

In vitro Anti–Oxidation and Anti–Cholinesterase Activities of Tuna and Chicken Hydrolysates and their Maillard Reaction Products

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Abstract:

Objective: To evaluate the anti-oxidation and anticholinesterase activities and cell viability of chicken and tuna hydrolysates and their Maillard reaction products.

Material and Methods: Maillard reaction products (MRPs) derived from chicken or tuna hydrolysates were prepared by heating the hydrolysate with glucose at 84°C for 90 minutes. Physical characteristics, ultraviolet (UV) absorbance, browning intensity, protein concentrations, molecular weight distribution, and amino acid profiles of the hydrolysates and MRPs were determined. The anti-oxidation properties of the hydrolysate and MRPs were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Anti-cholinesterase activities were evaluated using Ellman's method. Cell viability of the samples was evaluated in RAW 264.7 cells.

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Results: The MRPs presented as brown to dark brown liquids with Brix values ranging from 61.33 to 64.17 °Brix. The pH value ranged from 5.31 to 6.20. The UV absorbance at 294 nm and browning intensity at 420 nm of the MRPs were less than the absorbance of the corresponding hydrolysates. The protein concentrations were 41–44% (hydrolysates) and 18–20% (MRPs). The major constituents of the hydrolysates and MRPs were small molecules (MW<300 Da). The samples exhibited anti-oxidative activity (EC_{50} 2.03 – 3.51 mg/mL) and anti-acetylcholinesterase activity (45–52% inhibition at 0.5 mg/mL). Both hydrolysates and MRPs showed no toxicity to RAW 264.7 cells at concentrations up to 1 mg/mL.

Conclusion: Chicken and tuna hydrolysates and their MRPs exhibited mild anti-oxidative and moderate acetylcholinesterase inhibitory activities.

Keywords: bioactive peptides, Maillard reaction products, anti-oxidation, acetylcholinesterase inhibitors

Introduction

“Functional foods” are defined as “natural or processed foods that contain biologically-active compounds which, in effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers to promote optimal health, reduce the risk of chronic or viral diseases, and manage their symptoms”¹. Given its wealth of natural resources, Thailand has long been referred to as “the kitchen of the world.” Thailand is among the top five global chicken exporters, and it is the world’s leading exporter of processed foods, including canned tuna, frozen seafood, shrimp, and poultry². However, many by-products of processed food production, which are high-quality nutritional resources, are discarded as waste.

Bioactive peptides and proteins are among the by-products of the poultry and seafood processing industries^{3,4}. The biological activities of these bioactive molecules include antihypertensive, antioxidant, anti-cholinesterase, antimicrobial, anti-inflammatory, anticancer, antithrombotic, and hypocholesterolemic properties^{5–8}. These biological activities can be beneficial to health and lower the risk of some chronic diseases.

The Maillard reaction is a non-enzymatic chemical reaction between carbonyl groups of reducing sugars and the amino groups in amino acids, peptides, or proteins. A complex array of compounds known as Maillard reaction

products (MRPs) are formed during this reaction and have been shown to contribute to flavor and color, as well as the bioactive qualities of the involved foods^{9,10}. Dietary protein-derived peptides are commonly used as functional components or as ingredients in processed foods^{11,12}. MRPs produced from various sugar-protein hydrolysates have been generated and described in several investigations, demonstrating the favorable effects and functional properties of the Maillard reaction products, such as anti-oxidation, anti-cholinesterase, and anti-inflammatory activities^{13–15}.

Cholinesterases are serine hydrolases that catalyze acetylcholine (ACh) metabolism. Centrally acting cholinesterase inhibitors can increase ACh levels and have been used to slow the progress of Alzheimer’s disease (AD)¹⁶. Oxidative stress and systematic chronic inflammation can increase the risk of hypertension, hyperglycemia, dyslipidemia, cancer, and neurodegenerative disease^{17,18}. In this work, tuna and chicken hydrolysates and their MRPs were prepared, and their *in vitro* functional properties, such as anti-oxidative, anti-cholinesterase, and anti-inflammatory activities, were evaluated. The findings from this work can be beneficial for further development of these by-products by the food industry to create the functional foods for health promotion and reduce the risk of chronic diseases.

