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Sulfite Determination by a Biosensor Based on Bay Leaf Tissue Homogenate: Very Simple and Economical Method

Mustafa Teke
Muğla University, Science and Art Faculty, Chemistry Department, Muğla Turkey

Mustafa Kemal Sezgintürk and Erhan Dinçkaya
Ege University, Faculty of Science, Biochemistry Department, Bornova-Izmir, Turkey

Abstract: Of all the food additives for which the FDA has received adverse reaction reports, the ones that most closely resemble true allergens are sulfur-based preservatives. Sulfites are used primarily as antioxidants to prevent or reduce discoloration of light-colored fruits and vegetables, such as dried apples and potatoes, and to inhibit the growth of microorganisms in fermented foods such as wine. This work aims to prepare an electrochemical biosensor based on bay leaf tissue homogenate that contains polyphenol oxidase enzyme abundantly for sulfite detection in foods. The principle of the biosensor is based on the inhibition effect of sulfites on polyphenol oxidase in the bioactive layer. Optimum conditions for the biosensor, such as temperature and pH, were investigated. Some stability parameters of the biosensor were also identified. The biosensor showed a linear calibration graph in the range of 25–100 μM sulfite. The biosensor presents a very simple, economical, reliable, and feasible method for sulfite detection in foods.

Keywords: Sulfite, food additives, biosensor, allergic additives

INTRODUCTION

Sulfites are a group of sulfur-based compounds that may occur naturally or may be added to food as an enhancer and preservative. The FDA estimates that one out of 100 people is sensitive to the compounds [1]. A person can develop sensitivity to sulfites at any time in life, and the cause of sensitivity is unknown. For a person who is sensitive to sulfites, a reaction can be mild or life-threatening. In 1986, the FDA banned the use of sulfites on fruits and vegetables that are eaten raw, such as lettuce or apples [2]. Regulations also require manufacturers who use sulfites in their processed products to list the compounds on their product labels. Although sulfites are no longer used on most fresh foods, they still can be found in a variety of cooked and processed foods [3]. They also occur naturally in the process of making wine and beer. Avoiding foods that contain or are likely to contain sulfites is the only way to prevent a reaction. If you are sensitive to sulfites, be sure to read the labels on all food items. When eating out, ask the chef or server if sulfites are used or added to food before or during preparation. Though most people don't have a problem with sulfites, they are a hazard of unpredictable severity to people, particularly asthmatics, who are sensitive to these substances. FDA uses the term "allergic-type responses" to describe the range of symptoms suffered by these individuals after eating sulfite-treated foods. Responses range from mild to life-threatening [4].

Because of the importance of sulfite, many alternative methods have been proposed. A direct iodometric titration, which was open to interferences from iodine-reactive compounds, was proposed [5]. A Colorimetric detection has extensively been used [6–8]. Electrochemical-based systems were also constructed for the detection of sulfite [9–11]. One of the most important drawbacks of these methods was the need for a pretreatment of the sample before sulfite determination. However, some methods have been reported that showed good and improved sample throughput [12–20].

The aim of the work reported in this paper is to develop an amperometric inhibitor biosensor based on bay leaf (*Laurus nobilis* L.) tissue homogenate for quantifying sulfite in foods. Bay leaf tissue homogenate

Address correspondence to: Mustafa Teke, PhD., Muğla University, Faculty of Science and Art, Chemistry Department, 48000, Muğla, Turkey. E-mail: mteke@mu.edu.tr

contains polyphenol oxidase enzyme abundantly. Polyphenol oxidase (tyrosinase) is a bifunctional, copper-containing oxidase having both catecholase and cresolase activity. Polyphenol oxidase (PPO) enzymes catalyze the *o*-hydroxylation of monophenols to *o*-diphenols. They can also further catalyze the oxidation of *o*-diphenols to produce *o-quinones*. It is the rapid polymerization of *o*-quinones to produce black, brown, or red pigments (polyphenols) that is the cause of fruit browning.

It is well known that sulfites are used to inhibit browning by acting as a reducing agent [21]. This mechanism is based on the inhibition effect of sulfite on polyphenol oxidase [22]. Copper-catalyzed oxidation of catechol by the polyphenol oxidase is retarded in the presence of sulfite. The principle of the measurements was based on the determination of consumed oxygen level by the reaction of polyphenol oxidase in biological material and a decrease in the consumed oxygen level related to the sulfite concentration injected to the reaction cell.

Measurements were carried out by standard curves that were obtained by the determination of decrease in the consumed oxygen level related to sulfite concentration.

