Nutritional Composition and α-Glucosidase Inhibitory Activity of Five Chinese Vinegars

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Abstract

Shanxi aged vinegar (SAV) and Zhenjiang aromatic vinegars (ZAV) are representative of minor grain crop vinegars in the north of China and rice vinegars in the south, respectively. The nutritional composition of five Chinese vinegars, including three typical SAV and two ZAV, were determined. Investigations showed that these vinegars differed from each other in terms of the organic acid content (75.32-175.95 g/L), and amino acids (1215.9-2878.8 mg/100 g). SAV, especially oat vinegar, were 9-40, 2-211, 4-210, 1-3 and 1-1.5 times higher than ZAV in calcium, phosphorus, iron, zinc, and manganese contents, respectively. Nicotinic acid, nicotinamide, phenolics, alkaloids and saponin in these vinegars were shown to be 2.33-37.83 mg/100g, 1.23-35.03 mg/100g, 382.4-4518.1 μg/mL, 0.088-1.209 mg/mL, and 0.183-0.662 mg/mL, respectively. SAV seemed relatively better than ZAV in terms of essential amino acids, minerals and vitamins and also exhibited strong α-glucosidase inhibitory activities. These results suggested that both types of typical Chinese vinegars could be used as health foods, despite their different raw materials and processing technologies.

Introduction

Vinegar has been consumed as a food condiment and preservative in the Chinese diet for more than 3,000 years. There are currently at least 14 main types on the market for different raw material and processing technologies, including Zhenjiang aromatic vinegar (ZAV), Shanxi aged vinegar (SAV), Jiangzhe rose vinegar (JRV), and Baoning bran vinegar (BBV)[20]. Among these, ZAV and SAV are the most famous and mainly popular in the south and north of China, respectively.

ZAV generally employs rice and rice bran as major raw materials, whereas SAV uses minor grain crops (sorghum, oat, and tartary buckwheat) as the main raw materials with a very large dosage of great koji (composed of wheat bran and pea, and comprising around half the raw materials). These minor grain crops are traditionally treasured as health foods for their special nutritive value and functional activity. The manufacturing flowcharts for both types of vinegars are shown in Fig. 1. With respect to processing procedures, ZAV employ liquid-state fermentation in saccharification and alcohol fermentation, and solid-state fermentation in acetic acid fermentation respectively, while the entire SAV process takes place in the solid state. In particular, ZAV has only an aging process of less than three months without a roasting process following acetic acid fermentation, while SAV has a 6-day fuming (roasting) period and at least 18 months of aging. These processes endow SAV with increased concentrations of organic acids, flavor and nutrients due to chemical and enzymatic reactions[20], which explains the great variation in quality and nutri-

Raw material

Koji preparation → **Koji** →↓

Saccharification

Yeast culture →↓

Alcohol fermentation

Acetic-acid bacteria →↓←**Rice hull and wheat bran**

Acetic acid fermentation (solid)

↙   ↘

Roasting (80℃ for 6 days)   **Maturation (less than 3 months)**

↓   ↓

Maturation (18 months)   **Zhenjiang Aromatic Vinegar (ZAV)**

↓

Shanxi Aged Vinegar (SAV)

Fig. 1. Flowcharts for manufacturing SAV and ZAV used in the present study

ents among both types of vinegars.

Besides as an acid seasoning, vinegar has also been used for medicinal purposes in traditional Chinese medicine[30]. Recently, the health benefits of vinegar have attracted much attention. Studies have shown that vinegar possesses a wide spectrum of physiological effects, including regulating blood pressure[19], assisting calcium absorption[18], improving blood fluidity[31], antioxidant activities[26], and antithrombotic and fibrinolytic activity[11]. A steady rise in consumer demand has seen an increase from 2.5 million tons in 2004 to 3.4 million tons in 2009. However, information concerning the detailed chemical composition of these Chinese vinegars remains extremely limited, despite such widespread consumption of SAV or ZAV.

Diabetes mellitus has become a common disease in recent years. An α-Glucosidase inhibitor is usually used to prevent or medically treat typeII diabetes by combining with intestinal α-glucosidase and blocking the uptake of postprandial blood glucose. A recent study has also demonstrated that vinegar can significantly regulate postprandial hyperglycemia[10]. The mechanism by which

vinegar reduces postprandial blood glucose levels has been suggested as the inhibition of amylases and disaccharidase by acetic acid[22]. However, whether the acetic acid is the only constituent of vinegar with α-glucosidase inhibitory activity remains to be found.

As there are no defined microorganisms, major raw materials and standard procedures for vinegar processing, different varieties of vinegar have wide-ranging flavors and nutritional compositions, despite being the same type of vinegars in China. The objective of this study was to determine the detailed composition of five kinds of Chinese vinegars, including three typical SAV and two typical ZAV, including their proximate composition (moisture, crude protein, carbohydrate, lipid, ash, and titratable acidity) and nutritional composition (organic acids, amino acids, minerals, vitamin B complex and total phenolics, alkaloids, and saponin). In addition, the α-glucosidase inhibitory activities of the vinegars were also determined.

