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Comparison of Lipid Extraction Methods of Food-Grade Sorghum *(Sorghum bicolor)* Using Hexane

K. L. Christiansen, C. L. Weller, V. L. Schlegel, I. M. Dweikat

Abstract. *Much has been reported concerning sorghum phytochemicals, such as policosanols, but little literature exists on how various extraction methods might be used to optimize the recovery of such phytochemicals. The purpose of this study was to compare three extraction methods for recovery of different sorghum lipids. Lipids were extracted from whole kernel and ground Macia, a food-grade sorghum hybrid, with hexane by a Soxtec method, a refluxing method (RB), and a bench-scale recirculated solvent (RC) method. Comparisons of total lipid yield and lipid class yields extracted from the grain by each method were made. Total lipid yield for the three extraction methods ranged from 0.04% to 3.59%. The extraction method affected the total lipid recovered and the composition of the extract. The Soxtec method yielded greater total lipid content and yielded greater amounts of policosanols than the RB and RC methods.*

Keywords. *Extraction, Lipids, Sorghum.*

The interest in phytochemicals, plant-derived compounds, is growing as the link between diet and coronary heart disease (CHD) becomes increasingly understood. Phytochemicals such as policosanols (long-chained alcohols), phytosterols (plant compounds similar in structure to cholesterol), and long-chained fatty acids have been shown to positively influence low-density lipoprotein (LDL) cholesterol levels, a factor contributing to CHD (Carr et al., 2005). Many phytochemicals are present in grain sorghum, a drought-resistant cereal crop. Carr et al. (2005) showed that concentrations of non-high-density lipoprotein (HDL) cholesterol were reduced in hamsters fed lipid extract from grain sorghum whole kernels. To further develop grain sorghum lipids as a potential prevention for CHD, it is necessary to explore how lipid extraction methods affect phytochemical class recovery and thus determine how to achieve optimal extraction of these beneficial phytochemicals.

Solvent extraction of lipids from a solid matrix is defined as leaching. Conventional leaching methods include Soxhlet extraction, maceration, percolation, high-speed mixing referred to as turbo-extraction, and sonication (Kaufmann and Christen, 2002). Newer leaching methods include supercritical fluid extraction, microwave-assisted extraction, and pressurized solvent extraction (Kaufmann and Christen, 2002). The

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Soxhlet extraction method is recognized as the standard to which other extraction methods are typically compared (Luque de Castro and Garcia-Ayuso, 1998). Another extraction method based on Soxhlet is Soxtec, which achieves similar results in less time, using less energy, and has proven extraction efficiency (Sporring et al., 2005; Giner et al., 1996). A comprehensive explanation of the novel techniques, including ultrasound-assisted, microwave-assisted, supercritical fluid, and accelerated solvent extraction, along with discussions of their advantages, disadvantages, and budding uses, is provided by Wang and Weller (2006).

Schmidt (2002) compared two extraction methods, refluxing in a round bottom flask (RB) and a bench-scale recirculated extraction system (RC), for extracting grain sorghum wax but made no comparisons to Soxhlet or Soxtec extractions. Interestingly, Schmidt (2002) reported that the amount of wax extracted did not differ between the RB and RC methods but that the chemical compositions of the waxes obtained by each extraction method differed. For example, RB yielded higher amounts of aldehydes and acids, while the RC method yielded higher amounts of alcohols. Wang et al. (2005) compared the total lipid yields, not just wax, from a Soxtec extraction system and RC extraction method using sorghum dried distillers grain with solubles (DDGS), noting only that the waxy and oily fractions of the lipid extract varied between the DDGS extracts and the whole kernels extracts.

Chukwumah et al. (2007) found that phytochemical recovery from peanuts differed between extraction methods. However, how different extraction methods could affect the yield of lipid classes from unprocessed grain sorghum has not yet been reported. Therefore, the objectives of this study were to quantify and compare the total lipid class yields isolated from both ground and whole kernel sorghum using three different extraction methods. The methods examined were Soxtec, refluxing (RB), and a bench-scale recirculated extraction system (RC). Lipid compositions were analyzed and compared to assess the capability of the extraction method and the effects of grain state (whole kernel and ground) on lipid class yields.

Materials and Methods

Sample Collection, Storage, and Preparation

Mature food-grade sorghum (*Sorghum bicolor*), variety Macia, was harvested in the fall of 2005 in Lincoln, Nebraska. Macia, a cultivar of the caudatum type originating from Zimbabwe, was selected for its drought resistance and yield under stress conditions. The seed contains about 70% starch and has a non-waxy endosperm. The sorghum kernel fat content is ~3.3%. A sample of 100 g of Macia grain was milled (model 4E grinding mill, Straub Co., Hatboro, Pa.), and the fines were analyzed with a sieve shaker (Ro-Tap, W.S. Tyler, Cleveland, Ohio). The moisture contents of the samples were determined using ASAE Standard S352.2 (ASAE Standards, 2003) for whole kernels and AACC Method 44-10 (AACC, 2000) for the ground sorghum. The grain was stored at -12°C until use for extraction.

