Antioxidant activities of a peptide derived from chicken dark meat

Yoko Fukada¹ · Saki Mizutani¹ · Sarika Nomura¹ · Wakana Hara¹ ·
Riko Matsui¹ · Kumiko Nagai¹ · Yuki Murakami¹ · Nanami Washio¹ ·
Narumi Ikemoto¹ · Masaaki Terashima¹

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Abstract Antioxidant activities against hypochlorite ions and peroxy radicals of a chicken dark meat hydrolysate digested with pepsin were examined with the myoglobin method based on the structure change of myoglobin due to redox reaction with reactive oxygen species (ROS). A peptide that showed strong antioxidant activity against the peroxy radical was isolated from the hydrolysate using HPLC equipped with a hydrophobic-interacting column. The sequence of the first five amino acid residues of the peptide was determined as YASGR (Tyr-Ala-Ser-Gly-Arg), and this sequence matched with the amino acid residues 143–147 of chicken β -actin (GenBank: CAA25004.1). The synthetic peptide YASGR showed very high antioxidant activity against the peroxy radical. Antioxidant activities of the free amino acids, confirmed that the tyrosine residue of this peptide was possibly responsible for antioxidant activity.

Keywords Peptide · Antioxidant · Peroxy radical · Myoglobin · Chicken meat

Introduction

Various reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by the metabolism of oxygen in vivo (Villamena 2013). Super oxide anion, hydrogen peroxide, hydroxyl radicals, peroxy radicals, and hypochlorite ions are typical ROS. A number of studies have provided

evidences that excess oxidative stress is closely related to aging processes and various diseases including cancer, Alzheimer disease and other life-style related diseases (Vina et al. 2011; Chen and Keaney 2012; Castillo 2009). Excess oxidative stress is caused by the excessive production of ROS and/or impaired antioxidant removability in vivo (Gupta et al. 2014). The dietary intake of antioxidants, therefore, may be helpful to maintain a good antioxidant capacities and is strongly recommended by medical doctors. Based on this, for decades the researchers in various fields have examined numerous food compounds as potential candidates for antioxidants (Zaknun et al. 2012; Taghvaei and Jafari 2015). A large number of studies have reported the characteristics of polyphenols in plants as antioxidants, and their functional roles in vivo (Li et al. 2014).

Recent studies also have revealed that peptides derived from food proteins have various functions such as antihypertensive, antioxidant, and antithrombotic activities (Najafian and Babji 2012; Ryan et al. 2011; Chakrabarti et al. 2014; Kim et al. 2012). These peptides consist of 2–20 amino acids and their molecular masses are less than 6000 Da. Antioxidant peptides have been found in the hydrolysates of meat muscles and byproducts (Lafarga and Jayes 2014; Centenaro et al. 2011; Onuh et al. 2014; Mann et al. 2014; Sun et al. 2011; Sowmya et al. 2014), and also in the proteins of various plants (Zhang et al. 2010; Chen et al. 2007; Zhu et al. 2008; Xie et al. 2008; Siswoyo et al. 2011). The amino acid side chains most likely determine the antioxidant activities of the peptides, because thiol in cysteine, thioether in methionine, the indole group in tryptophan, the phenolic hydroxyl group in tyrosine, imidazole group in histidine are relatively easily oxidized (Houglund et al. 2013). The amino acid sequence also has significant effects on the antioxidant activity strength of the peptides (Saito et al. 2003). The effects of antioxidant peptides have also demonstrated in cultured cells (Sheih et al. 2010;

✉ Masaaki Terashima
terasima@mail.kobe-c.ac.jp

¹ Department of Biosphere Sciences, Kobe College, 4-1, Okadayma, Nishinomiya, Hyogo 662-8505, Japan

Himaya et al. 2012). For example, two peptides, CAAP (Cys-Ala-Ala-Pro) and VCSV (Val-Cys-Ser-Val), derived from a flounder fish protein scavenged intracellular reactive oxygen species in Vero cells (Ko et al. 2013). The specific amino acid sequence might be a key factor in the functional effects of the peptide in vivo. Moreover, egg white digested with trypsin had an increased effect in plasma radical scavenging in spontaneous hypertensive rats (SHR) (Manso et al. 2008).

