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Antioxidant activity of vinegar melanoidins

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Abstract

Melanoidins, the brown polymers formed through Maillard reaction during vinegar process, are one of major high-molecular-weight fractions of vinegar. In this study the antioxidant activity of high-molecular-weight fractions (MW > 3500 Da) separated from ethanol-supernatant extraction of concentrated Zhenjiang aromatic vinegar was evaluated by different *in vitro* tests: the DPPH radical scavenging activity, the reducing power, total phenolic content, and the inhibitory effect on hydroxyl radical. Each individual fraction was found to have antioxidant activity in all the model systems tested. The high-molecular-weight fractions of vinegar (MW > 3500 Da) were separated into different fractions by DEAE-Sepharose fast flow. The fractions eluted by 0.2 mol/L NaCl and 0.3 mol/L NaCl with higher phenolic content have stronger DPPH radical scavenging activity and reducing power. Antioxidant activity in hydroxyl radical system was not correlated with phenolic content. Two phases which have stronger effects on Maillard reaction products (MRPs) in production process were examined. Decoction, storing and aging may affect vinegar's antioxidant activity. The present results support the concept that melanoidins formed during vinegar production process may have health promotion activity.

Keywords: Vinegar; Melanoidin; Antioxidant activity

1. Introduction

Brewed vinegar, a commonly used condiment of food, also has medicinal uses by virtue of its physiological effects, such as promoting recovery from exhaustion (Fushimi et al., 2001), regulating blood glucose (Ebihara & Nakajima, 1988), blood pressure (Kondo, Tayama, Tsukamoto, Ikeda, & Yamori, 2001), aiding digestion (Liljeberg & Bjorck, 1998), stimulating the appetite, and promoting calcium absorption (Kishi et al., 1999). According to *Dictionary of Chinese Materia Mediaca* (Jiangsu New Medical College, 2004), vinegar can be made from various sources and rice vinegar is better for medicinal uses. In China, different areas have their characteristic species. Zhenjiang vinegar, which is produced from sticky rice through static surface acetic acid fermentation, is one of the most common traditional vinegars in China. Vinegar made from grain is rich in polysaccharides, phenolic compounds and protein, which undergo profound molecular changes during decoction and storing and aging. Melanoidins are also one of major components formed in vinegar. According to our previous study (Xu, Tao, & Ao, 2005a), Zhenjiang aromatic vinegar has antioxidant activity *in vitro*, ethanol-supernatant extraction of vinegar has higher antioxidant activity. Ethanol-supernatant extraction was further fractionated into different molecular weights (MW), MW below 3500 Da, MW 3500–7000 Da, MW 7000–14000 Da and MW above 14000 Da, by means of dialysis. The trends of browning, reducing power and DPPH radical scavenging activity of Zhenjiang vinegar fractions were generally of the order of MW above 3500 Da > unfractioned > MW bellow 3500 Da (Xu, Ao, & Tao, 2004). It showed that the antioxidant activity had a certain relationship with the absorbance at 420 nm, the antioxidant activity of high-molecular-weight fractions may be due to the Maillard reaction products (MRPs), e.g., melanoidins.

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are forms of activated oxygen and nitrogen, respectively, which include free radicals such as superoxide ion, hydroxyl and nitric oxide radicals, as well as non-free-radical species such as hydrogen peroxide and nitrous acid. Free radicals can damage diverse cellular macromolecules, including proteins, carbohydrates, lipids, and nucleic acids due to their high reactivity. Lipid peroxidation is thought to be closely associated with aging, atherosclerosis and carcinogenesis. It is now thought that dietary antioxidants are essential to health. There have been a few studies on the antioxidant activity of vinegar. Nishidai, Nakamura, and Torikai (2000) found an ethyl acetate extract of Kurosu (a kind of rice vinegar) exhibited antioxidant activity in linoleic acid autoxidation model and the DPPH radical system. Dihydroferulic acid and dihydrosinapic acid isolated from Kurosu were the major constituents responsible for Kurosu's radical scavenging activity (Shimoji et al., 2002). Previous study in our lab showed that Zhenjiang aromatic vinegar has a higher content of tetramethylpyrazine (He, Ao, Wu, Li, & Tao, 2004) which may come from MRPs in vinegar, while tetramethylpyrazine also has antioxidant activity (Li, Zhang, Gao, Hou, & Wei, 2004).

The objective of the present investigation is to evaluate the antioxidant activity of high-molecular-weight fractions, melanoidins, obtained from ethanol-supernatant extraction of concentrated Zhenjiang aromatic vinegar. Melanoidins were separated by dialysis and DEAE-Sepharose fast flow. Their antioxidant properties were determined by different methodologies. The reducing power, DPPH radical scavenging activity, hydroxyl radical inhibiting effect and total phenolic content were assessed. Also effects of main production processes in which melanoidins were formed on antioxidant activity of vinegar were examined. It proved the idea that MRPs produced in vinegar production process have antioxidant activity.

