FINAL REPORT
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Bioreactive Proteins: II. Screening Bioactive Peptides from Animal By-product Proteins

Principal Investigator:	Feng Chen Department of Food Science and Human Nutrition Clemson University Clemson, South Carolina 29634
Co-Principal Investigators:	Bo Li Department of Genetics, Biochemistry and Life Science Clemson University, Clemson, South Carolina 29634
	Xi Wang Department of Genetics, Biochemistry and Life Science Clemson University, Clemson, South Carolina 29634

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INTRODUCTION

Various plant or animal proteins have been found to be able to produce the so-called bioactive peptides [1] that have multiple biological functions in light of their antihypention [2,3], antibacterial [4-6], immuno-modulatory [7-9] and taste-stimulatory activities [10,11,12]. Advances in bioactive peptides are driven by a molecular understanding of biological process, and modern-state-of-art instrumental analysis. Nevertheless, bioactive peptides nowadays have been found with many exciting applications as dietary supplements, antimicrobial agents, and pharmaceutical alternatives, as well as their promising application as animal feed ingredients. Antioxidant activities have been found to be closely associated with many above mentioned biofunctions [13, 14] in light of the facts that cancer, coronary heart disease, and Alzheimer's diseases are reported to be caused in part by oxidation or free radical reactions in the body [16-19]. However, in light of the safety concerns of many artificial antioxidants, such as BHA, BHT, and n-propyl gallate, in food systems, the use of artificial antioxidants foodstuffs is restricted or prohibited in some countries. Therefore, it is necessary to develop more safe alternative antioxidants, besides the α-Tocopherol, carotenoids, catechins, and polyphenols, [21-24], from natural resource.

Recently, some protein hydrolysates have been reported to exhibit antioxidant activities (25-28). However, there is little information about antioxidants developed from animal rendering waste. Collagen is an animal by product, cheap and resourceful. In the phase I of the present study, we investigated the antioxidant activity of porcine collagen hydrolysates in several antioxidant models as the foundation for further phase II study on antimicrobial and pharmacological rsearch.

MATERIALS AND METHODS

Materials. pork collagen powder was obtained from Protein Products GmbH+Co KG.,U. S. Triton X-100 (peroxide and carbonyl free), butylated hydroxytoluene (BHT) and all of proteolytic enzymes were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade.

Preparation of Collagen Hydrolysates. Collagen was dissolved in distilled water and adjusted to pH 2.0 with 1.0 N HCl. After addition of pepsin, the mixture was incubated at 37 °C for 0.5,1,2,4,6,8,10,16,20,24 h. The resulting hydrolysates were heated in boiling water for 4 min to inactivate the protease, ant then neutralized, and centrifuged (10 min at 20000*g*), stored for further tests.

Measurement of antioxidant activity in a Peroxidation System. Antioxidant activities of the hydrolysates were measured in two bioassays: peroxidation determination and the DPPH radical scavenging capacity (RSC) determination [29].

Purification of Antioxidant Peptide. Hydrolysates were applied to a Sephadex column (100-200 mesh, Φ2 cm×90 cm; Bio-Rad, Hercules, CA) equilibrated with deioned water for purification. The sample fractions were collected and detected at 257 nm. They are also assayed by the DPPH test.

Statistical Analysis. All assays were carried out in triplicate. Data were expressed as means with standard deviations. Student's *t*-test ($p < 0.05$) was calculated to compare the mean of each sample with that of the control (represented as none in the figures) sample.

RESULTS AND DISCUSSIONS

Antioxidant Activities of Collagen Hydrolysates.