To screen for effective antioxidants from food, the evaluation protocol that is used to determine the antioxidant activities of foods is a very important factor (Moon and Shibamoto 2009). The oxygen radical absorption capacity (ORAC) assay proposed by Wu et al. (2004a, b) is a very popular method, but it takes 1 to 2 h to measure the antioxidant activity. Terashima et al. (2007, 2010a) proposed the myoglobin method to evaluate antioxidant activities against hypochlorite ions, hydroxyl radicals, peroxy radicals, and peroxynitrite. This protocol was applied to evaluate the antioxidant activities of various standard substances (Terashima et al. 2010a), flavonoids (Terashima et al. 2012), vegetables and beans (Terashima et al. 2013), and traditional Japanese miso seasoning (Morikawa et al. 2014). Because this protocol is simple and quick, it is suitable to screen antioxidants against hypochlorite ions, hydroxyl radicals, peroxy radicals, and peroxynitrite.

Effects of amino acid residues and amino acid sequences on the antioxidant activities of the antioxidant peptides are an important issue to clarify the functional roles of the antioxidant peptides. The purpose of this work is to identify antioxidant peptides in chicken dark meat hydrolysate digested with pepsin, and characterize their antioxidant activities. A novel antioxidant peptide, YASGR (Tyr-Ala-Ser-Gly-Arg), was found in this work and this amino acid sequence matched with those from residues 143 to 147 of chicken β -actin. This peptide showed very strong antioxidant activity against the peroxy radical. The contribution of the amino acid residues of the peptide YASGR on the antioxidant activity was discussed based on the experimental results.

Materials and methods

Materials

Chicken dark meat was purchased at a local market. Pepsin from porcine gastric mucosa (Sigma-Aldrich, St. Louis, USA) was used to prepare hydrolysates. Myoglobin (equine skeletal muscle, 95–100 %) and peroxynitrite were purchased from Sigma-Aldrich (USA) and Dojin Chemicals (Japan), respectively. The synthetic peptide, YASGR, was purchased from Funakoshi Corporation (Japan). All other reagents were of reagent grade.

Preparation of hydrolysate

Chicken dark meat (18 g) was boiled for 6 min, and ground with a mortar and pestle. After the ground meat was added to 200 ml MilliQ water containing 0.35 g NaCl and 0.2 g pepsin, the pH of the solution was adjusted to pH 2.0 with 1.0 M HCl. The solution was then incubated at 37 °C, and 20 ml of the sample was collected at 0, 15, 25, 30, 45, 60, and 90 min. To terminate pepsin digestion, the pH of the sample was adjusted to 7.0 with 1 M NaOH (Terashima et al. 2010b). The sample was centrifuged with a table top centrifuge (TX-201, Tomy, Japan) for 10 mins at 10,000 rpm, and the recovered supernatant was filtered with a filter (Minisart, (pore size 0.2 μ m), Sartorius Stedim Biotech, Germany). The sample was divided into two aliquots (about 10 ml), and were stored at -80 °C for future use.

Fig. 1 Effect of digestion time on myoglobin protection ratio **a** Myoglobin protection ratio against peroxy radicals ($n = 3$), **b** Myoglobin protection ratio against hypochlorite ions ($n = 3$)

Table 1 Myoglobin protection ratio of the fractionated samples

Retention time (min)	Myoglobin protection ratio against peroxy radicals (%)	Myoglobin protection ratio against hypochlorite ions (%)
0–20	27.0	56.6
20–40	20.1	19.2
40–60	25.3	23.0
60–80	9.0	5.7
80–100	0.0	0.6
100–120	6.1	0.4