2. Materials and methods

2.1. Vinegar samples and materials

Fresh-made vinegar, undecocted vinegar, decocted vinegar, and two-years-vinegar were obtained from Jiangsu Hengshun Vinegar-industry Co., Ltd. (Jiangsu, People's Republic of China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, MO). All solvents/chemicals used were of analytical grade and obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of Zhenjiang aromatic vinegar extracts and Zhenjiang aromatic vinegar melanoidins

One batch of fresh-made vinegar about 100 kg were obtained from vinegar plant (Jiangsu Hengshun Vinegar-industry Co., Ltd., Jiangsu, China) and stored for the following experiments.

Preparation of Zhenjiang aromatic vinegar extracts (ZVE) was carried out (in triplicate) according to the scheme given in Fig. 1. Aliquots (2.0 g) of ZVE were dissolved in distilled water (20 mL) and separated by dialysis into two fractions: low-molecular-weight fractions (LMW, MW $<$ 3500 Da) and high-molecular-weight fractions (HMW, MW $>$ 3500 Da). HMW was put onto the top of a glass column (20 $\times$ 2.2 cm i.d.) filled with DEAE-Sepharose fast flow in water (Pharmacia, Uppsala, Sweden). Elution was done with distilled water, 0.1 mol/L NaCl, 0.2 mol/L NaCl, 0.3 mol/L NaCl, and 0.6 mol/L NaCl, at a flow rate of 2 mL/min in turn. The effluent was monitored at 280 nm, and four fractions were collected and desiccated by vacuum. The same steps were repeated for at least 10 times and fractions eluted by NaCl of different concentration were collected, respectively, and dried in vacuum for antioxidant activity test.

2.3. Wavelength spectra of vinegar melanoidins (effluents of 0.2 mol/L and 0.3 mol/L NaCl)

Wavelength spectra of vinegar melanoidins were recorded on a UV/Vis U-3000 photometer, wavelength 190–700 nm. Samples were dissolved in deionized water at a concentration of 0.25 mg/mL.

2.4. Determination of total phenolic content

Total phenolic content was determined as described previously (Zhu, Hackman, Ensunsa, Holt, & Keen, 2002). To a 200 μL sample of the Zhenjiang aromatic vinegar extracts (ZVE), 1 mL of Folin–Ciocalten reagent (diluted 10 times) and 1 mL sodium carbonate (7.5%) reagent were added. Fix volume to 5 mL with distilled water. Subsequently, the mixture was incubated in the dark for 30 min. After incubation, the absorbance was measured at 760 nm. Gallic acid was used as a standard for the calibration curve. The phenolic content is reported as gallic acid equivalents (μg) using the following linear equation based on the calibration curve: $A = 0.0077C + 0.0105$, $R^2 = 0.9994$, where A is the absorbance and C is gallic acid equivalents (μg).

2.5. DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to Xu et al. (2004) and Yokozawa et al. (1998) with a slight modification. Briefly, a 0.2 mmol/L solution of DPPH radical solution in ethanol was prepared, and then 1 mL of this solution was mixed with 1 mL of a test sample dissolved in 100 mmol/L Tris–HCl buffer (pH 7.4); the mixture was then shaken up vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. This activity is given as percent DPPH radical scavenging and is calculated as

$$\%\text{DPPH radical scavenging} = [1 - (\text{Ai} - \text{Aj})/\text{Ac}] \times 100;$$

