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ABSTRACT

The objectives of this research were to investigate the fermentation performance of US sorghum varieties for the production of distilled spirits as well as their associated coproducts and to study the formation of volatile compounds that are related to the flavor quality of the spirits. Three US sorghum varieties (red, white, and waxy sorghums) and four yeast strains (DADY, Ethanol Red, GR-2, and 71B) were used for distilled spirit production. Both sorghum variety and type of yeast strains had effects on alcohol concentration and alcohol yield. The alcohol concentration varied from 10.26 to 11.34% (v/v) while alcohol yield varied from 80.93 to 90.33%. Using Ethanol Red yeast achieved consistently the highest average alcohol concentration (11.10%, v/v) and yield (87.33%) regardless of variation in sorghum variety. Waxy sorghum demonstrated significantly higher average alcohol concentration (11.20%, v/v) and yield (89.65%) than white sorghum (10.74% for concentration and 84.7% for yield) and red sorghum (10.28% for concentration and 82.27% for yield). Alcohol fermentation also produces other metabolites as byproducts. Glycerol and lactic acid are the two major byproducts found from sorghum spirit fermentation. DADY produced the highest level of glycerol (~1.4–1.5%, v/v) during fermentation, while GR-2 produced the lowest level of glycerol (0.9–1.1%, v/v). For all conditions, the lactic acid level was less than 1.2% (v/v). Eight volatile compounds were identified in sorghum spirits which mainly relate to fruity, sour, sweet, floral, buttery, and creamy flavors of the spirits.

1. Introduction

Distilled spirits are an alcoholic beverage distilled from grains, fruits, or other fermentable ingredients. They include brandy, gin, rum, tequila, whiskey, vodka, baijiu, and various flavored liqueurs. The global distilled alcohol beverage market was $1475 billion in 2021. With projected annual growth of 9.1%, the global alcoholic beverages market size is expected to reach $2797 billion by 2028 [1]. Spirits market sales account for more than 30% of total alcohol sales in the United States with nearly 97 billion U.S. dollars [2]. Vodka is the most popular spirit in terms of volume sales of the spirits industry, followed by whiskey and rum. Currently, the distilled spirits, such as whiskey, vodka, or gin are mainly produced from corn, wheat, and rye grains, although some vodkas are made from potatoes.

Sorghum-based spirits have been around for a while, such as sorghum rum, vodka, and beer, and some small brand sorghum spirits available locally, such as Golden Biscuit Sorghum Spirit (Nashville Craft Distillery, Nashville, Tennessee), New Southern Revival Sorghum Whiskey (High Wire Distilling Co., Charleston, SC), Queen Jennie Sorghum Whiskey (Riley's Wines of The World, Madison, WI), Rocktown Sorghum Arkanas Whiskey (Rock Town Distillery, Little Rock, Arkansas), etc. Sorghum grain has been used as a main or only ingredient for Chinese baijiu production in China, especially for high-quality baijiu, also known as "sorghum spirit". Solid-state fermentation technology is commonly used to produce the most well-known Chinese baijiu with different flavors and characteristics, in which microbial cultures are grown on a solid matrix. Meanwhile, liquid-state fermentation has also been used to produce baijiu in China [3].

Liquid-state fermentation generally uses corn as the raw material to produce baijiu, with all processes performed in the liquid state. Some sorghum grains contain a significant level of phenolic compounds, such as tannin which is considered as one of the primary factors influencing sorghum spirit fragrance [4–6]. Han et al. [7] compared sorghum spirit with the ones produced from other grains (wheat, corn, rice, and barley),

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and concluded that the flavors and quality of the sorghum baijiu were the best followed by wheat. They suggested that Chinese baijiu should use sorghum as the main raw material, and may mix with wheat. Xu et al. [8] evaluated five Australian sorghum varieties for baijiu production using solid-state fermentation. In general, solid-state fermentation technology is commonly used to produce most well-known baijiu beverages with desired flavors and characteristics. Szambelan et al. [9] conducted research on Poland sorghum distilled spirit using liquid fermentation with focus on volatile compounds generated during fermentation. Although there are some sorghum spirits beverages available from some local brewers and some research conducted in the foreign countries, the scientific information about fermentation performance of the US sorghum varieties for distilled spirits is not available. The objectives of this research were to investigate fermentation performance of US sorghum varieties for distilled spirit production as well as coproducts and to study the formation of volatile compounds that are related to flavor quality of the distilled spirits.