Fractionation of hydrolysate by ultrafiltration and dialysis

In order to prepare the sample to be used for the screening of antioxidant peptides, chicken meat (202.5 g) was processed by scaling up the above mentioned protocol. The hydrolysate (2250 ml) digested for 60 mins with pepsin was centrifuged

with a centrifuge (Himac CR22E, Rotor PRP12–2, Hitachi, Japan) at 10,000 rpm for 20 mins. Then, the recovered supernatant was fractionated with filtration and dialysis (Centenaro et al. 2014). An ultrafiltration unit (Vivaflow 50, Sartorius Stedim Biotech, Germany) was used. The molecular cut off of the membrane was 3000 Da, and the membrane area was 50 cm². The filtrate (1700 ml) was freeze-dried with a freeze dryer (FDU-2200, EYELA, Japan), and the resulting solid was dissolved in 50 ml of Milli-Q water. The prepared solution was divided into 5 aliquots (10 ml each), and each aliquot was dialyzed against 1000 ml of Milli-Q water with a Spectropore® dialysis membrane (MWCO 500 Da, SpectrumLabs, USA) for 24 h. The dialyzed hydrolysates were collected, and freeze-dried. Finally, the obtained solid was dissolved in 10 ml of Milli-Q water.

Separation of peptides in hydrolysate with HPLC

The peptides in the fractionated hydrolysate were separated with a HPLC system (LC10A, Shimadzu, Japan) equipped

Fig. 2 Chromatogram of hydrolysate (Top: Fraction No. 1 (retention time: 0–20 min), bottom: Fraction No. 2 (retention time: 20–40 min))

with a hydrophobic interacting column (Cosmosil 5C18-MS-II, 4.6 mm × 150 mm, Nacalai Tesque, Japan). Solution A (Milli-Q water containing 0.1 % (v/v) trifluoroacetic acid) and solution B (acetonitrile containing 0.1 % (v/v) trifluoroacetic acid) were used as the mobile phases. The following gradient program was applied to separate the peptides: B concentration 30 % at 90 min, B concentration 50 % at 100 min, B 0 % at 105 min, and stop at 120 min. The peptides in the effluent were monitored at 215 nm with a spectrophotometer (SPD-20 A, Shimadzu, Japan). While 20 µl of the sample was injected for analytical purpose, 200 µl of the sample was applied to the HPLC for the fractionation. The fractionated samples were freeze-dried, and the resulting solids were dissolved in Milli-Q water.

Myoglobin protection ratio

The antioxidant activities of the samples (hydrolysate, peptides, and free amino acids) against ROS (peroxyl radicals and hypochlorite ions) were evaluated with the myoglobin method proposed by our group (Terashima et al. 2010a). In the case of the peroxyl radical, all the solutions were pre-incubated at 47 °C. To 2.5 ml of myoglobin solution, 0.1 ml of a test sample, and 1.0 ml 2, 2'-azobis(2-methylpropionamide) dihydrochloride solution were added, and incubated at 47 °C for 15 min before absorbance measurement. For antioxidant activity measurement against the hypochlorite ion, 3.0 ml of myoglobin solution (0.25 mg/ml), 0.1 ml test sample, and 0.5 ml of hypochlorite solution (0.02 %) were mixed, and the absorbance of the reaction mixture was measured at 409 nm. In the myoglobin method, antioxidant activities were evaluated based on the changes of absorbance at 409 nm of myoglobin because of the reaction with ROS.

Table 2 Myoglobin protection ratio of peak fractions against peroxyl radicals

Peak no.	Myoglobin protection ratio against peroxyl radicals (%)
1	2.5
2	0.7
3	13.5
4	0.0
5	10.5
6	9.0
7	5.4
8	7.4
9	5.8

Fig. 3 Effect of peptide concentration on myoglobin protection ratio against peroxyl radicals ($n = 3$)

Myoglobin protection ratio (%) for the sample was defined by the following equation:

Myoglobin protection ratio (%)

$$= \left\{ 1 - \frac{ABS^0 - ABS^R \text{ (with antioxidant)}}{ABS^0 - ABS^R \text{ (without antioxidant)}} \right\} \times 100$$

where, ABS^0 is the absorbance of the myoglobin solution (control), ABS^R (without antioxidant) is the absorbance of the measurement solution containing only ROS, and ABS^R (with antioxidant) is the absorbance of the measurement solution containing both the test sample and ROS. Average values were obtained from triplicate measurements.

Fig. 4 Myoglobin protection ratio of amino acids against peroxyl radicals ($n = 3$) ○ Tyr, □ Ala, △ Ser, ◇ Gly, ▽ Arg

Amino acid sequences of isolated antioxidant peptides were determined with a protein sequencer ABI Procise 491HT (Applied Biosystems, USA).

