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Evolution of Volatile Compounds Generated During One-Stage and Two-Stage Fermentation and Aging Processes of Banana Vinegars Using HS-SPME-GC-MS

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Abstract

Volatile compounds generated during one-stage and two-stage fermentation and aging processes of banana vinegars were determined using headspace/gas chromatography-mass spectrometry analysis. A total of 35 volatile compounds were detected in the one-stage fermented banana vinegars with acetic acid and 1-butanol (2, 2 dimethyl) produced at high levels, while a two-stage fermentation process yielded a total of 48 volatile compounds with acetic acid and benzeneethanol detected abundantly. During a 100-days aging process, acetic acid and butanoic acid (3-methyl) were detected at high levels in the one-stage fermented vinegars while the two-stage fermented vinegars contained abundant amounts of acetic acid, benzeneethanol and acetoin.

Keywords: Aging process; Fermentation process; HS-SPME-GC-MS; Vinegars; Volatile compounds

Introduction

Fruit vinegar is one of the most popular food products that are widely available in the market. It is an acidic liquid that is produced by fermentation of fruit pulps or juices with the help of bacteria, consisting mainly of acetic acid, water and other trace chemicals to give flavor and taste [1]. In addition to being consumed directly, fruit vinegar is commonly used as a preservative, an antiseptic and a cleaning agent [2]. Fruit vinegar is a highly beneficial drink, as it helps to promote different kinds of beneficial effects to consumers, such as having antidiabetic effects and lowering cholesterol levels in blood by inhibiting the oxidation of low density lipoproteins (LDLs), among other benefits [3,4].

There has been a growing demand for fruit vinegars in recent years because of their health benefits proven by many studies [5,6]. As health benefits of fruit vinegars depend on their phenolic compounds that act as antioxidant agents [7], a variety of fruits that are rich in phenolics have been utilized for the production of fruit vinegars including banana [8-11].

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Aroma is among the essential aspects to consider when developing a new food product. The volatile composition of banana fruit has been studied in recent years and approximately 146 volatile compounds have been identified in banana extract, with 31 odorants considered as odor-active compounds in banana fruit [12].

The key odorants of fruit vinegars have been studied to a lesser extent, particularly those of banana vinegar. In rabbiteye blueberry (*Vaccinium ashei*) vinegars, acetic acid, 2/3-methyl-butanoic acid, phenethyl acetate, 2-phenylethanol, octanoic acid, eugenol, and phenylacetic acid were reported to be the most important aroma-active compounds [13]. In Zhenjiang aromatic vinegar, which is one of the four famous China-style cereal vinegars, esters (ethyl acetate, acetic acid 2-phenylethyl ester and dihydro-5-pentyl-2 (3 H)-furanone), acids (acetic acid and 3-methyl-butanoic acid) and carbonyl compounds including ketones and aldehydes (2, 3-butanedione and 3-hydroxy-2-butanone) were reported as the key odorants [14].

It is well documented that the overall aromas of vinegars are enriched when they are aged in barrels as a result of water loss through the wood pores, transfer of aromatic compounds from the wood, and formation of new compounds via chemical reactions [15-17]. According to this, changes in the volatile compounds of fruit vinegars as a consequence of aging processes should be investigated.

For this purpose, this study was undertaken to evaluate changes in the volatile compounds in banana vinegars produced from banana puree via one-stage and two-stage fermentation, and aging processes using headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS).

Materials and Methods

Chemicals

Solvents for GC analysis were of chromatographic grade. All other chemicals were analytical grade, provided by local chemical manufacturers. Deionized water was prepared by a Milli-Q Water Purification system (Millipore, MA, USA).

Banana samples, and vinegar fermentation and aging processes

Banana (*Musa acuminata* 'Gros Michel' cv. ‘Hom Thong’) fruits were purchased from local markets of Mahasarakham, Thailand. Banana vinegars were produced from banana puree via one-stage and two-stage fermentation. Banana puree was made by mixing 1 kg of banana pulp with 1 kg of distilled water with potassium metabisulfite (0.2 g/L) added to avoid undesirable microbial growth. The obtained banana puree was allowed to settle overnight at ambient temperature before use. One-stage vinegar fermentation was carried out by adding absolute alcohol to banana puree (1380 mL) to yield a final volume of 1500 mL, resulting in the initial alcohol content of 8%. The obtained mixture was then inoculated with *Acetobacter pasteurianus* TISTR 521 at a ratio of 10% (v/v) and kept for 20 d at 30°C on an incubator shaker (150 rpm). For two-stage fermentation, sucrose syrup was added to banana puree (2000 mL) to obtain a brix value of 18%, with the pH of the mixture adjusted to 4.47 using acetic acid. The obtained mixture was then inoculated with *Saccharomyces cerevisiae* TISTR 5049 at a ratio of 10% (v/v) to produce wine. The obtained wine with an adjusted alcohol content of 8% was inoculated with *A. pasteurianus* TISTR 521 at a ratio of 10% (v/v) and kept for 20 d under the same conditions. Sampling was performed at the end of the fermentation process to determine the volatile compounds of banana vinegars. A 100-day aging process was also performed to determine changes in the volatile compounds.

