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Research Article

Study on the Fluorescence Spectra Characteristics of Vinegar-Water Solutions

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Fluorescence spectra and polarization spectra of vinegar-water solutions with different concentration of CH_3COOH have been studied. The characteristics and mechanism of fluorescence spectra are discussed, and polarization degree is calculated. Vinegar-water solutions are excited by ultraviolet (UV) light at 380 nm. The characteristic fluorescence peaks of the solution were identified at 445 nm and 470 nm. The type of emission fluorescence is $\pi^* \rightarrow$ n. With the increasing concentration of CH_3COOH/pH value, the peak intensity is enhanced first, and then fluorescence quenching occurs. The polarization degree confirms the molecular orientation of different sample solutions. This research provides theoretical and experimental basis for the physical/chemical properties and quality of vinegar detection by florescence spectroscopy.

1. Introduction

Although it is one of the most important sour fermented liquid seasonings, mature vinegar can be used for medicinal purposes. It is helpful to reduce the risks of hypertension, hepatitis, and skin disease. However, in recent years, excessive additives and industrial acetic acid are added into vinegar in order to lower the cost of production. It is known that ingesting excess additives or inedible industrial acetic acid would cause physical harm to the human body [1, 2]. More seriously, long-term ingestion could cause major damage to health. Therefore, in order to ensure the quality of vinegar, studies of vinegar safety are necessary to be carried out. Numerous scientific researches on vinegar have been done in recent years [3–6]. With the combination of factor analysis of multiplex fluorescence in parallel, Callejón et al. classified different Shirley vinegar. Their method demonstrates a good way to extract relevant chemical information about the vinegars as well as classify and discriminate them considering the different ageing [3]. Wu et al. studied the biodiversity of lactic acid and acetic acid bacteria in the process of Shanxi mature vinegar's fermentation, and they found *Acetobacter pasteurianus* showing great variety in both genotypic and phenotypic tests [4]. Nagashima carried out comprehensive analysis on the antioxidant activity of a new type of black vinegar named "IZUMI." The results suggest that "IZUMI" increases antioxidant activity and reduces oxidative stress and blood filtration time in female subjects [5]. Wang et al. compared and determined the acetic acid of plum vinegar via visible/near infrared (Vis/NIR) spectroscopy and multivariate calibration; the results indicated that Vis/NIR spectroscopy combined with chemometrics could be used as an inexpensive and efficient way for the determination of acetic acid of plum vinegar [6]. All these studies have built a good foundation for the development of vinegar safety. However, there are a few quantitative researches on the fluorescence spectral characteristics and group structure of vinegar that have extensive application for scientific research and engineering practices of vinegar.

Fluorescence spectrometry is a detection method which is convenient, quick, and accurate. After excitation by ultraviolet or visible light, substances such as amidogen or hydroxyl would generate fluorescent light, which can reveal specific characteristics of the material. Fluorescence spectrometry can carry out the qualitative and quantitative analysis of the fluorescence. It is one of the most effective ways to study molecular structure and structural features [7]. For example,

Guzmán et al. evaluated the quality of olive oil via fluorescence analysis, and the results presented demonstrate the ability of the fluorescence technique to characterize olive oils on the basis of all the quality parameters studied [8]. Maki et al. used perylene diimide and nitrogen oxides (PBILN) body as fluorescence reagent, with flow injection fluorescence method to measure ascorbic acid. The analytical results were in good agreement with those obtained using a conventional method [9]. Iizuka et al. performed fluorescence determination of melatonin by high-performance liquid chromatography (HPLC), and they obtained the structure of fluorescent products of melatonin [10]. Zhang et al. classified phytoplankton with a fluorescent method, and the sinusoidal-amplitude-modulation (SAM) technique was used in this method. Results revealed that this method has advantages of better detection limit and shorter detection time compared with the existing method [11]. Zhao and Li performed colorimetry and fluorescent detection of anionic surface active agent with water soluble sensors. The study found that the detection has the advantage of visual detection and high selectivity and sensitivity [12]. But so far, fluorescence spectrum analysis of vinegar has not attracted enough attention due to the complex ingredient of vinegar. Studies about fluorescence spectra of vinegar are rarely reported.

