Biological Properties of Peptide Released by in-vitro Stimulated Digestion of Cooked Meats

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Abstract The aim of this work was to evaluate potential health-related properties of bioactive peptide derived from in-vitro gastrointestinal digestion of cooked meats. During the simulated gastrointestinal digestion process, the soluble liquid sample from different time points representing different steps of gastrointestinal digestive tract were analysed for biological activities; namely, antioxidant, angiotensin-1-converting enzyme (ACE) inhibition and anti-inflammation activities. All digested meats showed increased DPPH antioxidant property with increasing digestion time and contained ability to protect oxidative stress in H2O2-induced CaCo-2 cells with significantly suppressed the formation of malondialdehyde. The ACE inhibitory activity increased covered digestion time indicating anti-hypertension properly. Result indicated that the IC50 of ACE inhibition activity of white meat tuna is significantly lower than other meats in the intestinal phase. Anti-inflammatory effect by nitric oxide (NO) inhibition in macrophages cells revealed that NO inhibition of all digested meats increased with the digestion time. Digested tuna (white and dark meat), and pork at 165 min digestion were among the lowest IC50, hence more inflammatory inhibition than digested beef and chicken.

Keywords: cooked meats, tuna, in-vitro simulated digestion, anti-inflammation, antioxidant, ACE inhibition


1. Introduction

Meat plays an increasingly role in the diet of the world’s population. Dietary meats have been recognized for their nutritional properties as an essential source of amino acids upon digestion and absorption [1]. Meat proteins are not only important source of essential amino acids, but of bioactive peptides as well [2,3] such as protein-derived peptides, creatine, carnosine, L-carnitine, glutathione, conjugated linoleic acid and taurine [4]. It is known that bioactive peptides are generated naturally in mammals within the gastrointestinal tract during the metabolisms of dietary meat proteins [5,6]. During gastrointestinal proteolysis, ingested meat-derivative proteins are attacked by stomach-secreted digestive enzymes, such as pepsin, followed by trypsin, chymotrypsin, elastase, and carboxypeptidase secreted in the small intestine with a consequent generation of biological peptides [7]. Meat derived bioactive peptides have been shown to exert a beneficial health effect including antihypertensive, antioxidant, antithrombotic, antimicrobial, or anticancer activities [3,8]. Examples of meat-derived bioactive peptides were illustrated in some literatures [8,9,10,11] which included angiotensin converting enzymes (ACE) inhibitor peptides isolated from the beef rump (VLAQYK) [12], porcine muscle (AAATP and RPR) [13], chicken (KPLLCS and ELFTT) [14], tuna dark muscle (WPEAAELMMEVDP) [15] and tuna muscle (PTHIKWGD) [16]. Bioactivities of peptides depend on the sequence, amino acid composition, and molecular mass [17]. In addition, literature has been reported that length of peptide could affect the intensity of the bioactivity, with smaller peptides characterized by greater bioactivity [18,19].

Meat protein from different sources with different textures would be digestible at the different rates, leading to difference in bioavailability of particular types of amino acids and peptides [20]. The quantity of peptides released upon digestion is hardly predictable. However, some authors reported that the potential yield of bioactive peptides is relatively high during digestion of dairy proteins. For instance, the theoretical yield of opioid peptides encrypted in milk proteins was estimated ranged between 2% and 6% [21]. Consumption of meat proteins is well known in benefit of nutrition but additional functional effects have not been much studied. Potentially meat and other proteins consumption could enhance certain physiological functions. In order to study potentially-functional peptides from studying cooked meats, the gastrointestinal digestive system was therefore...
simulated to generate peptides similar to those released in a physiological digestion process. The functional activities of the peptides obtained from digestion of five types of cooked meat (dark and white tuna meats, pork, chicken, and beef) at the different time points were determined and compared in term of anti-oxidative, anti-inflammatory and ACE inhibitory functions. The outcome for this study could lead to better understanding of functional protein and peptides in our common diets.

