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Yield Comparisons of Different Methods of Waxy Fraction Extraction from Grain Sorghum

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Abstract Three solvent extraction techniques were used to recover waxy fractions from grain sorghum kernels. Yield and chemical composition of the waxy fractions obtained by reflux, bench scale (recirculated solvent), and countercurrent extraction methods were compared. Waxy fraction yield from countercurrent extraction (0.200%) was significantly greater ($p<0.05$) than the yields of wax from both reflux (0.184%) and bench-scale (0.179%) methods. The waxy fraction extracted using the bench-scale method showed the greatest relative amount of long-chained (primarily C:28 and C:30) alcohols while the countercurrent-extracted wax showed the greatest relative amount of long-chained fatty acids and fatty aldehydes. Countercurrent extraction removed a higher additive percentage of fatty aldehydes, acids, and alcohols than reflux or bench-scale extraction method.

Keywords: cereal, solvent extraction, hexane, lipids, natural wax

Introduction

The extraction of waxy fractions (primarily C:28 and C:30 long-chained fatty aldehydes, fatty alcohols, and fatty acids) from grain sorghum has been studied since the 1940's (1, 2). Numerous properties of these naturally-occurring waxy materials are similar to those of carnauba wax. A large amount of carnauba wax and other natural waxy fractions, including limited amounts of rice bran oil wax and spent barley grain wax, are used each year in such products as automobile waxes, fruit and candy coatings, cosmetics, and chemical bases. Additionally, more than 1.3 million metric tons of grain sorghum are used annually in the United States to produce ethanol (3). Approximately 8 kg of dry residue in the form of distillers dried grain (DDG) remains from every 25 kg of grain sorghum used to produce ethanol. The DDG is sold solely as animal feed. Sorghum DDG contains considerable amounts of lipid materials, including long-chained fatty acids, fatty aldehydes, and fatty alcohols. Removal and recovery of a waxy fraction could be of great benefit by creating value-added products from grain sorghum, and providing another source of a natural waxy material (4, 5).

Compounds in the waxy fraction of grain sorghum are generally classified as non-polar lipids, and can therefore be extracted from the grain using non-polar solvents such as hexane (2). Historically, researchers studying sorghum wax have used relatively simple laboratory apparatuses to extract the waxy fraction from sorghum for their studies (1, 6, 7). This generally involved Soxhlet extraction or refluxing sorghum kernels with a solvent. The information gained from these studies has provided yield, melting point, chemical composition, and other characteristics of a typical grain sorghum waxy fraction.

Full-scale commercial solvent extraction operations, such as those of the soybean and rice bran processing industries, utilize high-capacity continuous countercurrent extraction technology. It is assumed that a commercially viable grain sorghum processing operation could make use of similar equipment and unit operations for extraction and recovery of the waxy fraction from whole kernels. In an effort to build on the research of others, and in order to move towards the realization of a commercial grain sorghum extraction operation, we decided to test the countercurrent extraction techniques against the batch extraction apparatuses used in earlier research. Results from this study could then be used in subsequent studies on more advanced extraction methods such as microwave-assisted extraction, ultrasound-assisted extraction, pressurized extraction, and supercritical fluid extraction. Supercritical fluid extraction is currently in use in the recovery of wax from spent barley grains from alcoholic beverage production.

A study conducted by Reuber (8) of Iowa State University featured extraction of oilseeds using a semi-continuous extractor constructed with laboratory glassware, tubing and fittings. The extractions were conducted in batches, but the grain and solvent/lipid fractions (miscella) were used in further extractions and a simulated countercurrent extraction was performed. The design by Reuber was considered highly applicable for a realistic yet economical simulation of countercurrent extraction using grain sorghum kernels. The experimental bench-scale extraction apparatus of this study was based on Reuber's design, but some design and operational changes were necessary to accommodate the unique properties of grain sorghum.

Using the bench-scale extraction apparatus and traditional

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refluxing equipment, three methods of extraction were examined in this study: 1) reflux extraction using techniques developed by other researchers, 2) recirculated solvent extraction using the bench-scale laboratory extraction apparatus with recirculation of solvent in the system, and 3) simulated countercurrent extraction using the bench-scale laboratory extraction apparatus with a single pass of solvent through simulated successive stages of extraction. Waxy fraction yields were calculated as percentages of dry grain sorghum kernels and the percent yields from the three methods compared.