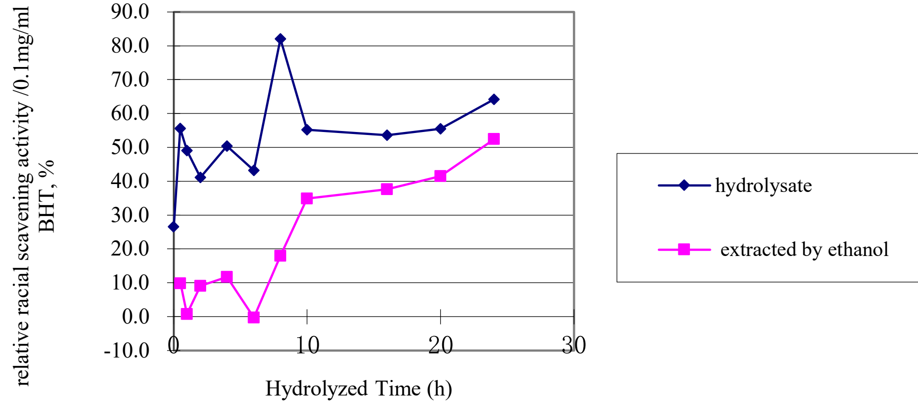

Fig. 1 The DPPH radical scavenging activity of collagen hydrolysate at different hydrolyzed hours

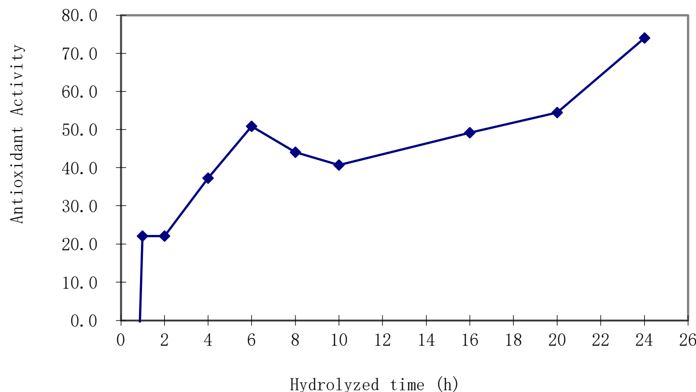

Figure 2: Antioxidant activity of collagen hydrolysate in linolenic acid Peroxidation model

Antioxidant activities of the collagen hydrolysates hydrolyzed by pepsin from 0.5 to 24 h have been shown in **Figure 1** and **2**. The curses show that general antioxidant activity of the hydrolysates increased with the hydrolysis time, which means shorter peptides exhibited higher antioxidant activities than the parent protein

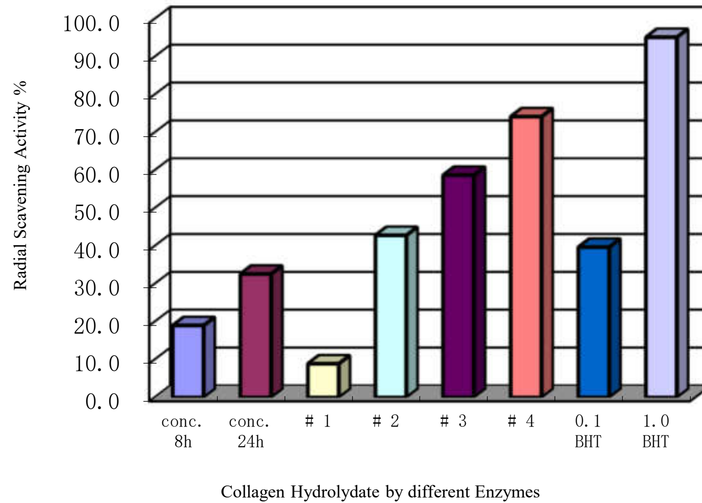

Fig 3 The DPPH radical scavenging activity of the ethanolic extracts of the collagen hydrolysates by different protease treatment

The collagen hydrolysate (hydrolyzed by pepsin for 24 h) was further hydrolyzed by the papain or other proteases (see **Figure 3**). The # 4 sample has the strongest RSC. But in linolenic acid peroxidation system, the # 2 sample has shown the strongest antioxidant activity (**Figure 4**). Meanwhile #3 sample among the ethanolic extraction of the hydrolysates also exhibited a very strong antioxidant capacity. Also, all the extracts except # 1 sample showed an stronger antioxidant activity than that of the control, 1.0 mg/ml BHT.

