REVIEW

Properties, Composition, and Analysis of Grain Sorghum Wax

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ABSTRACT: Grain sorghum wax has been judged to be a potential source of natural wax with properties similar to carnauba wax. Approximately 0.16–0.3% (w/w) wax can be extracted from grain sorghum depending on the efficiency of the organic solvents. Although the melting points of carnauba wax and sorghum wax are similar, i.e., 78–86 and 77–85°C, respectively, they differ in acid values, i.e., 2–10 and 10–16, respectively, and saponification numbers, i.e., 77–95 and 16–49, respectively. Improved knowledge of the properties, composition, and analysis of grain sorghum wax would assist in efforts for industrial application of this product. Major components of sorghum wax are hydrocarbons, wax esters, aldehydes, free fatty alcohols, and FFA. The hydrocarbons consist mainly of C_{27} and C_{29}, and the aldehydes, alcohols, and acids are mainly C_{28} and C_{30}. The wax esters are mostly esters of C_{28} and C_{30} alcohols and acids.

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Grain sorghum has historically been grown in dry climates where maize is less adapted. The production of grain sorghum in the United States fluctuated between 12 and 22 million metric tons per year during 1990–2000 (1). Overall, production has decreased, as maize is being bred to be more adaptable to drier climates. Throughout history the use of grain sorghum has been restricted to feed (6.7–13.1 million metric tons per year) (1). Nevertheless, there is a need to maintain the crop for diversity purposes within cereal grains. To help sustain interest in grain sorghum, practical and realistic uses unique from those of maize are needed. One such use involves a lipid material, generally known as wax, which can be removed from the outer surface of grain sorghum kernels. It was first noted in the 1940s and has been judged to be a potential source of natural wax, with properties similar to carnauba wax. Carnauba wax accounted for approximately 87% of total vegetable wax imports (5,000 metric tons) into the United States in 1998, and these wax imports continue to increase (2).

Grain sorghum wax has been studied in terms of its recovery, physicochemical properties, and application. Although sorghum wax has physical properties similar to carnauba wax, the chemistry of sorghum wax is not fully understood. Furthermore, the few studies on the chemical composition of the wax that have been published are not in agreement. Improved knowledge on the properties, composition, and analysis of grain sorghum wax would assist in efforts for industrial application of this product.

This paper discusses the structure of grain sorghum, analytical recovery of wax from grain sorghum, physiochemical properties of sorghum wax, methodology for wax analysis, and chemical composition of sorghum wax. The conflicting literature related to wax chemistry is highlighted and discussed. Also, methods of analysis become available for use by future researchers.

STRUCTURE OF GRAIN SORGHUM

Grain sorghum species are members of the grass family, the Gramineae (3). It is an important cereal that, unlike wheat, barley, and rice, can be grown economically in the semi-arid regions of the world. Because of its tolerance to drought and adaptation to tropical and subtropical ecosystems, sorghum constitutes a major source of calories and proteins for millions of people in Africa and Asia: Sorghum provides 329 kcal and 8–16 g protein per 100 g (4). About 16 kg per capita per year is consumed as food in Africa, and about 18 kg per capita per year is consumed in Asia (5). Although sorghum is used primarily as an animal feed in the United States, there has been considerable interest in its potential use as a brewing material and as human food for the replacement of maize or a partial replacement of wheat (3). However, structure and chemical function of grain sorghum have not been studied to the same extent as grains that grow readily in the more developed regions of the world (3).

The structure of grain sorghum can be separated into three distinctive anatomical components: bran (pericarp-testa, 7%), germ (embryo, 9%), and endosperm (storage tissue, 84%) (6). The bran consists of pericarp and seed coat (or testa). The outer layer or pericarp originates from the ovary wall (7) and is divided into three histological tissues: epicarp, mesocarp, and endocarp (8). The outermost layer or epicarp is generally covered with a thin layer of wax. The epicarp is two or three layers thick and consists of rectangular cells often containing

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pigmented material. Like other surface waxes from leaves, sheaths, stems, bark, flowers, fruits, and husks (9), grain sorghum surface wax appears to play an important role in preserving the water balance of the plant by reducing evaporation from its surface, serving as a barrier to the passage of water into and out of cells (10). Other protective functions may include minimizing mechanical damage to cells and inhibiting fungal and insect attack.

