Phenolic and Volatile Compounds in the Production of Sugarcane Vinegar

Gan-Lin Chen,*,# Feng-Jin Zheng,*,# Bo Lin, Shui-Bing Lao, Jie He, Zhi Huang, Yuan Zeng, Jian Sun, and Krishan K. Verma

ABSTRACT: This study aimed to explore the dynamic variations in the phenolic and volatile organic compounds of sugarcane vinegar subjected to different production processes. The determination of phenolic and volatile organic compounds was performed by UPLC-MS and solid phase micro extraction (SPME) coupled with gas chromatography combined with mass spectrometry (GC–MS). The complete fermentation process of sugarcane lasted nine days, and production of vinegar of up to 3.04% (w/v), total acids, and 4.1% alcoholicity was accomplished. Various phenolic compounds of sugarcane juice (non-sterilized) and those of alcoholic and acetic acid fermentation were obtained after nine days of fermentation. These were benzoic acid (2.024, 1.002, and 1.027 mg L\(^{-1}\)), furmeric acid (0.060, 0.205, and 1.124 mg L\(^{-1}\)), quinic acid (0.019, 0.074, and 0.031 mg L\(^{-1}\)), chlorogenic acid (0.349, 1.635, and 1.217 mg L\(^{-1}\)), apigenin (0.002, 0.099, and 0.004 mg L\(^{-1}\)), kaempferol (0.003, 0.336, and 0.003 mg L\(^{-1}\)), caffeic acid (−, 0.005, and 0.005 mg L\(^{-1}\)), luteolin (0.003, 0.323, and 0.005 mg L\(^{-1}\)), and p-coumaric acid (0.018, 0.015, and 0.027 mg L\(^{-1}\)). Forty-five volatile organic compounds were also identified. The sugarcane juice can be commercialized as an alternative to wine as it presents characteristics of an alcoholic fermented beverage.

1. INTRODUCTION

Vinegar is one of the most popular and valuable food products throughout the globe. Vinegar is a traditional acidic condiment in China and has drawn more attention for its several health benefits. It acts as an antioxidant, antibacterial, anti-inflammatory, and anticancer agent; helps in preventing cardiac disorders; regulates high blood pressure and glucose and lipid metabolism; improves cognition; and promotes weight loss.\(^1\)–\(^4\) It is used as a seasoning and preservative for preparing foods and sometimes as a beverage.\(^5\) It is obtained from raw materials containing mainly carbohydrates by a two-step fermentation process: one involves ethanol formation by yeasts (usually Saccharomyces spp.) by the conversion of fermentable sugars (alcoholic fermentation) and the second involves the oxidation of ethanol to acetic acid (acetic acid fermentation or acetylation).\(^5\)–\(^7\) Due to its health benefits, the Chinese have developed various types of vinegar products in recent years, like apple cider and other fruits vinegars, which are most popular. Fruit vinegar is brewed with artificially planted or wild-type fruits as raw materials; the production cost of the raw materials determines the scale of research and development and production of vinegar. Recently, new products linked with various fruits have arisen, that is, fruit juice with added vinegar and fruit vinegar, which improve and/or maintain the organoleptic and health-promoting benefits.\(^1\)–\(^4\),\(^8\)–\(^10\)

There are few research organizations and/or industries which are involved in the production of sugarcane-based vinegar beverages. Therefore, the agro-food industries are focusing their research and development on unique food products with a higher nutritional value, based on traditional processes.\(^11\) The quality of these food products and their acceptance by users depends on their various characteristics, the most important one being aroma. In wine-based vinegars and derived products, aroma is due to the presence of various volatile organic compounds (VOCs) which belong to various chemical classes. The VOCs may come from the products like red wines, fruits, cider, malted barley, honey, and others and/or may be formed during fermentation production and storage.\(^8\),\(^10\)–\(^14\) VOCs are present to a large extent in fruits and aromatic/medicinal plants and significantly affect the

Received: September 15, 2020
Accepted: November 4, 2020
Published: November 17, 2020
quality of vinegars. Importantly, the production of sugarcane vinegar involves various processes like alcoholic and acetic acid fermentation and fumigation. These processes affect the total phenolic contents of the vinegars. Few studies have been carried out to determine the levels of the phenolic acids and volatile organic compounds generated during the production of sugarcane vinegar.

Sugarcane is cultivated in about 102 countries of arid and semi-arid areas covering an area of about 24 million hectares. In China, sugarcane has been used for sugar production for a long time, but only recently winemaking from sugarcane has drawn the attention of the Chinese researchers/scientists. Sugarcane provides sugar and alcohol and its cultivation originates from Southeast Asia. Globally, China is the third largest producer of sugarcane and is also its exporter. China’s sugarcane production is forecast at 9.25 million metric tons for the year 2020−2021, up by 450,000 metric tons from the previous year. The predicted increase is mainly due to the expected return of normal atmospheric conditions as well as stable sugarcane prices. A total of 9.445 million tons of sugarcane was produced during the year 2018−2019 crushing season. Guangxi province in China is the largest producer of sugarcane (11.54 million mu in 2018−2019), which produces 6.34 million tons of cane sugar, amounting to 67.1% of the country’s total cane sugar production (source: www.chinasugar.org.cn).

