading of SDG (μg/g CO_2) at ethanol concentration of 7.8 mol% (Design-Expert 7.1.6, Stat-Ease, Minneapolis, MN)

Table 2 CO_2 loading of SDG from different preparations of flaxseed in SC-CO_2 modified with 7.8 mol% ethanol at 60 °C and 45 MPa[a]

Treatment	CO_2 loading (μg SDG/g CO_2)
T DF WS	0.16 ± 0.08^b
CO_2 DF WS	0.06 ± 0.05^b
FF H	0.29 ± 0.16^b
DF H	0.12 ± 0.1^b
HY WS	3.80 ± 0.6^c

T DF WS ground seed defatted with petroleum ether, *CO_2 DF WS* ground seed defatted with SC-CO_2, *FF H* ground full-fat hulls, *DF H* ground hulls defatted with petroleum ether, *HY WS* ground hydrolyzed seed, defatted with petroleum ether

[a] Means ± standard deviation based on triplicate extractions ($n = 3$)

[b, c] Means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$)

7.8 mol% ethanol, 60 °C and 45 MPa. As seen in Table 2, this was not the case. The CO_2 loading at these conditions was actually found to be 0.16 μg/g CO_2. One reason was that the model generated previously, which was used to predict the maximum CO_2 loading, was not found to be significant. Regardless, because the extraction conditions were kept constant for the pre-treatment portion of the study, it should be possible to determine the optimal starting material to maximize CO_2 loading based on relative comparisons. Again, it is important to point out that it is the CO_2 loading that is reported, not solubility. For the samples which underwent alkali hydrolysis prior to SC-CO_2 extraction it is likely that the CO_2 loading would be approaching "apparent solubility"; however, confirmation of "apparent solubility" requires further research.

The CO_2 loading of SDG obtained from T DF WS, CO_2 DF WS, FF H and DF H were found to be similar ($p > 0.05$) (Table 2). This may be due to the high variability between replicates of each treatment, as parameters such as flow rate and pressure were difficult to control precisely in the presence of ethanol. However, the CO_2 loading obtained from the above mentioned treatments were significantly ($p \leq 0.05$) less than that from HY WS, which was more than 13 times greater. The alkali treatment hydrolyzes the large SDG macromolecules into smaller macromolecules and free SDG. Because solubility in SC-CO_2 depends on molecular mass and size, the relatively smaller molecules in the hydrolyzed sample, as expected, resulted in higher CO_2 loading.

It was expected that the CO_2 loading of SDG from the flax hulls would be significantly higher than that from the whole seed, considering that SDG is mostly concentrated in the hulls [24]. However, this was not the case. The increased complexity of the hull matrix, including the existence of waxes and mucilage [16], in comparison to

Table 3 Average material balance for SDG extractions (6 h) for 4 g of each flaxseed treatment using SC-CO_2 + 7.8 mol% ethanol at 60 °C and 45 MPa[a]

Treatment	SDG in starting material (mg)	SDG in residue (mg)	Total SDG in extract (μg)	SDG gained after SC-CO_2 extraction (μg)
T DF WS	60.7 ± 7.2[d]	60.8 ± 20.6[d]	15.9 ± 5.4[b]	106 ± 92.9
CO_2 DF WS	54.7 ± 5.7[c]	54.8 ± 15.1[c]	6.1 ± 3[b]	9.5 ± 7.7
FF H	90.6 ± 11.4[f]	90.6 ± 26.1[f]	21.6 ± 13.1[b]	7.2 ± 8.7
DF H	80.8 ± 6.7[e]	80.7 ± 21.7[e]	19.4 ± 15.3[b]	5.1 ± 13.7
HY WS	37.1 ± 3.0[b]	36.8 ± 7.3[b]	171.8 ± 36.1[c]	−125.3 ± 609

T DF WS ground seed defatted with petroleum ether, *CO_2 DF WS* ground seed defatted with SC-CO_2, *FF H* ground full-fat hulls, *DF H* ground hulls defatted with petroleum ether, *HY WS* ground hydrolyzed seed, defatted with petroleum ether

[a] Means ± standard deviation based on triplicate extractions ($n = 3$)