The endosperm has two distinct layers. The first layer is the aleurone layer, or the outer coat of the endosperm. It contains rectangular cells with thick cell walls, and these contain large amounts of protein, minerals, and oil. The peripheral tissue in the endosperm is compacted with several layers of cells containing mainly protein with little starch. Under this dense layer of protein cells is the corneous and floury endosperm cells containing the starch (6).

The germ consists of two sections: the embryonic axis and the scutellum. The embryonic axis becomes the new plant. The scutellum is the germ reserve tissue with large amounts of oil, protein, enzymes, and minerals (6).

The chemical composition of sorghum is approximately 75% starch, 12% protein, 3.6% oil, 2.7% fiber (cellulose and hemicellulose), 1.6% ash, and 0.2% wax. Chemical composition of anatomical parts of sorghum was reviewed in detail by Serna-Saldivar and Rooney (6).

EXTRACTION OF WAX FROM GRAIN SORGHUM

Various methods for extracting waxes, using relatively nonpolar solvents, have been described. Because surface waxes are largely in a hydrophobically associated form, they may be extracted with solvents like hexane (11–22), benzene (23–26), chloroform (12,25,26), light petroleum ether (27), or acetone (17).

Small amounts of wax have been removed from the sorghum plant for taxonomical classification by just dipping plant leaves or whole plants in a solvent (light petroleum ether, hexane, or chloroform) for less than 1 min (28–30). For the purpose of collecting the wax, sorghum kernels have usually been refluxed in a solvent for an extended time. Waxy materials and oily residuals of the extract have been separated mainly in two ways. In one, the initial solvent was evaporated from the extract containing wax and oils before a more polar solvent such as acetone or alcohol was added and cooled, and wax was collected by filtration. In the other, the extracted solvent was stored in a freezer, and crystallized wax was collected by filtration.

The former method was primarily employed in the earlier studies at Kansas State University (14,15,31–34). Dalton and Mitchell (17) also employed the former method. Bunger and Kummerow (13) and Cannon and Kummerow (16) employed the latter method for wax recovery from grain sorghum.

Recent sorghum wax recovery methods at the University of Nebraska have focused mainly on sources of sorghum wax: whole sorghum kernels (19,27,35,36), bran from abrasive decortication (22), and bran and suspended solids from wet-peeled sorghum (21). Saraiva (27) initially extracted wax using petroleum ether refluxed with whole kernels, filtered the solution to remove the sorghum kernels from the solvent, and then lowered the solvent temperature, which allowed wax to precipitate.

Lochte-Watson and Weller (19) and Lochte-Watson *et al.* (21,22) continued with Saravia's general procedure (27) using hexane as the solvent and reducing the reflux time from 90 to 30 min. Lochte-Watson *et al.* (20) extracted wax using hexane and reported that more wax was recovered from three successive 10-min reflux periods than from a single 30-min reflux period.

Lochte-Watson (37) studied the quality and quantity of wax from grain sorghum co-products, such as dry-milled bran, wet-peeled bran, and distillers dried grain (DDG) from ethanol production and found that the wax recovered from wet-peeled bran had qualities most similar to carnauba wax whereas wax recovered from DDG was the least similar.

Weller *et al.* (38) attempted to extract wax from grain sorghum using ethanol. Yields of the wax were similar for ethanol and hexane. However, the hexane-extracted wax, which contained whiter flakes, seemed to have a higher quality than the ethanol-extracted wax, which was pink in color. Furthermore, during the extraction process, the ethanol miscella looked like a creamy slurry after chilling for crystallization and was very difficult to cold-filter.