Soxtec Extraction

A Soxtec HT6 system and 1046 heating unit (Foss, Eden Prairie, Minn.) were used to perform extractions on the whole kernel and ground sorghum samples using the procedure developed by Foss and printed in the Soxtec HT6 operation manual. Technical-grade hexane (Fisher Scientific, Pittsburgh, Pa.) served as the extracting solvent. Three sets of mass-to-solvent ratios were used, along with three different extraction times. Samples of approximately 5 g (M1) of ground or whole kernel sorghum were placed in

Table 1. Treatment combinations of grain state, solvent volume, and extraction time for Soxtec extractions on Macia grain sorghum.

Grain State	Hexane (mL)	Extraction Time (h)
Ground	15	1
Ground	25	1
Ground	35	1
Kernel	15	1
Kernel	25	1
Kernel	35	1
Ground	15	2
Ground	25	2
Ground	35	2
Kernel	15	2
Kernel	25	2
Kernel	35	2
Ground	15	4
Ground	25	4
Ground	35	4
Kernel	15	4
Kernel	25	4
Kernel	35	4

60 × 20 × 1 mm cellulose thimbles (Whatman International, Ltd., Maidstone, U.K) for extraction in 15, 25, and 30 mL of hexane. The mass of each extraction cup was determined (M2), and then each extraction cup was filled with the appropriate, randomly assigned solvent volume for lipid collection. The thimble with grain was placed in the boiling hexane for the first half of the extraction period and was above the boiling hexane for the second half of the extraction. For the 1 h extraction, the thimble was in the hexane for the first 0.5 h and held above the hexane for the second 0.5 h. The other extraction times were 2 and 4 h. Table 1 presents the treatments for the Soxtec extractions.

After the extractions, the miscellas were transferred from the extraction cups to 40 mL glass vials of known masses (M4), and the samples were dried in a convection oven at 40°C until steady masses were reached and recorded (M5). The mass of each extraction cup after extraction was determined (M3) to document any residual lipid recovered but not transferred. The mass of the extracted lipid was calculated using:

$$(M3 - M2) + (M5 - M4) \tag{1}$$

The yield of the extract was calculated using:

$$\left(\frac{[(M3 - M2) + (M5 - M4)]}{M1}\right) \times 100 \tag{2}$$

Each extraction, consisting of each grain sample with each of three volume levels at one extraction time period, was completed in triplicate. The dried samples were stored at -12°C until use for lipid analysis.

Refluxing Extraction

For the RB method, a 2 L round bottom flask and mantle heater were used to perform 0.5 h extractions with 300 mL of technical-grade hexane at 64°C and 100 g (M1) of the ground or whole kernel sorghum. The extraction time and the solvent-to-mass ratio were decided upon after referring to the results of Wang et al. (2005). They concluded that there

was no apparent increase in yield beyond a 300 mL to 100 g solvent-to-solid ratio and a half-hour extraction time for extractions at the boiling point of hexane. After the extractions, the spent grain and miscella were separated with a Buchner funnel, and the miscella was captured in 250 mL round bottom flasks of known masses (M2). The miscella was then concentrated using a rotary evaporator (SafetyVap R-144, Büchi, Flawil, Switzerland) under vacuum at 40°C. The miscella was transferred to a 40 mL glass vial of known mass (M4) and placed in a convection oven at 40°C until a constant mass (M5) were reached. The mass of the 250 mL round bottom flask was determined a second time to document any lipid not transferred (M3).

The mass of the extracted lipid was calculated using equation 1. The yield of the extract was calculated using equation 2. The dried samples were stored at -12°C until use for lipid analysis. The RB extractions of ground and whole kernel sorghum were completed in triplicate.

Recirculated Solvent Extraction

The RC method, which recirculates heated solvent using a peristaltic laboratory pump (MasterFlex model 7520-00, Cole-Parmer Instrument Co., Chicago, Ill.) to pump heated solvent over a grain bed for the length of the extraction, was used to extract lipids from

