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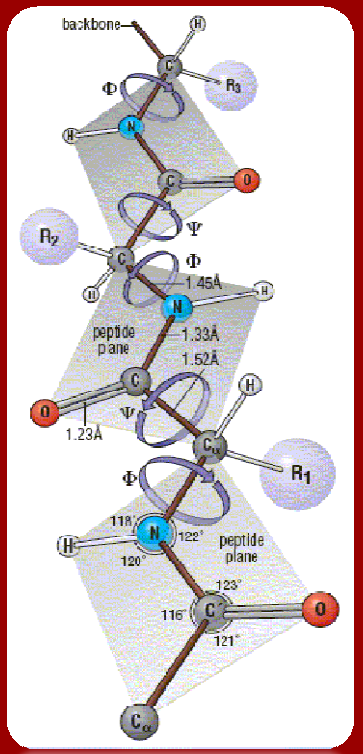

Evaluation of radical Scavenging of peptides after *in vitro* digests of chicken protein

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PEPFUN

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1 Introduction

Major food protein sources

Animal protein sources ~ 20%:

- Meat, collagen, plasma
- Milk, caseins, whey proteins
- Eggs, fish, marine invertebrates

Plant protein sources ~ 80%:

- Favabeans
- Peas, other legumes

Cereal proteins/grains:**~ 57%**
Wheat, barley, rye, rice, maize

- Other, e.g. quinoa

Oilseeds~ 16%:

- Soy bean
- Sunflower
- Rapeseed/canola
- Flaxseed
- Other oilseeds

Other proteins ~ 1%:

- Algea
- Microbial/single cell

Protein functionalities in food

1. TECHNOLOGICAL FUNCTIONALITY

- **Solubility, Water holding,**
- **Gelling, Foaming,**
- **Emulsifying capacity**

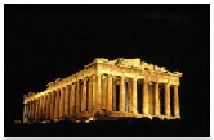
2. SENSORY QUALITY

- **Color**
- **Texture & juiciness**
- **Flavour**

3. PHYSIOLOGICAL EFFECTS

- **Nutrition** – amino acids
- **Antinutritional and toxic compounds**
- Enzyme inhibitors
- allergy, substrate for toxic compounds
- **Health promoting compounds**

Substrate for bioactive peptides

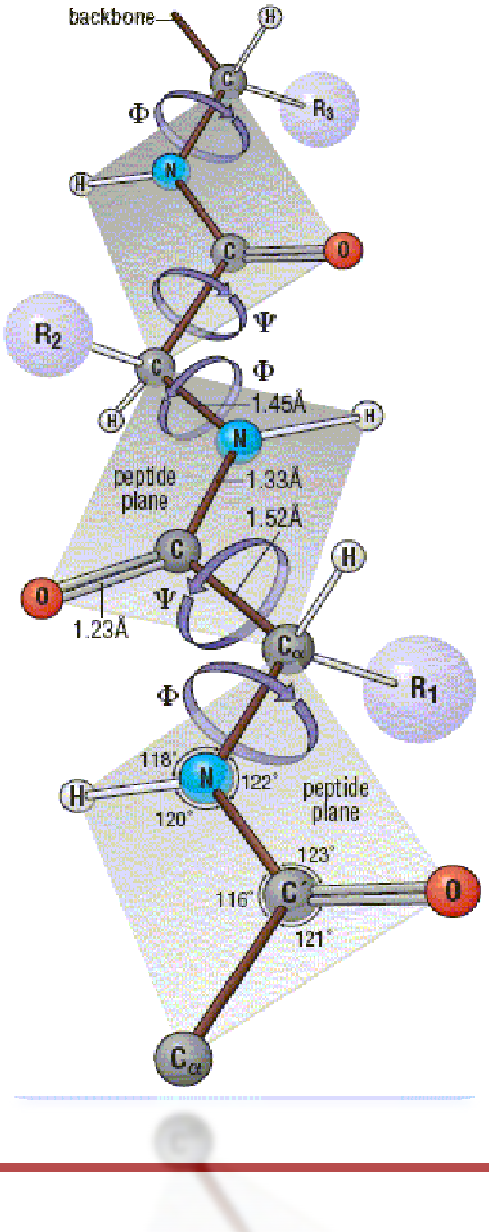


PEPTIDE

The word “peptide” comes from the Greek term “πεπτός” *peptós*, “small digestibles”

Peptides are short polymers of amino acids linked by peptide bonds.