CHARACTERISTICS OF SORGHUM WAX

Commercial waxes are characterized by a number of properties such as color, melting point, specific gravity, penetration, acid value, saponification number, and ester value (39). Greener-Donhowe and Fennema (40) obtained a melting point of 81.7 ± 0.3°C for refined yellow #1 carnauba wax using DSC. In general, the melting points ranged from 82 to 86°C (41) and from 78 to 85°C (39) for different grades of carnauba wax. Weller *et al.* (38) reported that the wax from DeKalb 48 (grain sorghum), extracted with petroleum ether, had a melting point of 84.9 ± 0.1°C compared to that of carnauba wax of 82.2 ± 0.1°C (Table 1).

In earlier studies, slightly lower melting points of 81°C (11), 80–84°C (12,17), 77–82°C (13), and 78°C (18) were obtained for sorghum grain surface wax using techniques other than DSC. Carnauba wax is often added to other waxes to increase their melting points. Sorghum wax had a higher melting point than carnauba wax, indicating that sorghum wax could possibly be used as well to increase the melting points of other waxes (38).

Acid value indicates the amount of FFA present in a wax. Acid values of grain sorghum wax extracted with Skellysolve B were 10.8 (11), 10.5–16.22 (13), and 11.1–12.2 (16). Carnauba wax had acid values of 4.5 (42), 2–10 (41) and 2.9–9.7 (39). Weller *et al.* (38) reported acid values of carnauba wax and grain sorghum wax were 9.9 ± 0.1 and 9.2 ± 0.1, respectively (Table 1). The small differences in the values among sorghum waxes might result from different sources of sorghum, different extracting solvents, and different extracting techniques.

TABLE 1
Characteristics of Laboratory-Extracted Grain Sorghum Surface Wax and Commercial Carnauba Wax (refined yellow #1)[a] (38)

	Sorghum	Carnauba
Melting point (°C)	84.94 ± 0.12	82.19 ± 0.06
Acid value	9.16 ± 0.1	9.93 ± 0.1
Saponification number	31.48 ± 0.1	81.78 ± 0.1
Hunter color values		
L	95.44 ± 0.90	73.95 ± 0.80
a	0.97 ± 0.05	1.33 ± 0.10
b	5.52 ± 0.11	27.69 ± 0.43

[a]Presented values are means of three replicates ± SD.

Saponification number, which represents the amount of free and esterified FA, for sorghum wax has been reported to be much lower than for carnauba wax: The saponification numbers were 16–44 (13) and 30–49 (16) for sorghum wax and 78 (13,16), 81.9 (42), 77–95 (41), and 79–95 (39) for carnauba wax. Weller *et al.* (38) confirmed the differences, reporting saponification numbers for sorghum wax and carnauba wax of 31.5 ± 0.1 and 81.8 ± 0.1, respectively (Table 1). Saponification number and acid value can estimate the weight ratio of esters and free acids, which is approximately [(saponification number – acid value) × (2/acid value)] assuming the esters are all wax esters and the carbon numbers on the alcohol moiety are almost the same as those on the acid moiety in an ester. Similarity in acid value and difference in saponification number between sorghum wax and carnauba wax indicates that sorghum wax has fewer esters than carnauba wax.

Sorghum wax from DeKalb 48 was whiter (greater L value in Hunter Lab color scale) and had less yellow (lower +b value) than carnauba wax (Table 1) (38). Less intense coloration of grain sorghum wax may be beneficial in certain potential applications such as formulation of glazings and protective coatings. However, the color depends greatly on refining steps through which the waxes pass.

CHEMICAL COMPOSITION OF SORGHUM WAX

Wax is chemically defined as an ester of a long-chain carboxylic acid and a long-chain alcohol, which is also called a wax ester to differentiate it from other compounds. In a broad sense, hydrocarbons, long-chain acids, long-chain alcohols, and other long-chain nonpolar compounds as well can be included as a part of wax if they contribute a high melting point and imperviousness to water (39,43). According to Hamilton's review (43), the major component in a refined-grade carnauba wax is wax esters, representing 84–85%. The other constituents are 3–3.5% FFA, 2–3% alcohols, 2–3% lactides, 1.5–3% hydrocarbons, 4–6% resins, and 0.5–1% moisture and inorganic residue.

