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Microbiological Characteristics and Volatile Components of Deastringent Persimmon Vinegar

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Abstract

Acetic acid producing bacteria were isolated from deastringent persimmon vinegar and the major bacterium was identified using morphological and biochemical tests. *Acetobacter* sp. AH-1 was motile, gram negative rod with catalase positive and oxidase negative. The strain can grow up to 5% ethanol and 2% NaCl as well as 25% glucose. Optimum temperature and pH for growth were 30°C and 5.0, respectively. Volatile constituents of persimmon vinegar were analyzed by purge and trap sampling. Acetic acid and alcohol were the largest volatile compounds quantitatively in persimmon vinegar. Among alcohols, 2-methyl-1-propanol, isoamyl alcohol and amyl alcohol were detected. Isovaleraldehyde and benzaldehyde for aldehyde, isoamyl acetate, ethyl formate, propyl acetate, and ethyl acetate for esters were likely to contribute to persimmon vinegar flavor.

Key words: persimmon, vinegar, *Acetobacter*, flavor

INTRODUCTION

Vinegar is a very important spice for food preparations throughout the world. In Korea it was primarily produced from rice wine residue and various fruits. Theoretically vinegar is dilute solution of acetic acid produced by ethanol formation from fermentable sugars by the action of yeasts, normally strains of *Saccharomyces cerevisiae*, followed by acetic acid formation from ethanol by *Acetobacter* sp. (1).

Recently persimmon vinegar has drawn consumer's attraction as health foods; however, the poor qualities of persimmon vinegar sometimes resulted in consumer claims. This is because the fermentation process is not clearly established in terms of raw materials and microorganisms as well as processing conditions. Our previous report suggested fast and reproducible fermentation process for persimmon vinegar (2). Two strains of acetic acid forming bacteria were isolated from the deastringent persimmon.

We selected one strain for this study and the microbiological characteristics were evaluated and identified. In addition, deastringent persimmon vinegar was produced using the same strain to evaluate its flavor characteristics were evaluated.

MATERIALS AND METHODS

Culture media

Acetic acid producing bacteria were isolated from traditional persimmon vinegar of excellent quality. After 10-fold serial dilution of 1ml vinegar, the samples were plated on the isolation medium. The composition of isolation medium was glucose 3%, yeast extract 0.5%, ethanol 2%, $CaCO_3$ 1%, agar 2%, and ethanol 3% (3). In addition, culture medium for acetic acid production was Henneberg medium (4) which contains glucose 3%, peptone 1%, and ethanol 4%.

Identification of acetic acid producing bacteria

Based upon the previous study (2), we selected the strain AH-1, which showed rapid growth at the initial fermentation period. Morphological and biochemical characteristics of the AH-1 strain was evaluated and the identification was carried out by the general method of the bacterial identification as described in Bergey's manual of systematic bacteriology (5) and Bergey's manual of determinative bacteriology (6). After 3 day incubation using SM medium (yeast extract 0.5% and glucose 5%, physiological and biochemical characteristics were determined by comparing with control.

Persimmon vinegar fermentation

Using selected strain AH-1, starter was prepared. Medium for starter was Henneberg medium and incubation was carried out at 30°C for 36 hr. For persimmon vinegar fermentation, deastringent persimmon was softened by placing persimmons under daylight for 6 days. They were mashed and starter and ethanol were added at the ratio of 5 and 4% to the weight of mashed persimmon, respectively. Fermentation was carried out at 25°C for 2 weeks and aging at 10°C for 2 weeks. At the end of aging persimmon vinegar was sterilized at 80°C for 20min followed by rapid cooling at 15°C.

Qualitative analysis by gas chromatography/mass spectroscopy

Volatile components of persimmon vinegar were analyzed

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by gas chromatograph (HP 5890II Plus) using HP-5 column (50 m×0.2 mm i.d, 0.1 μ) in combination with gas chromatograph/mass spectrometer (Hewlett Packard Model 5989A) based on retention times of authentic standards. GC/MS condition was as follows: ionization potential 70 eV and scan range 30 to 500 m/e. Identification was achieved by comparing MS data with NIH/EPA reference spectra (7). Sample introduction included dynamic purge-and-trap sampling and direct injection.

A 3 ml sample was heated in a covered 25 ml needle sparge glass tube immersed in boiling water for 2 min. HPLC grade water (Fisher Scientific, Springfield, NJ) was added (10 ml) and mixed well with a stainless steel rod. The tube was attached to a Tekmar LSC-3 headspace concentrator (Tekmar Corp., Cincinnati, OH) and wrapped with heat tape. Purging was carried out with ultra high purity nitrogen. Volatiles were collected on a 177 mm×6.3 mm Tenax trap (Anspec Co., Ann Arbor, MI) packed with Tenax TA (Alltech Associates Inc., Deerfield, IL). After preliminary heating at 50°C volatiles were thermally desorbed at 200°C for 5min onto an identical capillary column as described above. Initial oven temperature (50°C) was held for 6min after which the temperature was programmed to 280°C at 2°C/min

Identification of volatile components in persimmon vinegar

Major volatile components were identified by comparing with the retention time of corresponding standard compound and confirmed with mass spectral data. Minor volatiles were tentatively identified by Willey library. 1-Octanol was used as internal standard.