Table 1. The raw materials, koji, major manufacture processing of vinegars

	SV	ZHV	LRV
Materials (%, w/v)	Sorghum (45%)	Rice (30%)	Rice (15%)
	Wheat bran (14%)	Wheat bran (40%)	Starch (24%)
	Rice hull (14%)	Rice hull (28%)	Wheat bran (15%)
	Great koji (27%, barley, oats and peas)	Wheat koji (2%)	Rice hull (15%)
			Wheat koji (6%)
			Yeast powder (3%)
Microorganisms in koji	*Aspergillus*	*Rhizopus*	*Rhizopus*, yeast
Saccharification	Solid state	Liquid state	Liquid state
Alcohol fermentation	Solid state	Liquid state	Liquid state
Acetic acid fermentation	Solid state	Solid state	Solid state
Roasting	Yes	No	No
Aging	18 months	3 months	2 months
Production period (days)	540	90	60

Materials and methods

1. Materials

Three typical SAV, including sorghum vinegar (SV), oat vinegar (OV), and tartary buckwheat vinegar (TBV), were obtained from Shanxi Ziyauan Microorganism R & D Co., Ltd. (Shanxi, China). Two typical ZAV, including Zhenjiang Hengshun vinegar (ZHV) and Liubiju rice vinegar (LRV) were purchased from a local market. The raw materials, koji and major manufacture processing, are shown in Table 1. The SAV use minor grain crops as the main raw material with a large dosage of great koji (27% of raw materials). Their production takes 18 months. The ZAV, ZHV and LRV employ rice as the main raw material with a small dosage of koji (less than 3%) and with aging periods of less than three months. In particular, besides rice, LRV also uses starch as a fermentation substrate to cut costs, and adds yeast powder to rice to partly replace koji and accelerate fermentation. The change ratios of the main raw material to vinegar product are 1:3 for SAV, 1:4 for ZHV, and 1:6 for LRV. Only salt is added to these vinegars to avoid over-fermentation after acetic acid fermentation.

Intestinal acetone powders of rat, 4-nitrophenyl α-D-glucopyranoside (4-NPG), Folin-Ciocalteu (FC) reagent, and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other reagents were of analytical grade.

2. Proximate composition

Moisture and ash were estimated using the standard AOAC[2] methods. Protein content was calculated from the nitrogen content (%N × 6.25), as analyzed by the Kjeldahl method. The carbohydrate content was examined using the phenol-sulfuric acid colorimetric method[9]. The lipid content was determined using a Soxhlet apparatus according to the procedure described by Huang[16] and the pH was measured by a digital pH meter. Titratable acidity was determined as acetic acid per 100 mL using the standard method[1].

3. Organic acids

Before analysis, a 6 mL aliquot of vinegar was diluted to 20 mL with water. To eliminate interference, 5 mL of this solution were passed through a C18 SPE cartridge, which had been previously activated with 2 mL of a 9:1 (v/v) $H_2O:CH_3OH$ mixture. The cartridge was then washed with 2 mL of water and the collected eluates were diluted to 25 mL. A 10 μL aliquot of this solution was loaded onto a Shimadzu HPLC system to analyze the organic acids (acetic, citric, tartaric, gluconic, malic, succinic, and lactic acids). The HPLC system was equipped with a high pressure pump (0.6 mL/min), at 45 °C, using an ionic exchange Aminex HPX-87H column (300 × 7.8 mm, 9 μm) from Bio-Rad and a diode array detector (210 nm) was used for the organic acid. The mobile phase was a 0.005 mol/L H_2SO_4 solution (pH 2.1).

4. Amino acids

For the analysis of amino acid in vinegar, thirty milliliters of vinegar was filtered and concentrated under reduced pressure into a gummy residue, 1 mL of which was mixed with 3 mL of 6 N HCl and hydrolyzed at 110°C for 24 h. Subsequently, the hydrolysate was filtered and an aliquot of 0.5 mL was dried under a vacuum. Three milliliters of the citrate buffer (pH 2.2) were added to reconstitute the sample and 10 μL was used in loading into the amino acid analyzer (S433, SYKAM Co. Ltd., Munich, Germany).

5. Minerals

Calcium, phosphorus, iron, zinc, iodine and manganese were determined using an atomic absorption spectrophotometer (Spectra-AA220, Varian Co., Palo Alto, CA, USA) after digestion in mixed acids (nitric acid : perchloric acid = 4 : 1). The selenium content was determined using the fluorometric method of Huang[16].

6. Vitamin B complexes

Thiamine and riboflavin were analyzed using a Fluorometer (RF5300PC, Shimazu, Kyoto, Japan) and niacin (nicotinic acid and nicotinamide) was analyzed by a colorimetric method using a spectrophotometer (UV mini-1240, Shimazu, Japan) by following the standard methods of the AOAC[2].

7. Total phenolics

A sephadex C-18 column was used to separate the phenols from these compounds. Briefly, 0.2 mL of vinegar was passed through the column, which was then washed 3 times with 2 mL of water and the adsorbed phenols were eluted 3 times with 3 mL of methanol on the basis of UV monitoring. At the end of the separation, the methanolic fractions were incorporated and dried and the total phenolic content in the extract was measured spectrophotometrically using the Folin-Ciocalteu method[24].