Material and Methods

Preparation of tuna and chicken hydrolysates and the MRPs

The hydrolysates derived from tuna (*Katsuwonus pelamis*) and chicken (*Gallus domesticus*) were prepared at i-Tail Corporations (Songkhla, Thailand) from tuna and chicken broth, respectively. Raw tuna was purchased from F.C.F. Fishery Co. Ltd., Bangkok, Thailand. The raw chicken was purchased from Betagro, Bangkok, Thailand, and was identified by Mr. Kasem Noorit, Assistant Quality Control Manager, i-Tail Corporation. The tuna broth was obtained by steaming whole tuna, and the chicken broth was produced from chicken breasts, using the following process. Firstly, sediments in the broth were separated through a sieve (150 μ m). The broth was then heated at 55°C and then allowed to cool to room temperature. Serine endopeptidase (EC 3.4.21.62) from *Bacillus licheniformis* (Soufflet Biotechnologies, Colombelles, France) (0.7% by weight of solid weight in the broth) was added and the mixture was stirred for 60 minutes. Then the temperature was raised to 90°C for 10 minutes to inactivate the enzymes. The solution was concentrated with a shell and tube evaporator at 100–120°C until the concentration of the hydrolysate remained at 60 \pm 5°Brix. Water activity was controlled at <0.8. The freshly prepared hydrolysates were kept in a sealed aluminum pouch at ambient temperature and used for the preparation of the MRPs within 2 weeks. For the study experiments, a mixture of tuna (or chicken) hydrolysate (40 g) and glucose (40 g) was dissolved in distilled water (800 mL) in a reactor. The solution was heated to 84°C for 90 minutes. The temperature inside the reactor was monitored with a dial thermometer (TEL-TRU, Rochester, NY, USA). Then, the reaction mixture was cooled down to room temperature and concentrated using a rotary evaporator (Heidolph Hei-VAP Core, Schwabach, Germany) with a water bath temperature controlled at 50 \pm 5°C. The mixture was allowed to evaporate until the residual volume became constant and the concentration of

the residue was controlled at 60 \pm 5°Brix. The concentrated MRPs were stored in a sealed aluminum pouch at –18°C until further investigations (within 2 weeks).

Physical properties

The Brix values of the hydrolysates and MRPs were measured with a refractometer (ATAGO, Master-93H, Tokyo, Japan). The pH values of the samples were determined using a pH meter (Mettler Toledo FE20, Shanghai, China) at room temperature. The ultraviolet (UV) absorbance (294 nm) and browning intensity (420 nm) were analyzed by a microplate reader (UV-2401PC, Shimadzu Co., Ltd., Japan) in triplicate¹⁹. For UV absorbance analysis, the hydrolysates and the MRPs were diluted 1000-fold with distilled water. For browning intensity analysis, the hydrolysates and the MRPs were diluted 50-fold with distilled water.

Molecular weight distribution, amino acid profiles, and protein concentrations

Molecular weight (MW) distribution was detected with an in-house method modified from the national standard GB/T 22729–2008 oligopeptide powder of marine fish at the Thai Union's Global Innovation Center (GIC). High performance column chromatography (Agilent; 1200 Infinity series with diode-array detector (DAD) was equipped with a TSKgel® G200SWXL size exclusion column (7.8 mm ID \times 30 cm, pore size 5 μ m). The sample (20 μ L) was injected into the system and eluted with 0.1% trifluoroacetic acid, 45% acetonitrile, and 55% Milli-Q water at a flow rate of 0.5 mL/min. Standard proteins and peptides with MWs ranging from 12,000 to 132 Da were used to prepare a calibration curve. The % MW distributions were calculated from the relative areas under curves of the peaks at the retention times corresponding to the standard proteins and peptides by using Agilent ChemStation software. The amino acid content was determined at ALS Laboratory Group (Thailand) Co., LTD, based on the Association of Official Analytical Chemists (AOAC) (2019) 994.12 official method. Protein

concentrations were determined with the AOAC 992.15 official method for the determination of crude proteins in meat and meat products at i-Tail Corporation Laboratory, Songkhla, Thailand.