Although the waxy fraction from grain sorghum has properties similar to carnauba wax, the chemistry of sorghum wax is not fully understood. Furthermore, the few studies published on the chemical composition of sorghum wax are not in agreement (2). Recent work by Hwang *et al.* (9) utilized high performance liquid chromatography to identify and confirm key chemical compounds in the sorghum waxy fraction. In addition to determining waxy fraction yields, the objective of this research was to examine the waxy fraction obtained by each extraction method for the presence and quantity of long-chained fatty acids, aldehydes, and alcohols.

Materials and Methods

Grain sorghum preparation A variety of grain sorghum, NC+7R37E, grown and harvested by the University of Nebraska Foundation Seed Division in Mead, Nebraska, USA in 1996 was used in this experiment. Kernels were stored in 100 kg brown paper bags at 5°C until use. Grain sorghum kernels were divided into individual 6 kg batches, transferred to a rectangular washtub, and combined with cool water (at approximately 15°C) until the grain was covered to a depth of about 2 cm. The kernels were agitated by hand for 5 min, and any floating materials were poured off while keeping the grain in the container.

Cool water was again added to the kernels until it was fully submerged. The slurry was agitated by hand for approximately 3 min. Water and kernels were poured into a grain sieve to separate the water from the kernels. The grain was allowed to drain in the sieve for approximately 10 min. The kernels were then transferred to drying screens within a forced-air, heated drying box. The kernels were placed on the screen to a depth of 1.5 cm and allowed to dry for approximately 24 hr at 40°C. The moisture of the grain was spot-checked using a Steinlite moisture meter (Model SS250; Fred Stein Laboratories Inc., Atchison, KS, USA) to ensure that it was close to the target of approximately 10%(wb). The cleaned kernels were stored in a covered 20 L plastic bucket in a commercial refrigerator at 10°C until needed for experimentation.

Reflux extractions A sample of 600 g of clean grain sorghum was placed in a 2,000 mL spherical flask. Six hundred mL hexane solvent (Industrial Grade Hexane, C_6H_{14}, HX0299-3; EM Science, Gibbstown, NJ, USA) was carefully added to the flask. The grain and solvent mixture was placed on a mantle heater (Electromantle UM 2000; Electrothermal Engineering Ltd., Essex, UK) and heated to 65°C. A Liebig-type glass condenser was directly coupled to the top of the flask both to condense vapors liberated through heating the hexane, and also to protect against possible accidental boiling. A thermometer was inserted down the condenser and into the solvent to monitor the temperature of the refluxing operation.

When the target temperature of 65°C (the temperature used in commercial extraction) was reached, a 30 min reflux cycle was started. This reflux time was based on earlier work by Lochte-Watson (1). The procedure was closely monitored to ensure that boiling did not occur. Minor adjustments were made to the temperature in order to maintain it as close to the target temperature as possible. After 30 min had elapsed, the vessel was allowed to cool down for approximately 2 min. The flask contents were immediately poured through a Büchner funnel lined with a coffee filter sitting atop a 1,000 mL screw-top flask. The filtered hexane and wax, or miscella, were collected in a flask, capped, and placed in a -16°C refrigerator for a period of 18 hr to precipitate the wax. This procedure was repeated until 6 miscella samples were recovered.

Construction of the bench-scale extractor The bench-scale extractor consisted of two major vessels: a solvent reservoir and an extraction chamber. These vessels were custom-made by the University of Nebraska Glass Shop. They had a 75 mm inside diameter and featured an outer jacket in which hot water was circulated to maintain a consistent temperature throughout the extraction.

The vessels and associated equipment were set up on a laboratory stand and placed under a vapor hood to ensure that any hexane vapors were removed from the experimentation area. The extraction chamber and solvent reservoir were arranged so that there would be gravity drainage from the extraction chamber to the solvent reservoir (see Fig. 1).