2. Materials and Methods

2.1. Chemicals

Porcine pepsin (EC 3.4.23.1, from porcine gastric mucosa, > 250 units·mg⁻¹ solid), pancreatin from porcine pancreas (4 × USP specifications, EC number 232-468-9), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH), minimum essential medium eagle (MEM), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), RPMI-1640 medium, lipopolysaccharide (LPS, from *Escherichia coli*), L-nitroarginine (L-NA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in this study were of analytical grade.

2.2. Cooked Meats Preparation

Five types of meat, skipjack tuna (white and dark muscle), chicken, pork, and beef were selected to study in this work. Fish muscle generally consists of white and dark muscle, the dark muscle is a band of dark tissue that lies beneath the skin throughout the body. In tuna, dark muscle is also located near the backbone [22]. Different color between white and dark muscle was previously observed [23]. The L*, a* and b* values of white and dark muscle were 46.90±1.27, 2.36±0.75, 5.53±1.67 and 31.32±2.34, 4.89±0.68, 1.74±0.29, respectively [23]. In this study, fresh white and dark muscles from skipjack tuna were obtained from Thai Union Frozen Group PCL., Samutsakhan, Thailand. Chicken breast, pork tenderloin, and beef tenderloin were purchased from local market. After removing the obvious fat and connective tissue, all samples were cut into a size of 10 cm x 15 cm x 1.5 cm and placed in a zip-locked plastic bag before boiled in boiling water for 10 mins. These types of meat were selected based on preliminary composition evaluation to obtain protein, fat and ash content as similarly as possible. All samples were cut in at least three lots of meats each underwent simulated gastrointestinal digestion and analyses separately.

2.3. In-vitro Simulated Digestion

Each cooked meat (20 g) was mixed with 300 ml pepsin solution [0.462% pepsin (Sigma P6887) (w/v), 49 mM NaCl, 12 mM KCl, 10 mM CaCl₂, 2.4 mM MgCl₂, and 2.5mM K₂HPO₄]. Adjusted pH to 5.5 (using either 3M HCl or 3M NaOH) and 3 ml of the mixture was collected as a sample at time 0 (T₀). Meat and pepsin mixture was further incubated with stirring at 220 rpm in a water-bath set at 37°C. The second sample (T₃0) of 3 ml was collected at 30 min prior to adjusting the pH to pH 3.8 and continued incubation for a 30 min. The third sample (T₅0) was collected and then the pH was adjusted to pH 2.2 and left incubated for further 15 min. The forth sample (T₇5) was collected prior to the addition of 30 ml of bile and pancreatic solution [0.2 g pancreatine (Sigma P1750), 1.25 g bile extract (Sigma B8631) and 0.1 M NaHCO₃ in 50mL distilled water]. After adjusted to pH5.0 and further incubated for 30 min, the fifth sample at 105 min digestion (T₁05) was collected. The digestion mixture was then adjusted to pH 6.5 and incubated for 60 min then the last sample at 165 min (T₁65) was collected. The digested samples at T₀-T₇5 were referred to gastric phase and over T₇5-T₃0 were referred to small intestine phase. Each collected sample was stopped by heating up to 95°C for 10 min, cooled in an ice bath and then centrifuged in a refrigerated centrifuge at 4500x g for 15 min [24]. The supernatant was transferred to an amber bottle and was kept at -20°C for further study.

2.4. Nutrient Contents Analysis

Cooked meat samples were subjected to evaluate their nutrient contents including protein, ash, moisture and fat content according to AOAC method [25].

2.5. Degree of Hydrolysis (DH) Determination

Soluble peptides from simulated GI digestion (T₀, T₃₀, T₅₀, T₇₅, T₁₀₅ and T₁₆₅) were determined for DH following the method of Adler-Nissen (1979) and Wanasundara et al. (2002) with slight modification [26,27]. The DH is defined as percent ratio of the number of peptide bonds broken to total number of bonds per unit weight. The α-amino group was expressed in terms of L-leucine concentration. Degree of hydrolysis was calculated based on the number of α-amino groups before and after the hydrolysis.

