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# Changes in composition and thermal transition temperatures of grain sorghum wax during storage

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**Abstract**

Grain sorghum (*Sorghum bicolor*) wax is composed mainly of aldehydes, alcohols, and acids. Aldehydes, comprising about one-half of the wax, are readily converted to acids in presence of air. In this study, whole sorghum wax and an aldehyde fraction from sorghum wax were subjected to oxidative conditions. Changes in the major components and thermal transition temperatures were determined using HPLC and differential scanning calorimeter (DSC), respectively. The aldehyde fraction was oxidized markedly to acids over 4 months in storage at room temperature. Acid content, in the fraction, was initially 5–7% and increased to 42–51% after 135 days in storage. Consequently, thermal transition apex and end temperatures of the fraction, which were initially 73–74 and 76–77 °C, respectively, increased to 80–81 and 83–85 °C, respectively, after 135 days. Whole sorghum wax, composed initially of 55% aldehydes, 37% alcohols, and 7% acids, slightly increased acid level to 8–12% during storage over 5 months under various conditions. Thermal transition temperatures of the wax changed little over all storage conditions during 5 months of storage with 83–84 °C for apex temperatures and 86–87 °C for end temperatures.

**Keywords:** aldehyde, composition, grain sorghum wax, melting point, storage, transition temperature

## 1. Introduction

About 0.2% of grain sorghum (*Sorghum bicolor*) can be recovered as wax by extraction with hexane (Lochte-Watson et al., 1999). Wax extracted from grain sorghum has been noted to have physical properties similar to carnauba wax (Weller et al., 2000). Carnauba wax is a major imported vegetable wax in the United States and other countries. Carnauba wax is used in various industries to manufacture polishing wax (cars, shoes, floors, etc.), paper and packaging materials, paints, cosmetics (lipsticks, enamels, etc.), foods (bubble gum, chocolate, fruit and vegetable coatings, cheese coatings, etc.), and pharmaceutical products.

Carnauba wax is known to be mostly composed of wax esters (84–85%) (Hamilton, 1995a). However, the chemistry of grain sorghum wax is not fully defined. Until recently, studies on the chemical composition of grain sorghum wax, with the first published around 1950, were not in agreement (Bunger and Kummerow, 1951; Cannon and Kummerow, 1957; Dalton and Mitchell, 1959; Seitz, 1977; Bianchi et al., 1979; Avato et al., 1990). Only Bianchi et al. (1979) and Avato et al. (1990), among the references listed, reported that aldehydes were one of the major components in grain sorghum wax. Hwang et al. (2002a) recently confirmed that the major components in the sorghum wax were aldehydes, alcohols, and acids. Their studies indicated that the major components of grain sorghum wax were not wax esters, compounds that have been traditionally considered as wax. In particular, high concentration of aldehydes is a unique chemical characteristic of grain sorghum wax.

HPLC has not been widely used to analyze waxes due to the low solubility of the waxes in solvents at room temperature, the lack of UV-detectable materials in the waxes, and the difficulties in separation of the components (Hamilton, 1995b). Hwang et al. (2002b) developed an HPLC method using a silica column and an evaporative light scattering detector (ELSD) to analyze the major components of sorghum wax. They reported that grain sorghum wax was composed of 46% (w/w) aldehydes, 41% alcohols and 8% acids. Aldehydes were not detected in carnauba wax (Hwang et al., 2002b).

Aldehydes in grain sorghum wax are expected to be easily oxidized to form acids when exposed to air before and after application of the wax on various surfaces, whereas the other components are stable. Autoxidation of aldehydes to acids in sorghum wax is different from the traditional autoxidation in highly unsaturated lipids, which typically affects rancidity, odor, and flavor of lipid-containing materials. Because aldehydes in the wax are mostly long-chained, the converted acids are not expected to affect odor and flavor. Also, because the wax most likely consists of saturated compounds (Bianchi et al., 1979; Avato et al., 1990), the traditional autoxidation usually taking place around double bonds is not likely to occur.