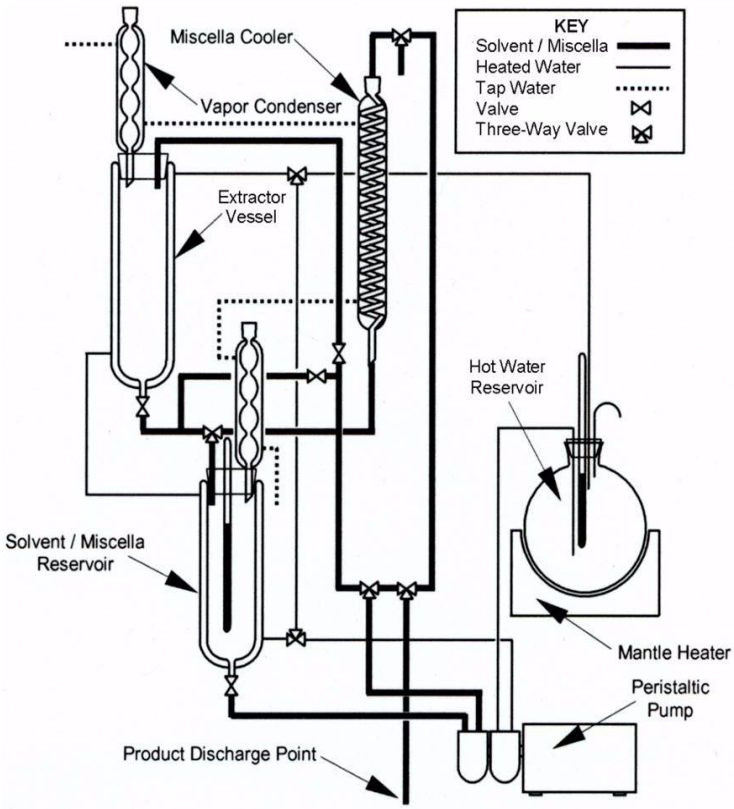

Figure 1. Bench-scale solvent extractor diagram.

the ground and whole kernel sorghum samples. The RC extractor was constructed by Schmidt (2002) and is shown in figure 1. Hexane heated to ~64°C was recirculated for the length of the extraction. The RC method was used to perform 0.5 h extractions with 300 mL of technical-grade hexane and 100 g (M1) of the ground or whole kernel sorghum. The extraction time and the solvent-to-mass ratio were decided upon after referring to the results of Wang et al. (2005). The miscella was recovered from the RC system in a 250 mL round bottom flask of known mass (M2). The miscella was then concentrated using a rotary evaporator (SafetyVap R-144, Büchi, Flawil, Switzerland) under vacuum at 40°C. The miscella was transferred to a 40 mL glass vial of known mass (M4) and placed in a convection oven at 40°C until a constant mass (M5) were reached. The mass of the 250 mL round bottom flask was determined a second time to document any lipid not transferred (M3).

The mass of the extracted lipid was calculated using equation 1. The yield of the extract was calculated using equation 2. The dried samples were stored at -12°C until use for lipid analysis. The RC extractions of ground and whole kernel sorghum were completed in triplicate.

Lipid Analysis

Thin-layer chromatography (TLC) was used to separate extracted lipids. Samples in 10 μL volumes were spotted on 20 × 20 cm analytical normal-phase silica gel TLC plates along with a lipid standard. The lipid standard was a mixture of a four-lipid standard containing cholesterol, free fatty acids, triacylglycerides, and tocopherol (Sigma Chemicals, St. Louis, Mo.) and a three-lipid standard containing triacylglycerides, 1,2 diacylglycerides, 1,3 diacylglycerides, and monoacylglyceride (Supelco, St. Louis, Mo.). The plates were resolved in a hexane : diethyl ether : acetic acid (85:15:2) solvent system. The plates were submerged in a solution of 10 g cupric sulfate dissolved in 100 mL of 8% phosphoric acid to develop the lipid bands. The plates were allowed to dry under a chemical hood and then charred at 166°C for 11 min. The plates were analyzed by densitometry to quantify the triacylglycerides (TAG), policosanols (PA), free sterols (FS), and diacylglycerides (DI). The densitometry data were obtained with a Kodak Gel Logi 440 imaging system interfaced to Kodak image analysis software (Kodak Image Sensing, Rochester, N.Y.). Samples were resolved, charred, and analyzed by densitometry in triplicate.

Experimental Design

A total of 18 treatments (replicated three times) was used to observe fixed effects of grain state, volume of extracting solvent, and extraction time on total lipid yield and lipid class yields for Soxtec extractions. The intent behind varying the parameters of the Soxtec extractions was to observe the effects on lipid class yields.

Total lipid yield and lipid class yields from each Soxtec extraction time were pooled together, resulting in six Soxtec treatments (3 volumes × 2 grain states) for three separate statistical comparisons with the RC and RB extractions. A total of six treatments (replicated three times) was used to observe fixed effects of grain state and extraction method on total lipid yield and lipid class yields from the Soxtec, RC, and RB extractions.

Statistical Methods

The data were analyzed using the programming application R, version 2.3.0 (R Foundation, 2006). Analysis of variance (ANOVA) procedures were used to test for significant ($\alpha = 0.05$) mean differences among treatments for total lipid yield and lipid class yields. The Tukey HSD (honest significant difference) test was used to find the treatments that differed from each other when significant differences were found by ANOVA. Tukey HSD in R creates a set of confidence intervals on the differences between the means of the levels of a factor and an adjusted p-value for multiple comparisons.

