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Supercritical carbon dioxide extraction, purification, and characterization of wax from sorghum and sorghum by-products as an alternative natural wax

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Abstract

The objective of this work was to obtain high purity natural wax from sorghum and by-products of sorghum processing (sorghum dried distillers grains with solubles [DDGS] and sorghum bran) using a green process based on supercritical carbon dioxide (SC-CO_2). SC-CO_2 extractions were carried out at varying temperatures (50, 70°C) and pressures (30, 40 MPa) at a CO_2 flow rate 1 L/min for 120 min. Significantly higher wax yield (4.9%) from DDGS was obtained by SC-CO_2 at 40 MPa/70°C compared with whole kernel (0.6%) and bran (3.3%) ($p < 0.05$). The yield of the extracts obtained by SC-CO_2 extraction was higher than that of the conventional hexane extraction for all three sorghum sources. The highest fraction of wax in the SC-CO_2 extracts was obtained from whole kernel extracts (89%), whereas it was 53.3% from the DDGS and 26.8% from the bran at the same extraction conditions. SC-CO_2 and hexane extracts from sorghum whole kernel shared a similar melting peak temperature of 76.3–77.9 and 79.7°C, respectively, while DDGS and bran extracts by SC-CO_2 showed a much lower melting temperature in the range of 50.7–61.9°C, indicating the presence of lower melting point components such as triacylglycerols. However, the melting points of the DDGS and bran extracts after ethanol purification were significantly increased with the observed peak temperature of 80.8 and 82.0°C, respectively. While the wax yield from DDGS and bran was higher than that of whole kernel, the sorghum whole kernel feedstock was found to be a more feasible feedstock to obtain higher purity wax.

KEYWORDS
extraction, purification, sorghum, supercritical carbon dioxide, wax

INTRODUCTION

The global wax market was over USD 7.3 billion in 2020 and is projected to reach USD 8.9 billion by 2025, at a compound annual growth rate of 4.0% from 2020 (https://www.marketsandmarkets.com). There is a demand for alternative wax sources due to growing industrial, food, pharmaceutical, and cosmetic applications of waxes, dwindling waxes supply, and increasing wax prices. There is a shift toward eco-friendly alternative natural waxes in industry due to growing consumer preference for "natural" products in food, food packaging, and cosmetics applications. For example, the use of natural waxes in food packaging industry is increasing due to health concerns regarding paraffin wax. Similarly, due to the growing concerns about health and wellness, the demand for natural waxes in the cosmetic products is growing in the developed countries. Currently, United States imports Carnauba wax from Brazil and Candelilla wax from Mexico to meet natural wax demands. Carnauba wax, which is obtained from Brazilian palm, is currently the major commercially imported wax. Therefore, there is a need for alternative wax sources that can be produced and processed in the United States.

Grain sorghum is a promising alternative natural wax source that has been proposed as a domestically

grown, viable substitute for Carnauba or as a similar additive (Hwang et al., 2002). The 2018 production of grain sorghum in the United States was 9.24 million metric tons (National Agricultural Statistics Service, 2018), which makes the United States the largest producer and exporter of grain sorghum in the world, consisting of approximately 80% of the total domestically produced crop exported to China (Foreign Agricultural Service/USDA, 2018).

In the United States, grain sorghum is primarily used for ethanol production (Harron et al., 2017). Ethanol production generates sorghum dried distillers grains with solubles (DDGS). DDGS is the dried fraction obtained after the starch in sorghum is fermented to produce ethanol. Then, ethanol is separated by distillation, and the wet material is centrifuged to separate solids from the solubles. The solubles are concentrated by evaporation and dried with the solids to obtain DDGS (Ciftci et al., 2012). Another utilization of sorghum is flour production, which generates sorghum bran as a co-product. Sorghum has been globally marketed for food consumption due to its gluten-free, high antioxidant properties (Awika et al., 2005). Sorghum kernel itself and also sorghum processing by-products such as sorghum DDGS from ethanol production and sorghum bran from flour production are promising sources for natural wax.

Despite the potential of sorghum and its processing by-products to be a sustainable natural wax source, several challenges must be overcome for sorghum wax to be brought to the market. These challenges are: (i) lack of information on the effect of sorghum feedstock on wax extraction and wax quality; (ii) absence of a separation process to obtain pure sorghum wax that meets natural wax industry specifications; and (iii) absence of a "benign" extraction method that will generate wax for food and cosmetics applications. Without bringing solutions to the outlined challenges, sorghum wax's potential will remain untapped. So far, various organic solvents have been used to extract waxes from sorghum, including hexane (Hums et al., 2018; Hwang et al., 2004; Lochte-Watson et al., 2000), chloroform (Jenks et al., 2000), petroleum ether (Saraiva, 1995), ethanol (Hums et al., 2018; Nghiem et al., 2018; Weller et al., 2000), and methanol (Hums et al., 2018). However, the use of the above-mentioned solvents results in considerable amounts of solvent waste that are mostly toxic and not environmentally benign (Sin et al., 2014). Although a few studies reported grain sorghum wax in terms of its recovery, physicochemical properties of the extracts, and the applications of the extracts (Hwang et al., 2002), there is no specific processing method to obtain high purity sorghum wax to meet industry's natural wax standards.

