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Statistical Significant Differences between Aroma Profiles of Beer Brewed from Sorghum

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Abstract: There is currently an increased demand for foodstuffs that are classified as gluten-free including beer. Beer produced using gluten-free grains has a distinct flavor profile that differs greatly from that of beer produced from gluten-containing grains. The chemical difference between beers made from these two different grain sources has been explored and some key differences have been identified. Here malt sources containing gluten (barley) and malt without gluten (sorghum) were used to determine which compounds are statistically different based upon their concentrations. A total of 14 (7 barley and 7 sorghum) small-batch beers were made from malt extract. The aroma profile was sampled using SPME with chemical separation and identification and quantification using GC-MS. As expected, the differences were not the result of unique compounds but compounds present in differing amounts. A total of 17 compounds were found to be present in beer brewed from both extracts but in amounts that were highly significantly different.

Keywords: sorghum; malt; GC-MS

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1. Introduction

Beer is one of the oldest fermented beverages currently being sold on the market. It is also one of the most widely consumed alcoholic beverages in the world [1]. Beer is traditionally brewed using malted barley. Unfortunately, people with known gluten-sensitivities or diagnosed with celiac disease (CD) are unable to drink conventional beer. For a naturally gluten-free beer, brewers can use pseudo-cereals or gluten-free grains such as rice, corn, sorghum, or millet. These grains are distantly related to wheat, rye, and barley therefore these products are considered safe to consume by individuals who have CD or who are gluten intolerant [2].

Those who follow a gluten-free diet still want to have the option of selecting from the highest quality of gluten-free products available to them. This demand for higher quality gluten-free products is the reason why the gluten-free product market is estimated to be worth more than 7.59 billion USD [3]. Strict adherence to living a gluten-free lifestyle can be extremely difficult and often compromising the quality of life. People will oftentimes forgo their strict diets and accept the side effects of consuming gluten to take part in popular activities such as eating out and consuming a beer [4–6]. Although one can argue that beer is not required and does not necessarily play an important role in providing someone with their necessary nutrients, however, a person's diet does encompass a number of food items that provide more than just the physiological need for nutrients. Beer is one of these products that is consumed throughout the world on a large scale. Therefore, it is important that individuals with CD are also able to safely consume and purchase good-tasting gluten-

free beers, due to the impact it would have on improving a person's well-being and ability to participate in social life [2].

Sorghum (*Sorghum bicolor* (L.) Moench) is a tropical cereal grown throughout the world in India, China, Brazil, Africa, Australia, and the United States [7]. The use of sorghum as a brewing material has been around for thousands of years in a number of different countries throughout Africa [8]. By the late 1980s, Nigeria was manufacturing clear sorghum beer to mimic traditional lager and stout styles as opposed to the traditionally opaque beer of Africa [9]. Nigeria produces in excess of over 18 million hectoliters of beer annually, much of which is produced using sorghum grains [10]. The use of sorghum as a brewing substrate has grown in popularity throughout Africa as well as the United States. The increase in popularity within the United States has to do more with people following a gluten-free diet and brewers wanting to accommodate that sector of the market.

Beer's flavor plays a significant role in consumers' acceptance or rejection of the product. The flavors produced during the fermentation process are the result of a number of complex reactions between a wide variety of chemical compounds [11]. Beer consists of a number of different flavor compounds such as alcohols, esters, aldehydes, ketones, esters, carboxylic acids, organic acids, sulfur compounds, amines, phenols, and all of these different compounds at their varying levels of concentration will influence the aroma and flavor of the final product [12,13]. A number of these volatile compounds play an important role in the overall beer's flavor, while others will merely build up the background flavor of the product [14]. These compounds are derived from a combination of the fermenting grain, yeast metabolism, and the addition of bittering hops.

The objective of this study was to compare the volatile and semi-volatile compounds between barley and sorghum beer using SPME-GC-MS. To focus on the compounds originating from the grain source, only one yeast strain was used and no hops were added. Therefore, any changes observed in this project originate from the source grain. A better understanding of these differences could help brewers create higher quality, consumer acceptable beers from gluten-free grains.

