



A comparison of oil and fat content in oilseeds and ground beef—using supercritical fluid extraction and related analytical techniques*

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A supercritical fluid extraction (SFE) has been applied for the determination of total fat content of five different oilseed matrices (soybeans, sunflower, safflower, cottonseed and rapeseed) and ground beef samples containing approximately 10, 20 and 30% fat by weight. Lipid content was determined using both gravimetric analysis as well as the sum of all fatty acids, expressed as triglycerides, from the gas chromatography (GC) profiles of the fatty acid methyl esters (FAMES). The latter analysis is required by the Nutritional Labeling and Education Act of 1990 which redefined the determination of fat for nutritional labeling purposes. The oilseed results are compared to data from a collaborative study by the American Oil Chemists Society (AOCS) and the Association of Official Analytical Chemists International (AOAC). The collaborative study data were determined by both AOCS Official Methods and by SFE. All of our data yielded higher oil recoveries than the collaborative study data obtained via AOCS official methods and SFE with neat carbon dioxide (CO₂). However, our results are in excellent agreement with the collaborative study data obtained by SFE with ethanol-modified CO₂ and the Federation of Oil, Seeds and Fats Association International method. The ground beef results are compared to previously published reports from our laboratory. They show that fat determination using GC-FAME analysis is equivalent to the gravimetric analysis results and has the additional benefit that different types of fat (i.e. saturated and monounsaturated) can also be determined in addition to total fat. Hence, the results from this study advocate the use of SFE as a suitable replacement for traditional organic solvent extraction in the determination of fat/oil content in agriculturally-derived products. Published by Elsevier Science Ltd

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INTRODUCTION

Analytical determination of oil or fat content has historically been achieved with Soxhlet-based methods

*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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employing organic solvents. Lumley and Colwell (1991) have reviewed the methodology that has been used, including the many solvent systems that have been utilized for these determinations. Even with the advent of pulsed nuclear magnetic resonance and near infrared methodologies, solvent extraction has remained the most commonly employed technique to date.

Recently, supercritical fluid extraction (SFE) has been investigated as an alternative technique for the analytical-scale determination of oil in seeds (Taylor *et al.*, 1993; Walker *et al.*, 1994; King *et al.*, 1996; Snyder *et al.*, 1996). In addition, a joint American Oil Chemists'

Society/Association of Official Analytical Chemists International (AOCS/AOAC) collaborative study has been undertaken to determine the oil content of seeds employing SFE with subsequent comparison of the data to that obtained by AOCS Official Methods. This collaborative study was designed to meet two requirements of the fats and oils industry: (1) a determinative method similar to that used for the process-scale extraction of vegetable oils, and (2) the determination of total oil content. In this regard, neat carbon dioxide (CO₂) and ethanol (EtOH) modified-CO₂ were utilized, respectively, for the above mentioned requirements.

The Nutritional Labeling and Education Act (NLEA) of 1990 has redefined fat consistent with nutritional labeling requirements. It requires that total fat be calculated as the sum of fatty acids from a total lipid extract, expressed as triglycerides. Also, classes of fat (e.g. saturated fat) may be calculated and expressed as free fatty acids (House *et al.*, 1994). The NLEA protocol consists of three basic steps: (1) hydrolysis to produce free fatty acids and release bound lipids, (2) solvent extraction, and (3) gas chromatographic analysis of the fatty acid methyl esters (FAMES). Saturated, monounsaturated and total fat are then calculated from the resulting FAME profile and expressed as triglycerides (Snyder *et al.*, 1996). This allows the extract to be chemically analyzed for fat content and does not depend on the fat content being measured gravimetrically.