So far, Athukorala and Mazza (2010) extracted wax from triticale straw using SC-CO_2. They reported that the triticale wax extracted by SC-CO_2 had thermal properties similar to the one extracted by hexane. In another study, Morrison et al. (2006) used SC-CO_2 to extract cuticular wax from flax processing waste with the aid of ethanol as a co-solvent. Their results showed that the SC-CO_2 extraction yielded 7.4% wax compared with 4.0% with hexane extraction. So far, only conventional solvents have been investigated for wax extraction from sorghum feedstocks (Hums et al., 2018; Hwang et al., 2004; Jenks et al., 2000; Lochte-Watson et al., 2000; Nghiem et al., 2018; Saraiva, 1995; Weller et al., 2000). To the best of our knowledge, there is no reported study on the extraction and purification of sorghum wax using SC-CO_2.

In this study, it was hypothesized that SC-CO_2 can extract higher purity wax from sorghum and its by-products that have similar melting properties to that of Carnauba wax. The specific objectives were to: (i) determine the effect of extraction method, namely, hexane extraction and supercritical carbon dioxide (SC-CO_2) extraction, on the extract yield and quality; (ii) determine the effect of SC-CO_2 extraction on the amount of wax yield and quality of the extracts obtained from different sorghum feedstocks, namely, sorghum whole kernel, sorghum DDGS, and sorghum bran; (iii) determine the wax content of the SC-CO_2 extracts from sorghum whole kernel, sorghum DDGS, and sorghum bran; and (iv) purify the wax extracts using a simple purification process.

MATERIALS AND METHODS

Materials

Whole sorghum kernel and sorghum bran were obtained from a local farm in Nebraska, NE. Sorghum DDGS was obtained from Western Plains Energy LLC (Oakley, KS). The sorghum feedstocks were sealed and stored at −20°C until used in the described extractions. High purity (99.99% purity) liquid CO_2 was purchased from Matheson (Lincoln, NE). Solvents and reagents were acquired from Fisher Scientific International, Inc. (Hampton, NH) and were of HPLC grade.

Hexane extraction

Whole sorghum kernel, sorghum DDGS, and sorghum bran were extracted using a hexane reflux method. Approximately 150 ml of hexane and 100 g of sorghum feedstocks were mixed in a 5-L round bottom flask that was coupled with a condenser to return the evaporated hexane into the flask continuously. The flask was heated at 65°C for 1 h. After 1 h of extraction, the sorghum and hexane mixture was vacuum filtered through a Whatman No. 2 filter paper. The filtrate was stored at −20°C for 24 h to precipitate the wax fraction. After cold-storage, the solution was vacuum filtered through

a Whatman No. 42 filter paper. The filter paper that included the solid wax fraction was removed and allowed to dry at room temperature for 2 h. The dried wax along with filter paper was weighed, and the wax yield was expressed as the mass percentage of the sorghum feedstock used for extraction.

SC-CO_2 extraction

SC-CO_2 extractions were carried out in a laboratory-scale supercritical fluid extraction system (SFT 110, Supercritical Fluids, Inc., Newark, DE). The details and operation of the system were reported somewhere else (Belayneh et al., 2017).

Sorghum feedstock (approximately 60 g of sorghum whole kernel, 30 g of sorghum bran, and 30 g sorghum DDGS) was loaded into the extraction vessel, and the air in the vessel was flushed out by opening the CO_2 cylinder before each run. The shut-off valve was closed and the extraction vessel was heated while controlling its temperature with the oven that had a built-in temperature controller. After reaching the set extraction temperature, CO_2 was pumped into the extraction vessel using the CO_2 pump. Extraction pressure was monitored and maintained at a constant using the CO_2 pump. The system was operated in a semi-continuous mode where SC-CO_2 continuously flowed through the extraction vessel that was packed with the feedstock. A static extraction time of 20 min was employed by keeping the shut-off valve closed. Then, the shut-off valve was opened and the extracts were collected continuously in a glass sample collection vial held in a cold trap at -10°C. Extractions were performed at 50 or 70°C and 30 or 40 MPa pressure. Samples were collected every 30 min for a total extraction time of 210–300 min. At the end of each sampling time, a new vial replaced the previous one. The CO_2 flow rate was maintained at 1 L/min (measured at ambient conditions) with a heated micrometering valve, and measured by the gas flow meter placed after the sample collection vial. The amount of each fraction was determined gravimetrically, and the extract yield was expressed as mass percentage of the sorghum feedstock used in the extraction. The extracts were stored at -20°C after extraction until analyzed.