2. Materials and Methods

2.1. Chemicals

All chemicals were used as received without further treatment or purification. Sodium chloride and ethanol (chromatography grade) were purchased BDH (Radnor, PA, USA). Guaiacol and 1-heptanol were purchased from TCI (Tokyo, Japan).

2.2. Brewing

The experimental beer was brewed using a 37 L Worthog electric brewing system located on the Coastal Carolina University campus (Conway, SC, USA). Each batch of beer was brewed using a liquid malt extract (LME): Maillard Malts® Sorghum extract syrup or Maillard Malts® Amber Malt extract syrup. The LME was added to either 11 or 22 L of water, respectively, and boiled for 60 min. The wort was allowed to cool and brought back to an 18 L volume using distilled water. All brews had a target of 10.5° Plato. After diluting the wort with water, 3 L of the wort was aliquoted into separate clean, sterile one-gallon fermenters. Each fermenter was inoculated with Fermentis Safale™ US-05 and was dry pitched without hydration at 2 g per 3 L. The fermenter was sealed with an airlock. Each beer was allowed to ferment at room temperature (~22 $\pm$ 2 °C) for two weeks. Ten-milliliter samples were aseptically taken from each fermenter on days 0, 3, 7, and 14. A total of 7 different brews with each extract type was performed. The first brew of each type was separated into three separate fermenters, the remaining brews into four separate fermenters. This gave a total of 27 separate fermentations for both barley and sorghum for a total of 54 individual fermentations.

2.3. Volatile Analysis

The extraction and analysis of the volatile and semi-volatile compounds in the beer samples were conducted using solid-phase microextraction (SPME). The SPME-GC-MS analysis was adapted from the literature [15] and is summarized as follows. Ten milliliters of beer samples from each time point (0, 3, 7, and 14 day) were placed into a 20 mL headspace vial with 3 g of NaCl. In addition, 50 µL of the internal standard (200 mg/L 2-heptanol and 100 mg/L guaiacol in ethanol) was added to the headspace vial. The sample was stirred and allowed to equilibrate at 40 °C for 10 min prior to exposure of the fiber.

A divinylbenzene-carboxen-polydimethylsiloxane 50/30 µm (DVB-CAR-PDMS) fiber was selected for the analysis because it has been shown to provide reasonably high extraction efficiencies for a wide range of chemical compounds [16]. The fiber was conditioned according to the manufacturer's instructions by inserting it directly into the GC-MS injector at 250 °C for 30 min. The fiber was exposed to the headspace within the vial for 30 min at 40 °C with agitation (250 rpm). The fiber was then inserted into the gas chromatography-mass spectrometry (GC-MS) injector and de-absorbed for a total of 2 min at 250 °C to allow for sample analysis.

2.4. GC Parameters

Gas chromatography-mass spectrometry (GC-MS) was carried out using a Shimadzu GC-2010 coupled to a QP2010 SE quadrupole mass spectrometer. A Rxi-5Sil MS column (30 m × 0.25 µm I.D.) with a film thickness of 0.25 µm was used. The GC was equipped with a split-splitless injector which was held at 250 °C. The analysis was performed with a splitless injection over the 2 min desorption time. The GC oven was initially set to 30 °C with a 2 min hold and then was raised in three steps: 30–70 °C at 10 °C min^{-1} and held for 1 min; 70–220 °C at 4 °C min^{-1} and 220–270 °C at 20 °C min^{-1} and finally held at 270 °C for 6 min. The response of the mass spectrometer was monitored in TIC mode from 35–280 m/z. Compounds were identified via match to the NIST Mass spectra library.

