Evaluation of antioxygenic property of honey based on electrochemical sensing method

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Abstract. Antioxidant capacity of antioxidants is very important in food science. In this paper, we proposed a fast and low-cost antioxidant capacity evaluation method based on an electrochemical detection platform. Silver ions and chitosan were used to synthesize a hydrogel as the electrochemical detection platform. The depolymerization of hydrogel caused by Fenton solution can be hindered by the antioxidants. Therefore, the electrochemical signals of silver ions can be used to measure the antioxidant properties of honey. Jujube honey, linden honey, locust honey and wattle honey were submitted to evaluation. Results indicates the antioxidant capacities of the four honeys follows following order: Jujube honey > linden honey > locust honey > wattle honey.

1 Introduction

Natural antioxidants mainly refer to the antioxidants contained in fruits and vegetables. All fruits and vegetables contain very high natural antioxidants, such as vitamin A, C, E, P, polyphenols and so on [1-3]. Studies have shown that natural antioxidants in fruits and vegetables have protective effects. For example, vitamin E and beta-carotene can protect cell membranes; vitamin C can excrete free radicals in cells. Natural antioxidants can help people prevent heart disease, cancer and other diseases, and can enhance brain power, delay aging. Therefore, the total antioxidant capacity (TAC) has become an important index to measure the antioxidant capacity of food, fruit juice and additives [4-6]. But in recent years, many studies have come to the opposite conclusion that excessive intake of antioxidants may bring health risks. For example, Lu and co-workers found that intake of antioxidants has the risk of promoting the development of autoimmune diseases. Although the role of antioxidants has been controversial in academia, whether it is beneficial or harmful to health, there is a wide demand for an efficient method to detect TAC in the fields of nutrition, pharmacology, physiology and food processing.

Nowadays, the conventional detection methods for TAC include: oxygen free radical absorptive capacity test (ORAC method), total oxidant scavenging capacity test (TOSC method), ROO-induced beta-carotene fading method, iron reduction capacity test (FRAP method) and Folin-Ciocalteu colorimetric method [7-9]. These optical detection methods have some insurmountable shortcomings, such as the need for a longer detection time, the need to decolorize the samples in advance, and the lack of anti-interference ability. In contrast, the electrochemical method for TAC detection has become a highly anticipated alternative method because of its simple, highly sensitive, portable and other advantages [10].

Among them, the direct electrode detection method and the chemically modified electrode detection method are susceptible to the interference of other components in complex samples, and are more suitable for evaluating the antioxidant capacity (AC) of a single oxidant. Enzyme method and the latest DNA damage method can effectively solve the interference and provide more sensitive signals, but enzymes and DNA are easy to inactivate, expensive and difficult to be stably immobilized on the surface of the electrode. We recently proposed a screening method for electrochemical oxidants based on polysaccharide-metal ion crosslinked hydrogels. This method combines the depolymerization of polysaccharide-metal crosslinked hydrogels by ROS and the scavenging effect of antioxidants on ROS, and utilizes the difference of electrochemical behavior of metal ions before and after the depolymerization of hydrogels [11,12]. This method can be used for rapid determination of TAC with low cost, easy operation and no need of electrode modification.

In this work, we tested TAC of honey from four different honey source plants using established methods. Silver ions have been used as an electrochemical probe, while chitosan has been used as cross-linked polymer.

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2 Experimental

2.1 Materials

Silver nitrite, chitosan, acetic acid, hydrogen peroxide and potassium ferricyanide were all analytical grade. Four floral sources of honeys include locust honey (Tongren Tang), linden honey (Tongren Tang), jujube honey (Tongren Tang) and wattle honey (Cisheng Tang) were purchased from online shop. Acetic acid has been used as electrode using the measurement.

2.2 Chitosan hydrogel preparation

Chitosan hydrogel has been prepared based on our previous report with a minor modification [11]. More specifically, a certain amount of silver nitride solution (10 mM) was added to 2 ml of a 1 wt% chitosan solution (in 1% acetic acid). After vigorous shaking for 30 s, 0.1 M NaOH was added dropwise until the gelation process was initiated. The pH of the final chitosan-silver ions hydrogel (denoted chitosan hydrogel) was 6.8.

2.3 Chitosan hydrogel depolymerization

Depolymerization was conducted by injecting a certain amount of Fention solution into the chitosan hydrogel. Sonication in a bath was performed for 30 s to accelerate the diffusion process.