Antioxidative properties

The hydrolysate and MRP samples were dissolved in absolute ethanol (EtOH) and serially diluted to achieve 8–10 concentrations ranging from 0.14 to 75 mg/mL. The solution being tested (100 μ L) was transferred to an Eppendorf tube. A 100 μ L solution of 6×10^{-5} M 2,2-diphenyl-1-picrylhydrazyl (DPPH) in absolute EtOH was added and carefully mixed to give the final concentration of the test sample of 0.07–37.5 mg/mL. The reaction mixture was agitated for a few minutes and left to stand at room temperature in the dark for 30 minutes. The absorbance of the mixture was determined at 517 nm by comparing 100 μ L of the sample solution to 100 μ L of absolute EtOH as the blank solution. Trolox was used as the reference standard. The percentage inhibition value was calculated by using the equation: % inhibition = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, where A_{control} = absorbance of DPPH solution without sample and A_{sample} = absorbance of DPPH solution with sample solution. Percent inhibitions were plotted against sample concentrations to create a dose-response curve. A linear regression analysis was performed to calculate the sample concentrations required for a 50% reduction of the DPPH radical (EC_{50} value)²⁰.

Acetyl- and butyrylcholinesterase inhibition

Acetylcholinesterase from *Electrophorus electricus* (eeAChE), butyrylcholinesterase from equine serum (eqBChE), the enzyme substrates, acetylthiocholine iodide (ATCI) and butyrylthiocholine iodide (BTCl), and the reagent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. All assays were performed in 96-well plate according to Ellman's method previously described^{21,22}. Briefly, the reaction mixture (250 μ L) contained 50 mM Tris-HCl buffer (pH 8.0, 50 μ L), 1.5 mM ATCI or BTCl solution

in distilled water (25 μ L), 5 mg/mL of test compounds in EtOH (25 μ L), and 3 mM DTNB (125 μ L) were added. Then, 25 μ L of eeAChE (or eqBChE) in 50 mM Tris-HCl buffer containing 0.1% (w/v) BSA (pH 8.0) was added. The final concentration of the test compounds in the wells equaled 0.5 mg/mL. The enzymatic cleavage and the consequent reaction resulted in the development of a yellow color, which was measured at 405 nm every 11 seconds for 2 minutes in a Microplate Scanning Spectrophotometer. Each experiment was repeated in triplicate. Rivastigmine ($\geq 98\%$ purity, Sigma-Aldrich, Steinheim, Germany) at the concentration of 0.5 mg/mL was taken as the reference drug. The following expression was used to calculate the proportion of enzyme inhibitory activity (% Inhibition): % inhibition = $[(\text{mean velocity of the blank} - \text{mean velocity of the sample}) \times 100] / \text{mean velocity of the blank}$. The results were reported as % inhibition \pm S.D.

Cell viability²³

The hydrolysate and MRP samples were subjected to cell viability assays. Briefly, RAW 264.7 cells (ATCC®TIB-71™) passage 12 were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and a 1% streptomycin/penicillin cocktail at 37°C in a 5% CO₂. The cells were then trypsinized and seeded into a 96-well plate (25,000 cells/well). Hydrolysate and MRP samples at 50, 100, 200, 400, and 1000 μ g/mL were incubated with the cells for 24 hours, then replaced with a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (MTT) solution (5 μ g/mL) and incubated for 4 hours. A formazan crystal was dissolved with *N,N*-dimethylsulfoxide (DMSO) and UV absorption determined at 590 nm.

Statistical analysis

The data were expressed as means \pm standard deviations (S.D.) Statistical analyses were performed using the t-test (two tails) in Microsoft Excel. A p-value < 0.05 was considered statistically significant.

Results

Physical properties

The MRPs from the tuna and chicken hydrolysates were obtained as dark brown and brown liquids, with Brix values ranging from 61.33 to 64.17 °Brix and pH values from 5.31 to 6.20. The MRPs could mask the fish and chicken odors and give a mild and sweet odor (Table 1). The UV absorbance at 294 nm and browning intensity of the MRPs were slightly less than those of the original hydrolysate (Figure 1).

MW distribution, amino acid profiles, and protein concentrations

The MW distribution was analyzed by gel filtration chromatography and the relative amounts of peptides with different molecular weights were calculated. The fitting equation of the standard curve was $y = -0.0657x^2 + 0.1554x + 0.5509$ ($R^2 = 0.9822$). The MW distribution and protein concentrations of the hydrolysates and that of the MRPs were analyzed and are shown in Table 2. The protein concentrations of the hydrolysates ranged from approximately 41–44% and of the MRPs ranged from approximately 18–20%. The major components in the hydrolysates and MRPs were small molecules (MW 300–100 Da and <100 Da). Amino acid contents were determined and are shown in Table 3.