2. Materials and methods

2.1. Sorghum samples

Three varieties of grain sorghum from Nu Life Market company (Scott City, KS, USA) were used for this project. All sorghum samples were cleaned using a Gamet sieve shaker (Dean Gamet Mfg. Co., Minneapolis, MN) with a 1.98-mm (5/64-in) triangular-hole sieve to remove broken kernel and small foreign materials. Large broken kernels and foreign materials were manually removed. An Udy cyclone mill (Udy, Fort Collins, CO) equipped with a 1.0-mm screen was used to grind clean samples into flour. The ground sorghum was sealed in plastic bags and stored in a sealed plastic box at 25 °C until used.

2.2. Characterization of chemical, physical, and thermal properties of grain sorghum

Moisture content was determined using AOAC standard method (AOAC 930.15). Starch was determined with a kit from Megazyme (Bray, Ireland) according to AACC approved method 76/12. Crude protein content was determined using nitrogen content analysis method (AOAC 990.03). Briefly, sample was combusted for nitrogen measurement, then nitrogen was converted to protein using a conversion factor of 6.25. Crude fat and ash contents were determined by following standard methods of AOAC 920.39 and AOAC 942.05, respectively. Crude fiber was analyzed by using the Filter Bag Technique (ANKOM Technology, Macedon NY). Tannin content of sorghum grains was determined by following the modified vanillin assay procedures for measurement of condensed tannin [10]. Kernel hardness, kernel weight, and kernel size were analyzed using the single kernel characteristics system 4100 (Model SKCS4100, Perten Instrument, Inc., Reno, NV, USA). A rapid visco analyzer (Model RVA-3c, Newport Scientific Ltd., Warriewood, Australia) was used to determine pasting properties of sorghum flours. In detail, sorghum flour (4.0 g with 14% moisture content) and water (25 mL) were mixed at 50 °C. The slurry was held at 50 °C for 1 min and heated 95 °C. The hot paste was held at 95 °C for 2.5 min, cooled to 50 °C, and held at 50 °C for 2 min. The total process was 13 min.

2.3. Selection of yeast strains

Four yeast strains from the same species, *Saccharomyces cerevisiae* that are commercially used for vodka and alcohol production were selected for sorghum spirit fermentation in this study, including Lalvin 71B (71B) (Lallemend Co., Lallemand, Australia), Safspirit GR-2 (GR-2) (Fermentis Co., Marcq-en Baroeul, France), Red Star Distillers' active dry yeast (DADY) (Fermentis Co., Milwaukee, WI, USA), and Red Star Ethanol Red (Ethanol Red) (Lesaffre, Milwaukee, WI, USA). These four strains have been considered as optimal yeasts for vodka and high concentration alcohol fermentation [11]. 71B is an active dry yeast for neutral spirits, vodka, and gin. GR-2 is good for neutral alcohol production, especially for vodka. DADY is a specially selected strain of *Saccharomyces cerevisiae* designed for distillers' use in grain mash fermentations, and it has been used in the fermentation for light whiskey and neutral spirits. Ethanol Red with high ethanol tolerance is developed for ethanol industry. It also can be used for "Very High Gravity" fermentation. Detailed properties of these selected yeast strains are presented in Table 1.

2.4. Liquefaction and simultaneous saccharification and fermentation (SSF) process

Prior to the fermentation, the sorghum flour underwent a liquefaction process. Flour slurry with 30% sorghum flour (db) (from Section 2.1) was prepared in flasks, and then α-amylase (240 KNU/g starch, ~1.26 g/mL, Novozymes, New York, NY) was added. The slurry was placed in a rotary water-bath shaker at 70 °C. Temperature of the water bath was then raised from 70 to 90 °C, kept at 90 °C for a few minutes with agitation speed of 180 rpm, and then lowered to 85 °C and kept for 60 min. After the liquefaction was done, flasks containing mashes were removed from the water-bath shaker.