Methodology of quantification for wax classes. Class fractionation of a wax is not an easy task. Wax can be separated into classes using column chromatography, TLC, and HPLC. Hamilton (43) and Misra and Ghosh (44) reviewed the methods for wax class separation in detail.

Most studies (17,25,26) employed column chromatography to fractionate and quantify wax classes by gathering eluate, evaporating solvent, and weighing. In column chromatography it is hard to separate classes cleanly. Wax is not readily dissolved in solvents at room temperature, so it must be liquified with heat and dissolved before applying it on the column. Solvents and column should also be heated. Therefore, it is difficult to set uniform conditions, and inconsistent fractionation results.

The Bianchi group (25,26,45) fractionated sorghum wax and maize wax using silica gel column chromatography with carbon tetrachloride to elute alkanes, esters, and aldehydes in that order; chloroform to elute alcohols; and chloroform/acetic acid (99:1, vol/vol) elute acids. Tulloch and his group used hexane containing increasing proportions of chloroform (46) or containing diethyl ether (47–49) as column chromatography solvents. Hwang *et al.* (50) also developed a column chromatography method to fractionate waxes with separation of the major components accomplished by a hexane/chloroform/acetic acid/methanol system. Column and solvents were heated during fractionation in their study.

TLC has been used as a fairly reliable tool to visualize the whole pattern of wax classes by using different solvent systems such as carbon tetrachloride, chloroform, chloroform with 1% ethanol (26,42,47–49,51), and light petroleum ether/diethyl ether/acetic acid (80:20:1) (52). However, it should not be overlooked that the wax is readily solidified and may not move well with any solvent at ambient temperature. Quantification with densitometry also is not an easy task since uniform charring is not obtained. Iodine vapor, a visualization method, is not suitable for wax identification since wax contains few double bonds. For the most part, charring methods have been adopted in TLC visualization only if the samples do not need to be recovered for further analysis.

HPLC could be the most reproducible technique to quantify classes in a lipid such as wax. However, as Hamilton (43) indicated, HPLC methodology for wax analysis has been developing slowly because of the need to find a suitable detector, since waxes have no useful UV chromophore. Another reason for the slow progress likely has been solidification of wax in HPLC lines and columns, resulting in unreliable data. If HPLC methodology develops, quantification will be much easier and more timely than using column chromatography. For HPLC analysis, an appropriate detector and a heating chamber or pad around the column and lines are needed. Difficulties in separation of wax esters and steryl esters also hinder the use of HPLC in wax analysis (43). These two components were found to elute with aldehydes in most HPLC systems (Moreau, R., personal communication). Stewart and Downing (53) pioneered an HPLC method for the separation of wax esters and steryl esters using magnesium hydroxide as an adsorbent. Nordbäck and Lundberg (54) also successfully separated steryl esters from wax esters using an HPLC system with an alumina column. To the authors' knowledge, separation of the two components from aldehydes using HPLC has not been studied.

Although GC is not usually used for whole wax classes, Tulloch (42) analyzed some commercial waxes, finding

hydrocarbons, monoesters, free acids as methyl esters, and free alcohols as acetates, with a single chromatography system.

Inconsistencies in chemistry of sorghum wax. Papers concerning the chemical composition of sorghum wax have shown inconsistencies among research groups. Bunger and Kummerow (13) separated sorghum wax into ethanol-soluble (11%) and -insoluble fractions (89%) and saponified the insoluble fraction, reporting that the latter contained 48% hydrocarbons, 19% fatty alcohols (esterified and free forms), and 16% fatty acids (esterified and free forms) of the entire wax. Cannon and Kummerow (16) reported acetyl number, ester number, and acid number for the grain sorghum wax extracted by hot Skellysolve B and suggested the wax contained wax esters, FFA, and probably free fatty alcohols. Dalton and Mitchell (17) reported that grain sorghum wax extracted with hot Skellysolve B and precipitated with acetone appeared to be composed of 5% hydrocarbons, 49% wax esters, and 46% free fatty alcohols. They also concluded it did not contain carbonyl compounds on the basis of an unsuccessful reaction between the wax and 2,4-dinitrophenylhydrazine, which is supposed to react with aldehydes or ketones, producing a yellow 2,4-dinitrophenylhydrazone precipitate. Seitz (24) reported that the wax extracted from grain sorghum using hot benzene consisted of 4–5% hydrocarbons, 46–50% wax esters, 40–45% fatty alcohols, and about 8% other lipid components.