8. Total alkaloid extraction and analysis

Vinegar (30 mL) was filtered and concentrated under reduced pressure into a gummy residue, which was extracted twice with HCl (1 N, 25 mL) by refluxing on a water bath for 30 min. After cooling, the solution was filtered into a volumetric flask (250 mL). Alkaloids were liberated at pH 9.8 from the filtrate by adding an aqueous solution of 0.7 M sodium carbonate and extracted with methylene chloride (3 × 30 mL) in a separator funnel. The latter extract was dried over anhydrous sodium sulfate to yield the total alkaloid, which was quantified by bromophenol blue colorimetry[21] using berberine hydrochloride as standard.

9. Total saponin extraction and analysis

Extraction and estimation of total saponin were accomplished as described by He et al.[14]

10. α-Glucosidase inhibiting activity

The inhibitory activity of vinegar against rat α-glucosidase was determined by measuring the formation of 4-nitrophenol by α-glucosidase following the reaction with 4-nitrophenyl α-D-glucopyranoside (4-PNP) as described by Yamaki and Mori[32]. The inhibitory activity of vinegar was measured according to the protocol of a micro-well kit (0.4 ml × 96 flat-bottom wells, Sumitomo Bakelite Co., Ltd., Tokyo, Japan). Vinegar was neutralized to pH 7.0 by using 2 N NaOH to eliminate the effect of the acetic acid on α-glucosidase. A serial twofold dilution of the neutralized vinegar in microtiter flat-bottom 96-well plates were mixed with 50 μL α-glucosidase (25 mg/mL), 50 μL 4-NPG (0.9133 mg/mL) and 120 μL 0.5 M phosphate buffer (pH 6.7). The mixtures were then incubated at 37°C for 60 min and sodium carbonate (50 μL, 0.67 M) was added to stop the reaction. The absorbance of the reactants was measured at 405 nm using a microplate reader (Model 550, BIO-RAD Lab., Tokyo, Japan). The α-glucosidase inhibitory activity of vinegar was then computed as the slope value from the curve of absorbance versus the vinegar concentration. The higher the slope value, the stronger the anti-α-glucosidase activity of the vinegar.

11. Statistical analysis

Each experiment was performed in triplicate, with the results expressed as a mean ± SD. Statistical comparisons were made via one-way analysis of variance, followed by a Duncan multiple-comparison test. Differences were considered significant when the p values were 0.05.

Results and discussion

1. Proximate composition

The results of the proximate chemical analysis of the five vinegars are shown in Table 2. The moisture contents of these vinegars ranged from 67.53% in OV to 88.10% in LRV, and SAV showed lower moisture content than ZAV, which could be related to its longer aging period. The variations in this parameter implied proportional increases or decreases in all nutrients on a wet weight basis. Crude protein contents varied from 1.7% in LRV to 10.47% in TBV, and all the SAV were significantly higher than ZAV in this parameter ($p <$

Table 2. Chemical components of vinegar (%, w/v) [A, B]

Component	SV	OV	TBV	ZHV	LRV
Moisture	68.10 ± 2.85 [a]	67.53 ± 0.26 [a]	83.24 ± 0.77 [b]	86.74 ± 0.02 [c]	88.10 ± 0.27 [c]
Crude protein	3.86 ± 0.01 [a]	6.48 ± 0.08 [b]	10.47 ± 0.09 [c]	2.27 ± 0.11 [d]	1.70 ± 0.02 [e]
Carbohydrate	0.76 ± 0.09 [a]	0.59 ± 0.007 [b]	0.33 ± 0.03 [c]	0.27 ± 0.02 [c]	0.13 ± 0.005 [d]
Total lipid	0.24 ± 0.07	0.31 ± 0.05	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.00
Ash	4.57 ± 0.68 [a]	4.93 ± 0.20 [a]	2.58 ± 0.15 [b]	3.16 ± 0.12 [c]	2.41 ± 0.17 [b]
pH	3.60 ± 0.44	3.71 ± 0.29	3.68 ± 0.16	3.38 ± 0.18	3.59 ± 0.25
Titratable acidity	6.01 ± 0.02 [c]	7.78 ± 0.04 [a]	6.77 ± 0.02 [b]	6.75 ± 0.04 [b]	5.27 ± 0.02 [d]

[A]: Each value is expressed as a mean ± SD (n = 3); Values in a row with different superscripts differ significantly ($p <$ 0.05).

[B]: SV, OV, TBV, ZHV and LRV represent sorghum vinegar, oat vinegar, tartary buckwheat vinegar, Zhenjiang Hengshun vinegar and Liubiju rice vinegar, respectively.

Table 3. Organic acid contents (g/L) of vinegars [A, B]

Organic acid	SV	OV	TBV	ZHV	LRV
Tartaric acid	1.54 ± 0.16	7.32 ± 1.15	3.45 ± 0.21	1.92 ± 1.11	3.04 ± 0.09
Formic acid	1.80 ± 0.21	4.51 ± 0.88	2.90 ± 0.14	0.3 ± 0.21	0.27 ± 0.16
Malic acid	4.50 ± 0	8.57 ± 0.45	6.50 ± 0.71	1.92 ± 1.01	1.09 ± 0.06
Lactic acid	25.50 ± 4.24	54.38 ± 0.88	22.50 ± 2.12	9.38 ± 3.71	33.04 ± 11.67
Acetic acid	57.05 ± 1.06	98.13 ± 2.65	73.5 ± 7.78	59.25 ± 26.52	62.63 ± 6.89
Citric acid	0.33 ± 0.08	1.05 ± 0.06	1.25 ± 0.21	1.43 ± 0.74	2.29 ± 0.16
Succinic acid	0.41 ± 0.14	0.63 ± 0.25	0.23 ± 0.02	0.69 ± 0.30	0.67 ± 0.11
Total	91.1 ± 1.03	175.95 ± 7.73	111.43 ± 6.05	75.32 ± 2.71	103.39 ± 3.49

[A]: Each value is expressed as a mean ± SD (n = 3).