After the mashes cooled down to room temperature, the pH of the mashes was adjusted to around 4.2 with 2 M HCl. Before SSF, the dry yeast was activated by adding 1.0 g of active dry yeast into 19 mL of preculture media (containing (per liter): 20 g glucose, 5.0 g peptone, 3.0 g yeast extracts except 71B, 1.0 g KH_2PO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$) and incubated in an incubator at 38 °C for around 30 min at 200 rpm. For 71B, inoculation condition was 35 °C for 20 min at 200 rpm. An aliquot of 1.0 mL of activated yeast culture (~2.8×10^8 cells/mL), 100 μL of Spirizyme (Glucoamylase, Novozymes, Franklinton, NC), and 0.30 g of yeast extract were added into each flask that contained mashes. Flasks were sealed with an S-airlock filled with mineral oil. Fermentation was conducted at 30 °C and 150 rpm in a shaking incubator for 72 h. After 72 h of fermentation, fermentation broth was sampled for chemical composition analysis. Finished mash was distilled for alcohol yield evaluation. The finished mash in each 250 mL flask was entirely transferred to a 500-mL distillation flask and the Erlenmeyer flask was washed 4 times with 100 mL (25 mL × 4) of distilled water. The contents were distilled on a distillation unit and the distillates were collected into a 100-mL volumetric flask that was immersed in ice water. When the distillates in the volumetric flask approaching the 100 mL mark (<0.5 mL to the mark), the volumetric flask was removed from the distillation unit and the distillation process was stopped. The distillates in the volumetric flask were equilibrated for a few hr in a 25 °C water bath,

Table 1
Commercially available yeast strains used in this study for the production of spirits.

Company	Strains	Species	Fermentation temperature	Final products
Lallemand, Australia	Lalvin 71B	*S. cerevisiae*	15–30 °C	Neutral spirits, vodka, and gin.
Fermentis, France	SafSpirit GR-2	*S. cerevisiae*	20–32 °C	Very neutral alcohol, especially vodka.
Fermentis, United States	Red star distillers' active dry yeast DADY	*S. cerevisiae*	32 °C	Ethanol, light spirits, and whiskeys
Lesaffre, United States	Ethanol Red	*S. cerevisiae*	30–40 °C	Ethanol

then brought to the 100 mL mark with distilled water. Ethanol concentrations in the distillates were analyzed by HPLC with a Rezex RCM column (Phenomenex, Torrance, CA) and refractive index detector [12].

2.5. Analytical methods

After fermentation, alcohol concentration, glycerol, lactic acid, and residual sugars (glucose, maltose, and maltotroise) contents were quantitatively analyzed on a high-performance liquid chromatography (HPLC) (1260 system, Agilent Technologies Inc., Santa Clara, CA). This HPLC was equipped with a HPX-87H organic acid column (7.8 × 300 mm) (Bio-Rad Laboratories, Hercules, CA) and a refractive index detector (RID). The separation temperature was 60 °C and the mobile phase was 5 mM H_2SO_4 with a flow rate of 0.6 mL/min. The RID temperature was 45 °C. Alcohol yield was calculated as the ratio of the actual alcohol yield (gram of alchol determined by HPLC) to the theoretical alcohol yield (g) (=starch grams × 1.11 × 0.511) [13].

The volatile compounds in the fermentation broth were analyzed using a gas chromatography-mass spectrometry (GC-MS) according to Pinu and Villas-Boas [14] with modifications. Briefly, triplicate 1-mL aliquots were taken from each post-fermentation broth, in which approximately 100–300 mg of anhydrous sodium chloride was added to reach saturation. The mixture was then extracted by adding 0.5 mL of ethyl acetate containing 0.2 mg/mL internal standard (phenol, not present in any sample) followed by vigorous mixing with a vortex mixer for 30 s. The ethyl acetate layer was sampled for GC-MS analysis (GC 6890 coupled with MS 5973, Agilent Technologies, Santa Clara, CA, USA) with a quadrupole mass selective detector (electron impact ionization; positive mode) operated at 70 eV. The analytical column was Zebron ZB-1701 (30 m × 250 μm × 0.25 μm) (Phenomenex, Torrance, CA) together with a 5-m guard column (7AG-G000-00-GZK, Phenomenex, Torrance, CA). The GC was operated at split mode with a 50:1 split ratio, and the injection volume was 1 μL. Helium was used as carrier gas with a flow rate of 0.5 mL/min. The GC oven temperature was initially held at 50 °C for 1 min and increased to 200 °C at a rate of 20 °C/min and held for 2 min. The total running time for this method was 10.5 min. The interface and quadruple temperatures were 230 °C and 150 °C, respectively. The MS detector was turned off between 2.9 to 3.1 min and 3.3–3.5 min to offload alcohol peak and ethyl acetate peaks, respectively. The MS was operated in the scan mode over a mass range of 30–250 a.m.u.