2.4 Evaluation of TAC of honey

Electrochemical method has been used for analysing the TAC of the honey. Three electrodes system has been used, where a Pt wire, a Ag/AgCl (3M) and a glassy carbon electrode (GCE) have been used as counter electrode, reference electrode and working electrode, respectively.

Cyclic voltammetry (CV) was conducted from 1.0 V to 0 V with a scan rate of 50 mV/s. Differential pulse voltammetry (DPV) was conducted from 1.0 V to 0 Vwith an amplitude of 0.03 V, a pulse width of 0.05 s, a pulse period of 0.1 s and a quiet time of 5 s. For honey analysis, a certain amount of honey solution has been introduced along with the Fenton solution. The difference of the silver signal between the absence and presence of honey has been used for representing the TAC of honey.

3 Results and discussion

Polysaccharides and metal ions were crosslinked to form hydrogels, which were directly used as electrochemical analysis platform for TAC detection. The detection mechanism is as follows:

(1) Metal ions cannot effectively diffuse to the surface of the electrode when they are crosslinked, thus producing weak electrochemical signals.

(2) Quantitative ROS was added to the hydrogel for oxidative depolymerization, and the fragments of low molecular weight polysaccharides containing metal ions were produced due to the breakage of some polysaccharide chains. This kind of polysaccharide fragments containing metal ions are easy to diffuse to the electrode surface in voltammetry scanning, and then produce strong electrochemical signals.

(3) Quantitative ROS and antioxidants were added to the hydrogel. The depolymerization rate of the hydrogel was slowed down in varying degrees due to the scavenging effect of antioxidants. Therefore, less fragments of polysaccharide containing metal ions diffused to the surface of the electrode in voltammetry scanning, and then the TAC of the antioxidant was introduced by comparing with the electrochemical signals for quantitative analysis.

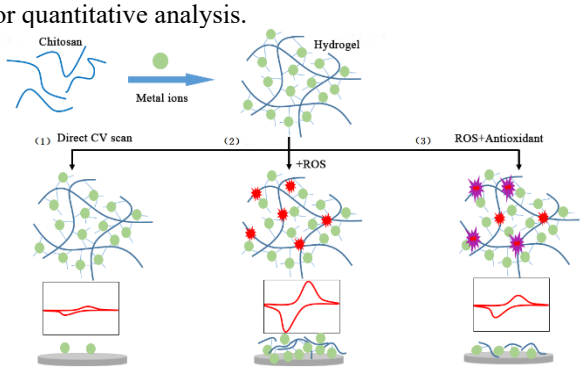

Fig. 1. Schematic diagram of electrochemical detection of polysaccharide metal ion crosslinked hydrogels

Figure 1A displays the SEM observation of silver ions crosslinked chitosan hydrogel. The morphology of the hydrogel exhibits a three-dimensional porous network structure. Figure 1B shows the CV profiles of chitosan and hydrogel. It can be seen that no characteristic peaks can be observed for chitosan solution, suggesting the chitosan shows no electrochemical reaction in the scan range. In contrast, the hydrogel shows a distinct oxidation and reduction peak 0.5 V and 0.3 V, respectively, corresponding to the oxidation and reduction of metallic silver. In this work, the oxidation of silver has been chosen for representing the TAC of honey.

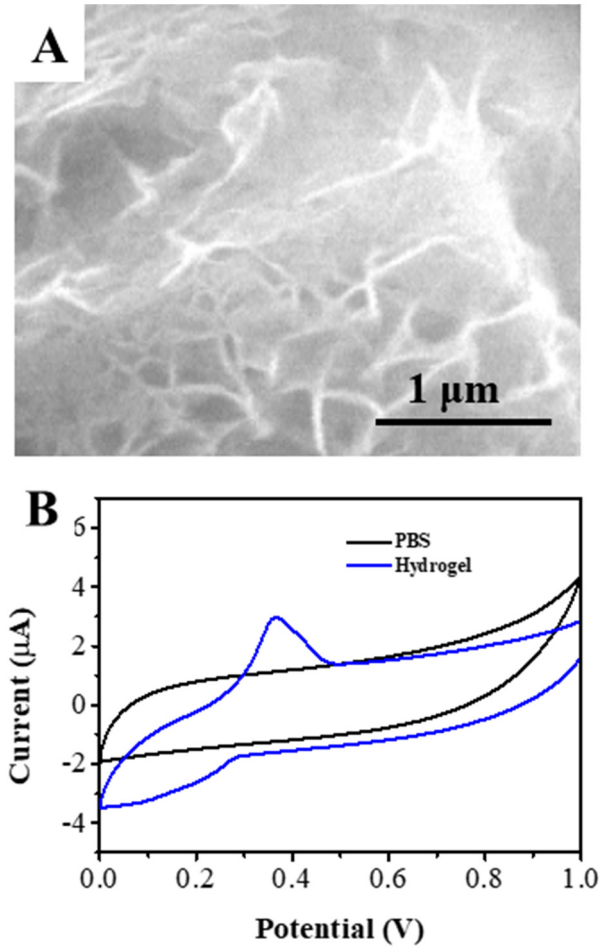

Fig. 2. (A) SEM image of hydrogel. (B) Cyclic voltammetry profiles of chitosan and hydrogel.