Functional properties

The antioxidative activity of the MRPs was evaluated by the DPPH free radical scavenging assay using Trolox as the reference compound. The MRPs and the original hydrolysates showed mild anti-oxidative activity with EC_{50} values ranging from 2.03 to 3.51 mg/mL, whereas Trolox exhibited an EC_{50} value of 10.11 µg/mL (Table 4). The anti-oxidative properties of the hydrolysates and MRPs were significantly different from Trolox (p -value < 0.05). However, the MRPs did not show different anti-oxidative activities

than the corresponding hydrolysates. The cholinesterase enzyme inhibition effects of the hydrolysates and the MRPs are shown in Table 4. The hydrolysates and MRPs at 0.5 mg/mL showed moderate inhibitory activity against AChE with 45.91–52.03% inhibition. Rivastigmine at the same concentration inhibited AChE function by 82.32%. Statistical analysis indicated that the MRPs exhibited lower AChE inhibitory properties than the corresponding hydrolysates. All samples at 0.5 mg/mL did not affect BChE function (% inhibition 3.29–16.82) (Table 4).

Cell viability

RAW 264.7, a macrophage cell, can be stimulated by amyloid-β plaques (Aβs). RAW 264.7 exposed to Aβs was previously studied mimicking an inflammatory event that occurs in AD²⁴. Therefore, a safety profile of RAW 264.7 cells when treated with the hydrolysate and MRPs was determined in this study. The results from the cell viability assays showed that hydrolysates and MRPs were not harmful to macrophage cells (Figure 2). Both chicken and tuna hydrolysates and MRPs maintained cell viability above 90% up to a concentration of 1 mg/mL (p -value > 0.05 compared to the control). At 500–1,000 µg/mL, the samples showed slightly increased cell viability due to the amino acids contained in the samples as a source of nutrition for cell growth. However, all compounds were unable to reduce inflammation in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells (data not shown).

Table 1 Physical characteristics of the hydrolysates and MRPs

Sample	Brix (°Bx)	pH
Tuna hydrolysate	61.33±0.29	5.60±0.00
Chicken hydrolysate	63.00±0.00	6.20±0.01
Tuna MRP	64.17±0.29	5.31±0.06
Chicken MRP	62.83±0.29	5.50±0.01

MRP=Maillard reaction product

Table 2 Molecular weight distribution of the soluble protein and peptides in the hydrolysates and MRPs

Product	Protein (%)	Molecular weight distribution (%)								Average MW
		>10,000 Da	10,000 – 5,000 Da	5,000 – 3,000 Da	3,000 – 1,000 Da	1,000 – 500 Da	500 – 300 Da	300 – 100 Da	<100 Da	
Tuna Hydrolysate	41.08	0.135	2.171	3.709	11.803	8.906	6.638	16.209	50.429	696.667
Chicken hydrolysate	44.21	0.207	0.869	1.165	4.262	5.237	9.606	21.112	57.541	331.000
Tuna MRP	18.34	0.156	2.593	4.266	12.723	9.193	6.737	17.320	47.012	782.000
Chicken MRP	19.83	0.140	0.775	1.120	4.111	6.028	10.427	23.289	54.111	326.333

MRP=Maillard reaction product, MW=Molecular weight, DA=Dalton

Table 3 Amino acid content of the hydrolysates and MRPs

Amino acid	Content (g/100g)			
	Chicken hydrolysate	Chicken MRP	Tuna hydrolysate	Tuna MRP
Aspartic Acid	1.16	0.52	1.93	0.74
Cysteine	0.03	0.01	0.03	Not detected
Glutamic Acid	2.72	1.27	3.02	1.27
Glycine	1.5	0.9	2.84	1.56
Histidine	2.94	1.65	3.07	1.23
Hydroxylysine	0.12	0.06	0.36	0.08
Hydroxyproline	0.59	0.33	1.67	0.73
Isoleucine	0.37	0.16	0.46	0.18
L-Alanine	1.68	0.85	2.98	1.27
L-Arginine	1.92	1.04	2.85	1.17
Leucine	0.74	0.35	1.13	0.46
Lysine	1.88	0.95	2.24	0.83
Methionine	0.13	0.07	0.57	0.16
Phenylalanine	0.34	0.16	0.6	0.22
Proline	1.09	0.58	2.34	1.02
Serine	0.68	0.3	1.03	0.41
Threonine	0.55	0.24	0.92	0.36
Tryptophan	Not Detected	Not Detected	<0.01	Not Detected
Tyrosine	0.37	0.13	0.37	0.07
Valine	0.54	0.25	0.73	0.3

