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Isolation of small molecular peptide extract from chicken breast muscle using gastrointestinal enzymes and characterization of its antioxidant activity (#567)

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Introduction

Chicken breast is well accepted by consumers all over the world. Chicken-derived protein is known to be rich in essential amino acids with highly biological value. Food derived peptides with their bioactivities (e.g., antioxidant) have been reported in a variety of foods such as, milk, eggs, fish and a few animal meat muscles (Erdmann *et al*. 2008; Ryan *et al*. 2011). By using common isolation methods (e.g., autolysis with buffer), researchers have reported that peptides isolated from chicken sources (e.g., breast and skin etc.) possess a wide range of bioactivity (Jamdar *et al*. 2012; Onuh *et al*. 2014). To the best of our knowledge, however, there is no study isolating peptides from chicken breast by using gastrointestinal enzymes such as, pepsin and pancreatin. Thus, the objective of this study was to isolate peptides from chicken breast muscle using digestive enzymes and characterize their antioxidant activity.

Methods

Samples preparation

Chicken breast samples purchased from a local market were used. The samples were cooked in a boiling water bath until inner temperature reached to 75. After cooling at room temperature, the cooked chicken breast samples were hydrolyzed by (i): pepsin at pH 2.0 and 37℃ for 2 h, and (ii): pancreatin at pH 8.0 and 37℃ for 8 h (Chang *et al*. 2006) and separated by the time of enzyme treatment as follow; (P0): pepsin 0 h, (P2): pepsin 2 h, (PP1): pepsin 2 h + pancreatin 1 h, (PP2): pepsin 2 h + pancreatin 2 h, (PP3): pepsin 2 h + pancreatin 3 h, (PP4): pepsin 2 h + pancreatin 4 h, (PP5): pepsin 2 h + pancreatin 5 h, (PP6): pepsin 2 h + pancreatin 6 h, (PP7): pepsin 2 h + pancreatin 7 h, (PP8): pepsin 2 h + pancreatin 8 h. Hydrolysates were centrifuged at 10,000 × *g* for 20 min. The supernatant was collected and filtered with Whatman filter paper (No. 1), the filtrates were then kept at -78℃ in a deep freezer until use.

Fractionation of hydrolysates

Three fractions with different molecular weight ranges: >10 kDa, 3-10 kDa and <3 kDa were prepared by ultracentrifuging using a filtration membrane with molecular weight cutoff of 10 kDa (Millipore). The fractionated samples were concentrated and then re-separated on a C_{18} column (2.2 cm × 10 cm, GRACE VYDAC, USA) connected to a high-performance liquid chromatography (HPLC) (Jasco, Easton, MD, USA). Peaks were collected and concentrated by freezing-dried before using for antioxidant activity assay.

Antioxidant activity

The free radical-scavenging activity of the crude peptide extracts (CPEs), fractions, and peaks were determined using the 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid, $ABTS^{+}$) radical cation according to method of Chang *et al*. (2013), and using a ORAC assay kit (Cell Biolabs).

Statistical analysis

Statistical analysis was performed with the SAS program for Windows V9.2 (SAS Institute, Cary, NC, USA). General linear model (GLM) with Duncan's multiple range test was carried out to analyze significant differences among the treatments ($p<0.05$).

Results

The antioxidant activities of crude peptide extracts (CPEs) measured using ABTS radical scavenging activity and ORAC assay kit are presented in Figure 1. The radical scavenging activities of CPEs were significantly higher in the samples treated by the gastrointestinal enzymes (above 60%) compared with control (7.29±0.54%) and P0 (8.34±0.45%) ($p<0.05$). No differences in antioxidant activities of CPEs occurred between the two enzymes used ($p>0.05$, Fig. 1a). In the case of ORAC assay, the antioxidant activity of PP2 showed the highest value (105.10±0.23 mg TE) whereas, the control showed the lowest value (32.11±0.86 mg TE) ($p<0.05$, Fig. 1b).

The small molecular weight (<3 kDa) fraction of PP2 showed the highest antioxidant activity in both ABTS (15.71±0.05 μM GE) and ORAC (189.09±0.41 μM GE) assays compared with the larger molecular weight fractions ($p<0.05$, Fig. 2a and b).

