Effect of processing conditions and simulated gastrointestinal digestion on the activity of angiotensin I-converting enzyme (ACE) inhibitory peptide derived from duck meat hydrolysate

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ABSTRACT
In this study, the ACE-inhibitory peptide (<3 kDa) was obtained through ultrafiltration of duck meat enzymatic hydrolysate. The research investigated the effect of processing conditions (heating, pH, NaCl, glucose) and simulated gastrointestinal digestion on the activity of peptide. It was found that contents of NaCl and glucose, heating temperatures (up to 100°C), and heating times (20–120 min) had no significant effect on the activity of peptide. The peptide attained highest activity at pH = 7, while sharply declined at alkaline condition. The activity of peptide significantly increased by 9.08% than before in vitro digestion. Peptides were cleaved into smaller fragments after pepsin digestion and leading to increased exposure of internal hydrophobic residues. With further treatment of trypsin, more free amino acids were released and surface hydrophobicity was declined. The study showed that the activity of ACE-inhibitory peptide has ideal tolerance for different processing conditions and in vitro gastrointestinal digestion.

1. Introduction
In recent years, many dietary proteins are proven to exert beneficial influence on body functions once bioactive peptides are released from proteins by either enzyme hydrolysis or food processing including ripening and fermentation (Toldra, Reig, Aristoy, & Mora, 2018; Zhang, Zhang, Wang, Chen, & Luo, 2017). Bioactive peptides typically range within 2–30 amino acids in length and are characterized with low molecular weight, stable structure, and high absorbptivity (Ryder, Bekhit Ael, McConnell, & Carne, 2016). Bioactive peptides derived from foods have been shown to have diverse bioactivities, such as antioxidant, opioid, antimicrobial, anti-hypertensive, anti-thrombotic, anti-inflammatory, and immunomodulation, etc. (Wang, Chen, Fu, Li, & Wei, 2017). Additionally, these peptides are usually characterized with less side-effects compared with the synthetic peptides and drugs. Based on the above advantages, bioactive peptides are usually valued as promising nutraceutical ingredients in functional foods.

Angiotensin I-converting enzyme (ACE) is a key enzyme in the controlling of blood pressure in vivo (Bonesi et al., 2010). It converts angiotensin I to angiotensin II (a potent vasoconstrictor), and simultaneously, inactivates the vasodilating peptide bradykinin (Jr, Kahn, & Shumway, 1956). Therefore, the inhibition of ACE is valued as an effective strategy to treat hypertension. In recent years, the safer and milder ACE-inhibitory peptides derived from food proteins have attracted great interest among researchers. For example, ACE-inhibitory peptides have been generated from chicken (Sangsawad, Roytrakul, & Yongsawatdigul, 2017), pork (Muguruma et al., 2009), beef...
A 0.1% formic acid gastrointestinal digestion on the activity of ACE-inhibitory peptide. Thus, the objective of the present study was to investigate the effect of heating on the activity of ACE-inhibitory peptide derived from duck meat is almost blank.

Duck meat has received high appreciation in consumers worldwide since the recommendation of reducing red meat intake, which may be associated with the happening of cardiovascular disease (Zelber-Sagi et al., 2018). The global consumption of duck meat account for about 4.53 million tons per annum, of which the Asian countries take up 82.6% of total consumption amount (Khan et al., 2019). Duck meat is a great dietary protein resource, and it has an ideal ratio of omega 6 to omega 3 fatty acids, and also abundant in essential amino acids and microelements, including methionine, lysine, iron, selenium and niacin (Wang, Zhang, Zou, Sun, & Xu, 2018). Apart from the basic nutritional value, the development of new functional substances from duck meat is an innovative thinking in food industry, the research about bioactive peptide is one of them. The study on bioactive peptide derived from duck meat is mainly antioxidant peptide to date, and the production of ACE-inhibitory peptide is rarely reported. Thus, the study of ACE-inhibitory peptide derived from duck meat has important innovative value. On the other hand, although ACE-inhibitory peptide are widely studied and used as functional food component in reducing blood pressure, its activity may be affected by some processing conditions (heating, pH, and food ingredients) when served as functional ingredient in food industry (Hwang, 2010). In addition, enzymes in the digestive tract may change the activity of peptide by modifying their structure (Shimizu & Ok Son, 2007). Therefore, the peptide must be resistant to the gastrointestinal digestion, only in this way can be absorbed and distributed to target organs and tissue in active form.

