Optimization of the Extraction and Fractionation of Corn Bran Oil Using Analytical Supercritical Fluid Instrumentation

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Abstract

Supercritical fluid extraction (SFE) is combined with supercritical fluid chromatography (SFC) in an analytical mode to develop a system for fractionating and enriching high value ferulate-phytosterol esters (FPE) contained in corn bran oil. Corn bran is initially extracted with neat supercritical carbon dioxide (SC-CO_2) at various pressures (13.8, 34.5, and 69 MPa) and temperatures (40, 60, and 80°C) to see if the FPE can be enriched in the extracts. These initial studies show the greatest percentage of FPE could be extracted under two sets of conditions: 69 MPa at 80°C and 34.5 MPa at 40°C. Both sets of parameters yield an extract containing ~1.25% FPE. A stock supply of corn bran oil is then produced by scaled-up SFE at 34.5 MPa and 40°C for subsequent chromatographic fractionation. The SFE-obtained corn bran oil is then applied to the head of a minichromatographic column containing an amino-propyl sorbent. SFC is than commenced using neat SC-CO_2 at 69 MPa and 80°C to remove the majority of the triglyceride-based oil. Pressure and temperature are then lowered to 34.5 MPa and 40°C, respectively, and ethanol is added as a modifier. The modifier is added in an increasing stepwise gradient program, and fractions are collected at equal volume intervals. The resultant fractions are analyzed by analytical high-performance liquid chromatography with evaporative light-scattering detection and show that FPE could be enriched to a 14.5% (w) level.

Introduction

High value nutraceutical components in seed oil matrices usually occur at low levels, either in the bulk oil or concentrated on the residual protein meal (flakes, bran, fiber, etc.). The isolation and enrichment of such oil–seed components is desired to increase their effectiveness, although commercial preparations are frequently sold as concentrates in a coextracted oil matrix. Methods for concentrating such nutraceuticals should be benign so as not to affect the desired components; therefore, supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) using supercritical carbon dioxide (SC-CO_2) as a processing agent are excellent techniques for this purpose. In addition to being environmentally benign, SC-CO_2 processing can also have considerable appeal to consumers of such products.

Two-step fractionation processes, such as those just proposed, require considerable effort to optimize. The cost of materials, time and labor, and equipment can be substantial in screening for the best conditions for either extraction or fractionation (chromatography). With the advent of modern, automated analytical SFE instrumentation, such modules can be used to address the optimization of such separation processes. With slight modification, the described equipment can be changed into a small-scale preparative chromatograph employing lower cost sorbent material in place of the sample matrix used in traditional analytical SFE. Such units can be used to optimize the SFE stage that is conducted prior to SFC.

Recently, it has been reported that the hexane-derived extract from corn bran contains high levels of ferulate-phytosterol esters (FPE) similar in composition and function to oryzanol, an ingredient with nutritional functionality that is found in rice bran and rice bran oil (1–3). Oryzanol has been shown to lower the levels of serum cholesterol in laboratory animals and humans (4,5). Recently, ferulate-phytosterol esters, and in particular the sitostanyl ester, have also been shown to have similar cholesterol-lowering activity (6,7).

Corn bran and corn fiber are obtained as byproducts from the dry- and wet-milling of corn, respectively—processes that are used in converting corn into numerous products. Moreau et al. (8) reported that both corn bran and corn fiber yield oils that contain FPE. Moreau et al. performed hexane and SC-CO_2 extractions and found that the corn fiber oil contained higher percentages (3–6%, w) of FPE than corn bran oil (1.5%, w). They also noted that corn fiber, which contains similar sterol components, produced lower levels of oil than corn bran. Unfortunately, in both cases, the nutraceutically-active components are at low levels in a predominately triglyceride-containing oil extract. Enrichment of these active components in an extract or concentrate would be a

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welcomed improvement to the current situation.

In this study, we have expanded upon earlier research that has incorporated a two-step process consisting of SFE and SFC in combination to fractionate and enrich high value nutraceutical components (9–11). Here, an integrated procedure for the extraction of oil from corn bran was developed and combined with SFC to obtain fractions enriched in the FPE components. Such reconnaissance experiments have utilized commercial, analytical-scale equipment for designing and optimizing the fractionation process. The knowledge gained in such studies can be applied to scaling up the process with respect to parameters such as pressure, temperature, modifier, and sorbent type.