Water was heated in a 5,000 mL round bottom flask on

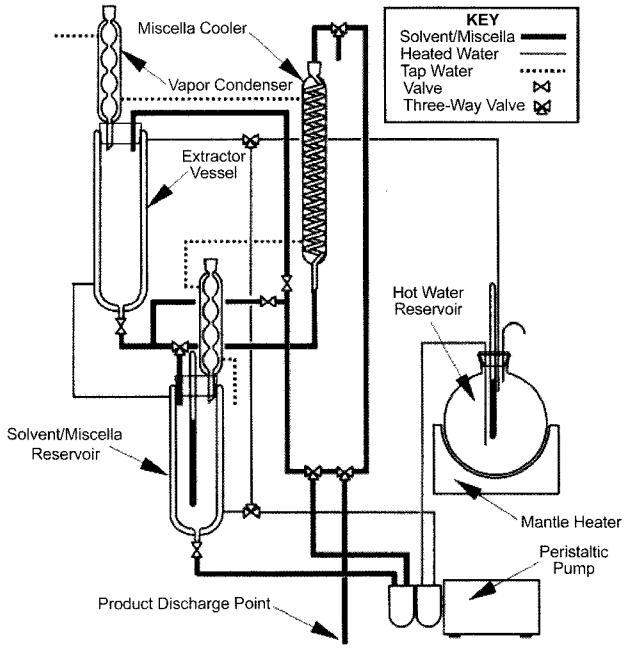

Fig. 1. Diagram of the bench-scale solvent extractor apparatus.

a mantle heater (Electromantle EM 5000/CE; Electrothermal Engineering Ltd.) and circulated through the extraction chamber and solvent reservoir vessels to keep the extraction temperature constant. A peristaltic laboratory pump was used for moving solvent and hot water through the bench-scale extractor equipment. Tandem pump heads were used on a single variable speed driver. The valves of the solvent and the hot water systems were arranged so that portions of each system could be bypassed while the pump was working to move fluids for the other system.

Flexible Viton® 1/4 inch (0.635 mm, i.d.) tubing was used for hexane lines. Hot water lines were constructed of 1/4 inch (0.635 mm) flexible silicone tubing, and cold water lines were of 1/4 inch (0.635 mm) polypropylene tubing. All valves were Teflon® plug valves.

Recirculated solvent extractions The aforementioned bench-scale solvent extractor was used in the laboratory to study the effects of recirculating solvent during an extraction rather than refluxing the sorghum and solvent together. For this experiment most of the variables were held constant in order to compare the waxy fraction obtained by each of the methods. Six hundred g sorghum grain was placed in the extraction chamber. Six hundred mL hexane solvent was placed into the solvent reservoir. After the water had been heated to approximately 90°C, the system was configured to circulate hot water and solvent in their respective systems as a pre-heating step.

When the solvent had reached the target temperature of 65°C, the solvent system valve direction was changed to divert hexane up to the extraction chamber. The hot water system valve direction was also changed to allow the extractor vessel jackets to be heated. Once the solvent had covered the bed of sorghum, the valve at the discharge of the extraction chamber was adjusted to maintain the level of hexane right above the top of the grain bed. This ensured that all of the grain would be in constant contact with the solvent, and also served to mimic the resistance to hexane flow in a deep-bed commercial extractor. Covering the grain also marked the beginning of the 30 min extraction time.

After 30 min, the pump was stopped and the miscella was allowed to drain from the extraction chamber into the reservoir. The miscella was circulated through the cooler and back to the reservoir. After a few minutes of flow through the cooler, the miscella was discharged to a flask, capped, and placed in a -16°C refrigerator for 18 hr to precipitate the wax. The extracted grain sorghum kernels were discharged and the system was readied for another extraction. This procedure was repeated to collect 6 miscella samples in total.

Countercurrent extractions The number of stages necessary for the countercurrent extraction process to reach a target concentration of 1.3%(w/w) waxy fraction in miscella was calculated to be three (10). This number was determined using a semi-graphical calculation technique to simulate the equilibrium of solute in solvent in both the solution and inert solids phases in countercurrent leaching stages (11). A procedure was then devised to simulate the multiple stages using only the extraction chamber and the miscella reservoir of the bench scale extraction apparatus.

Typically, a commercial extractor is divided up into sections or stages with a catch basin and a recirculation pump located beneath each stage to recirculate miscella through the grain bed and advance it to the next stage. This advancement to the next stage is in the opposite direction of material travel, and gives the countercurrent flow of materials.

Since there was only one extraction chamber (section) and one miscella reservoir (catch basin) in the bench-scale extraction apparatus, the extractions needed to be staged to simulate the movement of sorghum to subsequent sections, and for the miscella to increase in concentration. For the experimental countercurrent extraction, materials moved through the system according to the diagram shown in Fig. 2. Fresh grain was introduced on the left side of the diagram, and fresh solvent was introduced on the right. After an individual extraction was completed, the sorghum and miscella moved on to their next use in the system according to the diagram. Thus, a countercurrent flow of materials was established, and a reasonable simulation of a commercial extractor was attained.