Bianchi and his group extensively studied waxes from maize and sorghum plants (25,26,30,45,51,55–58). They reported that most of the plant waxes observed contained aldehydes in large quantities as well as other traditional wax components. Maize kernel wax was reported to contain no aldehydes (45), but grain sorghum kernel wax had 32 (25) or 21% aldehydes (26). Further, they stated, "TLC detection of aldehydes was readily accomplished using 2,4-dinitrophenylhydrazine, previously heating in a test tube an ethanolic solution of the reagent with the wax. The 2,4-dinitrophenylhydrazones formed were clearly visible as yellow spots on developed plates" (56). This was contrary to Dalton and Mitchell's observation (17).

Tolluch and his group have also worked extensively on waxes from plant leaves for taxonomical purpose. They observed the presence of β-diketones in quite high amounts in many species of grasses and grain plants (28,29,46–49,59,60), although they did not study the grain kernel waxes intensely. Tulloch (49) did report that 6–19% aldehydes were detected in some grass waxes.

Aldehydes in sorghum wax. The inconsistency of the reports on sorghum wax chemistry limited the reliability of the information available to understand the wax's properties. Most previous papers reported that grain sorghum wax contained hydrocarbons, wax esters, acids, and alcohols, as described above. Only the Bianchi group consistently reported that sorghum wax had aldehydes. Hwang *et al.* (50) observed a dark spot in sorghum wax whose R_f values did not match any of the traditional wax or oil standards in different TLC developing solutions. The column chromatography fraction of the dark spot represented more than 40% (w/w) of the total wax. They attempted several chemical reactions to identify the dark spot. Reaction with 2,4-dinitrophenylhydrazine (61), Benedict's test (62), chromic acid reaction (63), and reaction with KOH did not provide solid conclusions. The dark spot fraction reacted with borohydride (64), producing alcohols. The fraction also was exposed to room temperature air for 5 d and applied on TLC, resulting in a very dark acid band. They concluded that the dark spot was aldehydes on the basis of the results of borohydride reduction and autoxidation, and this was confirmed by NMR spectroscopy and GC–MS analyses. These results support the observations of the Bianchi group (25,26).

If we assume the presence of aldehydes in sorghum wax, then acid value and saponification number are not necessarily confined to describing the amount of free acids and their esters. Aldehydes in sorghum wax can be readily oxidized during storage in contact with air to produce acids (65), giving a higher acid value for oxidized sorghum wax than for freshly extracted sorghum wax. In particular, saponification number does not measure only wax esters and acids, because under the hydrolysis conditions of the analysis, aldehydes can be partly converted to acids as discussed above, resulting in a higher saponification number than the one observed before the wax is subjected to hydrolysis.

Compositional analysis of individual wax classes. After determining the wax classes, chain lengths and double bonds of the individual wax classes, fractionated by column chromatography, can be analyzed by GC. High-temperature-limit columns may be needed for longer chains in waxes than in normal lipids. The Bianchi group (25,26,45) used a packed or capillary column. They did not derivatize hydrocarbons and aldehydes while they transformed alcohols and acids into acetates and methyl esters, respectively. Esters, as such, were subjected to GC analysis as well as transesterification followed by acetylation. Other GC methods were reviewed by Misra and Ghosh (44). Unidentified compounds were identified by MS (25,26,43,66), NMR (47,48,67), and IR spectroscopy (68).