2.6. Statistical analysis

Data were collected and calculated using Microsoft Excel (Microsoft Corporation, Redmond, WA). Significant differences among means ($p < 0.05$) were recognized using the least significant difference (LSD) method.

GC-MS data were analyzed using JMP (JMP Statistical Discovery LLC, Cary, NC). A linear regression model with an interaction term was fitted with p-values less than 0.0001 and R^2 more than 0.92 for all volatile compounds quantified except for methyl isobutyrate due to the lack of factor effect significance for this compound. ANOVA was performed for each quantified compound to evaluate the significance of sorghum variety, yeast strains, and their interaction on integrated total ion current (TIC) peak area of the compound.

3. Results and discussion

3.1. Physical and chemical properties of grain sorghum

Table 2 shows the physical properties of the three sorghum varieties. Significant variations in kernel hardness index (55.66–74.09), average 100 kernel weight (23.44–33.39 mg), kernel diameter (2.05–2.81 mm), and test weight (56.67–58.71 lb/bu) were observed among the three sorghum varieties. In general, white sorghum has a higher kernel hardness index and test weight than red and waxy sorghums. It was also noticed that red sorghum has the lowest kernel diameter among the three sorghum varieties.

Table 2
Physical properties of grain sorghum.

Type of grain sorghum	Kernel hardness index	Single kernel weight (mg)	Kernel diameter (mm)	Test weight (lb/bu)
White sorghum	74.09±1.29[a]	27.61 ± 0.35	2.57 ± 0.01	58.71 ± 0.01
Waxy sorghum	69.31 ± 0.94	33.39 ± 0.63	2.81 ± 0.04	58.64 ± 0.10
Red sorghum	55.66 ± 0.96	23.44 ± 0.25	2.05 ± 0.03	56.67 ± 0.06

[a] Mean ± standard deviation. Kernel hardness, single kernel weight, and kernel diameter were based on 300 kernels. Test weight was based on three replicates.

Table 3 shows the chemical composition of the three sorghum varieties. Starch content ranged from 64.50 to 67.83% (db), which is in the range of the results (61.0–74.8%, db) from previous studies [8,15–18]. White sorghum had slightly higher starch content (67.83%, db) than red sorghum and waxy sorghum (66.46% and 64.50% (db), respectively). In terms of protein content (9.75–11.07%, db), white sorghum had a higher protein content than waxy and red sorghums. Crude fat contents of the sorghum ranged from 1.91 to 2.68% (db). All of three sorghums have the similar crude fiber content (~1.65%, db). Red sorghum had a significant higher tannin content (3.61%, db) than white sorghum (0.06%, db) and waxy sorghum (0.30%, db). Red sorghum can be considered as a high tannin sorghum variety.

3.2. Alcohol concentration and yield

Table 4 shows the effects of sorghum variety and type of yeast strains on alcohol concentration and yield. The alcohol concentration varied from 10.26 to 11.34% (v/v) while alcohol yield varied from 80.93 to 90.77%, indicating both sorghum variety and yeast strains had effects on alcohol fermentation performance. Although the p-values regarding to the effect of yeast strains on alcohol concentration and yield were about 0.15 (Table 5), it is still apparent that Ethanol Red achieved the consistently highest average alcohol concentration (11.10%, v/v) and highest alcohol yield (87.33%) regardless of variation in its substrate (Table 4). This agrees with the fact that Ethanol Red is designed to produce alcohol with high efficiency and has been widely used for industrial ethanol production. GR-2 and 71B had similar average alcohol yields (85.12% vs 85.80%). DADY had the lowest average alcohol yield (83.94%) among the four yeast strains. Yield consistency is an important part of providing a consistent product with desired quality. For vodka production, high alcohol concentration and consistency are more desirable than others. Therefore, Ethanol Red is an advisable choice of yeast strain for vodka production.

The yield of distilled spirits can be regulated with yeast [11]. However, the substrates for spirit fermentation are also important. Results showed that sorghum variety had a significant effect on alcohol concentration and yield with p-value less than 0.05 (Table 5). Previous research showed that waxy sorghum varieties have a higher alcohol yield than non-waxy varieties at the same starch level [19–22]. Our results were consistent with the previous findings. Waxy sorghum demonstrated significantly higher average alcohol concentration (11.20%, v/v) and yield (89.65%) than white (10.74% for concentration and 84.7% for yield) and red sorghums (10.28% for concentration and 82.27% for yield). The differences in the alcohol yields are largely due to the relative properties of their starch. Waxy sorghum has high amylopectin content, resulting in high starch availability [21]. This can be explained by the adverse effects of higher amylose content in regular sorghum varieties during gelatinization process. In general, waxy

Table 3
Chemical composition of grain sorghum.