Results and Discussion

Soxtec Extraction Yields

Total lipid (TL) yields from the Soxtec extractions ranged from 0.04% to 3.59% for the 18 treatments. Higher levels of TL were extracted from ground sorghum than from whole kernels, probably because there was more surface-to-solvent contact. Figure 2 presents the various yields for the different treatments. The variability of the extracts may be related to the small volumes of solvent used. Wang et al. (2005) reported using 50 mL of hexane to 5 g of sample in a thimble for Soxtec extraction and did not document great variability. Because greater variability was observed with the ground sorghum TL than with whole kernels, particle size (fig. 3) could account for some variability. Wang et al. (2005) found a positive correlation between lipid yield and DDGS particle size: the smaller the particle, the greater the yield.

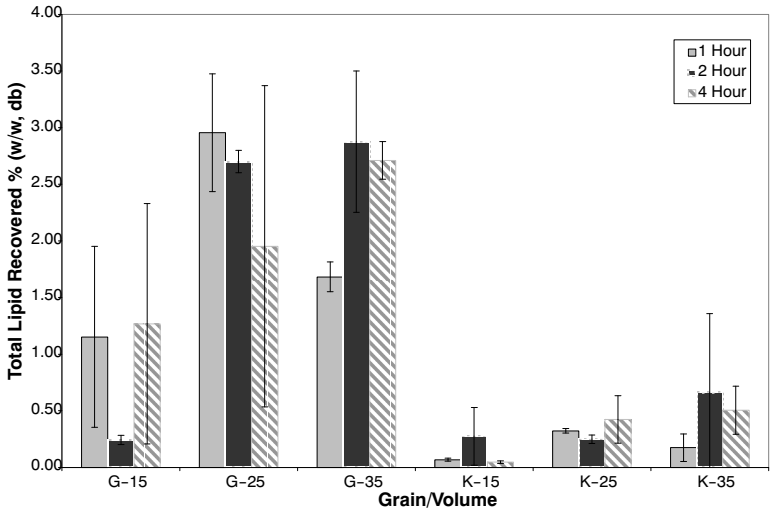

Figure 2. Total lipid yield by the Soxtec extraction method for given grain (G = ground and K = whole kernel) to solvent volume (15, 25, and 35 mL of hexane) ratios at each extraction time (1, 2, and 4 h).

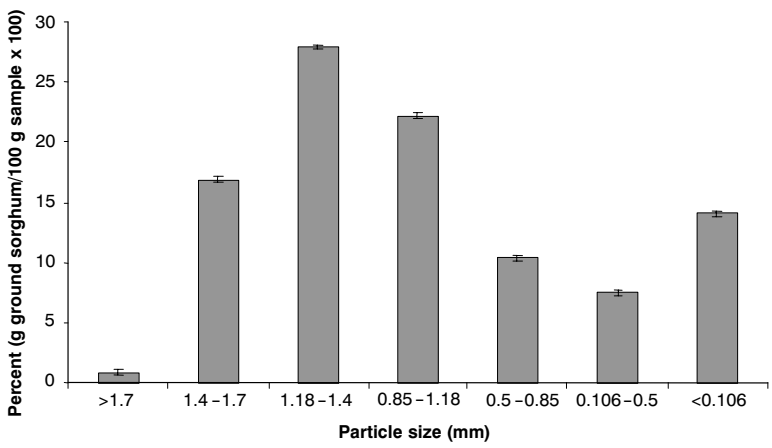

Figure 3. Characteristic particle size distribution in ground grain sorghum Macia samples.

Table 2. Confidence intervals and adjusted p-values between grain state and hexane volume for total lipid recovered by Soxtec extraction as determined by Tukey HSD (G = ground, K = whole kernel).

Comparison	Difference	Lower	Upper	p Adjusted
K-G	-1.57	-2.19	-0.94	<0.0001
35-15 mL	0.95	0.02	1.87	0.04
25-15 mL	1.05	0.12	1.97	0.02
25-35 mL	0.10	-0.83	1.02	0.96

An ANOVA was performed in R to evaluate the effects imparted by grain state, solvent volume, and extraction time on TL in order to draw parallels to lipid class yields. The ANOVA revealed that the length of extraction time did not significantly affect TL yields. However, the ANOVA also revealed that grain state and solvent volume had significant effects on TL: grain state Pr (>F) <0.0001, and solvent volume Pr (>F) 0.015. The Tukey HSD test revealed that significant differences existed between grain states in TL yield; ground sorghum resulted in greater TL yield. A significant difference was also found between solvent volumes; the 35 and 25 mL volumes recovered more TL than 15 mL. No significant difference was found between 35 and 25 mL in TL yield. Table 2 summarizes the Tukey HSD test results.

Extraction Method Yield Comparison

Because time did not significantly affect TL yield when using the Soxtec extraction method, the extraction times for each solvent volume and grain state combination were pooled to create three means for each grain state and volume. This pooling of extraction times allowed a balanced comparison between the Soxtec method and the RC and RB methods at each of the three volumes.

