Current trends in bioactive peptides from muscle foods and their potential application

J Yongsawatdigul and A Hamzeh

School of Food Technology, Institute of Agricultural Technology
Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand
E-mail: jirawat@sut.ac.th

Abstract. Muscle protein is a valuable source of energy and essential amino acids, which are needed for growth and maintenance of physiological functions. It also provides peptides known as bioactive peptides exerting some health benefits, including antihypertension, antioxidant activity, immunomodulatory activity, and improving brain function, among others. Bioactive peptides can be generated from digestion or enzymatic reaction. Cooking condition is found to critically affect digestibility of chicken breast and release of bioactive peptides. Extreme thermal treatment at 121°C/15 min reduces simulated gastrointestinal digestion (GI) of chicken breast. In addition, release of peptides inhibiting angiotensin converting enzyme (ACE) that regulates blood pressure via renin-angiotensin system decreases. This implies that extreme thermal process of muscle food lower nutritional values and available ACE inhibitor peptides. Enzymatic hydrolysis is also an effective means to produce bioactive peptides, providing appropriate proteases is applied under the optimal condition. Hydrolysates or bioactive peptides would likely be modified through GI digestion. Thus, changes of bioactivities upon GI digestion should be taken into consideration for optimization of protein hydrolysate production. Structural changes of bioactive peptides further take place during transepithelial transport. This would definitely affect bioactivities of peptides reaching the target organ in either positive or negative manner. To further develop functional food or nutraceutical products, efficacy of bioactive peptides should be tested in vivo to assure their health benefits.

1. Introduction
Bioactive peptides are protein fragments composed of 2-20 amino acids which can exert a physiological effect, such as antioxidant, antihypertension, immunomodulatory activity [1-3]. These peptides generated from various sources including egg, milk, bean, meat and fish, after being exposed to hydrolysis process. The hydrolysis can be carried out using either enzymes or chemicals, in which chemical hydrolysis of proteins via acid or alkali is less effective due to harsh condition destroying some amino acids [4]. Enzymatic process can be conducted in two categories including addition of exogenous proteases or fermentation by protease-producing microorganisms (Table 1).

Addition of exogenous proteases is more preferable because many factors affecting hydrolysis can be effectively controlled. Production of peptides with high ACE inhibition from Pacific hake can be
achieved using Protamex at pH 6.5 for 125 min at 3% enzyme [5]. The optimum conditions to produce antioxidant peptides from duck meat were at 50 °C, pH 7 for 8 h by Protamex [6]. In addition, crude proteases from hepatopancreas of Pacific white shrimp was used to generate antioxidant peptides from splendid squid [7].

Table 1 Bioactive peptides derived from muscle foods by exogenous proteases and fermentation

| Origin                | Bioactivity       | Peptide generation | Reference |
|-----------------------|-------------------|--------------------|-----------|
| Dry-cured beef        | ACE-inhibitory    | Aging              | [8]       |
|                       | Antioxidant       |                    |           |
| Beef                  | ACE-inhibitory    | Aging              | [9]       |
|                       | Antioxidant       |                    |           |
| Duck meat             | Antioxidant       | Aging              | [10]      |
| Dry sausage (pork)    | ACE-inhibitory    | Fermentation       | [11]      |
|                       | Antioxidant       |                    |           |
| Shrimp                | ACE-inhibitory    | Fermentation       | [12]      |
|                       | Antioxidant       |                    |           |
| Fish                  | Antioxidant       | Fermentation       | [13]      |
| Pork                  | ACE-inhibitory    | Pepsin             | [14]      |
| Fish                  | DPP-IV and ACE inhibitory | Alkalase, Flavorzyme, Corolase PP and Promod 144 | [15] |
| Chicken               | Antioxidant       | Papain             | [16]      |

Bioactive peptides can also be released by gastrointestinal digestion of muscle foods. In this regard, food processing and preparation play an important role in generation of peptides with certain bioactivities [17]. Sangsawad, Roytrakul and Yongsawatdigul [18] reported that peptides with higher ACE inhibition ability could be generated from chicken breast as exposed to a mild heat at 70 °C for 30 min, while an extreme heat at 121 °C resulted in peptides with lower activity.

2. Purification and identification of bioactive peptides

Hydrolysates prepared from a proteinous substrates contain a mixture of active and inactive peptides with different properties of size, charge, hydrophobicity, and others [19]. To separate and purify bioactive peptides, chromatographic techniques are used, in which a consecutive chromatography including size exclusion chromatography (SEC), ion exchange chromatography (IEC), hydrophobic interaction chromatography (HIC) and reversed phase chromatography (RPC) can be applied to obtain the most active peptides. SEC, IEC were applied to separate peptides based on their size and charge characteristics, respectively. Hydrophobicity separation of peptides can be conducted by HIC and RPC. Liquid chromatography tandem-mass spectrometry (LC-MS/MS) is a common method to identify peptide sequence. Some purification and identification methods used in bioactive peptides derived from muscle foods are shown in Table 2.