HS-SPME-GC-MS analyses

Volatile compounds in banana puree and those produced during one-stage and two-stage vinegar fermentation and aging processes were identified by HS-SPME-GC-MS analyses. A Bruker autosampler fitted with an SPME sampling system was used to extract and introduce in splitless mode 0.5 mL aliquots of the volatile constituents onto an Agilent DB-WAX capillary column (60 m × 0.25 mm i.d. film thickness 0.25 µm) installed in a Bruker model 450-GC chromatograph coupled to a Bruker model 320-MS mass selective detector.

The SPME system consisted of an SPME fiber holder, a fiber conditioning station and a sample incubation block capable of heating and agitation of the sample. Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was used for the analysis. The autosampler was controlled by MS Workstation software.

Samples were incubated at 60°C with pulse-agitation at 500 rpm. After 10 min of sample equilibration, the SPME fiber was inserted and exposed to the sample headspace for 30 min of incubation. The fiber was then injected into the GC inlet for 5 min to desorb the analytes before injection.

Helium was delivered as the carrier gas at a constant pressure with a flow rate of 0.8 mL/min. The temperature of the GC oven was programmed to hold at 28°C for 10 min, increased to 160°C at 3°C/min, then ramped up to 230°C at 4°C/min and finally held at this temperature for 10 min, giving a total run time of 81.5 min. The injector temperature was set at 60°C. Mass spectra were recorded at 70 eV in electron impact (EI) ionization mode. The temperature of the quadrupole mass detector, ion source and transfer line was 40°C, 250°C and 230°C, respectively. Mass spectra were scanned in the m/z range of 25 amu to 400 amu with 2.0 scan/s and a solvent delay of 3 min. Identification of the volatile compounds was done by comparison of the mass spectra of the samples with the data system library and retention indices. The volatile compounds were identified by using MS Workstation. The peak areas were used directly to give the percentage volatile composition of the vinegars by dividing the area of each peak to the total area under all of the peaks.

Results and Discussion

Volatile compounds identified during two-stage vinegar fermentation and aging processes

It was noted that the volatile constituents of banana puree were altered by brix and pH adjustment, and the volatile profiles of the two-stage fermented banana vinegars and aged vinegars were different. Banana puree utilized as a substrate for the fermentation process was found to contain a total of 17 volatile compounds. On the other hand, brix and pH adjustment resulted in the formation of other volatiles including 2-(3-methyl-1-butyloxy)-ethyl acetate; DI-2/3-methylbutyl maleate; butanoic acid (anhydride); 2-methyl-propanoic acid (pentyl ester); acetic acid (ethyl ester); 4-butoxy-1-butanol; 1, 3-butanediol and pentane were detected.

It is interesting to note that only 2-(3-methyl-1-butyloxy)-ethyl acetate, DI-2/3-methylbutyl maleate, butanoic anhydride, propanoic acid (2-methyl, pentyl ester), acetic acid (ethyl ester), 1, 3-butanediol, 1-butanol (4-butoxy) and pantane were detected in brix-and pH-adjusted banana purees which was well-supported by Reineccius G, 2013 [18] who stated that discussed in source book of flavors about volatile compounds from in sugars, his results showed that acetic acid and propanoic acid came from fructose,butyric acid, acetates butyrates and butanol came from sucrose.

The findings obtained in our study were in good agreement with previous studies reporting various volatile compounds in different banana cultivars, which included 1-butanol (3-methyl) and benzeneethanol, 3-hydroxy-2-butanone (acetoin), elimicin (benzene, 1, 2, 3-trimethoxy-5-(2-propenyl), acetic acid, isovaleric acid (butanoic acid 3-methyl) and butanoic acid in dwarf banana [12,19-21], and ethanol in giant banana [12].

Wine was found to contain a total of 36 volatile compounds. It is interesting to note that only Propanoic acid (2-methyl, 1-methylbutyl), Acetic acid (2-methylpropyl ester), Acetic acid (hexyl ester), Nonanoic acid, Decanoic acid (ethyl ester), Formic acid (hexyl ester), Dodecanoic acid (ethyl ester), Ethanol, 2-(2-butoxyethoxy) and Benzaldehyde 2-methyl were detected in banana wine.