Type of grain sorghum	Moisture (%)	Starch (%, db)	Crude protein (%, db)	Crude fat (%, db)	Crude fiber (%, db)	Ash (%, db)	Tannin (%, db)
White sorghum	11.33±0.08[a]	67.83 ± 1.30	11.07 ± 0.13	2.68 ± 0.07	1.65 ± 0.04	1.64 ± 0.01	0.06 ± 0.009
Waxy sorghum	10.17 ± 0.07	64.50 ± 1.40	10.92 ± 0.01	1.95 ± 0.10	1.67 ± 0.01	1.71 ± 0.01	0.30 ± 0.042
Red sorghum	11.52 ± 0.04	66.46 ± 0.72	9.75 ± 0.01	2.01 ± 0.06	1.63 ± 0.01	1.75 ± 0.01	3.61 ± 0.041

[a] Mean ± standard deviation. All data were based on two replicates.

Table 4
Effect of sorghum variety and type of yeast on alcohol concentration and fermentation efficiency.

Sorghum sample	Alcohol concentration (%, v/v)				
	DADY	Ethanol Red	GR-2	71B	Average
White sorghum	10.85 ± 0.16b[a,b]	11.16 ± 0.19b	10.87 ± 0.05b	10.09 ± 0.07b	10.74
Waxy sorghum	10.90 ± 0.12b	11.34 ± 0.02b	11.29 ± 0.14c	11.27 ± 0.30b	11.20
Red sorghum	10.26 ± 0.09a	10.81 ± 0.25a	10.30 ± 0.15a	10.36 ± 0.33a	10.28
Average	10.67	11.10	10.82	10.57	
Sorghum sample	Alcohol Yield (%)				
	DADY	Ethanol Red	GR-2	71B	Average
White sorghum	83.63 ± 1.31b	86.00 ± 1.54a	83.78 ± 0.38b	85.47 ± 0.51a	84.74
Waxy sorghum	87.25 ± 0.89c	90.77 ± 0.17b	90.35 ± 1.05c	90.22 ± 2.32b	89.65
Red sorghum	80.93 ± 0.73a	85.21 ± 2.02a	81.24 ± 1.22a	81.71 ± 2.62a	82.27
Average	83.94	87.33	85.12	85.80	

[a] Mean ± standard deviation. All data were based on three replicates.
[b] In each column, means with different letters are significantly different at $p < 0.05$.

Table 5
Analysis of variance for the effects of sorghum variety, yeast strain, and their interaction on the final ethanol concentration and ethanol yield.

Source of Variation	Ethanol concentration (%, v/v)			Ethanol yield (%)		
	df	F-value	P-value	df	F-value	P-value
Sorghum variety	2	18.1792	0.0002	2	25.9201	0.0001
Yeast strains	3	2.1061	0.1529	3	2.1022	0.1534
Interaction	6	1.2968	0.3294	6	1.3121	0.3234

sorghum has lower peak gelatinization temperature and lower ending gelatinization temperature than normal sorghum, which makes waxy sorghum starch easily hydrolyzed [20–22]. In addition, waxy sorghum usually has lower viscosity than normal sorghum during liquefaction, which also renders waxy sorghum achieve high hydrolysis efficiency. Fig. 1 shows the pasting properties of three sorghum varieties. The final viscosity of waxy sorghum (2079.0 cP) was much lower than that of red sorghum (3154.0 cP) and white sorghum (3413.0 cP), and the setback viscosity of waxy sorghum was much lower than that of red (1675.5 cP) and white sorghum (2018.5 cP). In addition, waxy sorghum required less time (5.00 min) to reach the peak viscosity than red (5.37 min) and white sorghums (5.80 min). RVA results indicated that starch in waxy sorghum flour is much easier to swell and disintegrate than that in normal red and white sorghum flours.