Figure 4 presents the TL yield of each of the methods: RB, RC, and Soxtec including the three different volumes used in the Soxtec extractions. The TL yields for the five methods of extraction ranged from 0.04% to 3.59%. ANOVAs were performed in R to determine the effects, if any, of grain state and/or extraction method on TL yield to draw parallels to lipid class yields. The ANOVAs were constructed to compare each of the Soxtec extrac-

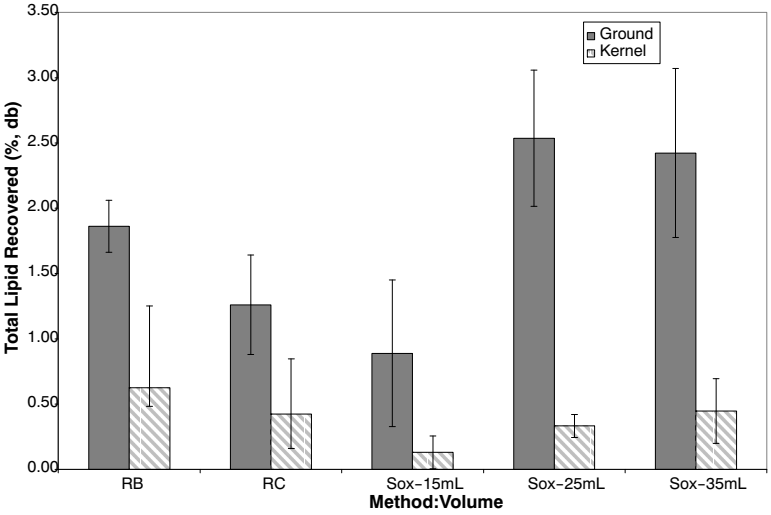

Figure 4. Total lipid recovered (%, d.b.) from ground and whole kernel food-grade sorghum with hexane by three extraction methods, refluxing (RB), recirculated (RC), and Soxtec (Sox) at each of the three different volumes (15, 25, and 35 mL).

tions at each volume (15, 25, and 35 mL) to the RB and RC methods. The effects of grain state and extraction method on TL were significant (grain state Pr (>F) <0.0001, and extraction method Pr (>F) 0.002) between the 15 mL Soxtec, RB, and RC extractions.

The ANOVA comparing the 25 mL Soxtec, RB, and RC extractions confirmed significant effects of grain state and extraction method on TL yield (grain state Pr(>F) <0.0001, and extraction method Pr (>F) 0.006). Lastly, a significant effect of grain state (Pr (>F) <0.0001) on TL was detected between the 35 mL Soxtec, RB, and RC extractions. Table 3 presents the Tukey HSD test results for the significant differences at specific levels of grain state and extraction method. Figure 5 presents a histogram of total lipid yield frequency from the whole kernel and ground sorghum extracts. The histogram supports the statistical findings that ground samples yielded more lipid than whole kernels.

Simple Lipid Class Content from Soxtec Extractions

Figure 6 shows the TLC results of the lipids extracted from the grain samples using the Soxtec, RB, and RC methods. Only the yield of TAG, PA, FS, and DI levels could be estimated with the lipid standards used for densitometry.

Table 3. Confidence intervals for ground and whole kernel sorghum and extraction method on total lipid (% d.b.) recovered as determined by Tukey HSD (G = ground sorghum, K = whole kernel sorghum, Sox = Soxtec method, RB = refluxing, RC = recirculated).

Level	ANOVA			
	S (15 mL) : RB : RC			
	Difference	Lower	Upper	p Adjusted
Sox-RB	-0.55	-0.89	-0.22	0.001
RC-RB	-0.34	-0.68	-0.013	0.04
K-G	-0.99	-1.21	-.076	<0.001
Level	S (25 mL) : RB : RC			
	Difference	Lower	Upper	p Adjusted
Sox-RB	0.49	-0.08	1.066	0.09
Sox-RC	0.84	0.27	1.40	0.004
K-G	-1.13	-1.51	-0.75	<0.0001
Level	S (35 mL) : RB : RC			
	Difference	Lower	Upper	p Adjusted
K-G	-1.18	-1.544	-0.82	<0.0001

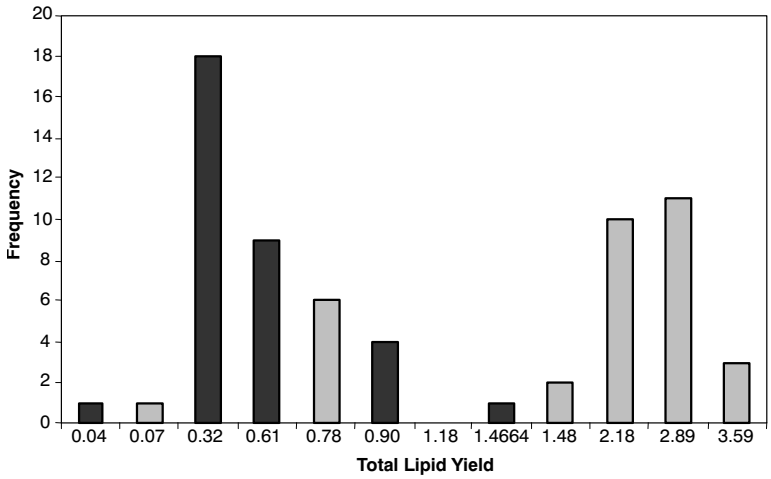

Figure 5. Histogram of total lipid yield frequency from all whole kernel (black) and ground (gray) sorghum extracts.

