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Preparation of peptides from oyster shells and investigation of their in vitro antioxidant activities

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Abstract

Oysters are the important marine biological resources available to human beings. The oyster shells contain important organic matters, such as amino acids, proteins and peptides. In the present study, the oyster shell peptides (OSP) were prepared, and the in vitro antioxidant activities of OSP were investigated. OSP were obtained by water refluxing extraction, followed by anion-exchange chromatography and reverse-phase C8 column chromatography. Using vitamin C as control, the capacity of OSP in scavenging 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), superoxide anion, hydroxyl and 1,1-diphenyl-2-picrylhydrazine (DPPH) radicals was determined. In addition, using disodium ethylenediamine tetraacetate as control, the capacity of OSP in chelating Fe^{2+} was determined. Results showed that, OSP had the capacity of scavenging ABTS, superoxide anion, hydroxyl and DPPH radicals and chelating Fe^{2+}. In conclusion, OSP have the antioxidant activities, and can be further developed into functional health food or medicine with related activities.

Keywords: oyster shells; peptides; preparation; antioxidant.

Practical Application: Oyster shell peptides have the antioxidant activities, and can be further developed into functional health food or medicine with related activities.

1 Introduction

Oysters are the famous and common shellfish, and the largest cultured shellfish in the world. Oysters are not only delicious and nutritious, but also have unique health care function, and they are the important food resource available to human beings (Caffrey et al., 2016). The oyster processing is an important branch of food industry (Muth et al., 2013). The oyster shells mainly contain the calcium carbonate. They also contain a small amount of calcium phosphate, calcium sulfate, iron oxide, and aluminum, magnesium, silicon and other trace elements. Therefore, the oyster shells are a valuable inorganic salt resource. In addition, the oyster shells contain important organic matters, such as amino acids, proteins and peptides (Tsukamoto et al., 2004). In recent years, the bioactive peptides have attracted much attention because of their special physiological functions and high medicinal value (Shori et al., 2021; Betancur-Ancona et al., 2022; Tekle et al., 2022). At present, there are reports on the separation and extraction of peptides from oyster meat (Bigot et al., 2012; Liu et al., 2019). As the oyster shells contain more calcium carbonate, there is less report on the extraction of bioactive peptides from oyster shells, which undoubtedly hinders the further development and utilization of oyster shell resources.

In the process of normal aerobic metabolism in body, many reactive oxygen species (ROS) are formed. In addition, the body can produce ROS through exogenous invasion such as infection, ionic radiation and air pollution (Hatoum et al., 2006; Sun et al., 2008). ROS have certain functions in body, such as immune and signal transduction processes, but excessive ROS will have the destructive behaviors (Zorov et al., 2014). Natural antioxidants have gradually become a research hotspot, especially the antioxidant peptides with the ability to inhibit macromolecular peroxidation and scavenge ROS in the body (Sun et al., 2004; Zhang et al., 2012). In this study, the oyster shell peptides (OSP) were prepared, and the in vitro antioxidant activities of OSP were investigated. The objective was to provide a reference for preparation of OSP and studying their activities.

2 Materials and methods

2.1 Preparation of OSP

According to the optimized extractions parameters, 1 kg of oyster shell powder (60 mesh) was added to the extraction kettle, followed by adding 10 kg of distilled water. The refluxing extraction was performed for 1 h. After filtering, the filtrate is concentrated by rotating evaporation at 60 °C, followed by filtering using 0.45 μm microporous membrane. After freeze drying, the crude extract was obtained. The crude extract was dissolved in Tris-HCl buffer, followed by anion-exchange chromatography. The elution solution of main peaks was collected. After rotating evaporation at 60 °C and freeze drying, the preliminary purified extract was obtained. It was dissolved in distilled water, followed by reverse-phase C8 column chromatography. The elution solution of main peaks was collected. After rotating evaporation at 60 °C and freeze drying, the final OSP product was obtained.

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According to the biuret reaction method, OSP were preliminarily determined as dipeptides or tripeptides. The quantitative analysis showed that the peptide content was 31.44%.

