Capability of Several Marine Active Substances to Eliminate Hydroxyl Radicals

Yongli Zhang
Logistics Department, Guangzhou College of Technology and Business, Guangzhou, China
670511263@qq.com

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 4g/L, the elimination rate of para-hydroxyl radicals reached 91.71%.

1. Introduction
One of the serious poisons of free radicals is that it interacts with unsaturated fatty acids in a free or bound state, causing them to undergo a peroxidation reaction (lipid peroxidation), which leads to the destruction of biofilms, and ultimately to the destruction of cell functions and various physiological disorders and pathological changes [1]. To reduce the harm of free radicals to the human body, in addition to relying on the free radical scavenging system in the body, exogenous free radical scavengers are also needed to be found and discover. Use these substances as substitutes so that they interact with free radicals before they enter the body Combined to block external free radical attacks and protect the body from harm.

There are four common Marine active substances: trehalose, carboxymethyl chitosan, glucosamine hydrochloride and corrugated paphia sinensis.

Trehalose is made up of two glucose molecules to alpha, alpha, 1, 1 - glycosidic bond of the reducing sugar, its nature is very stable, has a unique biological characteristics, the protection of biological dehydration resistance, resistance to freeze protection and high anti-permeability protection, at the same time gives to prevent aging, prevent protein denaturation starch, inhibition of lipid rancidity, inhibiting the production of fishy taste and odor correction, the correction action, inhibiting rice, rice bran, fresh and stable material of superoxide dismutase (sod), moth-proofing teeth and complementary energy and other features. However, other sugars in nature, such as sucrose and glucose, have no magical protective effect on a variety of bioactive substances. This unique features that trehalose in addition can be used as a protein, enzyme, vaccines and other biological products excellent activity of protectant, or keep the cell activity, moisturizing cosmetics important component, prevent more can be used as food deterioration to keep the food fresh flavor and unique food
ingredients to improve the quality of food, expand the trehalose as the function of the natural edible sweet sugar [2].

Carboxymethyl chitosan is an important derivative of chitosan. Compared with chitosan, its physical and chemical properties are greatly improved: it has 10% water solubility, film formation and strong chelation of heavy metals. This kind of neutral, pure natural and completely nontoxic product shows more superior characteristics in the fields of medical care, industry and agriculture. At present, it has been applied in many aspects such as advanced cosmetic additives, medical health products, heavy metal chelating agents, drug sustained release agents, plant growth agents and industrial wastewater treatment. However, studies have shown that many unique physiological activities and functional characteristics of carboxymethyl chitosan are closely related to its molecular weight [3].

Glucosamine Hydrochloride (GAH for short), white crystalline powder, molecular formula C_{6}H_{13}NO_{5}HCl, in the glucose structure, c2-hydroxyl is replaced by amino group, hydrochloric acid and amino group into salt, is an important derivative of chitin. Glucosamine and closely related to the human body, widely exists in the body, can reduce the production of nitric oxide in plasma and prostaglandin E2, inhibit platelet aggregation, but both exist in combination type without free type, GAH stable properties, are widely used in chemical, pharmaceutical, food and other industries, and can be used in cosmetics, feed additives. It has anti-inflammatory, detoxification, antisepsis, antibacterial and other functions in living organisms, and is also an important raw material for the synthesis of related drugs [4].

Paphia undulata (Paphia undulata) is an important Marine shellfish in China. It is abundant in the whole coastal area south of zhejiang province. Paphia undulata is not only rich in nutrition and delicious in taste, but also has good health care function and has important edible and medicinal value [5]. The small molecule peptides obtained by enzymatic hydrolysis from paphia undulate have many unique functions and have a broad application prospect.

Based on the four kinds of Marine active substances of oxidation resistance research, known Marine active substances with good antioxidant capacity, looking for Marine natural antioxidants have certain help, to reduce disease, improve people's physical quality, has the very vital significance, at the same time, in order to further the study of Marine active substances and application provides a theoretical basis.