Results and discussion

Figure 1 shows the effects of digestion time with pepsin on the myoglobin protection ratio of the hydrolysate against peroxyl radicals and hypochlorite ions. The myoglobin protection ratio against peroxyl radicals and hypochlorite ions increased from 60 % to 100 % and 50 % to 90 % respectively during the 90 min digestion (Fig. 1a and 1b). The relatively high myoglobin protection ratio at reaction time zero was probably because of pre-existing amino acids, peptides, and proteins in the sample. Although digestion process contributes about 40 % of the antioxidant activity of the hydrolysate, these results clearly indicated that the amino acids and/or peptides generated by the pepsin digestion had antioxidant activities against hypochlorite ions and peroxyl radicals. Based on these results, the screening of antioxidant peptides in the hydrolysate digested with pepsin for 60 min in the following study was done.

The hydrolysate containing peptides with molecular weights in the range of 500–3000 Da was fractionated using HPLC. The effluent from the column was fractionated every 20 min. The myoglobin protection ratio of the fractions against peroxyl radicals and hypochlorite ions is shown in Table 1. A relatively high myoglobin protection ratio against both peroxyl radicals and hypochlorite ions was observed in fraction No. 1 (0–20 min.), No. 2 (20–40 min), and No. 3 (40–60 min). The peaks were not well separated in the chromatogram. Therefore, fractions No. 1 and No. 2 were used. Furthermore, to focus on the antioxidant activity of the peptides against peroxyl radicals was worked out, because the peroxyl radicals causes peroxidation of various polyunsaturated fatty acids, and is closely related to many diseases. Nine well separated peaks were observed in the chromatograms of fractions No. 1 and No. 2 (Fig. 2). Peak 3, 5, 6, 8, and 9 showed high myoglobin protection ratios against peroxyl radicals (Table 2).

Amino acid sequences of the first five residues of the peptides from peak fractions 3, 5, 6, and 9 were analyzed. The amino acid sequence of the first five residues of the peptide in peak fraction No. 5 was determined as YASGR. This sequence corroborated with the amino acid sequence of residues 143–147 of chicken β -actin (GenBank: CAA25004.1).

The concentration effects of the synthesized peptide YASGR on the myoglobin protection ratio against peroxyl radicals is shown in Fig. 3. The myoglobin protection ratio increased with the concentration of the peptide YASGR, and reached 89.8 % at the peptide concentration of 10 mM

(278 μ M in the reaction mixture). The previous works (Terashima et al. 2010a; 2012) showed that the myoglobin protection ratios of ferulic acid, Trolox, ascorbic acid, chlorogenic acid, and glutathione at the same concentration were 100 %, 98.3 %, 95.7 %, 58.3 % and 32.1 %, respectively. Thus, the antioxidant activity of the peptide YASGR against peroxyl radicals was almost the same as Trolox and ascorbic acid. The antioxidant activity of the peptides may be attributed to the amino acid side chains. To elucidate which amino acid residue contributed to the antioxidant activity of the peptide YASGR, the myoglobin protection ratio of the five free amino acids, tyrosine, alanine, serine, glycine, and arginine against peroxyl radicals was determined (Fig. 4). Although alanine, serine, glycine, arginine, and threonine showed very low myoglobin protection ratio, tyrosine showed a 40 % myoglobin protection ratio at 1.0 mM (27.8 μ M in the reaction mixture). The myoglobin protection ratio of tyrosine was determined for only concentrations of 0.5 mM (13.9 μ M in the reaction mixture) and 1.0 mM (27.8 μ M in the reaction mixture) because of its low water solubility. The side chain of tyrosine is known to easily undergo oxidative additions in the presence of ROS and RNS (Maskos et al. 1992; Gieseg et al. 1993). These results strongly suggest that tyrosine is the key amino acid residue responsible for the antioxidant activity of the peptide YASGR against peroxyl radicals.

In conclusion, the antioxidant peptide YASGR derived from β -actin of chicken meat was identified. This peptide showed very high antioxidant activity against the peroxyl radical because of its tyrosine residue.