MRP=Maillard reaction product

Discussion

Bioactive peptides derived from food proteins have great promise as constituents in functional foods and nutraceuticals^{14,15}. The Maillard reaction is a chemical and non-enzymatic browning process that begins with the conjugation of compounds containing free amino and carbonyl groups. In some foods, the browning reaction plays an essential role in the development of sensory characteristics such as colors, aromas, and flavors. In this study, the chicken and tuna hydrolysates were enzymatically prepared from chicken and tuna broths. The hydrolysates underwent Maillard reactions with glucose under mild conditions to obtain the MRPs. The physical characteristics, components, and bioactivities of the proteins and peptides as well as their MRPs, were evaluated to determine their functional properties.

Table 1 shows the physical characteristics of the hydrolysates and MRPs produced in this study. The Brix values of the MRPs ranged within the same degree range ($60 \pm 5^\circ$ Brix) indicating similar concentrations of the solutes in the samples. The hydrolysates and MRPs were found to be slightly acidic. It has been well known that protein hydrolysates and peptides contribute to the flavor and aroma of foods^{25,26}. Peptides are acknowledged as crucial flavor enhancers and precursors to the Maillard reaction^{27,28}. Therefore, MRPs derived from protein hydrolysates, peptides, and carbohydrates can affect the flavor characteristics of food products. In this study, the MRPs were able to mask the strong fish and chicken odor and gave a good odor. The UV absorbance of the MRP solutions was monitored at 294 and 420 nm, which indicated the presence of the early-intermediates and degree of the Maillard reaction, respectively²⁸. Previous studies suggested that the UV absorbance and browning intensity increased with longer heating times²⁹. In this work, both absorbance of the intermediate products and browning intensity of the MRPs decreased from the original hydrolysates (Figure 1). This

indicated a lower degree of the Maillard reaction occurred under our conditions of low temperature (84°C) and short reaction time (90 minutes). These findings corresponded with the less intense color of the MRPs when compared with the corresponding original hydrolysates.

From Table 2, the protein concentrations in the MRPs were less than half of that found in the hydrolysates. In the preparation of MRPs, glucose was added to the hydrolysate in a 1:1 ratio. This suggests that some portions of the proteins underwent a Maillard reaction and transformed into other Maillard reaction products. The MW distribution analysis of the soluble protein and peptides showed that the major constituents of both hydrolysates were less than 300 Da (Table 2). This finding reflected the fact that the non-specific protease hydrolyzed the protein and peptides primarily into single amino acids and dipeptides. In MRPs, the abundance of small molecules (MW<100 Da) decreased by approximately 3% compared to the original hydrolysates, while the abundance of the higher MW molecules increased. This might result from the reaction of the amino acids with glucose to form higher MW MRPs. The most abundant amino acids in the tuna and chicken hydrolysates were histidine, glutamic acid, and L-alanine (3.07, 3.02, and 2.98 g/100 g, respectively) and histidine, glutamic acid, and L-arginine (2.94, 2.72, and 1.92 g/100g, respectively). In the MRPs, the amino acid contents were decreased from the original hydrolysate. These findings suggest that some amount of the amino acids underwent the Maillard reaction with glucose to form the MRPs (Table 3).

Some constituents of the Maillard reaction products, such as melanoidins and Amadori rearrangement products (ARPs), can scavenge oxygen radicals³⁰. In this work, the antioxidative properties of the hydrolysates and MRPs were evaluated with the DPPH radical scavenging assay in comparison to Trolox. The hydrolysates and MRPs showed only mild anti-oxidative activity (Table 4). This result was consistent with a recent report that suggested the DPPH

Table 4 Functional properties of the hydrolysates and MRPs

Sample	DPPH assay (EC ₅₀ ±S.D., mg/mL)		AChEI activity (% inhibition±S.D.) ^a		BChEI activity (% inhibition±S.D.) ^a	
	Hydrolysate	MRP	Hydrolysate	MRP	Hydrolysate	MRP
Tuna	2.352±0.452 ^{Aa}	2.032±0.115 ^{Aa}	52.03±0.62 ^{Aa}	49.83±0.71 ^{Ab}	3.29±0.31 ^{Aa}	5.36±0.15 ^{Ab}
Chicken	3.282±0.308 ^{Aa}	2.248±0.133 ^{Aa}	50.64±0.45 ^{Ba}	45.91±0.46 ^{Bb}	8.08±0.39 ^{Ba}	16.82±0.33 ^{Bb}
Rivastigmine	N.D.		82.32±0.76		85.8±0.64	
Trolox	0.010±0.001		N.D.		N.D.	

