Determination of Oil Content in Oilseeds by Analytical Supercritical Fluid Extraction[1]

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The total oil content of soyflakes, canola seed and wet-milled corn germ were determined by analytical supercritical fluid extraction (SFE) with carbon dioxide as the extraction solvent. Results obtained by SFE were in excellent agreement with those obtained by a conventional Soxhlet technique with organic solvents. The analytical-scale SFE technique yielded average means within one standard deviation of the means derived from the organic solvent-based methodology. Matrices containing both high and low oil content were successfully extracted with carbon dioxide at comparable precision to that obtained with the standard procedure. The supercritical fluid-based technique appears to be a suitable replacement for traditional extraction methods with organic solvents, thereby potentially eliminating the costs associated with solvent disposal and exposure of laboratory personnel to toxic and flammable solvents.

KEY WORDS: **Oil content analysis, oilseeds, supercritical fluid extraction.**

The analytical determination of oil content in oilseeds has been traditionally accomplished through the use of a Soxhlet-based extraction method with organic solvents as the extraction medium (1-3). An excellent review of the methodology that has evolved is provided by Lumley and Colwell (4), including the plethora of solvent systems that have been utilized in oil/fat determinations. Improvements in apparatus (5), sample preparation (6,7) and methodology (8,9) for determining oil content of seeds continues to be documented in the current literature, and specialized symposia (10) have been held on the above subject.

Although alternative methods, such as pulsed nuclear magnetic resonance (11) and near infrared (12), have been used for the evaluation of the oil content of seeds, the solvent extraction method remains the most commonly employed methodology, due in part to its similarity with the industrial-scale solvent-based process for extracting seed oils. However, concerns have recently been raised regarding the use of carcinogenic and flammable solvents in the analytical laboratory environment (13). These have been documented by the American Oil Chemists' Society through method revisions (14).

An alternative technique to the organic solvent-based extraction methods is supercritical fluid extraction (SFE). The efficacy of this technique for the extraction of oils from a variety of seed matrices has been demonstrated by researchers in this laboratory (15-17) and by other investigators throughout the world (18-20). However, the emphasis of this prior research has been to develop an alternative option for the large-scale processing of oilseeds, rather than to use SFE as an alternative analytical technique for the determination of oil content in seeds.

In this study, multiple quantitative extractions with SC-CO_2 have been performed on several seed matrices to determine the accuracy and precision of the technique for the routine determination of oil levels in commodity oilseeds. Soybean flakes, canola seeds and wet-milled corn germ were extracted to establish the efficacy of the method on matrices representing a wide range of oil and moisture contents. Several experimental parameters were optimized to attain reproducible results, including the effect of water dissolution in the SC-CO_2 and dissolved gas in the extracted oil sample. This study also compares the results from SFE to those obtained with petroleum ether as the extraction solvent.

EXPERIMENTAL PROCEDURES

The apparatus used for the SFEs has been previously described in the literature (21). Welding-grade carbon dioxide was obtained in 60-lb, nonsyphon tube cylinders from Matheson Gas Products (Joliet, IL). Rotary evaporation of extracts was accomplished on a Buchi Rotavapor R, rotary evaporator (Brinkmann Instruments Co., Westbury, NY).

The soybeans used in this study were acquired from Illinois Seed Co. (Pekin, IL). They were cracked, dehulled and flaked to a thickness of 0.015-0.020″. The flakes were stored at −20°C to prevent enzymatically induced deterioration until they were extracted. Flaked canola was obtained from CSP Foods Ltd. (Altona, Manitoba, Canada), while the wet-milled corn germ was procured from A.E. Staley Co. (Decatur, IL). These seed moieties were also stored at −20°C until they were extracted. Prior to extraction, the canola flakes and the wet-milled corn germ were ground in a Model MC-170 Miracle Mill (Markson Science, Inc., Phoenix, AZ) into a fine powder.

Samples of the above seeds ranging between 3.0-3.5 g were utilized for the SFE. Samples were weighed and poured into the extraction cells, and glass wool plugs were used to retain the seed in the cell. An extraction-enhancing agent (22), Chem-Tube Hydromatrix (Analytichem International, Harbor City, CA), was mixed with the wet-milled corn germ to adsorb excessive water, which interferes with the SFE of the corn oil. The granular diatomaceous earth-based adsorbent, Hydromatrix, not only adsorbs excessive moisture from the sample matrix, but disperses the seed sample, thereby promoting effective contact between the extraction fluid and seed matrix. Approximately two grams of the Hydromatrix were used for each corn germ extraction.

