Study on the Metabolism of Sesamin to Ethanol and the Preparation of Sesamin Microcapsules

Xiue Ren*, Shuyi Zeng, Xiuxiu Xie, Kemin Zhu, and Xiuhua Chen

School of Chemical Engineering and New Energy Materials, Zhuhai College of Jilin University, Zhuhai, Guangdong, China

*Corresponding author e-mail: renxiue19@jluzh.edu.cn

Abstract. Hydroxyl radicals were obtained by simulating ethanol metabolism in vitro. The absorbance of sesamin was measured by reacting with sesamin solutions of different concentrations. The ability of sesamin sample to scavenge hydroxyl radicals decreased with the increase of dilution ratio in a certain range. A comparative experiment between sesamin and water was designed to verify that sesamin has a significant promoting effect on alcohol dehydrogenase (ADH). The results showed that sesamin could promote the metabolism of ethanol. It indicated that the sesamin can dispel alcohol and protect the liver. In addition, the sesamin was encapsulated by the coacervation method to optimize the preparation method of sesamin microcapsules, which laid a foundation for the practical application of sesamin.

1. Introduction
China's sesame resources are very rich, and sesame contains high sesamin, accounting for about 0.5% to 1%. Sesamin has many advantages, such as lowering human cholesterol, having anti-hypertension, antibacterial and anti-oxidation, and promoting the metabolism of ethanol and other biological activities. Therefore, sesamin can strengthen the metabolism of ethanol and enhance the detoxification activity to protect the liver. Some articles have also pointed out that sesamin can inhibit breast cancer and immune activation, eliminate physiological effects such as harmful elements of immune cells, and play a synergistic role with vitamin E [1]. Therefore, sesamin has potential application prospects in the fields of medicine, health care and food.

After drinking alcohol in the living, most of the ethanol ingested in the body will be oxidized and decomposed into acetaldehyde in the liver, then converted into acetic acid by acetaldehyde dehydrogenase and then reacted with coenzyme A to form acetyl-CoA, and finally passed through the tricarboxylic acid cycle. Oxidation to carbon dioxide and water, and then excreted. The enzymes involved in the oxidative decomposition of ethanol are mainly alcohol dehydrogenase (ADH), microsomal alcoholase oxidation system (MEOS) and catalase. The oxidative decomposition of ethanol mainly passes through the ADH pathway. If the activity of ADH is lowered, it will increase the burden on the liver and accelerate the development of alcoholic liver disease. Sesamin can promote the activity of ADH and protect the liver [2,3].

In the experiment, hydroxyl radicals were generated by in vitro simulated ethanol metabolism, and reacted with different concentrations of sesamin solution to measure the absorbance, so that the ability of sesamin samples to scavenge hydroxyl radicals was decreased within a certain range with the increase of dilution factor. And the control experiment of sesamin and water was set up to verify that sesamin has a significant promoting effect on alcohol dehydrogenase (ADH) to prove that sesamin has
the effect of promoting ethanol metabolism. Sesamin and its active ingredients can act on the human liver, which has the effect of protecting the liver and hangover.

In the field of functional foods, microcapsule technology can not only reduce the loss of functional factors during processing or storage, but also effectively transfer functional factors to the human gastrointestinal tract, thereby reducing toxicity, improving efficacy, and improving food quality. Therefore, microencapsulation technology provides a new theory and application platform for the research and development of functional foods, which is very beneficial for the development of functional foods [4].

In recent years, for the preparation of microcapsules by complex coacervation, most people choose gelatin/arabum as the wall material, and form microcapsules by curing the cross-linking of glutaraldehyde. In this experiment, chitosan with good biocompatibility and antibacterial properties is used. The natural sodium alginate polysaccharide is used as the experimental wall material, and calcium chloride is used as the curing agent to avoid the residual toxicity of the organic solvent, improve the biological activity of the composite microcapsule, and provide an experimental basis for its commercial application.

2. Materials and methods

2.1. Materials, reagents and instruments
Constant temperature water bath; ultrasonic cleaning machine; electronic analytical balance; ultraviolet-visible spectrophotometer (UV-1750); optical microscope; electric furnace; magnetic stirrer; sesamin standard; 0.0018 mol/L FeSO₄; 0.3% H₂O₂; mol/L salicylic acid-ethanol; sodium pyrophosphate decahydrate (pH=8.8); 11.5% ethanol solution; 0.027 mol/L oxidized coenzyme I; 0.25 U/mL alcohol dehydrogenase; 1.5% (m/V) Sodium alginate; 0.75 % (m/V) chitosan; 5% (m/V) calcium chloride; 1 mol/L NaOH; 2% (V/V) acetic acid; monoglyceride; dilute sulfuric acid; Hydrochloric acid; the water used is deionized water.