2. Experimental method
Detection of the ability to remove hydroxyl radical: with reference to the Fenton reaction system model, a fixed-time reaction method was used to determine the hydroxyl radical scavenging capacity. The reaction system contained 8.8mmol/L H_{2}O_{2}, 9mmol/L FeSO_{4}, 9mmol/L salicylic acid anhydrous ethanol solution and marine active substances of different concentrations, and the volume was 1mL. With distilled water as a reference, H_{2}O_{2} was added to start the reaction, and the reaction was performed at 37°C for 30 minutes. The absorbance Ax of the reaction system was measured at 510nm. The marine active substance was replaced with distilled water, and the absorbance A_{0} of the model control group was measured. Taking into account the absorbance value of the marine active substance itself, the background absorption value of the active substance is taken as an equivalent volume (1 mL) of 9mmol/L FeSO_{4}, 9mmol/L salicylic acid-ethanol, a mixture of different concentrations of the marine active substance solution and distilled water. Ax. The absorbance of the positive control Vc was measured in parallel three times and averaged. The formula for the scavenging rate of hydroxyl radicals of the sample is as follows:

\[
\text{Scavenging rate of hydroxyl radical by the sample} = \left[1 - \frac{A_x}{A_0}\right] \times 100\%
\]

Where Ax is the Absorbance of hydroxyl radical at 510nm; A_{0} is the Absorption value at 510 nm background. A0 is the Model control group absorbance at 510nm.

3. Result analysis
Hydroxyl radical (OH) is an important reactive oxygen species, which is formed by the loss of an electron by hydroxide (OH) from the molecular formula. Hydroxyl radicals have a very strong
electron-gathering ability, which is the oxidation ability, with an oxidation potential of 2.8 V. It is second only to oxidants in nature. Compared with other free radicals, hydroxyl radicals are the most active reactive oxygen radicals in the body. They are generated during the oxidative metabolic process of life activities, which can cause oxidative damage to large molecules such as amino acids, proteins, fats and nucleic acids, and induce apoptosis. Or make the transformed cells out of the control of normal cells to grow indefinitely and become cancerous [6]. Operate according to the above experimental method. The results are shown in Table 1, Table 2, Table 3, Table 4, and Table 5.

**Table 1. Ascorbic acid for hydroxyl free radical elimination rate**

| Concentration (g/L) | Absorbance Ax | Absorbance Ao | Absorbance Ax₀ | Elimination rate (%) |
|---------------------|---------------|---------------|----------------|---------------------|
| 0.5                 | 0.976         | 2.041         | 0.001          | 52.23               |
| 1                   | 0.018         | 2.043         | 0.004          | 99.31               |
| 2                   | 0.016         | 2.045         | 0.007          | 99.56               |
| 3                   | 0.014         | 2.042         | 0.008          | 99.71               |
| 4                   | 0.011         | 2.044         | 0.010          | 99.95               |

**Table 2. Trehalose on hydroxyl free radical elimination rate**

| Concentration (g/L) | Absorbance Ax | Absorbance Ao | Absorbance Ax₀ | Elimination rate (%) |
|---------------------|---------------|---------------|----------------|---------------------|
| 0.5                 | 2.020         | 2.022         | 0.017          | 0.94                |
| 1                   | 1.996         | 2.021         | 0.022          | 2.33                |
| 2                   | 1.928         | 2.020         | 0.023          | 5.69                |
| 3                   | 1.888         | 2.024         | 0.024          | 7.91                |
| 4                   | 2.020         | 2.022         | 0.017          | 29.78               |

**Table 3. Carboxymethyl chitosan for hydroxyl free radical elimination rate**

| Concentration (g/L) | Absorbance Ax | Absorbance Ao | Absorbance Ax₀ | Elimination rate (%) |
|---------------------|---------------|---------------|----------------|---------------------|
| 0.5                 | 1.321         | 2.121         | 0.024          | 38.85               |
| 1                   | 0.976         | 2.120         | 0.039          | 55.81               |
| 2                   | 0.754         | 2.124         | 0.045          | 66.62               |
| 3                   | 0.531         | 2.122         | 0.051          | 77.38               |
| 4                   | 0.232         | 2.123         | 0.056          | 91.71               |

**Table 4. Glucosamine hydrochloride on hydroxyl free radical elimination rate**

| Concentration (g/L) | Absorbance Ax | Absorbance Ao | Absorbance Ax₀ | Elimination rate (%) |
|---------------------|---------------|---------------|----------------|---------------------|
| 0.5                 | 1.900         | 1.971         | 0.018          | 4.52                |
| 1                   | 1.712         | 1.980         | 0.101          | 18.64               |
| 2                   | 1.651         | 1.982         | 0.103          | 21.90               |
| 3                   | 1.550         | 1.985         | 0.107          | 27.30               |
| 4                   | 1.431         | 1.975         | 0.109          | 33.06               |