EXPERIMENTAL

Chemicals

Sodium dihydrogenphosphate (NaH_2PO_4), disodium hydrogenphosphate (Na_2HPO_4), phenol, pyrogallol, naphtol, Tris, HCl, thiourea, and catechol were purchased from E. Merck (Germany). Glutaraldehyde (Grade II, 25% aqueous solution), gelatin (type 3, 225 Bloom), were purchased from Sigma (St. Louis, USA). Bay leaves (*Laurus nobilis* L.) used in the experiments were collected from a shrub of a certain variety cultivated in a garden of Ege University at Izmir in Turkey. All reagents used were of analytical grades.

Apparatus

YSI 54 A, 57 A model oxygen meters, and YSI 5700 series dissolved oxygen (DO) probes (YSI Co. Inc., Yellow Springs, Ohio, USA) were used. A water bath was used for preparation of bioactive material (Stuart Scientific Linear Shaker bath SBS 35) (UK). All the measurements were carried out of constant temperature using a thermostat (Haake JF, Germany). A magnetic stirrer (IKA-Combimag, RCO) and pH meter with electrode (WTW pH 538, Germany) for preparing buffer solutions were used. The temperature was maintained at constant in the reaction cell by circulating water at appropriate temperature around the cell compartment during the experiment.

Preparation of the Bioactive Layer Material

One hundred milligrams of Bay leaves were weighed and homogenized with 1 ml working buffer (0.05 M, pH 8.5, Tris-HCl buffer) in a porcelain bowl. Then 10 mg gelatin was weighed and added to a test tube and Bay leaf tissue homogenate (300 µl) was pipetted into the test tube. The mixture of Bay leaf tissue homogenate and gelatin was incubated at 38°C for 15 min to dissolve the gelatin. Bay leaf tissue homogenate-gelatin mixture (200 µL) for preparation of the biosensor was dispersed over the dissolved oxygen probe membrane surface and allowed to dry at 4°C for 1 h. After that, for crosslinking with glutaraldehyde, the probe carrying bioactive layer was immersed into 2.5% (v/v) glutaraldehyde solution and was allowed to wait for 5 min. Finally, the biosensor was washed with bi-distilled water.

Measurement Procedure

The biosensors based on Bay leaf tissue homogenate were put in to the thermostatic reaction cell containing working buffer (pH 8.5, 0.05 M Tris-HCl buffer) and the magnetic stirrer was fixed at a constant speed. A few minutes later, dissolved oxygen concentration was equilibrated because of the diffusion of dissolved oxygen between the working buffer and dissolved oxygen probe. At this time, catechol was injected into the thermostatic reaction cell. The dissolved oxygen concentration started to decrease and a few minutes later it reached the constant dissolved oxygen concentration due to the enzymatic reaction equilibration below.

$$\text{Catechol} + 1/2O_2 \xrightarrow{\text{Polyphenoloxidase}} \text{o-quinone} + H_2O$$

At this moment, dissolved oxygen concentration was recorded. Then, a sulfite standard or a sample contained sulfite was injected into the reaction cell. Dissolved oxygen concentration started to increase because of the inhibition of the reaction of polyphenol oxidase shown above. Measurements were carried out by monitoring the increase in dissolved oxygen concentration occurring after sulfite injection.

RESULTS AND DISCUSSION

Optimum Temperature for the Biosensor

As known, the activity of biosensors is strongly affected by changes in temperature. Each biosensor works best at an optimum temperature. The activity of biosensors usually decreases away from the optimum values. This is due to the importance of the tertiary structure of the

enzyme in its function. Because of this the optimization studies of temperature were very important. The optimum temperature studied ranged from 20 to 40°C. The results are given in Figure 1.

Above the temperature of 30°C, the activity of the biosensor decreases, as the increasing kinetic energy could negatively cause the tertiary structure of polyphenol oxidase in the bioactive layer of the biosensor. The biosensor signals obtained at 25°C were lower than the signals obtained at 30°C. The difference between two temperatures was determined as 25%. Finally, the optimum temperature for the biosensor was accepted as 30°C.

Optimum pH for the Biosensor

Since the activity of the biosensor was also dependent on pH, the influences of pH on biosensor were investigated from pH 6–9.5. The response of the biosensor increased until pH 8.5 and remainede constant between 8 and 8.5. Figure 2 shows the pH graphs for the biosensor.