Oxidation of the aldehydes in the wax may alter the composition of the wax by conversion of the al-dehydes to acids. Thermal transition temperature of an aldehyde is lower than that of an alcohol or acid with the same number of carbons (Linstromberg and Baumgarten, 1974). Consequently, the oxidation of the aldehydes may affect the thermal transition temperatures of the wax.

In this study, grain sorghum wax and an aldehyde fraction from the wax were stored in contact with air under various storage conditions. Determination of changes in composition and thermal transition temperatures of the sorghum wax and its component aldehydes under these various conditions was the objective of this study.

## 2. Materials and methods

### 2.1. Materials

The grain sorghum was Golden Harvest H512, grown and harvested in the 1996 and 1999 crop years in Nebraska. Carnauba wax (T-1) was obtained from Strohmeyer & Arpe Co., Inc. (Short Hills, NJ).

### 2.2. Extraction of wax from grain sorghum

Wax was extracted and collected from 10 subsamples of grain sorghum as previously reported (Hwang et al., 2002a). Washed and dried grain sorghum (800 g) was refluxed with 800 ml hexane for 30 min. The contents were filtered through coffee filter paper lying on top of a Whatman no. 2 filter paper. The filtrate was placed in a −18 °C freezer for at least 8 h. Wax precipitate was collected by filtering the cold miscella with a Whatman no. 42 filter paper and desolvented under vacuum. Three subsamples of the collected wax were evaluated for composition, two subsamples were evaluated for thermal transition temperatures, and five subsamples underwent fractionation.

### 2.3. Fractionation of grain sorghum wax components

Major grain sorghum wax components were fractionated and collected from subsamples of the collected wax using column chromatography (Hwang et al., 2002a) with silica gel (particle diameter: 2–25 µm; average pore diameter: 60 Å, Aldrich, Milwaukee, WI).

The eluting solvent mixtures were applied on the column in the sequence of 50 ml hexane, 30 ml hexane:chloroform (containing 0.75% ethanol) (29:1), 30 ml hexane:chloroform (5:1), 60 ml hexane:chloroform (1:1), 30 ml hexane:chloroform (1:2), 180 ml chloroform, 102 ml chloroform:acetic acid (50:1), 84 ml chloroform:acetic acid (20:1), and 100 ml methanol. Fractions were identified using TLC (silica gel, 20 cm × 20 cm, particle size: 250 µm, Aldrich) with a developing solvent of hexane:diethyl ether:acetic acid (85:15:2) following a previous report (Hwang et al., 2002a). The fractions were stored in the eluted solvent at −18 °C. Solvent in the fractions was removed using a nitrogen stream before storage trials. Three subsamples of collected aldehyde fraction were evaluated for composition and two subsamples were evaluated for thermal transition temperatures.

## 2.4. Storage of aldehyde fraction of sorghum wax and whole sorghum wax

The aldehyde fraction was redissolved in hexane (Ald–Hex) or chloroform (Ald–Chl). The solvent was removed using a nitrogen stream. It was stored at room temperature, in sunlight, for up to 135 days. Grain sorghum wax samples were stored at room temperature and at an elevated temperature (45 °C ± 1.0). For the room temperature sample, a part was

stored in the dark (Wax-R-D) for up to 161 days and the other part was stored in the dark for 15 days and exposed to sunlight (Wax-R-S) for another 146 days. For the elevated temperature sample, one part was stored at 45 °C in the dark (Wax-A-H) for up to 161 days. The other part was stored at 45 °C in the dark for 25 days and redissolved with chloroform on the 25th day followed by solvent removal using a nitrogen stream. This was re-stored again at 45 °C in the dark (Wax-A-C) for another 136 days. Storage of sorghum wax for 5 months was anticipated to be enough time to monitor the changes in composition and transition temperatures of the wax as related to quality because 5 months is an acceptable period for storage and transportation of wax after extraction before use. The storage conditions for the samples are summarized in Table 1.