[B]: SV, OV, TBV, ZHV and LRV represent sorghum vinegar, oat vinegar, tartary buckwheat vinegar, Zhenjiang Hengshun vinegar and Liubiju rice vinegar, respectively.

0.05), while the carbohydrate contents were low for all vinegars. They followed the order: SV (0.76%) > OV (0.59%) > TBV (0.33%) and ZHV (0.27%) > LRV (0.13%). The saccharides in these vinegars were also much lower than those in some Japanese vinegars, ranging from 1 to 5%[19]. Fermentation was generally more thoroughly conducted in Chinese vinegar in order to obtain the considerably sour taste. The five vinegars had lower lipid contents ranging from 0.14 to 0.31%, with no significant difference emerging. The ash contents ranged from 2.41 to 4.93%, with OV and SV having higher ash contents than the others. Titratable acidity is an important index of vinegar and must exceed 3.5% (w/v) in accordance with the China State Standard. The vinegars had far higher titratable acidities (5.27-7.78%).

2. Organic acids

The contents of organic acids in the vinegars analyzed are presented in Table 3, with totals ranging from 75.32 to 175.95 g/L. OV and TBV contained more than the others, while ZHV contained less.

Organic acids are important in imparting vinegar with a suitable taste and flavor. Recent studies showed that organic acids also have important physiological activities, including antihypertensive[19] and antihyperglycemic effects[35]. The concentrations of acetic acid in the vinegars ranged from 5.7% (w/v) to 9.8% (w/v) (Table 3). According to Cheng et al.[7], when food containing 10-150 mmol/L of acetic acid was eaten, the concentration of acetic acid possibly reached the millimolar level in the small intestine. Such a level can have physiological effects, including disaccharidase and angiotensin-I converting enzyme inhibitory activities in the small intestine[22]. The vinegars contained so much acetic acid that they could still reach the millimolar level in the small intestine, even after dilution 6-11 times.

Lactic acid was found to be the second dominant organic acid (0.9%-5.4%) next to acetic acid in the vinegars. This nonvolatile acid would generally intensify the stimulus of acetic acid and make sourness taste more persistence. Tartaric acid showed an obvious difference in different vinegars and the order was: OV (7.32 g/L) > TBV (3.45 g/L) and LRV (3.04 g/L) > ZHV (1.92 g/L) and SV (1.54 g/L). Formic and malic acids were high in SAV (1.80-4.51 and 4.50-8.57 g/L, respectively), which were 6-20 and 2-8 times higher than that in ZAV (0.27-0.3 and 1.09-1.92 g/L, respectively). As regards succinic acid, it was present at low concentration and showed no obvious difference between SAV and ZAV.

Citric acid, which is known to be good for health, ranged from 0.33 to 2.29 g/L in the vinegars, with LRV containing more (2.29 g/L) than the others (0.33-1.43 g/L). This concentration was less than that of Japanese vinegars (2.73-7.66 mg/mL)[34].

3. Amino acids

Table 4 shows the concentrations of various amino acids in the five vinegars, with their totals in order: SV (2878.8 mg/100 g) > ZHV (2465.1 mg/100 g) > TBV (2332.3 mg/100 g) > OV (1532.1 mg/100 g) > LRV (1215.9 mg/100 g).

The total amino acids of several Japanese vinegars including rice vinegar, malt vinegar, apple vinegar and onion vinegar reportedly ranged from 30.6 mg/100 mL to 210 mg/100 mL[15]. Compared with these Japanese vinegars, the Chinese vinegars tested in the present study were more abundant in total amino acids, namely 5.8-94 times more than Japanese vinegars. The formation of amino acids in vinegar depends on the raw materials used, the particular fermentation condition and the action and autolysis of the bacteria involved in the process[5].

These vinegars are rich in all kinds of amino acids. As shown in Table 4, glutamic acid was the most abundant amino acid in these vinegars, and ranged from 294.9 mg/100 g in LRV to 887.5 mg/100 g in SV. Some amino acids, including asparagine, glycine, alanine, valine, leucine, threonine, serine, isoleucine and lysine dominated and accounted for about 55 to 62% of all the amino acid. Cystine, methionine, tyrosine, phenylalanine, histidine and arginine in these vinegars were mi-

Table 4. Amino acid contents (mg/100g) of vinegar [A, B]