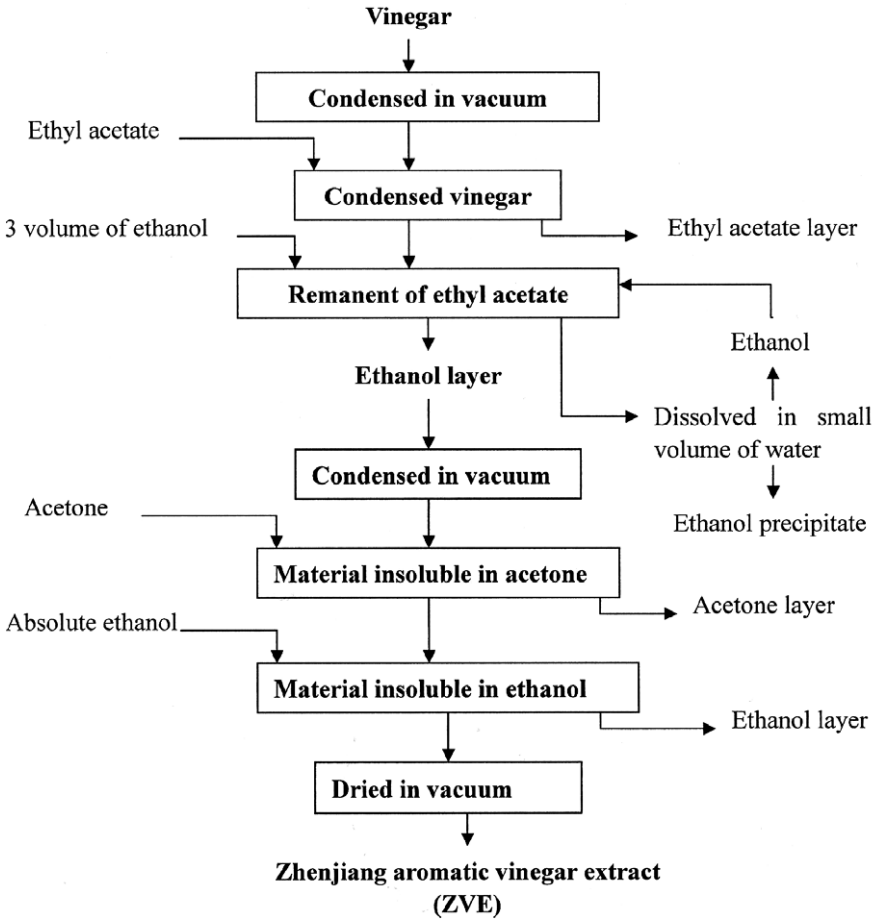

Fig. 1. Scheme for preparation of Zhenjiang aromatic vinegar extracts (ZVE).

Ai: Absorbance of mixture of 1 mL sample and 1 mL 0.2 mmol/L DPPH;
Aj: Absorbance of mixture of 1 mL sample and 1 mL ethanol;
Ac: Absorbance of mixture of 1 mL 0.2 mmol/L DPPH and 1 mL Tris–HCl buffer.

2.6. Reducing power

Total reducing power was determined as described previously with slight changes (Zhu et al., 2002). Briefly, 0.4 mL test sample (25–250 mg/L) was mixed with 1 mL phosphate buffer (0.2 mol/L, pH 6.6) and 1% potassium ferricyanide (1 mL); then the mixture was incubated at 50 °C for 30 min. Afterward, 1 mL of trichloroacetic acid (TCA) (10%) was added to the mixture. The upper layer solution (1 mL) was mixed with distilled water (1 mL) and 0.1% ferric chloride (0.2 mL), and the absorbance was measured at 700 nm. The higher absorbance of the reaction mixture inferred a higher reducing power.

2.7. Inhibition of hydroxyl radical

The assay was performed according to the method of Klein, Cohen, and Cederbaum (1981), and Singh, Chidambara Murthy, and Jayaprakasha (2002). Various concentrations of extracts 0.6 mL in distilled water were taken in different test tubes. Iron-EDTA solution (0.8 mL; 0.021% ferrous ammonium sulphate and 0.037% ethylene diamine tetraacetic acid), ethylene diamine tetraacetic acid (EDTA, 0.4 mL; 0.022%), and dimethyl sulphoxide (DMSO, 0.8 mL; 0.88% v/v in 0.1 mol/L phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.4 mL of 0.264% ascorbic acid. Test tubes were capped tightly and heated in a water bath at 80–90 °C for 15 min. The reaction was terminated by the addition of 1 mL of ice-cold TCA (17.5% w/v). 3 mL of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank. The inhibition rate of hydroxyl radical is calculated by the following formula: % inhibition rate of hydroxyl radical = (1 − (difference in absorbance of sample/difference in absorbance of blank)) × 100.

2.8. Statistical analysis

The results reported here are the means of at least three measurements and standard deviation (SD). Means of

treatment (decoction or storing and aging) on DPPH radical scavenging activity were compared with untreated control vinegar by Student's *t*-test. The level of significance was set at $P < 0.05$(*).

3. Results and discussion

3.1. Preparation of Zhenjiang aromatic vinegar extracts and Zhenjiang aromatic vinegar melanoidins

High-molecular-weight fractions (HMW, MW > 3500 Da) were acquired by dialysis of Zhenjiang aromatic vinegar extracts (ZVE) which were obtained by the scheme described in Fig. 1.