One or more polypeptide subunits constitute a protein molecule.



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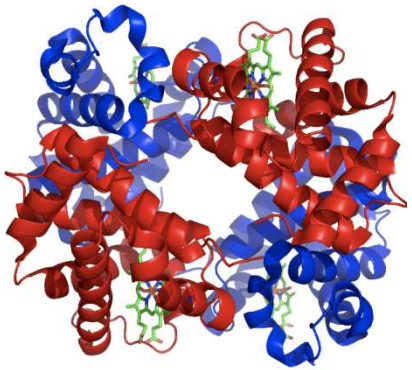
BIOACTIVE PEPTIDE

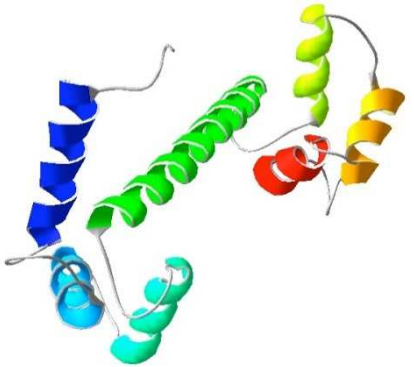
Peptides with a biological activity in addition to the nutritional value

Sequences of amino acids which are inactive within the original protein but which display specific properties once they are released by enzymatic hydrolysis



Native protein molecule

Enzymatic hydrolysis



BIOACTIVE PEPTIDES

Food protein substrates for bioactive peptides

1. **Plant protein sources:** soy protein, cereal protein, rice, pea protein, barley, sunflower meal, flaxseed.

2. **Other proteins:** algae.

3. **Animal protein sources:**

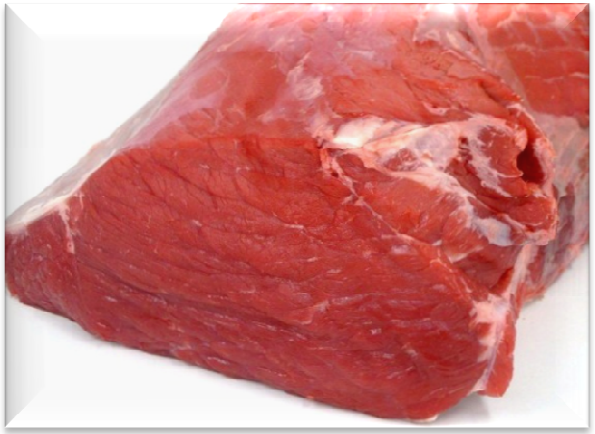
   - Milk protein, eggs, fish protein, marine invertebrates.
   - **Meat:** muscle (porcine, chicken muscle) beef sarcoplasmic proteins, haemoglobin, plasma (porcine, bovine)

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.

While hydrolyzed proteins have good antioxidant activity, it is still not well-understood how the composition of peptides influences their ability to inhibit lipid oxidation.

Generation bioactive peptides from meat

- Porcine myofibrillar proteins (Li et al., 2007; Saiga et al., 2003)
- Porcine meat (Arihara et al., 2001)
- Bovine skin and bovine blood (Kim et al.,2001; Daoud et al., 2005)
- Bovine sarcoplasmic proteins (Jang & Lee, 2005)