After the sequencing of the extractions was planned, they were executed in the same manner as the recirculated solvent extractions. Sorghum and hexane samples for each stage were prepared. Solvent was placed inside the

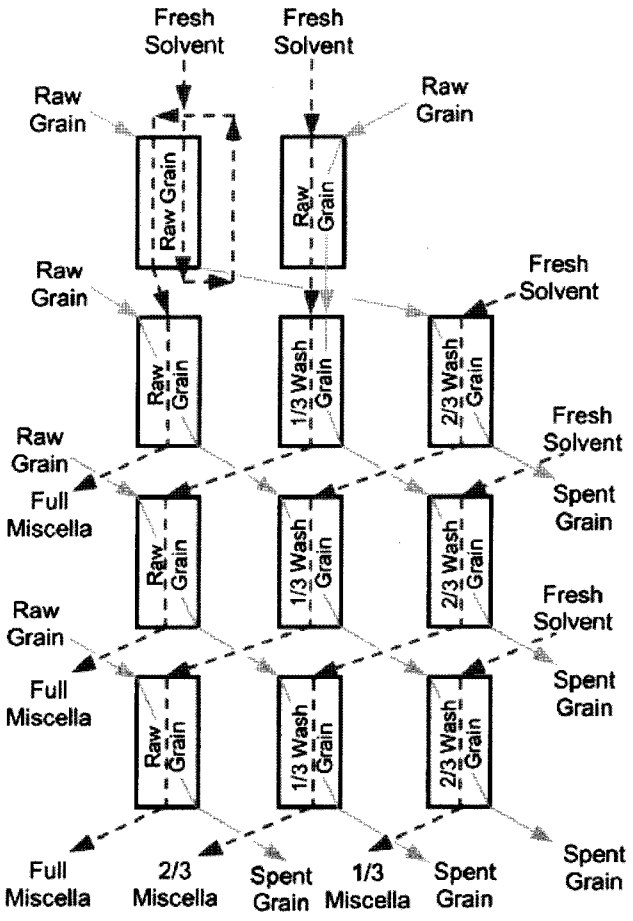

Fig. 2. Diagram of the countercurrent solvent extractor system.

reservoir vessel, and the sorghum kernels were placed in the extraction chamber. Once the solvent had reached the target temperature of 65°C, the solvent was directed to the extraction chamber. When the level reached the top of the grain bed, the miscella was allowed to flow back to the reservoir. The total extraction time comprised the filling time to the top of the grain bed and the discharge time and was about 5 min. After the miscella had completely drained back into the solvent reservoir it was discharged into a screw-top flask and labeled for future use or cold storage. The extractions were sequenced so as to minimize the removal of partially extracted grain. The entire procedure was repeated 4 times, yielding 12 full miscella samples. The first two samples were discarded because they were 'starters' and did not represent extraction of three distinct grain samples. The remaining samples were placed in the -16°C refrigerator for 18 hr to precipitate the wax.

Waxy fraction recovery After 18 hr in the -16°C freezer, the samples had changed from clear to opaque in appearance. A cloudy layer of wax precipitate could be seen at the bottom of the flasks. The apparatus for separating the waxy fraction from the hexane consisted of a filtering flask and a Büchner funnel. The funnel was placed in the neck of the flask and a rubber adapter added to ensure a seal. The vacuum tap on the flask was attached to a sink tap vacuum eductor via a neoprene hose.

A pre-weighed 90-mm Whatman no. 42 ashless filter paper was placed in the funnel and the sample of miscella was poured into the funnel. The precipitated waxy fraction was retained on the surface of the filter paper. The filter paper was carefully removed and placed in a desiccant chamber to remove trace amounts of solvent. The miscella filtrate was returned to the screw-top flask that it came from, and placed in the freezer for another 18 hr. The filtration and chilling procedure was repeated twice, giving a total of three filtrations of each sample of miscella (Fig. 3).