Table I. Typical Parameters Used for an SFF Experiment

	Pressure	Temperature	Time	Flow rate	Solvent
Fraction 1	69.0 MPa	80°C	60 min	2 mL/min	CO_2
Fraction 2	34.5 MPa	40°C	60 min	2 mL/min	1% EtOH–CO_2
Fraction 3	34.5 MPa	40°C	60 min	2 mL/min	2% EtOH–CO_2
Fraction 4	34.5 MPa	40°C	60 min	2 mL/min	3% EtOH–CO_2
Fraction 5	34.5 MPa	40°C	60 min	2 mL/min	5% EtOH–CO_2
Fraction 6	34.5 MPa	40°C	60 min	2 mL/min	7% EtOH–CO_2
Fraction 7	34.5 MPa	40°C	60 min	2 mL/min	10% EtOH–CO_2
Fraction 8	34.5 MPa	40°C	60 min	2 mL/min	15% EtOH–CO_2
Fraction 9	34.5 MPa	40°C	30 min	2 mL/min	20% EtOH–CO_2

Table II. Weight Percent and Mass Recoveries of Components from the SFE of Corn Bran

	13.8 MPa					
	80°C		60°C		40°C	
Compound	Weight (%)	Mass (mg)	Weight (%)	Mass (mg)	Weight (%)	Mass (mg)
FASE*	19.85	0.10	6.89	0.40	3.92	3.41
TG†	53.40	0.27	60.95	3.54	90.11	78.49
FFA‡	23.68	0.12	28.23	1.64	4.04	3.52
FS§	2.40	0.01	3.19	0.19	1.10	0.96
FPE"	0.67	0.01	0.74	0.04	0.83	0.72
	34.5 MPa					
	80°C		60°C		40°C	
Compound	Weight (%)	Mass (mg)	Weight (%)	Mass (mg)	Weight (%)	Mass (mg)
FASE	3.10	2.79	3.60	3.75	3.15	3.46
TG	87.40	78.40	87.67	91.44	90.91	100.09
FFA	3.83	3.44	4.88	5.09	3.57	3.93
FS	4.62	4.14	2.73	2.85	1.15	1.26
FPE	1.04	0.94	1.12	1.17	1.23	1.35
	69 MPa					
	80°C		60°C		40°C	
Compound	Weight (%)	Mass (mg)	Weight (%)	Mass (mg)	Weight (%)	Mass (mg)
FASE	4.10	4.32	3.48	3.24	3.67	2.99
TG	88.96	93.86	88.16	81.99	87.26	71.11
FFA	4.54	4.80	5.40	5.02	3.55	2.89
FS	1.13	1.20	2.05	1.91	4.44	3.62
FPE	1.26	1.33	0.92	0.85	1.09	0.89

* FASE, fatty acid-phytosterol esters.
† TG, triglycerides.
‡ FFA, free fatty acids.
§ FS, free sterols.
" FPE, ferulate-phytosterol esters.

Experimental

Corn Bran

The corn bran was a gift from Mr. Rex Winter of Illinois Cereal Mills, Inc. (Paris, IL).

SFE

The SFE screening studies were performed with an Isco (Lincoln, NE) model SFX 3560 automated extractor. Corn bran was extracted with all possible combinations of three pressures (13.8, 34.5, and 69 MPa) and three temperatures (40, 60, and 80°C). The extractions were conducted for 120 min at a pump delivery flow rate of 2 mL/min liquid CO_2. The unit's restrictor was heated to 80°C using pressurized collection at 0°C.

The corn bran oil stock supply was obtained by extraction with SC-CO_2 at 34.5 MPa and 40°C using the National Center for Agricultural Utilization Research SFE pilot plant (12). The extract was centrifuged to separate the oil from the waxes in the extract. The separated oil was decanted into a funnel and filtered through glass wool. The resultant oil was then stored at 4°C until use.

Supercritical fluid fractionation

Supercritical fluid fractionation (SFF) studies were also performed with an Isco model SFX 3560 automated extractor. The sorbents tested for the SFF were as follows: silica gel (60–200 mesh, J.T. Baker Chemical, Phillipsburg, NJ), amino-propyl bonded silica (40 µm, Varian Associates, Harbor City, CA), neutral alumina (60–325 mesh, Fisher Scientific, Fair Lawn, NJ), and diol-modified silica (37–55 µm, Millipore Corporation/Waters Chromatography, Milford, MA). The sorbents were added to a 10-mL extraction vessel, and corn bran oil (~0.4 g) was manually applied to the top of the sorbent bed. The extraction/fractionation procedure was then commenced with fractions collected at the timed intervals noted in Table I. The first fraction was intended to remove most of the triglyceride-based oil. The parameters for subsequent fractions were designed to fractionate and enrich the collection of FPE.