According to Saiga *et al*, the increased antioxidant activity might accompany the increased hydrophobicity of the peptides. Since the hydrophobic amino acid content in the protease hydrolysates was greater than that in the papain hydrolysates, the hydrophobicity of the protease hydrolysate was larger than that of the protease hydrolysate. On the other hand, the general hydrolysate has the stronger antioxidant activity than its ethanolic extraction counterpart. According to the RSA and antioxidant activity in linolenic acid system, # 4 sample was selected for further separation and purification for next tests.

Separation of Antioxidant Peptides. #4 hydrolysate was isolated and characterized by the Sephadex LH-20 and ion exchange column chromatography (see Figure 5). Sephadex column chromatography provided three peptide fractions from this hydrolysate, all of which have in a certain degree some antioxidant activity in lipid peroxidation system and DPPH radical scavenging activity compared compared to the control (no peptides). Fraction I showed the strongest activity among all fractions (Figure 5). The peptides in fraction I were further separated by anion exchange column chromatography and a reversed-phase HPLC column in order to isolate and purify more purified antioxidant peptides. This work will continue in the next project.

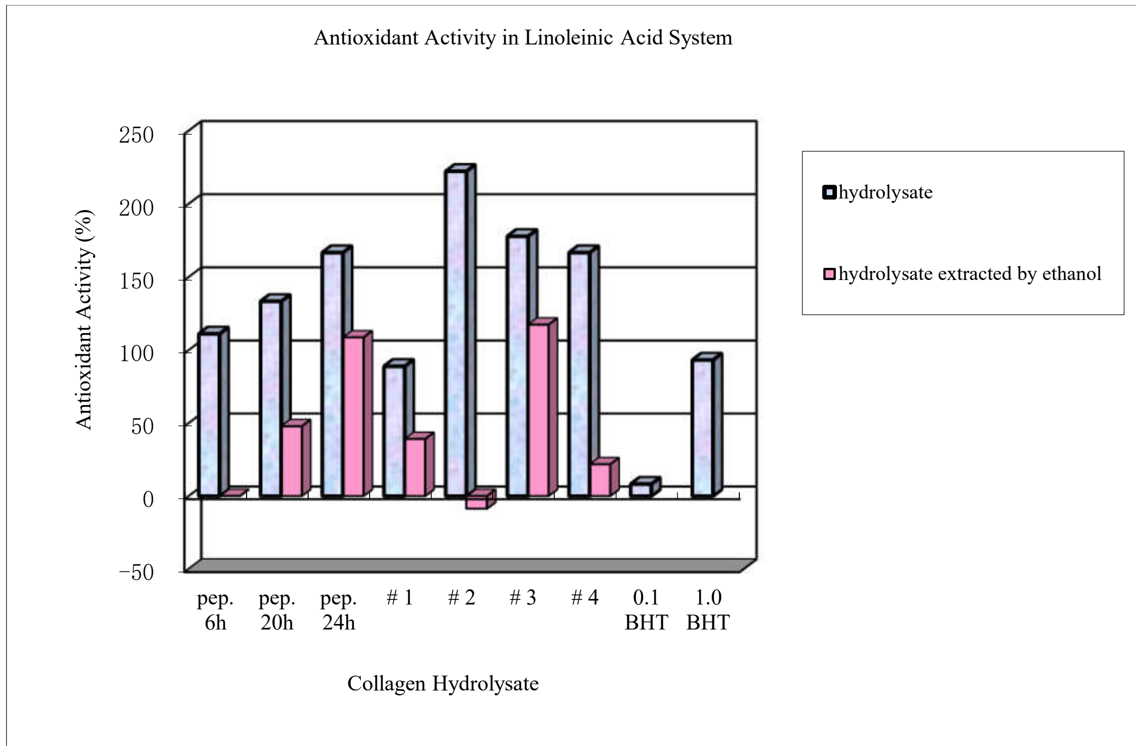

Fig. 4 The antioxidant activity of collagen hydrolysates by different protease treatment in linolenic acid peroxidation system

fig.7 Purification of antioxidant peptide from the third hydrolysate of collagen