Sugarcane juice is a drink which is commonly consumed in various countries and is rich in carbohydrates and various electrolytes. It has been shown to be more effective as a sports and rehydration drink. Sucrose is the main component of cane juice, is a more suitable carbon source for microbial sports and rehydration drink. Sucrose is the main component.

Figure 1. Changes in total phenolic contents in the extract of sugarcane juice (NS—non-sterilized) and vinegar fermentation during alcoholic and acetic acid fermentation processes. Results are expressed as the mean ± SE (n = 3). Sugarcane juice (0 days, NS—non-sterilized) and alcoholic and acetic acid fermentation.

The quantification of phenolic compounds gives an estimate of the content of all compounds belonging to the subclass of phenolic compounds present in a sample (Figure 1). The phenolic compounds which were quantified in the beverage were benzoic acid, ferulic acid, quinic acid, chlorogenic acid, apigenin, kaempferol, caffeic acid, luteolin, and p-coumaric acid (Table 1). Significant differences were observed in the levels of phenolic compounds produced during fermentation. In this study, benzoic acid content was the highest followed by chlorogenic and ferulic acid in sugarcane juice (non-sterilized), alcoholic fermentation was found highest in chlorogenic acid followed by benzoic and kaempferol, and the chlorogenic acid level was found highest followed by ferulic and benzoic acid in acetic acid fermentation. Before fermentation of sugarcane juice (non-sterilized), mainly eight phenolic compounds were identified. During fermentation, nine phenolic acids were identified (Figure 1 and Table 1).

The phenolic acids in sugarcane vinegar fermentation substrates under different production processes were subjected to principal component analysis (PCA) (Figure 2A). The first three principal components explained 219.91% of the total variation (PC1 = 53.44%, PC2 = 77.44%, and PC3 = 89.03%, respectively). PC3 was highly affected by chlorogenic acid, quinic acid, benzoic acid, apigenin, kaempferol, and luteolin acid. PC 1 and PC2 were primarily correlated with p-coumaric acid, caffeic acid, and ferulic acid. The relatively dispersed distribution of the data points in the PCA plot revealed the differential changing pattern of phenolic acids produced during the sugarcane vinegar fermentation substrates and those produced during alcoholic and acetic acid fermentation production processes. The similarities of phenolic acids in vinegar fermentation substrates were evaluated through hierarchical cluster analysis (HCA) (Figure 2B). Similarly, phenolic acids were also obtained in the extracts of sugarcane vinegar during alcoholic and acetic acid fermentation.

The extracted VOCs of aromatic substances in sugarcane juice, wine, and vinegar were between 95 and 115 species. The...
Table 1. Phenolic Compounds in the Sugarcane Juice (Non-sterilized) and Fermented Sugarcane Juice (Raw Vinegar) (mg L⁻¹)ᵃ