2.6. Amino acid Composition

Amino acid compositions of peptides released from simulated GI digestion (0.5 μl) were analyzed by HPLC equipped with ZORBAX Eclipse Plus C18 column (Agilent Technologies, Palo Alto, California). Column size was 4.6×250 mm with a dimeter of 5 μm. The o-phthalaldehyde (OPA) was used for pre-column derivatization of primary amino acids and 9-fluorenylmethyl chlororformate (FMOC) was used for secondary amino acids. The mobile phases were A: 10 mM Na₂HPO₄, 10 mM Na₂B₄O₇, 32 mg NaN₃; and B: Acetonitrile: methanol: water (45:45:10, v/v/v). The gradient was set as follows: 0-0.5 min, 0% B; 23.0 min, 57% B; 23.1 min, 100% B; 27.0 min, 100% B; 28.0 min, 0% B; 30.0 min, 0% B. Flow was 1.5 ml/min. The primary amino acids (OPA-derivatized) were monitored at 338 nm, while the secondary amino acids (FMOC-derivatized) were monitored at 262 nm. Amino acid standard (10 pmol/μL to 1 nmol/μL) calibration curves were used.
2.7. DPPH free Radical Scavenging Activity

The scavenging effect on DPPH free radical was measured as described by Brand-Williams et al. (1995), with modification [28]. A volume of 1.0 ml of samples from simulated GI digestion (T0, T30, T60, T75, T105 and T165) in different concentrations (0-10 mg protein/ml) was added to 1.0 ml of 0.2 mM DPPH in 95% ethanol. The mixture was homogenized in a vortex and kept for 30 min at room temperature in the dark. The absorbance of the solution was measured at 517 nm using a microplate reader. The percentage inhibition was calculated and results were expressed as a value of IC50 which was a concentration that caused 50% reduction in anti-oxidation from maximum.

2.8. Cellular Antioxidant Activity

2.8.1. Cell Culture

Caco-2 human intestinal cells were used as a model of the intestinal epithelium, and this cell line was obtained from American Type Culture Collection (Rockville, MD, USA). The cells were cultured in growth medium (MEM; Minimum Essential Medium; Gibco, Burlington, ON, Canada) with 20% fetal bovine serum (FBS; Hyclone, UT, USA) and 50 units/mL of penicillin-streptomycin (Gibco, NewYork, USA). The medium was changed twice per week. The cell were grown in a flask for 5-8 days after NewYork, USA). The medium was changed twice per week. The cell were grown in a flask for 5-8 days after

2.8.2. Induction of Oxidative Stress

The oxidative stress was induced to confluent cells by adding 1mM H2O2 [30]. Prior to treatment, Caco-2 monolayers were washed twice with Hank’s balanced salt solution (HBSS) without calcium and magnesium. The cells were then incubated with soluble peptides from simulated GI digestion (T0, T30, T60, T75, T105 and T165) at final concentration of 1 mg/ml for 24 h. Cells were washed with HBSS followed by the addition of 1 mM H2O2 for 24 h. At the end of 48 h, the cells were further washed twice with phosphate-buffered saline (PBS) and cell were lysed with cell lysis buffer consisting of 50 mmol/L in the Tris-HCl, pH 7.4, 150 mmol/L NaCl, 5 mmol/L dithiothreitol and Triton X-100 (1%, v/v) for 5 min on ice and centrifuged at 14000 g and 4 °C for 10 min. Use this clear supernatant for the assay total antioxidant activity.

2.8.3. Total Antioxidant Capacity Analysis

The cellular antioxidant capacity was determined by QuantiChrom™ Antioxidant Assay kit (Bioassay System, USA). This kit measured total antioxidant capacity in which Cu2+ is reduced by antioxidant to Cu+. Chemicals and assay procedure was followed manufacture instruction and measured OD at 570 mM. Trolox was used as a standard.