2.2. Sesamin clearance of hydroxyl radicals
Preparation of hydroxyl radicals according to Fenton reaction

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\bullet
\]

\[
\text{C}_7\text{H}_5\text{O}_3\text{OH}^- + \text{C}_7\text{H}_5\text{O}_4\text{H}^\bullet \rightarrow \text{C}_7\text{H}_5\text{O}_4^- + \text{C}_7\text{H}_5\text{O}_4^\bullet
\]

The role of salicylic acid: with the remaining hydroxyl radicals to form dihydroxybenzoic acid at 510 nm absorption and formation of a stable, easy to determine its absorbance.

(1) Determination of \( A_0 \): Take 2 mL of sesamin solution, add 7.2 mL of deionized water, shake well, place in a cuvette, and measure the absorbance at 510 nm.

(2) Determination of \( A_0 \): 4 mL of 0.0018 mol/L FeSO₄ solution, 3 mL of 0.0018 mol/L salicylic acid-ethanol solution, 0.2 mL of 0.3% H₂O₂, and finally 2 mL of deionized water were added to the test tube. Then placed in 37 °C water bath for 30 min. Using water as a reference, transfer the solution from the tube to the cuvette and measure the absorbance at 510 nm and record the data [5,6].

(3) The deionized water in step (2) was replaced with sesamin solution at a concentration of 628 μg/mL, 314μg/mL, 157 μg/mL, 104 μg/mL, and 78.54 μg/mL, respectively. The absorbance value was measured at the nm wavelength, and recorded in the table and the hydroxyl radical scavenging rate was calculated according to the Equation (1).

\[
f = \frac{A_0 - A_f}{A_0} \times 100\%
\]  \tag{1}

In Equation (1), \( f \) is a hydroxyl radical scavenging rate; \( A_0 \) is the absorbance of a blank tube (with no sesamin added); \( A_f \) is the absorbance of adding sesamin.
2.3. Promotion of sesamin on alcohol dehydrogenase (ADH)

Under the action of alcohol dehydrogenase (ADH) and oxidized coenzyme I, ethanol decomposes NADH. NADH has an absorption peak at 340 nm.

(1) Determination of $A_{\text{Sesamin}}$: Add 3 mL of sodium pyrophosphate buffer with a pH of 8.8, 2 mL of 0.027 mol/L oxidized coenzyme I, 1 mL of 11.5% ethanol solution, 0.2 mL 314 ug./mL Sesamin solution, shake well, and let it sit at room temperature for 5 min. Add 0.2 mL of 0.25 U/mL (1 mL of 0.25 units) of alcohol dehydrogenase (ADH), shake well, and measure the absorbance immediately at 340 nm. Record every 10 s for up to 70 s. The distilled water was used as a reference to zero.

(2) Measurement of $A_{\text{without Sesamin}}$: The sesamin solution in (1) was replaced with an equal amount of distilled water as a blank control group, and the absorbance was measured. The method is the same as (1).

(3) Calculate the activation rate of alcohol dehydrogenase under the action of sesamin according to the Equation (2).

$$\text{activation rate} = \frac{A_{\text{Sesamin}} - A_{\text{without Sesamin}}}{A_{\text{without Sesamin}}} \times 100\%$$  \hspace{1cm} (2)

In Equation (2), $A_{\text{Sesamin}}$ is the absorbance when sesamin is added; $A_{\text{without Sesamin}}$ is the absorbance when no sesamin is added.

2.4. Preparation of Sesamin Microcapsules

(1) Preparation of core material solution: 0.02 g of sesamin solid was weighed and dissolved in 10 mL of distilled water containing 0.08 g of monoglyceride, and uniformly mixed under ultrasonic conditions for use.

(2) Mixing the sodium alginate solution with the core solution: Mix 8 mL of 1.5% (m/V) sodium alginate solution and 5 mL of Sesamin emulsion. Stir at a constant temperature of about 70 °C for 1 h.

(3) Mixing chitosan solution with calcium chloride solution: taking 30 mL of 0.75% (m/V) chitosan solution, adding 3 mL of 5% (m/V) calcium chloride solution, mixing at room temperature for mixing Uniform and adjusted to pH = 5.4 with 1 mol/L NaOH solution.

(4) Preparation of microcapsules: a mixed solution of sodium alginate and core solution was taken up with a 2.5 mL syringe, and a syringe needle with a pore diameter of 0.45 mm was selected and added to the chitosan and chlorinated at a distance of 3 cm from the liquid surface. In the mixed solution of calcium, the mixed solution was shaken at a low speed during the dropwise addition.

(5) Curing of the capsule: During the dropwise addition, 1.7 mL of a 5% (m/V) calcium chloride solution was added and solidified for 30 minutes.

