

Effect of active carbon on decoloration of antler base collagen peptides

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Abstract. The antler base protein hydrolyze solution was decolorized through activated carbon. Based on the decolorizing rate and loss rate of peptide, the influence of peptide pH, active carbon content, treatment temperature and reaction time on the decolorization of active carbon were studied. The best conditions of decolorization were determined as follow: 2% active carbon content, pH 3, treatment temperature of 50°C, reaction time of 3h, the decolorizing rate and loss rate of antler base collagen peptides were 87.5% and 10.57±0.57%, respectively.

1. Introduction

Antler base is the rudimental antler on the pedicle of male sika deer (*Cervus nippon*) or red deer (*cervus elephus*) after sawing off the velvet antler, which then falls off by itself when the new velvet antler begins to germinate in the next year [1,2]. It looks like a plate, so it is named 'antler base'. Antler base slag was the residue which water-soluble protein was extracted or was extracted with 50% alcohol, more than 80% of antlers base protein was retained in residue, which was mainly collagen. The collagen could be prepared antioxidant activity polypeptide used protease.

More bitter flavour would be made when the more exposed hydrophobic amino groups by protease hydrolysis, at the same time, the color of hydrolysate would darken with the increase of the hydrolysis going[3]. The bitterness and yellowness of peptides solution not only affected the appearance and taste, but also had great limitations on the application of peptides. So the decolorization was an essential part in the process of purification of peptides. Activated carbon had high surface area, strong adsorptivity, low cost and no influence on the physicochemical characterization and biological activity of the product[4,5]. Therefore, active carbon was widely used in decolorizing treatment of industrial products.

In this study, antler base collagen peptides were prepared by alkaline protease, the peptides were decolorized used active carbon, and the influence of decolorizing temperature, time, pH and the adding amount of activated carbon on decolorizing rate were investigated. It would provided a theoretical basis for the deep application of antlers base collagen peptide.

2. Material and Methods

Antler base: Jilin Sino-ROK Institute of Animal Science (Changchun, China).

Alkaline protease: 20U/mg, Shanghai Baoman Biology Science & Technology Co.

Powder activated carbon: Tianjing Huadong Reagent Co.

TU-1810 Ultraviolet spectrophotometer, Beijing Purkinje General Instrument Co.

SHB-III Water ring vacuum pump, Zhengzhou Great Wall Industry & Trade Co.

2.1 The Preparation of Antler Base Collagen Peptides. The antler base slag concentration of 15%, alkaline protease concentration of 6%, pH8.5, temperature 53°C, reaction time 4h were chosen, after the reaction, boiling water bath was used to inactivate the enzyme and keep 10 min, then cooled down to room temperature used ice water, centrifugal (10000 g, 4 °C, 10 min), at the end the enzymatic hydrolysate would be dried into power by spray drier.

2.2 Decolorization Process. The different activated carbon content (1, 2, 3, 4, 5%), temperature (20, 30, 40, 50, 60 °C), decoloring pH (3, 4, 5, 6, 7) and decoloring time (30, 60, 90, 120, 150 min) were

reviewed.

2.3 Determination of Decolorizing Rate. Ultraviolet spectrophotometry was used to determine the content of peptides[6]. The maximum absorption peak (OD_1) of polypeptide solution was determined by using ultraviolet spectrophotometer at the full wavelength to determine the maximum absorption peak (OD_1) of polypeptide solution. After decolorizing, the absorption value (OD_2) of the polypeptide solution in the wavelength was measured, and the decolorizing rate of the polypeptide solution was calculated according to equations (1).

$$\text{Decolorizing rate (\%)} = (OD_1 - OD_2) / OD_1 \times 100\% \quad (1)$$

2.4 Determination of Loss Rate of Peptides. Coomassie brilliant blue was used to determine the content of peptides[7], the pretreatment protein content was recorded as P_1 , the protein content by decolorizing was P_2 , and the peptide loss rate was calculated according to equations (2).

$$\text{Loss rate (\%)} = (P_1 - P_2) / P_1 \times 100\% \quad (2)$$

3. Results and Discussion

3.1 The Influence of pH on Decolorization. The activated carbon content of 3%, decolorizing temperature of 40 °C, decolorizing time of 90min. The influence of different pH (3, 4,5,6,7) on decolorization of antler base peptides were investigated. The results were shown in figure 1.

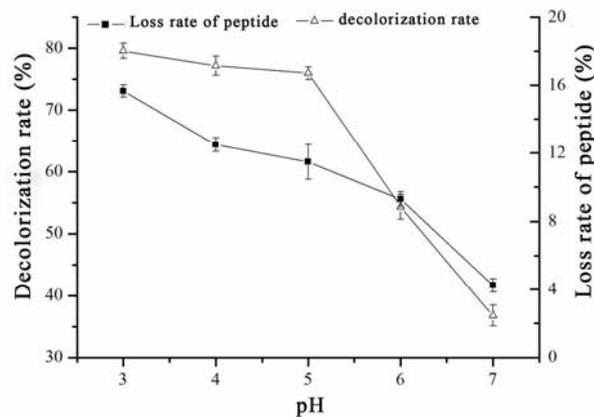


Fig.1 The influence of different pH on decolorization of antler base peptides

As could be seen from the figure, decolorization rate of antler base peptides was gradually reduced with the increase of the decolorizing pH. The pH of peptide solution had an appreciable effect on the decolorization effect of activated carbon, just activated carbon had the best decolorization effect in an acidic environment. The loss rate of peptides was inversely correlated with decolorization effect, loss rate was decreased with the increase of pH. Synthesize two kinds of test index, when pH was 5.0, the decolorization rate and loss rate of peptides reached the optimal combination. At this time, the decolorization rate and loss rate of peptides were $75.97 \pm 1.024\%$ and $11.48 \pm 1.03\%$, respectively.