2.8.4. Lipid Peroxidation Determination

The cellular malondialdehyde (MDA) was used to determine the extent of lipid peroxidation under oxidative stress using QuantiChrom™ TBARS Assay kit (Bioassay System, USA). Chemicals and assay procedure was followed manufacture instruction and measured OD at 535 mM.

2.9. ACE-inhibitory Activity Analysis

Peptide samples collecting from simulated GI digestion (T0, T30, T60, T75, T105 and T165) were analyzed ACE-inhibition activity assay following as described by Cushman and Cheung (1971) with slightly modification [31]. The absorbance was measured at 228 nm and the percentage of ACE inhibition (%) was calculated as equation below;

\[ \text{The inhibition rate (\%)} = \left( \frac{E_c - E_s}{E_c - E_b} \right) \times 100 \] (1)

Where Es is the absorbance when the sample is added to the post-reaction mixture, Ec is the absorbance with a buffer (instead of the sample) added and Eb is the absorbance when a stop solution is added before the reaction occurs. IC50 was calculated.

2.10. Anti-inflammatory Activity Analysis

Anti-inflammatory activity was determined by the inhibition ability of peptide released samples obtained from simulated GI digestion (T0, T30, T60, T75, T105 and T165) against NO production in LPS-stimulated RAW 264.7 macrophage cell [32]. Briefly, the RAW264.7 cell line was cultured in RPMI-1640 medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine, penicillin G (100 units/ml), streptomycin (100 g/mL) and 10% fetal bovine serum (FBS). The cells were harvested with trypsin-EDTA and diluted to a suspension in a fresh medium. The cells were seeded in 96-well plates with 1x104 cells/well and allowed to adhere for 2 h at 37°C in a humidified atmosphere containing 5% CO2. After that the medium was replaced with a fresh medium containing 100 g/mL of LPS together with the test samples at various concentrations and was then incubated for 24 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent (Sigma-Aldrich, USA). The L-nitroarginine (L-NA) was used as positive controls. The stock solution of each digested sample was dissolved sterilized water and the solution was added to the medium RPMI-1640. Inhibition (%) was calculated using the following equation and IC50 values were determined.
Inhibition (%) = \frac{(A - B)}{(A - C)} \times 100 \quad (2)

Where \( A \): concentration (µg/ml) [A: LPS (+), sample (−)]; \( B \): LPS (+), sample (+); \( C \): LPS (−); sample (−).

2.11. Statistical Analysis

All the data are presented as mean ± standard deviation (SD), and all the statistical analyses were performed by the SPSS statistics program (Version 16.0). An analysis of variance (ANOVA) was conducted, and the differences between the means were examined using Duncan’s Multiple Range Test at \( P < 0.05 \), while all the samples were measured in triplicate.

3. Results and Discussion

3.1. Chemical Composition of Cooked Meats

Chemical composition of five types of cooked meats (dark and white tuna meats, pork, chicken and beef) was presented in Table 1. The results revealed that white and dark meat from tuna had a similar level of protein content as beef, which was slightly lower than those in pork and chicken. As expected, tuna white meat contained the least fat content; i.e. about more than 3 times less than those of chicken, pork and beef. On the contrary, tuna dark meat contained higher fat content that of the chicken breast meat while did at 0.2 and 0.8 time less than those of pork and beef, respectively. In the case of ash content, it was higher in both tuna white and dark meats than that in other meats (p<0.05).