| phenolic acid | sugarcane juice (non-sterilized) | alcoholic fermentation (days) | acetic acid fermentation (days) |
|--------------|----------------------------------|------------------------------|-------------------------------|
|              |                                  | 1   | 3   | 5   | 7   | 9   | 1   | 3   | 5   | 7   | 9   |
|               |                                  |     |     |     |     |     |     |     |     |     |     |
| benzoic acid | 2.024 ± 0.018ᵇ                  | 1.188 ± 0.012ᵇ              | 1.286 ± 0.141ᵇ              | 0.925 ± 0.232ᵇ              | 0.025 ± 0.008ᵇ              | 0.001 ± 0.007ᵇ              | 1.016 ± 0.025ᵇ              | 0.002 ± 0.004ᵇ              | 0.968 ± 0.003ᵇ              | 1.027 ± 0.099ᵇ              |
| ferulic acid | 0.060 ± 0.004ᵇ                  | 0.343 ± 0.000ᵇ              | 0.264 ± 0.017ᵇ              | 0.134 ± 0.017ᵇ              | 0.219 ± 0.017ᵇ              | 0.205 ± 0.015ᵇ              | 1.160 ± 0.017ᵇ              | 1.154 ± 0.057ᵇ              | 1.340 ± 0.002ᵇ              | 1.458 ± 0.009ᵇ              | 1.24 ± 0.003ᵇ              |
| quinic acid  | 0.019 ± 0.002ᵇ                  | 0.104 ± 0.008ᵇ              | 0.090 ± 0.012ᵇ              | 0.081 ± 0.012ᵇ              | 0.011 ± 0.009ᵇ              | 0.016 ± 0.0004ᵇ             | 0.007 ± 0.0004ᵇ             | 0.009 ± 0.0004ᵇ             | 0.015 ± 0.0004ᵇ             | 0.032 ± 0.0003ᵇ             | 0.073 ± 0.0003ᵇ             |
| chlorogenic acid | 0.349 ± 0.004ᵇ              | 3.687 ± 0.117ᵇ              | 3.595 ± 0.054ᵇ              | 3.589 ± 0.076ᵇ              | 1.857 ± 0.0007ᵇ             | 1.635 ± 0.0071ᵇ             | 1.185 ± 0.003ᵇ              | 1.425 ± 0.0023ᵇ             | 1.365 ± 0.0033ᵇ             | 1.258 ± 0.0011ᵇ             | 1.217 ± 0.0063ᵇ             |
| apigenin     | 0.002 ± 0.0001ᶜ                 | 0.024 ± 0.0004ᵈ             | 0.053 ± 0.003ᵈ             | 0.059 ± 0.0011ᶜ             | 0.018 ± 0.0003ᵈ             | 0.099 ± 0.0045ᵇ             | 0.003 ± 0.0007ᵇ             | 0.004 ± 0.0003ᵈ             | 0.007 ± 0.0001ᶜ             | 0.007 ± 0.0001ᶜ             | 0.0004 ± 0.0000ᶜ             |
| kaempferol   | 0.003 ± 0.0005ᵈ                 | 0.081 ± 0.0012ᵈ             | 0.181 ± 0.0013ᵈ             | 0.197 ± 0.0045ᵇ             | 0.267 ± 0.0017ᵇ             | 0.336 ± 0.00098ᵇ            | 0.005 ± 0.00004ᵈ             | 0.002 ± 0.0003ᵈ             | 0.002 ± 0.00004ᵈ             | 0.003 ± 0.00004ᵈ             | 0.0000 ± 0.00000ᵈ             |
| caffeic acid | –                                | 0.005 ± 0.0005ᵈ             | 0.006 ± 0.0004ᵈ             | 0.005 ± 0.0005ᵇ             | 0.005 ± 0.0006ᵈ             | 0.005 ± 0.0006ᵈ             | 0.005 ± 0.0006ᵈ             | 0.005 ± 0.0006ᵈ             | 0.005 ± 0.0006ᵈ             | 0.005 ± 0.0006ᵈ             | 0.005 ± 0.0006ᵈ             |
| luteolin     | 0.003 ± 0.001ᵈ                  | 0.079 ± 0.0002ᵈ             | 0.175 ± 0.0003ᵈ             | 0.188 ± 0.0007ᵈ             | 0.264 ± 0.0002ᵈ             | 0.323 ± 0.0002ᵈ             | 0.006 ± 0.0004ᵈ             | 0.004 ± 0.0003ᵈ             | 0.004 ± 0.0004ᵈ             | 0.004 ± 0.0004ᵈ             | 0.0006 ± 0.0000³ᵈ            |
| p-coumaric acid | 0.018 ± 0.003³ᵈ              | 0.014 ± 0.0028ᵈ             | 0.013 ± 0.0021ᵈ             | 0.013 ± 0.0018ᵈ             | 0.015 ± 0.0044ᵈ             | 0.015 ± 0.0019ᵈ             | 0.020 ± 0.0050ᵈ             | 0.0041ᵈ             | 0.022 ± 0.0000³ᵈ             | 0.0000 ± 0.0000³ᵈ             | 0.0000 ± 0.0000³ᵈ             |

ᵃ: ‘-‘: non-detection. Results are expressed as the mean ± SD (n = 3). In each row, different superscript letters indicate significant differences among different production processes.

Figure 2. Principal component analyses (PCA – A) and hierarchical cluster (B) of phenolic acid compounds of sugarcane fermentation during the alcoholic and acetic acid fermentation processes. NS – non-sterilized, AF – alcoholic fermentation, AAF – acetic acid fermentation.
the relative content of isobutanol and isoamyl alcohol gradually decreases. Isoamyl alcohol has an apple brandy aroma and pungent taste. It exists in sugarcane juice in the form of natural esters produced by the metabolism of sugar compounds in sugarcane juice by yeast, and their relative content increases to 13.48% and constitutes the main component of the characteristic flavor of sugarcane wine.

Phenylethanol has a variety of flavors such as rose, violet, jasmine, and so forth. Esters, the main components of fermented wines, are volatile compounds with an aromatic odor and play a key role in the formation of fermented wines. The types and relative content of ester compounds have increased significantly. The types have

vinder are presented in Table 2 and Figure 4 as a heatmap. The cluster analysis according to the squared Euclidean distance method was carried out to identify the similarity among the samples.

Alcohols are the main substances in fermented wines that give the sweetness and flavor to the wine. The alcohol and acid react to generate various esters, which constitute the special aroma of fermented wines. After sugarcane juice is completely fermented with alcohol, the type and the relative content of its alcoholic compounds, namely, n-pentanol, 2-heptanol, n-hexanol, and so forth, were estimated, and it was found that the relative content of isobutanol and isoamyl alcohol gradually decreases. Isoamyl alcohol has an apple brandy aroma and pungent taste. It exists in sugarcane juice in the form of natural esters produced by the metabolism of sugar compounds in sugarcane juice by yeast, and their relative content increases to 13.48% and constitutes the main component of the characteristic flavor of sugarcane wine.