RESULTS AND DISCUSSION

Identification of strain AH-1

Morphological characteristics of the strain AH-1 were as follows: gram negative, rod, and motile (Table 1). The colonies on the nutrient agar were pale and opaque. Judging from the result of oxidase negative and catalase positive, the strain requires oxygen as the terminal electron acceptor; in addition, the strain oxidized not only ethanol into acetic acid but also lactate and acetate into CO_2. Therefore the strain AH-1 was not classified into *Gluconobacter* sp. but *Acetobacter* sp. (6). Most *Acetobacter* sp. show low tolerance against NaCl (8,9); on the contrary the strain AH-1 grew very well even at 2% NaCl.

The strain AH-1 was able to grow up to 5% ethanol as well as 12% sodium acetate. But the growth was completely inhibited at 10% ethanol. In general, *Acetobacter* sp. was reported to be inhibited in the 20% glucose medium. Conversely the strain AH-1 exhibited good growth at 25% glucose.

The optimum temperature and pH for the growth of the strain AH-1 were 30°C and 5.0, respectively. The growth of strain AH-1 was largely affected by the types of carbon sources. Ethanol, sorbitol, and galactose were utilized for growth but other carbon sources including methanol, raffinose and starch were not utilized. Crystal violet was the only one that did not affect the growth of the strain among the dyes tested. $HgCl_2$ also inhibited the growth.

Judging from the above result, the strain AH-1 was similar to *Acetobacter aceti*; however, several differences cannot be overlooked. Therefore, the strain AH-1 was des-

Table 1. Morphological and biochemical characteristics of the strain AH-1

Morphology	
Shape	rod
Motility	motile
Gram stain	negative
Opacity	opaque
Pigmentation	pale
Catalase	+
Oxidase	−
Growth on SM medium	
+0.5% NaCl	++
+1.0% NaCl	++
+2.0% NaCl	++
+1.0% Ethanol	++
+2.0% Ethanol	++
+5.0% Ethanol	++
+10.0% Ethanol	−
+4.0% Sodium acetate	++
+8.0% Sodium acetate	++
+12.0% Sodium acetate	++
0.5% Yeast extract	
+20% D-glucose	+
+25% D-glucose	+
+30% D-glucose	−
Oxidation of	
Ethanol into acetic acid	+
Lactate into CO_2	+
Acetate into CO_2	+
Growth on carbon sources	
Methanol	−
Ethanol	±
L-Aranitol	−
D-Mannitol	−
Sorbitol	±
Dulcitol	−
D-Ribose	−
D-Xylose	−
D-Fructose	−
D-Glucose	−
D-Galactose	±
Sucrose	−
Maltose	−
Starch	−
Growth in the presence of	
0.001% Malachite green	−
0.0001% Crystal vilot	±
0.001% Brilliant green	−
0.001% $HgCl_2$	−
Growth conditions	
Optimum temperature (°C)	30
Optimum pH	5.0

− no growth; + growth; ++ growth abundant

ignated as *Acetobacter* sp. AH-1.

Identification of volatile components in deastringent persimmon vinegar

Total of 37 volatiles were positively identified (Table 2). They were grouped into 8 classes which included 4 acids, 9 alcohols, 4 aldehydes, 2 alkanes, 13 esters, 2 ethers, 2 ketones, and 1 miscellaneous component. Ion chromatogram of volatile components in persimmon vinegar was shown in Fig. 1. Major components of persimmon vinegar were alcohols with ethanol highest. The second was 2-methyl-1-propanol. Among individual components acetic acid was the highest.

Table 2. Identification of volatile components in deastringent persimmon vinegar

Retention time(min)	Compounds	Area%
	Acids	
20.56	Acetic acid	17.824
30.91	Lactic acid	0.451
32.61	Valeric acid (Pentanoic acid)	0.075
37.29	Hexanoic acid	0.072
	Alcohols	
15.07	Ethanol	12.352
18.38	Propanol	2.431
20.97	2-Methyl-1-propanol	4.260
26.15	3-Methyl butanol (Isoamyl alcohol)	3.005
26.40	2-Methyl butanol (Amyl alcohol)	2.028
29.62	2,3-butanediol	0.132
42.18	Octanol (IST)	4.399
43.04	Heptanol	0.057
47.37	1-Decanol	0.066
48.48	4-Terpineol	0.098
	Aldehydes	
13.65	Acetaldehyde	0.729
22.76	Isovaleraldehyde (3-methyl butanal)	2.445
39.73	Octanal	0.260
40.54	Benzaldehyde	0.318
	Alkanes	
41.49	Undecane	0.101
45.42	Dodecane	0.512
	Esters	
14.29	Methyl formate	0.221
16.51	Ethyl formate	2.736
19.90	Ethyl acetate	1.809
22.26	Isopropyl acetate	0.307
25.10	Propyl acetate	2.472
27.14	Ethyl isobutyrate	0.652
28.00	Isobutyl acetate	3.151
29.47	Ethyl butyrate	0.125
30.20	n-Butyl acetate	0.424
31.95	Ethyl isovalerate	0.145
33.22	Isoamyl acetate	2.889
38.82	Ethyl caproate	0.079
39.42	1-Hexyl acetate	0.116
	Ethers	
23.22	3-Ethoxy 1-propene	2.135
24.34	Ethyl isobutyl ether	0.222
	Ketones	
16.13	2-Propanone	0.993
26.65	3-Hydroxy 2-butanone	1.157
	Miscellaneous	
33.87	Furfural	0.865