Table 1. Nutrients of all cooked meats

<table>
<thead>
<tr>
<th>Type of meats</th>
<th>Protein (%)</th>
<th>Fat (% dry basis)</th>
<th>Ash (% dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna (White meat)</td>
<td>84.93±1.65a</td>
<td>1.45±0.35a</td>
<td>6.50±1.00a</td>
</tr>
<tr>
<td>Tuna (Dark meat)</td>
<td>88.70±0.14a</td>
<td>5.30±0.30a</td>
<td>4.82±0.75a</td>
</tr>
<tr>
<td>Chicken (breast)</td>
<td>92.61±3.66a</td>
<td>4.62±0.59a</td>
<td>4.09±0.95a</td>
</tr>
<tr>
<td>Pork (tender loin)</td>
<td>90.50±1.28a</td>
<td>6.33±1.94a</td>
<td>3.89±0.71a</td>
</tr>
<tr>
<td>Beef (tender loin)</td>
<td>84.45±0.21b</td>
<td>9.65±0.63a</td>
<td>3.66±1.09a</td>
</tr>
</tbody>
</table>

Different superscripts (a, b, c) indicated the significant difference among the data in the same column.

3.2. Degree of Hydrolysis (DH) and Free Amino Acid Composition

After simulated GI digestion, DH of all meats increased with the extent of digestion time. The DH of each type of meats were similar (mostly) in all digestion intervals (Figure 1).

This result was similar trend with releasing of free amino acids, which was found that free amino acids also increased with the extent of digestion time and small intestinal phase were able to release more amino acids than gastric phase. At the end of digestion (T165), all essential amino acids, particularly leucine, isoleucine and valine are abundant in every meat types, especially valine more present in white meat tuna than other meat (Figure 2).

These three amino acids were called branched-chain amino acids (BCAAs), which have recently been recognized as having functions other than simple nutrition. The BCAAs play critical roles in the regulation of energy homeostasis, nutrition metabolism, gut health, immunity via special signaling network, especially phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signal pathway [33]. Additionally, Current evidence supports BCAAs and their derivatives as the potential biomarkers of diseases such as insulin resistance (IR), type 2 diabetes mellitus (T2DM), cancer, and cardiovascular diseases (CVDs) [33]. These diseases are closely associated with catabolism and balance of BCAAs. Hence, optimizing dietary BCAAs levels should have a positive effect on the parameters associated with health and diseases. Among digested meats in this study, the highest amount of BCAAs was observed in white meats tuna (5.25±0.006%), followed by dark meat tuna (3.86±0.04%), chicken (3.68±0.07%), pork (3.34±0.08%), and beef (3.11±0.07), respectively. Additionally, some other essential amino acids such as histidine, methionine and lysine were found to be abundant in white meats of tuna (Figure 2).

3.3 Antioxidative Activity

3.3.1. DPPH Scavenging Activity

Digestive enzyme can be hydrolyzed each type of meats and produced different sized peptide and amino acid sequence which can exhibit biological activity. Hydrolyzed short chain peptides containing water soluble hydrophilic peptides have been reported to possess antioxidative properties [34]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their electron or hydrogen donating ability [35]. Figure 3 shows the results of IC50 of DPPH radical scavenging capacity of digested meats. It was observed that IC50 of DPPH radical scavenging capacity in all digested meats decreased with the extent of digestion time. Among all digested meats, tuna white meat showed the lowest IC50 when entered small intestine at T105 and T165 indicating that white meat tuna provided the highest action as a good electron transfer and hydrogen atom transfer (antioxidant) and could react with free radicals to terminate the radical chain reaction than other type of meat.

3.3.2. Cellular Total Antioxidant Capacity

Cellular total antioxidant activities of all digested meats were determined in CaCo-2 cell culture under stress condition by hydrogen peroxide (H2O2) treatment. This was also done in comparison to that under none-stress condition; i.e. cell culture without H2O2 (cell; control) treatment. The results (Figure 4) indicated that all digested meats contained the ability to protect oxidative stress in cell. Nonetheless, the anti-oxidative activities did not significantly change during simulative gastrointestinal digestion. Recently, a number of studies demonstrated that peptides digested by enzyme from fish protein and other protein sources are potential source of antioxidant peptide [24,30,32,36].
Figure 1. Effect of digestion time on degree of hydrolysis obtained from digested meats. Different superscripts (a, b, c) indicated the significant difference (p<0.05) among the data in the same digestion time.

