Physicochemical Qualities and Flavor Patterns of Traditional Chinese Vinegars Manufactured by Different Fermentation Methods and Aging Periods

Yaping Gao¹, Yunhee Jo¹, Namhyeok Chung¹, Song-Yi Gu¹, Yong-Jin Jeong², and Joong-Ho Kwon¹

¹School of Food Science and Biotechnology, Kyungpook National University, Daegu 41566, Korea
²Department of Food Science and Technology, Keimyung University, Daegu 42601, Korea

ABSTRACT: Physicochemical properties of Fujian Yongchun aged vinegar (FYAV) and Shanxi mature vinegar (SMV) were compared in terms of the fermentation methods applied and aging periods (3, 5, 8, and 10 years), and combined E-nose/E-tongue analyses were performed to assess their flavors. Compared with submerged fermentation-derived FYAV, solid-state fermentation-derived SMV showed higher values of pH, brix, soluble solids, total phenolic content, and antioxidant activity, but not total acidity or total organic acids. Aging period resulted in an increase in pH, total phenolic content, and antioxidant activity. Principal component analysis based on E-tongue/E-nose analyses was performed to distinguish between the vinegars produced by different fermentation methods and under aging periods. Solid-state fermentation and an aging process were considered good techniques for vinegar brewing, considering the various organic acids and high levels of total phenolics and antioxidant activity.

Keywords: aged Chinese vinegar, physicochemical quality, E-tongue, E-nose, principal component analysis

INTRODUCTION

China has a long history of brewing vinegar, and approximately 26 million hectoliters of vinegar are produced each year from various raw materials, mainly cereals, such as rice, sticky rice, and wheat bran (1). Different regions of the country produce their own indigenous vinegars from local crops (2). Fujian Yongchun aged vinegar (FYAV) and Shanxi mature vinegar (SMV) are the most well-known types, along with vinegars from the Zhejiang and Sichuan provinces (3). FYAV, produced from sticky rice and red rice koji, is famous in southern China and is the only vinegar produced by submerged fermentation (SmF) (4). Similar traditional SmF processes are applied in Europe to produce high-quality wine vinegar (5). SMV is the most famous traditional vinegar in northern China; sorghum is the main raw material along with a very large dosage of koji, and this vinegar is made by solid-state fermentation (SSF) (6). Generally, the fermentation of Chinese vinegar may be divided into two systems: SSF, which involves microbial growth, metabolism, and product formation on solid particles in a very small volume of water; and SmF, which is based on microorganism cultivation in liquid medium containing nutrients (7). SSF, an alternative to SmF, has been important in Chinese vinegar manufacture for a long time. SSF is more cost-effective as it requires smaller vessels, lower water consumption, and lower energy consumption compared to SmF processes. The complicated composition and flavor of Chinese vinegars reflects the raw materials and production processes used in their manufacture.

Vinegar takes a long time to produce; the most time-consuming stage is the maturation process. The traditional production process involves a maturation period during which many substances that impart flavor, such as esters, are formed by chemical reactions. Therefore, each type of Chinese vinegar has its own distinct sensory characteristics. It is possible to evaluate vinegar taste and flavor profiles according to their types and aging periods. Generally, sensory analysis by a panel of experts is a costly process because it requires trained people; additional problems such as the subjectivity of the human response to odors and variability between individuals must also be considered. E-nose and E-tongue devices, which simulate human olfaction and taste, consist of an array of sensors and pattern recognition tools. Electronic
sensors in the form of the E-nose and E-tongue, which respond to odor and taste by using a simple, nonspecific sensor array and pattern-recognition software, are used widely for sensory analysis to determine the quality of foods. Compared with sensory evaluation, E-nose and E-tongue provide simple, quick, and accurate analyses.

Various studies have evaluated traditional Chinese vinegars to characterize their aromatic constituents (8-11). However, few studies have investigated the physicochemical qualities and sensory properties of Chinese vinegars according to the raw materials, fermentation methods, and aging periods. From the perspective of consumer preference for traditional aged vinegars, we selected two Chinese vinegars produced by different fermentation methods (SSF and SmF) for different aging periods (3, 5, 8, and 10 years) to compare their physicochemical qualities, antioxidant properties, and volatility and taste patterns.