As the pH of polyphenol oxidases is around 7–9, the biosensor can be employed to detect the sulfite of different samples [23–26]. Moreover, in pH values above 8.5, the biosensor activity was decreased. This result could be caused by two different possible factors. The first one was the possible inactivation of the enzymes at higher pH values. The other one was the dissociation of phenolic hydroxyl groups at high pH values such as 10.5. The optimum pH of the biosensor was 8.5. Therefore, in subsequent experiments, the pH was adjusted to 8.5 using Tris-HCl buffer.

Calibration Graph

A linear relationship between sulfite concentration and change of dissolved oxygen concentration was observed in the range of 25–200 μM sulfite solution. The detection limit of the sensor is 25 μM with a correlation coefficient of 0.9905. The calibration graph is shown in Figure 3.

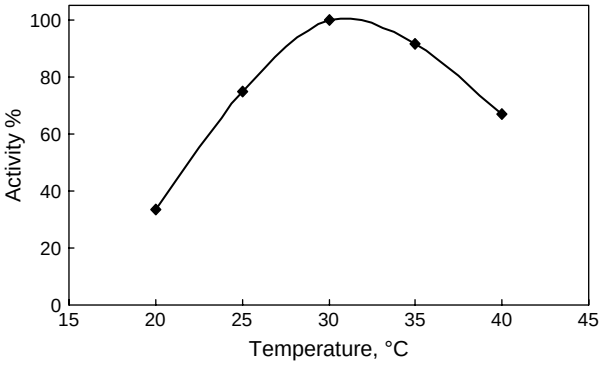

Figure 1. The effect of temperature on the biosensor response. [Tris-HCl buffer, 50 mM, pH 8.5. Catechol and sulfite concentrations were injected to a final concentrations of 200 μM. The response obtained at temperature of 30°C was set to 100%].

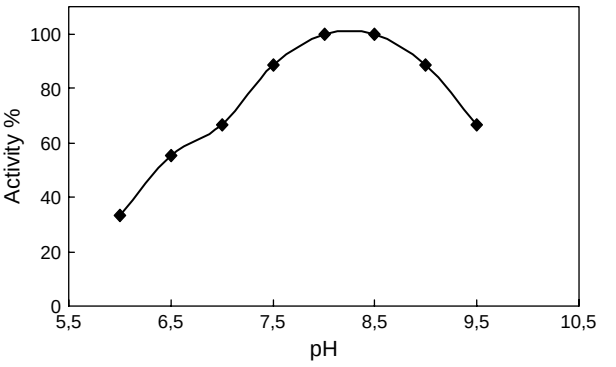

Figure 2. The effect of pH on the biosensor response. [Tris-HCl buffers, 50 mM and pH 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5. Catechol and sulfite concentrations were injected to a final concentrations of 200 μM. The activity at pH 8.5 was set to 100% in each buffer, *T*:30°C.].

Temperature Stability of the Biosensor

Thermal stability of the immobilized bay leaf tissue homogenate system was studied using a 50 mM Tris-HCl buffer solution of pH 8.5 and exposing the biosensor to the temperature of 35°C for various periods. The results are given in Figure 4.

As can be shown from the figure, the biosensor signal decreased with the increasing incubation period. At the end of the fifth hour, the biosensor showed 70.6% of its initial activity. This result was very good for a biosensor based on plant tissue homogenate.

pH Stability of the Biosensor

The pH stability is one of the most important properties of any immobilized enzyme in biosensors. For investigation

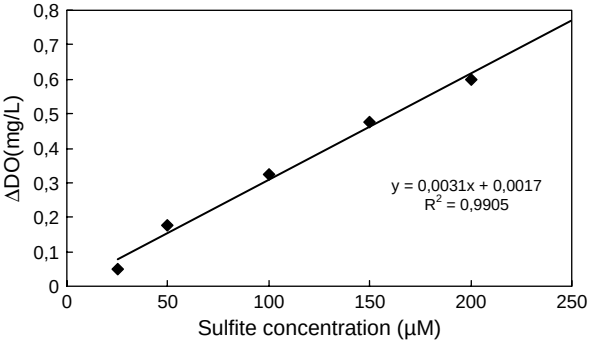

Figure 3. Calibration curve for sulfite. [Measurements were done in Tris-HCl buffer, 50 mM, pH 8.5, 35°C. Catechol concentration was used as 200 μM].