2.2 Detection of ABTS radical scavenging capacity

The samples of 0, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL OSP solution were prepared. The vitamin C (VC) with the same concentration was used as the positive control. A certain amount of 7 mmol/L 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) solution was added to the colorimetric tube, and an equal volume of 2.45 mmol/L potassium persulfate solution was added, followed by reaction under closed light and room temperature for 16 h. A 1 mL of reaction solution was taken and diluted with 5 mmol/L phosphate buffer (pH 7.0) to obtain the absorbance (734 nm wavelength) of 0.70. Then, 0.5 mL of sample solution and 0.5 ml of above reaction solution were mixed, followed by reaction for 6 min. The absorbance of final reaction solution was measured as at 734 nm wavelength. The distilled water was used as blank. The scavenging rate of ABTS radical was calculated as follows: scavenging rate (%) = $[1 - (A_1) / A_0] \times 100$ (A_0: absorbance of distilled water; A_1: absorbance of reaction solution).

2.3 Detection of superoxide anion radical scavenging capacity

A 1 mL of sample solution and 3.3 mL of distilled water were added to the colorimetric tube. Then, 4.5 mL of Tris-HCl buffer with pH 8.2 was added, followed by reaction at room temperature for 20 min. A 0.2 ml of 3 mmol/L of pyrogallol solution was added to the reaction solution. The reaction was performed for 4 min, and terminated by 6 mol/L hydrochloric acid solution. The absorbance of final reaction solution was measured at 320 nm wavelength. The distilled water was used as blank. The scavenging rate of superoxide anion radical was calculated as follows: scavenging rate (%) = $[1 - (A_1) / A_0] \times 100$ (A_0: absorbance of distilled water; A_1: absorbance of reaction solution).

2.4 Detection of hydroxyl radical scavenging capacity

A 1.5 ml of 2×10^{-5} mol/L crystal violet solution, 1 mL of 5×10^{-3} mol/L Fe^{2+} solution, 0.4 mL of 1% H_2O_2 solution, 1 ml of Tris-HCl solution (pH 5.5) and 2 mL of sample solution were added to the colorimetric tube, followed by dilution to 10 ml volume with distilled water. After reaction for 5 min, the absorbance of reaction solution was measured at 588 nm wavelength. The scavenging rate of hydroxyl radical was calculated as follows: scavenging rate (%) = $[(A_2 - A_0) / (A_1 - A_0)] \times 100$ (A_0: absorbance of reaction solution with H_2O_2 but without sample solution; A_1: absorbance of reaction solution without H_2O_2 or sample solution; A_2: absorbance of reaction solution with H_2O_2 and sample solution).

2.5 Detection of DPPH radical scavenging capacity

A 1 mL of 2×10^{-4} mol/L 1,1-diphenyl-2-picrylhydrazine (DPPH) solution and 1.0 mL of sample solution were added to the colorimetric tube, followed by dilution to 5 mL volume 5 mL using 95% ethanol aqueous solution. The mixture was incubated at 25 °C for 30 min. Then, the absorbance of reaction solution was measured at 517 nm wavelength. The scavenging rate of DPPH radicals was calculated as follows: scavenging rate (%) = $[1 - (A_1-A_2) / A_0] \times 100$ (A_0: absorbance of reaction solution with DPPH but without sample solution; A_1: absorbance of reaction solution with sample solution and DPPH; A_2: absorbance of reaction solution with sample solution but without DPPH).

2.6 Detection of Fe^{2+} chelating capacity

A 1 mL of sample solution, 3.7 mL of distilled water, 0.1 mL of 2 mmol/L $FeCl_2$ solution and 0.2 mL of 5 mmol/L ferrozine solution were added to the colorimetric tube. The mixture was incubated at 25 °C for 10 min. The absorbance of reaction solution was measured at 562 nm wavelength. Disodium ethylenediamine tetraacetate (EDTA-Na_2) was used as the positive control. The Fe^{2+} chelating rate was calculated as follows: Fe^{2+} chelating rate (%) = $[1 - (A_1-A_2) / A_0] \times 100$ (A_0: absorbance of reaction solution with ferrozine but without sample solution; A_1: absorbance of reaction solution with ferrozine and sample solution; A_2: absorbance of reaction solution with sample solution but without ferrozine).

2.7 Statistical analysis

All statistical analysis was carried out using SPSS 18.0 software (SPSS Inc., Chicago, USA). Each measurement was performed for three times, and the data were expressed as mean ± standard deviation. The comparison between two groups was performed using t test. Values of $P < 0.05$ were considered as significantly different.