Red sorghum had the lowest alcohol concentration and yield among the three sorghum varieties. One major possible reason is that red sorghum has a high tannin content (3.61%, db). Tannins have adverse effects on starch digestion because of their ability to interact with proteins (including hydrolytic enzymes) [23]. In addition, mash viscosity of

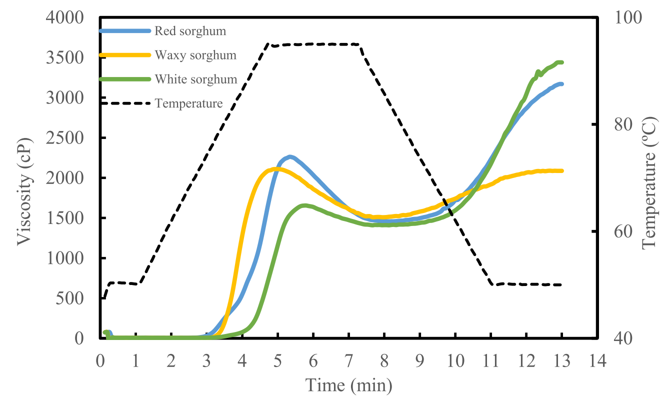

Fig. 1. RVA pasting profiles of the three sorghum samples.

tannin sorghum is higher than that of waxy sorghum and normal sorghum [20–22]. The peak viscosity of red sorghum (2277.5 cP) is higher than that of waxy sorghum (2106.5 cP) and white sorghum (1633.5 cP). Therefore, tannin sorghum generally has low ethanol yield.

3.3. Byproducts from alcohol fermentation

Alcohol fermentation is a complex metabolic process in which sugar is fermented into alcohol and CO_2 as well as other metabolites as byproducts. Glycerol and lactic acid are the two major byproducts from spirit fermentation [24,25]. Fig. 2 shows the effect of sorghum variety and yeast strains on glycerol formation in the spirit fermentation. Glycerol production is highly dependent on the yeast strains being employed, which is unique amongst the examined coproducts. DADY produced the highest level of glycerol (1.4–1.5%. v/v) during fermentation, while GR-2 produced the lowest level of glycerol (0.9–1.1%, v/v). There is little ambiguity or divergence in the glycerol concentrations being produced by the yeasts. Indeed, the only significant variance in production were the two outliers produced by from Ethanol Red. In general, sorghum variety does not have significant effect on glycerol formation, even though red sorghum has larger range of glycerol level (0.9–1.65%, v/v). This may indicate that glycerol production may be a valid indicator of the type of yeast strain present in a particular fermentation batch [19]. Such an assay could be performed by isolating out all yeast strains within a reactor, growing them in a broth, and then assaying the supernatant for glycerol. If a yeast strain was characterized, then it could be identified by its glycerol output, though glycerol has the potential to be toxic to yeast and humans depending on dosage [25,26]. It also has implications in the wine and baijiu industries [19,27]. Sorghum variety has much less effect on glycerol formation compared with type of yeast strains. Even though variety did not show significant effect on glycerol formation, it was observed that red sorghum had higher average glycerol level than waxy and white sorghums. Waxy sorghum had the lowest average glycerol level. Regardless of yeast strain and sorghum variety, the glycerol level of 0.9–1.7% is comparable to the results from previous studies. Du et al. [28] studied the effect of yeast strains on glycerol yield using 10 yeast strains during wine fermentation