Ethanol is frequently used for the precipitation of HMW polymers such as DNA, proteins and polysaccharide. Upon addition of ethanol, HMW compounds lose their solubility and precipitate. The higher the concentration of ethanol, the more LMW compounds also precipitate. In practice, a large proportion of ethanol-supernatant extraction of Zhenjiang aromatic vinegar is low-molecular-weight compounds (MW < 3500 Da, account for more than 90% in ethanol-supernatant extraction), and HMW fractions (MW > 3500 Da) account for only a small proportion (less than 10% in ethanol-supernatant extraction). Some studies demonstrate that HMW Maillard colorants solublize better in water than they do in ethanol. Maillard components from coffee were reported to give higher absorbance values in aqueous solutions than they did in ethanol solutions (Del Castillo, Ames, & Gordon, 2002). Content of melanoidins in ethanol precipitation are higher than in ethanol-supernatant extraction, at present we only studied the melanoidins in ethanol-supernatant extraction, future work would also focus on the melanoidins from ethanol precipitate of vinegar.

Various methods have been tried to separate melanoidins, it was showed that vinegar melanoidins could be absorbed onto anionic exchanger, e.g., DEAE-Sepharose fast flow. Ninety milligrams of HMW fractions (MW > 3500 Da) of ZVE were separated by DEAE-Sepharose fast flow in water (Pharmacia, Uppsala, Sweden) (Fig. 2). For each vinegar sample, four fractions (1–4) were collected as illustrated in Fig. 2. Fractions 1 contain the effluents of 0.1 mol/L NaCl (F1); fractions 2, fractions 3 contain the effluents of 0.2 mol/L NaCl (F2) and the effluents of 0.3 mol/L NaCl (F3), hereinafter as vinegar melanoidins, and fractions 4 contain the effluents of 0.6 mol/L NaCl (F4).

F2 and F3 have been further tried to be separated by Superdex G75, Superdex G200 or Sephadex G100, it was showed that F2 and F3 are mixture of different molecular weight between 3.5 Da and 100 kDa (elution maps were not shown here). Actually, the determination of melanoidins molecular weight by conventional techniques, such as gel filtration, ultrafiltration, and dialysis, is questionable because of the absence of standards which are structurally similar to melanoidins.

3.2. Wavelength spectra

Wavelength spectra of F2 and F3 are shown as Figs. 3 and 4, respectively, UV–Vis absorption spectra of these two samples were found to have a similar pattern. F2 and F3 exhibited featureless end absorption, becoming more intense as the wavelength decreased. The shape of this UV/VIS spectrum had also previously been found to be a characteristic of melanoidins (Hofmann, 1998).

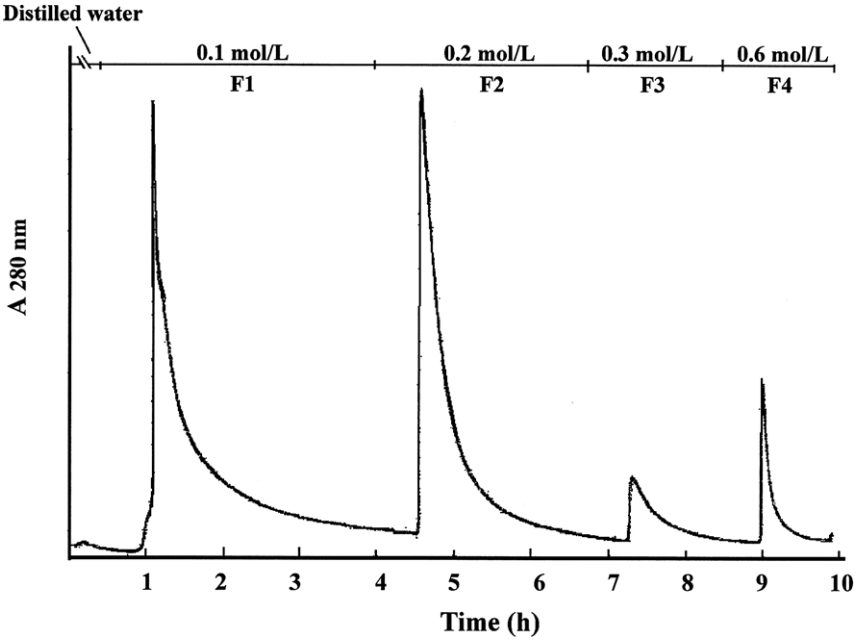

Fig. 2. Separation of high-molecular-weight fractions of Zhenjiang aromatic vinegar extracts (HMW, MW > 3500 Da) by DEAE-Sepharose fast flow.