Purification of wax

The sorghum extracts obtained from whole kernel, DDGS, and bran by SC-CO_2 at 40 MPa/70°C were mixed with ethanol at a ratio of 1:4 (extract:ethanol, w/v). The mixture was placed into a 50-ml centrifuge tube and vortexed for 5 min. The centrifuge tubes containing mixtures of samples and ethanol were then centrifuged at 4000 $\times$ *g* at 4°C for 5 min. Then, the supernatant was decanted and the same washing procedure was repeated four times. Then, the solid sample was dried in an oven at 40°C to remove ethanol completely. The amount of dried sorghum wax after purification was determined gravimetrically.

Melting profile

Melting properties of the sorghum extracts and the purified extracts were analyzed using a differential scanning calorimeter (DSC) (Diamond DSC, Perkin Elmer, Waltham, MA). Sorghum extract samples (5 mg) were placed in an aluminum DSC pan and hermetically sealed. An identical sealed empty aluminum pan was used as a reference. The pans were placed in the DSC and heated to 100°C at 5°C/min and kept at 100°C for 10 min, then cooled down to 40°C and kept at this temperature for 10 min. Then, the samples were heated again from 40 to 100°C at 5°C/min to obtain the melting curve of the samples.

Wax characterization

Wax characterization was performed using a reverse-phase HPLC (Agilent 1290 Infinity II series, Agilent Technologies, Inc., Santa Clara, CA) equipped with an evaporative light scattering detector (ELSD) according to Hums et al. (2018) with some modification. Wax extracts were dissolved in chloroform and an aliquot (10 μl) was injected onto a YMC column (250 mm $\times$ 4.6; 5 μm particle size, Leonberg, Germany) that was held at 40°C. A gradient system of solvent A (99.9% methanol and 0.1% formic acid) and solvent B (99.9% chloroform and 0.1% formic acid) was used as mobile phase at a flow rate of 0.65 ml/min. The gradient elution profile was started with 80% A and 20% B and changed to 20% A and 80% B over 10 min and then held at 20% A and 80% B for 20 min, and then changed to 80% A and 20% B in 15 min, and held at 80% A and 20% B for 10 min. The ELSD was operated at a nebulizer pressure of 3.5 bar and a temperature of 40°C.

A calibration curve was prepared using known quantities of commercial refined corn oil, and the wax extracted from whole sorghum grain by hexane as described in "Hexane extraction" section. A polar fraction was obtained by ethanol extraction from camelina oil. Camelina oil was extracted from camelina seed in the lab using the procedure of Belayneh et al. (2015). Standards to prepare calibration curves were generated using different concentrations of polar fraction, oil, and wax in chloroform as described by Hums et al. (2018). The weight percentage of each compound in the sample was calculated from the calibration curves. The yield of the chloroform-soluble materials was then

multiplied by the weight percentage of wax analyzed to obtain the yield of wax for each sample.

Statistical analysis

Data are presented as mean ± SD based on triplicate experiments and analyses. A single factor ANOVA was used to analyze differences in extracts yield among extraction conditions and feedstocks, DSC data (onset melting temperature, peak melting temperature, offset melting temperature, and enthalpy), and in composition among the sorghum extracts. SAS version 9.3 was the statistical software package used for all analyses (SAS Institute Inc., Charlotte, NC). An alpha level of <0.05 was used to denote significance. A post hoc test was performed using Tukey's multiple comparison.

RESULTS AND DISCUSSION

Effect of extraction parameters on extracts yield

The effect of pressure and temperature on the SC-CO_2 extraction kinetics of the three sorghum feedstocks (whole kernel, DDGS, and bran) is shown in Figure 1. The maximum yield of the sorghum extracts by SC-CO_2 ranged between 0.8% (whole kernel) and 11.9% (bran) depending on the extraction conditions. The lowest yields were obtained from the whole kernel. The maximum yield from whole kernel (0.8%) was obtained at 40 MPa/70°C. The extraction rates of the bran and the DDGS were similar, whereas the extraction rate of the whole kernel was slower than those of bran and DDGS. In the first 150 min of the SC-CO_2 extraction of the whole kernel, higher yields were obtained at 30 MPa/70°C, whereas the yield was higher after 150 min at 40 MPa/70°C. The extract yield of sorghum whole kernel significantly improved when pressure increased from 30 to 40 MPa at 70°C (Figure 2) ($p < 0.05$). This was due to an increase in the density of SC-CO_2, which in turn increased the solvation capacity (Ciftci et al., 2012).