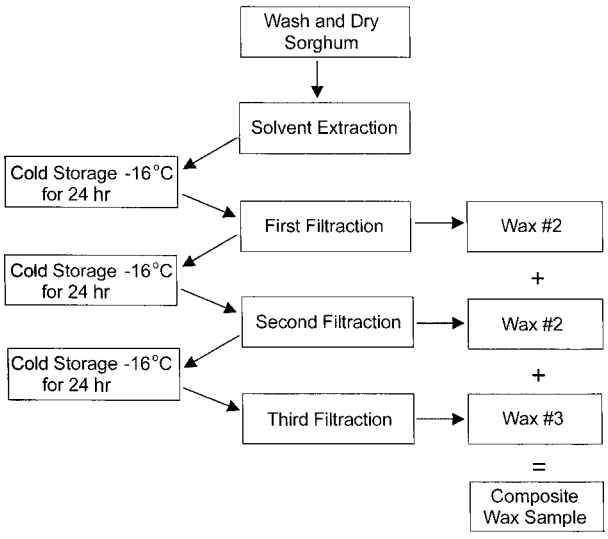

Fig. 3. Sorghum waxy fraction extraction and filtration flow chart.

After the sample had been held in the desiccant dryer for 24 hr, the filter paper was removed and weighed. The initial weight of the filter paper was subtracted from the weight of the filter paper with the waxy fraction. The masses of the waxy fraction for each of the three filtrations were added together to give the total weight of the waxy fraction (g) recovered from the grain sample.

In order to determine the yield percentage, the moisture of the sorghum used during the experiment was first determined using ASAE standard S352.2 (12). The waxy fraction yield was then calculated as:

$$\%\ \text{waxy fraction} = \left(\frac{\text{total waxy fraction mass(g)}}{\text{original mass of grain sorghum(g)}}\right) \times \left(\frac{1}{(1-\text{wet basis moisture content})}\right) \times 100\%$$

Chemical analysis Waxy fraction samples from the three extraction techniques were diluted in hexane solvent and analyzed in an HPLC system. The compounds of most interest were long-chained fatty acids, aldehydes, and alcohols (primarily C:28 and C:30). These are the three main types of compounds shown by research to be present in sorghum waxy fractions.

Two Waters 510 HPLC pumps (Waters Corp., Milford, MA, USA) were operated in gradient modes by a Waters Millenium Program (version 2.15.01). Flow rate of the mobile phase was 1 mL/min. A Rheodyne 7725i injector (Coati, CA, USA) with a 100-μL injection loop, and a Luna 5-μL column (250 mm long × 4.6 mm i.d.; Phenomenex, Torrance, CA, USA) were used. A guard column (4 mm long × 3 mm i.d. silica cartridge in a SecurityGuard cartridge system; Phenomenex) was connected to the column. The column and guard column were heated at 43 °C using a Waters column heater module. Exposed lines from injection loop to detector connection were maintained at about 38-40°C using a wrapped heating tape. The detector was a Varex ELSD II (Varex, Rockville, MD, USA) operated at 60°C with a nitrogen pressure of 930 kPa.

Samples were dissolved in hexane at 20 μg/100 μL and 100 μL of each sample injected into the system. The relation between concentration and area were plotted for calibration by injecting standards (aldehyde fraction for aldehydes; lignoceryl alcohol for alcohols; and lignoceric acid for acids) with different concentrations covering the levels of sample components for each HPLC system.

Samples of waxy fractions from each extraction method were tested for moisture content by placing them in a 100 °C forced draft oven and determining the water present in each waxy fraction. The chemical content of each waxy fraction then was adjusted for the respective moisture.

Safety emphasis The dangers associated with hexane use commercially and in laboratories are well documented (13-15). Great care was taken to ensure that the entire laboratory apparatus was built with materials compatible with hexane, and that operating scenarios were reviewed to minimize the risk of a hexane spill or fire.

Experimental design and data analysis Three extraction

treatments and their corresponding yields were observed in an unstructured, random design using type 3 analysis of variance and detection of significant differences with a p-value <0.05. Statistical analysis was performed on the waxy fraction yield data using PROCMIXED (Version 8.2; SAS Inc., Cary, NC, USA).

Results and Discussion

Wax yield comparison For the reflux extractions, the mean waxy fraction yield was 0.184% with a standard deviation of 0.003%. The recirculated solvent extraction mean waxy fraction yield was 0.179% with a standard deviation of 0.006% and the countercurrent mean waxy fraction yield was 0.200% with a standard deviation of 0.011%. There was a significant difference (p<0.05) between the yields of the three extraction methods.

Furthermore, the comparison of extraction method yields using the difference of least squared means showed the mean waxy fraction yield of the countercurrent extraction was significantly greater (p<0.05) than the mean waxy fraction yields from the other two extraction methods. This observation was anticipated as the successive stages were expected to allow for a more concentrated wax level in the final miscella while using an amount of solvent equivalent to that used in the other extraction techniques (11).

