Research on the Properties of Several Marine Active Substances to Eliminate Superoxide Anions

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Abstract. Using hydroxyl radicals as an in vitro antioxidant model and using ascorbic acid (Vc) as a positive control, four important marine activities of trehalose, carboxymethyl chitosan, glucosamine hydrochloride, and rippled paphia small molecule peptide were studied Antioxidant ability of the substance in vitro. The experimental results show that the four marine active substances have good antioxidant properties, and their antioxidant properties show a certain positive correlation with the concentration; carboxymethyl chitosan has a stronger ability to eliminate hydroxyl radicals than the other three marine active substances. When the concentration was 4 g/L, the elimination rate of para-hydroxyl radicals reached 91.71%.

1. Introduction
Some peculiar edible and medicinal plants in China contain a large amount of phenols. These materials are characterized by electrons that are easily taken away by free radicals, and they will become harmless to people after losing electrons of stable substances [1-2].

Trehalose, also known as yeast sugar, is a natural sugar. It is a non-reducing sugar composed of two glucose molecules with 1,1-glycosidic bonds and has good stability [3-4].

Paphia undulate, also known as flower snail, is one of the largest marine shellfish cultured offshore in southern China. It contains active substances with significant antioxidant and anti-fatigue activities. Carboxymethyl chitosan is a water-soluble chitosan derivative, which has many characteristics, such as strong antibacterial property, fresh-keeping effect, and an amphoteric polyelectrolyte.

Glucosamine hydrochloride (D-Glucosamine Hydrochloride, referred to as GAH), also known as glucosamine hydrochloride, is one of the natural amino monosaccharide derivatives. It is white crystal, odorless, easily soluble in water, slightly soluble in methanol and insoluble in organic solvents such as ethanol [5].

The research on the oxidation resistance of these four marine active substances shows that marine active substances have good antioxidant capacity, which provides new ideas for finding natural marine antioxidants.
2. Experimental method
Detection of the ability to remove superoxide anions: Take 4.5mL of 0.05mol/L Tris-HCl buffer solution, preheat in a 20℃ water bath for 20min, add 1mL sample and 0.4mL 25 mmol/L pyrogallop solution, and mix for 5min in a 25℃ water bath. Add 8 mol/L HCl 1 mL to stop the reaction. Take distilled water as reference, measure absorbance Ai at 420nm, blank control group Ao replace the sample with the same volume of distilled water. The absorbance of the positive control Vc was measured in parallel three times and averaged. The formula for the rate of elimination of superoxide anion radicals from a sample pair is as follows Formula (1):

\[
\eta = \left(1 - \frac{A_i}{A_o}\right) \times 100\%
\]  

In the formula, \( \eta \): Elimination rate of superoxide anion free radical
\( A_i \): the Absorbance of superoxide anion at 420 nm,
\( A_o \): the Absorbance of superoxide anion at 420 nm.

3. Results and discussion
Elimination of superoxide anion: Superoxide anion radicals can be used as the parent of most oxygen radicals, and generate other radicals through a series of reactions in the body. Not only are the derived free radical’s toxic to cells, but the superoxide anion radicals themselves are also toxic. According to the above experimental method, the results are shown in following Table 1~5 and Figure 1~5.

| Concentration(g/L) | Absorbance Ai | Absorbance Ao | Elimination rate(%) |
|-------------------|--------------|--------------|---------------------|
| 0.1               | 0.252        | 0.650        | 61.2308             |
| 1                 | 0.068        | 0.671        | 89.8657             |
| 3                 | 0.025        | 0.655        | 96.1832             |
| 7                 | 0.020        | 0.670        | 97.0149             |

| Concentration(g/L) | Absorbance Ai | Absorbance Ao | Elimination rate(%) |
|-------------------|--------------|--------------|---------------------|
| 0.1               | 0.293        | 0.320        | 8.4375              |
| 1                 | 0.283        | 0.321        | 11.8380             |
| 3                 | 0.279        | 0.323        | 13.6223             |
| 7                 | 0.268        | 0.327        | 16.7702             |
Figure 1. Ascorbic acid for eliminating rates.