Amino acid	SV	OV	TBV	ZHV	LRV
Asparagine	227.2 ± 10.8	130.1 ± 11.7	205.6 ± 4.1	421.8 ± 18.5	51.2 ± 5.0
Threonine	92.0 ± 13.1	50.0 ± 3.8	84.2 ± 2.3	136.9 ± 5.2	50.9 ± 2.6
Serine	111.7 ± 11.6	61.5 ± 3.4	88.3 ± 3.8	82.4 ± 6.3	49.9 ± 4.5
Glutamic acid	887.5 ± 53.5	462.6 ± 17.4	695.1 ± 14.9	659.1 ± 26.2	294.9 ± 17.1
Glycine	175.0 ± 24.0	99.6 ± 7.9	141.6 ± 3.1	131.1 ± 18.5	80.8 ± 13.9
Alanine	250.9 ± 14.4	119.0 ± 9.4	186.8 ± 11.5	153.3 ± 14.1	166.9 ± 7.7
Cystine	29.9 ± 5.2	20.5 ± 2.1	22.0 ± 3.6	52.9 ± 7.4	9.9 ± 1.4
Valine	215.5 ± 12.0	107.2 ± 9.1	170.5 ± 9.3	118.2 ± 11.1	105.7 ± 8.0
Methionine	20.4 ± 2.5	11.1 ± 1.4	19.9 ± 2.5	22.1 ± 3.7	20.8 ± 3.2
Isoleucine	105.9 ± 10.6	51.0 ± 3.3	85.9 ± 5.0	81.0 ± 11.6	57.2 ± 4.4
Leucine	175.3 ± 14.0	92.6 ± 4.4	130.6 ± 9.1	97.6 ± 9.2	95.5 ± 7.5
Tyrosine	51.0 ± 4.5	30.6 ± 1.7	41.5 ± 2.8	56.3 ± 7.9	12.0 ± 1.8
Phenylalanine	69.7 ± 4.7	53.5 ± 3.5	57.4 ± 6.3	66.2 ± 6.2	18.5 ± 2.3
Histidine	52.3 ± 3.4	29.9 ± 2.5	44.7 ± 4.7	74.5 ± 7.1	9.9 ± 1.4
Lysine	80.5 ± 5.9	45.7 ± 3.7	100.6 ± 8.5	47.6 ± 5.9	72.0 ± 4.6
Arginine	50.4 ± 2.3	36.7 ± 2.6	49.6 ± 4.2	84.5 ± 5.2	23.8 ± 1.3
Proline	293.8 ± 16.6	130.3 ± 7.8	207.9 ± 14.3	179.8 ± 14.6	96.2 ± 4.6
Total	2878.8 ± 110.9	1532.1 ± 25.1	2332.3 ± 10.3	2465.1 ± 18.1	1215.9 ± 12.1

[A] : Each value is expressed as a mean ± SD (n = 3).
[B] : SV, OV, TBV, ZHV and LRV represent sorghum vinegar, oat vinegar, tartary buckwheat vinegar, Zhenjiang Hengshun vinegar and Liubiju rice vinegar, respectively.
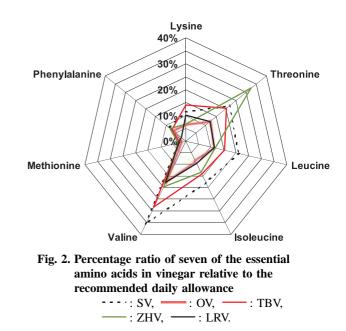
nor amino acids, accounting for about 6 to 13% of the total amino acid. The amino acids influenced the taste and bio-functionality of the vinegars[15, 29], while glutamic acid, asparagine, glycine, alanine and methionine could enhance the flavor of the vinegars thanks to their umami taste.

Figure 2 shows the essential amino acid supply, which indicates the percentage ratio of essential amino acids in vinegar (100 g) relative to its recommended daily allowance. Significant differences emerged in the essential amino acid supply between both types of vinegars, SAV and ZAV. SAV mainly provided valine, while ZHV could provide more threonine. Obvious differences also emerged in the essential amino acid supply among the three SAV. The total value of the essential amino acid supply of SV was the highest (118.4%) among these vinegars, followed by TBV (101%), while OV ranked third (63%). These data suggest that SV is superior in terms of essential amino acid content than the others. The essential amino acids in SV could be derived from its main raw materials, sorghum, which is rich in essential amino acids[27].

4. Minerals

The mineral contents of these vinegars are summarized in Table 5. The data indicated that calcium, phosphorus, iron, zinc and manganese were the major mineral constituents in these vinegars, while iodine and selenium were also detected in appreciable amounts. OV contained the highest amounts of minerals, and LRV the lowest.

Calcium was the predominant mineral in the five vinegars, ranging from 31.735 mg/kg in LRV to 1246 mg/kg in OV and its content differed significantly among the five vinegars ($p < 0.05$). It is necessary to

Fig. 2. Percentage ratio of seven of the essential amino acids in vinegar relative to the recommended daily allowance
- - - - : SV, ━━━ : OV, ─── : TBV,
─── : ZHV, ─── : LRV.

emphasize the high calcium content of SAV. All the values were higher in SAV (651-1246 mg/kg) than those in ZAV (31-134 mg/kg). SV (651 mg/kg), OV (1246 mg/kg), and TBV (696 mg/kg), meanwhile, were excellent sources of this mineral, even more so than milk (~500 mg/kg). Several clinical studies have shown calcium to be an effective pressure-reducing agent[23, 36]. It had been reported that leavening could improve the absorbability of calcium in flour-based products due to the phytate hydrolysis caused by yeast fermentation[27]. Dietary vinegar was also found to enhance intestinal calcium absorption by improving calcium solubility and due to the trophic effect of the acetic acid[25].