(6) Separation and drying of the finished product: After standing for about 1 h, the mixture was filtered with a cloth funnel, and the microcapsules were rinsed with distilled water, and the precipitate was naturally air-dried to obtain a finished product of sesamin microcapsules.

In order to allow the milk core to be better dispersed in the continuous phase, we emulsified sesamin with an emulsifier. After several experiments, we determined that the optimum dosage of related substances for the preparation of sesamin microcapsules by the complex coacervation method as follows.

- Core material ratio: Sesamin: monoglyceride (emulsifier) mass ratio = 1:4
- Wall material ratio: sodium alginate: chitosan mass ratio = 1:2
- Core wall ratio: 0.5:1~1:1
- Curing agent: calcium chloride solution accounts for 9.3% of the total volume

3. Results and analysis

3.1. Sesamin clearance of hydroxyl radicals

Figure 1. shows that sesamin does have a clear clearance effect on hydroxyl radicals. In the concentration range of sesamin with a dilution ratio of 2 to 3, the absorbance decreases with the increase of sesamin concentration, that is, the ability of sesamin to scavenge hydroxyl radicals
increases with the concentration of sesamin. However, the absorbance measured by sesamin at a too high or too dilute concentration is still abnormal. Therefore, within a certain sesamin concentration range (ie, 100 μg/mL to 400 μg/mL), as the concentration of sesamin increases, the ability to scavenge hydroxyl radicals increases. In general, different concentrations of sesamin did have a clearer effect on hydroxyl radicals.

| Dilution factor | Sesamin concentration μg/mL | \( A_f \) | \( f\% \) |
|-----------------|----------------------------|--------|--------|
| Stock solution  | 628                        | 0.725  | 29.20  |
| 2 times         | 314                        | 0.608  | 40.63  |
| 3 times         | 157                        | 0.710  | 30.66  |
| 4 times         | 104                        | 0.809  | 20.99  |
| 5 times         | 78.54                      | 0.762  | 25.59  |

\( A_0 = 1.024 \)

Figure 1. The scavenging rate of hydroxyl radicals at different dilution ratios of sesamin

3.2. Promotion of sesamin on alcohol dehydrogenase (ADH)

The experiment proves that sesamin has an activation effect on alcohol dehydrogenase from the Figure 2. It can be clearly seen that the activation of sesamin on alcohol dehydrogenase increases rapidly before 30 s in the reaction. And then the increase rate of activation rate tends to be gentle. At last the activation rate is maintained at 28% or so.

| time/s | \( A_{\text{Sesamin}} \) | \( A_{\text{without Sesamin}} \) | Activation rate % |
|--------|--------------------------|---------------------------------|-------------------|
| 10     | 0.776                    | 0.624                           | 24.36             |
| 20     | 0.824                    | 0.658                           | 27.96             |
| 30     | 0.887                    | 0.694                           | 27.81             |
| 40     | 0.932                    | 0.727                           | 28.20             |
| 50     | 0.970                    | 0.758                           | 27.97             |
| 60     | 1.020                    | 0.791                           | 28.95             |
| 70     | 1.058                    | 0.821                           | 28.89             |
3.3. Microcapsule quality inspection

Microcapsule morphology, particle size and distribution were observed under a microscope. Observe whether it is a capsule under the optical microscope. It can be seen that the surface of the microcapsules was smooth, and the adhesion between each other was less.

| Table 3. Sesamin microcapsules |  |
|-------------------------------|--|
| Microcapsule traits | Particle size and its distribution | Whether it is a capsule | Microcapsule morphology |
| Milky white, slightly transparent, elastic | Particle size: about 110 μm | Yes | The microcapsules are spherical, and some of the microcapsules do not stick, and very few will stick |
|  | Distribution: Most |  |  |

Figure 3. Microcapsules in a micrograph

4. Conclusion

In summary, it can be seen that sesamin can react with hydroxyl radicals, and decrease the accumulation of hydroxyl radicals. The elimination rate of hydroxyl radicals is as high as 40.63%. Sesamin has a synergistic effect with alcohol dehydrogenase, and the enzyme activation rate is up to 28.95%. By detecting the clearance rate of sesamin on hydroxyl radicals and the promotion of alcohol
dehydrogenase, it can be verified that sesamin can promote the metabolism of ethanol. Therefore, it can reduce the damage to the liver.

In addition, the sesamin microcapsules with smaller particle size were successfully prepared. Under the microscope, the surface of the microcapsules was smooth, and the adhesion between the microcapsules and the microcapsules was less, indicating that it can encapsulate the active ingredients well. In the capsule, it provides an experimental basis for the commercial application of sesamin.

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