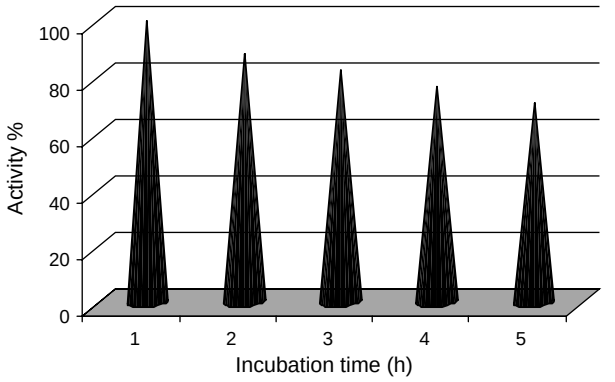

Figure 4. Thermal stability of the biosensor. [Measurements were done in Tris-HCl buffer, 50 mM, pH 8.5, 35°C. Catechol and sulfite concentrations were used as 200 μM].

of pH stabilities of the biosensor, it was incubated in pH 8.5, 50 mM Tris-HCl buffer for 5 hours at 4 °C.

According to the optimum pH study, the biosensor activity is optimum at pH 8.5. The stability of the biosensor was very good at the end of the 5-hour incubation period at pH 8.5, 50 mM Tris-HCl buffer. The biosensor lost 11.8% of its initial activity. The results are given in Figure 5.

Operational Stability of the Biosensor

For the quantification of operational stability at fixed temperature, 15 measurements were made consecutively. From the plot of percentage residual activity versus measurements number, the lifetime of the biosensor based on plant tissue was excellent. This plot is given in Figure 6.

As is obvious from the figure, the stability of the biosensor was the same as the initial at the end of the fifth measurement. Moreover, at the end of the 11th measurement, the biosensor retained 94% of its initial activity.

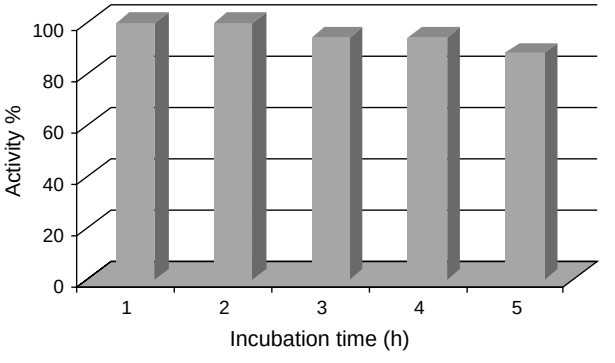

Figure 5. pH stability of the biosensor. [Measurements were done in Tris-HCl buffer, 50 mM, pH 8.5, 4°C. Catechol and sulfite concentrations were used as 200 μM].

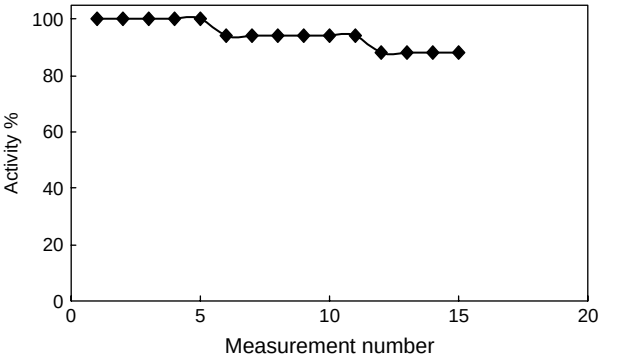

Figure 6. Operational stability of the biosensor. [Working conditions: Catechol and sulfite concentrations were injected to a final concentration of 200 μM. Measurements were done in Tris-HCl buffer, 50 mM, pH 8.5, 35°C.]

Sample Analysis

It was also demonstrated that pickle water analysis could be made without sample preparation or extraction step. Pickle samples were chosen as real samples because, in Turkey, the sulfites are used in this industry routinely.

The results of the developed method were in good agreement with those of the reference method. These results indicate that the proposed biosensor can be applied successfully for the determination of sulfite in food samples. The results are shown in Table 1.

CONCLUSIONS

In conclusion, the results presented in this work demonstrate that plant tissue homogenate biosensor, based on Bay leaf *(Lauris nobilis L.)*, is achievable for the detection of sulfite. The biosensor is readily prepared from inexpensive and commercially available materials through a general method. The biosensors have the good analytical properties such as fast response, long-time stability, and a good detection range. These advantages proposed that the biosensor based on sulfite oxidase could be applied successfully for determination of sulfite in foods.

Table 1. Sulfite analysis in some real samples by the biosensor and by the enzymatic reference method [27]

	Found sulfite (mg/ml)		
Sample	Reference[a]	Biosensor	% Relative error
Pickle water 1	1.52	1.6	+5.2
Pickle water 2	1.61	1.6	−0.6
Pickle water 3	1.57	1.6	+1.9

[a]Mean of three determinations.

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