Release of bioactive peptides from proteins

Fermentation in food

- Microorganisms/proteolytic enzymes, e.g. yogurt and cheese

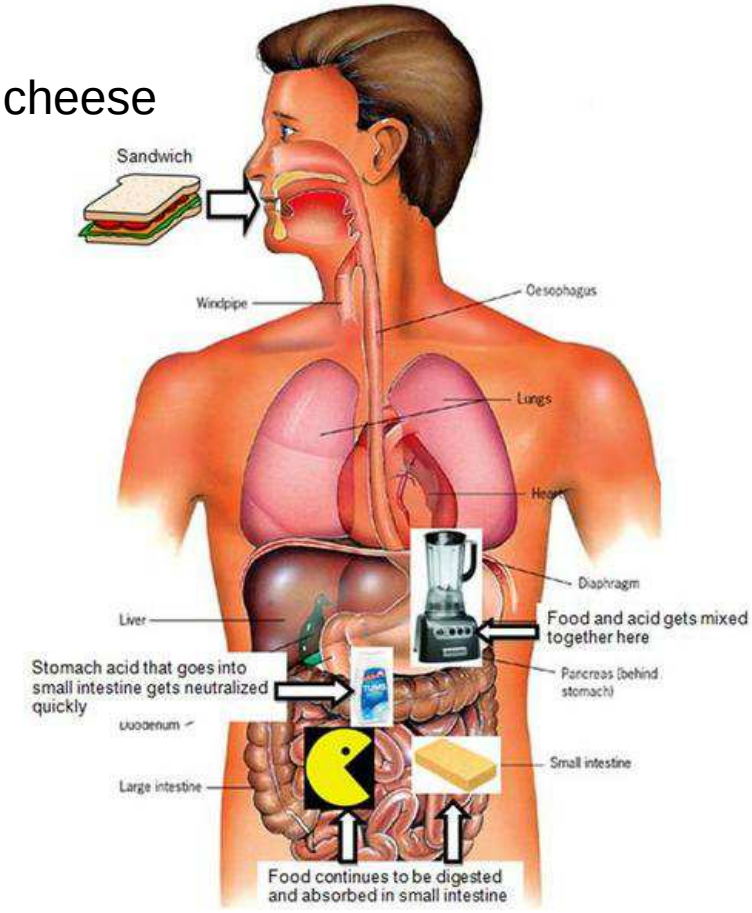
Hydrolysis of protein preparations *in vitro*

- Digestive enzymes (pepsin, trypsin, chymotrypsin)
- Microbial enzymes (thermolysin, proteinase K)
- Plant enzymes (papain)

During digestion *in vivo*

- Digestive enzymes (pepsin, trypsin, chymotrypsin**)**

Genetic engineering



Proteolytic enzymes in the digestive system

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Stomach (pH 1.5-5)

- Pepsin

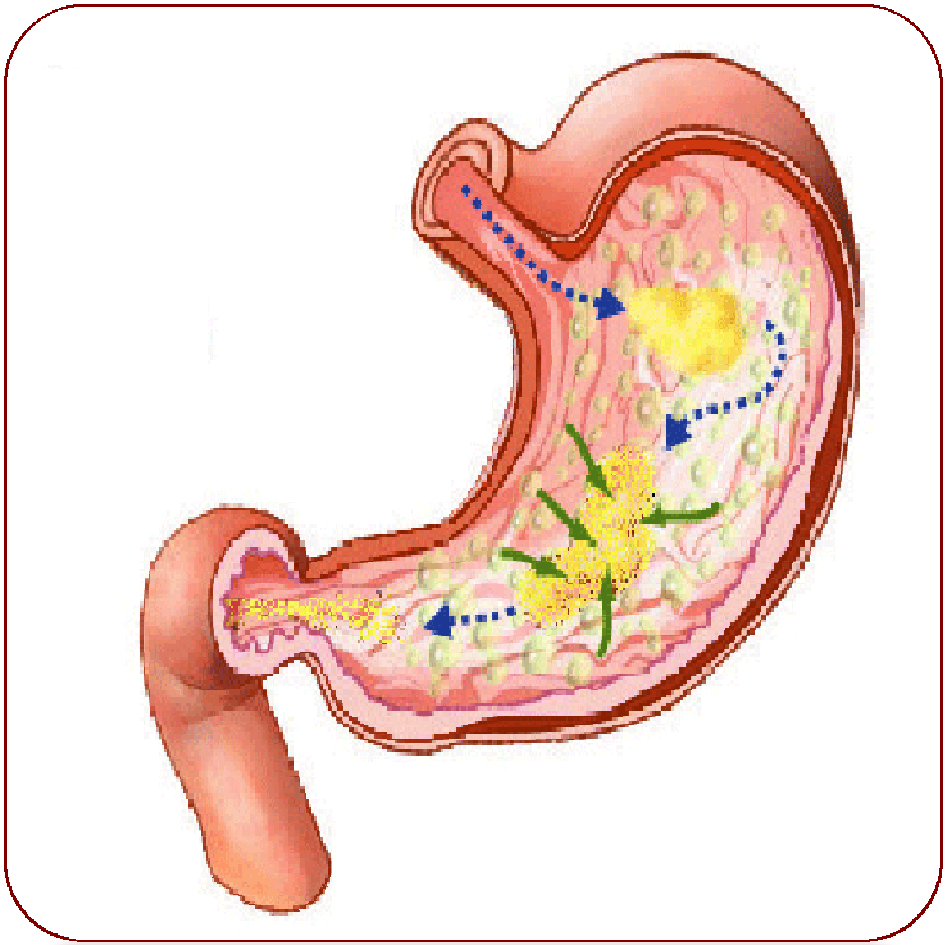
Duodenum (pH 5.7-6.4)