Phosphorus is a component of DNA, RNA, ATP, and also phospholipids, which form all cell membranes,

Table 5. Minerals in vinegar (mg/kg) [A, B]

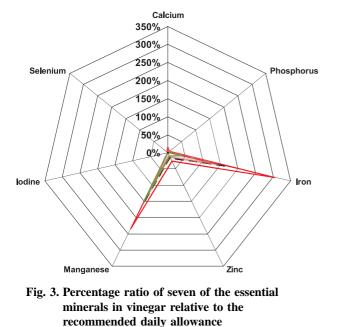| Mineral	SV	OV	TBV	ZHV	LRV
Calcium	651.370 ± 49.432 [a]	1246.365 ± 71.873 [b]	695.904 ± 70.839 [a]	133.897 ± 11.468 [c]	31.735 ± 6.104 [d]
Phosphorus	3334.577 ± 124.741 [a]	4722.563 ± 390.527 [b]	3465.652 ± 221.695 [a]	1968.038 ± 77.367 [c]	22.296 ± 2.663 [d]
Selenium	0.008 ± 0.006 [a]	0.015 ± 0.009 [a]	0.014 ± 0.001 [a]	0.012 ± 0.006 [a]	0.001 ± 0.003 [a]
Iron	161.991 ± 22.364 [a]	304.603 ± 28.671 [b]	139.636 ± 18.479 [a]	38.254 ± 4.728 [c]	1.452 ± 0.083 [d]
Zinc	22.207 ± 1.331 [a]	37.816 ± 1.918 [b]	17.704 ± 2.104 [c]	16.584 ± 1.427 [c]	0.243 ± 0.016 [d]
Manganese	45.168 ± 3.295 [a]	70.369 ± 5.623 [b]	39.775 ± 4.768 [a]	45.318 ± 4.442 [a]	0.038 ± 0.009 [c]
Iodine	0.173 ± 0.011 [a]	0.528 ± 0.107 [b]	0.098 ± 0.028 [c]	0.314 ± 0.027 [d]	0.066 ± 0.039 [c]

[A] : Each value is expressed as a mean ± SD (n = 3); Values in a row with different superscripts differ significantly ($p < 0.05$).

[B] : SV, OV, TBV, ZHV and LRV represent sorghum vinegar, oat vinegar, tartary buckwheat vinegar, Zhenjiang Hengshun vinegar and Liubiju rice vinegar, respectively.

making it an essential element for all living cells. Phosphorus also turned out to be the predominant mineral in these vinegars. In terms of phosphorus content, SAV was obviously higher than ZAV. The highest value was found in OV (4722.6 mg/kg), followed by SV (3465.6 mg/kg) and TBV (3334.6 mg/kg), ZAV (1968.0 mg/kg) ranked fourth, and the content of LRV (22.3 mg/kg) was the lowest.

SAV also seemed to be a richer source of iron (139.9-304.6 mg/kg), while OV had the highest iron content among the vinegars. Figure 3 also shows the mineral supply, the percentage ratio of minerals in vinegar (100 g) relative to the recommended daily allowance. As shown in Fig. 3, iron was the most noticeable mineral supplied by SAV. The iron supply was from 139.6% of TBV to 304.6% of OV. Consuming less than fifty gram of OV could provide sufficient iron to meet the recommended daily allowance of 15 mg per person. Cereals generally contain small amounts of phytate but sufficient to inhibit iron absorption. However, fermentation could cause the degradation of phytate, finally changing cereal with low iron bioavailability into a diet of high iron bioavailability[27]. Furthermore, vinegar and acetic acid could also increase iron absorption[25]. Therefore, the iron in vinegar was speculated as having high human bioavailability.

OV and SV contained to show relatively high amounts of zinc (37.8 and 22.2 mg/kg, respectively) (Table 5). TBV and ZHV contained almost equivalent amounts of zinc (17.7 and 16.6 mg/kg, respectively),

while LRV had the lowest zinc content (0.243 mg/kg). As recommended by the Food and Nutrition Board, National Research Council[12], the recommended daily amount is 16 mg. *In vivo* investigations of the effect of phytate on zinc bioavailability showed a similar relationship for iron and phytate, and food fermentation and processing that decreased the phytate content improved zinc absorption[27].

Vinegar was also a rich source of manganese. The richest source was OV (70.369 mg/kg), followed by SV (45.168 mg/kg) and ZHV (45.318 mg/kg) (Table 4). The value of TBV ranked fourth (39.775 mg/kg), and LRV was the lowest (0.038 mg/kg). The daily manganese requirement is 3-5 mg, which can be covered by 50 g of OV. The manganese supply was 234.6% for OV, 151.1% for ZHV, 152.6% for SV, and 132.6% for TBV, respectively (Fig. 3). These data show that consuming less than 100 g of vinegar can meet the entire manganese requirement of the body. The human bioavailability of manganese in vinegars may be compromised by the co-presence of some components such as calcium, iron and phytates that can render manganese unavailable for absorption in the intestine. Therefore, further research must be conducted to elucidate the human bioavailability of manganese in vinegar.

Variations in the contents of minerals for vinegars might be due to their raw materials and manufacturing methods. SAV is mainly made from minor grain crops, such as sorghum, oat, tartary buckwheat, and pea, which tend to be grown in drought and semi-arid areas. Mineral-efficient varieties of plants are more drought resistant and require less irrigation. In addition, SAV generally has a longer roasting and aging process (more than 18 months), which makes it condensed.