Pressure had a larger impact on the extraction of wax from whole kernel at a higher extraction temperature (70°C) compared with a lower temperature (50°C). There was no significant difference in the extraction yields obtained from whole kernel at 30 MPa/50°C (0.5%) and 40 MPa/50°C (0.4%) ($p > 0.05$); however, the yield significantly increased to 0.8% at 40 MPa/70°C (Figure 2) ($p < 0.05$). The effect of temperature was evaluated at different pressure levels, and it was observed that at relatively low pressure, temperature did not have a significant effect on the extracts yield (Figure 2) ($p > 0.05$). However, at higher pressure (40 MPa), the yield was significantly higher at an elevated temperature (Figure 2)

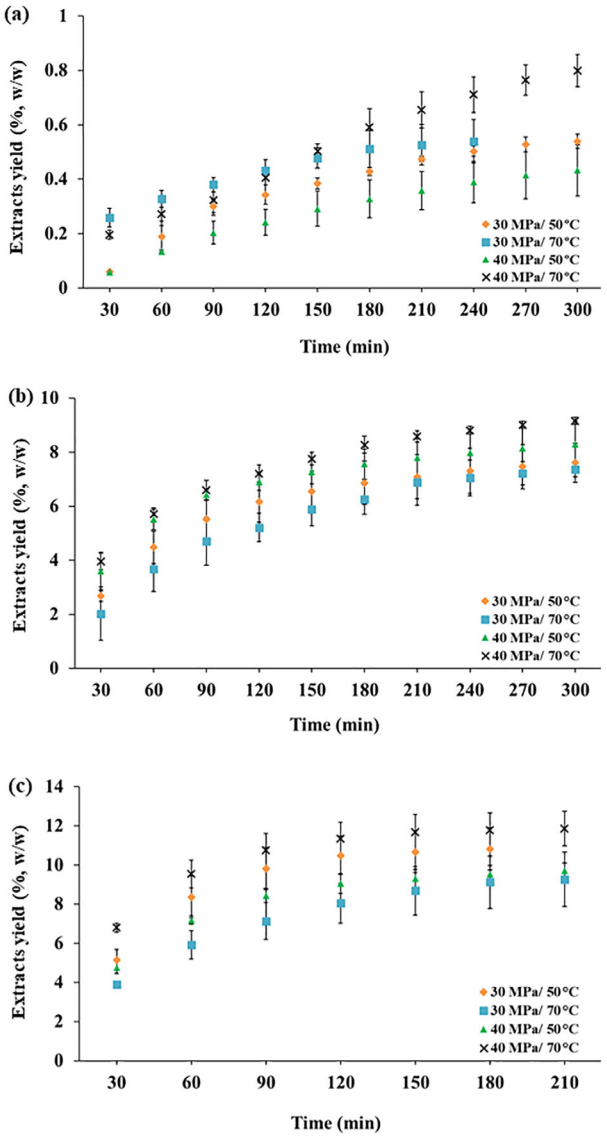

FIGURE 1 Extracts yield (%, w/w) from sorghum feedstocks at varying SC-CO_2 conditions. (a) Whole kernel; (b) DDGS; and (c) bran

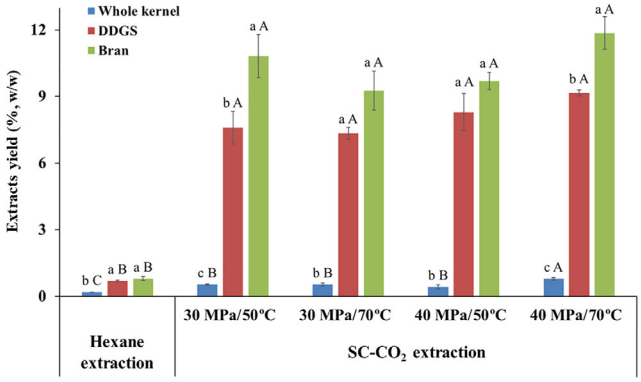

FIGURE 2 Extracts yield (%, w/w) from sorghum feedstocks by hexane and SC-CO_2 extractions. Means with different lowercase letters among sorghum feedstocks at the same extraction condition, and means with different uppercase letters within the same sorghum feedstocks among conditions are significantly different ($p < 0.05$). DDGS, dried distillers grains with solubles

($p < 0.05$). The solubility of the lipids in SC-CO_2 is a function of the solvent density and solute vapor pressure, and this relationship results in a crossover behavior. The crossover behavior of solubility isotherms observed in Figure 1 is attributed to two factors: an increase in wax solubility with temperature due to increased solute vapor pressure as well as a decrease in wax solubility due to the decreased CO_2 solvent density (Guclu-Ustundag & Temelli, 2004). Therefore, the "vapor pressure effect" predominated over "solvent density effect" at a higher temperature and pressure combination, yielding a higher extraction efficiency.

SC-CO_2 extraction was found to be a more efficient method to extract oil and/or wax from sorghum feedstocks when compared with hexane extraction (Figure 2). The extracts yield from sorghum whole kernel, DDGS, and bran by hexane extraction was 0.2%, 0.7%, and 0.8%, respectively, which were significantly lower than the SC-CO_2-extracted ones, which were 0.8%, 9.2%, and 11.9%, respectively (Figure 2) ($p < 0.05$). Similar results were previously reported for SC-CO_2 and hexane extraction of wax from triticale straw (Athukorala & Mazza, 2010) and lipids from sorghum DDGS (Wang et al., 2007).