- Trypsin
- Chymotrypsin
- Elastase

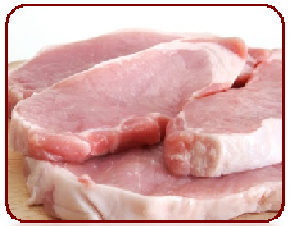
Jejunum (pH 7.4)

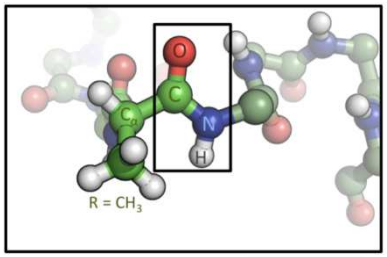
- Brush border peptidases
- Enterocyte peptidases

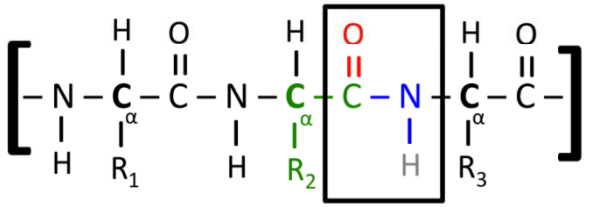
Ileum (pH 7.7)

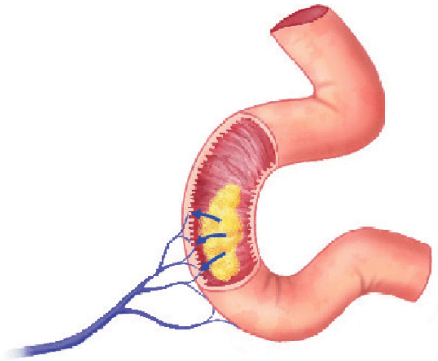


After digestion, bioactive peptides can be absorbed in the intestine and enter the blood stream directly, which ensures their bioavailability *in vivo* and a physiological effect at the target site.