3 Results

3.1 Capacity of OSP in scavenging ABTS radicals

Results of this study showed that, both OSP and VC had capacity of scavenging ABTS radicals, and the ABTS radical scavenging rate of OSP had obvious dose-dependence. When the concentration was 0.2, 0.4 and 0.6 mg/mL, the ABTS radical scavenging rate of OSP was significantly lower than that of VC, respectively ($P < 0.05$). When the concentration was 0.8 and 1 mg/mL, the ABTS radical scavenging rate had no significant difference between two substances ($P > 0.05$) (Figure 1).

3.2 Capacity of OSP in scavenging superoxide anion radicals

As shown in Figure 2, both OSP and VC also had capacity of scavenging superoxide anion radicals. The superoxide anion radical scavenging rate in each group obviously increased with the increase of concentration. When the concentration was 0.6, 0.8 and 1 mg/mL, the superoxide anion radical scavenging rate of OSP was significantly lower than that of VC, respectively ($P < 0.05$). When the concentration was 0.2 and 0.4 mg/mL, the superoxide anion scavenging rate had no significant difference between two substances ($P > 0.05$).

3.3 Capacity of OSP in scavenging hydroxyl radicals

OSP and VC had capacity of scavenging hydroxyl radicals. The hydroxyl radical scavenging rate in OSP group increased with the increase of concentration. When the concentration was 0.2, 0.4, 0.6 and 0.8 mg/mL, the hydroxyl radical scavenging rate of OSP was significantly lower than that of VC, respectively ($P < 0.05$). When the concentration was 1 mg/mL, the hydroxyl scavenging rate had no significant difference between two substances ($P > 0.05$) (Figure 3).

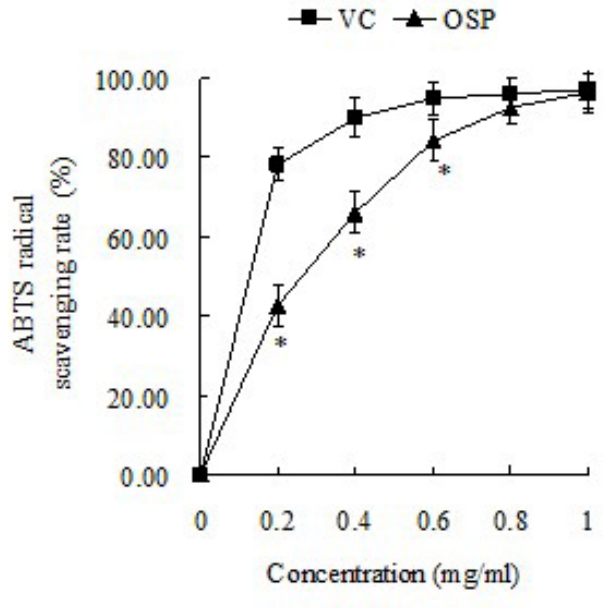

Figure 1. Comparison of ABTS scavenging rate between OSP and VC. *P < 0.05 compared with VC. ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; OSP, oyster shell peptides; VC, vitamin C.

3.4 Capacity of OSP in scavenging DPPH radicals

Figure 4 showed that, both OSP and VC had good capacity of scavenging DPPH radicals, and the DPPH radical scavenging rate of OSP had obvious dose-dependence. When the concentration was 0.2, 0.4 and 0.6 mg/mL, the DPPH radical scavenging rate of OSP was significantly lower than that of VC, respectively ($P < 0.05$). When the concentration was 0.8 and 1 mg/mL, the DPPH radical scavenging rate had no significant difference between two substances ($P > 0.05$).

3.5 Capacity of OSP in chelating Fe^{2+}

Both OSP and EDTA-Na_2 also had capacity of chelating Fe^{2+}. The Fe^{2+} chelating rate in OSP group obviously increased with the increase of concentration. At each concentration, the Fe^{2+} chelating rate of OSP was significantly lower than that of EDTA-Na_2 ($P < 0.05$) (Figure 5).