Figure 2. Free amino acid compositions of digested meats at 165 min digestion (T₁₆₅). WM: tuna white meat, DM: tuna dark meat, PM: Pork, CHM: chicken, BM: Beef.

Figure 3. Effect of digestion time on IC₅₀ of DPPH inhibition activities from digested meats. Different superscripts (a, b, c) indicated the significant difference (p<0.05) among the data in the same digestion time.
It was earlier shown that lipid peroxidation was involved in the cleavage of polyunsaturated fatty acids at its double bonds, resulting in cellular membrane damage and increase in MDA production [37]. The H\textsubscript{2}O\textsubscript{2} was used for inducing the oxidative stress and caused lipid peroxidation. The concentration of the MDA released from the oxidation was measured as a biomarker of oxidative stress in Caco-2 cells. The MDA level in CaCo-2 cells pre-incubated with digested meats (1 mg/ml) under the stimulation of 1 mM H\textsubscript{2}O\textsubscript{2} are represented in Figure 5. Under none-stress induction condition (cell; control), CaCo-2 cells exhibited a basal level of MDA due to the normal oxidative metabolism. On the contrary, under the oxidative stress condition caused by the addition of 1 mM H\textsubscript{2}O\textsubscript{2} (cell+ H\textsubscript{2}O\textsubscript{2}; negative control), MDA was significantly increased (P < 0.001) from that of the control (cell), indicating the cellular lipids were oxidized by H\textsubscript{2}O\textsubscript{2}. Addition of digested meats in the H\textsubscript{2}O\textsubscript{2}-induced CaCo-2 cells mostly significantly (P < 0.05) suppressed the formation of MDA as compared to that of the negative control group (cell+ H\textsubscript{2}O\textsubscript{2}), suggesting the protective effects from the digested meats against the lipid peroxidation. These protective effects could be due to the radical scavenging activities of digested meats or the activation of the cellular defense system against oxidative stress by pre-incubation digested meats. The tendency of MDA value is reduced when increasing digestion time (Figure 5). The lowest of MDA value was found in all digested samples at T\textsubscript{165}, and tuna white meat showed a trend of the lowest amount of MDA. However, the produced MDA from tuna white meat is not significant different from tuna dark meat and beef. This might be due to these digested meats contained more hydrophobic amino acid such as Val, Leu, Trp, His and small peptide more than other hydrolyzed meats (Figure 2). Previous studies reported that typically, peptides with anti-oxidative properties often contain residues of hydrophobic amino acid include Val or Leu at the N-terminus of the peptides, and Pro, His, Tyr, Trp, Met, and Cys in their sequences [38].

### 3.4. ACE inhibitory Activity

Angiotensin-converting enzyme (ACE) plays a major role in the regulation of blood pressure. It indirectly increases blood pressure by causing blood vessels to constrict [39]. Any compounds that contain the activity of ACE inhibition would be benefit for treatment of cardiovascular diseases. In this study, the ACE inhibition activity was found to increase with digestion time. The
The strongest activity was at digested time of 165 min (T165) with DH of 79-81%. This result was consistent with the previous report stating that ACE-inhibitory peptides are usually small peptides with sizes comprising between 2 and 20 amino acids [40], approximate molecular weight of 220-2,200 Da. Half maximal inhibitory concentration (IC50) values of digested meat was shown in Figure 6. Among all meats, tuna white meat showed lowest IC50 of ACE inhibition activity indicating that digested tuna white meat exhibited strongest ACE inhibition activity.