5. Vitamin B complexes

Table 6 shows the nicotinic acid, nicotinamide, thiamine and riboflavin contents of the five vinegars. According to the results, thiamine and riboflavin were low in SAV and ZHV, and not detected in LBV. However, large amounts of nicotinic acid and nicotinamide were present at contents ranging from 2.33 to 37.83 mg/100 g and 1.23 to 35.03 mg/100 g, respectively.

The two niacin vitamers, nicotinic acid and nicotinamide, contribute to the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) which are involved in the physiological redox reactions of carbohydrates, fatty acids and amino acid metabolism. Riboflavin acts as a catalyst for the mitochondrial electron transport chain in its coenzymes. Interestingly, SAV was much richer than ZHV in nicotinic acid, nicotinamide and thiamine, mak-

Fig. 3. Percentage ratio of seven of the essential minerals in vinegar relative to the recommended daily allowance
- - - - : SV, ——— : OV, ——— : TBV,
——— : ZHV, ——— : LRV.

Table 6. Vitamins, total phenolics, alkaloids and saponin in vinegar [A, B]

	SV	OV	TBV	ZHV	LRV
Nicotinic acid (mg/100 g)	29.24 ± 2.63 [a]	37.83 ± 1.48 [b]	34.12 ± 1.53 [a]	11.91 ± 0.15 [c]	2.33 ± 0.03 [d]
Nicotinamide (mg/100 g)	24.48 ± 1.24 [a]	35.03 ± 2.74 [b]	13.37 ± 0.30 [c]	13.52 ± 1.28 [c]	1.23 ± 0.05 [d]
Thiamine (μg/100 g)	0.37 ± 0.09 [a]	0.71 ± 0.06 [b]	0.46 ± 0.07 [a]	0.13 ± 0.01 [c]	– [c]
Riboflavin (mg/100 g)	1.45 ± 0.05 [a]	1.63 ± 0.34 [a]	0.89 ± 0.04 [b]	2.1 ± 0.05 [c]	– [c]
Phenolics (μg/mL)	3964.7 ± 64.2 [a]	2812.1 ± 52.8 [b]	4518.1 ± 107.5 [c]	1564.2 ± 103.7 [d]	382.4 ± 20.7 [e]
Alkaloids (mg/mL)	0.819 ± 0.066 [a]	1.209 ± 0.261 [b]	0.648 ± 0.054 [c]	0.979 ± 0.137 [d]	0.088 ± 0.002 [e]
Saponin (mg/mL)	0.593 ± 0.061 [a]	0.662 ± 0.019 [a]	0.604 ± 0.048 [a]	0.290 ± 0.033 [b]	0.183 ± 0.026 [c]

[A]: Each value is expressed as a mean ± SD (n = 3); Values in a row with different superscripts differ significantly ($p < 0.05$).

[B]: SV, OV, TBV, ZHV and LRV represent sorghum vinegar, oat vinegar, tartary buckwheat vinegar, Zhenjiang Hengshun vinegar and Liubiju rice vinegar, respectively.
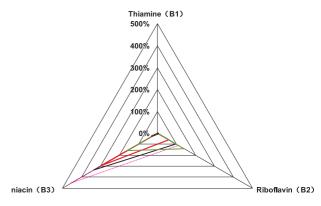
[C]: Not detected.

ing it a good source of dietary niacin. The VB supply, namely the percentage ratio of VB in vinegar (100 g) relative to the recommended daily allowance of the same, was given in Fig. 4. As shown here, one hundred grams of vinegar can supply 22.3-455.4% and 59.3-140% of the daily requirements of niacin and riboflavin daily for the human body. On average, a 35 g aliquot of OV would provide almost all an adult's daily niacin requirement (25 mg), and 1/3 of an adult's daily riboflavin requirement, as recommended by the Food and Nutrition Board, National Research Council[12].

Nicotinic acid and nicotinamide are mainly present in plant-based and animal foods, respectively and abundant niacin was found in SAV in the present study. The raw materials (sorghum, oat, tartary buckwheat and particularly wheat bran) used to produce SAV are rich in niacin[33]. For example, wheat bran has 12.5 mg/100g of niacin, which is the highest among these raw materials. However, according to the change ratio of raw materials to vinegar products (generally, one kilogram of raw materials can produce three kilograms of vinegar), niacin in vinegar products will decrease to 1/3 of its raw material quantity. Therefore, we speculated that microorganisms involved in vinegar making could synthesize this vitamin.

6. Total phenolics, alkaloid and saponin

Generally speaking, phenolics, alkaloids and saponin are considered major active fractions for curative effects in traditional Chinese medicine and food, hence the quantities contained in these vinegars were also determined.

The total phenolic contents decreased in the order of TBV (4518.1 μg/mL), SV (3964.7 μg/mL), OV

Fig. 4. Percentage ratio of three of the vitamin B in vinegar relative to the recommended daily allowance

- - - - : SV, ·········· : OV, ——— : TBV, ——— : ZHV, ——— : LRV.

(2812.1 μg/mL), ZHV (1956.2 μg/mL) and LRV (382.4 μg/mL) (Table 6). SAV were significantly higher than ZAV in total phenolics ($p < 0.05$), which could be attributed to the high phenolic contents in minor grain crops[11].