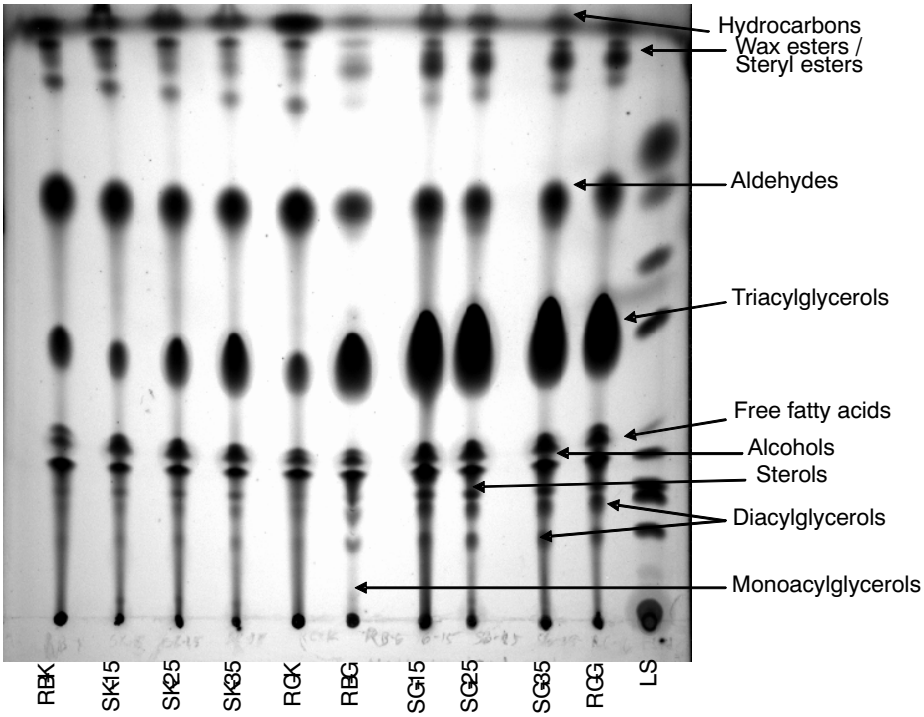

Figure 6. TLC plate with individual lanes revealing the lipid profile of each extraction method, refluxing (RB), recirculated (RC) and Soxtec (S) from each volume of hexane, from whole kernel and ground sorghum (G = ground, K = kernel, and LS = lipid standard).

ANOVAs were performed in R to observe effects of solvent volume and grain state on each lipid class yield extracted by Soxtec. The grain state had an effect on the yield of PA (Pr (>F) 0.04) and on the yield of FS (Pr (>F) 0.04). The Tukey HSD test discerned that more PA and FS were recovered from whole kernels than from ground samples in Soxtec extractions. The solvent volume had no significant effect on the yields of the four lipid classes; volume affected the TL, but the lipid classes in each extract were recovered in similar ratios.

Simple Lipid Class Comparison among Extraction Methods

Figure 7 shows a graphical comparison of the estimated lipid class yield (calculated as [lipid class mass / total lipid extract mass × 100]) by densitometry for each of the extraction methods. The lipid profiles of the Soxtec extracts are similar for the ground and whole kernel samples, but the amounts of each lipid class differ. The lipid profiles of the RB extracts differ between ground and whole kernel samples, as no detectable DI and FS are present in the whole kernels. The lipid profiles from the RC method also differ, with the whole kernels lacking detectable DI and FS.

ANOVAs were performed in R to observe the effects of extraction method and grain state on each lipid class yield. Extraction method had a significant effect on the yield of PA (Pr (>F) 0.03) and FS (Pr (>F) 0.05) between the 15 mL Soxtec, RB, and RC extraction methods. The ANOVA on the 25 mL Soxtec, RB, and RC lipid class yields confirmed significant effects of extraction method on yield of PA (Pr (>F) 0.006) and of grain state on yield of DI (Pr (>F) 0.02) and an interaction effect between grain state and method on