3.2 The Influence of Active Carbon Content on Decolorization. The decolorization temperature of 40°C, decolorization time of 90 min, pH5, The influence of different active carbon concentration (1%, 2%, 3%, 4%, 5%, 6%) on decolorization of antler base peptides were investigated. The results were shown in figure 2.

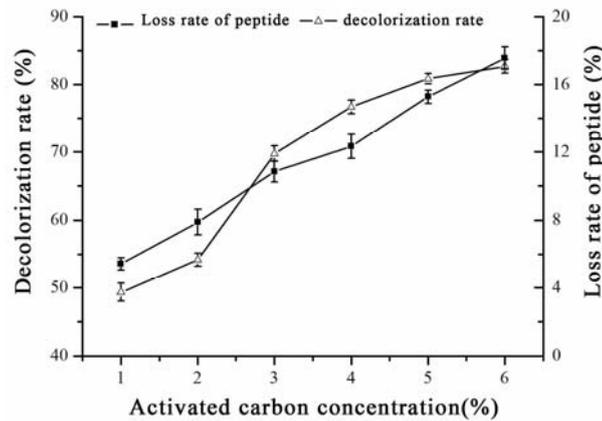


Fig.2 The influence of active carbon concentration on decolorization of antler base peptides

As was shown in the figure, the decolorization rate of activated carbon and the loss rate of the peptides were increasing with the increase of activate carbon concentration. When activate carbon concentration was 6%, the decolorization rate was up to $82.69 \pm 1.03\%$, but the loss rate of peptides was $17.56 \pm 0.65\%$. When activate carbon concentration over 4%, the rate of decolorization tended to upward trend, and the loss rate of peptides tended to upward tendency in a straight line. Synthesizing two kinds of test index, 4% activated carbon concentration was selected.

3.3 The Influence of Temperature on Decolorization. The activated carbon concentration of 4%, pH5, and decolorizing time of 90min, the influence of different reaction temperature (20, 30, 40, 50, 60°C) on decolorization of antler base peptides were investigated. The result was shown in Figure 3.

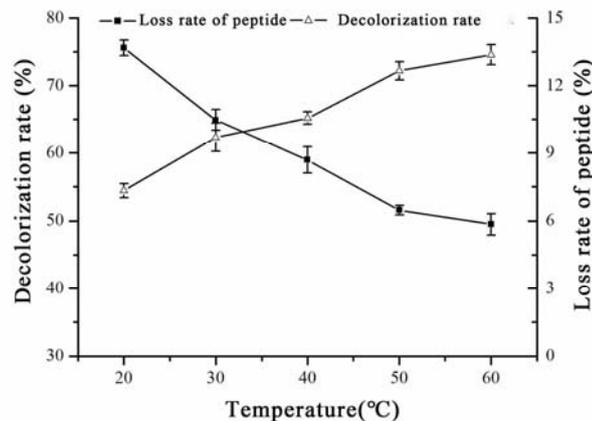


Fig.3 The influence of temperature on decolorization of antler base peptides

As was shown in the figure, the decolorization rate of active carbon was increasing and the loss rate of peptides was decreasing with the increase of reaction temperature. The viscosity of polypeptide solution was decreasing, with the increase of decolorization temperature was increased, and the movement of pigment in the solution increased in the same time, which accelerated the peptides contact opportunity with activated carbon and promoted the decolorization effect. The decolorization rate change was not evident when the temperature exceed 50 °C, and increased the loss rate of peptides. So 50 °C was chosen as the best decolorizing temperature.

3.4 The Influence of Reaction Time on Decolorization. The decolorization temperature of 50°C, activated carbon concentration of 4%, pH5, the influence of different reaction time (30, 60, 90, 120, 150min) on decolorization of antler base peptides were investigated. The result was shown in Figure 4.

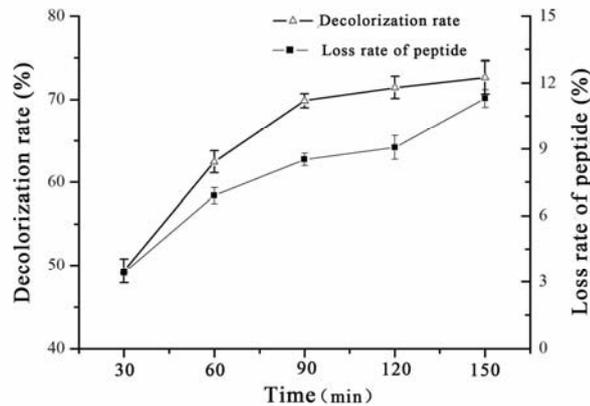


Fig.4 The influence of reaction time on decolorization of antler base peptides

As was shown in the figure, the decolorization rate and loss rate of peptide were increasing with the increase of decolorization in the same time. When reaction time was 90min, the decolorization rate tended to be stable. Combined with two indexes, we chosen decolorization time 90min as the best decolorizin. At this time, the decolorization rate of antler base peptides was $79.86 \pm 0.26\%$, and the loss rate of peptides was $10.57 \pm 0.57\%$.

4. Summary

Activated carbon was used to adsorb and decolor antler base collagen polypeptide solution. The optimal decoloration conditions of antler base collagen peptides as follow: pH5, decolorization temperature of $50\text{ }^{\circ}\text{C}$, activated carbon content of 4%, decolorization of 90min. Under these condition, the decolorization rate of collagen polypeptide was $79.86 \pm 0.26\%$, the loss rate of peptides was $10.57 \pm 0.57\%$. We expect the thesis may had theoretical basis for the further research of peptides.

Acknowledgements

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