2.5. Identification of Volatile Compounds

LRI Values—The compounds identified by mass spectra were confirmed based upon their RI values calculated using nonpolar (DB-5). The RI values were compared to literature values. Aliphatic hydrocarbon standards were analyzed in the same manner using a DB-5 column to calculate RI:

$$RI = 100N + 100n\ (t_{Ra} - t_{Rn})/(t_{R(N+n)} - t_{RN}) \quad (1)$$

N is the carbon number of the lowest alkane and n is the difference between the carbon number of the two n-alkanes that are bracketed between the compound; t_{Ra}, t_{Rn}, and $t_{R(N+n)}$ are the retention times of the unknown compound, the lower alkane, and the upper alkane [17].

Any compounds where the LRI values were not either found or failed to match the literature value are tentatively assigned based on the MS spectral match in the NIST library. While confident in the attribution, it is possible, they could be erroneously assigned and further investigation using additional methods would be needed to confirm identity.

2.6. Compound Response

The GC-MS peak area of each identified compound was normalized against the peak area of the internal standard 2-heptanol in each chromatographic run. This relative response was compiled for each compound and used in the statistical analysis.

2.7. Characterization

For each brew, both the wort and the final beer (14 day) were characterized in terms of color and free amino nitrogen (FAN). The color was determined using ASBC Standard Method Beer-10 and reported in units of SRM. The analysis was conducted using a Shi-

madzu UV-1800, using a disposable 1 cm plastic cuvette. FAN was determined by following the ASBC Standard Method Wort-12: Free Amino Nitrogen with ninhydrin method. The calorie and ABV were calculated using the change in the specific gravity.

2.8. Statistics

Sample comparisons were made using the Wilcoxon Rank Sum Test (Mann-Whitney U Test). The nonparametric counterpart to the Student-*t*-test is more appropriate given the smaller sample size and nonnormality of the data. As a result, estimates provided represent the median of the distribution for the relative index as opposed to the mean. All *p*-values were adjusted using the Benjamini and Hochberg adjustment to minimize false discovery rates due to multiple testing [18]. The open-source software, R, was used for analysis, including the nparcomp and exactRankTests packages.

3. Results

3.1. Color

There were significant differences between the color of the beer brewed using barley vs. sorghum. The average color measurement for barley was 27.5 SRM (n = 27) versus the sorghum beer at 4.9 SRM (n = 27).

3.2. Free Amino Nitrogen (FAN)

The range for a typical 10.5° Plato malt wort is between 150–230 mg L^{-1} FAN. The malted barley sample was within that range with a 95% confidence interval of 161.6 (±43) mg L^{-1} FAN (n = 27), while the sorghum sample has a 95% confidence interval of 24.8 (±2.8) mg L^{-1} FAN (n = 27). Following fermentation, FAN levels at the 95% confidence interval were 143.7 (±35) mg L^{-1} FAN (n = 27) for the malted barley beer and 19.5 (±2.7) mg L^{-1} FAN (n = 27) for the sorghum beer.

3.3. Volatile Analysis

An initial 10 mL sample of wort was taken prior to inoculating the wort with yeast, and additional samples were taken on days (3, 7, and 14) during and following the completion of the fermentation process. The purpose of this work was to compare the volatile and semi-volatile analysis of beer brewed using the two different grain (malted barley vs. sorghum) sources prior to and following the fermentation process, therefore the focus was on the comparison between 0 day and 14 day. To help normalize and reduce run variability the response reported here is the peak area relative to the peak area of the internal standard, 2-heptanol. To address the aims of this project, the following questions were posed: (1) What compounds showed a significant change following fermentation—regardless of grain type? (2) What compounds were present exclusively from each grain type? And (3) What compounds were present in both grain types and were present in statistically different amounts?

When examining the compiled data, and disregarding the grain source, there were a total of 2345 unique compounds found in the analysis across all brewed samples. From the list of unique compounds identified, 152 compounds showed significant differences following fermentation ($p = 0.01$). Of these 152 compounds, 39 compounds within the data set showed highly significant changes ($p < 0.01$). Table 1 shows the list of these 39 compounds. These compounds ranged in type from long-chain carboxylic acids (5 compounds), higher-order alcohols (7 compounds), a small number of aldehydes (4 compounds), alkanes (7 compounds), esters (12 compounds), furans (1 compounds), and ketones (2 compounds).