The hydrolytic procedure of the NLEA protocol not only produces free fatty acids but releases bound lipid matter and generally increases the amount of lipid matter detected by gravimetric analysis (King *et al.*, 1996). Lemke and Engelhardt (1993) have successfully determined total fat by SFE on acid-hydrolyzed meat and cheese samples. King (1994) determined the total fat content both gravimetrically and by GC-FAME analysis on ground beef samples that had been hydrolyzed and dehydrated, showing at this time, that gravimetry yielded only slightly higher results. Recently, Bøwadt *et al.* (1996) reported employing SFE with EtOH modified-CO₂ on acid-hydrolyzed livestock feeds, dry pet food and snack food samples.

Reports have detailed the detection and quantitation of FAMES by GC in which fatty acids have been reacted with NaOMe or BF₃/MeOH to form the methyl esters (Lanza *et al.*, 1980; Gildenberg and Firestone, 1985; Matter *et al.*, 1989; Eder *et al.*, 1992; Ulberth and Henninger, 1992). Recently, FAME formation under supercritical conditions has been reported (Berg *et al.*, 1993; Jackson and King, 1996; Snyder *et al.*, 1996). These investigations used an immobilized lipase to enzymatically catalyze the transesterification of the lipids with methanol. Other lipase-catalyzed reactions of lipids under supercritical conditions suggest that such reactions exhibit benefits, such as improved reaction equilibrium, enhanced incorporation and faster reaction times

(Pasta *et al.*, 1989; Adschiri *et al.*, 1992; Shishikura *et al.*, 1994).

In this study, SFE with an in-line lipase-catalyzed reaction as reported by Snyder *et al.* (1996) for fat determination in meats was modified and used on oilseed matrices. The resultant FAME extracts were analyzed by GC to calculate the total fat content. The SFE technique of Bøwadt *et al.* (1996) was employed on the oilseed matrices and previously characterized ground beef samples, using both gravimetric and GC-FAME analyses for total fat determination. Additionally, SFE with neat and ethanol-modified CO₂ was conducted on the oilseed matrices according to the AOCS/AOAC oilseed collaborative study protocol.

MATERIALS AND METHODS

Oilseed samples

Five oilseeds (soybean, safflower, sunflower, rapeseed, and cottonseed) were prepared by Mike Kennedy of Cargill Analytical Services (Minnetonka MN, USA) by milling to a fine powder and passing through a USA no. 20 sieve and were included in the 1995-1996 AOCS Smalley Laboratory Proficiency Program. There were two sets representing two separate lots of each oilseed type. Each sample within a set was analyzed in duplicate.

Ground beef samples

The ground beef samples with three nominal levels of fat content (10, 20, and 30%) were prepared by the Department of Animal Science at the University of Illinois, USA, and have been previously described by King *et al.* (1996).

Supercritical fluid extraction

Supercritical fluid extraction/supercritical fluid reaction (SFE/SFR) with an in-line lipase catalyst was performed with a Hewlett-Packard Model 7680T SFE unit (Hewlett-Packard, USA) as previously reported by Snyder *et al.* (1996). However, the amount of Novozym SP 435 (Novo Nordisk, USA) enzyme was adjusted to 1.8 g and the amount of extraction matrix was ~0.5 g (prior to a 30 min freeze-drying procedure). Each extract was injected once and the total oil content was calculated from gas chromatographic data of the resulting fatty acid methyl esters as reported by House *et al.* (1994). The GC conditions were those reported by Snyder *et al.* (1996).

Additional SFE of the oilseed matrices was conducted with an Isco, Inc. Model SFX 3560 automated extractor (Isco, Inc., USA). SFE with neat CO₂ was performed at 7500 psi and 100°C for 30 min with a flow rate of 3.2 ml min⁻¹ liquid CO₂ (LCO₂). SFE with

ethanol-modified CO₂ was performed at 7500 psi and 100°C with 15% (v/v) ethanol for 60 min at a flow rate of 2 ml min⁻¹ (LCO₂). Under both sets of conditions, the restrictor was heated to 150°C and the receiver (containing 1 g of glass wool) was heated to 60°C. Total oil content was then determined gravimetrically after the extract was dried to a constant weight as outlined in AOCS Official Method Ca 2c-25 (1990).