4 Discussion

ROS are produced by aerobic cells in the metabolic process. In some pathological states, ROS are related to a variety of diseases and injuries. They directly or indirectly participate in the pathological process of cardiovascular diseases such as atherosclerosis, ischemic heart disease and hypertension, and are an important cause of lipid peroxidation, aging and carcinogenesis (Kojda & Harrison, 1999; Touyz & Schiffrin,

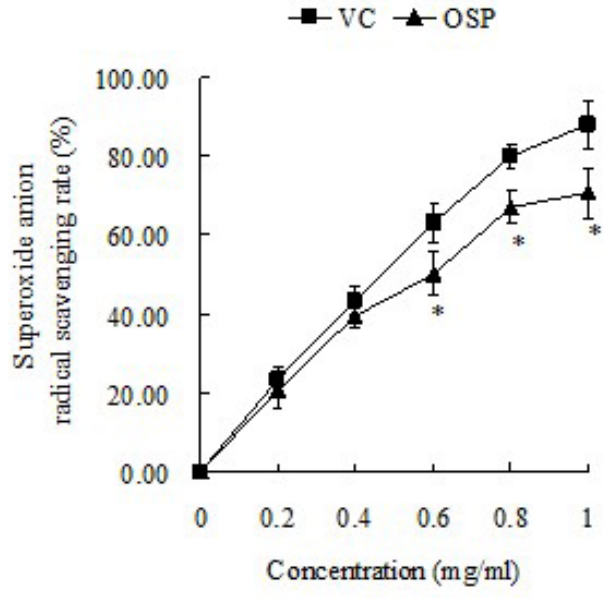

Figure 2. Comparison of superoxide anion radical scavenging rate between OSP and VC. *P < 0.05 compared with VC. OSP, oyster shell peptides; VC, vitamin C.

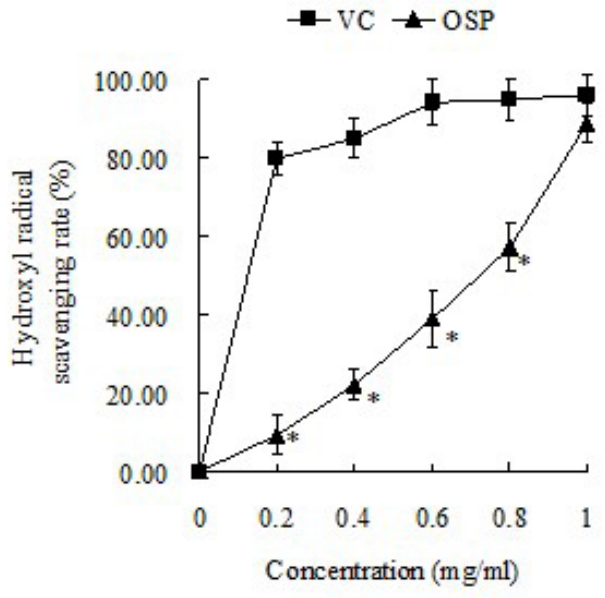

Figure 3. Comparison of hydroxyl radical scavenging rate between OSP and VC. *P < 0.05 compared with VC. OSP, oyster shell peptides; VC, vitamin C.

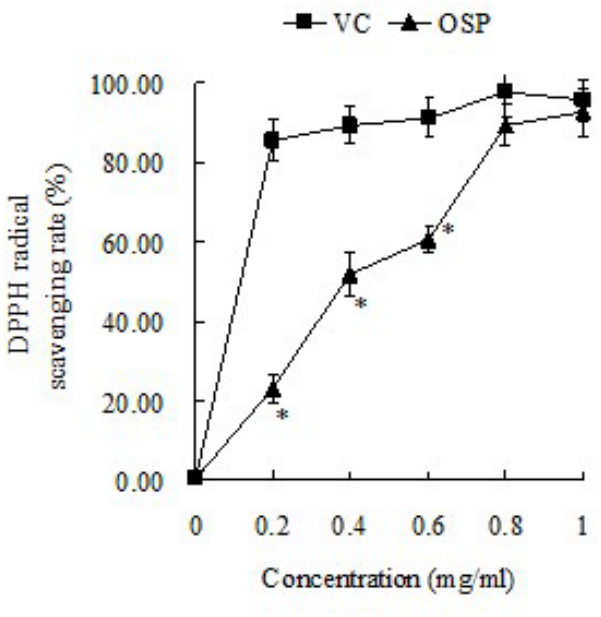

Figure 4. Comparison of DPPH scavenging rate between OSP and VC. *P < 0.05 compared with VC. DPPH, 1,1-diphenyl-2-picrylhydrazine; OSP, oyster shell peptides; VC, vitamin C.