**MATERIALS AND METHODS**

**Materials**

Two types of traditional Chinese vinegars, namely FYAV and SMV, with aging periods of 3, 5, 8, and 10 years, were purchased from a wholesale market in Beijing, China. Three sets of 500-mL bottles were purchased for physicochemical and sensory evaluations and were kept at 4°C during the experiments. Information regarding the raw materials, total acidity, and fermentation methods was obtained from the vinegar bottle labels and is listed in Table 1.

**Determination of pH, total acidity, brix, total soluble solid content, and Hunter’s color value**

The pH value was measured using a pH meter (Thermo Scientific Orion 3-star, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total acidity was determined by titrating the vinegars to pH 8.35 with 0.1 N NaOH and was expressed as the acetic acid equivalent. The brix of the vinegars was measured using a refractometer (Master M, ATAGO Co., Ltd., Tokyo, Japan), which was calibrated with distilled water. The total soluble solid content was determined by the hot air oven method at 105°C. The Hunter’s color values of the vinegars were measured using a colorimeter (CM-3600D, Konica Minolta, Osaka, Japan) and expressed as L* (white/dark), a* (red/green), b* (yellow/blue), and ΔE (overall color difference). Chinese vinegars are normally dark, and thus the samples were diluted at a ratio of 1:2 for determination of the Hunter’s color value.

**Analysis of organic acids**

Organic acids were analyzed by high-performance liquid chromatography (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) after passing through a 0.45-µm membrane filter to remove the protein components. Chromatographic analysis was performed at 35°C using an Aminex HPX-87H column (7.5×300 mm; Bio-Rad Laboratories, Hercules, CA, USA) and UV detector (214 nm), with 5 mM sulfuric acid as the mobile phase running at a flow rate of 0.6 mL/min. Sample concentrations were quantified using 6 external standards from Sigma (St. Louis, MO, USA) and expressed in mg/100 mL.

**Determination of total phenolic content and antioxidant activity**

The total phenolic content of the vinegars was determined by the Folin-Ciocalteu assay (12). First, vinegar samples (0.2 mL) and distilled water (1.8 mL) were mixed with 0.2 mL of Folin-Ciocalteu phenol reagent. After incubation for 6 min, 2 mL of Na₂CO₃ was added and the mixed solution was incubated for an additional 90 min. Thereafter, the absorbance of the mixture was measured at 750 nm using a spectrophotometer (Optizen 2120UV, Mecasys Co., Ltd., Daejeon, Korea). Total phenolic content was expressed as mg gallic acid equivalent (GAE)/100 mL. The antioxidant activities of the vinegars were determined from their 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical-scavenging activity (RSA) (13,14). For the DPPH assay, sample was mixed with freshly prepared DPPH radical solution (absorbance of 1.000±0.050 at 517 nm), and the absorbance of the resulting solution was determined using a spectrophotometer. For the ABTS assay, a 7 mM ABTS stock solution was first mixed with 2.45 mM potassium persulfate and incubated in the dark at room temperature for 16 h. The resulting ABTS radical solution was then diluted in ethanol to an absorbance of 0.700±0.050 at 734 nm. The vinegar sample and diluted ABTS solution were mixed together and the reaction absorbance

**Table 1. Chinese traditional vinegars with their specifications**

| Sample | Region       | Raw material           | Fermentation method         | Total acidity (%) | Aging period (year) |
|--------|--------------|------------------------|-----------------------------|-------------------|---------------------|
| FYAV   | Fujian province | Glutinous rice, red yeast rice, sugar, salt | Submerged fermentation       | >6.50             | 3, 5, 8, 10         |
| SMV    | Shanxi province | Sorghum, barley, peas, bran, salt | Solid state fermentation     | >6.00             | 3, 5, 8, 10         |

FYAV, Fujian Yongchun aged vinegar; SMV, Shanxi mature vinegar.
was detected using the spectrophotometer. Trolox was used as a standard for the calibration curve and antioxidant activity was expressed as mg Trolox equivalent (TE) /100 mL.