Table 1. Semi-volatile compounds showed a highly significant change in median amount relative to the internal standard regardless of grain material. A highly significant change in median amount relative to internal standard per grain material for barley ([a]) or sorghum ([b]). LRI values that are bold were not confirmed and the identities are therefore only tentatively assigned.

Compound	LRI	Initial	Final	*p*-Value	Flavor Descriptor
Acids					
Acetic acid [b]	669	0.0016	0.0311	<0.001	Vinegar
Isovaleric acid	861	0.0009	0.0112	<0.001	Sweat, acid, rancid
2-Methylbutanoic acid	866	0.0005	0.0097	0.002	Cheese, sweat
1,2-Dimethyl-cyclopent-2-enecarboxylic acid	**994**	0.0019	0.0276	0.001	N/A
Octanoic (caprylic) acid [b]	1203	0.0022	0.0769	<0.001	Sweat, cheese
Alcohols					
2-Methyl-1-propanol (iobutyl alcohol) [b]	643	0.0295	0.5106	<0.001	Wine, solvent, bitter
2-Methyl-1-butanol (active amyl alcohol)	712	0.0657	2.0574	0.005	Malt
3-Methyl-1-butanol (isoamyl alcohol)	786	0.0014	4.4535	<0.001	Whiskey, malt, burnt
1-Pentanol [b]	788	0.001	0.0665	<0.001	Balsamic
Phenylethyl alcohol [b]	1113	0.0049	0.5571	<0.001	Honey, spice, rose, lilac
3-(1,3,3-Trimethylbutoxy)- 2-Butanol	1339	0.0021	0.0083	0.005	N/A
2,2-Dimethyloct-4-en-3-ol	1343	0.0005	0.0054	0.005	N/A
Aldehydes					
Isovaleraldehyde [a]	662	0.0198	0.0045	0.003	Ethereal, aldehydic, fatty
Diethyl acetal	725	0.0009	0.0096	<0.001	Earthy, green
Nonanal [a]	1105	0.0018	0.0056	<0.001	Fat, citrus, green
Decanal [a]	1207	0.0011	0.0037	0.002	Soap, orange, peel, tallow
Alkanes					
Cyclohexane, 1,1,3,5-tetramethyl-, cis- [b]	**1050**	0.0008	0.0018	0.007	N/A
2,2,11,11-Tetramethyl-dodecane [a]	**1020**	0.001	0.0047	<0.001	N/A
2,2-Dimethyldecane [a]	1025	0.0008	0.0041	<0.001	N/A
3-Methyl-5-propylnonane	1032	0.0025	0.0064	0.005	N/A
5-Ethyl-2,2,3-trimethyl-heptane	**1052**	0.001	0.0064	0.003	N/A
5-(2-Methylpropyl)-nonane	**1091**	0.0003	0.0046	<0.001	N/A
Tridecane	1300	0.0009	0.0525	0.002	N/A/
Esters					
Ethyl acetate	631	0.0005	0.4686	<0.001	Fruity
Ammonium acetate [a]	**689**	0.0042	0.1486	<0.001	N/A
Ethyl propionate [b]	709	0.0006	0.0076	<0.001	Fruity
Butyl acetate [b]	771	0.0009	0.0726	0.008	Ethereal
Isoamyl acetate [b]	873	0.0011	1.0757	<0.001	Sweet, banana