High-performance liquid chromatography

All analyses were 15-µL injections of 5-mg/mL solutions. They were performed using a Spectra-Physics SP8800 pump (Spectra Physics Analytical, San Jose, CA) connected to a SpectraSYSTEM AS3000 autosampler equipped with a Rheodyne 7010-151 loop injector (100 µL) (Thermo

Separation Products, San Jose, CA), a Bio-Rad (Richmond, CA) model 1250424 column heater at 30°C, an Alltech (Deerfield, IL) model 500 ELSD evaporative light-scattering detector (ELSD) at 40°C with 1.5 SLPM of N_2, and a ChromQuest Chromatography Data System (ThermoQuest, San Jose, CA). The analytical HPLC column was a 5-µm Chromsep Cartridge, Lichrosorb DIOL (3 × 100 mm, Chrompack, Raritan, NJ). The mobile phase was a linear gradient of solvent A (hexane:acetic acid, 1000:1, v/v) and solvent B (hexane:2-propanol, 100:1, v/v) at a flow rate of 0.5 mL/min. The linear gradient timetable was as follows: at 0 min, 100:0; at 8 min, 100:0; at 10 min, 75:25; at 40 min, 75:25; at 41 min, 100:0; at 50 min, 100:0 (%A:%B, respectively).

Results and Discussion

The initial SC-CO_2 extractions were performed to check if FPEs were preferentially extracted from the corn bran. As can be seen in Table II, the FPE ranged between 0.67 and 1.26% (w) of the extracts, indicating that they were not being selectively extracted. All major classes of lipid-type compounds were present in each extract.

Table II also shows the extraction parameters that produced the most enrichment of FPE. This is noted in terms of weight percent of the extract and the resultant mass of each component. There were two sets of extraction parameters that produced almost identical results: 34.5 MPa at 40°C and 69 MPa at 80°C. SFE at 34.5 MPa and 40°C yielded an extract containing 1.23% (w) FPE, which was equivalent to 1.35 mg of a total extract of 110.1 mg. SFE at 69 MPa and 80°C produced an extract containing 1.26% (w) FPE, which equated to 1.33 mg from a total extract of 105.5 mg. These two sets of pressure and temperature combinations were then used for the production of two stock supplies of corn bran oil employing the SFE pilot plant cited in the experimental section. Corn bran oil produced by SFE at 34.5 MPa and 40°C was then utilized for the SFF studies.

For the SFF experiments, an extraction cell was filled with sorbent, and the corn bran oil was applied to the top (inlet) of the cell. SFF commenced with neat CO_2, and then ethanol (EtOH) was added in a stepwise gradient to effect elution of the corn bran oil components. Ethanol was selected as the cosolvent, because it has Generally Regarded As Safe (GRAS) status in the U.S. for use in food processing.

SFF trials using silica gel yielded mass balances where ~95% of the starting corn bran oil was recovered. The vast majority (~90%) of the triglyceride portion of the oil eluted in the first three fractions. However, the overall FPE recovery was low (42%) and partitioned over many fractions, rather than being concentrated in one particular fraction.

SFF with diol sorbent provided nearly a total mass balance recovery (98%), with ~94% of the triglycerides eluting in the first fraction. However, approximately half (46%) of the FPE also eluted in the first fraction. The FPE then proceeded to elute in the subsequent fractions. The total FPE recovery was slightly more than 80%.

SFF data obtained using neutral alumina as the fractionating sorbent proved to be complex. Mass recovery was only about two-thirds of the starting material, and individual component recovery was erratic: triglyceride recovery was low (17.5%), but diglyceride and free fatty acid recoveries were substantially elevated. This may not be all that unexpected, as earlier research by King et al. (13) reported chemical reactions occurring with an alumina sorbent. They noted that transesterification of triglycerides occurred using SC-CO_2 over a packed bed of alumina that had been pretreated with methanol. Also, FPE recovery was approximately five times the starting amount, a result difficult to account for.

The amino-propyl sorbent was found to afford the best fractionation/enrichment of corn bran oil, and the fractionation was able to be simplified to four steps: neat CO_2, 1% (v) EtOH–CO_2, 2% (v) EtOH–CO_2, 10% (v) EtOH–CO_2. Total mass balance of the components in the extract was demonstrated, and 90% of the triglycerides eluted in the first fraction. All of the FPE were recovered, with 99% contained in a single fraction (10% EtOH–CO_2).