## 2.5. HPLC of sorghum wax

The major components in sorghum wax were determined using HPLC as previously reported (Hwang et al., 2002b): Two Waters 510 HPLC pumps (Waters Corp., Milford, MA) were operated in gradient modes using mixtures of hexane, methyl *tert*-butyl ether and acetic acid as mobile phases. Flow rate of

**Table 1.** Extraction methods and storage conditions of sorghum wax samples

| Sample | Sample code | Pretreatment | Storage condition | | |
| | | | Temperature | Sunlight/ dark | Lengths |
| --- | --- | --- | --- | --- | --- |
| Aldehyde fraction separated from grain sorghum wax using column chromatography | Ald–Hex | Dried fraction was redissolved in hexane, and the solvent was evaporated | Room temperature | Sunlight | Up to 135 days |
| | Ald–Chl | Dried fraction redissolved in chloroform, and the solvent was removed | Room temperature | Sunlight | Up to 135 days |
| Grain sorghum wax (extracted using hexane) | Wax-R-D | Dried powder wax | Room temperature | Dark | Up to 161 days |
| | Wax-R-S | Dried powder wax was stored in dark at room temperature for 15 days | Room temperature | Sunlight | Up to another 146 days |
| | Wax-A-H | Dried powder wax | 45 °C | Dark | Up to 161 days |
| | Wax-A-C | Dried powder wax was stored in dark at 45 .C; on 25th day it was redissolved in chloroform, and the solvent was removed | 45 .C | Dark | Up to another 136 days |

mobile phase was 1 ml/min. Column was a Luna 5µ silica column (250 mm $L$×4.6 mm i.d.; Phenomenex, Torrance, CA) connected to a guard column (4 mm $L$×3 mm i.d. silica cartridge in a SecurityGuard cartridge system; Phenomenex). The column and guard column were heated at 40 °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 (Rockville, MD) operated at 50 °C with nitrogen pressure of 930 kPa. All the standards and samples were dissolved in hexane and 100 µl of each injected.

## 2.6. Determination of thermal transition temperatures

Thermal transition temperatures of sorghum wax and the major fractions of the wax were measured using a Pyris 1 Perkin–Elmer differential scanning calorimeter (DSC) (Norwalk, CT) according to ASTM D 4419-90 (ASTM, 2000) with minor modification as follows: 10 mg (±1 mg) of sample were weighed in an aluminum pan (Part No. 122292 and 100793; TA Instruments, New Castle, DE). The sample pan and an empty reference pan were placed in the DSC sample and reference compartments. The test cell was precycled by holding it for 1 min at 30 °C, heating it from 30 to 105 °C at 10 °C/min, and cooling it to 30 °C at 10 °C/min. After holding the test cell for 1 min at 30 °C, it was heated from 30 to 105 °C at 10 °C/min and held for 1 min at 105 °C. DSC curve of the last heating cycle was recorded. Transition peak apex temperature was obtained by the intersection of tangents to slopes of the largest peak. The onset and end temperatures were located by the intersection of the peak tangents with the base line.

## 2.7. Statistical analysis

Composition values of the aldehyde fractions and the waxes were means of three determinations. Thermal transition temperatures were means of two determinations. For aldehyde fraction means, between-subjects effects were observed with a two treatments for 5 days factorial design and the means in each treatment were compared performing one-way ANOVA using SPSS for Windows Release 10.0.5 (1999) Standard Version (SPSS, Inc., Chicago, IL). Tukey's HSD

test was employed for comparison of means where significant differences occurred between days. For wax means, one-way ANOVA was performed to compare the means of 13 treatment-day combinations. Tukey's HSD test was employed for comparison of means where significant differences occurred between combinations. The significant differences were determined at $P < 0.05$.