Figure 2. Trehalose for eliminating rates.

Table 3. Carboxymethyl chitosan for ultra-oxygen anion eliminating rates.

| Concentration (g/L) | Absorbance Ai | Absorbance Ao | Elimination rate (%) |
|---------------------|---------------|---------------|----------------------|
| 0.1                 | 0.320         | 0.330         | 3.0303               |
| 1                   | 0.312         | 0.335         | 6.8657               |
| 3                   | 0.295         | 0.340         | 13.2353              |
| 7                   | 0.280         | 0.338         | 17.1597              |

Table 4. Glucosamine hydrochloride for ultra-oxygen anion eliminating rates.

| Concentration (g/L) | Absorbance Ai | Absorbance Ao | Elimination rate (%) |
|---------------------|---------------|---------------|----------------------|
| 0.1                 | 0.303         | 0.325         | 6.7692               |
| 1                   | 0.286         | 0.326         | 12.2699              |
| 3                   | 0.261         | 0.330         | 20.9091              |
| 7                   | 0.249         | 0.327         | 23.8532              |
Figure 3. Carboxymethyl chitosan for eliminating rates.

Figure 4. Glucosamine hydrochloride for eliminating rates.

Table 5. Rates of glucosamine hydrochloride on DPPH free radical elimination.

| Concentration(g/L) | Absorbance Ai | Absorbance Ao | Elimination rate (%) |
|-------------------|--------------|--------------|----------------------|
| 0.5               | 0.381        | 0.390        | 2.3077               |
| 1                 | 0.297        | 0.389        | 23.6504              |
| 3                 | 0.251        | 0.393        | 36.1323              |
| 7                 | 0.155        | 0.388        | 60.0515              |
Figure 5. Rates of glucosamine hydrochloride on DPPH free radical elimination.

It can be known from Table 1 to Table 5, and Figure 1 to Figure 5 that as the concentrations of the four marine active substances increase, the absorbance $A_i$ for eliminating superoxide anions becomes smaller, and the smaller the absorbance $A_i$, the stronger the oxidation resistance; Change, because $A_0$ is the distilled water instead of the sample, the absorbance of the blank control group; the elimination rate of superoxide anion of the four marine active substances all increases with increasing concentration.

According to the data of Table 1 to Table 5, the following Figure 6 is obtained:

Figure 6. Four kinds of Marine active substances to eliminate super oxygen anion.

It can be seen from Figure 1 that compared with Vc, the four marine active substances have not strong elimination effect on the superoxide anion produced by the pyrogallol auto-oxidation system. When the sample concentration was 0.1 g/L, the elimination rates of superoxide anion by Vc, trehalose, carboxymethyl chitosan, glucosamine hydrochloride, and rippled paphia small molecule peptides were 61.23%, 8.44%, 3.03%, 6.77%, and 2.31%. When the concentration of Vc is 3 g/L, almost all the superoxide anion radicals in the reaction system can be eliminated. However, after the concentration of carboxymethyl chitosan and glucosamine hydrochloride reaches 3 g/L, the elimination rate changes with increasing concentration. Not big. With increasing concentration of trehalose, its elimination ability increases more slowly than the other three marine active substances. With the increase of the concentration, the elimination ability of the small-molecule peptide of C. bafii was more obvious. After the concentration was 1 g/L, the elimination ability was stronger than the other three marine
active substances at the same concentration.

4. Conclusions
During the normal metabolism of the biological body, superoxide anion, an active oxygen radical, is generated. Too much free radical can damage the body. Under normal circumstances, excessive free radicals produced by the body are quickly removed by the body's scavengers, so the body's free radicals are in a dynamic equilibrium. If the imbalance or clearance is too slow, too many free radicals will attack biological macromolecules, accelerate the aging process of the body, and induce a variety of diseases such as inflammation, malignant tumors, and immune disorders. Therefore, it is of great significance to study natural antioxidants and foods that can remove active oxygen free radicals in the body. Studies have shown that polysaccharides have strong antioxidant activity both in vitro and in vivo. Different types of polysaccharides have different antioxidant effects on different reaction systems, and they are also selective for the types of free radicals removed.

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