SFE was also conducted with a Leco Corporation Model FA-100 SFE module (Leco Corporation, St Joseph, MI). The oilseed or ground beef matrix (1.0 g) and 1.0 ml of sulfuric acid (10% v/v) were added to a 50 ml beaker and allowed to soak for 10–15 min. One and one-half grams of Leco Dry, a moisture adsorbent and matrix dispersant, (Leco Corporation, USA) were mixed with the acid soaked sample prior to filling the extraction thimble. Just before placement in the extractor, 1.0 ml of ethanol (100%) was added to the thimble. SFE was performed at 9000 psi and 100°C at a flow rate of 21 min⁻¹ (measured at NSTP) for 25 min after an initial 5 min static hold. The variable restrictor was heated at 100°C. Collection was performed in a vial packed with glass wool (1.5 g). Total fat was determined by gravimetric analysis after evaporating any residual solvents. Supercritical Fluid Extraction/Supercritical Fluid Chromatography grade CO₂ (Air Products and Chemicals, Inc., USA) was used for all SFE experiments.

Statistical analyses

For both the oilseed and ground beef extractions, the experiments were setup as complete block designs. Analyses of variance (ANOVAs) were performed on the calculated percent total fat and the means were compared using least significant differences (LSD)

using Statistix 4.1 software (Analytical Software, USA).

RESULTS AND DISCUSSION

Results for the extractions of the oilseed matrices are tabulated in Table 1. The first four columns represent data from the current study. The other columns of data are from the AOCS/AOAC collaborative study on the determination of total fat in oilseeds by SFE (unpublished data) and are presented here for comparison. The analysis of variance (ANOVA) indicated that there were significant differences ($F_{4,40} = 17,124$; $p < 0.001$) in oil content between the five oilseeds examined. In addition, for four of the five oilseeds (sunflower, safflower, rapeseed and cottonseed), the individual lots were significantly different from each other. The two lots of soybeans were statistically equivalent. There were also significant differences ($F_{3,40} = 106.7$; $p < 0.001$) between the four extraction methods. The SFE/SFR method yielded significantly higher results than the other three extraction methods and SFE with neat CO₂ gave lower results than any of the other methods. SFE with ethanol-modified CO₂ using the Leco Corporation and Isco, Inc. systems provided intermediate results, but they were equivalent to each other.

Our data exhibited higher oil recoveries than the collaborative study data obtained by SFE with neat CO₂ and all of the AOCS official extraction methods except for rapeseed. Inclusive of both data sets, our oil yields ranged from 0.3–4.5% higher than the collaborative study data. These results were not unexpected. There were four different AOCS Official Methods, Ac 3-44 (soybeans), Ag 1-65 (safflower), Ai 3-75 (sunflower, rapeseed) and Aa 4-38 (cottonseed), utilized for

Table 1. Mean percentage ($n = 2$) total oil content (wt%) of oilseed samples

Matrix ^a	SFE/SFR ^b	SFE ^c	CO ₂ ^d	EtOH/CO ₂ ^d	AOCS ^e	FOSFA ^f
Set I						
Soybean	20.1	21.0	20.2 (19.2) ^g	21.2 (20.5) ^g	19.1	21.7
Safflower	40.2	39.3	36.9 (35.7)	38.6 (37.1)	36.4	39.1
Sunflower	42.5	40.2	40.4 (38.8)	41.5 (40.2)	38.7	41.8
Rapeseed	40.6	41.5	38.8 (37.7)	41.2 (40.2)	40.3	42.7
Cottonseed	21.4	21.1	19.4 (19.1)	20.7 (19.7)	18.2	20.6
Set II						
Soybean	20.5	20.2	20.1 (19.3)	21.3 (20.4)	19.4	21.9
Safflower	41.4	38.9	38.4 (36.9)	39.8 (38.0)	38.0	40.6
Sunflower	45.6	43.4	43.8 (42.3)	45.0 (43.0)	42.7	46.0
Rapeseed	44.3	44.1	40.9 (39.8)	43.9 (43.5)	43.7	45.2
Cottonseed	20.5	20.5	18.8 (18.4)	19.3 (19.3)	17.5	20.0

^aSet I and Set II represent different lots of each oilseed.