Phenylethanol has a variety of flavors such as rose, violet, jasmine, and so forth. Esters, the main components of fermented wines, are volatile compounds with an aromatic odor and play a key role in the formation of fermented wines. The types and relative content of ester compounds have increased significantly. The types have
also determines the fl

Ethanol is on ethanol during the two-step acetic acid fermentation. to the action of ethanol dehydrogenase of acetic acid bacteria

content is 27.914%, these are acetic acid, propionic acid, valeric higher than wine. There are six types of acids whose relative types and relative content of acids in sugarcane vinegar are

during fermentation.

Maillard reaction and microbial metabolism of sugarcane juice.

These compounds are closely related to those involved in the formation of esters. The relative content of acidic compounds in sugarcane wine decreased to 4.529%. Among them, the relative content of acetic acid was reduced to 1.736%, but it is still the predominant compound. 2-Hydroxycinnamic acid was not detected in sugarcane wine. Although the relative content of acetic acid was reduced to 1.736%, but it is still the flavoring agent in sugarcane wine. Propionic acid appears in the sugarcane wine after fermentation and is a by-product of yeast protein metabolism. The types and relative content of hydrocarbons, phenols, and other heterocyclics were significantly reduced, especially \( \text{o-methoxyphenol} \) and \( \text{4-vinyl-2-methoxyphenol} \). The relative contents of \( \text{3-dihydrobenzofuran} \) and \( \text{1,3-di-tert-butylbenzene} \) were reduced to less than 0.2%. These compounds are closely related to those involved in the Maillard reaction and microbial metabolism of sugarcane juice during fermentation.

Sour taste is the main characteristic of fruit vinegar, and it also determines the flavor and quality of the product. The types and relative content of acids in sugarcane vinegar are higher than wine. There are six types of acids whose relative content is 27.914%, these are acetic acid, propionic acid, valeric acid, \( \text{n-octanoic} \) acid, \( \text{2-hydroxyacetic} \) acid, and caprylic acid. Acetic acid has a strong vinegar fragrance. The acetic acid content in sugarcane vinegar increases significantly, mainly due to the action of ethanol dehydrogenase of acetic acid bacteria on ethanol during the two-step acetic acid fermentation. Ethanol is first oxidized to form acetaldehyde, and then acetaldehyde is oxidized to form acetic acid. These acids impart the flavor to fruit vinegar.

Esters are important in imparting the aroma characteristics to fruit vinegar. The aromas are floral, fruity, wine, and honey. The types of esters in sugarcane vinegar increased compared with sugarcane wine, and the relative content decreased (59.01%), mainly because the relative content of ethyl lactate decreased, while the relative content of ethyl acetate increased (13.42%). Ethyl acetate has a fruity aroma when isobutyl acetate and ethyl isovalerate are added to it. These ester compounds are used during fermentation of sugarcane by acetic acid bacteria. The relative content and types of alcohol in sugarcane vinegar have been reduced, but they still contribute to the flavor of sugarcane vinegar, and they are mainly isobutanol, isomyl alcohol (3.703), 2-heptanol, \( \text{n-hexanol} \), and so forth. In the process of acetic acid fermentation, the ventilation and aging of the processing technology also reduce the content of some volatile components of raw sugarcane vinegar.

3. DISCUSSION

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have shown that the final product should be one having considerable standards of safety and is an important element for safety purposes. The moisture content in sugarcane juice was 860 ± 103 g L\(^{-1}\). This value is in accordance with the guidelines of the Organization for Economic Co-operation and Development (OECD) which states that the extracted sugarcane juice has nearly 86% water. The moisture content of the sugarcane juice helps in the fermentation for the preparation of beverages. The nutritional value of sugarcane juice is associated to its higher sugar level while its protein and lipid levels are very low. Mineral nutrients such as calcium, potassium, and phosphorus are mainly found in sugarcane juice. The method of harvesting and that of the filtration process determines the mineral content of cane juice.

The changes in the chemical composition of cane juice can occur due to various reasons, namely, due to varietal differences in the crop, soil conditions, climatic changes, harvesting time, and the method of extraction and filtration of the juice. The pH of cane juice indicates that the product can be consumed or not. The variation in pH of cane juice can be attributed to the harvesting period of the crop and the method of extraction of the juice. The variation in the soluble solid content (SSC) of the juice may be due to environmental conditions, soil profile, harvesting time, crop variety, as well as the way the crop was harvested. The crop with higher soluble solids content was more suitable for fermentation. The level of sugarcane juice in a crop will depend on the crop variety, harvesting time, and other factors. During fermentation, a declining trend in the pH of cane juice was observed. The pH values were found to be reduced on the third day of fermentation, and then it was found to be more or less constant throughout the fermentation process. A similar trend was seen in \( \text{Ananas comosus} \) L. Merr. fermented beverage from Angola. The important factor in determining the final quality of fermented drink is the presence of volatile acids. The presence of acetic acid is not desirable in alcoholic fermentation because besides changing the flavor and aroma of the drink, it also indicates contamination by acetic acid bacteria.

