BIOACTIVE PEPTIDES FROM CHICKEN MEAT

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Abstract – The objective of this study was to identify bioactive peptides from *in vitro* digests of chicken protein. For that, Chicken protein (CP) was subjected to a two-stage in vitro digestion (1 h pepsin followed by 2-h pancreatin at 37 ºC). The pepsin digest was subjected to Sephadex G-25 gel filtration. LC–MS/MS identified Ile-Glu-Cys-His-Val (III); Tyr-Val-Lys-Gln (II) and Arg-Glu-Ser-Ile-Gly, Asp (I) to be the prominent peptides/ amino acid in these fractions. Of the three fractions collected, fractions II (734 Da) and III (730 Da) showed the highest ABTS+ scavenging activity and were 30-32% superior to mixed chicken protein digest ($P < 0.05$). Fraction III was most effective in neutralising ·OH and was 89% more efficient ($P < 0.05$) than mixed chicken digest.

Key Words –Chicken protein, Gel filtration, Tandem mass spectrometry.

I. INTRODUCTION

Increased oxidative stress and its downstream effects can lead to various conditions such as cardiovascular diseases [1], Alzheimer's disease [2], aging [3] and cancer [4]. Dietary intake of antioxidant compounds can reinforce the body's oxidant status and help to maintain a balanced condition in terms of oxidant/antioxidant in the body. Given this background, there is increasing interest in food proteins and their constituent peptides as potential candidates for use as antioxidants. The production of peptides through hydrolytic reactions seems to be the most promising technique to form proteinaceous antioxidants since peptides have substantially higher antioxidant activity than intact proteins. In this study, peptides of chicken protein were obtained, after in vitro digestion, and identified using liquid chromatography/tandem mass spectrometry (LC/MS/MS). In addition the antioxidant properties of the fractions obtained with Sephadex G-25 gel filtration were characterized by the following techniques: $ABTS^{\cdot+}$ and $^{\cdot}OH$ radical-scavenging.

II. MATERIALS AND METHODS

2.1.Experimental design. Both sides of the chicken breast (Pectoralis major) were purchased from a local meat purveyor. Cooking process was performed by placing the one piece of each meat sample in a convection-steam oven (Küppersbusch CPE 110, Küppersbusch Großküchentechnik GmbH, Gelsenkirchen, Germany) set to 120 °C. The heating treatment was considered complete when all the meat samples had reached a temperature of 75 ± 3 °C at the center of the muscles (approximately 20 min). The internal temperature of the muscles was monitored using a thermometer. After thermal treatment, the muscles were immediately cooled in an ice batch. The raw and cooked pieces were minced, vacuum packed and stored at −20 °C until analyzed.

2.2. Preparation of protein digests. Chicken protein *in vitro* digests was prepared according to the method of Lo and Li-Chan [5]. The suspension of chicken protein (5%, w/v) in nano pure deionized water was adjusted pH 2.0 with 1 M HCl, followed by the addition of pepsin (4%, w/w, protein basis). The mixture was incubated 1 h in a shaking water bath set at 37 °C to allow pepsin digestion. Subsequently, the pH was adjusted to 5.3 using 0.9 M $NaHCO_3$. After the addition of pancreatin (4% w/w, protein basis), the pH was adjusted to 7.5 with 1 M NaOH. The digestion was re-started and continued in the 37 °C shaking water bath for another 2 h. Aliquots of hydrolysates were at 180 min during the pepsin/ pancreatin sequential digestion, adjusted to neutrality (pH 7.0) with 1 M NaOH/HCl, and heated at 96 °C for 5 min to inactivate the enzymes. Each aliquot was kept at –20 ºC before use.

2.3. Isolation and purification of peptides (Size exclusion chromatography (SEC)). A 2 mg/mL of gastrointestinal solution (i.e., 180 min total digestion time), were dissolving in the elution buffer (0.02 M phosphate, pH 7.4), was clarified and sterilized through a 25-mm syringe filter with a 0.22 µM membrane (Fisher Scientific, Pittsburgh, PA). The purified solution (10 mL) was loaded to the Sephadex G-25 fine column (Pharmacia XK 26/70, Piscataway, NJ, USA) and eluted in a 4 ºC cold room with the elution buffer at a 0.9 mL/min flow rate. Peptide fractions were collected using an automated fraction collector, and the absorbance (215 nm) of the eluents was measured. A fixed

amount of sample (1.5 ml) at a protein concentration of 35 mg/ml was applied to the column, and 3 ml fractions were collected. The absorbance of the effluent was measured at 280 nm. Three fractions were separated. The effluent peptide solutions were immediately subjected to the antioxidant activity test as described below.

2.4. Tandem mass spectrometry. Molecular mass and peptide sequence of the 3 fractions were determined using an Agilent 1100 liquid chromatography/tandem mass spectrometry (LC/MS/MS) (Agilent Technologies, Santa Clara, CA, USA) equipped with a thermostatted □-wellplate autosampler and a capillary pump, and connected to an Agilent Ion Trap XCT Plus Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA) using an electrospray (ESI) interface. A volume of 40 □l of each sample was injected into a Waters Xbridge BEH300 C18 (5 μm, 1.0x150 mm) HPLC column for peptide analysis, thermostatted at 40 °C, at a flow rate of 10 μL/min.

2.5 Antioxidant capacity. The antioxidant potential of the four fractions was compared by assessing their capacity to scavenge 2, 20-azinobis (3-ethylbenzothiszoline-6-sulphonic acid) ($ABTS^{+}$) [6] and (hydroxyl $^{\cdot}OH$) radicals [7].

III. RESULTS AND DISCUSSION

The antioxidant potential of the CP digests was compared by assessing their capacity to scavenge 2, 20-azinobis (3-ethylbenzothiszoline-6-sulphonic acid) ($ABTS^{+}$) and hydroxyl $^{\cdot}OH$ radicals. The pepsin digests were subjected to Sephadex G-25 gel filtration. LC–MS/MS identified Ile-Glu-Cys, His,Val (III); Tyr, Val, Lys, Gln (II) and Arg, Glu, Ser, Ile, Gly, Asp (I) to be the prominent peptides/ amino acid in these fractions. Of the four fractions collected, fractions II (734 Da) and III (730 Da) showed a similar $ABTS^{\cdot+}$ scavenging activity and were 30-32% superior to mixed chicken protein digest ($P < 0.05$). Fraction IV was most effective in neutralising ·OH and was 89% more efficient ($P < 0.05$) than mixed chicken digest.

Table 1. Antioxidant activity ($^{\cdot}OH$ and ABTS) of gel filtration fractions of the *in vitro* digest of chicken protein hydrolysate (CP) by a Sephadex G-25 gel filtration.

Fraction	Molecular weight (KDa)	$^{\cdot}OH$ Scavenging (μM Trolox)	$ABTS^{+\cdot}$ Scavenging (μM Trolox)
I	>40	12.3 ± 2.5^{c}	200 ± 7.5^{c}
II	0.734-40	72.3 ± 2.5^{b}	1300 ± 1.5^{b}
III	0.1-0.730	76.3 ± 1.7^{b}	1150 ± 5.5^{b}
IV	<0.1	88.3 ± 4.2^{a}	2050 ± 4.5^{a}

Means (n = 3) without a common letter differ significantly ($P < 0.05$).

IV. CONCLUSION

Understanding the relationship between peptide composition and antioxidant activity could lead to the development of new class of extremely effective, multifunctional, generally recognized as safe antioxidants that could be used in many food applications.

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