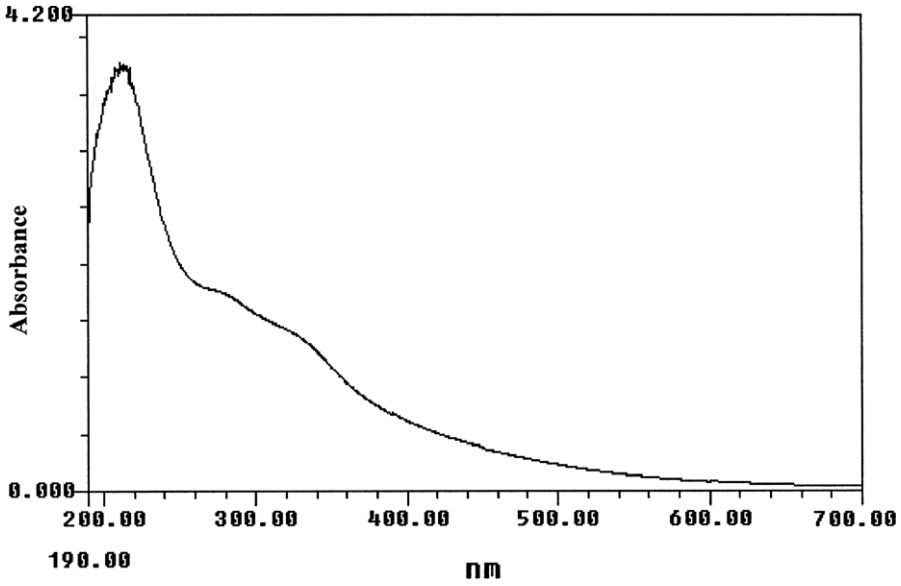

Fig. 3. Wavelength spectrum of F2.

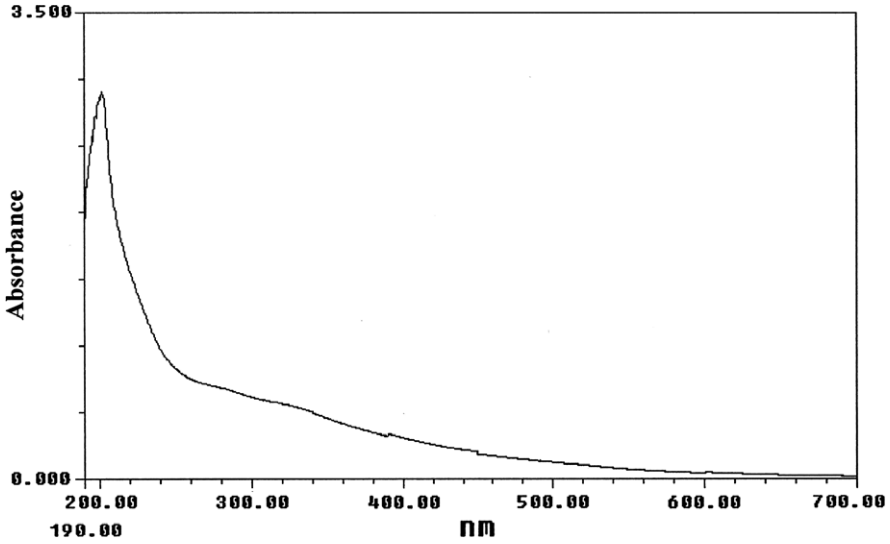

Fig. 4. Wavelength spectrum of F3.

F2 and F3 were brown powder when dried in vacuum, and sponginess when freeze-dried. They can dissolve in water or 70% (v/v) ethanol, but they can't dissolve in organic solvent such as absolute ethanol, ether, acetone and acetic ester, etc.

Melanoidins are materials formed by interactions between reducing sugars and compounds possessing a free amino group, such as free amino acids and the free amino groups of peptides. The complex network of interactions, resulting in melanoidins, the brown and high-molecular-weight products, as the final reaction products, is commonly referred to as the Maillard reaction products (MRPs). From the characteristic and wavelength spectra scan, F2 and F3 were speculated as MRPs, i.e., vinegar melanoidins.

3.3. Total phenolic content

Total phenolic content of Zhenjiang aromatic vinegar extracts (ZVE) and different fractions of ZVE are shown in Table 1. Total phenolic content of fraction F2, F3 was much higher than that of F1 and F4. F1 was the lowest among the Zhenjiang aromatic vinegar fractions. Total phenolic content of 1 mg of F1, F2, F3 and F4 were 34.36, 98.97, 99.08 and 41.53 μg, respectively, determined as the gallic acid equivalent. Therefore, total phenolic content of F2 and F3 were much higher than that in any other vinegar fractions.

From literature data it is clear that the composition of melanoidins strongly differs with food composition and the technological conditions. Folin–Ciocalten reagent is

Table 1
Total phenolic content in Zhenjiang aromatic vinegar extracts (ZVE) and individual Zhenjiang aromatic vinegar fractions (F1–F4, MW > 3500 Da)

Samples	ZVE	F1	F2	F3	F4
Gallic acid equivalents (μg/mg)	53.7 ± 0.91	34.36 ± 0.82	98.97 ± 1.23	99.08 ± 1.67	41.53 ± 0

All values are presented as the mean ± SD ($n = 3$).

very unstable in basic condition. Phenolic groups deoxidize it easily. During vinegar production it is likely that phenolic compounds, e.g., tyrosine residues that have pheno1ic nature also participate in the reaction, becoming part of the brown, water-soluble polymers called vinegar melanoidins. And the amount of phenolic groups which are incorporated into the brown polymer may have a certain relationship with the antioxidant activity of vinegar melanoidins.