In wine, there are two groups of phenolic acids, namely, hydroxybenzoic and hydroxycinnamic acid. Gallic acid, a type of phenolic acid, is found in the plants in the form of free

![Figure 3. Classes of volatile compounds in the sugarcane juice, the sugarcane wine and the sugarcane vinegar. Results are presented as the mean ± SE (\( n = 3 \)).](https://dx.doi.org/10.1021/acsomega.0c04524)
acids, esters, catechin derivatives, and hydrolysable tannins.\textsuperscript{44} Gallic acid and its derivatives showed good biological activity as an antimicrobial, antioxidant, and antidiabetic agent. The content of gallic acid in sugarcane wine is comparable to that of regular wine.\textsuperscript{45} Tian et al.\textsuperscript{45} showed that the phenolic compounds, namely, ferulic acid, chlorogenic acid, caffeic acid, and $p$-coumaric acid of dry wine obtained from grapes were also found in the wine produced in the present study.

The phenolic compounds in vinegar are mainly derived from the raw materials. However, the changes in phenolic contents occurred during the different stages of fermentation in the production of vinegar.\textsuperscript{46} As expected, the phenolic contents in vinegar fermentation substrates steadily enhanced as alcoholic and acetic acid fermentation progressed. The production of phenolic acids is due to specific chemical changes during acetic acid fermentation.\textsuperscript{47} The level of phenolic acids, flavonoids, and aroma components changes during vinegar production.\textsuperscript{16,48,49}

In fermented sugarcane juice, forty-five VOCs were quantified. The higher alcohol concentration is the main

Figure 4. Hierarchical clustering of volatile organic compounds detected in sugarcane juice, wine and vinegar.
precursors for the formation of esters and related aromas.\textsuperscript{42} 1-octanol contributes to a fruity aroma in beverages and significantly contributes to their flavor by enhancing sweetness and improving after taste.\textsuperscript{50} Acetal (1,1-diethoxyethane) is a VOC formed during fermentation in the production of sugarcane wines and plays a significant role in imparting a sweet cookie flavor to the wine.\textsuperscript{51}

In conclusion, the optimized method based on HS-SPME/GC–MS was found to be a suitable tool for the identification of the volatile organic compounds of sugarcane vinegar obtained from sugarcane juice. A total of forty-five VOCs were quantified. The VOCs of sugarcane vinegar may be influenced by environmental conditions, light intensity, and agronomic methods used for the farming of sugarcane crop. The VOCs impart the flavor and aroma to the beverage. Different phenolic compounds such as benzoic acid, ferulic acid, quinic acid, chlorogenic acid, apigenin, kaempferol, caffeic acid, luteolin and p-coumaric acid were identified, and they were found in concentrations which could be compared with those present in other wine varieties. This method helps to identify the authenticity of composition of wine as well wine-based aromatc vinegars. The findings suggest that sugarcane juice can be used as an alternative to produce wine once it is optimized to have appropriate characteristics for an alcoholic fermented beverage. Therefore, fermented sugarcane juice may eventually find a place in the global agro-industrial sector.

4. EXPERIMENTAL SECTION

The mature stalks of sugarcane (\textit{Saccharum officinarum} L. spp. Hybrid) were collected from the experimental area of the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences (GxAAS), Nanning, Guangxi, China. After harvesting, they were transported to the laboratory, washed, and crushed in the Agricultural Products Processing Research Institute, GxAAS, Nanning, Guangxi, China. The collected sugarcane juice was filtered to remove suspended solids.

4.1. Fermentation Conditions. The alcoholic and acetic acid fermentations were carried out in three steps. The total soluble solid content of sugarcane juice was standardized to 20 °Brix (pH 5.5). The inocula were activated by solubilization of 10 g of yeast in 100 mL of water at 40 °C, and the solution was stirred manually, and then the yeast was added to the wort at 10 gL\textsuperscript{-1} concentration, as per the manufacturers’ instructions. The fermentation process following inoculation was performed in a container (1000 L) fitted with a hydraulic bung outlet for removing CO\textsubscript{2}. The vessel was kept under a controlled temperature (27 °C) in an incubator, until the soluble solid content reached 5 °Brix or up to constant. After fermentation, the medium was filtered through a 0.22 \textmu m microporous membrane into glass bottles previously cleaned and sterilized, and the bottles were then kept in a refrigerator under a controlled temperature. Production of vinegar of up to 3.04% (w/v) total acid and 4.1% alcoholicity was accomplished.\textsuperscript{52} In the current study, the fermentation substrates of sugarcane vinegar during different production periods, including raw materials, alcoholic and acetic acid fermentation products (1–9th day) were collected for further analysis.

4.2. Determination of Phenolic Compounds by Ultra-High Pressure Liquid Chromatography Tandem Mass spectrometry (UPLC-MS). The phenolic compounds were analyzed according to the method\textsuperscript{53} by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS), (Waters EVEO TQ-S, Waters Corporation, USA) with an electrospray ionization source. The nebulizer gas was nitrogen, and the collision was argon. MS/MS detection was performed in negative and positive-ion modes and by using multiple reaction monitoring. The mobile phase consisting of deionized water with 0.1\% formic acid and acetonitrile with 0.1\% formic acid was pumped at a flow rate of 0.25 mL min\textsuperscript{-1}. The gradient elution program was as follows: 95% A from 0 to 0.88 min, 95 to 78% A from 0.88 to 1.28 min, 78% A from 1.28 to 4.48 min, 78 to 55% A from 4.48 to 9.08 min, 55% A from 9.08 to 13.88 min, 55 to 95% A from 13.88 to 14.50 min, and 95% A from 14.50 to 15.40 min. The column temperature was maintained at 40 °C, and the injected sample volume was 2 \textmu L. Individual phenolic compounds were identified by comparing the retention time with respective standards and quantified using external standard methods.