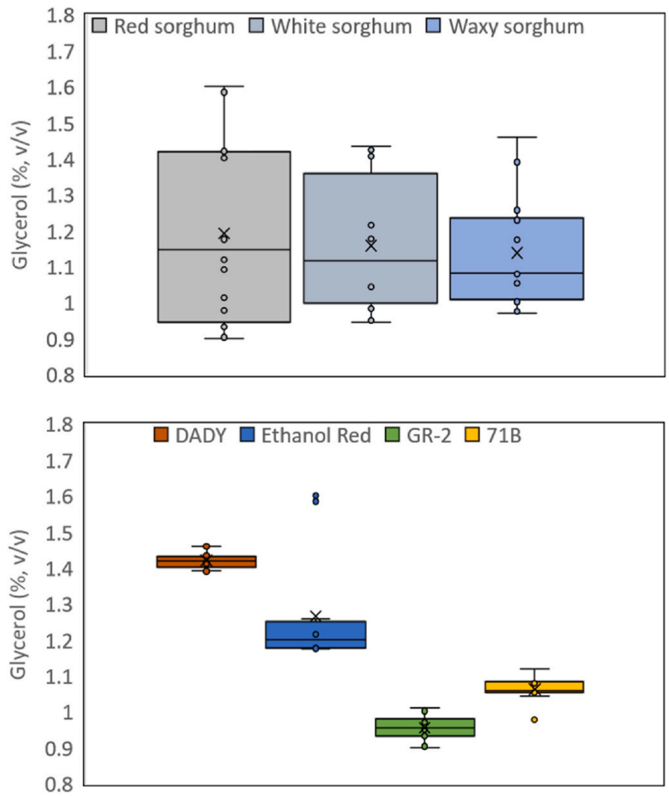

Fig. 2. Effects of yeast strain and sorghum variety on glycerol formation during spirit fermentation.

and found that glycerol levels ranged from ~7.5 to 10.6 g/L. Wei et al. [29] reported that glycerol concentration can range from 0 to 31.33 g/kg of Chinese baijiu depending on the type of yeast strains and filamentous fungi.

Lactic acid is a universal metabolite and often appears in alcohol fermentation. For example, the lactic acid level in the Moutai-flavor baijiu was found up to 36.20 g/kg of fermented grain [30]. Because most yeasts don't naturally produce lactic acid, we would assume that lactic acid was produced by microbes other than yeasts [31]. In fact, the acid formation was a result of oxidation during the fermentation process and contamination by lactic acid bacteria [3,15]. It is also true that lactic acid can cause stress on yeasts and affect alcohol yield [32]. Fig. 3 shows the effects of sorghum variety and yeast strain on lactic acid formation. While red sorghum supports lactic acid formation slightly stronger than white sorghum or waxy sorghum, the difference is minor based on average lactic acid level. However, yeast strain had a significant effect on lactic acid formation. Ethanol Red yielded significantly higher lactic acid level than the other three yeast strains, and the lowest level of lactic acid was obtained from DADY fermentation. As mentioned before, yeasts don't naturally produce lactic acid, there might be something indirectly corelated to yeast strains and further research is needed to understand this phenomenon. In general, the formation of lactic acid could inhibit alcohol fermentation and reduce the alcohol yield. However, lactic acids' general correlation to yield implies that lactic acid level does not have significant effect on the alcohol yield. Ethanol Red produced both the highest level of lactic acid (up to 1.2%, v/v) and the highest alcohol yield (87.33%). DADY achieved both the lowest level of lactic acid (less than 0.5%, v/v) and the lowest alcohol yield (83.94%). The relationship between alcohol yield and lactic acid level may be affected by other factors. This is a topic which remains largely unstudied but may have significant implications [32,33].

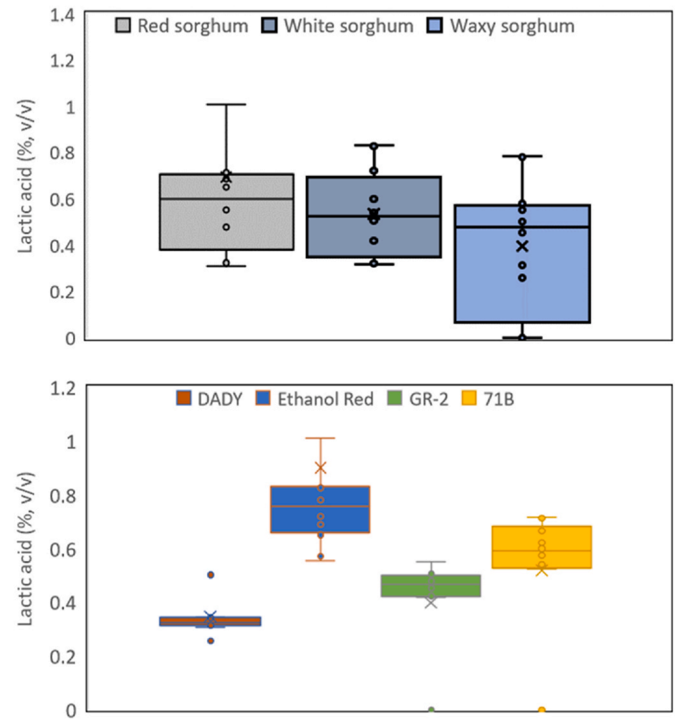

Fig. 3. Effects of yeast strain and sorghum variety on lactic acid formation during spirit fermentation.

