〔J.Inst.Enol.Vitic.Yamanashi Univ.**26**, 1～4 1991〕

Decomposition of Bound Sulfite into Free Sulfite and Determination of Total Sulfite in Wine Using a Microbial Sensor

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Abstract

Total sulfite in wine was determined by use of a microbial sensor consisting of a sulfur-oxidizing autotrophic bacterium(*Thiobacillus thiooxidans* JCM7814) and an oxygen electrode. Although this microbial sensor selectively detected free sulfite, it could not directly apply to the determination of total sulfite. When the microbial sensor method was applied for the determination of total sulfite, bound sulfite had been previously decomposed into free form by treating with alkaline or acid. By treating wine samples with 4 N KOH for 5 min at room temperature, the microbial sensor could determine total sulfite in wines with similar accuracy to the modified Rankine method. The recoveries of total sulfite were 95.5% and 100% in white wine and red wine, respectively. Good recoveries were attained in the same manner by treatment with heating at 85℃ for 10 min under acid condition of 0.5 N H_2SO_4.

Introduction

Sulfite is traditionally used as a preservative in wine industry to protect wine from undesirable oxidation and microbial spoilage. When sulfite is added to wine, it readily binds with aldehydes, ketones, sugars, pigments, tannins, etc. to form bound sulfite. Such bound sulfite is no more effective as the preservative. Since free sulfite in wine decreases due to the binding reactions with the substances in wine, periodic additions of sulfite are required to maintain the reasonable concentration of free sulfite during fermentation and storage. However, sulfite has harmful effects on health if excessive sulfite is accumulated in wine. To prevent adversary effects of sulfite on health, total sulfite content in wine should be regulated from the view point of food hygiene. Therefore, a simple and accurate method for total sulfite determination has been requiring as well as free sulfite determination.

Some methods including the Rankine (1) and the Monier-Williams (2) have been recommended for the determination of sulfite in wine. These methods are complicated even for skilled workers and require a large amount of samples and a long period of time for analysis. Alternatively, there are a few methods using enzyme such as sulfite oxidase isolated from chicken liver(3) or a microbial sensor consisting of whole cells of *Thiobacillus*

thiooxidans and an oxygen electrode(4,7). The enzymatic method did not provide reliable results because the presence of pigments or ascorbic acid in sample interfered with an accurate quantitation of sulfite. In addition, the enzyme itself is unstable in such sample. The microbial sensor(4,7) is preferable for determination of sulfite due to its specificity to sulfite, stability of the cells, and rapid measurement. The microbial sensor, however, could not apply to total sulfite determination in sample, or it was useful only for measuring free sulfite.

In this study, we have investigated pretreatment methods of wine samples for decomposing bound sulfite into free form in order to apply the microbial sensor to total sulfite determination.

Materials and Methods

Reagents. The following reagents were prepared freshly before use. Standard free sulfite solution: 406.5mg of sodium bisulfite ($NaHSO_3$) was dissolved in distilled water. The solution was then put into a 250ml volumetric flask and filled up to the mark with distilled water. This solution contains 1000 μg/ml of sulfite as SO_2. The solution was properly diluted to prepare lower concentrations of sulfite. Standard bound sulfite solution: 240.8mg of glutaraldehyde sodium bisulfite addition compound (Aldrich Chemical Co. Inc. USA) was dissolved in distilled water and then filled up to 1000ml. This solution contains 100 μg/ml of sulfite as SO_2. Glutaraldehyde sodium bisulfite addition compound was abbreviated to GANa–SO_2.

Alkaline treatment for decomposing bound sulfite. Various concentrations of sodium hydroxide and potassium hydroxide were used as alkaline solutions to decompose bound sulfite. An alkaline solution described by Hamano *et al.* (5) was also prepared as follows. (A) 5% ferrous sulfate solution: 5g of $FeSO_4 \cdot 7H_2O$ was dissolved in 0.01N H_2SO_4 and filled up to 100 ml. (B) Alkaline extractant: 40 g of sodium hydroxide and 25g of potassium sodium tartrate (Rochelle salt) were dissolve in distilled water and filled up to 1000ml. (C Alkaline solution for treatment: 25ml of th solution (A) was added to 50ml of th extractant (B). Hereafter, this mixed alkalin solution (C) is referred to as the Hamano' solution.

Five milliliter of sample solution wa mixed with 7.5ml of alkaline solution or th Hamano's solution described above, and th mixture was left for various period at roo temperature for decomposing bound sulfite.

Acid treatment for decomposing bound sulfi

Bound sulfite was decomposed into fre form by heating under acid condition in th same manner as described by Kawamura *et al.* (6). Samples were heated at 85℃ for 10mi (holding time) under the acid condition of 0. N H_2SO_4 and then cooled down to roo temperature.