4.3. Determination of Volatile Compounds by SPME. The volatile compounds were extracted by solid phase micro extraction (SPME) and determined by gas chromatography. Sample analysis was performed on a gas chromatograph coupled with a mass spectrometer (Bruker, USA) and a mass detector, as per the method.\textsuperscript{54} The instrument conditions were as follows: injector temperature 250 °C, detector temperature 300 °C, flow rate of hydrogen as a carrier gas at 3.3 mL min\textsuperscript{-1}, and nitrogen as a make-up gas at 30 mL min\textsuperscript{-1}. The flow rates of detector gas (hydrogen) and air were 40 and 400 mL min\textsuperscript{-1}, respectively. The programmed temperature was 50 °C (3 min), increased to 90 °C at the rate of 5 °C min\textsuperscript{-1}, and held at 230 °C for 7 min at 10 °C min\textsuperscript{-1}. The mass spectra were compared with those of the literature and NIST Standard Reference Database and Willey 8 Library.

4.4. Statistical Analysis. Data were presented as the mean ± SD. One-way ANOVA was performed using SPSS 23.0 statistical software. Differences were considered significant at p < 0.05. PCA and HCA were used to analyze the interrelationship between the variables and the clustering characteristics in collected samples using MetaboAnalyt 3.0, respectively.

\section*{AUTHOR INFORMATION}

Corresponding Authors
Gan-Lin Chen — Institute of Biotechnology, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China; Email: ganlin-chen@gxaas.net
Feng-Jin Zheng — Institute of Agro-Products Processing Science and Technology, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China; Email: zhengfengjin@gxaas.net

Authors
Bo Lin — Institute of Agro-Products Processing Science and Technology, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Shui-Bing Lao — Institute of Agro-Products Quality Safety and Testing Technology, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Jie He — Institute of Agro-Products Quality Safety and Testing Technology, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Zhi Huang — Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China
Yuan Zeng — Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China

https://dx.doi.org/10.1021/acsomega.0c04524
ACS Omega 2020, 5, 30587–30595
Jian Sun − Institute of Agro-Products Processing Science and Technology, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China

Krishan K. Verma − Key Laboratory of Sugarcane Biotechnology and Genetic Improvement (Guangxi), Ministry of Agriculture and Rural Affairs/ Guangxi Key Laboratory of Sugarcane Genetic Improvement/ Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530 007, Guangxi, China; orcid.org/0000-0002-5501-7905

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c04524

Author Contributions

#G.-L.C. and F.-J.Z. have contributed equally to this work.

Funding

This study was financially supported by The China Spark Program (grant go. 2015GA790013), Guangxi Key Technology R&D Program (grant no: GK-AB16380244), Nanning Science and Technology Program of Guangxi (grant no. 201720103), Guangxi Academy of Sciences, Nanning, Guangxi, China for providing the necessary facilities for this study. All authors read, revised, and approved the manuscript for publication.

ACKNOWLEDGMENTS

All of the authors are grateful to the Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, China for providing the necessary facilities for this study. All authors read, revised, and approved the manuscript for publication.