The antioxidant activity of peak fractions (I to X) was measured using the ABTS assay (Fig. 3a & b), the antioxidant activity ranged from 9.88% to 65.72% for the chicken breast muscle CPE PP2-S (<3 kDa). Among all the peak fractions collected on the RP-HPLC, the highest activity was found in the peak fraction III (65.72%), followed by fraction IV (61.20%) ($p<0.05$).

Conclusion

Based on the results obtained from the present study, it may be concluded that the peptides fraction with small molecular weight (<3 kDa) isolated the chicken breast muscle by hydrolyzing with digestive enzymes exhibited strong antioxidant activity. Further study to identify the amino acids sequence of these peptides and test for their other bioactivities is needed.

Notes

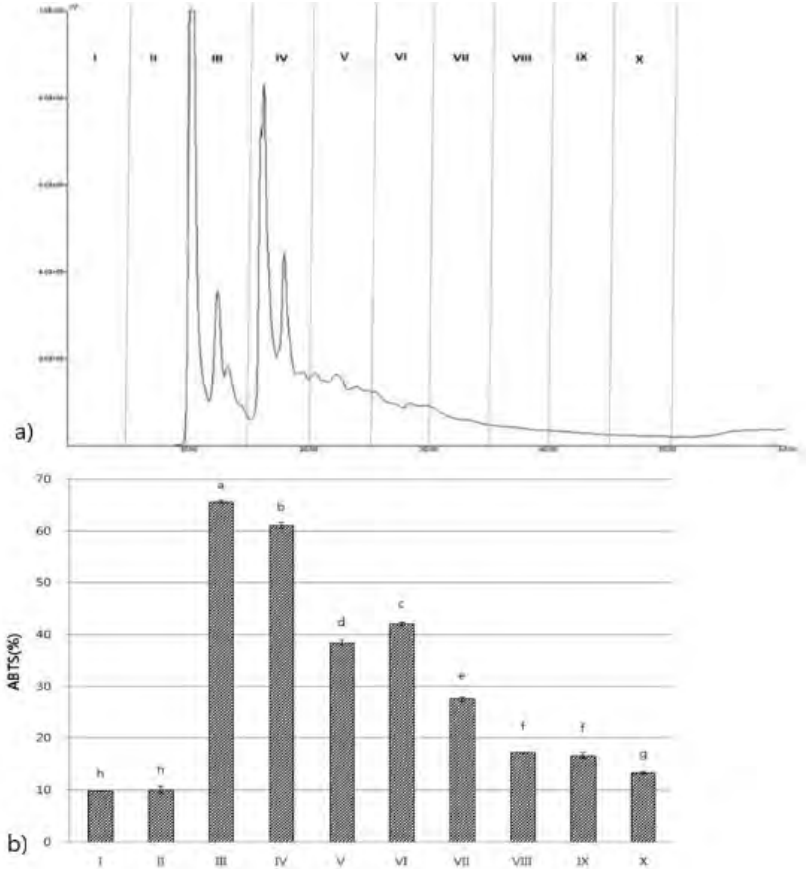

Fig. 3. The chromatogram (a) and antioxidant activity (b) of smll molecular weight fractin PP2.
Different letters (a-g) indicate significant differences in antioxidant activity among the peak fractions ($p<0.05$).

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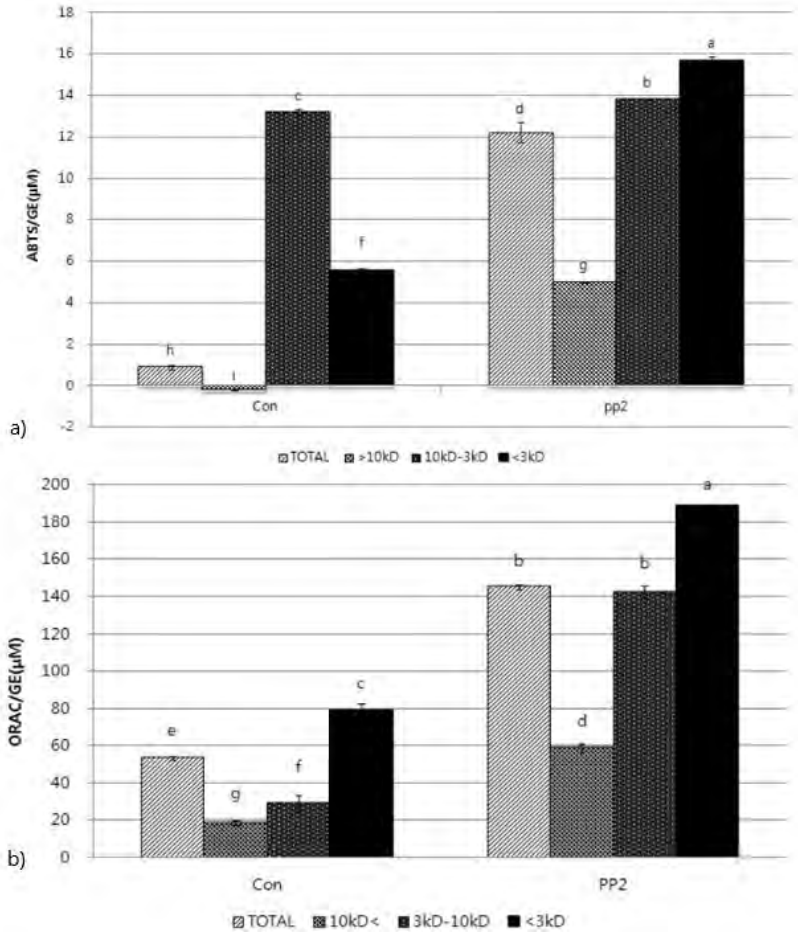

