Supercritical Fluid Extraction to Determine the Oil Content in Copra and Extracted Meal

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ABSTRACT: Supercritical fluid extraction (SFE) was used as a primary method to determine the oil content of copra and its meal. Determination of the oil content of copra takes 9 h, and for meal 6 h are necessary with Soxhlet extraction, whereas SFE determined oil contents in about 10 min. The extremely high oil content of copra makes grinding difficult, as the sample becomes very gummy and it is difficult to remove the entire sample from the grinder. Adding diatomaceous earth to the sample before grinding eliminated the difficulties of cleaning the grinder and also enabled very fast SFE extractions. The variances for Soxhlet and SFE were not significantly different from each other ($P > 0.10$) in both copra and meal oil contents. The 95% confidence interval around the mean differences (SFE-Soxhlet) was (–0.35, 0.90) and (0.08, 0.26) and for copra and meal, respectively. Although the SFE meal oil content (9.81%) was significantly higher than the Soxhlet meal oil content (9.64%), the size of the average difference (0.17%) was relatively small. This small difference was considered acceptable owing to the ability to use SFE in real-time process control. Therefore, SFE can be used to determine the oil content in copra and its meal in less than 10 min.

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Supercritical fluid extraction (SFE) has been used for determining the oil content in a number of seed oil matrices, such as soybean and canola (1–5) and rapeseed by-products (6). However, information on using SFE for determining the oil content of copra appears to be limited.

Copra, or coconut kernel sun-dried to 5–12% moisture before expelling (5–6% is preferred for extracting the oil), has an oil content of 60–65% w/w. The oil content of the associated meal after extraction is typically 7.5–8.5% oil. A fast, reliable method is needed for complete oil extraction. Current Soxhlet extraction technology takes 9 h to determine the oil content of copra and 6 h to determine that of meal. Owing to these long extraction times, there is no real time process control, and only historical data (data that are characteristic of a process but not used in real-time control) can be acquired.

SFE can be divided up into three steps: (i) sample preparation, which includes grinding, (ii) extraction, and (iii) analyte (lipid) trapping. For high-lipid samples, grinding to an appropriate particle size can be difficult. In the case of copra, the matrix becomes a gum in the grinder, making it very difficult to remove and thereby affecting the homogeneity of the sample. Therefore, the use of diatomaceous earth (DE) has been investigated as a homogenization or grinding aid (7). However, the extraction times were still very long.

The objective of this study was to investigate the use of SFE as a fast extraction while retaining the extraction efficiency of current commercial liquid solvent extraction technology. In addition, the development of a technique to grind the sample properly and easily was carried out.

MATERIALS AND METHODS

Supercritical fluid extraction (SFE) sample preparation. Copra was mixed with DE at a ratio of 1 part (10 g) copra (W_C) to 3 parts (30 g) DE (W_{DE}) in a 500 mL polypropylene beaker. This ratio was chosen because the copra appeared to be dispersed sufficiently in the DE before grinding, and this ratio of sample to DE also cleaned the grinder best (compared with 1:1 and 1:2 ratios). This mixture was then thoroughly mixed with a spatula before transfer to a Retsch ZM-100 grinder (Haan, Germany), where it was ground with a 12-tooth rotor through a 10-mesh (2.00-mm) sieve at 18,000 rpm. The mixture was then transferred back to the beaker, and mixed again to ensure homogeneity. The ratio of $W_C/(W_{DE} + W_C)$ was used in the calculation of the copra oil weight percent content.

Copra meal was ground as received in a Retsch ZM-100 grinder with a 12-tooth rotor through a 20-mesh (0.850-mm) sieve at 18,000 rpm.

Collection apparatus (traps). The traps are prepared by cutting 8-mesh glass wool half the length of 20 × 150-mm glass culture tubes. Coarser glass wools should not be used, as they do not trap as efficiently. Trapping analytes with glass wool is a common practice (5,6). The glass wool is torn almost in half and then inserted the full length of the culture tubes with a Teflon-coated stirring rod. The collection tubes are weighed before (W_{CTB}) and after the extraction (W_{CTA}), as the oil content is determined on a gravimetric basis.

The tube into which the sample was collected during the extraction time study was weighed warm and not allowed to cool to room temperature before reweighing. The important criterion for this study was to determine when no additional oil was

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TABLE 1
Comparison of Oil Contents Between Supercritical Fluid Extractions (SFE) and Soxhlet Extractions (both with a middle grind) for Copra and Extracted Meal

Technique	Samples	*n*	% Oil	SD	Std. Error	% rsd[a]
SFE	Copra	36	64.72	0.72	0.12	1.11
Soxhlet	Copra	6	64.45	0.58	0.24	0.90
SFE	Meal	54	9.81	0.11	0.02	1.13
Soxhlet	Meal	8	9.64	0.15	0.05	1.60

[a]% rsd, percent relative standard deviation.

extracted, so we could choose an appropriate time (>90% of the oil extracted) for the first SFE extraction. Once the extraction time was optimized, collection tubes were typically stored in a desiccator until they reached room temperature (about 5–10 min).