## 3. Results and discussion

### 3.1. Thermal transition temperatures of carnauba and grain sorghum waxes

Thermal transition temperatures of carnauba and grain sorghum waxes were measured to determine similarities in thermal properties. The DSC curves for carnauba and sorghum waxes were considerably different (Figure 1). Transition peak of carnauba wax was wider than that of sorghum wax and had a shoulder on the left slope of the peak. Thermal transition onset, apex and end temperatures of carnauba wax were 73.6 °C (±0.1 °C), 86.5 °C (±0.1 °C), and 88.4 °C (±0.1 °C), respectively, whereas those of grain sorghum wax were 77.0 °C (±0.1 °C), 83.6 °C (±0.2 °C), and 86.3 °C (±0.2 °C). Carnauba wax started melting at a lower temperature and ended melting at a higher temperature than grain sorghum wax. The difference in thermal transitions of the two waxes was attributed to the totally different compositions of the two waxes (Hwang et al., 2002a, 2002b). Wax esters are the major components in carnauba wax, whereas aldehydes and alcohols are the major components in sorghum wax. The fact that sorghum wax has a narrower melting temperature range than carnauba wax would give advantage to sorghum wax in applications where a fairly defined melting or softening temperature is desirable.

### 3.2. Composition and thermal transition temperatures of aldehyde fraction from sorghum wax during storage

The aldehyde fraction, separated from wax using column chromatography, was dissolved in hexane or chloroform, and the solvent was removed (aldehydes were eluted with chloroform plus a small amount of hexane in column chromatography; Hwang et al.,
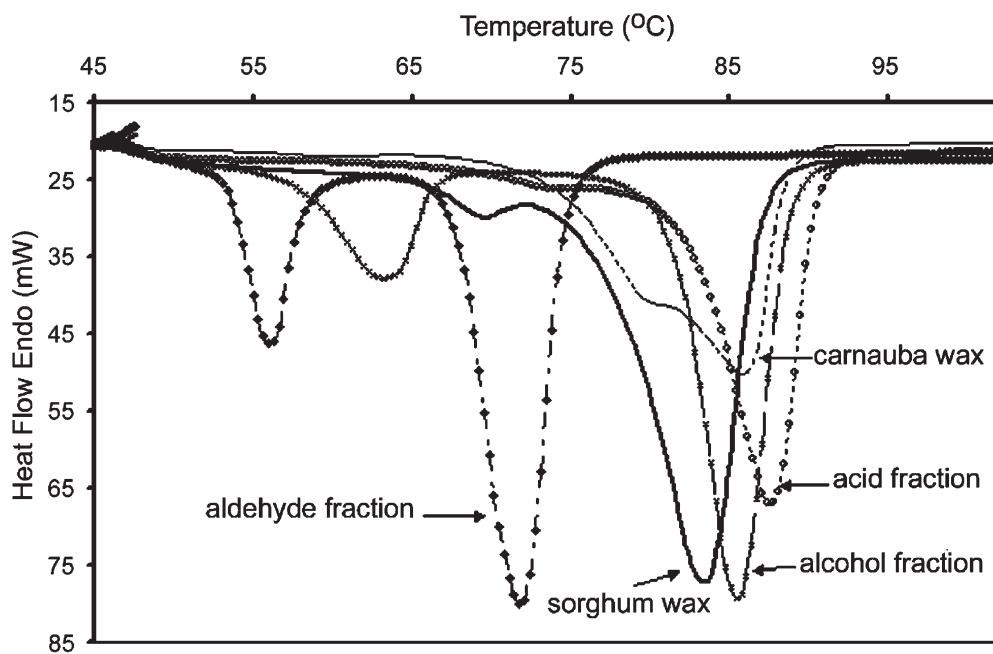
**Figure 1.** DSC curves of carnauba wax, sorghum wax, and major fractions of sorghum wax.

2002a). When the fractionating solvent (mostly chloroform) was removed from the aldehyde fraction, a faster oxidation of the fraction was noticed in a preliminary study, compared with the aldehyde fraction that was desolvented, redissolved with hexane, and desolvented again. This was the reason for redissolving the aldehyde fraction in hexane and in chloroform for comparison, and the reason for redissolving sorghum wax in chloroform.

The hexane and chloroform redissolved aldehyde fractions (Ald–Chl) initially contained 5 and 6.8% acids, respectively, and the rest was aldehydes (Table 2). Acid content increased slowly at room temperature and in sunlight over the 135 days in storage. The chloroform-redissolved fraction (Ald–Hex) showed significantly ($P < 0.05$) higher acid production than the hexane-redissolved fraction (Ald–Chl) on Day 135, reaching 50.9% acids in the former and 41.7% acids in the latter. Whole sorghum wax and aldehyde fraction were more easily dissolved in chloroform than in hexane. The faster oxidation of the chloroform-redissolved aldehyde fraction may have been due to the arrangement of aldehyde groups in a form more exposed to the air when the aldehyde fraction was dissolved once with chloroform than with hexane. Chloroform being more polar than hexane might orient the more polar aldehyde groups outwards.