Significantly higher extract yields were obtained from both DDGS and bran samples compared with whole kernel (Figure 2) ($p < 0.05$). Extract yields obtained from sorghum DDGS and bran by SC-CO_2 at all conditions were not significantly different (Figure 2) ($p > 0.05$). The significantly different extract yields between the whole kernel and the DDGS and bran samples were due to the different oil contents of the feedstocks. Naturally, wax is concentrated on the surface of the sorghum kernel. When the whole kernel is extracted with SC-CO_2, only the wax on the surface of the kernel is extracted. However, sorghum DDGS and bran contain triacylglycerol-based oil in addition to wax; therefore, when the sorghum DDGS and bran are extracted, the oil fraction is also extracted with the wax, which results in higher extract yields. The SC-CO_2 extraction was completed in a shorter time for bran (210 min) compared with DDGS (300 min) (Figure 1). This difference can be explained by the differences in the lipid compositions of those feedstocks.

Characterization of the sorghum extracts

The lipid classes (polar, oil, and wax) of the sorghum extracts obtained from the three sorghum feedstocks obtained by both SC-CO_2 and hexane are shown in Table 1. The compositions of the hexane extracts from whole kernel and bran were similar and contained primarily wax followed by polar compounds. The wax content of the hexane extracts from whole kernel and bran was 99.3% and 92.9%, respectively. However, the wax content was significantly lower for the hexane extracts from DDGS (53.1%) ($p < 0.05$). In addition, the hexane

TABLE 1 Composition of chloroform-soluble extracts represented as a weight fraction determined from HPLC-ELSD

Extraction method	Extraction condition	Composition (%, w/w)		
		Polar	Oil	Wax
		Sorghum whole kernel		
SC-CO_2	30 MPa/50°C	$0.3 \pm 0.0_{b}$	$19.4 \pm 0.0_{c}$	$74.8 \pm 0.0_{c}$
	30 MPa/70°C	$0.3 \pm 0.0_{b}$	$19.9 \pm 0.0_{c}$	$89.1 \pm 0.1_{abc}$
	40 MPa/50°C	$0.3 \pm 0.0_{b}$	$18.7 \pm 0.0_{cd}$	$81.4 \pm 0.0_{bc}$
	40 MPa/70°C	$0.3 \pm 0.0_{b}$	$15.6 \pm 0.0_{cde}$	$75.5 \pm 0.1_{bc}$
Hexane	60°C	$0.8 \pm 0.0_{b}$	ND	$99.3 \pm 0.0_{a}$
		Sorghum DDGS		
SC-CO_2	30 MPa/50°C	ND	$49.2 \pm 0.1_{ab}$	$44.5 \pm 0.0_{de}$
	30 MPa/70°C	ND	$44.2 \pm 0.0_{ab}$	$53.3 \pm 0.0_{d}$
	40 MPa/50°C	ND	$51.0 \pm 0.1_{a}$	$42.4 \pm 0.0_{de}$
	40 MPa/70°C	ND	$46.5 \pm 0.0_{ab}$	$53.5 \pm 0.0_{d}$
Hexane	60°C	$6.5 \pm 0.0_{b}$	$40.4 \pm 0.0_{b}$	$53.1 \pm 0.0_{d}$
		Sorghum bran		
SC-CO_2	30 MPa/50°C	$54.3 \pm 0.1_{a}$	$11.1 \pm 0.0_{cde}$	$28.0 \pm 0.0_{ef}$
	30 MPa/70°C	$54.2 \pm 0.1_{a}$	$9.5 \pm 0.0_{de}$	$26.8 \pm 0.0_{ef}$
	40 MPa/50°C	$64.3 \pm 0.0_{a}$	$8.2 \pm 0.0_{e}$	$24.3 \pm 0.0_{f}$
	40 MPa/70°C	$56.1 \pm 0.0_{a}$	$10.8 \pm 0.0_{cde}$	$28.1 \pm 0.0_{ef}$
Hexane	60°C	$7.1 \pm 0.0_{b}$	ND	$92.9 \pm 0.0_{ab}$

Note: Results are expressed as mean ± SD. Means with different subscript letters within the same column are significantly different ($p < 0.05$).
Abbreviation: ND, not detected.

extracts from DDGS contained a significantly higher oil fraction (40.4%) compared with whole kernel (15.6%) and bran (10.8%) ($p < 0.05$).

The compositions of the SC-CO_2 extracts showed that there was no significant effect of extraction conditions on the obtained polar, oil, and wax fractions from the same feedstock. However, the used sorghum feedstocks had a significant effect on the compositions of the extracts. The whole kernel extracts primarily of wax, ranging between 74.8 and 89.1% (w/w). This amount was significantly higher than that of DDGS (42.4%–53.5%) and bran (24.3%–28.1%) extracts ($p < 0.05$), due to SC-CO_2 extracting only the wax on the surface of the whole kernel. SC-CO_2 has limited diffusion into the whole kernel, which resulted in a high purity wax extract. On the other hand, the DDGS and bran contained high amount of easily accessible oil, which was extracted by SC-CO_2. The polar and oil fractions did not exhibit significant differences among the studied SC-CO_2 extraction conditions for all sorghum feedstocks (Table 1). However, the DDGS extracts contained a higher amount of oil ($p < 0.05$) than whole kernel and bran extracts; the bran extracts contained a significantly higher amount of polar compounds ($p < 0.05$) than whole kernel and DDGS extracts.