Table 1. *Cont.*

Compound	LRI	Initial	Final	*p*-Value	Flavor Descriptor
Ethyl hexanoate (caproate) [b]	1000	0.28922	0.9731	<0.001	Fruity, sweet
Ethyl octanoate (caprylate) [b]	1197	0.0105	2.6329	<0.001	Fruit, fat
Phenethyl acetate [b]	1254	0.0008	0.0631	<0.001	Rose, honey
Ethyl decanoate (caprate) [a]	1395	0.0064	0.5295	<0.001	Grape
Isoamyl octanoate	1450	0.0006	0.0466	<0.001	Fruity
Ethyl dodecanoate (laurate)	1587	0.0043	0.1026	<0.001	Leaf
Ethyl hexadecanoate (palmitate)	1950	0.0007	0.0183	0.008	Waxy
Furans					
2,5-Dimethyl-furan [a]	701	0.0002	0.0056	<0.001	Meaty
Ketones					
β-Damascenone	1380	0.0018	0.0112	<0.001	Floral, woody
Benzophenone	1624	0.0012	0.0353	0.007	Balsamic
Phenols					
4-Vinylguaiacol	1310	0.002	0.0112	0.004	Spicy, clove

(Based on analysis of 54 different fermentations of sorghum (n = 27) and barley (n = 27)).

When looking at specific grain types, 18 compounds in barley and 18 in sorghum showed highly significant differences. As with the compounds that changed during fermentation (Table 1), these compounds range in type from long-chain carboxylic acids, higher-order alcohols, a small number of aldehydes, esters, furans, and ketones. Most of these compounds increased in concentration, suggesting that they are products of yeast metabolism. Exceptions to this increase are 3-methylbutanal, in barley and 2-methylbutanal in sorghum, both of which are known to originate from malt and have been shown to decrease with age [19,20]. Furfural in barley beers has also been shown to decrease in concentration.

The compounds seen in the analysis are typically what would have been expected, primarily esters, longer chain alcohols, and very few aldehydes. This was in part due to the function of the fiber that was used, which provides for a wide range of compound types but is least selective for aldehydes [16]. This in part also impacts the compounds that were observed to be changing. It should be noted also that the types of compounds observed are known to be related to yeast metabolism. Except for one compound (butanal, 3-methyl-) all were shown to increase in relative concentration. The compound 3-methylbutanal, a Strecker aldehyde, is typical of wort flavor and it is a product of the Maillard reactions produced in the boil, however, it is also known to decrease as beer ages.

While it was of special interest to examine the compounds that were exclusively present in beer based on the grain type, the compounds only found in one grain but not in the other was a small number (9), eight only in barley and one only in sorghum (Table 2). It is possible there were others especially originating from the sorghum malt, which showed relatively small peak areas for all compounds detected. However, the majority of the compounds detected were present in both the brews from the barley and sorghum extract but were present in different relative amounts.

Table 2. Semi-volatile compounds were only observed in beers brewed from either barley or sorghum.

Compound	Barley	Sorghum
2,6-Di-tert-butyl-P-benzoquinone	X	
2-Methyltetrahydrofuran-3-one	X	
Decyl acetate	X	
Heptyl acetate	X	
Propyl decanoate	X	
Propyl hexanoate	X	
Isobutyl decanoate	X	
Ethyl 4-methylpentanoate	X	
2-Methyltetrahydrothiophen-3-one		X

There were 40 compounds that were present in both grain types but that had statistically different relative amounts. The level of statistical difference varied for these 40 compounds with 17 compounds having a highly significant difference (Table 3). These compounds ranged in type from long-chain carboxylic acids (isovaleric acid), higher-order alcohols (isohexanol and 1-octanol), aldehydes (nonanal), benzene (vinylbenzene), esters (ethyl butyrate, isoamyl acetate, hexyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, phenethyl acetate, ethyl nonanoate, isobutyl octanoate, ethyl 9-decenoate), furans (5-methyl-2-furanmethanethiol), and a ketone (6-tetradecanone).

Table 3. Semi-volatile compounds that were present in beers brewed from barley and sorghum and were found to be highly significantly different in the median amount relative to the internal standard. LRI values that are bold were not confirmed.