3.4. Reducing power

Methods developed to measure the efficiency of dietary antioxidants were focused on different mechanism of the oxidant defense system, i.e., scavenging active oxygen species and hydroxyl radical, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions. In most cases, irrespective of the stage in the oxidative chain in which the antioxidant action is assessed, most non-enzymatic antioxidant activity (scavenging of free radicals, inhibition of lipid peroxidation, etc.) is mediated by redox reactions. The reducing power of different fractions of HMW in vinegar to reduce ferric ions was determined in this study. The higher amount of reducing power was observed in the F3, and F2 fractions, followed by F4 and F1 fractions (Fig. 5).

The reducing power of each fraction using ascorbic acid and gallic acid as reference compounds were also measured. As is shown in Fig. 5, the reducing power of these extracts was markedly lower than that of ascorbic acid and gallic acid. F3 and F2, which had higher reducing power, had reducing power that was only about 10% that of ascorbic acid and only about 9% that of gallic acid at a concentration of 250 μg/mL. The reducing power of each individual fractions of HMW (F1–F4) in vinegar to reduce ferric ions was lower than ZVE, suggesting that F2 and F3 have weak reducing power and they are not main responsible fractions for vinegar's reducing power.

3.5. DPPH radical scavenging activity

This method is based on the reduction of DPPH, a stable free radical. Because of the odd electron of DPPH, it gives a strong absorption maximum at 517 nm (purple color). Antioxidant reacts with DPPH, and the odd electron of the radical is paired off. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. This reaction has been widely used to test the ability of compounds to act as free radical scavengers and to evaluate the antioxidant activity of foods and plant extracts. DPPH radical scavenging activity of ZVE, as well as that of all four fractions, was dose-dependent (Fig. 6). The highest DPPH radical scavenging activity was shown by F2 and F3, followed by F4. Fractions F2 and F3 have a DPPH radical scavenging activity about 30% at a concentration of 50 μg/mL, close to 5 μg/mL ascorbic acid or gallic acid. When at 150 μg/mL, both F2 and F3 have DPPH radical scavenging activity about 70%. The lowest DPPH radical scavenging activity was shown by F1, which is only about 21% when at 150 μg/mL. Interestingly, the F2 and F3 fractions also contained the highest phenolic content, with the F4 the second highest, and F1 the lowest in phenolic content (Table 1). Given the above, we suggest that there may be a relationship between the total phenolic content and DPPH radical scavenging activity or the DPPH radical scavenging activity may have a relationship with the phenolic groups bounded to melanoidins.

3.6. Inhibition of hydroxyl radical

The hydroxyl radical is a powerful oxidizing agent, and by virtue of this characteristic, it is considered to be highly toxic in biological systems. This radical has the capacity to

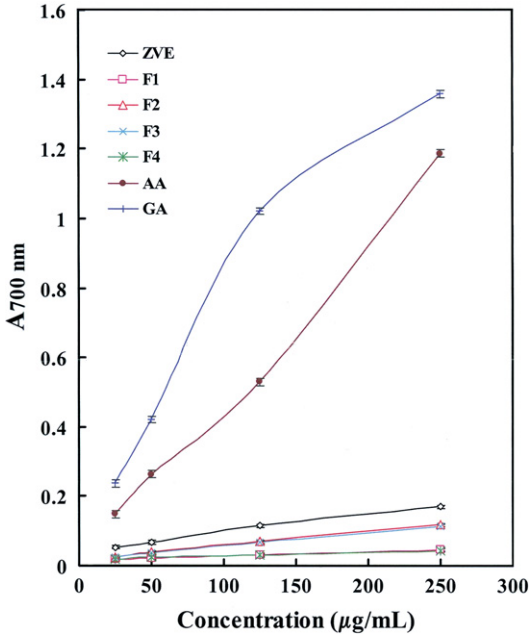

Fig. 5. Reducing power of Zhenjiang aromatic vinegar extracts (ZVE) and individual Zhenjiang aromatic vinegar fractions (F1–F4, MW > 3500 Da). Ascorbic acid (AA) and gallic acid (GA) were used as positive controls. All values are presented as the mean ± SD ($n = 3$).