Table 2 presents the wax content of sorghum feedstocks' extracts obtained by hexane and SC-CO_2 based on the extract yields and compositional analysis. The yield of wax extracted from whole kernel by SC-CO_2 was similar to the extracts yield (Figure 1a) due to extraction of only wax from the kernels' surface. However, DDGS and bran had a significantly higher wax content than the whole kernel. In addition, the results showed significant effects of the SC-CO_2 pressure and temperature on the wax composition ($p < 0.05$). A significantly higher amount of wax from DDGS (4.9%) was obtained by SC-CO_2 extraction at 40 MPa/70°C compared with other SC-CO_2 extraction conditions ($p < 0.05$). The wax content ranged between 3.4% and 3.9% in the extracts obtained at other SC-CO_2 extraction conditions. SC-CO_2 extractions yielded significantly higher wax contents compared with hexane extractions for all sorghum feedstocks (Table 2). The highest wax content by hexane extraction was from bran (0.7%), whereas it was 0.2% from whole kernel and 0.4% from DDGS. Previously, Morrison et al. (2006) showed that the wax from flax processing waste using SC-CO_2 at 55 MPa

TABLE 2 The wax content (%, w/w) of three sorghum feedstocks based on the extracts yield and wax composition analysis

	Wax content (%, w/w)		
Condition	**Whole kernel**	**DDGS**	**Bran**
30 MPa/50°C	$0.4 \pm 0.0_a{}^b$	$3.4 \pm 0.3_b{}^a$	$3.0 \pm 0.3_{ab}{}^A$
30 MPa/70°C	$0.5 \pm 0.1_a{}^c$	$3.9 \pm 0.1_b{}^a$	$2.5 \pm 0.4_{ab}{}^B$
40 MPa/50°C	$0.4 \pm 0.1_a{}^c$	$3.5 \pm 0.4_b{}^a$	$2.3 \pm 0.1_b{}^B$
40 MPa/70°C	$0.6 \pm 0.0_a{}^c$	$4.9 \pm 0.1_a{}^a$	$3.3 \pm 0.2_a{}^B$
Hexane	$0.2 \pm 0.0_b{}^c$	$0.4 \pm 0.0_c{}^b$	$0.7 \pm 0.0_c{}^A$

Note: Results are expressed as mean ± SD. Means with different subscript letters within the same column, and means with different superscript letters among sorghum feedstocks within the same row are significantly different ($p < 0.05$).

TABLE 3 Differential scanning calorimeter (DSC) melting points of the sorghum extracts from whole kernel, bran, and dried distillers grains with solubles (DDGS)

		DSC melting temperature (°C)			
Feedstock	**Extraction condition**	**Onset**	**Peak**	**Offset**	**Enthalpy (J g^{-1})**
Whole kernel	C1	$72 \pm 1_{ab}$	$76 \pm 0_b$	$78 \pm 0_{ab}$	$104 \pm 0_b$
	C2	$72 \pm 0_{ab}$	$77 \pm 1_{ab}$	$79 \pm 1_{ab}$	$114 \pm 14_b$
	C3	$73 \pm 0_a$	$78 \pm 1_{ab}$	$79 \pm 1_{ab}$	$125 \pm 12_b$
	C4	$73 \pm 1_a$	$77 \pm 1_{ab}$	$79 \pm 1_{ab}$	$119 \pm 1_b$
	Hexane	$75 \pm 0_a$	$80 \pm 0_a$	$81 \pm 0_a$	$192 \pm 24_a$
Bran	C1	$52 \pm 1_d$	$58 \pm 1_d$	$61 \pm 1_c$	$11 \pm 1_d$
	C2	$52 \pm 0_d$	$58 \pm 0_d$	$61 \pm 1_c$	$11 \pm 3_d$
	C3	$52 \pm 1_d$	$57 \pm 2_d$	$61 \pm 3_c$	$5 \pm 1_d$
	C4	$52 \pm 2_d$	$58 \pm 1_d$	$62 \pm 1_c$	$12 \pm 2_d$
	Hexane	$69 \pm 0_c$	$75 \pm 0_c$	$78 \pm 1_b$	$103 \pm 5_b$
DDGS	C1	$52 \pm 1_d$	$57 \pm 1_d$	$60 \pm 3_c$	$5 \pm 5_d$
	C2	$53 \pm 0_d$	$57 \pm 1_d$	$62 \pm 2_c$	$4 \pm 2_d$
	C3	$51 \pm 1_d$	$56 \pm 0_d$	$60 \pm 0_c$	$3 \pm 2_d$
	C4	$51 \pm 2_d$	$57 \pm 0_d$	$60 \pm 1_c$	$3 \pm 1_d$
	Hexane	$67 \pm 1_c$	$73 \pm 0_{bc}$	$76 \pm 1_{ab}$	$59 \pm 2_c$