In this study, vinegar-water solutions emit fluorescence with ultraviolet excitation. The spectral properties and fluorescence changing rules of those vinegar-water solutions are studied. The relationship between fluorescence spectra of different samples and their molecular clusters is also explored. Their characteristics are explained with polarized spectra of 0° and 90°. The experimental results provide references for future studies about physical/chemical properties and cluster structures of vinegar.

2. Materials and Methods

2.1. Sample Preparation. The experimental samples of vinegar solutions were produced by Hengshun Vinegar Industry Co., Ltd. The ingredients of vinegar are water, sorghum, barley, and peas. The vinegar was diluted with distilled (DI) water ($\rho > 0.5$ MΩ·cm) into different samples with volume percentage of 1%~10% (the step is 1%). According to the label of vinegar, the concentration of the acid is ~45 g/L; thus, the acetic acid concentrations of each sample were figured out in the range of 0.45~4.5 g/L (the step is 0.45). Samples were stored at room temperature.

2.2. Experimental Apparatus and Method. A steady-state and time-resolved fluorescence spectrometer FLS920 (The Edinburgh Instruments Co., Ltd) was used in the experiment, wherein the light source power of the steady-state spectrum measurement is a Xenon lamp of 450 W. The tunable wavelength range is 200~900 nm, and there are Glan Thompson prism instruments with the polarizer and analyzer in the excitation incident light path and the fluorescence emission light path, respectively, which can be manually set angle and detection angle. The instrument's parameter settings were set as follows. The slit band width of excitation light was 3 nm. The slit band width of emission light was 1 nm. Correction

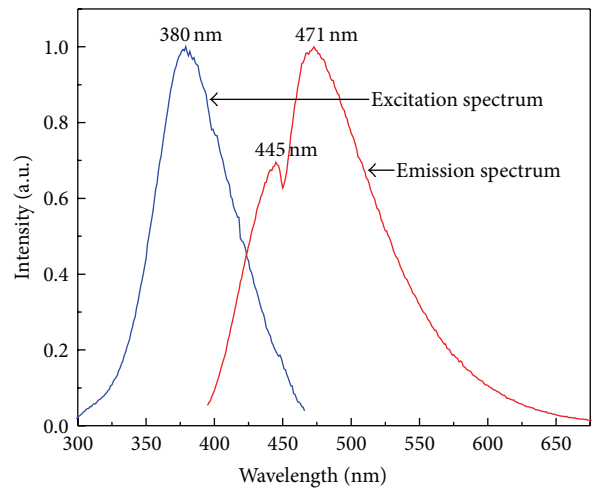

Figure 1: Normalized excitation spectrum and emission spectrum of vinegar-water solutions.

was used to eliminate the detector's error caused by the different time of different excitation light response. The scan time of each wavelength was 0.1 s.

1 mL as-prepared samples were added into the fluorescent quartz cuvettes with a size of 10 mm × 10 mm × 40 mm. In order to ensure the comparability of the results, the parameters of fluorescence spectrum detecting of all samples were the same. The scanning range was 200~700 nm, and the scan time of each wavelength was 0.1 s. Samples were shaken to ensure homogeneity of the solution before each test. All fluorescence detections were performed at room temperature.

3. Results and Discussion

3.1. The Excitation Spectrum and Fluorescence Spectrum of Vinegar-Water Solutions. Figure 1 shows the excitation and emission spectra of vinegar-water solutions. It is found that light with wavelength of about 380 nm is absorbed by vinegar-water solutions. And fluorescence spectrum with a peak at ~ 470 nm is emitted. In order to obtain the best excitation wavelength, multiband excitation spectrum scanning was performed with regard to the fluorescence peak at 470 nm. The scanning range is 300~450 nm, and scanning interval is 1 nm. According to Figure 1, the maximum absorption appears at 380 nm. Thus, the best excitation light wavelength for vinegar-water solutions is 380 nm. The emission spectrum of vinegar-water solutions in Figure 1 is obtained by 380 nm UV light excitation. The peaks in 400~600 nm are the main fluorescence peaks of vinegar-aqueous solutions. The main peak is located at 471 nm, and there is a weaker peak at 445 nm.