SFE. A FastFat™ SFE (Teledyne Isco, Lincoln, NE) was used for the extractions. Extraction parameters were a pressure of 7500 psi, an extraction temperature of 120°C and a restrictor temperature of 150°C. The restrictors were coaxially heated, and the flow rate was 2 mL/min (at 5000 psi). The restrictors appear to have an average lifetime of 60 extractions for the copra, and 100 for the meal before they need to be replaced, even with a cleaning program for them in place (60 min with CO_2 at 200°C). Failure to replace defective restrictors will result in low oil recoveries.Industrial-grade carbon dioxide with a dip tube was used for all of the extractions. It has also been reported that carbon dioxide cylinders with helium headspaces should not be used (8).

A 10-mL polymer cartridge was then completely filled with 5–6 g of the copra/DE mixture. The exact weight of the sample was recorded (W_{CS}). For meal, about one-third of the cartridge was filled with DE (unground), tared, then approximately 2 g of meal was loaded onto the DE in the cartridge and the exact weight recorded (W_{CM}). The DE selected provides a more tortuous solvent path than smaller sorbent particles, thereby minimizing plugging of the extraction cartridge frits and allowing solvent to flow without backpressure problems.The remaining extraction void volume was filled with DE. The copra was extracted for 5 min, then ground in a mortar and pestle, and then re-extracted for 5 min for a total of 10 min. After these extractions, samples were placed into a desiccator until they reached room temperature (5–10 min) before they were weighed. The percent oil extracted from the copra was determined by the following equation: $[(W_{CTA} - W_{CTB}) \times 100/W_{CS}]/[W_C/(W_{DE} + W_C)]$ and for meal $[(W_{CTA} - W_{CTB}) \times 100/W_{CM}]$.

Sample preparation and Soxhlet extraction. Copra was ground with a fabricated cutter type grinder to 10-mesh particle size at the Cargill Philippine plant laboratory. Five grams of ground copra (W_{SC}) was extracted with petroleum ether (150 mL) in a Soxhlet apparatus at a rate of 150 drops per min. The sample was extracted for 4.5 h, then the thimble containing the sample was removed and the solvent allowed to evaporate. The copra was carefully transferred to a motorized pulverizer (Retsch Model MG2A), and ground to a powder. This was returned to the filter paper and placed in the Soxhlet apparatus and extracted for another 4.5 h. At the completion of the extraction, the sample was dried for 2 h at 115°C, then cooled to room temperature. The flask was weighed before (W_{FB}) and after (W_{FA}) extraction. The oil content was determined by the following equation: $[(W_{FA} - W_{FB})/W_{SC}] \times 100$.

The meal was extracted in a similar fashion to the copra. The sample was ground with a Retsch ZM 100 grinder to a size of less than 20 mesh (0.850 mm) and extracted for only 3 h before regrinding, then extracting another 3 h. The oil content was calculated as previously described.

Statistics. In the first phase of the experiment (Table 1), Soxhlet extraction with a middle grind was compared with SFE with a middle grind. Large samples of copra and meal were collected in the Philippines processing plant. The Philippines plant laboratory split the copra and meal samples, retained some for analysis, and sent the remainder to the Cargill Laboratory in the United States. Owing to logistical constraints, the Philippines laboratory was able to analyze only six and eight samples of copra and meal, respectively. On receipt of the copra, the staff of the U.S. laboratory ground the entire sample with DE and divided it into subsamples.Thirty-six of these subsamples were then analyzed using SFE with a middle grind. Similarly, mealsamples were homogenized and divided into subsamples. Fifty-four of these subsamples were then analyzed using SFE with a middle grind.

In the second phase of the experiment (Table 2), Soxhlet extraction of copra samples with a middle grind was compared with SFE with a vent step. A set of 10 samples collected as part of the normal plant laboratory procedure was split into two parts. One part was analyzed in the Philippine plant lab using Soxhlet extraction with a middle grind and the second part was analyzed in the U.S. laboratory using SFE with a vent step. Variances were calculated according to Hahn and Meeker (9).

RESULTS AND DISCUSSION

An extraction time study was completed to determine the optimum extraction time.SFE extractions typically take 30–60 min. Figure 1 showed that most of the oil in copra was extracted in a very short time (2 min). Therefore, an extraction time of 5 min was chosen. This was a very significant advantage, especially since the time for the first Soxhlet extraction of for copra was 4.5 h.