Figure 6. IC50 of ACE inhibitory activities of digested meats at 165 min digestion (T165). Different superscripts (a, b, c) indicated the significant difference (P<0.05)

According to the literature, ACE inhibitory peptides typically contained aromatic ring or hydrophobic amino acid side chain on carboxyl end (Phe, Tyr, Leu, Met, Pro and Trp) showed high potent of this biological activity because it can react at active site of ACE [41]. From the result, Pro, Phe, Met in tuna white meat (Figure 2) may contribute to ACE inhibitory activity. Additionally, ability to decrease blood pressure of these digested meats may be come from taurine which is a neutral β-amino acid derived from the metabolism of Sulphur containing amino acids. The Main mechanism through which taurine may decrease blood pressure is thought to be the attenuation of angiotensin II signaling, which causes vasoconstriction and consequently increases blood pressure [42]. This decrease blood pressure through enhancement of the kinin-kallikrein system in the kidney that causes vasodilation [43] and may also lower blood pressure by decreasing levels of epinephrine (which increases heart rate) and norepinephrine (which causes vasoconstriction) [44]. Among tested meats, some literatures have been reported that the highest amount of taurine was found in tuna followed by pork, chicken and beef with a value of 1721.92±8.0 mg/kg [45], 610 mg/kg, 337 mg/kg and 43 mg/kg, respectively [46].

3.5. Anti-inflammatory effect in LPS-induced RAW264.7 macrophage cell

Prior to evaluation NO inhibition, the cytotoxicity of the digested soluble peptides was assessed in RAW264.7 macrophage cell using MTT assay [47] in order to investigate whether these digested meats affect cell survival. All digested meats collecting at T0, T75 and T165 did not show any cytotoxic effect on RAW264.7 macrophage cell up to 500 µg protein/ml. This was due to cell viability of all tested samples was higher than 80% of that in the control group [48].

Anti-inflammatory activity of digested meats was determined by measuring inhibitory effect on nitric oxide (NO) production from macrophage cells. NO is a major mediator of inflammation [49]. Any substances showing NO inhibitory activity would indicate its anti-inflammatory activity. The soluble digests from the simulated GI of the five meats; namely, tuna white and dark meats, pork, chicken and beef, were tested for the inhibitory effect on the NO production in RAW264.7 macrophage cell. The results revealed that NO inhibition increased with the digestion time (P<0.05). Tuna dark meat exhibited the highest NO inhibitory effect among all types of meat.

The IC50 value of NO inhibition, which was the concentration that causes 50% reduction of NO production, was evaluated from the five meat digestion samples at 165 min. The results (Figure 7) showed that there was no significant difference in IC50 value among digested tuna dark meat, tuna white meat and pork (p> 0.05). These IC50 values were significantly lower than the other two digested samples (beef and chicken). However, these IC50 values were higher than L-Nitroarginine which is wildly known as a strong NO inhibitor [50].

Figure 7. IC50 of NO inhibitory activities of digested meats at 165 min digestion (T165). Different superscripts (a, b, c) indicated the significant difference (P<0.05)

4. Conclusion

Functional properties of tuna protein were found to be derived by the digestion of indigenous enzyme in human GI system. Digested peptides and free amino acids exerted some bioactive and potential functional properties such as anti-oxidant activities, anti-hypertensive and anti-inflammatory activity in cellular levels. Cellular antioxidants in CaCo-2 cells showed higher anti-oxidation activity (lower MDA) in digested tuna than other meats, and successfully inhibited lipid peroxidation and damage and inflammation at sub-cellular level particularly for tuna (white and dark) meat and pork. Angiotensin-converting enzyme plays a crucial role that plays a fundamental role in blood pressure
homeostasis as well as fluid and salt balance in mammals [51]. All meats studied significantly inhibited ACE inhibitor. Digested white and dark meat tuna and pork could significantly inhibit ACE inhibitory enzyme decrease blood pressure (at IC50 of 1.7-1.8 µg protein/ µL) more than chicken and beef (p<0.05), significantly. Contribute to anti-inflammatory properties on macrophage cell line more extensively than digested chicken and beef. Eating meats do have several functional effects to our bodies differently.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

- NO: Nitric oxide
- ACE: Angiotensin-1-converting enzyme
- DPPH: 1,1-diphenyl-2-picryl-hydrazyl
- H2O2: Hydrogen peroxide
- MTT: 3 -(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide
- LPS: lipopolysaccharide
- L-NA: L-nitroarginine
- FCS: Fetal calf serum

References