%AChE and BChE inhibition of test compounds and rivastigmine at the concentration of 0.5 mg/mL

Capital letters are used to describe differences in % inhibition in each column.

Lowercase letters are used to describe differences in % inhibition in each row.

Different letters correspond to significant differences (p-value<0.05) between values.

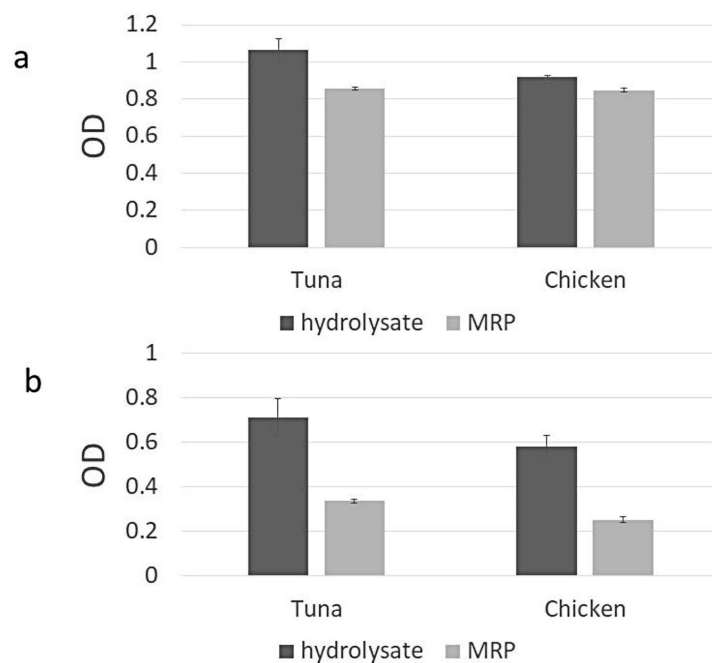
N.D.=not determined, MRP=Maillard reaction product, DPPH=.2,2-diphenyl-1-picrylhydrazyl

free radical scavenging capacity of MRPs in the early stages of the Maillard reaction was low, but progressively increased as the reaction time increased³¹. Antioxidant assays conducted in the laboratory can serve as preliminary screening methods, but they have limited application in the context of human nutrition. This is due to the fact that these assays, such as the 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (DPPH) and 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radicals, do not accurately represent the physiologically relevant radicals present in the human body under physiological conditions. Cell-based antioxidant studies such as lipid peroxidation and deoxyribonucleic acid (DNA) damage assays can be considered to further investigate a substance's antioxidant potential.

In Ellman's assay, at a concentration of 0.5 mg/mL, the hydrolysates and MRPs specifically inhibited acetylcholinesterase inhibitory activity, while butyrylcholinesterase inhibition was negligible (Table 4). Although low-molecular weight (MW<300 Da) peptides and MRPs were found to be the major constituents of the hydrolysates and MRPs, their ability to cross the blood-brain barrier to reach the target enzyme AChE in the brain still needs to be investigated.

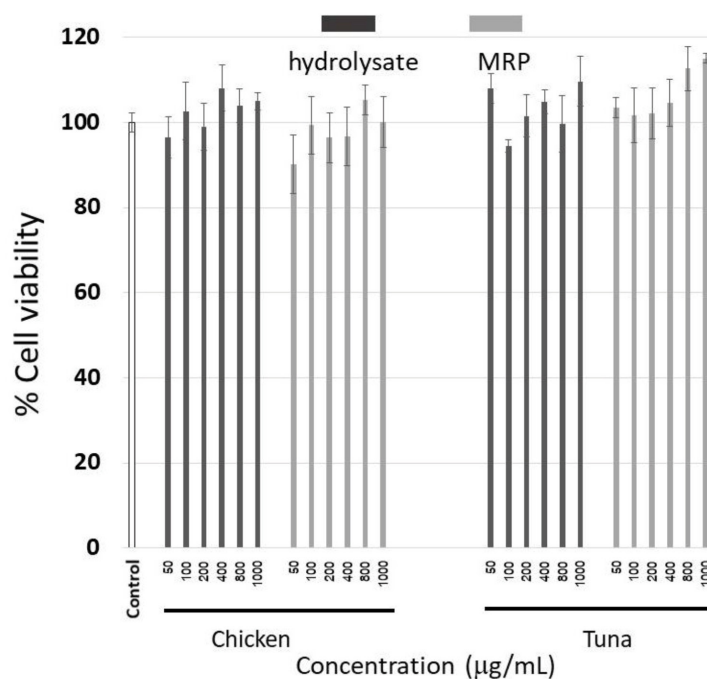
The pathology of AD has been well investigated. Amyloid-β plaques (Aβs) plays important role in the initiation