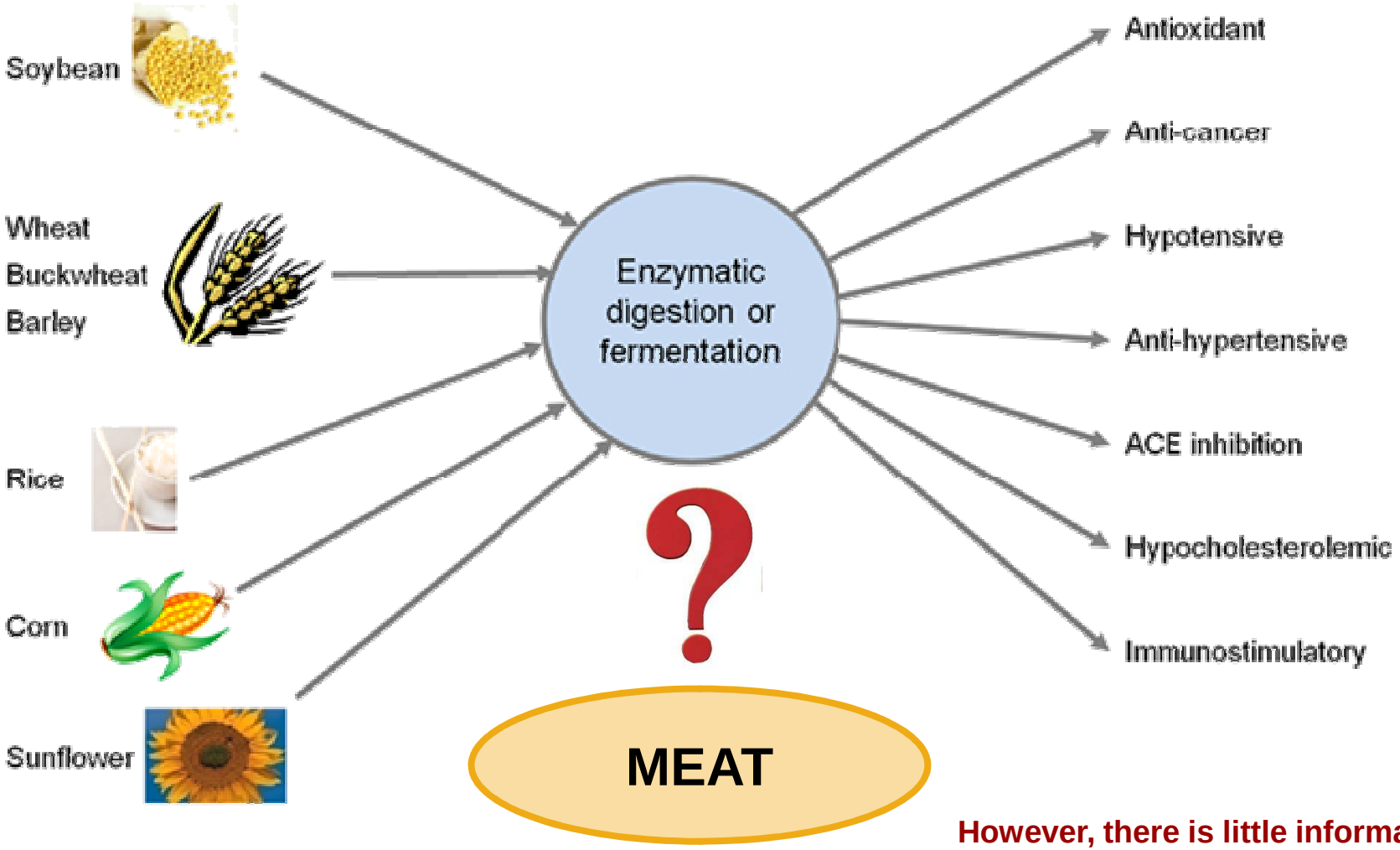






Important role in metabolic regulation and modulation, suggesting their potential use as functional food ingredients for health promotion and disease risk reduction.

Types of bioactivity



However, there is little information about antioxidant peptides generated in meat.

Meat derived peptides have a myriad of bioactive potential including:

1. Antioxidant
2. Antimicrobial
3. ACE-I-inhibitory
4. Anti-thrombotic
5. Cytomodulatory functions

ACE: angiotensin I-converting enzyme

1. Antioxidant

IMPLICATED DISEASE STATES

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Table 1. Antioxidant protein hydrolysates and peptides from muscle and by-products.

Amino acid sequence	Species	Source	Reference
D–S–G–V–T, I–E–A–E–G–E, D–A–Q–E–K–L–E, E–E–L–D–N–A–L–N, V–P–S–I–D–D–Q–E–E–L–M	Porcine	Muscle	Saiga et al. (2003)
D–L–Y–A, S–L–Y–A, V–W	Porcine	Muscle	Arihara (2006)
Q–G–A–R, L–Q–G–M, L–Q–G–M–Hyp, Hyl–C	Porcine	By-product	Li et al. (2007)
M–Q–I–F–V–K–T–L–T–G, D–L–S–D–G–E–Q–G–V–L	Venison	Muscle	Kim et al. (2009)
G–E–Hyp–G–P–Hyp–G–A–Hyp, G–P–Hyp–G–P–Hyp–G–P–Hyp–G, G–P–Hyp–G–P–Hyp–G–P–Hyp	Bovine	By-product	Kim et al. (2001)
P–S–K–Y–E–P–F–V	Grass carp	Muscle	Ren et al. (2008a)
–	Mackerel	Muscle	Wu et al. (2003)
–	Yellow stripe trevally	Muscle	Klompong et al. (2007)
N–A–D–P–G–L–N–G–L–E–G–L–A, N–G–L–E–G–L–K	Giant squid	Muscle	Rajapakse et al. (2005)

2 Hypothesis

Proteins in raw and processed foods can possess antioxidant peptide sequences and structural domains; the active fragments are released during the GI digestion process.