The SFE experiments were performed at 10,000 psig (680 bar) and 80°C in a 316 stainless-steel extraction cell measuring 6″ in length with an internal diameter of 5/8″. The flow rate and the amount of CO_2 used in each

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[1] Presented at the 4th International Symposium on Supercritical Fluid Chromatography and Extraction, Cincinnati, Ohio, May 20-22, 1992.

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extraction were measured by a dry test meter under ambient conditions. The flow rate was maintained at 5 L/min, and the amount of CO_2 ranged from 200–600 L (expanded volume), depending on the type of seed being extracted. The amount of CO_2 needed to ensure complete extraction was determined by multiple extractions with varying CO_2 volumes until no further oil was extracted. Approximately 200 L were used in the extraction of the soybean flakes. The canola seed extractions required 450 L of CO_2, due to the necessity to regrind the sample after the initial extraction period, and perform a subsequent second extraction. Wet-milled corn germ required even more CO_2, 600 L, due to its high oil content.

The collected oils were transferred to tared 250-mL round-bottom flasks after SFE and subjected to rotary evaporation for 75 min at 50°C to remove any residual carbon dioxide or water from the collected oil. The percent oil in the seeds was then determined by gravimetric measurement of the collected oil and expressed as a weight percent relative to the initial weight of the oilseeds.

The organic solvent-based extractions were conducted according to official methods (23) with petroleum ether as the extraction solvent in a Butt-type extraction apparatus.

RESULTS AND DISCUSSION

The results for the extraction of the three types of oil seeds by SFE and Butt-type techniques are tabulated in Table 1. The SFE results for soybean flakes *vs.* those obtained on the Butt extractor are in good agreement. Both techniques have excellent precision, showing less than 1% relative standard deviation (RSD) on the flaked seeds. A similar comparison of the sample means for the canola oil extraction indicates good agreement between the SFE and Butt-tube method. Although different, the canola sample means overlap within one standard deviation, their relative standard deviation averaging about 1%.

The largest recorded discrepancy between the sample means of the two techniques occurs for the extraction of the wet-milled corn germ. The SFE-derived result is slightly lower than the mean of the Butt extractor data despite the low standard deviation (less than 1%) associated with SFE of the corn oil. However, the two-sample means overlap within one standard deviation, due in part to the somewhat larger RSD (2.66%) associated with the organic solvent-based technique. The results in Table 1 suggest, however, that the SC-CO_2-based technique could be utilized in place of the traditional organic solvent-based method.

Several factors can impact on the accuracy and precision of the above SFE technique. For example, it was initially noticed that the wt% oil from the SFE method was always higher than that recorded with the Butt-tube method. Subsequent intermittent weighings of the collection flask after termination of the SFE revealed an appreciable weight loss (4%) over a 72-h period. This was due to the gradual release of absorbed CO_2 or water from the oil matrix. This effect could be eliminated by subjecting the collected oil sample to a rapid degassing *via* rotary evaporation.

The extraction of water from the sample matrix with SC-CO_2 cannot be ignored when using the above method. The solubility of water in SC-CO_2 has been determined by Evelein *et al.* (24) and is plotted as a function of pressure and temperature in Figure 1. For the temperature utilized in the SFE, 80°C, the solubility of H_2O in the SC-CO_2 is approximately 1 mol%, or 0.4 wt%. Hence, the loss of water from the sample matrix into the SC-CO_2 can also reduce the weight of the sample in the extraction vessel. Therefore, when using an SFE-based method for oil determination, we recommend against determining the oil content by weight loss of the sample before and after the SFE.

Carbon dioxide can also become imbibed in the viscous oil after decompression of the SC-CO_2/triglyceride mixture. For example, Brunner and Peter (25) have shown that the solubility of CO_2 in triglyceride-based oils is about 30 wt% at 300 bar and 15 wt% at 100 bar. Therefore, it is not unreasonable to expect some dissolution of the CO_2 in the collected oil. For this reason, as in conventional solvent extraction procedures, we recommend a quick rotary evaporation to remove dissolved gas and coextracted water.