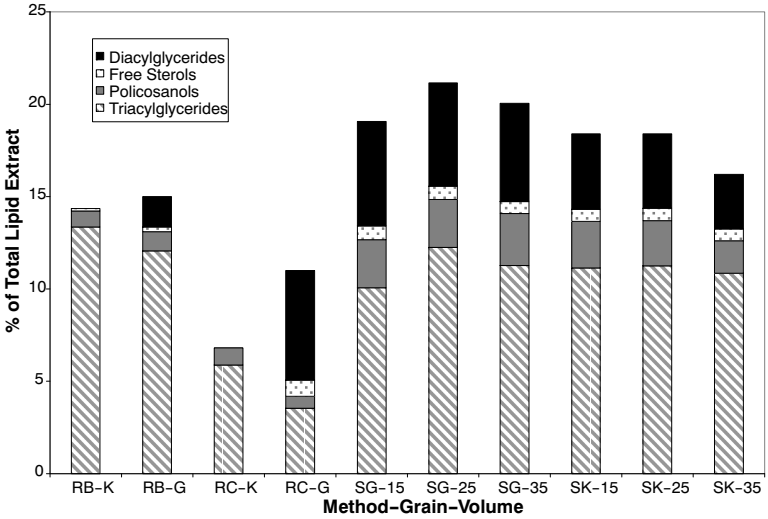

Figure 7. Yields of four simple lipid classes (diacylglycerides, free sterols, policosanols, and triacylglycerides) from ground (G) and whole kernel (K) sorghum by three extraction methods, refluxing (RB), recirculated (RC), and Soxtec (S) at three different volumes (15, 25, and 35 mL).

Table 4. Confidence intervals for extraction methods and grains on lipid class yield (PA = policosanols, FS = free sterols, DI = diglycerides, S = Soxtec, RB = refluxing, RC = recirculated).

Lipid Class	Level	ANOVA			
		S (15 mL) : RB : RC			
		Difference	Lower	Upper	p Adjusted
PA	Sox-RB	2.87	-0.162	5.91	0.06
PA	Sox-RC	3.04	0.01	6.08	0.05
FS	Sox-RB	1.02	-0.04	2.08	0.06
DI	K-G	-3.16	-6.69	0.37	0.07
Lipid Class	Level	S (25 mL) : RB : RC			
		Difference	Lower	Upper	p Adjusted
PA	Sox-RB	2.76	0.57	4.95	0.01
PA	Sox-RC	2.93	0.74	5.12	0.01
FS	K:Sox-G:RB	1.14	0.21	2.08	0.01
FS	K:Sox-K:RB	1.27	0.03	2.21	0.006
FS	K:RC-G:RC	-0.89	-1.83	0.04	0.06
FS	K:Sox-K:RC	1.40	0.47	2.34	0.003
DI	K-G	-3.02	-5.47	-0.56	0.02
Lipid Class	Level	S (35 mL) : RB : RC			
		Difference	Lower	Upper	p Adjusted
PA	Sox-RB	2.27	0.14	4.39	0.03
PA	Sox-RC	2.44	0.31	4.56	0.03
FS	K:Sox-G:RB	1.05	0.23	1.87	0.004
FS	K:Sox-K:RB	1.18	0.37	2.01	0.006
FS	K:RC-G:RC	-0.89	-1.71	-0.07	0.03
FS	K:Sox-K:RC	1.31	0.49	2.13	0.002
DI	K-G	-2.45	-4.79	-0.11	0.04

yield of FS (P(>F) 0.004). Similarly, significant effects of extraction method on yield of PA (Pr (>F) 0.02) and of grain state on yield of DI (Pr (>F) 0.04) and an interaction effect between grain state and method on yield of FS (P (>F) 0.002) were found between the 35 mL Soxtec, RB, and RC extraction methods. Table 4 presents the Tukey HSD test results of the significant differences. These results suggest that yields for particular lipid classes are contingent not only on grain state but also on extraction method.

Relative Efficiencies Between Methods and Grain State

The relative efficiencies were calculated using normalized lipid class yield (mean of three yield values from one method and grain state) ratios (i.e., mean yield of whole kernel RB extractions / mean yield of ground RC extractions). Tables 5 through 8 present the relative efficiencies.

The lipid class yields from the Soxtec extractions are similar among the varying parameters. The RB method appears to be more efficient for recovery of TAG than RC. It is difficult to discern a more efficient method and grain state for policosanol recovery. The RC method with ground sorghum resulted in more free sterols than other methods and grain states, and more diacylglycerides as well. Further treatments, such as longer

Table 5. Comparison of normalized triacylglyceride yield by one method to each other method expressed as a ratio (G = ground, K = whole kernel, S = Soxtec (volume of hexane in mL), RB = refluxing, and RC = recirculated).[a]

	RB-K	RB-G	RC-K	RC-G	S15-G	S25-G	S35-G	S15-K	S25-K	S35-K
RB-K	1.00									
RB-G	1.11	1.00								
RC-K	2.27	2.05	1.00							
RC-G	3.78	3.41	1.66	1.00						
S15-G	1.33	1.20	0.59	0.35	1.00					
S25-G	1.09	0.98	0.48	0.29	0.82	1.00				
S35-G	1.19	1.07	0.52	0.31	0.89	1.09	1.00			
S15-K	1.20	1.08	0.53	0.32	0.90	1.10	1.01	1.00		
S25-K	1.19	1.07	0.52	0.31	0.89	1.09	1.00	0.99	1.00	
S35-K	1.23	1.11	0.54	0.33	0.93	1.13	1.04	1.02	1.04	1.00

[a] Mean of three values for one method compared to the mean of three values for each other method (column method/row method).