Table 2 Purification schemes and identification applied for bioactive peptides derived from various muscle foods.
### Table

| Source           | Bioactivity     | P\(^a\) and I\(^b\) Methods                     | Identified peptide | Reference |
|------------------|-----------------|-------------------------------------------------|--------------------|-----------|
| Tuna dark muscle | Antiproliferation| SEC (Sephadex G-25), RPC\(^c\) (C-18) LC-MS/MS | LPHVLTEAGATP TAEGVYMVT | [20]      |
| Croceine croaker | Antioxidant     | UF\(^d\), AEC (DEAE-52 cellulose), SEC (Sephadex G-15), RPC (C-18) LC-MS/MS | YLMSR VLYEE MILMS | [21]      |
| Beef             | ACE-inhibitory  | UF, SEC (Sephadex G-25), RPC (C-18) Peptide sequencer | VLAQYK            | [22]      |
| Silver carp      | DPP-IV inhibitory| UF, TLC (silica gel), RPC (C-18) LC-MS/MS | LPIIDILV AQPAPA    | [23]      |
| Chicken          | ACE-inhibitory  | IEC\(^f\), SEC, RPC LC-MS/MS | KPLLCS ELFTT KPLL | [18]      |
| Alaska pollock   | Immunomodulatory| SEC, RPC LC-MS/MS | PTGADY            | [24]      |

\(^a\)Purification. \(^b\)Identification. \(^c\)Reverse phase chromatography. \(^d\)Ultrafiltration. \(^e\)Thin layer chromatography. \(^f\)Ion exchange chromatography.

### 3. Gastrointestinal (GI) digestion of bioactive peptides

GI digestion and intestinal epithelial absorption in human tract is the great challenges for bioavailability of peptides [25]. After oral ingestion, gastrointestinal enzymes may break down peroteins/peptides, thereby increasing or decreasing their activity. It has been reported that the GI digestion improved bioactivities of pork and chicken meat [26,18]. Peptides prepared from Tilapia showed a reduction in ACE inhibitory after GI digestion [3]. Some researchers reported an increase in bioactivities of peptides after in vitro GI digestion such as antioxidant activity of hydrolysates prepared from splendid squid [7] and poultry proteins [27]. Peptides can be hydrolyzed in stomach by pepsin, but the main hydrolsis takes place in intestine by pancreatin. You et al. [28] reported an increase in DH of fish protein hydrolysates from 37.4 to 38.7% at stomach digestion in 2 h, while the DH raised up to 46.6% after pancreatin digestion at the same incubation time.

### 4. Peptide transport

Transport of peptides across intestine into blood is another challenge for bioactive peptides to reach to target organ and exert a physiological function. Peptides can pass through intestinal cells via four mechanisms including para-cellular, passive diffusion, endocytosis and carrier mediated transport. Smaller peptides (di or tri) can pass easily, however there is some differences between adults and infants due to incomplete development of GI tract in infant [3,5,29]. Toopcham et al. [3] reported that tri and tetra-peptides of tilapia hydrolysate could pass through Caco-2 cell line Sangsawad et al. [30] identified 9 transepithelial transported tri-peptides from chicken breast, in which APP had the greatest ability to inhibit ACE. Peptides structure can also be transformed during transport by brush border enzymes. Sangsawad et al. [30] reported that KPLL was degraded to KP and LL by brush border proteases. Peptides derived from dry-cured ham, AAATP, AAPLAP and KPVAAP have been affected
by brush border proteases, resulting in generation of mostly di and tri-peptides. Escudero, Toldrá, Sentandreu, Nishimura and Arihara reported that KAPVA, PTPVP, and RPR from pork meat could decrease blood pressure in rats. Three peptides, ALTA, SLTA and VT, from pork actomyosin showed antioxidant effects in vitro and in vivo in rats.

5. Conclusions

Bioactive peptides can be applied in food formula as nutraceutical compounds, exerting various health benefits. Among various substrates, muscle food can be a good candidate to use for bioactive peptides generation because of their high protein content. The challenge with these peptides is that their structure is likely to be affected by digestive proteases and epithelial transportation. Study of transport would shed lights on how peptides are modified. Although peptides might be modified in GI tract, bioactivities of peptides have been shown in vivo, suggesting that permeated peptides in blood in spite of possible structural change could still exhibit functions.