^bSupercritical fluid extraction/supercritical fluid reaction with an in-situ lipase with GC-FAME analysis.

^cSupercritical fluid extraction with the Leco Corporation Model FA-100.

^dSupercritical fluid extraction with the Isco, Inc. Model SFX 3560.

^eAOCS Official Methods Ac 3-44 (soybeans), Ag 1-65 (safflower), Ai 3-75 (sunflower, rapeseed), and Aa 4-38 (cottonseed).

^fAOCS Official Method Am 2-93.

^gData in parentheses from an AOCS/AOAC collaborative study.

the extractions depending on the type of oilseed matrix. However, all of the methods employed petroleum ether in a Butt tube extractor. Extraction times ranged from 4–8 h depending on the type of oilseed being analyzed.

For the collaborative study, SFE with neat CO₂ was conducted at 7500 psi and 100°C. Extractions performed on the Leco system not only utilized ethanol as a modifier, but acid hydrolysis of the oilseed matrix prior to SFE. The SFE/SFR technique reported by Snyder *et al.* (1996) employed methanol as both a cosolvent and reactant. Thus, by incorporating acid hydrolysis and polar alcohols into the SFE process, higher oil recoveries would be expected as noted previously by Carpenter *et al.* (1993) and King (1994).

As seen from the data of the current study in Table 1, the percent oil recovered by SFE utilizing ethanol modified-CO₂ was less than that recovered from the Federation of Oil, Seeds and Fats Association International (FOSFA) method, AOCS Official Method Am 2-93 (1990), for all oilseed samples except one (set I cottonseed). The organic solvent extractions yielded 0.7–3% more oil than achieved via SFE using a cosolvent. These results are to be expected because the FOSFA extraction method involves three 4-h organic solvent extractions with sample regrinds before the second and third extractions, while the SFE procedure was one 60-min extraction. It has been previously noted by Taylor *et al.* (1993), that to achieve exhaustive SFE of oil from canola (rapeseed), a sample grind was required because of the high oil content. This finding can also be extended to other oilseed matrices containing high percentages of oil.

The data acquired with the Leco SFE unit showed similar oil recoveries to those obtained when SFE is performed with the Isco SFE unit using ethanol as a modifier. However, when compared to the FOSFA data, 70% of the Leco oil recovery data were lower by 0.7–2.6%, again reflecting the impact of the multiple sample regrinds used in the FOSFA procedure.

Total oil content determined by FAME analysis (SFE/SFR) was higher than the collaborative study data determined by EtOH-modified CO₂ in all cases but

one (Table 1—set I, soybean), which was only 0.4% lower. However, oil recovery determined by the FAME analysis compared to the FOSFA method showed half of the samples with higher recoveries and half with lower recoveries. All soybean and rapeseed data and the sunflower data of set II yielded lower oil amounts via FAME analysis. The soybean and rapeseed matrices exhibited lower total oil results by SFE/SFR on the average of 1.5 wt%, whereas the sunflower data were only 0.4 wt% less. The discrepancy between the two methods does not seem to be dependent on the amount of oil present in the matrix, as rapeseed contains twice the amount of oil as soybeans (40% versus 20%), but is more dependent on the matrix that is being extracted. The safflower and sunflower samples contain ~40 wt% oil, similar to rapeseed, however, both safflower samples and one sunflower sample exhibited higher oil recoveries by FAME analysis.