Table III. Percent Recoveries from Corn Bran Oil Using SFF with Amino-propyl Sorbent

Mass	FASE*	TG	FFA	FS⁄	1,3 DG"	FPE#	1,2 DG**
Individual runs							
101.87	114.94	102.19	77.11	105.44	123.65	101.60	107.96
101.02	95.58	102.45	85.84	102.00	111.91	96.11	78.15
98.85	95.05	98.96	85.84	127.01	133.68	106.53	93.27
98.91	95.90	99.72	77.33	116.85	115.76	106.73	107.00
95.61	93.77	95.70	78.95	121.94	133.92	106.66	94.71
Average recoveries							
99.25	99.05	99.80	81.01	114.65	123.78	103.53	96.22

* FASE, fatty acid-phytosterol esters.
† TG, triglycerides.
‡ FFA, free fatty acids.
§ FS, free sterols.
" 1,3 DG, 1,3 diglycerides.
FPE, ferulate-phytosterol esters.
** 1,2 DG, 1,2 diglycerides.

Because these fractionation/enrichment studies would be used for future research on a semi- or preparative-scale, it was decided to conduct multiple SFF experiments using the same sorbent bed. The required maximum sample load was not examined, but these experiments were required, because it would be necessary to use the sorbent at least several times to prevent the generation of large amounts of waste sorbent.

The same amino-propyl sorbent bed was tested for five SFF runs employing ~0.4 g of corn bran oil each time. A sorbent reconditioning step was incorporated between each fractionation. It consisted of SC-CO_2 at 69 MPa, 80°C, and a flowrate of 2 mL/min for 60 min to remove any excess EtOH from the sorbent charge. As shown in Table III, the data are very encouraging.

The average mass balance showed approximately total recovery (99.25%), and the FPE

yielded an average recovery of 103.5%. The occurrence of FPE recovery over 100% is not surprising when the small FPE mass isolated is taken into consideration, considering the possibility of weighing errors and chromatographic integration errors.

The first fraction contained the majority of the fatty acid-phytosterol esters and triglycerides and averaged 89.2% (w) of the mass for the five SFF runs. The fourth fraction mainly consisted of free fatty acids, free sterols, diglycerides, and ferulate-phytosterol esters and averaged 9.2% (w) of the mass for the five SFF runs. The FPEs were collected in the third (2% EtOH–CO_2) and fourth (10% EtOH–CO_2) fractions. However, they were concentrated in the latter, where on average, 99.6% (w) were present. The FPE composed on average 14.5% (w) of the fourth fraction for the five SFF runs, whereas they only represented 1.29% (w) of the starting corn bran oil. Thus, an enrichment factor of 11.24 can be attained from this fractionation/enrichment process.

The individual runs show a small but noticeable decline in mass recovery for the fifth SFF run, and this may indicate a slight decline in sorbent efficiency. However, the individual components of the corn bran oil all show approximately 100% recoveries, except for the free fatty acids, free sterols, and 1,3 diglycerides. In retrospect, the decrease in mass recovery may be due to an instrumentation error in collection efficiency. An instrumentation error is proposed, because worn extraction chamber seals were replaced after the fifth run and a small but noticeable amount of oil was found when the extraction chamber was cleaned, thus indicating a small loss of sample.

The high recoveries associated with the free sterols and 1,3 diglycerides warrant further investigation. This could possibly be related to the analytical chromatography system, which is not specifically optimized for their analysis. The peaks tended to be broad and had shoulder peaks and thus were not the ideal peak shape for chromatographic analysis. Likewise, the low recoveries of free fatty acids are still unaccounted for.

It was noted earlier that a sorbent reconditioning step was carried out between each SFF experiment. Fractions collected during these reconditioning runs yielded an average of 0.46 mg. Therefore, carryover from one run to the next does not seem to be problematic.

Conclusion

In this study, a two-step process of SFE and SFC on an analytical scale to enrich and fractionate ferulate-phytosterol esters from corn bran oil has been successfully demonstrated. These studies, using analytical-scale equipment, have provided valuable information that will be needed for process scale-up. In addition, only environmentally-benign (carbon dioxide) and a GRAS cosolvent (ethanol) have been used for the extraction and fractionation (chromatography) of these nutraceutical and high value components, a process that should also have consumer appeal. The described process provides a new way of isolating these components from milling byproducts, rather than the more conventional phytosterol isolation methods involving fatty oil residues such as deodorizer distillate (14). It also illustrates an expanded use of supercritical fluid-based analytical instrumentation that, when utilized in the combinatorial mode, can save considerable resources in the development of new fractionation technology (15).

Acknowledgments

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the products to the exclusion of others that may also be suitable.

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Manuscript accepted January 19, 2000.