Reported high-efficiency radical scavenging peptides released through in vitro pepsin and pancreatin digestion include those from:

1. **Casein (Hernandez-Ledesma, Amigo, Ramos, & Recio, 2004)**

1. **Amaize zein (Zhu, Chen,Tang, & Xiong, 2008)**

2. **Oyster protein (Crassostrea gigas) (Qian, Jung,Byun, & Kim, 2008)**

3. **Mussel protein (Mytilus coruscus) (Jung et al., 2007).**

HYPOTHESIS

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it is hypothesized that hydrolysis of chicken protein can release the peptide fragments capable of stabilising ROS and inhibiting lipid oxidation.

****The specific peptides or peptide fractions responsible for the antioxidant functions have not been elucidated.**

3 Objectives

OBJECTIVES

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In the present study, the ability of mixed as well as individual fractions of *in vitro* pepsin–pancreatin sequential digests of chicken protein to stabilise ·OH, DPPH and ABTS+· radicals was investigated.

The objective was to identify the most effective antioxidant peptide fraction(s) from chicken meat *in vitro* digests.

4 Methodology

METHODOLOGY

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1. Initially, the digest with the highest radical scavenging capacity was fractionated by means of gel filtration.

2. The ability to stabilise hydroxyl radical by each post-column fraction with Sephadex G-25 was subsequently examined

3. And the prominent peptides in active fractions were sequenced by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

METHODOLOGY

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Chicken breast

Gastric digestion. Pepsin. pH=1.8

Gastric Intestinal Digestion.

Pancreatin (pH= 7.5) and bile salt used for hydrolysis protein

Antioxidant activity assay:

-$ABTS^{+\cdot}$ radical scavenging activity

-DPPH

-·OH radicals.

Purification step techniques: Gel filtration chromatography

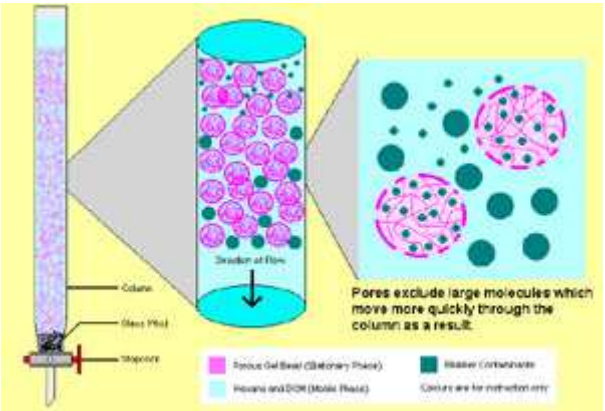

Figure 1. Experimental design for the generation and characterisation of bioactive peptides.

The 2 h pancreatin digest, which demonstrated the strongest activity against both radicals, was subjected to Sephadex G-25 gel filtration.

METHODOLOGY

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DPPH: is a stable free radical that shows maximum absorbance at 517 nm.

When DPPH radicals encounter a proton-donating substrate such as an antioxidant, the radicals would be scavenged and the absorbance is reduced.

The decrease in absorbance is taken as a measure for radical-scavenging activity and thus, antioxidant activity.

The filtered samples were separated and analyzed by HPLC–MS/MS

Then the MS and MS/MS scans were analyzed for Sherenga de novo sequencing and the probably peptides were listed.

Data were also compared with different NCBInr databases with enzymatic digestion restriction.