E-nose and E-tongue analysis
The electronic nose (GC type E-Nose Heracles II, Alpha MOS, Toulouse, France) was applied to monitor the volatility profiles from vinegar samples. Vinegar samples (1 mL) were placed in 10-mL headspace vials (22.5×75 mm, polytetrafluoroethylene/silicon septum, and aluminum cap). Each vial was placed in an ordered manner in the automatic sampler in the headspace system. The samples were heated to 40°C and 1 mL of headspace gas from the gaskets was sampled with a syringe and injected into the equipment. The measurement phase was 120 s, while the clean phase was 240 s. The Alpha ASTREE E-tongue (Alpha MOS) is composed of an automatic sampler unit, array of chemical sensors with cross-selectivity, and chemometrics software package. The sensor set comprised seven working electrodes (silicon-based potentiometric sensors: sensor for sourness, metallic, saltiness, umami, spiciness, sweetness, and bitterness) and a reference electrode (Ag/AgCl). Each 25-mL sample was taken from the bottle immediately after opening, placed in the beakers provided by Alpha MOS, and analyzed for 120 s. Up to five replicate measurements were performed for each sample and the mean was calculated. The E-nose and E-tongue results were analyzed by principal component analysis (PCA).

Statistical and correlation analyses
The results were expressed as the mean±standard deviation. Data analyses were performed using Statistical Analysis System software (version 8.1, SAS Institute Inc., Cary, NC, USA). Differences between the means of variables were reported by one-way analysis of variance. Significance was determined by Duncan’s multiple range test at P<0.05.

RESULTS AND DISCUSSION

Comparison in pH, total acidity, brix, total soluble solid content, and Hunter’s color value
All data regarding pH, total acidity, brix, and soluble solid content of the samples are presented in Table 2. FYAV showed lower pH values (3.01∼3.11) than SMV (3.47∼3.56). The pH values significantly increased with prolonged aging (P<0.05). All aged vinegars showed 6.50∼6.91% total acidity, in accordance with the standard level (minimum total acidity criterion: 5%) specified by the China State Bureau of Standards (15). A significantly negative effect of aging years was observed in FYAV (r=−0.8927) and SMV (r=−0.9317), which were more apparent in vinegar samples aged for more than 8 years. Sugar is produced from the fermentation of different types of cereals including rice, sorghum, barely, and bran. FYAV brewed by SmF showed significantly low brix compared with SMV by SSF (P<0.05). The explanation for this phenomenon is related to a dynamic balance between supply and demand for sugars in the SmF process, causing the sugar concentration to be low (16). Higher yield in SSF enables obtaining higher concentration of products, which is a main advantage over SmF. The brix of vinegars during the aging period gradually decreased in both FYAV (r=−0.8982) and SMV (r=−0.8868), and was most dramatic in the 10-year-old SMV sample. Similar results were observed for soluble solid content, which was much lower in FYAV than in SMV, and was significantly reduced in the 10-year-old vinegars (P<0.05). In traditional Chinese vinegars, black color is an important factor because it directly affects consumer perception. Hunter’s color parameters indicated that L* (lightness), a* (redness), and b* (yellowness) values were much higher in the FYAV samples than in the SMV samples (Table 3). A brighter-colored FYAV sample darkened during the aging process, resulting in a higher overall color difference (ΔE) than in SMV. The color difference of FYAV was most apparent in the 10-year-old vinegar (P<0.05). Overall, vinegars produced

Table 2. Physicochemical properties of traditional Chinese vinegars aged for different periods

| Sample | Aging year | pH     | Total acidity (%) | Sugar content (°Bx) | Soluble solid content (%) |
|--------|------------|--------|-------------------|---------------------|--------------------------|
| FYAV   | 3          | 3.01±0.01a | 6.88±0.01a       | 9.20±0.00a          | 4.78±0.09c              |
|        | 5          | 3.02±0.01a | 6.91±0.02a       | 8.40±0.00a          | 4.11±0.13d              |
|        | 8          | 3.10±0.02a | 6.82±0.02a       | 8.40±0.00a          | 4.09±0.13d              |
|        | 10         | 3.11±0.01c | 6.77±0.02       | 8.00±0.00c          | 3.80±0.12d              |
| SMV    | 3          | 3.47±0.01a | 6.77±0.00       | 17.60±0.00a         | 12.37±0.43a             |
|        | 5          | 3.47±0.01a | 6.74±0.02       | 17.57±0.06b         | 12.48±0.53a             |
|        | 8          | 3.56±0.01a | 6.67±0.01       | 17.20±0.00b         | 12.50±0.72a             |
|        | 10         | 3.55±0.01a | 6.50±0.01       | 15.83±0.06c         | 11.50±0.17b             |