and progression of AD. Aβs display multiple unwanted effects on the brain, including inflammation³². Microglia are immune cells that reside in the brain, like macrophages, which are derived from monocytes. RAW 264.7 was referred to as an inflammatory model for study the inflammation contributing to AD²⁴. Thus, most of the components of both chicken and tuna hydrolysates and MRPs consisted of amino acids (Table 3), which were capable of being nutrients for the cells. A cell viability assay demonstrated that murine macrophage cells grew in the presence of hydrolysate and MRP treatments (Figure 2). Although certain amino acids underwent the Maillard reaction (Table 3), the MRP products remained non-toxic to the cells (p-value>0.05). The results suggest that the RAW 264.7 cells were unaltered when treated with the hydrolysates and MRPs. Utilizing the functionality of the MRP products is unlikely to be disadvantageous to the macrophage cells. Alzheimer's disease involves various cell types, each having a distinct role in its development and progression. These cell types include neurons, astrocytes, microglia, endothelial cells, and oligodendrocytes. Therefore, when evaluating the safety profile of a substance developed for Alzheimer's treatment, it is advisable to consider the safety profile specifically for these cell types.



OD=Optical density, MRP=Maillard reaction product

Figure 1 UV absorbance (294 nm) (a) and browning intensity (420 nm) (b) of the hydrolysates and the MRPs



MRP=Maillard reaction product

Figure 2 Cell viability of the hydrolysates and MRPs

Conclusion

The hydrolysates and MRPs of chicken and tuna displayed mild antioxidant and acetylcholinesterase-inhibiting properties. These activities could be a result of bioactive peptides with low MWs and their MRPs. These processed food by-products can be further developed as value-added functional foods.

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Conflict of interest

This project was funded by i-Tail Corporation Public Company Limited. M.J. and P.P. are staff members of i-Tail Corporation Public Company Limited.

References

1. Definition of Functional Food [homepage on the Internet] Texas: Functional Food Center, Inc.; 2023 [cited 2023 Apr 2]. Available from: <https://www.functionalfoodcenter.net/>
2. Thailand: food industry [homepage on the Internet] Bangkok: Thailand Board of Investment; 2023 [cited 2023 Apr 4]. Available from: https://www.boi.go.th/upload/content/Food%20industry_5aa7b40bd758b.pdf
3. Sarmadi BH, Ismail A. Antioxidative peptides from food proteins: a review. *Peptides* 2010; 31:1949–56.
4. Himaya SWA, Ngo DH, Ryu BM, Kim SK. An active peptide purified from gastrointestinal enzyme hydrolysate of Pacific cod skin gelatin attenuates angiotensin-1 converting enzyme (ACE) activity and cellular oxidative stress. *Food Chem* 2012;132:1872–82.
5. Betoret E, Betoret N, Vidal D, Fito P. Functional foods development: trends and technologies, *Trends Food Sci Technol* 2011;22:498–508.
6. Pihlanto-Leppälä A. Bioactive peptides derived from bovine whey proteins: opioid and ACE-inhibitory peptides. *Trends Food Sci Tech* 2001;11:347–56.
7. Ngo DH, Vo TS, Ngo DN, Wijesekara I, Kim SK. Biological activities and potential health benefits of bioactive peptides derived from marine organisms. *Int J Biol Macromol* 2012;51:378–83.
8. Li-Chan ECY. Bioactive peptides and protein hydrolysates: research trends and challenges for application as nutraceuticals and functional food ingredients. *Curr Opin Food Sci* 2015;1:28–37.
9. Fu Y, Zhang Y, Soladoye OP, Aluko RE. Maillard reaction products derived from food protein-derived peptides: insights into flavor and bioactivity. *Crit Rev Food Sci Nutr* 2020;60:3429–42.
10. Horvat S, Roscic M. Glycosylation of lysine-containing pentapeptides by glucuronic acid: New insights into the Maillard reaction. *Carbohydr Res* 2010;345:377–84.
11. Karnjanapratum S, O'Callaghan YC, Benjakul S, O'Brien NM. In vitro cellular bioactivities of Maillard reaction products from sugar–gelatin hydrolysate of unicorn leatherjacket skin system. *J Funct Foods* 2016;23:87–94.
12. Song R, Shi QQ, Yang PY, Wei RB. Identification of antibacterial peptides from Maillard reaction products of half-fin anchovy hydrolysates/glucose via LC–ESI–QTOF–MS analysis. *J Funct Foods* 2017;36:387–95.
13. Zhao T, Zhang Q, Wang S, Qiu C, Liu Y, Su G, et al. Effects of Maillard reaction on bioactivities promotion of anchovy protein hydrolysate: The key role of MRPs and newly formed peptides with basic and aromatic amino acids. *LWT* 2018;97:245–253.
14. Nie XH, Zhao LM, Regenstein JM, Xu D, Meng XH. Antioxidant capacity of Maillard reaction products' fractions with different molecular weight distribution from chicken bone hydrolysate – galactose system. *Int J Food Sci Technol* 2017; 52:1632–38.
15. Oh JG, Chun SH, Kim DH et al. Anti-inflammatory effect of sugar–amino acid Maillard reaction products on intestinal inflammation model in vitro and in vivo. *Carbohydr Res* 2017; 449:47–58.
16. Holzgrabe U, Kapková P, Alptüzün V, Scheiber J, Kugelmann E. Targeting acetylcholinesterase to treat neurodegeneration. *Expert Opin Ther Targets* 2007;11: 161–79.
17. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V,