Thermal transition onset, apex, and end temperatures of aldehyde fraction from sorghum wax were initially 68–69, 73–74, and 76–77 °C, respectively (Figure 1). Thermal transition apex temperatures of acid and alcohol fractions were 87.8 and 85.7 °C, respectively. It was expected that the oxidation of aldehydes to acids would increase thermal transition temperatures of the aldehyde fraction with time. However, thermal transition temperatures of aldehyde fraction changed little during 21 days of storage (Table 2, Figure 2), because the conversion level of aldehydes to acids was low. Thermal transition apex and end temperatures of the hexane-redissolved aldehyde fraction increased significantly ($P < 0.05$) during 135 days of storage, with a remarkable increase in acids (Table 2). Onset temperature (66.7 °C) of the hexane-redissolved aldehyde fraction (Ald–Hex) on day 135 was lower than the initial temperature (69.1 °C), showing wider transition peak (Figure 2). Thermal transition onset, apex and end temperatures of the chloroform-redissolved aldehyde fraction increased significantly ($P < 0.05$) during 135 days of storage. The chloroform-redissolved aldehyde fraction showed significantly ($P$
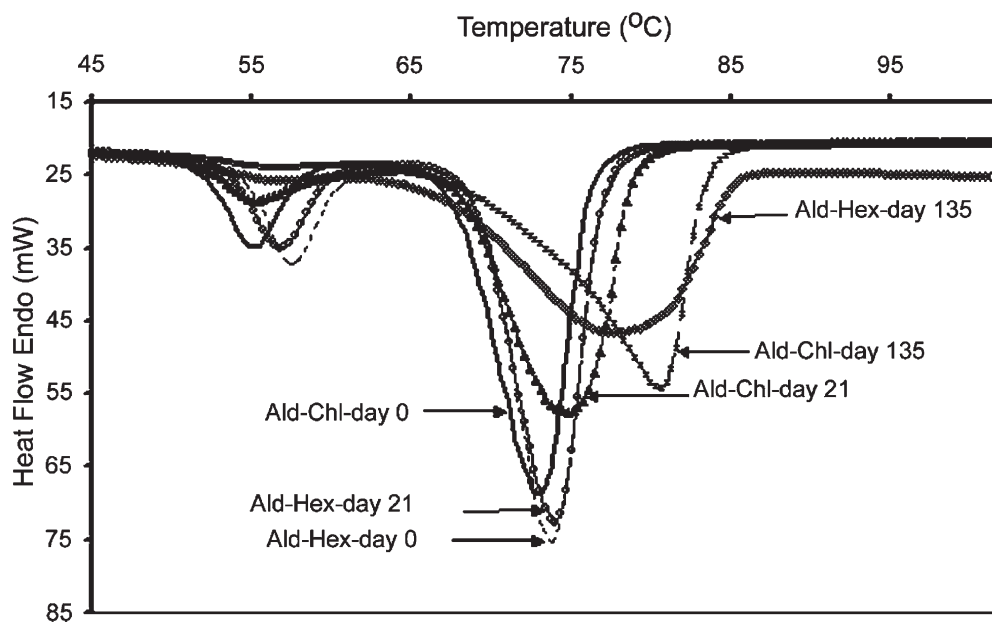
**Figure 2.** DSC curves of aldehyde fractions during storage at room temperature and in sunlight. See Table 1 for sample codes.