**Table 5. Undulate small peptides on the elimination rate of hydroxyl radicals**

| Concentration (g/L) | Absorbance Ax | Absorbance Ao | Absorbance Ax₀ | Elimination rate (%) |
|---------------------|---------------|---------------|----------------|---------------------|
| 0.5                 | 1.724         | 1.882         | 0.087          | 12.06               |
| 1                   | 1.652         | 1.878         | 0.137          | 19.33               |
| 2                   | 1.576         | 1.879         | 0.171          | 25.23               |
| 3                   | 1.541         | 1.881         | 0.205          | 28.97               |
| 4                   | 1.519         | 1.880         | 0.241          | 32.02               |
From Table 1 to Table 5, it can be known that as the concentration of the four marine active substances increases, the absorbance $A_x$ for eliminating hydroxyl radicals becomes smaller, and the smaller the absorbance $A_x$, the stronger the oxygen resistance; the absorbance $A_o$ remains basically unchanged. Because $A_o$ changed the marine active substance into water, the absorbance of the model control group was measured; the absorbance $A_{xo}$ increased with increasing concentration, indicating compliance with Lambert's law; the elimination rate of hydroxyl radicals by four marine active substances both increase with increasing concentration.

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

![Figure 1. Four kinds of marine active substances eliminating hydroxyl free radical ability](image)

**Figure 1.** Four kinds of marine active substances eliminating hydroxyl free radical ability

It can be seen from Figure 1 that only the carboxymethyl chitosan of the four marine active substances has a strong ability to eliminate hydroxyl radicals. As the concentration increases, the ability to eliminate hydroxyl radicals also increases, and the changes are more obvious. Shows a good dose-effect relationship. The ability of the other three marine active substances to eliminate hydroxyl radicals does not change significantly with increasing concentration, but also shows a good dose-effect relationship. When $V_c$ increased from 0.5 g/L to 1 g/L, the elimination rate increased rapidly from 52.23% to 99.32%. When the concentration of carboxymethyl chitosan was increased to 4 g/L, the elimination rate was 91.71%, and the growth rate of elimination ability was slower than $V_c$. Carboxymethyl chitosan has a much stronger ability to eliminate hydroxyl radicals than the other three marine active substances. When the concentration reaches 4 g/L, the elimination rates of the other three marine active substances are quite close. Although the elimination rate of trehalose increased slowly before the concentration of 3 g/L, when the concentration reached 4 g/L, the elimination rate of trehalose suddenly increased, indicating that the concentration had a better elimination rate after 3 g/L.

4. Conclusion

Carboxymethyl chitosan has a stronger ability to eliminate hydroxyl radicals than the other three marine active substances. Although the effect of four kinds of marine active substances in eliminating free radicals is much weaker than $V_c$, the four kinds of marine active substances can basically achieve the effects of eliminating free radicals and blocking the chain reaction of free radicals, and can suppress free radicals in a certain concentration range. This provides a theoretical basis for further research and application of marine active substances.
Acknowledgments
This work was supported by 2019 Lateral Project from Guangzhou College of Technology and Business.

References
[1] Wang X P, Ding Z Y. Effects of free radicals on human body and its scavenging methods [J]. Science research, 2003, 10 (5): 476-477. (In Chinese)
[2] Peng Y F, Zhou Y B, Li Q, et al. Characteristics and application of trehalose [J]. China food additive, 2008, 11 (7): 65-69. (In Chinese)
[3] Chen L Y, Du Y M, Liu Y. Study on the structure and antibacterial properties of carboxymethyl chitosan [J]. Journal of Wuhan university: natural science, 2000 (2): 191-194. (In Chinese)
[4] Chen X. Antiseptic and antimicrobial effects of glucosamine hydrochloride [J]. Fine chemical industry, 2001, 18 (2): 78-79. (In Chinese)
[5] Chen X, Yang Y P, Sun H L, et al. Effect of small molecule peptide of papavera sinensis on skin aging of acute aging rats induced by d-galactose [J]. Journal of Chinese medicine and pharmacology, 2011, 22 (4): 874-876. (In Chinese)
[6] Wang Ruizun. Isolation, Purification and Antioxidant Activity of Astragalus Polysaccharide [D]. He Nan: Zhengzhou University, 2010.