According to the excitation and emission spectra of vinegar-water solutions in Figure 1, vinegar-water solutions can absorb photons of UV light at 380 nm and then emit fluorescence. It demonstrates that vinegar-water solutions contain the corresponding absorption structure. In addition to water, the major ingredient of vinegar is acetic acid (CH_3COOH), which contains chromophores of carbonyl

Table 1: The fluorescence intensities and intensities ratio with different concentrations of two peaks. Among them, "a.u." indicates arbitrary units.

Fluorescence intensity/a.u.	Concentration/g/L									
	0.45	0.9	1.35	1.8	2.25	2.7	3.15	3.6	4.05	4.5
I_{445}	50230	71030	67850	60180	47520	40390	31530	25300	20660	14090
I_{470}	62310	90130	91540	83460	59430	59570	49150	40910	33660	24020
I_{470}/I_{445}	1.2405	1.2689	1.3492	1.3868	1.2506	1.4749	1.5588	1.6170	1.6292	1.7048

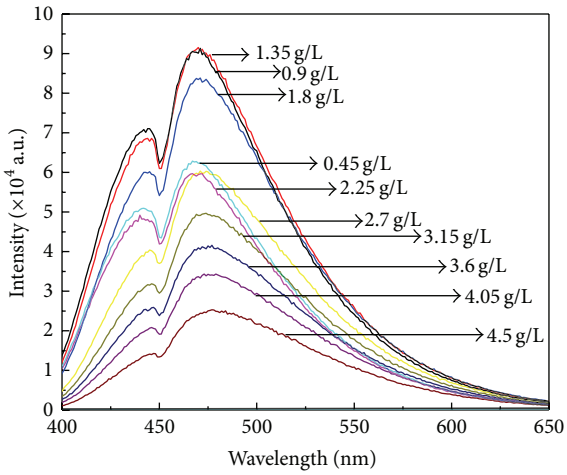

Figure 2: Fluorescence spectra of vinegar-water solutions with different CH_3COOH concentrations.

(C=O) and hydroxyl (–OH). It is known that organic molecules have four electron transition pathways in ultraviolet and visible light range. They are $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, and $\pi \rightarrow \pi^*$. The energy requirement of each transition follows this relationship: $\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$ [13]. The two electron transition ways including $n \rightarrow \sigma^*$ and $\pi \rightarrow \pi^*$ generally absorb light that has a wavelength shorter than 250 nm, while the light scanning range in this study is 350~700 nm. Then, the transition of $n \rightarrow \pi^*$ is detected [14]. At the same time, electron (n) in hydroxyl jumps to the first excited electronic state by absorbing the energy of incident photon, which is the transition way of $n \rightarrow \pi^*$. The two peaks in Figure 1 represent the fluorescence peaks emitted by carbonyl and hydroxyl after absorbing corresponding photons. When an electron jumps from the lowest vibration energy level of excited singlet state back to the ground state, it may transition to many different vibrational and rotational energy levels on the same electron energy level; then, fluorescence with different wavelength is emitted [15]. On the other hand, the thermal radiation effect also affects the fluorescence wavelength; thus, a wide fluorescent band forms in the range of 400~600 nm.

3.2. Fluorescence Spectra of Vinegar-Water Solutions Excited by Light at 380 nm. Figure 2 shows the fluorescence spectra of vinegar-water solutions with different concentration of CH_3COOH excited by UV light at 380 nm. The fluorescent peaks of all samples are in the range of 400~600 nm. The shapes of fluorescence emission spectra are unchanged, and the main peaks are located at ~470 nm. With the increasing concentration of CH_3COOH of the samples, the fluorescence intensity is gradually increasing until reaching a specific CH_3COOH concentration value. When the concentration of CH_3COOH reaches 1.35 g/L, the fluorescence intensity is maximized. However, the continuously increasing concentration of CH_3COOH causes the declination of fluorescence intensity, which is concentration quenching effect. The intensity of minor peak exhibits the same changing trend as the main peak. According to Lambert Beer's law [16], when the concentration of a certain solution is very low, the relationship between absorbance and concentration of the solution follows the following equation:

$$A = kdc, \tag{1}$$

where A is absorbance, k is absorption coefficient, d is optical path, and c is concentration of the solution. The fluorescence emission intensity is proportional to A. Therefore, the fluorescence intensities increase when the CH_3COOH concentrations increase at first. The fluorescence intensities decline when the concentration is higher than 1.35 g/L because of fluorescence quenching effect. The fluorescence quenching effect is caused not only by the enhanced energy dissipation of nonradiation transition, but also by the increased probability of nonradiation transition with increasing concentration [17].

According to the experimental data from Figure 2, we obtain the intensity ratio of two peaks corresponding to each concentration. In Table 1, each I_{470} is higher than the corresponding I_{445}. I_{470}/I_{445} slowly increases with the increasing CH_3COOH concentration at the beginning; when the concentration increases to 2.25 g/L, I_{470}/I_{445} decreases suddenly and continues to increase with the increasing concentration. First of all, sensitivities to the incident light of fluorescent chromophores corresponding to two peaks are different. Thus, the chromophores in solution are more likely to emit fluorescence under the irradiation with the wavelength of 470 nm, and I_{470} of any concentration is higher than the corresponding I_{445}. Secondly, in the early stages of the increasing CH_3COOH concentration, the number of fluorescent chromophores in solution increases, which leads directly to the increasing of I_{470}; thus, I_{470}/I_{445} is enhanced in the beginning. The decreasing of I_{470}/I_{445} when the concentration increases to 2.25 g/L is because the number of fluorescent chromophores increases to a certain amount and the collision probability increases, which promotes the nonradiation transition [18–20]. Therefore, I_{470} decreases, resulting in suddenly declination of I_{470}/I_{445}. After that, I_{470}/I_{445} continues

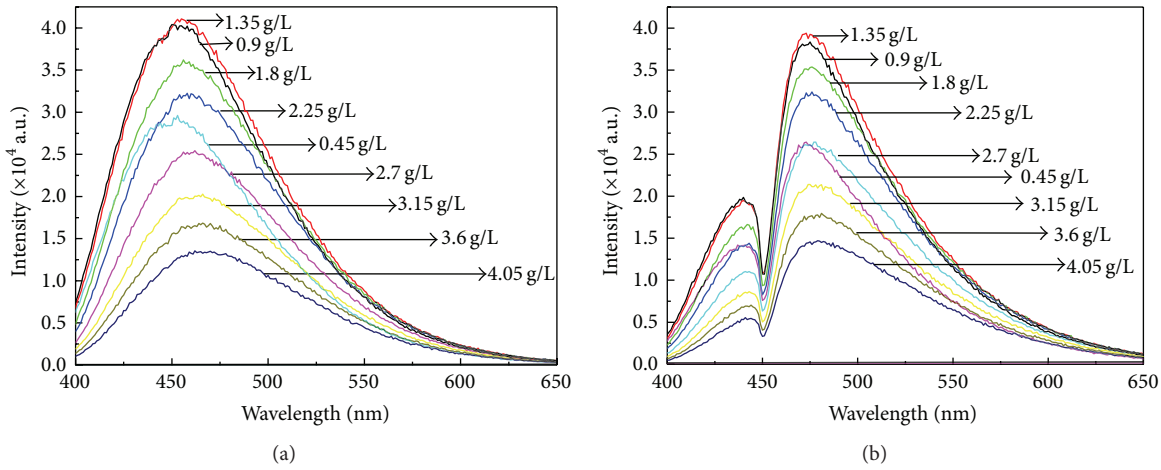

Figure 3: Polarization fluorescence spectra of vinegar-water solutions with different CH_3COOH concentrations: (a) 0° and (b) 90°.