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References

- Castillo R (2009) Metabolic syndrome. In: Rodrigo R (ed) Oxidative stress and antioxidants – their role in human disease. Nova Scientific Publishers, Inc., New York, pp. 159–192
- Centenaro GS, Mellado MS, Prentice-Hernández C (2011) Antioxidant activity of protein hydrolysates of fish and chicken bones. *Adv J Food Sci and Technol* 3:280–288
- Centenaro GS, Salas-Mellado M, Pires C, Btista I, Nunes ML, Prentice C (2014) Fractionation of protein hydrolysates of fish and chicken using membrane ultrafiltration: Investigation of antioxidant activity. *Appl Biochem Biotechnol* 172:2877–2893
- Chakrabarti S, Jahandideh F, Wu J (2014) Food-derived bioactive peptides on inflammation and oxidative stress. *BioMed Res Int* 608979: 1–11
- Chen K, Keaney JF Jr. (2012) Evolving concepts of oxidative stress and reactive oxygen species in cardiovascular disease. *Curr Atheroscler Rep* 14:476–483
- Chen GT, Zhao I, Zhao LY, Cong T, Bao SF (2007) In vitro study on antioxidant activities of peanut protein hydrolysate. *J Sci Food Agric* 87:357–361

- Gieseg SP, Simpson JA, Charlton TS, Duncun MW, Dean RT (1993) Protein-bound 3, 4-dihydroxyphenylalanine is a major reductant formed during hydroxyl radical damage to proteins. *Biochemistry* 32:4780–4786
- Gupta RK, Patel AK, Shah N, Chaudhary AK, Jha UK, Yadav UC, Gupta PK, Pakuwal U (2014) Oxidative stress and antioxidants in disease and cancer: a review. *Asian Pac J Cancer Prev* 15:4405–4409
- Himaya SWA, Ryu B, Ngo D-H, Kim S-W (2012) Peptide isolated from Japanese flounder skin gelatin protects against cellular oxidative damage. *J Agric Food Chem* 60:9112–9119
- Houglund JL, Darling J, Flynn S (2013) Protein posttranslational modification. In: Villamna FA (ed) *Molecular basis of oxidative stress—chemistry, mechanism, and disease pathogenesis*. John Wiley & Sons, Inc. Hoboken, pp. 71–92
- Kim S-K, Wijesekara I, Park EY, Matsumura Y, Nakamura Y, Sato K (2012) Proteins and peptides as antioxidants. In: Hettiarachchy NS, Sato K, Marshall MR, Kannan A (eds) *Bioactive food proteins and peptides – applications in human health*. CRC Press, Boca Raton, pp. 97–116
- Ko JY, Lee H, Samarakoon K, Kim JS, Jeon YJ (2013) Purification and determination of two novel antioxidant peptides from flounder fish (*Parlichthys olivaceus*) using digestive proteases. *Food and Chem Toxicol* 52:113–120
- Lafarga T, Jayes M (2014) Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. *Meat Sci* 98:227–239
- Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB (2014) Resources and biological activities of natural polyphenols. *Nutrients* 22:6020–6047
- Mann B, Kumari A, Kumar R, Sharma R, Prajapati K, Mahboob S, Athira S (2014) Antioxidant activity of whey protein hydrolysates in milk beverage system. *J Food Sci Technol* 52:3235–3241
- Manso MA, Miguel M, Even J, Hernández R, Aleixandre A, López-fandino R (2008) Effect of the long-term intake of an egg white hydrolysate on the oxidative status and blood lipid profile of spontaneously hypertensive rats. *Food Chem* 109:361–367
- Maskos Z, Rush JD, Koppenol WH (1992) The hydroxylation of phenylalanine and tyrosine: a comparison with salicylate and tryptophan. *Arch Biochem Biophys* 296:521–529
- Moon JK, Shibamoto T (2009) Antioxidant assays for plant and food components. *J Agric Food Chem* 57:1655–1666
- Morikawa M, Ando Y, Kita A, Sugita M, Matsumura S, Terashima M (2014) Comprehensive evaluation of antioxidant activity of miso with myoglobin method. *Food Sci and Technol Res* 20:1221–1228
- Najafian L, Babji AS (2012) A review of fish-derived antioxidant and antimicrobial peptides: their production, assessment, and applications. *Peptides* 33:178–185