REFERENCES

(1) Cejudo-Bastante, M. J.; Rodríguez-Dodero, M. C.; Durán Guerreo, E.; Castro Mejías, R.; Natera-Marin, R.; García Barroso, C.; Development and optimisation by means of sensory analysis of new beverages based on different fruit juices and sherry wine vinegar. J. Sci. Food Agric. 2013, 93, 741–748.
(2) Budak, N. H.; Ayki, E.; Seydmin, A. C.; Greene, A. K.; Guzel-Seydmin, Z. B. Functional properties of vinegar. J. Food Sci. 2014, 79, R757–R764.
(3) Cejudo-Bastante, C.; Castro-Mejías, R.; Natera-Marin, R.; García-Barroso, C.; Durán-Guerrero, C. Chemical and sensory characteristics of orange based vinegar. J. Food Sci. Technol. 2016, 53, 3147–3156.
(4) Chen, H.; Chen, T.; Giudici, P.; Chen, F. Vinegar functions on health: constituents, sources, and formation mechanisms. Compr. Rev. Food Sci. Food Saf. 2016, 15, 1124–1138.
(5) Tesfaye, W.; Morales, M. L.; García-Parrilla, M. C.; Troncoso, A. M. Wine vinegar: Technology, authenticity and quality evaluation. Trends Food Sci. Technol. 2002, 13, 12–21.
(6) Perestrello, R.; Silva, C.; Silva, P.; Câmara, J. Establishment of the volatile signature of wine-based aromatic vinegars subjected to maceration. Molecules 2018, 23, 499.
(7) Chen, G.-L.; Zheng, F.-J.; Sun, J.; Li, Z.-C.; Lin, B.; Li, Y.-R. Production and characteristics of high quality vinegar from sugarcane juice. Sugar Technol. 2015, 17, 89–93.
(8) Chininni, F.; Durán Guerreo, E.; Sonni, F.; Natali, N.; Natera Marin, R.; Riponi, C. Gas Chromatography–Mass Spectrometry (GC–MS) Characterization of Volatile Compounds in Quality Vinegars with Protected European Geographical Indication. J. Agric. Food Chem. 2009, 57, 4784–4792.
(9) Yu, Y.-J.; Lu, Z.-M.; Yu, N.-H.; Xu, W.; Li, G.-Q.; Shi, J.-S.; Xu, Z.-H. HS-SPME/GC-MS and chemometrics for volatile composition of Chinese traditional aromatic vinegar in the Zhenjia region. J. Inst. Brew. 2012, 118, 133–141.
(10) Shu, X.; Jiang, X.-W.; Cheng, B. C.-Y.; Ma, S.-C.; Chen, G.-Y.; Yu, Z.-L. Ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry analysis of the impact of processing on toxic components of Kansui Radix. BMC Complementary Altern. Med. 2016, 16, 73.
(11) Marrufo-Curtido, A.; Cejudo-Bastante, M. J.; Rodríguez-Dodero, M. C.; Natera-Marin, R.; Castro-Mejías, R.; García-Barroso, C.; Durán-Guerrero, E. Novel vinegar-derived product enriched with dietary fiber: Effect on polyphenolic profile, volatile composition and sensory analysis. J. Food Sci. Technol. 2015, 52, 7608–7624.
(12) Roberto, R. M.; García, N. P.; Hevia, A. G.; Valles, B. S. Application of purge and trap extraction and gas chromatography for determination of minor esters in cider. J. Chromatogr. A 2005, 1069, 245–251.
(13) Ubeda, C.; Callejón, R. M.; Hidalgo, C.; Torija, M. J.; Mas, A.; Troncoso, A. M.; Morales, M. L. Determination of major volatile compounds during the production of fruit vinegars by static headspace gas chromatography-mass spectrometry method. Food Res. Int. 2011, 44, 259–268.
(14) Jo, D.; Kim, G.-R.; Yeo, S.-H.; Jeong, Y.-J.; Noh, B. S.; Kwon, J.-H. Analysis of aroma compounds of commercial cider vinegars with different acidities using SPME/GC-MS, electronic nose, and sensory evaluation. Food Sci. Biotechnol. 2013, 22, 1559–1565.
(15) Ho, C. W.; Lázim, A. M.; Fazry, S.; Zaki, U. K. H. H.; Lim, S. J. Varieties, production, composition and health benefits of vinegars: A review. Food Chem. 2017, 221, 1621–1630.
(16) Yu, X.; Yang, M.; Dong, J.; Shen, R. Comparative analysis of the antioxidant capacities and phenolic compounds of oat and buckwheat vinegars during production processes. J. Food Sci. 2018, 83, 844–853.
(17) AFRIS (Animal Feed Resources Information System of FAO). Sugarcane Juice. 2015, http://www.feedipedia.org/node/560 (accessed 25 July, 2020).
(18) Farah, A. G. V. Brazilian Sugarcane Industry. The Brazil Business. 2013, http://thebrazilbusiness.com/article/brazilian-sugarcane-industry (accessed 24 April, 2020).
(19) Zhang, M.; Govindaraju, M. Sugarcane Production in China Sugarcane—Technology and Research. Alexandre Bosco de Oliveira; IntechOpen, 2018.
(20) United States Department of Agriculture Foreign Agricultural Service. Sugar Annual edited by Michael Francom; Gain Global Agricultural Information Network: Beijing, China. 2018.
(21) Kalpana, K.; Lal, P. R.; Kusuma, D. I.; Khanna, G. L. The effects of ingestion of sugarcane juice and commercial sports drinks on cycling performance of athletes in comparison to plain water. Asian J. Sports Med. 2013, 4, 181–189.
(22) Oliveira, E. R.; Callari, M.; Júnior, M. S. S.; Oliveira, A. R.; Duarte, R. C. M.; Boas, E. V. B. V. Assessment of chemical and sensory quality of sugarcane alcoholic fermented beverage. J. Food Sci. Technol. 2018, 55, 72–81.
(23) Nualsri, C.; Reungsang, A.; Plangklang, P. Biochemical hydrogen and methane potential of sugarcane syrups using a two-stage anaerobic fermentation process. Ind. Crop. Prod. 2016, 82, 88–99.
(24) James, B. J.; Ngarmmsak, T. Processing of Fresh-Cut Tropical Fruits and Vegetables: A Technical Guide; Food and Agriculture Organization of the United Nations: Bangkok, 2010.
(25) Kulkarni, M. S.; Kininge, P. T.; Ghasghase, N. V.; Mathapati, P. R.; Joshi, S. S. Effect of additives on alcohol production and kinetic studies of cultivar cerevisiae for sugar cane wine production. Int. J. Adv. Biotechnol. Res. Int. 2011, 2, 154–158.
(26) Tseng, D.-I.; Chia, Y.-C.; Tai, C.-Y.; Ou, A. S.-M. Investigation of Chemical Quality of Sugarcane (Saccharum Officinarum.) Wine During Fermentation Bysaccharomyces Cerevisiae. J. Food Qual. 2010, 33, 248–267.
(27) Le, V.-D.; Zheng, X.-W.; Chen, J.-Y.; Han, B.-Z. Characterization of volatile compounds in Fen-Daqu - a traditional Chinese liquor fermentation starter. *J. Inst. Brew.* 2012, 118, 107−113.