In terms of total alkaloids (Table 6), a wide variation was observed. The OV was the highest (1.209 mg/mL) in terms of total alkaloids, followed by ZHV (0.979 mg/mL), SV (0.819 mg/mL), TBV (0.648 mg/mL) and LBV (0.088 mg/mL). Raw materials and microorganisms used in manufacturing could have a profound effect on the alkaloid content of these vinegars. The total contents of saponin in SAV had no significant differences ($p > 0.05$), although significantly exceeding that of ZAV. Generally speaking, the raw materials used for SAV production have a high saponin content[33].

Moreover, almost the same raw materials were used in different forms of SAV, such as SV and OV, which may explain why SAV have almost equivalent saponin content.

Compared with SAV and ZHV, LRV was low in minerals, Vitamin B complexes, phenols, alkaloids, and saponin (Tables 5, 6), which could be attributed to its raw materials, microorganisms in the koji and the aging period. Although LRV shares its manufacturing processes with ZHV, replacing the rice with starch, adding yeast to accelerate fermentation, a short aging period and high moisture content lowered its nutrients.

7. α-Glucosidase inhibitory activity

It is well known that α-glucosidase is an exo-acting carbohydrase, the catalysis of which releases α-D-glucopyranose from the non-reducing ends of various carbohydrate substrates[13]. These enzymes play an important role in the biochemical processes of glycoproteins and glycolipids[4]. The presence of α-glucosidase inhibitor in diets can inhibit the activity of α-glucosidase and reduce the absorption of dietary carbohydrates, meaning α-glucosidase inhibitors might be useful in developing treatments for carbohydrate-mediated diseases such as diabetes, certain forms of hyperlipoproteinemia and obesity[3, 8]. It has been established that naturally occurring phenolics (catechin, Apigenin8-C-glucoside, anthocyanins, genistein, quercetin, chlorogenic acid), alkaloids (Berberine, 1,2-deoxynojirimycin) and saponin widely distributed in food have the ability to inhibit α-glucosidase[6]. To elucidate the influence of these bioac-
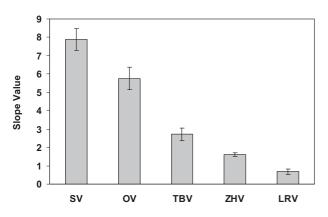
tive substances on α-glucosidase activity, the vinegars were neutralized and their α-glucosidase inhibitory activity subsequently compared (Fig. 5). The results showed that although all the vinegars exhibited α-glucosidase inhibitory activity, significant variations in the activity were found among them. The α-glucosidase inhibitory activity of these vinegars followed the order: SV (7.9), OV (5.8), TBV (2.7), ZHV (1.6), LRV (0.7). On the whole, SAV were higher than ZAV in terms of α-glucosidase inhibitory activity, which correlated to the higher contents of phenolics, alkaloids or saponins in the former. However, Pearson correlation analysis shows no relationship between phenolics, alkaloids or saponins and α-glucosidase inhibitory activity among these SAV. For example, TBV had a higher total phenolic content, whereas its α-glucosidase inhibitory activity was lower than SV, and OV (Fig. 5). Therefore, the α-glucosidase inhibitory activity could result from the integrative effects of these substances, phenolics, alkaloids and saponin. Moreover, different types of phenolics, alkaloids and saponin could have different α-glucosidase inhibitory activity, dependent on structure[17]. SV had been found to be rich in many phenolic acids, including gallic, 3,4-dihydroxybenzoic, chlorogenic, caffeic, vanillic, syringic and *p*-coumaric acids[11], which could explain the high activity of SV. However, data concerning alkaloids and saponins in SV and phenolics, alkaloids and saponins in OV and TBV are scarce. These vinegars possibly contain different types of phenolics, alkaloids and saponin with different α-glucosidase inhibitory activity. Further study should be conducted to elucidate the relationship between the α-glucosidase inhibitory activity and the structure of the bioactive substances. The effect of raw materials and manufacturing processing, such as fermentation and roasting, on the changes in certain substances in vinegar are being studied in our laboratory.

Conclusions

This is the first nutrient report for the two most typical Chinese vinegars, the nutritional composition of which shows some significant differences. SAV, especially OV, is more abundant than ZAV in nutrients owing to its special raw materials and manufacture processing, including organic acids, amino acids, calcium, phosphorus, iron, zinc, manganese, niacin, phenolics, alkaloids and saponin. Its abundance in nutrients may open up special applications, including supplementing minerals for some women deficient in calcium and iron, supplying niacin to some people with niacin deficiency and extracting organic acids and amino acid dressings.

Fig. 5. The α-glucosidase inhibitory activity of vinegar

Values represent the mean ± standard deviation (SD) of n = 3 duplicate assays. SV, OV, TBV, ZHV and LRV represent sorghum vinegar, oat vinegar, tartary buckwheat vinegar, Zhenjiang Hengshun vinegar and Liubiju rice vinegar, respectively.

The vinegars, especially SAV, were also found to have strong α-glucosidase inhibitory activity, which may be useful for diabetics. However, the stimulus of the sour taste of vinegar may prove a major obstacle to efforts to promote its consumption in large quantities. In summary, Chinese vinegar may be exploited as part of the development of various functional foods for these attributes.

Acknowledgments

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