FYAV, Fujian Yongchun aged vinegar; SMV, Shanxi mature vinegar.
Values with different letters (a-f) within the column are significantly different at P<0.05 based on Duncan’s multiple range test.
Organic acids contribute to the specific flavor and palatability of vinegars. The organic acid profile of FYAV showed that acetic acid was the most abundant (92.64–93.22%), followed by succinic acid (3.92–6.34%); however, oxalic and malic acids were detected in trace amounts or even at non-measurable levels (Table 4). SSF-derived SMV contained a greater variety and quantity of organic acids than SmF-derived FYAV. Recent studies showed that different types of secondary metabolites, such as food-grade pigments, organic acids, and flavor-imparting compounds, can be produced by SSF (17). Wang et al. (18) found significantly higher amounts of organic acids in SSF-derived vinegar than in SmF-derived vinegar, in strong agreement with our findings. FYAV showed no obvious change in organic acid content after prolonged aging, whereas SMV showed a significant decrease, particularly in the 5-year-old sample (r = −0.8910).

### Table 3. Hunter’s color value for traditional Chinese vinegars aged for different periods

| Sample | Aging year | Hunter’s color value |
|--------|------------|----------------------|
|        | L* | a* | b* | ΔE |
| FYAV   | 3 | 36.37±0.04 | 20.76±0.01 | 53.04±0.03 | 0.00 |
|        | 5 | 34.92±1.01 | 22.09±1.13 | 53.65±0.16 | 1.44 |
|        | 8 | 34.27±0.25 | 22.30±0.06 | 52.27±0.41 | 1.65 |
|        | 10 | 22.89±1.13d | 29.06±0.02d | 38.54±0.25d | 4.63 |
| SMV    | 3 | 2.15±0.04 | 14.48±0.20 | 3.43±0.01 | 0.00 |
|        | 5 | 2.33±0.03 | 15.22±0.11 | 3.75±0.04 | 0.72 |
|        | 8 | 1.64±0.04 | 11.82±0.06 | 2.55±0.05 | 1.79 |
|        | 10 | 1.36±0.06d | 10.27±0.25 | 2.11±0.10d | 2.20 |

FYAV, Fujian Yongchun aged vinegar; SMV, Shanxi mature vinegar. L*, degree of lightness (white +100−0 black); a*, degree of redness (red +100−0 red); b*, degree of yellowness (yellow +70−0−80 blue). ΔE = ((ΔL*)² + (Δa*)² + (Δb*)²)¹/². Values with different letters (a–g) within the column are significantly different at *P* < 0.05 based on Duncan’s multiple range test.

Comparison of organic acids

Organic acids contribute to the specific flavor and palatability of vinegars. The organic acid profile of FYAV showed that acetic acid was the most abundant (92.64–93.22%), followed by succinic acid (3.92–6.34%); however, oxalic and malic acids were detected in trace amounts or even at non-measurable levels (Table 4). SMV contained six organic acids; the major component was acetic acid with a content of 62.24–64.38%. Various organic acids were detected in SMV in relatively high amounts in the following order of abundance: succinic, malic, oxalic, citric, and tartaric acid (Table 4). SSF-derived SMV contained a greater variety and quantity of organic acids than SmF-derived FYAV. Recent studies showed that different types of secondary metabolites, such as food-grade pigments, organic acids, and flavor-imparting compounds, can be produced by SSF (17). Wang et al. (18) found significantly higher amounts of organic acids in SSF-derived vinegar than in SmF-derived vinegar, in strong agreement with our findings. FYAV showed no obvious change in organic acid content after prolonged aging, whereas SMV showed a significant decrease, particularly in the 5-year-old sample (r = −0.8910).