(28) Feng, Y.; Liu, M.; Ouyang, Y.; Zhao, X.; Ju, Y.; Fang, Y. Comparative study of aromatic compounds in fruit wines from raspberry, strawberry, and mulberry in Shaanxi area. *Food Nutr. Res.* 2015, 59, 29290.

(29) Duan, W.-F.; Zha, B.-Q.; Song, R.-R.; Zhang, B.; Lan, Y.-B.; Zhu, X.; Duan, C.-Q.; Han, S.-Y. Volatile composition and aromatic attributes of wine made with Vitissinifera L.Cabernet Sauvignon grapes in the Xinjiang region of China: effect of different commercial yeasts. *Int. J. Food Prop.* 2018, 21, 1423−1441.

(30) Rapp, A. Volatile flavour of wine: Correlation between instrumental analysis and sensory perception. *Nahrung* 1998, 42, 351−363.

(31) Rodríguez-Bencomo, J. J.; Conde, J. E.; Rodríguez-Delgado, M. A.; García-Montelongo, F.; Perez-Trujillo, J. P. Determination of esters in dry and sweet white wines by headspace solid-phase microextraction and gas chromatography. *J. Chromatogr. A* 2002, 963, 213.

(32) Salinas, M. R.; Garijo, J.; Pardo, F.; Zalacain, A.; Alonso, G. L. Color, polyphenol, and aroma compounds in rose wines after prefermentative maceration and enzymatic treatments. *Am. J. Enol. Vitic.* 2003, 54, 195−202.

(33) Li, H. *Wine Tasting*; Science Press: Beijing, China, 2006.

(34) Lorenzo, C.; Pardo, F.; Zalacain, A.; Alonso, G. L.; Rosario Salinas, M. Complementary effect of cabernet sauvignon on nonvolatile components with the total antioxidant capacity of Tartary buckwheat vinegar: Influence of the thermal processing. *Food Res. Int.* 2012, 49, 65−71.

(35) Zhang, Q.-H.; Huang, W.-D. Comparison of phenolic acids and flavan-3-ols during wine fermentation of grapes with different harvest times. *Molecules* 2009, 14, 827−838.

(36) Chen, S.; Su, J.; Liu, C.; Zhang, H. Y.; Heng, Y. W.; Liu, Y. B. Analysis of contents of total phenolic compounds and total flavones and DPPH radical scavenging activity during overmature vinegar production. *Food Sci.* 2009, 17, 038.

(37) Dávalos, A.; Bartolomé, B.; Gómez-Cordovés, C. Antioxidant properties of commercial grape juices and vinegars. *Food Chem.* 2005, 93, 325−330.

(38) Li, Y.-J.; Hu, J.-j.; Li, H.-m.; Shan, F.; Bian, J.-s.; Sun, Q.-y. Study on variations of main function ingredients in the Tartary buckwheat vinegar fermentation process with uncooked material. *Sci. Technol. Food Ind.* 2011, 12, 055.

(39) Wang, A.; Zhang, J.; Li, Z. Correlation of volatile and nonvolatile components with the total antioxidant capacity of Tartary buckwheat vinegar: Influence of the thermal processing. *Food Res. Int.* 2012, 49, 65−71.

(40) Zhang, M.; Pan, Q.; Yan, G.; Duan, C. Using headspace solid phase micro-extraction for analysis of aromatic compounds during alcoholic fermentation of red wine. *Food Chem.* 2011, 125, 743−749.

(41) Jewison, T.; Knox, C.; Neveu, V.; Djoumbou, Y.; Guo, A. C.; Lee, J.; Liu, P.; Mandal, R.; Krishnamurthy, R.; Sinelnikov, I.; Wilson, M.; Wishart, D. S. YMDB: the Yeast Metabolome Database. *Nucleic Acids Res.* 2012, 40, D815−D820.

(42) Chen, G.-L.; Zheng, F.-J.; Lin, B.; Wang, T.-S.; Li, Y.-R. Preparation and characteristics of sugarcane low alcoholic drink by submerged alcoholic fermentation. *Sugar Technol.* 2013, 15, 412−416.

(43) Peña-Neira, A.; Hernández, T.; García-Vallejo, C.; Estrella, I.; Suárez, J. A. A survey of phenolic compounds in Spanish wines of different geographical origin. *Eur. Food Res. Technol.* 2000, 210, 445−448.

(44) Blanco, P.; Mirás-Avalos, J. M.; Pereira, E.; Orriols, I. Fermentative aroma compounds and sensory profiles of Godello and Albariño wines as influenced by Saccharomyces cerevisiae yeast strains. *J. Sci. Food Agric.* 2013, 93, 2849−2857.