The reported SFE method not only eliminates the use of organic solvents in the laboratory environment but reduces the time and complexity associated with the standard method for oil determination (23). As noted in

TABLE 1

Percent Oil Recovery—SFE *vs.* Organic Solvent-Based Method[a]

Sample number	Soyflakes		Canola		Wet-milled corn germ	
	SFE	Butt-type	SFE	Butt-type	SFE	Butt-type
1	20.47	20.33	40.19	39.76	49.10	50.83
2	20.46	20.50	39.14	40.98	48.48	48.06
3	20.89	20.57	39.91	40.68	48.64	51.40
4	20.67	20.44	40.02	40.50	49.48	50.81
5	—	20.79	—	40.62	—	51.03
6	—	20.51	—	—	—	—
Average	20.62	20.52	39.82	40.51	48.93	50.43
SD	0.20	0.15	0.46	0.45	0.45	1.34
RSD	0.98	0.75	1.17	1.12	0.93	2.66

[a] All results in wt%. Abbreviations: SFE, supercritical fluid extraction; SD, standard deviation; RSD, relative standard deviation.

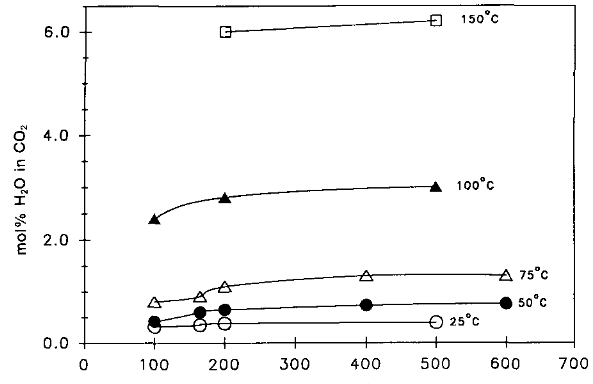

FIG. 1. Solubility of water in SC-CO_2 as a function of temperature and pressure.

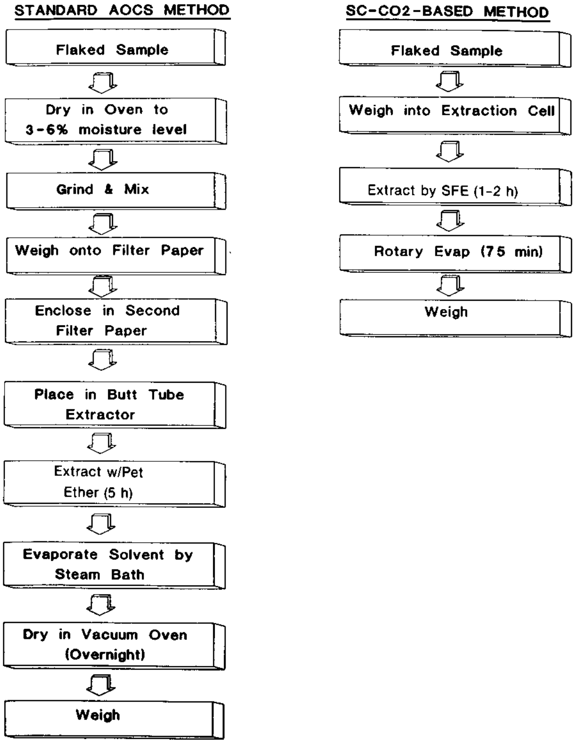

FIG. 2. Comparison of supercritical fluid extraction (SFE) method with standard American Oil Chemists' Society (AOCS) method.

Figure 2, many intermediate steps are eliminated by employing the SC-CO_2-based method. Particularly noteworthy is the reduction in extraction and solvent-evaporation time. The described procedure may also be used with slight modification as a replacement for other AOCS-approved analytical methods, such as the determination of total neutral oil and loss (26).

In conclusion, the described method based on extraction of the seed oil with SC-CO_2 offers a viable alternative to analytical methods employing organic solvents. With subtle modification, the technique can successfully extract either high or low oil-containing seeds of varying moisture content. The accuracy of the method yields equivalent values to those obtained by conventional organic solvent extraction within one standard deviation. The supercritical fluid-based technique offers considerable savings in solvent disposal costs and analysis time while also eliminating the exposure of laboratory personnel to toxic and flammable solvents.

ACKNOWLEDGMENT

We acknowledge the assistance of Imelda Schulte in providing the Soxhlet extraction of seed oils.

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[Received October 6, 1992; accepted January 3, 1993]