Table 2 compares total fat recovery from ground beef samples extracted by SFE and an organic solvent procedure (House *et al.*, 1994). All of the data were determined via GC-FAME analysis except that obtained using the Leco SFE system, which were determined by gravimetric analysis. Also, half of the data were acquired by extracting acid-hydrolyzed beef samples. The first four columns of data have been previously reported (King *et al.*, 1996; Snyder *et al.*, 1996) and are shown here for comparison to the present study. The gravimetrically determined data show a trend of slightly higher total fat recoveries. This parallels the findings of King (1994) and Snyder *et al.* (1996). King has stated that gravimetric analysis overestimated the total fat of ground beef samples by only 0.5–0.6 wt%. This current study produced gravimetric data ranging from 1.7–2.6 wt% higher. However, the current study utilized ethanol-modified CO₂ whereas King employed only neat SC-CO₂ for his extractions. In addition, Flickinger (1997) indicated that GC-FAME analysis of fat extracted from a wide variety of food samples gave lower results than gravimetric methods because the GC value quantitates only fat.

The ANOVA of percent total fat for all of the data in Table 2 indicated there was a significant effect of fat

Table 2. Mean ($n=3$) percentage total fat (wt%) from ground beef samples

Nominal fat%	GC-FAME analysis				Gravimetric analysis	
	H ⁺ , Et ₂ O ^a	SFE/SFR ^b		H ⁺ , SFE ^a	SFE ^c	
		Isco, Inc. SFX 2-10	Hewlett-Packard 7680T		EtOH/CO ₂	H ⁺ EtOH/CO ₂
10	12.8	11.5	11.2	11.7	12.9	13.2
20	21.8	22.1	20.6	21.9	23.1	23.2
30	28.6	29.4	28.8	27.2	29.4	29.0

H⁺ = Indicates acid hydrolysis prior to extraction.

^aData reprinted from King *et al.* (1996).

^bData reprinted from Snyder *et al.* (1996).

^cLeco Corporation Model FA-100.

determination method ($F_{5,36} = 9.27, p < 0.001$). The linear contrast comparing gravimetric methods (Leco data) to GC-FAME methods indicated the gravimetric methods were significantly higher than the GC-FAME methods ($T = 6.01, p < 0.001$). This finding supports that of King (1994) where a more polar solvent does not extract more lipid but removes other non-lipid coextractives, thus adding to the weight of the extract. The ANOVA of the gravimetrically determined percent total fat indicated no significant effect of acid hydrolysis on the meat samples prior to SFE.

However, our results are in contrast to those of Phillips *et al.* (1997). They quantitated the total fat in a total diet standard reference material, NIST SRM 1548, and mixed food composites using organic solvent extraction with gravimetric measurement of the resultant extract. The authors reported 3–11% lower total fat recoveries when acid hydrolysis was performed prior to extraction, a result contrasting to what is expected when one hydrolyzes a foodstuff.

CONCLUSION

In this study, we demonstrated that quantitating total fat by GC-FAME analysis provides a more exacting analysis, rather than gravimetric determination, to determine oil/fat content of oilseed and ground beef matrices. GC-FAME analysis not only gives comparable recoveries of lipids, but allows the analyst to chemically speciate the extract rather than just assuming that only fat is present in the extract. This research indicates that SFE is an alternative analytical technique for determining total fat in these matrices. The AOCS/AOAC collaborative study data, mentioned previously for determining the oil content in oilseeds, show equivalency between SC-CO₂ extraction and the AOCS Official Methods (1990), except for rapeseed. The SFE/SFR data in this study show greater oil recoveries than either SFE with neat CO₂ or the AOCS official extraction methods. The results obtained by SFE with EtOH-modified CO₂ are equivalent and are similar to the FOSFA method data. Total fat determination by the NLEA protocol (GC of FAMES) is nearly identical to the FOSFA method employing gravimetric analysis. Additionally, the SFE technique of Bøwadt *et al.* (1996) shows promise as a rapid method for utilizing SFE coupled with only gravimetric analysis of the resultant extract for oilseed matrices.

Finally, the total fat data from ground beef revealed that the gravimetric results from ethanol-modified SFE (with or without acid hydrolysis) were not equivalent to the previously reported GC-FAME analyses. This is further evidence that gravimetric analysis overestimates total fat content when compared to GC-FAME analysis of the resultant extract.

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