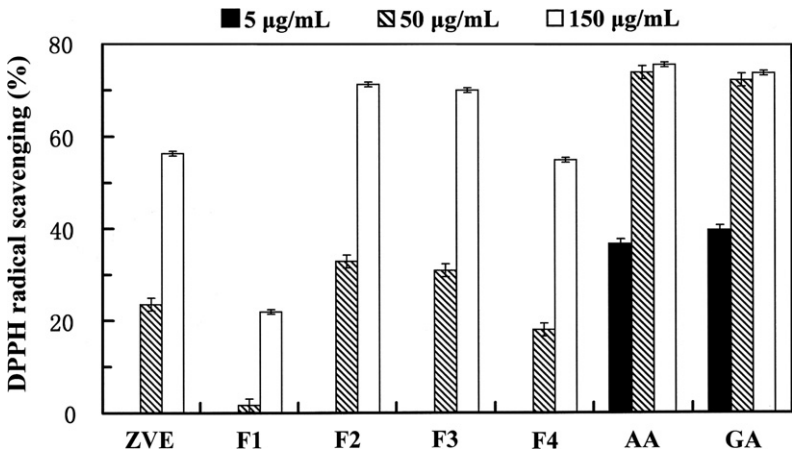

Fig. 6. DPPH radical scavenging activity of Zhenjiang aromatic vinegar extracts (ZVE) and individual Zhenjiang aromatic vinegar fractions (F1–F4, MW > 3500 Da). Ascorbic acid (AA) and gallic acid (GA) were used as positive controls. All values are presented as the mean ± SD ($n = 3$).

join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this species is considered to be one of the quick initiators of the lipid peroxidation process. Radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA model. The hydroxyl radicals formed by the oxidation react with dimethyl sulphoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent. The hydroxyl radical scavenging activity of ZVE and its fractions is shown in Fig. 7. Inhibition of ·OH of individual fractions of vinegar melanoidins (MW > 3500 Da), were dose-dependent (Fig. 7). The inhibition rate of ·OH of F1, F2, F3 and F4 was 23.74%, 18.97%, 11.31% and 9.18%, respectively, at a concentration of 60 μg/mL. Distinct from the result of DPPH radical scavenging activity and phenolic content, F2 and F3 didn't have the highest inhibition rate on ·OH. The inhibition rate of ·OH of ZVE was 8.72% at a concentration of 100 μg/mL, and 16.95% at a concentration of 200 μg/mL. The hydroxyl radical inhibition of ZVE couldn't be detected at low concentration, e.g., at 20 μg/mL or 60 μg/mL. Hydroxyl radical inhibition of ZVE and individual fractions tested for different times were not repeated well which may be due to the extreme instability of ·OH.

3.7. Influence of decoction on vinegar's DPPH radical scavenging activity

Vinegar is a mixture of numerous chemicals, including proteins, amino acids, carbohydrates, organic acids, lipids vitamins, and minerals, etc. It is well known that heat treatment promotes complex chemical reactions among food components. Among the many reactions occurring in processed foods, the non-enzymatic browning reaction or the Maillard reaction plays the most important role in the formation of various chemical.

Two phases of Zhenjiang aromatic vinegar production, decoction, storing and aging, which may have important influence on Maillard reactions, were examined. Decoction is the process of heating the vinegar under normal pressure. Nine vinegar samples before and after decoction were detected. Total phenolic content were lower after decoction. But total phenolic content were not significantly different from that of undecocted vinegar.

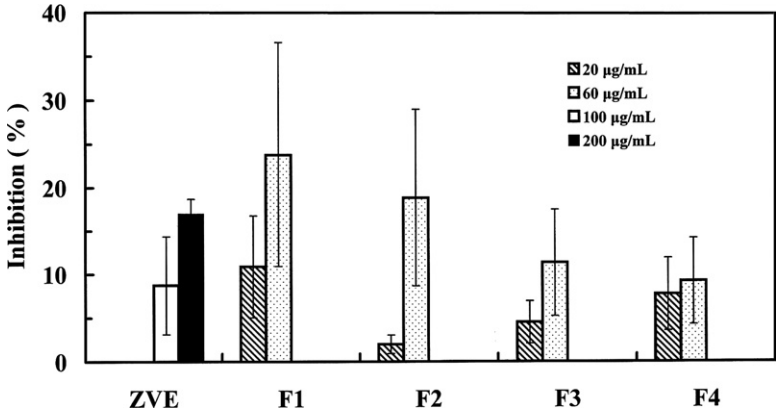

Fig. 7. Inhibition of ·OH of Zhenjiang aromatic vinegar extracts (ZVE) and individual Zhenjiang aromatic vinegar fractions (F1–F4, MW > 3500 Da). All values are presented as the mean ± SD ($n = 3$).

Influence of decoction on DPPH radical scavenging activity was determined. Table 2 shows that DPPH radical scavenging activity was higher after decoction. DPPH radical scavenging activity was significantly different from that of undecocted vinegar at 2.5 μL sample.