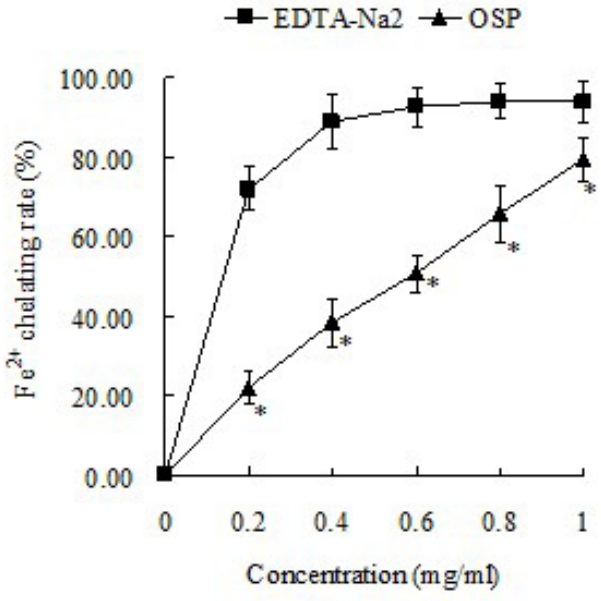

Figure 5. Comparison of Fe^{2+} chelating rate between OSP and VC. *P < 0.05 compared with VC. EDTA-Na_2, disodium ethylenediamine tetraacetate; OSP, oyster shell peptides; VC, vitamin C.

2004). Excessive ROS can induce the polyunsaturated fatty acid oxidation, destroy the structure and function of biofilm, and cause the pathological changes and death of cells and tissues in the body (Guo et al., 2019).

Many synthetic antioxidants such as 2,6-di-tert-butyl-4-methylphenol and butylated hydroxyanisole are commonly used as food additives to prevent food deterioration. Although these synthetic antioxidants have stronger antioxidant activity than natural antioxidants such as α-tocopherol and VC, consumers are more and more resistant to them due to the potential toxicity and side effects. Therefore, natural antioxidant substances have gradually become a research hotspot, especially the antioxidant peptides. Studies have shown that marine animal proteolytic peptides have significant antioxidant activity (Wu et al., 2015; Chai et al., 2017). Therefore, in the present study, the OSP were prepared, and the in vitro antioxidant activities of OSP were investigated.

There are many ways to detect the antioxidant activity of substances. ABTS radical scavenging method is widely used to determine the total antioxidant capacity of biological samples (Ilyasov et al., 2020). Superoxide anion radicals can cause the chain reactions and produce new free radicals and oxidants, leading to the toxicity to cells (Turrens, 2003). Hydroxyl radicals are the most active and toxic radicals. They can react with some biological macromolecules in cells, resulting in genetic mutation, membrane damage, enzyme inactivation, and mitochondrial oxidation (Huang et al., 2005). DPPH radicals are the model of fat-soluble radical. The antioxidant reacts with DPPH, and the reduced DPPH molecule is equal to the amount of hydroxyl provided by the antioxidant (Penkov et al., 2018). Fe^{2+} is the most effective pre-oxidant in the food system. Chelating excessive Fe^{2+} can also block the production of excessive radicals in body (Končić et al., 2011). Results of the present study showed that, OSP had the capacity of scavenging ABTS radicals, superoxide anion radicals, hydroxyl radicals and DPPH radicals chelating Fe^{2+}, with obvious dose-dependence. This indicates that, OSP have good antioxidant activity.

5 Conclusions

In this study, OSP are prepared, and the peptide content is 31.44%. The in vitro anti-oxidation experiments show that, OSP has the capacity of scavenging ABTS radicals, superoxide anion radicals, hydroxyl radicals and DPPH radicals and chelating Fe^{2+}. OSP can be further developed into functional health food or medicine with related activities. However, the total antioxidant activities of OSP are lower than VC. This may be related to the purity or dose of OSP used in this study, and this needs to be further investigated.

Conflict of interest

The authors declare that there is no conflict of interest.

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