Note: Results are expressed as mean ± SD. Means with different subscript letters within the same column are significantly different ($p < 0.05$). C1–C4 represent SC-CO_2 extraction conditions. C1: 30 MPa/50°C; C2: 30 MPa/70°C; C3: 40 MPa/50°C; C4: 40 MPa/70°C.

and 60°C had a much higher yield (7.4%) compared with hexane extraction (4.0%) ($p < 0.05$).

Melting point analysis

The melting behavior of waxes is an important physical property relative to their use. The melting temperature ranges can be used to identify wax fractions as well as to have an idea about their purity. DSC melting profiles of the hexane and SC-CO_2 extracts of the three sorghum feedstocks are presented in Table 3. The melting point (onset, peak, and offset) of the extracts from each sorghum feedstock was considerably different. The whole kernel samples extracted by SC-CO_2 and hexane had similar melting properties, and their melting peak temperatures ranged between 77.3 and 79.7°C, respectively ($p > 0.05$). Similar results were previously reported by Athukorala and Mazza (2010) for hexane or SC-CO_2-extracted crude waxes from triticale straw where the melting point ranged between 45 and 47°C. However, the melting points of the extracts from sorghum DDGS and bran by SC-CO_2 were significantly lower than those from sorghum whole kernel ($p < 0.05$).

The oil fraction is composed of mainly lower melting point triacylglycerols, free fatty acids, and small amount of monoacylglycerols and diacylglycerols. Therefore, the extracts with higher oil content have lower melting point compared with wax. Similarly, there was no oil in the hexane extracts of the sorghum bran, whereas SC-CO_2 extracts contained some oil (8.2%–11.1%). Both hexane and SC-CO_2 extracted mostly the wax fraction, which has a high melting point.

The extracts obtained from sorghum whole kernel, DDGS, and bran by SC-CO_2 were further purified by a simple step ethanol wash to obtain the wax fraction. The DDGS and bran extracts obtained from 40 MPa/70°C were selected based on their highest extracts yield (Figures 1 and 2) to perform the ethanol purification process. At the end of the ethanol wash

TABLE 4 Differential scanning calorimeter (DSC) melting points of the sorghum extracts from whole kernel, dried distillers grains with solubles (DDGS), and bran before and after ethanol purification

	DSC melting temperature (°C)			
Sample	**Onset**	**Peak**	**Offset**	**Enthalpy (J g^{-1})**
Purified whole kernel extract	$77 \pm 1_a{}^a$	$81 \pm 1_a{}^a$	$83 \pm 2_a{}^a$	$228 \pm 18_a{}^a$
Unpurified whole kernel extract	$73 \pm 1_b$	$77 \pm 1_b$	$79 \pm 1_b$	$118 \pm 1_b$
Purified DDGS extract	$77 \pm 0_a{}^a$	$81 \pm 0_a{}^a$	$83 \pm 0_a{}^a$	$226 \pm 19_a{}^a$
Unpurified DDGS extract	$51 \pm 2_c$	$57 \pm 0_c$	$60 \pm 1_c$	$7 \pm 1_c$
Purified bran extract	$78 \pm 1_a{}^a$	$82 \pm 1_a{}^a$	$84 \pm 1_a{}^a$	$209 \pm 18_a{}^a$
Unpurified bran extract	$52 \pm 2_c$	$58 \pm 1_c$	$62 \pm 1_c$	$10 \pm 1_c$

Note: Results are expressed as mean ± SD. Means with different subscript letters within the same column, and means with different superscript letters among sample groups (purified whole kernel extracts, purified DDGS extracts, purified bran extracts) after ethanol purification within the same column are significantly different ($p < 0.05$). Whole kernel extracts, DDGS extracts, and bran extracts obtained at 40 MPa/70°C were chosen based on their highest yield (Figures 1 and 2) to perform the ethanol purification process.