3.4. *Volatile compounds*

Alcoholic fermentation also yields many volatile compounds. The type and concentration of volatile compounds in the distillated spirits are highly related to spirit quality (e.g., flavor and aroma) and people's satisfaction level [34]. It has been reported that more than 1730 compounds have been found in Chinese baijiu [35]. The major volatile compounds in Chinese baijiu include acetals, acids, alcohols, aldehydes, esters, ketones, lactones, nitrogen-containing compounds, sulfur containing compounds, etc. [3]. The type and concentration of volatile compounds present in the distillated spirits are highly dependent on the fermenting culture, raw material used for fermentation, and fermentation conditions. Szambelan et al. [9] reported that aldehydes, esters, methanol, and higher alcohols are four major types of volatile compounds founded in Poland sorghum distilled spirits with total of 28 volatile compounds identified using two yeast strains (Ethanol red and fermiol) and two sets of enzymes for starch hydrolysis and saccharification. In this research, three sorghum varieties were fermented using four yeast strains including 71B, GR-2, DADY, and Ethanol Red. Eight major volatile compounds were identified from sorghum spirit fermentation with NIST (National Institute of Standards and Technology) library with match scores above 90. The peak area of each compound in total ion chromatograms was integrated by ChemStation software and normalized with the internal standard. The volatile compounds mainly relate to fruity, sour, sweet, floral, buttery, and creamy flavors of the spirits. In most cases, flavor compound profile was significantly affected by sorghum variety, yeast strain, and their interaction except for methyl isobutyrate, the level of which was not significantly affected by the factors (Table 6). Another exception is 1-propanol, 2-methyl, the concentration of which was not significantly affected by sorghum variety. In addition, phenylethyl alcohol level varied greatly among fermented spirits examined in this study (Supplemental information, Table S2). Most noticeably, spirits fermented by Ethanol Red yeast had significantly increased phenylethyl alcohol concentration as compared with spirits fermented by other yeast strains (Supplemental information, Table S1). The TIC peak area of phenylethyl alcohol in Ethanol Red

Table 6
Summary of ANOVA results. The significance of sorghum type, yeast type, and their interaction on total ion current peak area of each compound was assessed using partial *F*-test and *p*-values were reported in the table.

Volatile compounds[a]	*p*-values		
	Sorghum	Yeast	Sorghum*Yeast
1-propanol, 2-methyl	0.2792	<0.0001	<0.0001
Methyl isobutyrate	0.8175	0.9638	0.4077
Acetic acid	<0.0001	<0.0001	<0.0001
1-butanol, 3-methyl-	0.0017	<0.0001	<0.0001
2,3-butanediol	<0.0001	<0.0001	<0.0001
Phenylethyl alcohol	<0.0001	<0.0001	<0.0001

[a] 3-(methylthio)-1-propanol and benzeneethanol, 4-hydroxy were also detected in all sorghum spirits, however their TIC peaks were too small to be reliably integrated.

fermented spirits increased more than 6 folds as compared with DADY fermented sprits (Supplemental information, Table S2).

4. Conclusions

Fermentation performance of three US sorghum varieties using four yeast strains for distilled spirit production was investigated. The effects of sorghum variety and type of yeast strains on alcohol and coproduct yields and the formation of volatile compounds were studied. Both sorghum variety and type of yeast strains had effects on alcohol concentration and yield. The alcohol concentration varied from 10.26 to 11.34% (v/v) while alcohol yield varied from 80.93 to 90.33%. Ethanol Red achieved the consistently highest average alcohol concentration (11.10%, v/v) and yield (87.33%) regardless of variation in its substrate. Waxy sorghum demonstrated significantly higher average alcohol concentration (11.20%, v/v) and yield (89.65%) than white (10.74% for concentration and 84.7% for yield) and red (10.28% for concentration and 82.27% for yield) sorghums. Glycerol and lactic acid were the two major byproducts found from sorghum spirit fermentation. The glycerol formation was highly dependent on the yeast strain being employed. DADY produced the highest level of glycerol during fermentation, while GR-2 produced the lowest level of glycerol. For all conditions, the lactic acid level was less than 1.2% (v/v), which is comparable to the results from previous studies (∼2.5%, v/v). Eight volatile compounds were identified from sorghum spirit fermentation which mainly relate to fruity, sour, sweet, floral, buttery, and creamy flavors of the spirits.

Declaration of competing interest

There is no conflict of interest regarding this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2022.100314.

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