- Onuh JO, Girgih AT, Aluko RE, Aliani M (2014) *In vitro* antioxidant properties of chicken skin enzymatic protein hydrolysates and membrane fractions. *Food Chem* 150:366–373
- Ryan JT, Ross RP, Bolton D, Fitzgerald GF, Stanton C (2011) Bioactive peptides from muscle sources: meat and fish. *Nutrients* 3:765–791
- Saito K, Jin D-H, Ogawa T, Muramoto K, Hatakeyama E, Yasuhara T, Nokihara K (2003) Antioxidant properties of tripeptide libraries prepared by the combinatorial chemistry. *J Agric Food Chem* 51:3668–3674
- Sheih I-C, Fang TJ, Wu T-K, Lin P-H (2010) Anticancer and antioxidant activities of the peptide fraction from algae protein waste. *J Agric Food Chem* 58:1202–1207
- Siswoyo TA, Mardiana E, Lee KO, Hosokawa K (2011) Isolation and characterization of antioxidant protein fractions from melinjo (*Gnetum gnemon*) seeds. *J Agric Food Chem* 59:5648–5656
- Sowmya R, Ravikumar TM, Vivek R, Rathinaraj K, Sachindra NM (2014) Optimization of enzymatic hydrolysis of shrimp waste for recovery of antioxidant activity rich protein isolate. *J Food Sci Technol* 51:3199–3207
- Sun Q, Shen H, Luo Y (2011) Antioxidant activity of hydrolysates and peptide fractions derived from porcine hemoglobin. *J Food Sci Technol* 48:53–60
- Taghvaei M, Jafari SM (2015) Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. *J Food Sci Technol* 52:1272–1282
- Terashima M, Nakatani I, Harima A, Nakamura S, Shiiba M (2007) New method to evaluate water-soluble antioxidant activity based on protein structural change. *J Agri Food Chem* 55:165–169
- Terashima M, Watanabe R, Ueki M, Matsumura S (2010a) Comprehensive evaluation of antioxidant activity for various substances with 5-axe cobweb chart. *Food Chem* 120:150–155
- Terashima M, Baba T, Ikemoto N, Katayama M, Morimoto T, Matsumura S (2010b) Novel angiotensin-converting enzyme (ACE) inhibitory peptides derived from boneless chicken leg meat. *J Agric Food Chem* 58:7432–7436
- Terashima M, Kakuno Y, Kitano N, Matsuoka C, Murase M, Togo N, Watanabe R, Matsumura S (2012) Antioxidant activity of flavonoids evaluated with myoglobin method. *Plant Cell Rep* 31:291–298
- Terashima M, Fukukita A, Kodama R, Miki H, Suzuki M, Ikegami M, Tamura N, Yasuda A, Morikawa M, Matsumura S (2013) Evaluation of antioxidant activity of leafy vegetables and beans with myoglobin method. *Plant Cell Rep* 32:349–357
- Villamena FA (2013) Chemistry of reactive species. In: Villamena FA (ed) *Molecular basis of oxidative stress—chemistry, mechanism, and disease pathogenesis*. John Wiley & Sons, Inc., Hoboken, pp. 1–48
- Vina J, Gambini J, Lopez-Gruoso R, Abdelaziz KM, Borras C (2011) Females live longer than males: role of oxidative stress. *Curr Pharm Des* 17:3959–3965
- Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2004a) Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem* 52:4026–4037
- Wu X, Gu L, Prior RL, McKay S (2004b) Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. *J Agric Food Chem* 52: 7846–7856
- Xie Z, Huang J, Xu X, Jin Z (2008) Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chem* 111: 370–376
- Zaknun D, Schroecksnadel S, Kurz K, Fuchs D (2012) Potential role of antioxidant food supplements, preservatives and colorants in the pathogenesis of allergy and asthma. *Int Arch Allergy Immunol* 157:113–124
- Zhang J, Zhang H, Wang I, Guo X, Wang X, Yao H (2010) Isolation and identification of antioxidative peptide from rice endosperm protein enzymatic hydrolysate by consecutive chromatography and MALDI-TOF/TOF MS/MS. *Food Chem* 119:226–234
- Zhu L, Chen J, Tang X, Xiong YL (2008) Reducing, radical scavenging, and chelation properties of in vitro digests of alcalase-treated zein hydrolysates. *J Agric Food Chem* 56:661–667