5 Results

RESULTS

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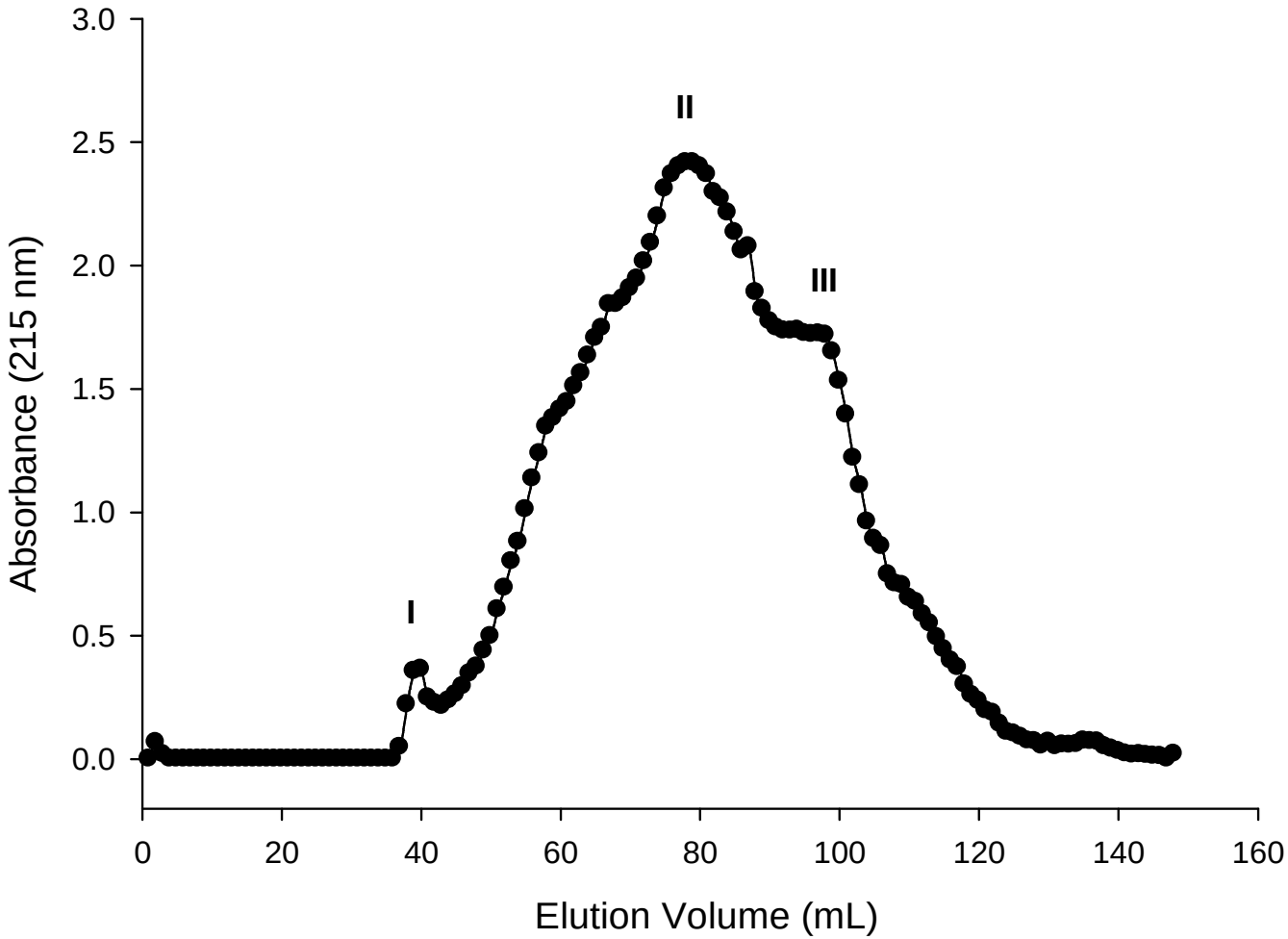

Figure 1. Sephadex G-25 gel filtration of the final in vitro digest (300 min total digestion time) of chicken meat (n = 3).

RESULTS

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Of the three fractions collected, fractions II (734 Da) and III (730 Da) showed the highest DPPH, ABTS+ scavenging activity and were 30-32% higher 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.

LC–MS/MS identified:

Fractions III: Ile, Glu, Cys, His, Val
Fractions II: Tyr, Val, Lys, Gln
Fractions I: Arg, Glu, Ser, Ile, Gly, Asp

to be the prominent peptides/ amino acid in these fractions.