The following methods are recommended for analyzing sorghum wax classes by GC: The hydrocarbon fraction may be dissolved in hexane and directly injected into the GC. Wax esters may be analyzed in two ways: The esters may be directly injected into GC, or the esters may be saponified to FFA and free fatty alcohols as per the American Oil Chemists' Society Official and Tentative Methods (AOCS) Ca 6b-53 (69) with modification. FA may be methylated according to AOCS Ce 2-66 (70) with modification and analyzed by GC. The modification for saponification of wax esters and methylation of FA should be further studied since it is very difficult to dissolve these compounds in the reacting solutions. Fatty alcohols may be derivatized to trimethylsilyl (TMS) ether derivatives for GC analysis as suggested by Christie (71). Fatty aldehydes may be directly injected into the GC or oxidized to form acids and the acids methylated as described above.

Composition of wax classes. The Bianchi group (25,26) reported that sorghum grain wax contained 1.3% hydrocarbons, 4% wax esters, 33.7% fatty alcohols, 24.4% FA, 31.9% aldehydes, and 4.7% unidentified; and 7% hydrocarbons, 13%

TABLE 2
Compositions of Wax Classes (%)

Carbon chain	Alkanes		Aldehydes		Alcohols		Acids	
length	*S. vulgare*[a]	*S. bicolor*[b,c]	*S. vulgare*[a]	*S. bicolor*[b]	*S. vulgare*[a]	*S. bicolor*[b]	*S. vulgare*[a]	*S. bicolor*[b]
21–25	2.5	8	0.1	—[d]	18.3	3	55.2[e]	45
26	4.2	3	2.5	3	12.7	7	2.4	5
27	34.0	33	0.1	—	1.6	—	0.6	—
28	4.2	3	46.7	37	44.6	39	20.6	37
29	53.2	38	0.3	—	0.7	—	0.6	—
30	0.4	1	49.9	57	22.1	48	20.6	13
31	1.5	3	Trace[f]	—	—	—	—	—
32–35	Trace	—	0.4	3	—	3	—	—

[a]Reference 25.
[b]Reference 26.
[c]Contained alkenes: Trace C_{24}, 5% C_{26}, 6% C_{28}, and Trace C_{30}.
[d]Not detected.
[e]Composed of 9% C_{16}, 25% $C_{18:1}$, 20% $C_{18:2}$, Trace C_{22}, 1% C_{24}, and 0.1% C_{25}.
[f]Trace, less than 0.1%.

wax esters, 32% fatty alcohols, 27% FA, and 21% aldehydes, respectively. They also reported compositions of individual wax classes (Table 2). The most abundant homologs of aldehydes, alcohols, and acids were C_{28} and C_{30}, whereas C_{27} and C_{29} were the major alkanes. Biochemical schemes and occurrences among these components have been reviewed by Bianchi (9). Oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) constituted almost half of the detected acids (25,26). In their studies, these FA were included in the wax, since they just dipped the sorghum in chloroform at room temperature and hot hexane, and used the whole extract as wax. Most of the FA with lower melting points were removed by recrystallization using acetone or alcohol or cold filtration as discussed above.

Avato *et al.* (26) also presented the following analysis of the composition of the esters: 1% C_{42}, 2% C_{44}, 3% C_{46}, 1% C_{48}, 1% C_{50}, 1% C_{52}, 4% C_{54}, 1% C_{55}, 19% C_{56}, 1% C_{57}, 37% C_{58}, 1% C_{59}, 25% C_{60}, and 2% C_{62}. This suggested that the esters of grain sorghum wax were mainly composed of acids and alcohols of C_{28} and C_{30}, which were also dominant in free forms.

Implication. Studies in the future related to the chemistry of sorghum wax should include further chemical analyses of the wax to refine the development of column chromatography, TLC, HPLC, and GC methods. Changes in levels of chemical components of waxes, especially aldehydes and acids, owing to autoxidation under various storage conditions need to be determined. Correlations between physical and functional properties with changes in chemical components of the waxes also need to be determined. Application work may now proceed since good methods for analysis exist and the changes of wax can be followed during function.

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