3.8. Influence of storing and aging on vinegar's DPPH radical scavenging activity

Storing and aging for Zhenjiang aromatic vinegar has two phases. When acetic fermentation is finished, vinegar should be sealed up for keeping for a period before vinegar drench. And finished-vinegar should also be sealed up until the consumer opens the package.

The first phase, lasted for about 20 days, doesn't have any significant influence on total phenolic content and DPPH radical scavenging activity of vinegar (Xu et al., 2005b). The second phase may last for several years, and it does have any significant influence on vinegar components.

Influence of storing and aging on DPPH radical scavenging activity of vinegar is shown in Table 3. Two-years-vinegar has higher DPPH radical scavenging activity than the fresh-made product. Total phenolic content of two-years-vinegar was not significantly different from fresh-made vinegar. DPPH radical scavenging activity of two-years-vinegar was significantly different from that of fresh-made vinegar at 5 μL sample.

The Maillard reaction is all-pervasive in food. It is most closely associated with foods which have been exposed to heat during industrial or domestic processing, though it also plays a role during storage, when foods are kept at ambient or even sub-ambient temperatures, but for extended periods. Most of the natural antioxidants present in foods are affected by oxidative stress promoted by processing and preservation methods (Anese, Manzocco, Nicoli, & Lerici, 1999; Ewold, Fjelkner-Modig, Johansson, Sjőholm, & Akesson, 1999). However, depletion of naturally occurring antioxidants of a processed food could be counteracted by the formation of novel compounds with antioxidant activity (Nicoli, Anese, & Parpinel, 1999). During decoction and storing and aging, the color of vinegar become darker and darker, while these two stages also have influence on antioxidant activity of Zhenjiang aromatic vinegar. Since vinegar has been sterilized by decocting under high temperature, new reaction products are not hosted by microbe.

In addition, storing and aging affect the content of tetramethylpyrazine (He et al., 2004), a kind of intermediate MRPs, suggesting that some kind of antioxidant activity compounds, e.g., vinegar melanoindins formed during these periods. Then, by optimizing the industrial processing conditions, it could be possible to keep the overall antioxidant activity by enhancing the formation of certain Maillard reaction products (MRPs) with antioxidant properties. Knowledge of the role of melanoidins in the prevention of lipid oxidation is limited, but they may act as other antioxidants at different levels in the oxidative sequence (Morales & Jiménez-Pérez, 2001, 2004), in a similar way to polyphenols.

Using DPPH radical scavenging activity, total phenolic content as indices, different batches of fresh-made-vinegar were compared, vinegar samples were not significantly different for various batches.

In summary, our observations demonstrate that Zhenjiang aromatic vinegar extracts (ZVE), and individual fractions of ZVE, vinegar melanoidins have DPPH radical scavenging activity, reducing power, and hydroxyl radical scavenging activity. Among the four Zhenjiang aromatic vinegar fractions, F2 and F3 exhibited the strongest antioxidant activity in DPPH system and reducing power system. ZVE and its four fractions have hydroxyl radical scavenging activity. F1 has the highest hydroxyl radical scavenging activity. F2 and F3 are speculated as Maillard reaction products, melanoidins. Our study demonstrated that vinegar melanoidins are important for antiradical properties

Table 2
Effects of decoction on total phenolic content and DPPH radical scavenging activity

Samples	Undecocted vinegar	Decocted vinegar
Total phenolic content (gallic acid equivalents mg/mL)	2.939 ± 0.294	2.927 ± 0.273
% DPPH radical scavenging activity of 2.5 μL sample	24.37 ± 3.46	27.25 ± 3.18[a]
% DPPH radical scavenging activity of 5 μL sample	47.70 ± 8.16	51.66 ± 1.50

All values are presented as the mean ± SD ($n = 9$).
[a] Student's t-test: $P < 0.05$, decocted vinegar *vs* undecocted vinegar.

Table 3
Changes of total phenolic content and DPPH radical scavenging activity during storing and aging

Samples	Fresh-made vinegar	Two-years-vinegar
Total phenolic content (gallic acid equivalents mg/mL)	3.944 ± 0.578	4.145 ± 0.233
% DPPH radical scavenging activity of 2.5 μL sample	37.59 ± 5.74	44.69 ± 5.71
% DPPH radical scavenging activity of 5 μL sample	66.36 ± 4.22	72.82 ± 1.91[a]

All values are presented as the mean ± SD ($n = 5$ or 6).
[a] Student's t-test: $P < 0.05$, two-years-vinegar *vs* fresh-made vinegar.

of vinegar. Future experiments should aim at purifying and characterizing the vinegar melanoidins that are responsible for the relatively high antioxidant activity of high-molecular-weight fractions.

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