**Table 2.** Changes in composition and thermal transition temperatures of aldehyde fraction of grain sorghum wax during storage at room temperature in sunlight

| Sample[a] | Storage length (days) | % (w/w)[b] | | | | Thermal transition temperatures (°C)[c] | | |
|---|---|---|---|---|---|---|---|---|
| | | Aldehydes | Acids | Alcohols | Hydrocarbons | Onset | Apex | End |
| Ald–Hex | 0 | 95.0 a | 5.0 a | –[d] | – | 69.1 a | 74.0 a | 76.9 a |
| | 7 | 95.0 a | 5.0 a | – | – | 68.7 a | 73.6 a | 75.9 a |
| | 14 | 93.4 b | 6.6 b | – | – | 68.8 a | 74.0 a | 77.0 a |
| | 21 | 90.6 c | 7.5 b | – | 1.9 a | 68.9 a | 74.3 a | 76.9 a |
| | 135 | 49.8 d | 41.7 c | 4.1 | 4.5 b | 66.7 b | 79.6 b | 85.1 b |
| Ald–Chl | 0 | 93.2 a | 6.8 a | – | – | 67.5 a | 73.3 a | 76.1 a |
| | 7 | 91.5 a | 8.5 a | – | – | 67.6 a | 74.5 ab | 77.4 ab |
| | 14 | 89.4 a | 10.6 ab | – | – | 67.9 a | 75.0 ab | 78.3 b |
| | 21 | 81.7 b | 14.2 b | 2.2 a | 1.8 a | 68.0 a | 75.4 b | 78.9 b |
| | 135 | 41.5 c | 50.9 c | 3.7 b | 3.8 b | 71.5 b | 81.3 c | 83.4 c |
| Tests of between-subjects effects (*P* value) | | | | | | | | |
| Samples | | 0.000 | 0.000 | 0.000 | 0.007 | 0.703 | 0.003 | 0.022 |
| Days | | 0.000 | 0.000 | 0.000 | 0.000 | 0.022 | 0.000 | 0.000 |
| Samples × days | | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.044 | 0.000 |

Means in each column in each sample with the same letters a–d are not significantly different *(P ≥ 0.05)*.

[a] See Table 1 for sample codes.

[b] Mean of three determinations.

[c] Mean of two determinations.

[d] Undetectable.

< 0.05) higher thermal transition temperatures than the hexane-redissolved fraction on day 135 and significantly ($P < 0.05$) higher acid level in the chloroform-redissolved fraction (Table 2). Industrial users who want higher melting point of a wax may, for example, at some point extract or dissolve sorghum wax in chloroform prior to incorporation in or on a product.

## 3.3. Composition and thermal transition temperatures of sorghum wax during storage

The sorghum wax sample, for the storage test, was initially composed of 55.4% aldehydes, 7.4% acids, and 37.2% alcohols (Table 3). The composition of the wax changed little during 5 months of storage in the dark and at room temperature (Wax-R-D). Exposure of the wax to sunlight for about 5 months at room temperature (Wax-R-S) slightly, but significantly ($P < 0.05$), increased the oxidation of aldehydes to acids (Table 3).

Hydrocarbons were not detected in the samples stored at room temperature (Wax-R-D and Wax-R-S) except for the sample stored in sunlight (Wax-R-S) for about 5 months. Storage of the wax at 45 °C for 42 days in the dark (Wax-A-H) did not notably affect the composition of the wax. The sorghum wax tested in the study was originally extracted with hexane. The aldehyde fraction of sorghum wax redissolved with chloroform (Ald–Chl) reached higher levels of acids sooner than hexane-redissolved aldehyde fraction (Ald–Hex) (Table 2) as discussed previously. However, whole wax did not show a noticeable difference in composition based on the redissolving solvents (Table 3). Sorghum wax stored at the elevated temperature (45 °C) in the dark (Wax-A-H) for about 5 months showed a slight significant ($P<0.05$) increase in acids and produced hydrocarbons (Table 3). Hydrocarbons were not detected in the wax sample that was redissolved in chloroform and stored at the elevated temperature (Wax-A-C) for about 5 months.