Table 6. Comparison of normalized policosanol yield by one method to each other method expressed as a ratio (G = ground, K = whole kernel, S = Soxtec (volume of hexane in mL), RB = refluxing, and RC = recirculated).[a]

	RB-K	RB-G	RC-K	RC-G	S15-G	S25-G	S35-G	S15-K	S25-K	S35-K
RB-K	1.00									
RB-G	0.83	1.00								
RC-K	0.93	1.13	1.00							
RC-G	1.34	1.62	1.44	1.00						
S15-G	0.33	0.40	0.36	0.25	1.00					
S25-G	0.33	0.40	0.36	0.25	1.01	1.00				
S35-G	0.31	0.37	0.33	0.23	0.92	0.92	1.00			
S15-K	0.34	0.41	0.37	0.25	1.03	1.02	1.11	1.00		
S25-K	0.35	0.43	0.38	0.26	1.06	1.05	1.15	1.03	1.00	
S35-K	0.49	0.60	0.53	0.37	1.49	1.48	1.61	1.44	1.40	1.00

[a] Mean of three values for one method compared to the mean of three values for each other method (column method/row method).

Table 7. Comparison of normalized free sterol yield by one method to each other method expressed as a ratio (G = ground, K = whole kernel, S = Soxtec (volume of hexane in mL), RB = refluxing, and RC = recirculated).[a]

	RB-K	RB-G	RC-K	RC-G	S15-G	S25-G	S35-G	S15-K	S25-K	S35-K
RB-K	1.00									
RB-G	0.49	1.00								
RC-K	0.13	0.26	0.00							
RC-G	0.14	0.29	0.00	1.00						
S15-G	0.17	0.35	0.00	1.20	1.00					
S25-G	0.17	0.36	0.00	1.23	1.03	1.00				
S35-G	0.20	0.40	0.00	1.38	1.15	1.12	1.00			
S15-K	0.19	0.39	0.00	1.35	1.13	1.10	0.98	1.00		
S25-K	0.19	0.39	0.00	1.34	1.12	1.09	0.98	0.99	1.00	
S35-K	0.19	0.40	0.00	1.37	1.15	1.12	1.00	1.02	1.02	1.00

[a] Mean of three values for one method compared to the mean of three values for each other method (column method/row method).

Table 8. Comparison of normalized diacylglyceride yield by one method to each other method expressed as a ratio (G = ground, K = whole kernel, S = Soxtec (volume of hexane in mL), RB = refluxing, and RC = recirculated).[a]

	RB-K	RB-G	RC-K	RC-G	S15-G	S25-G	S35-G	S15-K	S25-K	S35-K
RB-K	0.00									
RB-G	0.00	1.00								
RC-K	0.00	1.64	0.00							
RC-G	0.00	0.28	0.00	1.00						
S15-G	0.00	0.29	0.00	1.05	1.00					
S25-G	0.00	0.29	0.00	1.06	1.01	1.00				
S35-G	0.00	0.31	0.00	1.11	1.06	1.05	1.00			
S15-K	0.00	0.40	0.00	1.45	1.38	1.37	1.30	1.00		
S25-K	0.00	0.41	0.00	1.47	1.40	1.38	1.31	1.01	1.00	
S35-K	0.00	0.56	0.00	2.01	1.92	1.90	1.81	1.39	1.37	1.00

[a] Mean of three values for one method compared to the mean of three values for each other method (column method/row method).

extraction times, different solvent-to-mass ratios, and extractions on multiple sorghum varieties, should be performed to confirm the differences in lipid class yield between extraction methods.

Conclusions

The state of the starting materials affects the total lipid recovered and, most importantly, the composition of the extract. Extractions on ground sorghum resulted in higher total lipid yields than extractions on whole kernels. Extracts from ground sorghum yielded greater amounts of diacylglycerides, while extracts from whole kernels yielded greater amounts of policosanols. The method of extraction affects the total lipid yield and the lipid class yield. The Soxtec extractions yielded greater policosanol yields than the RC and RB methods, suggesting that other extraction methods should be analyzed for increasing policosanol recovery to approach that of Soxtec.

Further lipid analysis needs to be completed on the lipid extracts from the three methods. The policosanol, phytosterol, and tocopherol contents of the extracts need to be de-

termined using analytical methods, such as GC and HPLC, for a more accurate profile of their phytochemical composition and thus more accurate comparisons between grain states and extraction methods on lipid class yields.

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