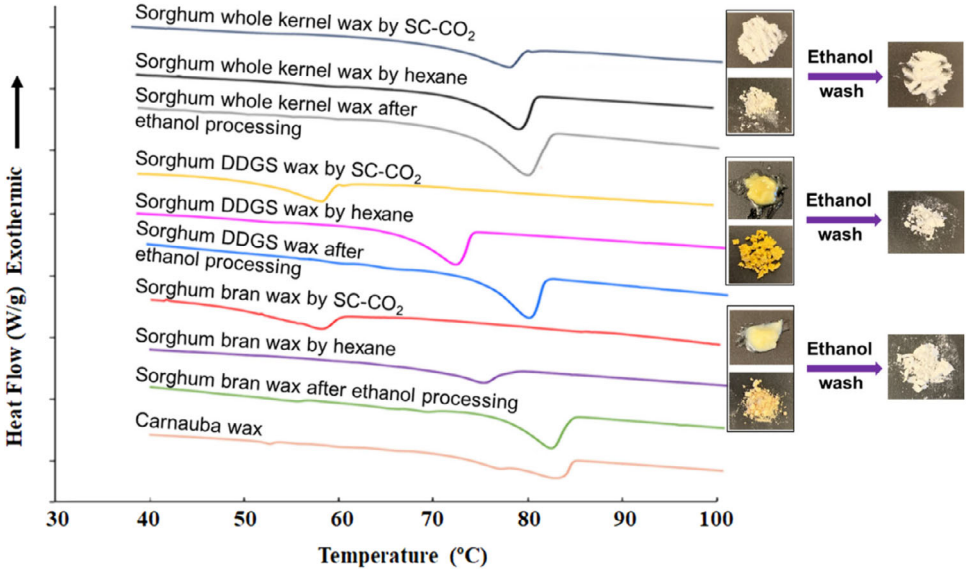

FIGURE 3 Differential scanning calorimeter melting curves of the recovered wax from sorghum extracts of whole kernel, dried distillers grains with solubles, and bran after ethanol purification. The extracts were extracted at 40 MPa/70°C, based on their highest yield (Figures 1 and 2)

process, approximately 85%, 42%, and 32% of the extracts were recovered from whole kernel, DDGS, and bran, respectively.

Table 4 reveals obtaining a higher melting point extract after the ethanol wash process. The onset, peak, and offset melting temperatures of the DDGS extracts increased from 51.1, 56.7, and 60.2°C to 77.1, 80.8, and 82.7°C, respectively, after the purification process. Similarly, onset, peak, and offset melting temperatures of the bran extracts increased from 52.1, 58.5, and 61.9°C to 78.1, 82.0, and 83.6°C, respectively, after ethanol purification. The increase in the melting point of the purified whole kernel extracts was lower compared with DDGS and bran because the whole kernel extracts were composed of mostly wax and had low amount of oil and negligible amount of polar compounds (Table 1). There was only about 4°C increase in the onset, peak, and offset melting temperatures of the whole kernel extracts after purification.

Figure 3 presents the DSC melting profiles of the sorghum whole kernel, DDGS, and bran extracts obtained by SC-CO_2 at 40 MPa/70°C before and after purification. Hexane extracts had higher melting points compared with SC-CO_2 extracts due to higher oil content of the SC-CO_2 extracts that were affected by the SC-CO_2 extraction pressure and temperature. As explained above, a slight increase in the melting temperature of the whole kernel after purification was due to its higher purity compared with DDGS and bran extracts. Ethanol purification was more effective on bran and DDGS extracts as they contained high amount of non-wax fractions. The ethanol purification generated high purity wax fractions that had similar melting curves and temperatures from all feedstocks (Figure 3). It was found that the purified sorghum wax from all feedstocks used in this study were very similar to Carnauba wax, which is the major natural wax utilized in industry, as shown by their similar DSC melting temperatures (Figure 3).

CONCLUSIONS

Sorghum and its by-products, namely, DDGS and bran, are found to be promising alternative natural wax sources. While the wax yield from DDGS and bran showed to be higher than that of whole kernel, sorghum whole kernel is a more feasible feedstock to obtain higher purity wax. The compositions of the extracts differed for the feedstocks and the solvent used for extraction. This study showed that the supercritical carbon dioxide (SC-CO_2) is a promising green alternative to traditional hexane to extract wax from sorghum feedstocks. Utilizing whole kernel has the potential to eliminate extensive purification processes. In addition, the whole kernels can be utilized for sorghum flour production after SC-CO_2 extraction because the extracted whole kernels will be food grade due to SC-CO_2 processing.

A simple purification process based on ethanol wash was able to obtain high purity sorghum wax from both DDGS and bran extracts. The purified sorghum wax from whole kernel, DDGS, and bran had similar melting properties, and they were very similar to Carnauba wax in terms of melting profiles. Hence, sorghum wax from whole kernel and also by-products of sorghum processing can be used as alternative natural candidates for various food and industrial applications, and in turn may reduce US dependence on non-renewable petroleum-derived waxes or natural wax imports.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Felipe Sperotto: Investigation; data acquisition; data analysis and interpretation; writing. **Junsi Yang**: Data acquisition; data analysis and interpretation; writing. **Loren Isom**: Research conception and design; data analysis and interpretation. **Curtis Weller**: Research conception and design; data analysis and interpretation. **Ozan N. Ciftci**: Research design; data analysis and interpretation; project supervision; writing.

ETHICS STATEMENT

The manuscript is prepared by following the Wiley's Publication Ethics Guidelines. All the authors declare that they have no conflict of interest and give their consent for authorship.

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