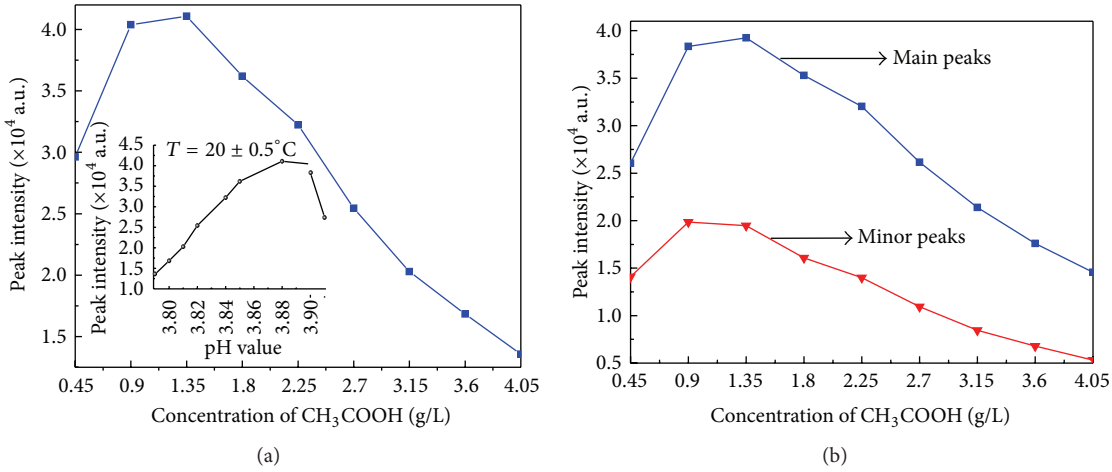

Figure 4: Line chart of polarized fluorescence peak intensities of vinegar-water solutions with different CH_3COOH concentrations: (a) 0° and (b) 90°.

to increase when CH_3COOH concentration increases. The reason is that as the concentration increases, the number of fluorescent chromophores is still growing. At this time, energy loss resulting from collisions between molecules is weaker than fluorescence intensities emitted by lots of fluorescent chromophores. Obviously, the energy loss does not affect the overall enhancement trend of the fluorescence emission intensity; then, I_{470}/I_{445} increases.

3.3. Polarization Fluorescence Spectra of Vinegar-Water Solutions. Figure 3 shows the polarization fluorescence spectra of vinegar-water solutions measured with inspection angles of 0° and 90°. When the solutions are detected with the same inspection angle, the shape and peak positions of each sample have barely changed. The peak in Figure 3(a) detected with 0° inspection angle reveals a peak at ~457 nm. Peaks in Figure 3(b) detected with 90° inspection angle are located at ~440 nm and ~475 nm. The results show that the fluorescence intensity gradually increases with the increasing concentration of CH_3COOH at first. Then, when the concentration reaches a certain value, the fluorescence intensity maximizes. However, the concentration quenching effect appears when the concentration continues to increase. The relationship of peak intensity versus CH_3COOH concentration is presented in Figures 4(a) and 4(b). The peak intensity differences between samples with CH_3COOH concentrations of 0.9 g/L and 1.35 g/L are not obvious. However, the fluorescence peak intensity increases notably when CH_3COOH concentration increases from 0.45 to 0.9 g/L. The peak intensity of other samples gradually decreases, but the attenuation amplitude is smaller than the enhancement. In addition, the relationship figure of peak intensity versus pH change is inserted in Figure 4(a), and the pH values are obtained by measuring vinegar solutions with different CH_3COOH concentrations ranging from 0.45 to 4.05 g/L. Obviously, fluorescence intensity gradually increases with the increasing pH value and reaches a maximum value when the pH value is 3.88. When the pH value reduces, the fluorescence quenching is caused by the proton of the acid group. On the other hand, the coordination ability of the hydrogen bond is strengthened and the solubility

Table 2: The fluorescence peak intensities of vinegar-water solutions with the polarization angle of 0° and 90°.

Fluorescence intensity/a.u.	Polarization angle/°	Concentration/g/L								
		0.45	0.9	1.35	1.8	2.25	2.7	3.15	3.6	4.05
I_{440}	0	28030	37620	37360	31400	27310	20920	15900	13080	9942
	90	14110	19850	19480	16080	14000	10940	8462	6792	5317
I_{475}	0	24270	34760	36040	32630	29810	23910	19440	16530	13080
	90	26050	38350	39250	35300	32030	26140	21390	17600	14570

Table 3: Polarization degree and ratio with different concentrations.

