FINAL REPORT
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Screening Bioactive Peptides from Animal By-product Proteins

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Start Date: ***June 15, 2006***

Duration of Project*:* *3 years (2006-2009)*

Objective (s):

The team will prepare, separate, screen, purify, identify, and if necessary, synthesize bioactive peptides as those in the collagen proteolytic hydrolysate. The proposed study is a 3-year plan to use *in vitro* models to test the following hypotheses. (1) there are short-chain peptides with strong antioxidant activities, most of which are composed of less than 10 amino acids with a similar core structure (amino sequence); (2) some of these short chain peptides possess anti-hypertension activities in animals; (3) some of these peptides also will have anti-inflammatory and anti-carcinogenic activities; (4) these bioactive peptides could be practically prepared via non-complex, cost-effective enzymatic reactions and produced in high reproducibility. If the first-phase objectives aforementioned can be finished in time, we plan to do *in vivo* animal test to confirm our results from the *in vitro* tests, and produce value-added products.

Project Overview:

Our preliminary experiment started from this summer (June) has resulted in some positive results as we have hypothesized. These results include (1) enzymatic hydrolysates from porcine collagen were found to possess strong antioxidant activities using *in vitro* DPPH and metal chelating methods; (2) chromatographic separation of these hydrolysates in an analytical scale tentatively seemed practical and feasible, which helped building a solid foundation for potential scale up. The aforementioned results are shown in the following details.

Porcine skin collagen was hydrolyzed by several proteases (e.g., pepsin, papain, protease from *Streptomyces*, etc.) singly or in combination. It was found that the types of enzymes and their combination significantly affected the antioxidant activities of the hydrolysates. Among them, one obtained through the treatment of using a cocktail mixture of three enzymes exhibited the highest antioxidant (DPPH and metal chelating) activities, which was stronger than that of 2mM BHT (see **Figure 1**).

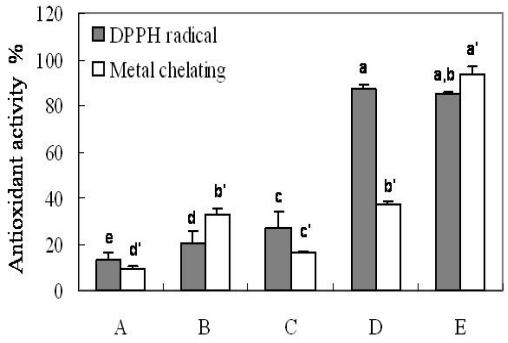

Figure 1. Comparison of the DPPH radical scavenging and metal chelating activities of standards (BHT for DPPH, EDTA for metal chelating) with those of the hydrolysates from porcine skin collagen, which was hydrolyzed by pepsin (A), further hydrolyzed with papain (B), protease from bovine pancreas (PP) (C), cocktail mixture of enzymes (D). BHT (2.0 mM in methanol) and EDTA (1.0 mM in distilled water) (E) were used as positive controls. Error bars show the standard deviations. Values are the means of triplicate analyses. a, b, c, d, e for DPPH radical, a', b', c', d' for metal chelating, are significantly different at $p < 0.05$ with Student's *t*-test. Means followed by the same letter are not significantly different

Further investigation found that proteolytic degree of hydrolysis and hydrolyzing time also had significant influence on the antioxidant activity of the collagen hydrolysates (see **Figure 2**).

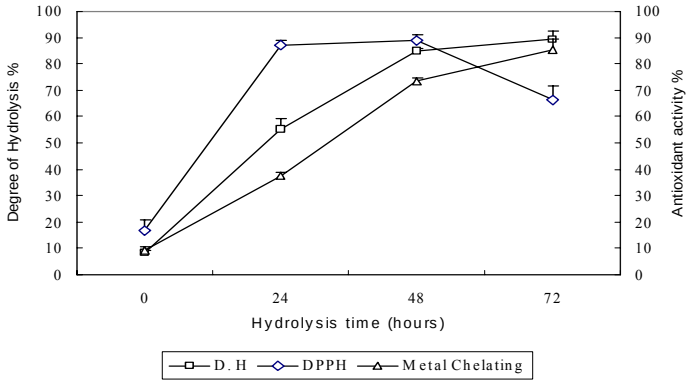

Figure 2: Degree of hydrolysis and antioxidant activities of porcine skin collagen hydrolysates by cocktail mixtures of three crude enzymes: PP, PS and PB at different hydrolysis time. PP: protease from bovine pancreas; PS: protease from *Streptomyces*; PB: protease fro *Bacillus polymyxa*

Such hydrolysates obtained from the cocktail enzymatic treatment were then separated by a Sephadex LH-20 gel filtration column into five fractions (P1-5) that showed different degree of antioxidant activities (see **Figure 3**).

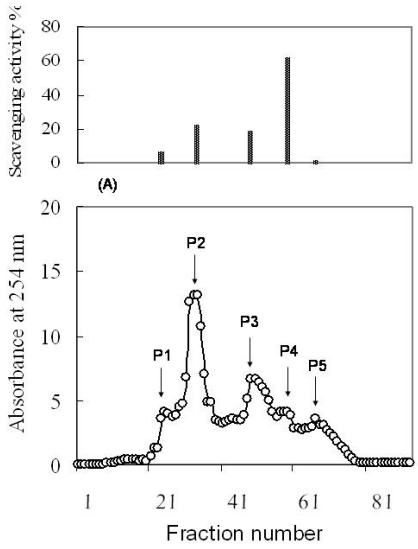

Figure 2: Antioxidant peptide fractions (P1-P5) that were obtained from the cocktail enzymatic treatment were separated by gel filtration chromatography on a Sephadex LH-20 column.

Research work on identifying the sequence of those bioactive peptides is on progress.

Impacts and Significance:

This research is based on our current knowledge on bioactive peptides that can be produced, either chemically or biologically, from some agricultural protein sources. Since bioactive peptides may be also able to be extracted from the rendered animal co-products, and developed as nutrient, safe, and disease-prevention products suitable for being used as pet foods and aquacultural feed, the rendered co-products are hopefully to be developed into novel products with high added-values. Also, a huge benefit (profits) is expected to the render industry because of the huge market for pet and aqualtural foods.

Publications: in preparation

Future Work: Within the next six months, we will focus on the following objectives: (1) selecting suitable chromatographic techniques to separate hydrolysates in larger quantities for screening potential bioactive peptides; (2) trying to use chromatographic techniques (e.g., HPLC and LC-MS) to identify one or two most potent bioactive peptides.

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