References

[1] Piyadhammaviboon P, Wonggam W, Benjakul S and Yongsawatdigul J 2012 J. Aquat. Food Prod. T. 21 265-78
[2] Lafarga T, O’Connor P and Hayes M 2014 Peptides 59 53-62
[3] Toopcham T, Mes JJ, Wichers HJ and Yongsawatdigul J 2017 Food Chem. 224 320-8
[4] Kristinsson HG and Rasco BA 2000 Crit. Rev. Food Sci. Nutr. 40 43-81
[5] Cinq-Mars CD and Li-Chan ECY 2007 J. Agric. Food Chem. 55 9380-8
[6] Lee SJ, Kim EK, Hwang JW, Oh HJ, Cheong SH, Moon SH, Jeon BT, Lee SM and Park PJ 2010 Food Chem. 123 216-20
[7] Hamzeh A, Benjakul S and Senphan T 2016 J. Food Sci. Technol. 53 3615-23
[8] Deniz E, Mora L, Aristoy MC, Candoğan K and Toldrá F 2016 Food Res. Int. 89 194-201
[9] Fu Y, Young JF and Therkildsen M 2017 Meat Sci. 123 134-42
[10] Liu D, Chen X, Huang J, Zhou X, Huang M and Zhou G 2017 Int. J. Food Sci. Technol. 52 2513-21
[11] Gallego M, Mora L, Escudero E and Toldrá F 2018 Int. J. Food Microbiol. 276 71-8
[12] Kleekayai T, Harney PA, O’Keefe MB, Poyarkov AA, Cunhaneeves A, Sunthornsuk W and Fitzgerald RJ 2015 Food Chem. 176 441-7
[13] Giri A, Nasu M and Ohshima T 2012 Int. J. Food Sci. Nutr. 1 13-22
[14] Katayama K, Anggraeni HE, Mori T, Ahimed AM, Kawahara S, Sugiyama M., Nakayama T, Maruyama M and Muguruma M 2008 J. Agric. Food Chem. 56 355-60
[15] Neves AC, Harney PA, O’Keefe MB, Alashi MA, Aluko RE and FitzGerald RJ 2017 Food Res. Int. 100 112-20
[16] Sun Y, Pan D, Guo, Y and Li J 2012 Purification of chicken breast protein hydrolysates and analysis of its antioxidant activity. Food Chem. Toxicol. 50 3397-404
[17] Dallas DC, Guerrero A, Parker EA, Robinson RC, Gan J, German JB, Barile D and Lebrilla CB 2015 Proteomics 15 1026-38
[18] Sangsawad P, Roytrakul S and Yongsawatdigul J 2017 J. Funct. Foods 29 77-83
[19] Sila A and Bougatéf A 2016 J. Funct. Foods 21 10-26
[20] Hsu KC, Li-Chan ECY and Jao CL 2011 Food Chem. 126 617-22
[21] Chi C F, Hu F Y, Wang B, Ren X J, Deng S G and Wu C W 2015 Food Chem. 168 662-7
[22] Jang A and Lee M 2005 Meat Sci. 69 653-61
[23] Zhang Y, Chen R, Chen X, Zeng Z, Ma H and Chen S 2016 *J. Agric. Food Chem.* **64** 831-9
[24] Hou H, Fan Y, Wang S, Si L and Li B 2016 *J. Funct. Foods* **24** 37-47
[25] Ao J and Li B 2013 *Food Res. Int.* **52** 334-41
[26] Escudero E, Sentandreu M A, Arihara K and Toldrá F 2010 *J. Agric Food Chem.* **58** 2895-901
[27] Anna T, Alexey K, Anna B, Mikhail T and Ulia M 2016 *Curr. Res. Nutr. Food Sci.* **4** 77-86.
[28] You L, Zhao M, Regenstein J M and Ren J 2010 *Food Chem.* **120** 810-6
[29] Vermeirssen V, Camp J Van and Verstraete W 2004 *Br. J. Nutr.* **92** 357-66
[30] Sangsawad P, Roiytrakul S, Choowongkomon K, Kitts DD, Chen X M, Meng G, Li-Chan ECY and Yongsawatdigul J 2018 *Food Chem.* **251** 77-85
[31] Gallego M, Mora L, Escudero E and Toldrá F 2018 *Int. J. Food Microbiol.* **276** 71-8
[32] Escudero E, Toldrá F, Sentandreu M A, Nishimura H and Arihara K 2012 *Meat Sci.* **91** 382-4
[33] Arihara K and Ohata M 2006 Advanced technologies for meat processing ed. Toldrá F (New York: Springer) pp. 245-74