Degree of polarization	Concentration/g/L								
	0.45	0.9	1.35	1.8	2.25	2.7	3.15	3.6	4.05
P_{440}	0.3303	0.3092	0.3146	0.3227	0.3222	0.3132	0.3053	0.3164	0.3031
P_{475}	0.0354	0.0491	0.0426	0.0393	0.0359	0.0446	0.0478	0.0314	0.0539
P_{475}/P_{440}	0.1072	0.1588	0.1354	0.1218	0.1114	0.1424	0.1566	0.0992	0.1778

is reduced when the pH value is low, so the fluorescence intensity is decreased. When the pH value is larger than 3.88, fluorescence quenching happens. It is because that the rise of alkalinity may cause long chain bending, which decreases fluorescence efficiency [21].

Compared with the polarized fluorescence spectrum in the condition of 0°, the spectrum in the condition of 90° has a second peak. This result occurs mainly because when polarized fluorescence excites the fluorescent system, chromophores in sample solutions select light according to the excitation light polarization direction and the relative orientation of itself. In Table 2, when the polarization angle is 0°, I_{440} is weaker than that of 90°. However, I_{475} is stronger than that of 90°. Because the fluorescent molecules in the state of emission at 475 nm are more than fluorescent molecules in the state of emission at 440 nm with the polarization angle of 0°, similarly, fluorescent molecules in the state of emission at 475 nm are less than fluorescent molecules in the state of emission at 440 nm with the polarization angle of 90°. The more the fluorescent molecules in emission state are, the stronger the fluorescence intensity will be emitted. Otherwise, weaker fluorescence intensity will be emitted.

Table 2 exhibits the experimental data of fluorescence peak intensities of vinegar-water solutions detected with two polarization angles. Under the condition of 0°, I_{457} is actually the superposition of I_{440} and I_{475} measured with polarization angle of 90°; then, I_{440} and I_{475} are chosen to perform analysis. In Table 2, it is clearly observed that I_{440} under the condition of 0° is greater than that of 90°, but I_{475} under the condition of 0° is weaker than that of 90°.

From Table 3, polarization degree of each concentration is also calculated according to the theoretical calculation formula of fluorescence polarization:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}. \quad (2)$$

$I_{\parallel}$ is fluorescence polarization peak intensity of 0°. $I_{\perp}$ is fluorescence polarization peak intensity of 90° [22]. Results are displayed in Table 3. P_{440} is larger than P_{475}, and P_{440} is ~10 times larger than P_{475}. Fluorescence polarization sensitivity is related to the collinear degree of excitation torque and emission torque. So intersection angle of absorption transition dipole torque and emission transition dipole torque under the irradiation at 440 nm is smaller than that under the irradiation at 475 nm, which leads to a higher collinear degree and unobvious depolarization effect at 440 nm compared to that at 475 nm.

4. Conclusions

After analysis and discussion, our conclusions are summarized as follows. (i) After being excited by UV light at 380 nm, vinegar-water solutions with different concentrations emit florescence. The fluorescence emission peaks are at 445 nm and 470 nm, and the emission type is $n \rightarrow \pi^*$. (ii) The relationship of fluorescence intensity versus CH_3COOH concentration follows Lambert Beer's law and fluorescence quenching effect. The relationship of fluorescence intensity versus pH values of vinegar-water solutions is related to the proton of the acid group, the coordination ability of the hydrogen bond, and the solubility or the situation of long chain. (iii) The relationship between the number/sensitivity of fluorescent chromophores and their collision probability decides the relationship between I_{445} and I_{470}. (iv) For each concentration of vinegar-water solutions, the intersection angle of absorption transition dipole torque and emission transition dipole torque under the irradiation at 440 nm is smaller than that under the irradiation at 475 nm. This result leads to a higher collinear degree and unobvious depolarization effect at 440 nm compared to that at 475 nm. Hence, the report has a great significance in future studies about physical/chemical properties as well as cluster structures of vinegar.

Competing Interests

The authors declare that they have no competing interests.

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