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Identification and verification of antioxidant active peptides derived from gastrointestinal enzymatic hydrolysis of chicken breast muscle (#568)

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Introduction

Biologically active peptides were defined as "food-derived peptides that exert, beyond their nutritional value, a physiological, hormone-like effect in humans" (Erdmann *et al*., 2008). Bioactive peptides consist of natural amino acid sequences (often 2 to 20 residues) encrypted in the parent protein molecule, and are usually inactive within the sequence of the protein. However, they are released during gastrointestinal digestion or *in vitro* hydrolysis with proteases and play important roles in the regulation and modulation of metabolism during digestion of food in the intestine. In the previous study about the isolation of antioxidant small molecular peptide extracts from gastrointestinal enzymatic hydrolysates of chicken breast muscle, there was a small molecular peptide fractions with radical scavenging activity above 60% (PP2-S-III and -IV). This study was performed to identify the amino acid sequence and evaluate the antioxidant activity of the identified peptides.

Methods

Samples preparation

The small molecular peptide fractions which showed the radical scavenging activity were selected as our previous study 'The isolation of antioxidant small molecular peptide extracts from gastrointestinal enzymatic hydrolysates of chicken breast muscle', and it could briefly explained as follow. Chicken breast muscles were cooked and hydrolyzed by pepsin at pH 2, 37℃ for 2 h, and pancreatin at pH 8, 37℃ for 2 h (Chang *et al*., 2006). Hydrolysates were centrifuged and the supernatant was filtered with Whatman No. 1 filter paper. The ultracentrifugal filtration and high-performance liquid chromatography were performed to separate peptides by molecular weight. Finally, small molecular peptides (PP2-S-III and IV) with antioxidant activity were fractionated and used as samples.

Peptide identification using LC-ESI-MS/MS

The peptides from hydrolysate fractions were analyzed by liquid chromatography tandom MS (LC-MS/MS, QStar Elite, Aplied Biosystems, Foster City, CA) equipped with a nano-electrospray ionization (ESI) source using a reverse phase C18 column (Agilent Technologies, Santa Clara, CA). Peptides were electrosprayed through a coated silica tip (PicoTip emitter, New Objective) at an ion spray voltage of 2,000 eV. Mass data were acquired automatically using Analyst QS 2.0 software (Applied Biosystems) with the range of *m/z* was 200 to 2,000).

Antioxidant activity

The free radical-scavenging activity of the chicken breast meat hydrolysates was determined using the 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid, $ABTS^{+}$) radical cation (Sigma-Aldrich, USA), according to a previously described method by Chang *et al*. (2013) and Re *et al*. (1999), and the ORAC assay kit (Cell Biolabs, Inc., USA), based on the method by Ou *et al*. (2001) and Dávalos *et al*. (2004).

Peptide synthesis

Identified peptides with antioxidant activity were chemically synthesized by GL Biochem (Shanghai , China).

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 the significant differences among the processes ($p<0.05$).

Results

The MS analysis via LC-ESI-Q-TOF was performed to identify peptides which have antioxidant activity from the PP2-S-III and PP2-S-IV. As a result, six *de novo* peptides were identified, EVELKE, ENKSELSQ, WILEE, QEVCGTDGVT, QDDVAVPQ, and TGSSNLWVP, respectively (Table 1 and Fig. 1).

Table 1. The result of identification of peptides contained in chicken breast muscle hydrolysate PP2-S using LC-ESI-MS/MS.

No.	Molecular weight (Da)	Amino acid sequence	Fraction No.
1	746.35706	EVELKE (Glu-Val-Glu-Leu-Lys-Glu)	PP2-S-FIII
2	934.44189	ENKSELSQ (Glu-Asn-Lys-Ser-Glu-Leu-Ser-Gln)	PP2-S-FIII
3	689.33948	WILEE (Trp-Ile-Leu-Glu-Glu)	PP2-S-FIII
4	1008.46710	QEVCGTDGVT (Gln-Glu-Val-Cys-Gly-Thr-Asp-Gly-Val-Thr)	PP2-S-FIV
5	871.42920	QDDVAVPQ (Gln-Asp-Asp-Val-Ala-Val-Pro-Gln)	PP2-S-FIII
6	960.45496	TGSSNLWVP (Thr-Gly-Ser-Ser-Asn-Leu-Thr-Val-Pro)	PP2-S-FIV