### Table 4. Organic acid content of traditional Chinese vinegars aged for different periods

| Sample | Aging year | Organic acid (mg/100 mL) | Acetic | Total | A/T |
|--------|------------|--------------------------|--------|--------|-----|
| FYAV   | 3 | 29±0 | ND | ND | 180±5 | 2,632±15 | 2,841±10 | 0.93 |
|        | 5 | 32±1 | ND | ND | 160±10d | 2,640±13 | 2,832±21 | 0.93 |
|        | 8 | 31±1 | ND | ND | 58±0 | 112±2 | 2,648±62 | 2,849±59 | 0.93 |
|        | 10 | 29±2 | ND | ND | 62±15 | 110±0 | 2,608±74 | 2,808±87 | 0.93 |
| SMV    | 3 | 214±7 | 79±10 | 30±2 | 283±0 | 817±13 | 2,368±39 | 3,791±48 | 0.62 |
|        | 5 | 206±2 | 71±1 | 28±0 | 259±0 | 821±14 | 2,286±71 | 3,671±5 | 0.62 |
|        | 8 | 201±8 | 70±1 | 28±0 | 260±2 | 834±0 | 2,290±12 | 3,684±10 | 0.62 |
|        | 10 | 186±1 | 62±1 | 21±0 | 241±1 | 737±3 | 2,256±10 | 3,504±14 | 0.64 |

FYAV, Fujian Yongchun aged vinegar; SMV, Shanxi mature vinegar.

1) Acetic acid content compared to total acid content.

2) Not detected.

Values with different letters (a–e) within the column are significantly different at *P* < 0.05 based on Duncan’s multiple range test.
Fig. 1. Principal component analysis plot of E-nose (A) and E-tongue (B) response data for traditional Chinese vinegars aged for different periods. FYAV, Fujian Yongchun aged vinegar; SMV, Shanxi mature vinegar; PC, principal component.

Table 5. Total polyphenol content (TPC) and radical scavenging activities (RSA) of traditional Chinese vinegars aged for different periods

| Sample  | Aging years | TPC (mg GAE/100 mL) | RSA (mg TE/100 mL) | DPPH | ABTS |
|---------|-------------|---------------------|---------------------|-------|-------|
| FYAV    | 3           | 72.46±0.77<sup>ae</sup> | 5.74±0.19<sup>e</sup> | 13.70±0.37<sup>ef</sup> |
|         | 5           | 69.33±0.67<sup>df</sup> | 8.00±0.25<sup>e</sup> | 18.36±0.16<sup>ef</sup> |
|         | 8           | 74.76±1.82<sup>df</sup> | 8.02±0.13<sup>ef</sup> | 18.36±0.58<sup>ef</sup> |
|         | 10          | 94.11±2.27<sup>df</sup> | 12.56±0.16<sup>ef</sup> | 32.56±0.25<sup>ef</sup> |
| SMV     | 3           | 398.74±1.70<sup>cd</sup> | 37.92±0.38<sup>cd</sup> | 226.30±2.10<sup>cd</sup> |
|         | 5           | 406.92±1.12<sup>ab</sup> | 40.94±0.48<sup>ab</sup> | 239.16±3.49<sup>ab</sup> |
|         | 8           | 402.08±3.92<sup>ab</sup> | 39.68±1.04<sup>ab</sup> | 234.18±1.21<sup>ab</sup> |
|         | 10          | 407.29±3.22<sup>ab</sup> | 40.37±0.91<sup>ab</sup> | 247.85±2.38<sup>ab</sup> |

FYAV, Fujian Yongchun aged vinegar; SMV, Shanxi mature vinegar.
DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid).
GAE, gallic acid equivalent; TE, Trolox equivalent.
Values with different letters (a-f) within the column are significantly different at \( P<0.05 \) based on Duncan’s multiple range test.