[b–f] Means in the same column followed by the same superscript letter are not significantly different ($p > 0.05$)

the matrix of the meal may have contributed to this outcome. Diffusion of SC-CO_2 and transfer of SDG through the hulls were less efficient compared to that in the meal. SDG may also exist in larger polymers in the hull than in the meal, and these larger polymers would be less soluble in SC-CO_2 compared to smaller polymers and individual compounds. As well, SDG polymers may be more tightly bound in the hull matrix. So, despite the higher concentrations, the CO_2 loading of SDG from hulls was not significantly greater. It was expected that the defatted hulls would result in a higher yield of SDG from traditional extraction than the full fat hulls, considering that hulls contain approximately 18.3% (w/w) lipid when extracted using petroleum ether [25], but this was also not seen (Table 3), possibly due to matrix changes or component degradation during defatting.

Due to the lengths of tubing and fittings of the extraction system, and the necessity to rinse these parts to recover all possible extract, some of the target component, in this case SDG may have been lost. An SDG mass balance was completed for each extraction, assuming that the final mass of residue was equal to the mass of the starting material, minus the mass of collected extracts and the mass of the fraction potentially lost. Results of the mass balance calculations are presented in Table 3. With the exception of the HY WS, more SDG was recovered after SC-CO_2 extraction (from the residue and the extract) compared to the amount in the starting material, although this difference was in the microgram range (Table 3). During SC-CO_2 extraction, the dense CO_2 diffuses into the matrix of the flax sample, expanding the sample. Upon depressurization the CO_2 dissipates from the sample, resulting in an irreversible opening of the matrix. Therefore, after the SC-CO_2 extraction, the SDG in the residue is more accessible to traditional extraction used for analysis purposes than the starting material is, and the resulting quantity is relatively greater. This phenomenon has been observed and taken advantage of in oil extraction. Dong and Walker [26] illustrated that canola flakes "exploded" through the SC-CO_2 depressurization process showed improvements in subsequent oil extractability. Potentially, rapid pressurization/depressurization could be a beneficial pre-treatment prior to traditional SDG extraction to enhance recovery.

Through traditional extraction used for analysis, significantly more SDG was extracted from the hulls than from the whole seed (Table 3). The alkali treatment was able to not only hydrolyze the SDG macromolecules, but also loosen the cell wall components, which previously limited the availability of the SDG. For the HY WS, less SDG was recovered from the residue after SC-CO_2 extraction compared to the starting material. This indicates that, although the effect of SC-CO_2 making the SDG more available to traditional extraction was most likely also present in these samples, there was a net loss. These extracts tended to be much stickier as a result of the hydrolysis, and this could have led to the increased deposit of extract on tubing, which was not possible to rinse and recover by ethanol. Interestingly, the SDG extracted using traditional methods from the hydrolyzed starting material was significantly ($p \leq 0.05$) less than that from the other treatments. These samples were treated similarly to those in a traditional extraction, with the exception of the use of food-grade ethanol rather than methanol, and the solvent was evaporated off before the sample was reground. For HPLC analysis, the hydrolyzed seed was soaked in 70% methanol for the same time and at the same temperature as in a traditional extraction. As previously mentioned, the alkali serves to not only hydrolyze the SDG macromolecule, but may also hydrolyze or solubilize other cellular constituents, which limit SDG availability. When the hydrolyzed sample was dried, even though cellular damage had already been caused by the alkali, the hardening of the sample may have created new barriers to availability. When the sample was soaked in 70% methanol in preparation for HPLC analysis, there was no alkali added to help in breaking up the newly formed matrix.

Conclusion

Overall, SC-CO_2 extraction resulted in much lower quantities of SDG compared to traditional extraction. The level of ethanol addition, temperature and pressure of extraction did not have a significant effect ($p > 0.05$) on CO_2 loading of SDG. The amount of SDG extracted and CO_2 loading were significantly ($p \leq 0.05$) enhanced by the pre-hydrolysis of the starting flax material. Additionally, although all of the SC-CO_2 extracts did not contain high levels of SDG, it was apparent that flax samples that had undergone pressurization and depressurization in the presence of SC-CO_2, had increased recovery of SDG using traditional extraction methods. Based on the findings, SC-CO_2 extraction is not recommended for the recovery of flax SDG under the conditions investigated; however, other treatments may enhance extraction yields.

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