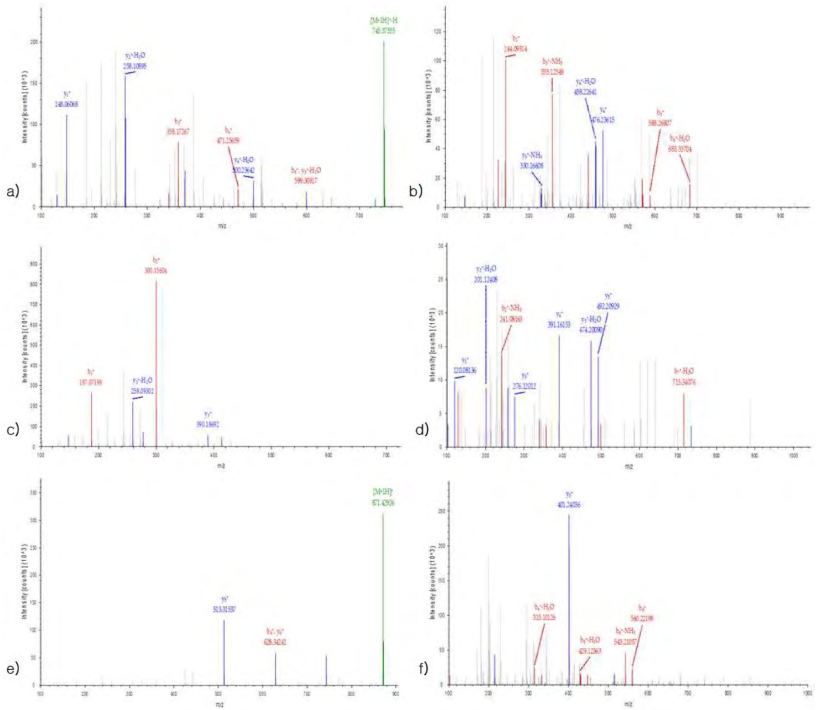
Notes

To investigate whether the peptides identified by MS analysis have antioxidant activity, these six peptides were chemically synthesized and the ABTS and ORAC assay were performed (Fig. 2). The peptide QEVCGTDGVT (92.50±0.15%) showed the highest radical scavenging activity in ABTS assay ($p<0.05$, Fig. 2a) and it has antioxidant activity corresponding to 16.6 μM GE/g. Peptides TGSSNLWVP (325.10±1.18 μM GE/g) and QEVCGTDGVT (324.24±1.44 μM GE/g) were significantly higher antioxidant activity than others in ORAC assay ($p<0.05$, Fig. 2b).

Conclusion

From these results, it could be concluded that the deca-peptide QEVCGTDGVT derived from chicken breast meat hydrolysate using gastrointestinal enzyme has high radical scavenging and oxygen radical absorbance capacity by their hydrophobic amino acid residues (Val and Thr), acidic amino acid residues (Gln, Glu, Asp, Asn, and Thr), and the nona-peptide TGSSNLWVP has high oxygen radical absorbance capacity. Further study on antioxidant activity by the identified peptides *in vivo* will be needed for their future use in the meat and meat products industry as functional biomaterials or a healthy dietetic method.

Notes



LC-ESI-MS/MS spectrum of peptides contained in chicken breast muscle hydrolysate PP2-S.
EVELKE (a), ENKSELSQ (b), WILEE (c), QEVCGTDGVT (d), QDDVAVPQ €, and TGSSNLWVP (f).

Notes

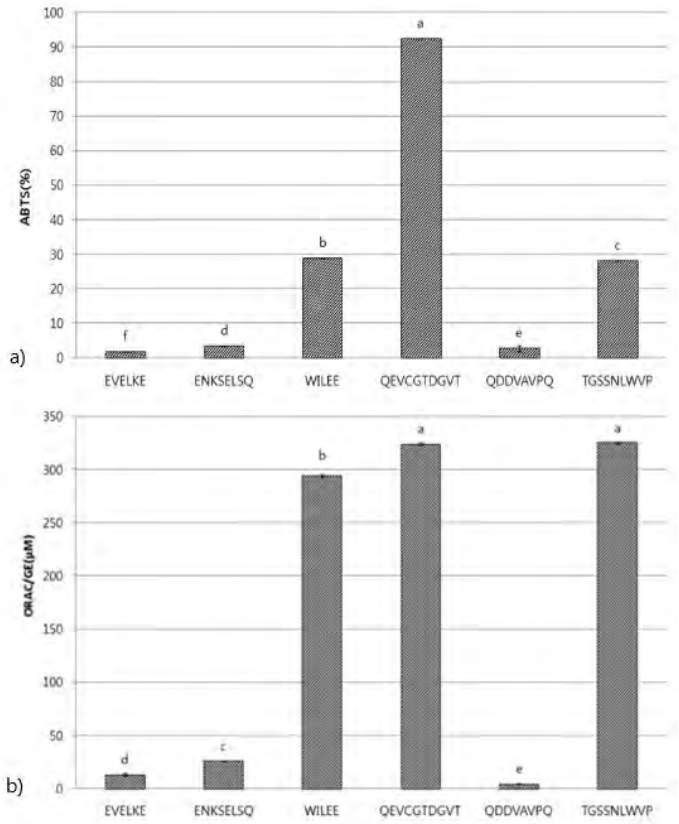


Fig 2. Antioxidant activities of chemically synthesized peptides identified from PP2-S.
ABTS assay (a) and ORAC assay (b). Different letters above bars indicate significant difference ($p<0.05$).

Notes