Determination of total sulfite. After dec mposing bound sulfite into free sulfite, th free sulfite formed was determined by microbial sensor (SO_2 METER NA–S01 Nakano Vinegar Co. Ltd., Japan). Th microbial sensor consists of a sulfur–oxidizin bacterium, *Thiobacillus thiooxidans* JCM781 and an oxygen electrode. Before samples wer applied to the microbial sensor, the pH of th samples was adjusted to below 2.0 by th addition of H_2SO_4. Further details of this mi robial sensor method were reported in the pr vious papers (6,7). As a comparison, tot sulfite concentration of untreated sample wa determined by the modified Rankine metho (8). The recovery percent of total sulfite by th microbial sensor method represented a relati value regarding the value mesaured by th modified Rankine method as 100%.

Sample. White wine and red wine wer purchased from market.

Results

Alkaline treatment with the Hamano's alkalin solution. Effect of treatments on recoverie

of total sulfite from GANa–SO_2 and wine samples by the microbial sensor are shown in Table 1. All of the three samples were treated with the Hamano's alkaline solution. When GANa–SO_2 sample was prepared in order to obtain 100 μg/ml of sulfite concentration, the recoveries of 80.7% and 94.1% were obtained by 5 and 15 min of treatment, respectively. Even when the samples were treated for 30 min, the recoveries from the wine samples were lower than 80%. These results indicate that the Hamano's alkaline solution was unsuitable to decompose bound sulfite in wine samples by the treatment less than 30 min.

Table 1. Recoveries of total sulfite from standard bound sulfite and wine samples treated with the Hamano's alkaline solution.

Sample	Treatment time [min]	Found SO_2 [μg/ml]	Recovery [%]
GANa-SO_2*1	5	80.7	80.7
	15	94.1	94.1
	30	94.1	94.1
White wine*2	5	49.8	69.8
	15	50.8	71.2
	30	54.7	76.7
Red wine*2	5	94.0	67.6
	15	81.7	58.8
	30	81.7	58.8

*1 Glutaraldehyde sodium bisulfite addition compound. Appiled sulfite content was 100 μg/ml.
*2 Total sulfite content estimated by the modified Rankine method:White wine;71.3 μg/ml, red wine;139 μg/ml.

Determination of total sulfite in wine treated with alkaline solution. To adopt the microbial sensor method as an alternative to the modified Rankine method, desirable treatment time should be less than 5 min. We investigated the composition of alkaline solution to improve the recoveries of total sulfite from wine. Table 2 shows the recoveries of total sulfite from white wine by the microbial sensor. All of the treatments were conducted for 5 min at room temperature. As can be seen in Table 2, alkaline solution of KOH was more effective to recover total sulfite than NaOH. When 4 N KOH was used in the treatment, the most favorable recovery of 95.5% was obtained. Addition of Rochelle salt to 4 N NaOH slightly improved the recovery, but the addition of the salt to 4 N KOH gave a negative effect to the recovery.

Table 2. Effect of composition of alkaline solution on recoveries of total sulfite from white wine*.

Alkaline solution	Found SO_2 [μg/ml]	Recovery [%]
Hamano's alkaline	70.7	61.5
1N NaOH	96.0	83.5
1N KOH	101.8	88.4
4N NaOH	96.0	83.5
4N KOH	109.8	95.5
4N NaOH + Rochelle salt	104.5	90.9
4N KOH + Rochelle salt	93.7	81.5

* Total sulfite content in white wine estimated by the modified Rankine method was 115 μg/ml.

Red wine was also treated with 4 N KOH for 5 min at room temperature, and then total sulfite was measured by the microbial sensor. The recoveries of total sulfite from wine samples were summarized in Table 3. The measured values of total sulfite concentration in wine samples by the two methods almost agreed.

Table 3. Determination of total sulfite in wine treated with alkaline of 4N KOH.

Sample	Found SO_2 [μg/ml]		Recovery [%]
	Rankine*	Sensor	
White wine	115.0	109.8	95.5
Red wine	69.0	69.3	100.4

* The sample was analyzed by the modified Rankine method without alkaline treatment by 4N KOH.

Determination of total sulfite in wine treated with heating under acid condition. Total sulfite in wine samples were decomposed into free form by acid treatment with heating(6), and then determined by the microbial sensor. As shown in Table 4, the recoveries were 104.2 % and 94.3 % in white wine and red wine, respectively. The acid treatment with heating, as well as the alkaline treatment with 4 N

Table 4. Determination of total sulfite from wine treated with heating at 85℃ under acid condition of 0.5N H_2SO_4.

Sample	Found SO_2 [μg/ml]		Recovery [%]
	Rankine*	Sensor	
White wine	120.0	125.0	104.2
Red wine	173.2	163.3	94.3

* The sample was analyzed by the modified Rankine method without acid treatment by 0.5N H_2SO_4.

KOH at room temperature, allowed the microbial sensor to determine total sulfite with good recovery.

Discussion

It was found that either alkaline or acid treatment was effective to decompose bound sulfite in wine samples. Although the time requiring for alkaline treatment was shorter than acid treatment, the pH of the alkaline treated sample had to be adjusted to below 2.0 before the measurement by the microbial sensor. The acid treatment did not need the pH adjustment before measurement, while heating and cooling operations were necessary. Employing any treatment way, total sulfite in wine was detectable by the microbial sensor in similar accuracy to the modified Rankine method.

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