Fig 2. Antioxidant activity of ultra-filtrated fractions from chicken breast meat hydrolysates PP2.
ABTS assay (a) and ORAC assay (b); Different letters (a-f) indicate significant differences ($p<0.05$). (Con): no enzyme treated, (PP2): pepsin 2 h + pancreatin 2 h

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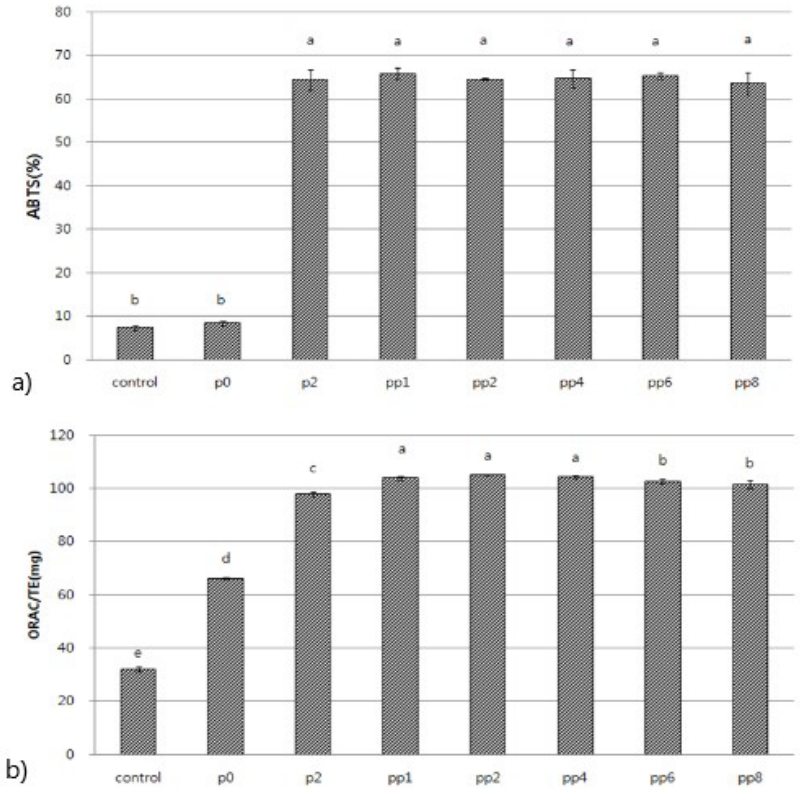

Fig 1. Antioxidant activity of chicken breast meat crude extracts.
ABTS assay (a) and ORAC assay (b); Different letters (a-e) indicate significant difference at $p<0.05$. (Con): no enzyme treated, (P0): pepsin 0 h, (P2): pepsin 2 h, (PP1): pepsin 2 h + pancreatin 1 h, (PP2): pepsin 2 h + pancreatin 2 h, (PP3): pepsin 2 h + pancreatin 3 h, (PP4): pepsin 2 h + pancreatin 4 h, (PP5): pepsin 2 h + pancreatin 5 h, (PP6): pepsin 2 h + pancreatin 6 h, (PP7): pepsin 2 h + pancreatin 7 h, (PP8): pepsin 2 h + pancreatin 8 h

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