for the preparation of koji containing various food-grade filamentous fungi, such as Aspergillus sp. and Rhizopus sp., has been shown to enhance antioxidant activity, possibly due to higher phenol and anthocyanin contents (7). SMV showed significantly higher antioxidant activities than FYAV (by 3–5-fold for DPPH RSA and by 8–14-fold for ABTS RSA), indicating that the SSF process involves longer production cycles and a more diverse range of materials (24). RSA increased with increasing TPC in the vinegar samples, suggesting a strong positive correlation between TPC and RSA using DPPH and ABTS. Xu et al. (25) also found that the antioxidant activity in Zhenjiang vinegar was correlated with the total phenolic and flavonoids content. The antioxidant activity changed with aging time; the activity of FYAV increased with age, and was particularly high in the 10-year-old sample, whereas SMV showed only a slight change in the DPPH \((r=0.8065)\) and ABTS \((r=0.5469)\) RSAs.

E-nose and E-tongue profiles
E-nose analysis was performed to evaluate the differences in the aromatic profiles of eight samples, and a PCA plot of the results is shown in Fig. 1A. Two principal components (PC) were used because they accounted for 87.52% of the variation in the data set. The plot consists of two axes, PC1 and PC2, where PC1 accounts for 64.51% of the sample variation and PC2 accounts for only 23.01%. FYAV and SMV were divided into clearly different areas on the right and left planes of the plot, respectively, indicating that the two samples could be distinguished by E-nose analysis based on their significantly different volatility profiles. Flavor compounds are formed chemically during fuming. Upon fermentation, SMV filtrate is transferred to a big jar and is exposed to the sun; in winter, surface ice is removed. Sun exposure and ice removal entail a concentration increase of acetic acid and flavor substances formed by chemical and enzymatic reactions. However, low concentrations of oxygen in the FYAV broth prevent rapid oxidation of ethanol. Consequently, each vinegar has its own flavor and taste characteristic (4). FYAV3, 5, and 8 were in the lower right region, whereas FYAV10 was in the middle upper region, without overlap. Therefore, the volatility profile of FYAV changed dramatically after 10 years of aging. However, four SSF-derived SMV samples overlapped with one another, showing no significant difference during aging. FYAV can be distinguished from SMV using E-nose analysis accompanied by PCA, and FYAV presented changes in volatility after 10 years of aging. Fig. 1B shows a PCA discrimination plot for the FYAV and SMV samples based on the E-tongue response data. The two principal components were retained because they accounted for 97% of the variation in the data set.
PC1 and PC2 accounted for 76.83% and 20.17% of the variation, respectively. Two types of vinegar were clearly differentiated: FYAV was positioned at the top right corner, whereas SMV was located in the lower position of the PCA plot. Four samples of FYAV overlapped with each other, indicating that no significant change in the taste profile of FYAV occurred during the aging process. However, the SMV taste profile showed a significant change after 5 years of aging and the SMV5~10 samples were positioned very close to one another, indicating that the taste remained stable without any obvious change after 5 years. Table 4 shows there were no significant differences among the aged FYAV samples with respect to total organic acid content; however, the content significantly decreased in SMV samples after 5 years of aging. Moreover, SMV showed a significantly greater quantity of organic acids than FYAV. Thus, PCA plots accompanied by E-tongue results coincide with the results of total organic acid content, demonstrating that E-tongue can be used to distinguish FYAV from SMV, and that SMV taste differed after aging for 5 years. E-tongue was found to be suitable for classifying differences in taste profiles among various vinegar samples.

CONCLUSION

Two traditional Chinese vinegars produced using different fermentation methods and for aging years were selected to compare their physicochemical qualities, antioxidant activities, and flavor patterns. FYAV produced by SmF showed lower levels of pH, brix, and soluble solid contents compared to SMV by SSF; however, total acidity was higher in FYAV than in SMV. These properties were significantly affected by the aging year from 8 years onwards. SSF-derived SMV contained different types of organic acids as well as higher levels of TPC and RSA. During aging, organic acids showed no change or decreased significantly from 10 years onwards, whereas TPC and RSA were generally increased. E-tongue and E-nose analyses provided complementary information for vinegar discriminations according to the vinegar types and aging periods with their distinctive flavors. FYAV showed changes in volatility after 10 years of aging, whereas SMV showed changes in taste after 5 years of aging.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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