The observed difference in acid formation between grain sorghum wax and the aldehyde fraction over time most likely can be attributed to the exposed area of the material to air. Assuming the wax and fraction were both in a powder form and of relatively the same particle size, proportionally more aldehydes in the aldehyde fraction would be exposed to air than aldehydes in the grain sorghum wax. In the wax, aldehydes are commingled with alcohols, acids, and other components such that less is exposed to air leading to

**Table 3.** Changes in composition and thermal transition temperatures of grain sorghum wax during storage

| Sample[a] | Storage length (days) | % (w/w)[b] | | | | Thermal transition temperatures (°C)[c] | | |
|---|---|---|---|---|---|---|---|---|
| | | Aldehydes | Acids | Alcohols | Hydrocarbons | Onset | Apex | End |
| Initial sample | | 55.4 a | 7.4 a | 37.2 a | –[d] | 77.0 a | 83.6 a | 86.3 a |
| Wax-R-D | 15 | 55.8 a | 7.8 ab | 36.3 a | – | 77.0 a | 83.3 a | 86.3 a |
| | 161 | 57.9 a | 7.8 ab | 34.2 a | – | 77.1 a | 83.5 a | 86.7 a |
| Wax-R-S | 9 | 55.3 a | 8.0 ab | 36.7 a | – | 77.4 a | 83.7 a | 86.2 a |
| | 27 | 56.7 a | 8.5 ab | 34.9 a | – | 77.1 a | 83.6 a | 86.4 a |
| | 146 | 47.9 b | 12.3 e | 39.7 a | 2.4 a | 76.8 a | 83.5 a | 86.4 a |
| Wax-A-H | 9 | 55.9 a | 9.1 abc | 35.0 a | – | 76.9 a | 83.3 a | 86.2 a |
| | 24 | 57.7 a | 8.2 ab | 34.2 a | – | 76.7 a | 83.5 a | 86.1 a |
| | 42 | 54.7 ab | 8.5 ab | 36.8 a | – | 76.6 a | 83.5 a | 85.9 a |
| | 161 | 54.6 ab | 11.0 cde | 34.4 a | 2.6 a | 77.0 a | 83.6 a | 86.4 a |
| Wax-A-C | 10 | 56.4 a | 8.4 ab | 35.2 a | – | 76.5 a | 83.4 a | 86.1 a |
| | 17 | 54.7 ab | 9.5 bcd | 35.8 a | – | 76.4 a | 83.5 a | 86.0 a |
| | 136 | 54.3 ab | 11.5 de | 34.3 a | – | 76.6 a | 83.0 a | 86.7 a |

Means in each column with the same letter a–e are not significantly different *(P ≥ 0.05)*.
[a] See Table 1 for sample codes.
[b] Mean of three determinations.
[c] Mean of two determinations.
[d] Undetectable.

less oxidation than in the aldehyde fraction. Further research is necessary to confirm this postulation.

Minor changes in composition of the whole sorghum wax during storage did not significantly ($P \geq 0.05$) influence the thermal transition temperatures of the wax, which ranged between 76.4 and 77.4 °C for onset, 83.0 and 83.7 °C for apex, and 85.9 and 86.7 °C for end, over all storage conditions (Table 3). This implies that sorghum wax has stable melting properties during storage for up to 5 months at room and elevated temperatures. Quality factors for final products that are dependent on stability of sorghum wax over time should be consistent. Further study is needed on performance of sorghum wax in products such as coatings and cosmetics. When a wax with a higher melting point is needed, one procedure for increasing it might be by developing a method to oxidize aldehydes to acids in sorghum wax.

## 4. Conclusion

The aldehyde fraction separated from sorghum wax showed notable conversion to acids and changes in the thermal transition temperatures during storage. In whole grain sorghum wax, however, a minor change in chemical composition of the wax was observed without affecting the thermal transition temperatures of the wax. It is noteworthy to mention that sorghum wax was very stable during storage even though it contained a large amount of aldehydes. This study will facilitate the utilization of sorghum wax for human and industrial uses by increasing the understanding of chemical and physical changes in grain sorghum wax during storage. Although sorghum wax is different from carnauba wax in composition, it has similar melting characteristics. The differences of sorghum wax over carnauba wax may provide advantages in industrial uses. Examining functional properties of sorghum wax, especially as a substitute for carnauba wax in part, industrial uses need to be the next research area of sorghum wax.

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