Maximum solubility was determined to be 1.9% (waxy fraction weight per miscella weight) (8) and the bench-scale extraction apparatus was designed to achieve a level of 1.3% waxy fraction in miscella. The 0.2% yield for the countercurrent extraction almost reached the 1.3% target with an observed concentration of 1.2% waxy fraction in final miscella from the third stage.

Chemical analysis of waxy fractions Chemical analysis of the waxy fraction samples using HPLC methods demonstrated that there are some differences in the long-chained lipid composition of the waxy fraction obtained from each extraction method. Table 1 shows the amounts of three lipid constituents and other constituents present in the waxy fractions obtained from the three extraction methods. The recirculated solvent method yielded a greater amount of fatty alcohols on a weight basis than the reflux or countercurrent extraction methods. Greater amounts of fatty aldehydes and fatty acids resulted from counter-current extraction than from the other two extraction methods on a weight basis.

Table 1 also shows the relative percentage of the three chemical components present in the waxy fraction samples. The recirculated solvent sample had the greatest percentage of fatty alcohols in the sample tested while the countercurrent extracted waxy fraction had the greatest percentage of both fatty acids and fatty aldehydes. Additionally, the countercurrent method showed the highest overall percentage for yield of the three desirable chemical components - fatty aldehydes, acids, and alcohols.

Countercurrent extraction appeared to limit extraction of constituents other than fatty aldehydes and fatty acids. It also seemed that countercurrent extraction preferentially extracted a greater amount of fatty aldehydes than fatty acids or alcohols. Perhaps the extraction of greater amounts of the relatively low polarity fatty aldehydes in a relatively low polarity solvent, hexane, during countercurrent extraction was due to its use of successive stages rather than the single stage of extraction for the reflux and recirculated solvent extractions.

Table 1. Quantity of lipids by both weight and percentage bases[1)] found in waxy fraction samples from reflux, recirculated solvent, and countercurrent extractions of grain sorghum kernels

Measured parameter	Reflux	Recirculated solvent	Countercurrent
Sample size (μg)	20.00	20.00	20.00
Moisture content (%wb)	0.34	0.15	0.02
Total collected lipids (μg)	19.93	19.97	19.99
Aldehyde (μg)	6.50	5.48	8.71
% of total collected lipids	32.5	27.4	43.6
Acid (μg)	1.61	1.60	1.78
% of total collected lipids	8.1	8.0	8.9
Alcohol (μg)	9.17	9.36	8.65
% of total collected lipids	45.9	46.8	43.2
Other (non-water) (μg)	2.65	3.53	0.85
% of total collected lipids	13.5	17.8	4.3

[1)]Reported chemical constituent is average of 3 replications.

The limited extraction of constituents other than fatty aldehydes and fatty acids may also be due to the short contact time of solvent with kernels in the countercurrent extraction where contact time of solvent with solids was approximately half of the time in reflux and recirculated solvent extractions. In the longer times of extraction, greater amounts of solvent were able to diffuse further into the interior of kernels from the exterior and greater amounts of miscella were able to diffuse from the interior of kernels to the exterior. Lipid materials from within the interior of the kernels were likely to be triglycerides from the germ, phytosterols, and steryl esters (i.e., *Other* in Table 1).

Further analysis of the values in Table 1 indicates a trend of decreasing moisture content in recovered waxy fractions as time of contact between solvent and kernels decreased. In the longer times of extraction, greater amounts of moisture were able to diffuse from the interior of kernels, thereby reducing the total amount of extracted lipid compounds.

Additional research studying the effects of extraction operating parameters (e.g., temperature, solvent to solid ratios, moisture content of solid, etc.) on physical and chemical properties of extracted lipid materials and oxidation or reduction of fatty aldehydes during multiple stage extraction is needed. Fatty aldehydes, acids, and alcohols have relatively high but slightly different melting points (16). They may therefore be useful in tailoring melting points of blends of long-chained compounds such as natural waxes. Application research could confirm the feasibility of tailoring melting points of blends through the

incorporation of grain sorghum waxy fraction compounds.

The countercurrent extraction method was shown to produce higher waxy fraction yields than either the reflux or recirculated solvent methods. The three stages used in countercurrent sorghum waxy fraction extraction nearly allowed the target of 1.3% (waxy fraction weight per miscella weight) to be met.

Acknowledgments

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