Acetic acid was major acid followed by lactic acid. Valeric and hexanoic acid were minor. This indicates that acetic acid mainly contributed to the strong acidic flavor. *Acetobacter* sp. produced the persimmon vinegar with total acidity of 6%, which was considerably high as compared to the traditional persimmon vinegar. Lactic acid should be the byproduct of acetic acid fermentation. 3-Methyl butanoic acid and 2-methyl propanoic acid, respective oxidized forms of 3-methyl-1-butanol and 2-methyl-1-propanol were not present.

The major alcohol in persimmon vinegar was ethanol followed by 2-methyl propanol (isobutyl alcohol), 3-methyl butanol (isoamyl alcohol), propanol, and 2-methyl butanol (amyl alcohol), 2,3-butanediol in that order. The high concentration of ethanol indicates either the insufficient oxidation to acetic acid or continuing hydrolysis of carbohydrates. Conversely Neuberg suggested that a dismutation reaction occurred in which one mole of acetaldehyde is reduced to ethanol while another is oxidized to acetic acid (10). In this case the both mechanisms were believed to be involved.

Pyruvic acid is formed during alcohol fermentation further converted to acetoin via α-acetolactate, or oxidized to diacetyl, or reduced to 2,3-butanediol (11). During persimmon vinegar fermentation, the concentration of 2,3-butanediol was reportedly unchanged (11); however, Yoon et al. detected only 1,3-butanediol in persimmon vinegar (12). In contrast, our persimmon vinegar showed 2,3-butanediol.

Amyl alcohol was also present in significant quantity, which was produced by a comparable series of reactions for the biosynthesis of keto-n-valeric acid from α-ketobutyric acid and, by decarboxylation and reduction (13). 3-Methyl-1-butanol (isoamyl alcohol) and 2-methyl-1-propanol were present in our vinegar, which were the metabolites of leucine and valine, respectively. These components were reported in the aging process of kochujang (14), takju (15), and beer (16). However, only 2-methyl-1-butanol were detected in traditional persimmon vinegar (12).

Despite the large portion of alcohols among volatiles, alcohols reportedly make minor contributions to flavor in food systems (17). That seemed to be persuasive in case of persimmon vinegar, for esters represent the persimmon vinegar

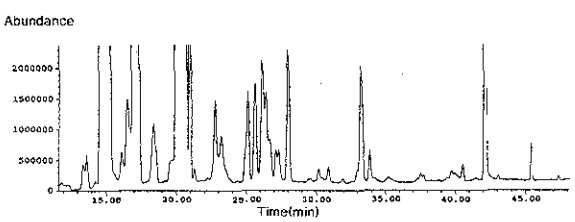

Fig. 1. Ion Chromatogram of volatile components in persimmon vinegar.

flavor upon sensory evaluation.

The oxidation of ethanol is reportedly accomplished in two steps (18). At the first step, ethanol is oxidized to acetaldehyde and converted into acetic acid at the second step. However, relatively low amount of acetaldehyde indicates that most of acetaldehyde was converted either into acetic acids or reduced to ethanol. Benzaldehyde and acetaldehyde was present in both our vinegar and traditional ones. Benzaldehyde, in particular, was identified as contributor to sweet cherry fruit flavor (19) as well as apricot flavor (20).

De Ley and Frateur obtained acetoin from D,L-lactate with 44 strains of *Acetobacter* (21). Acetoin was formed from pyruvate (22); and also from acetoactate. Acetoin was present in our persimmon vinegar but not in traditional vinegar (12). Presence of acetoin was reported mostly in vinegar either successive fermentation or mixed fermentation of ethanol and acetic acid (12). Since our persimmon vinegar was fermented with addition of ethanol to persimmon, the claim of Yoon et al. (12) contradicted our result. Most traditional persimmon vinegars go through ethanol and acetic acid fermentations, successively or at the same time.

Two alkanes, undecane and dodecane were identified. MacLeod and Cave (23) suggested saturated hydrocarbons from C_7 and C_{17}, were produced by decarboxylation of fatty acids from glycerides. Isobutyl acetate was the major ester in persimmon vinegar followed by isoamyl acetate, ethyl formate, propyl acetate, in that order. Ethyl acetate represent flavor of various products such as saurkraut (24), fresh pineapple (25), unifloral honey (26), and fig (27).

In conclusion, volatile components of persimmon vinegar were composed of the following: original flavor components, components produced during fermentation, and components produced during low temperature sterilization. To determine the origin of flavor, further research about each step of processing should be undertaken.

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