- et al. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev* 2017;2017:8416763.
18. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med* 2019;25:1822–32.
 19. Chen K, Yang Q, Hong H, Feng L, Liu J, Luo Y. Physicochemical and functional properties of Maillard reaction products derived from cod (*Gadus morhua* L.) skin collagen peptides and xylose. *Food Chem* 2020;333:127489.
 20. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical Analysis of *Ficus religiosa*. *Molecules* 2022;27:1326.
 21. Ellman GL, Courtney KE, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharm* 1961;7:88–95.
 22. Suwanhom P, Nualnoi T, Khongkow P, Lee VS, Lomlim L. Synthesis and evaluation of chromone-2-carboxamido-alkylamines as potent acetylcholinesterase inhibitors. *Med Chem Res* 2020;29:564–74.
 23. Joo T, Sowndhararajan K, Hong S, Lee J, Park SY, Kim S, Jhoo JW. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of *Ulmus pumila* L. *Saudi J Biol Sci* 2014;21:427–35.
 24. Ameruso A, Palomba R, Palange AL, Cervadoro A, Lee A, Di Mascolo D, Decuzzi P. Ameliorating amyloid- β fibrils triggered inflammation via curcumin-loaded polymeric nanoconstructs. *Front Immunol* 2017;8:1411.
 25. Temussi PA. The good taste of peptides. *J Pept Sci* 2012;18:73–82.
 26. Zhang Y, Dorjpalam B, Ho CT. Contribution of peptides to volatile formation in the Maillard reaction of casein hydrolysate with glucose. *J Agric Food Chem* 1992;40:2467–71.
 27. Van Lancker F, Adams A, De Kimpe N. Chemical modifications of peptides and their impact on food properties. *Chem Rev* 2011;111:7876–903.
 28. Fu Y, Zhang Y, Soladoye OP, Aluko RE. Maillard reaction products derived from food protein-derived peptides: insights into flavor and bioactivity. *Crit Rev Food Sci Nutr* 2020;60:3429–42.
 29. Zhu D, Damodaran S, Lucey JA. Physicochemical and emulsifying properties of whey protein isolate (WPI)-dextran conjugates produced in aqueous solution. *J Agric Food Chem* 2010;58:2988–94.
 30. Nooshkam M, Varidi M, Bashash M. The Maillard reaction products as food-born antioxidant and anti-browning agents in model and real food systems. *Food Chem* 2019;275:644–60.
 31. Chen K, Zhao J, Shi X, Abdul Q, Jiang Z. Characterization and antioxidant activity of products derived from xylose-bovine casein hydrolysate Maillard reaction: Impact of reaction time. *Foods* 2019;8:242.
 32. Hampel H, Hardy J, Blennow K. et al. The Amyloid- β Pathway in Alzheimer's Disease. *Mol Psychiatry* 2021;26:5481–503.