Honey is recognized as a natural food with many biological activities. It has been widely used in medical treatment, especially in the prevention and treatment of eye diseases such as apoplexy, burn, cataract, and gastrointestinal diseases. In the past, it was believed that honey's bacteriostatic properties were responsible for the effect of honey on these diseases. In recent years, with the deepening of honey research, it is found that there are a large number of phenolic compounds in honey. For example, sunflower honey from Spain contains camphor quercetin and citrus flavin. A large number of phenolic compounds have also been detected in honey from New Zealand.

Figure 2 displays the CVs of the silver ions linked hydrogel after addition of 0.1 mM Fenton solution in the absence and presence with 50 µL locust honey aqueous solution (1 mg/mL). It can be seen that the silver oxidation peak current in honey added solution is much smaller than the absence solution, indicating locust honey could result in an antioxidant activity to scavenging of ROS.

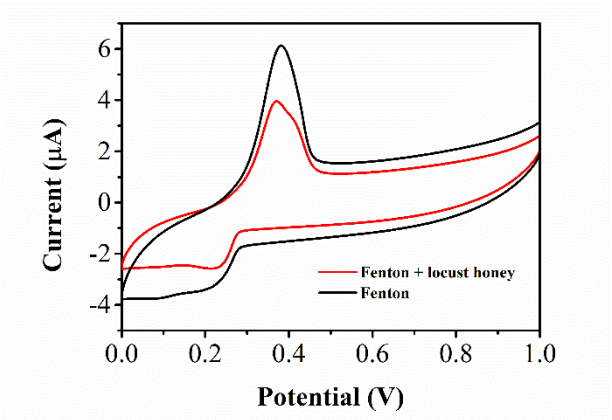

Fig. 3. Cyclic voltammetry profiles of hydrogel with Fenton solution and Fenton solution + locust honey aqueous solution.

The comparison of TAC performance for four different types of honey has been evaluated using a DPV method. Figure 4 shows DPVs of the TAC performance of locust honey, linden honey, jujube honey and wattle honey in the presence of 0.1 mM Fenton solution. It can be seen that the wattle honey shows the highest peak current among four samples, indicating the TAC of the wattle honey is quite low. In contrast, jujube honey shows a lowest peak current among these honeys, suggesting the TAC of the jujube honey is relatively higher than other three honeys. Based on the results, the TAC performance of the four floral sources of honey follows following order: Jujube honey > linden honey > locust honey > wattle honey.

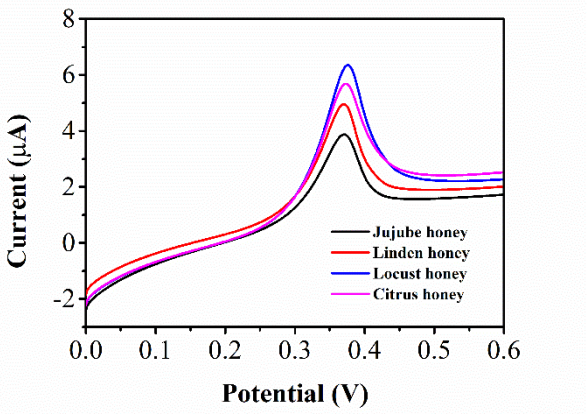

Fig. 4. Differential pulse voltammetry profiles of jujube honey, linden honey, locust honey and wattle honey in hydrogel with Fenton solution.

4 Conclusion

In conclusion, we proposed a new electrochemical sensing platform for evaluating the TAC performance for different types of honey. Silver ions and chitosan cross-linked hydrogel has been synthesized as the platform. The depolymerization of the hydrogel deduced from Fenton solution can be hindered when the presence of honey. The difference between the silver redox signals can be then used for TAC performance analysis. Jujube honey, linden honey, locust honey and wattle honey purchased from online shop have been submitted to evaluation as real samples. The results indicate the TAC performance of the

four floral sources of honey follows following order: Jujube honey > linden honey > locust honey > wattle honey.

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