Compounds	LRI	*p*-Value
Acids		
Isovaleric acid	861	1.33×10^{-4}
Alcohol		
Isohexanol	835	5.16×10^{-4}
1-Octanol	**1054**	1.48×10^{-5}
Aldehydes		
Nonanal	**1105**	7.4×10^{-7}
Benzene		
Vinyl benzene	889	1.51×10^{-6}
Esters		
Ethyl butyrate	778	2.62×10^{-10}
Isoamyl acetate	**873**	4.33×10^{-3}
Hexyl acetate	**990**	1.62×10^{-4}
Ethyl hexanoate (caproate)	1000	1.72×10^{-10}
Ethyl octanoate (caprylate)	1197	2.2×10^{-10}
Ethyl decanoate (caprate)	1395	4.3×10^{-9}
Phenethyl acetate	1254	1.17×10^{-8}
Ethyl nonanoate	**1288**	1.08×10^{-5}
Isobutyl octanoate	**1348**	3.06×10^{-4}
Ethyl 9-decenoate	**1360**	8.83×10^{-4}
Furans		
5-Methyl-2-furanmethanethiol	**701**	9.99×10^{-4}
Ketones		
6-Tetradecanone	1500	9.34×10^{-4}

(based on analysis of 54 different fermentations of sorghum (27) and barley (27)).

4. Discussion

Beer is a complex beverage system despite being made up of only four ingredients: yeast, water, hops, and malted grain. Typically, malted barley is used as the primary grain source for brewing, however alternative grains/pseudo-grains like sorghum are gaining popularity. However, the use of sorghum in beer manufacturing does have its issues, which are largely due to its low amylolytic activity (which is insufficient for complete saccharification), high gelatinization temperature, and low free amino content [21,22]. Sorghum malt has a higher concentration of alpha-amylase and a lower concentration of beta-amylase compared to malted barley. Due to the reduced enzymatic activity of sorghum this deficiency can lead to insufficient production of fermentable sugars, high dextrin content, and increased viscosity [23,24]. Due to sorghum's higher gelatinization temperature, the hydrolysis of sugars into fermentable sugars is only partially completed. Thus, resulting in fewer fermentable sugars for the yeast to metabolize for the production of ethanol, and volatile and semi-volatile compounds [25].

Wort, is composed of a number of different nitrogenous sources, however, yeast is only able to utilize the individual units (amino acids) and smaller peptides [26]. Free amino nitrogen is necessary for yeast health, growth, viability, vitality, fermentation efficiency, and beer stability and quality [27]. Optimum FAN levels can vary from yeast strain to yeast strain, batch to batch, and wort sugar levels and type. The general consensus is that anywhere from 100–140 mg L^{-1} FAN is required for a satisfactory fermentation, however, those values can vary based upon the fermentation matrix (wort vs. must) as well as the wort's starting gravity [28,29]. As expected, FAN levels will vary between malted barley and sorghum. Sorghum malts tend to have lower FAN levels as well as lower levels of valine [22,30]. This can lead to the production of higher levels of vicinal diketones (diacetyl) [31].

Research has shown that the initial FAN levels can have either a positive or negative effect on the development of certain aroma compounds produced by the yeast during fermentation [32]. While the concentration of fermentable sugars is important and can be a limited factoring, it is likely that for this experiment the FAN levels could have potentially reduced the yeasts' ability to produce a number of aroma active compounds [21]. If FAN levels are too low the yeast will produce lower concentrations of esters. Excessive levels of FAN can result in the overproduction of off-flavors such as diacetyl and higher (fusel) alcohols [33]. Due to the less than ideal starting FAN values in the sorghum wort, it is suspected that the FAN concentration played a negative role in the overall concentration of esters produced by the yeast. Despite not utilizing enzymes to help increase mash extraction efficiency, it would have not helped in this case. Malting and processing techniques will play a greater role in the overall concentration of FAN within the wort than in the utilization of enzymes [34]. Key differences between malted barley and sorghum could be associated with the steeping regime and the sorghum cultivar used to develop the LME [35].

Beer flavor is the result of a mixture of volatile, semi-volatile, and non-volatile compounds and their interaction with each other. The formation of flavor compounds is a complex and critical process that plays an important role in the quality of beer [36]. A number of flavor compounds have been identified in beer such as alcohols, esters, carbonyls, organic acid, sulfur compounds, amines, and phenol. A number of volatile compounds will contribute to the overall flavor and aroma of the final product, with other compounds playing a minor role in enhancing the flavor of the product [37].