After the initial 5-min dynamic SFE extraction, the copra was re-ground in a mortar and pestle, then re-extracted with SFE for 2 more min (last data point in Fig. 1); an additional 1.2% oil was recovered. This indicated that a certain amount of oil remained after the initial SFE, so the extraction was repeated. One sample of copra was then extracted by SFE for 5

TABLE 2
Comparison of Oil Contents Between SFE (with a vent step) and Soxhlet Extractions (with a middle grind) for 10 Different Copra Samples[a]

Samples	Soxhlet % oil	Vent SFE % oil
Copra 1	63.3	63.1
Copra 2	63.6	62.2
Copra 3	64.9	64.2
Copra 4	63.9	63.8
Copra 5	64.1	64.4
Copra 6	64.0	61.4
Copra 7	63.8	62.4
Copra 8	63.5	62.9
Copra 9	63.7	62.3
Copra 10	63.0	63.7

[a]Ten samples were run a single time for a comparison between Soxhlet and vent SFE analyses. For abbreviation see Table 1.

min, and the oil content was found to be 63.1%. This extracted sample was then ground with a mortar and pestle and extracted for an additional 5 min to yield 2% more oil for a total of 65% oil. The corresponding Soxhlet value for this sample was 65%. Hence, the first extraction accounted for approximately 97% of the oil, while a second extraction, after regrinding, was necessary to recover the residual oil. AOCS Official Method Am 2-93 also uses the middle grind option twice (10), to ensure a more complete extraction of oil.

Table 1 lists the comparison between SFE (with a middle grind) and Soxhlet (with a middle grind) extractions for determination of oil contents in both copra and meal sample. The variances for Soxhlet and SFE were not significantly different from each other ($P > 0.10$) in both copra and meal. The 95% confidence interval around the mean differences (SFE - Soxhlet) was (–0.35, 0.90) and (0.08, 0.26) for copra and meal, respectively. Although the SFE meal value (9.8%) was significantly higher ($P < 0.05$) than the Soxhlet meal value (9.6%), the size of the average difference (0.17%) was relatively small and was considered acceptable owing to the ability to use SFE in real-time process control.

Once the sample was defatted, the middle grind seemed to allow for more oil to be extracted. However, it is usually not

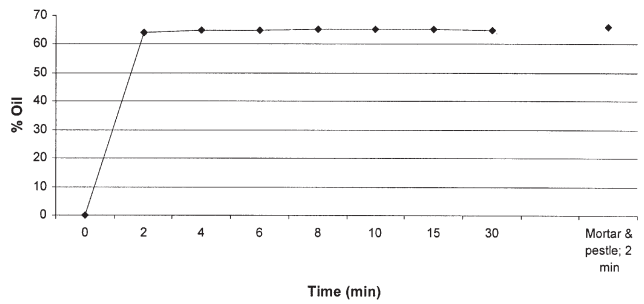

FIG. 1. Extraction time curve for the copra/diatomaceous earth mixture. A mortar and pestle grinding at the end of the initial extraction yielded an additional 1.2% oil and showed that an additional grinding was needed. The sample that resulted after this additional grinding was allowed to cool to room temperature before the determination was made, unlike the data points from the extraction time curve.

TABLE 3
Comparison of Oil Content Differences Between SFE (with a vent step) and Soxhlet Extractions (with a middle grind) for 10 Different Copra Samples in Table 2[a]

Data	*n*	Ave. difference	Ave. SD
All	10	0.74	0.98
Sample 6 dropped	9	0.53	0.77

[a]For abbreviation see Table 1.

the practice in crush plant laboratories to do the middle grinds. To characterize the complete amount of oil in a sample properly, a middle grind(s) were required. However, for process control in a plant environment, the described middle grind(s) option most likely will not be used. Therefore, an intermediate vent step was inserted into the SFE scheme. This step allowed for the extraction to be continued without removing the cartridge and regrinding the sample. The sample was extracted for 5 min, and then depressurized from 7500 psi to atmospheric pressure in 15 s. This technique allowed for extraction of additional oil, albeit not as much as the middle grind does, which was attributed to cellular disruption caused by the pressure drop (11–13). The oil content of the sample previously mentioned, for which the vent step was used, was found to be 64.5%, which was lower than the 65.2% (middle grind) but higher than the 63.1% obtained with just a 5-min extraction.

Table 2 shows the comparison between oil contents obtained *via* vent SFE and Soxhlet extractions using a middle grind for 10 different copra samples. The difference between the two methods was especially large for Copra sample 6, so the summary statistics were calculated with and without sample Copra 6 (Table 3). When sample 6 was included, the Soxhlet oil content was significantly higher than the vent SFE oil content ($P < 0.05$). The 90% two-sided tolerance to contain at least 80% of the Soxhlet-SFE values was –1.21, 2.69 (9). That is, we can be 90% confident that 80% of the time the difference between Soxhlet and SFE will be between –1.21 and 2.69. When sample 6 was removed, the Soxhlet oil content was borderline significantly higher than the vent SFE oil content ($P = 0.07$). The 90% two-sided tolerance to contain at least 80% of the Soxhlet-SFE values was –1.06, 2.13 (9). That is, we can be 90% confident that 80% of the time the difference between Soxhlet and SFE will be between –1.06 and 2.13.

If the SFE samples had been ground in the middle of the procedure, they would most likely have yielded higher results. Hence, the vent step was a compromise between experimental convenience, the amount of oil that is actually extracted during processing, and accuracy. Either way, a method has been developed to do very fast extractions (primary method), which are required to control processes or establish the oil content of a material.

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