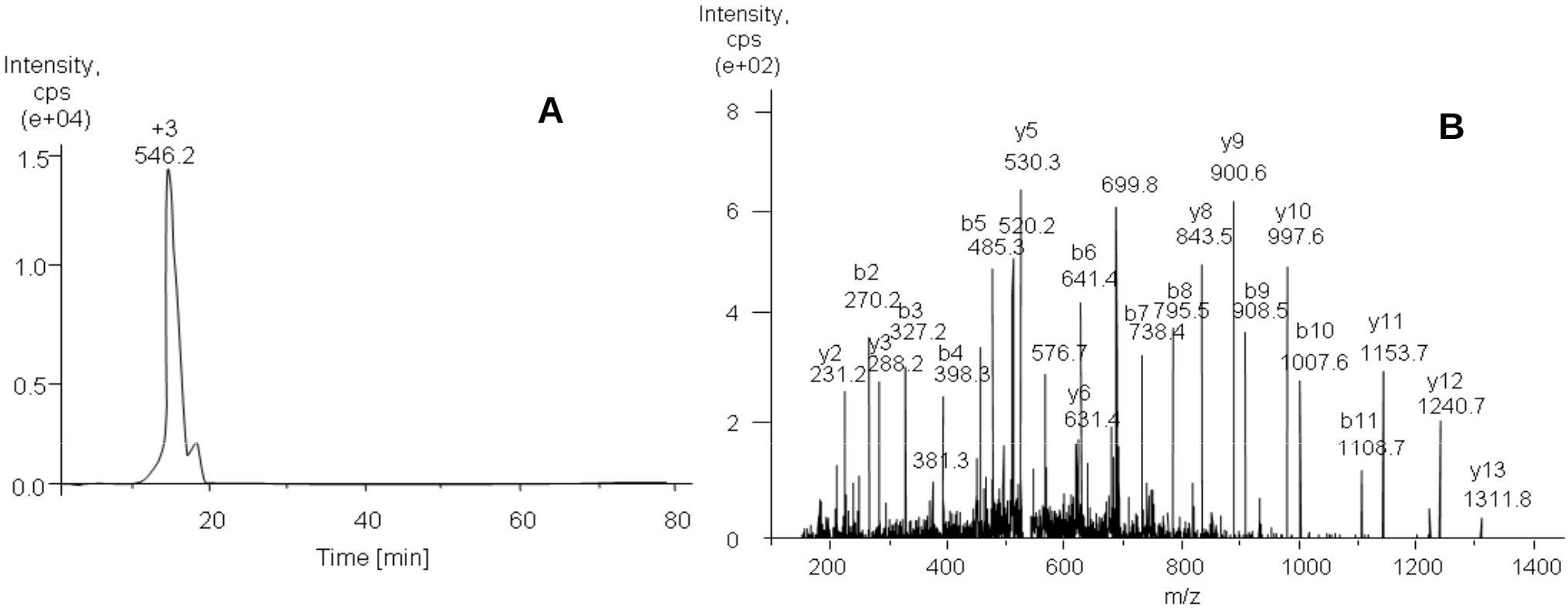

Figure 2. Extracted ion chromatogram (A) and tandem MS/MS spectrum (B) of the prominent peptide present in gel filtration Fraction I.

Proteins are unique antioxidants in that they can inhibit lipid oxidation though multiple pathways including:

- **Inactivation of reactive oxygen species**
- **Scavenging free radicals**
- **Chelation of prooxidative transition metals**
- **Reduction of hydroperoxides**
- **Alteration of the physical properties of food systems**

-Antioxidant peptides mostly contain below 20 amino acid residues per molecule and molecular masses of less than 6000 Da (Sun *et al.,* 2004).

-Also, the antioxidant activity of peptides is closely related to their aminoacid constituents, sequence (Grimble, 1994) as well as hydrophobicity (Chen *et al.,* 1998).

-In addition to the presence of proper amino acids, their correct positioning in peptide sequence plays an important role in antioxidant activity of peptides (Rajapakse *et al*., 2005)

DISCUSSION

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Li, Han & Chen (2008) indicated that antioxidant activity of peptides of molecular mass of 500-1500 Da is stronger than of peptides above 1500 Da and peptides below 500 Da.

On the other hand, antioxidant peptides with lower molecular weights have higher chance to cross the intestinal barrier and exert biological effects (Roberts et al.,1999).

Thus, peptides present in these collected fractions having molecular masses lower than 1700 Da might have biological effect.

6 Conclusion

CONCLUSION

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In conclusion, free radical scavenging activity of chicken protein was accentuated by *in vitro* digestion, especially after 2 h pancreatin digestion following the 1 h pepsin treatment.

On an equal weight concentration basis, fractions enriched with tetrameric peptides containing Cysteine, Histidine and Valine exhibited the strongest radical scavenging activity.

CONCLUSION

These short peptides are implicated in the protection of the upper digestive tract of humans from oxidative stresses and may partially explain why dietary protein promotes the health of the GI system.

CONCLUSION

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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 (GRAS) antioxidants**

That could be used in many food applications, including the development of functional foods fortified with oxidatively unstable, yet healthy, unsaturated fatty acids.

Nevertheless, our study has to be considered as a preliminary study so, further research is needed to purify and characterise the peptides that can exert antioxidant activity *in vitro* and then, to determine potential *in vivo* antioxidant activity.

Thanks for your attention