Ester compounds found in wine, which contains ethyl-4-hydroxybutanoate. Substance was caused by yeast. *Saccharomyces cerevisiae* during fermentation by-related activity at least three types of alcohol acetyl transferase, ethanol acetyltransferase isoamyl and alcohol acetyl transferase. Compounds in the acid found in wine, such as decanoic acid and dodecanoic acid, an acid smell on the positive side [22]. acetic acid smells sour, propanoic acid smells like soy sauce, butanoic acid and nonanoic acid smells like cheese [23]. Yeast turns sugar into alcohol through EMP pathway Fusel alcohol such as 3-methyl-1-butanol and 1-propanol was productivity of Amino acids and proteins in yeast cells. Benzene ethanol (phenylethanol) substance smell like the fragrance of flowers. It is caused by processes involving metabolites metabolism of amino acids. L-phenylalanine in yeast cells [24]. The substance of aldehyde compounds found in wine was nonanal (a positive smell), benzaldehyde (2-methyl). This compound is a precursor in the synthesis of the smell by working s of yeast enzyme (alcohol dehydrogenase using NAD^+ or possible decarboxylation interactions of acids [23]. Wanphen [25] study on wine aroma compounds found in banana wine, acetic acid (ethyl ester), octanoic acid (ethyl ester), decanoic acid(ethyl ester) and acetic acid (ethyl ester) smell good volatile in wine because there smells like a fruity floral scent of pineapple and pear ester compounds in wine. Elimicin (Benzene, 1, 2, 3-trimethoxy-5-(2-propenyl)), a substance that makes the wine taste with a texture (full bodied) and the smell of ripe bananas.

In 2 stage Vinegar Fermentation. Glucose as carbon source for the yeast to quickly use [26]. The process of making the first step. Yeast synthetic alcohol has two ways of metabolism of amino acids converted to 3-methyl-1-butanol (isoamyl alcohol). 1-propanol was synthesized by yeast, which involves metabolism of amino acid contain sulfur elemental. During vinegar fermentation Alcohol was converted into acetic acid by acetaldehyde intermediates by the work of bacteria through chemical oxidation stages, with the enzyme different types (alcohol dehydrogenase and aldehyde dehydrogenase) Zuobing et al. [23] study the composition of the substance essential in the cherry wine, the compound 3-methyl-1-butanol as flavoring agents in wine from the reaction of deamination and decarboxylation of amino acids. Ubeda et al. [27] studied the composition of essential nutrients in the fruit vinegar, 3-methyl-1-butanol as a precursor to acid isovaleric acid (3-methyl butanoic acid) or acetaldehyde was converted to acetoin (3-hydroxy butanone) which this material is an essential characteristic of vinegar.

2-stage vinegar aging was found to contain a total of 28 volatile compounds. It is interesting to note that only 3-methyl-thio propyl acetate, 4-Heptanol (3-methyl), 2-butanol (3-methyl acetate), 4-sec-butoxy-2-butanone and Benzaldehyde (2, 4-dimethyl) were detected in 2-stage vinegar aging.

Volatile compounds identified during one-stage vinegar fermentation and aging processes

A total of 21 volatile compounds were detected in banana puree mixed with absolute alcohol; banana puree itself contained acetic acid. Meanwhile, one-stage vinegar fermentation was found to cause a complete depletion of alcohol (ethanol) and changes in the volatile constituents of the mixture, in which 3-methyl butanoic acid, hexanoic acid, 2-ethyl-1-hexanol, benzeneethanol, 2-butanone, benzaldehyde, 2, 4-dimethyl benzaldehyde, 4-methyl benzaldehyde and nonanal were formed during the fermentation process, giving a total of 29 volatiles present in the one-stage fermented vinegar. It is interesting to note that acetaldehyde was detected at high concentrations at the end of the fermentation process, which might be due to the fact that this compound is an intermediary product during the conversion of ethanol into acetic acid.

At the end of a 100-day aging process, 2-(3-methyl-1-butyloxy)-ethyl acetate, 3-oxo, 1, 1-dimethylethyl butanoic acid, 2, 2-dimethyl-1-butanol, 1, 3-butanediol, phenol, pentane and nonanal were not detected. On the other hand, 3-methyl-2-butanol (acetate), *n*-propyl-*t*-butyl ether, 2-methoxy-2-methyl propane were generated during the aging process. The results obtained in our study indicated that the majority of volatile compounds present in banana puree were quite stable, most of which were responsible for the characteristic aroma of the vinegar.

Conclusion

This study has revealed changes in the volatile constituents generated during one-stage and two-stage fermentation and 100-day aging processes of banana vinegars as suggested by HS-SPME-GC-MS analyses. Our results showed that prior to the fermentation and aging processes, brix and pH adjustments resulted changes in the volatile constituents in banana purees. During a one-stage fermentation, a total of 35 volatile compounds were detected with acetic acid and 1-butanol (2, 2 dimethyl) produced abundantly. Meanwhile, a two-stage fermentation process yielded a total of 48 volatile compounds, in which acetic acid and benzeneethanol were detected at high levels. During a 100-day aging process, acetic acid and butanoic acid (3-methyl) were detected at high levels in the one-stage fermented vinegars whereas the two-stage fermented vinegars contained abundant amounts of acetic acid, benzeneethanol and acetoin. Our findings demonstrated that the two-stage fermented banana vinegars had higher volatile constituents as compared to the one-stage fermented vinegars. More importantly, a two-stage fermentation process gave rise to the production of butyrolactone, which is a positive aroma, while butyric acid and 2-propanol, which are a negative aroma, were generated in a one-stage fermentation and aging process. Our results indicated that two-stage fermentation process was more effective in the production of banana vinegars regarding the generation of positive odorants. To our knowledge, this is the first to identify changes in the volatile constituents generated during the fermentation and aging processes of banana vinegars.

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