The volatile and semi-volatile compounds produced during the fermentation process are the result of the yeast strain selected, the wort composition, and the brewing process [25]. The use of gas chromatography coupled with mass spectrometry (GC-MS) for the identification and quantification of the volatile and semi-volatile compounds found in beer to predict the flavor and aroma compounds is a common as well as established practice [38].

Long-chain carboxylic acids can contribute fruity, cheesy, and fatty odors and can also contribute to bitterness, astringency, and potentially rancidity. Alcohols contribute to

the strong and pungent smell and taste of beer. The higher-order alcohols are important precursors of flavor esters, and the presence and control of these alcohols can have an impact on the production of these flavor esters. Esters contribute fruity flavors and play an important role in balancing beer flavors. Esters contributed to the largest class of compounds in this study.

These mainly derive from yeast metabolism and not from the original malted grains and the majority of these compounds have been reported in previous studies of beer [14–16]. The compounds are a mixture of pleasant and unpleasant aromas, with many adding fruity or sweet character to the aroma, but several off aromas were noted. Compounds such as 1-octanol, and ethyl octanoate have unpleasant aromas from aromatic to fatty. The addition of these at levels that are at or above the perception threshold would certainly reduce the consumer experience. As the concentrations determined in this study are approximate based on an assumed response factor no relation to the perception thresholds could be determined. However, with the compounds being highly statistically different and primarily from yeast metabolism, changes in yeast strains could influence the relative concentrations of these key compounds.

The common flavors associated with malted barley are largely due to the roasting process [39]. Unlike malted barley, less is known about the compounds responsible for sorghum's flavor. There has been some research looking at the impact malting germination temperature has on fusel alcohol production for sorghum beers [36], but there is limited published data on the overall profile of sorghum brewed beers. Ma et al. 2016 is one of the few published papers looking at the volatile and semi-volatile compounds found in beer focusing on extruded and unextruded white sorghum used as a brewing adjunct. It should also be noted that utilizing 100% sorghum as your grain source for the production of beer could result in impaired yeast growth as well as impaired enzymatic activity [21], which is why some brewers will adjust their grain bill to incorporate malted barley as a way to overcome these issues [36]. Einfault (2020) looked at three different yeast strains to evaluate their fermentation activity and sensory characteristics. However, it is difficult to compare the two studies, since they focused on sixteen specific compounds, unlike this study that focused on complete characteristics. It also should be noted that Einfault's study utilized SafAle™ WB-06, which is a variant of *S. diastaticus* used to produce a high attenuating German Wheat beer. This strain of yeast is known for its subtle ester and phenol notes [40]. This particular study used SafAle™ US-05 American ale yeast, traditionally used for its neutral and clean-producing flavor profile.

Even though a number of the same chemical (esters, alcohols, aldehydes, etc.) compounds are found in malted barley and sorghum-based beer, the overall concentrations of these chemical groups will vary due to differences in the chemical composition (Total nitrogen, FAN, sugar concentration, etc.) of the grains used. These differences in the composition of barley and sorghum influence the yeast's ability to metabolize these metabolites for secondary metabolite production of the wort [26]. The final sensory profile of the beer is not solely reliant upon the volatile and semi-volatile compounds produced by the yeast, but by the combination of the hops used and their addition to the boil, along with the grain source as well as the compounds produced by the yeast during the fermentation process [21].

5. Conclusions

The analysis of beer brewed from extracts of sorghum and barley was undertaken to determine the chemical composition of the major fermentation compounds. A large number of compounds changed in apparent concentrations and the major differences were not in a set of unique compounds but in a larger set of compounds that are the constituents of both beer types. The concentrations of these compounds were approximated using the response relative to an internal standard and it would be of interest to continue this work by looking at the effect of different yeast strains on the observed aroma compounds.

In addition, a targeted analysis of some of the key compounds should be undertaken to determine actual concentrations in the resulting beers.

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