Until now, various studies have investigated the separation and identification of the ACE-inhibitory peptide, while there is little information regarding the influence of simulated gastrointestinal digestion and processing conditions on the activity of ACE-inhibitory peptide. Thus, the objective of the present study was to investigate the effect of heating temperature, heating time, pH, food ingredients (NaCl and glucose), and in vitro gastrointestinal digestion on the activity of ACE-inhibitory peptide derived from duck meat.

2. Materials and methods

2.1. Materials and chemicals

Frozen duck (Cherry valley) breasts were provided by Jiangsu Yi Guest Food Co. Ltd., China. Ducks were slaughtered and then the breast muscles were rapidly removed, packed under vacuum, and stored at −20°C until further use. Alcalase 2.4 L (2.4 AU-A/g) were provided by Novo Co. (Novozyme Nordic, Bagsvaerd, Denmark). Pepsin (400 U/mg proteins), trypsin (285 U/mg proteins), 1-aniline-8-naphthalene-sulphonate (ANS), angiotensin I-converting enzyme (from rabbit lung, 0.1 U/mL, hippuryl-L-histidyl-L-leucine (HHL), and hippuric acid (HA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Protein content was determined using bicinchoninic acid (BCA) protein assay kit from Thermo Fisher Scientific (Waltham, MA, USA). Acetonitrile and formic acid were HPLC grade. All other reagents used were of analytical grade.

2.2. Preparation of duck meat peptide

Frozen duck breasts were thawed at the cooling room (4°C) for 12 h, and then the connective tissue and skin were removed. The meat was minced and mixed with distilled water at the ratio of 1:3 (w/v). The mixture was then homogenized by a homogenizer at 8000 rpm for 1 minute. The pH of the homogenate was adjusted to 10.0, and Alcalase 2.4 L was added at an enzyme dosage of 2000 U/g and the reaction was carried out at 65°C for 4 h. During the reaction, 4 M NaOH was used to adjust the pH of the mixture to 10.0. The enzymatic hydrolysis was stopped by putting samples in a water bath at 100°C for 10 min, and then cooled to room temperature. The reaction mixture was then centrifuged at 10,000 g for 10 min. The supernatant was passed through an ultrafiltration membrane with molecular weight cut-off of 3 kDa on an Amicon stirred cell (EMD Millipore Co., Billerica, MA, USA). The obtained fraction was then lyophilized and stored at −20°C for further analysis.

2.3. Determination of ACE-inhibitory activity

The ACE-inhibitory activity of peptide was evaluated according to the method of Nakamura et al. (1995) with slight modifications. Briefly, 80 µL of peptide sample was incubated at 37°C for 10 min with 200 µL HHL (5 mM HHL in 100 mM borate buffer containing 300 mM NaCl at pH 8.3) in a 1.5 mL centrifuge tube. The blank control group was arranged by substituting the sample solution with borate buffer. Subsequently, 20 µL of ACE (0.1 U/mL) was added and incubated with the above mixture at 37°C for 60 min. Finally, the reaction was stopped by the addition of 150 µL of 1 M HCl. The obtained final mixture was filtered through 0.45 µm PES syringe filter, and 20 µL was analyzed. The generated hippuric acid (HA) was quantitated using an external standard, and determined via HPLC on a Sunfire C18 analytical column (4.6 mm × 150 mm; particle size 5 µm, Waters Scientific Inc., Milford, MA, USA) with a run time of 20 min at 228 nm. The column was eluted by an isocratic elution of 85% mobile phase (A) – 0.1% formic acid in ultra-pure water and 15% mobile phase (B) – 0.1% formic acid in acetonitrile at a flow rate of 2 mL/min. Three replicates for each sample were performed. The ACE inhibition rate was calculated using the following equation:

\[ \text{ACE Inhibition rate(%) = } \left[ 1 - \frac{A_{\text{inhibitor}}}{A_{\text{blank}}} \right] \times 100 \]

where \( A_{\text{inhibitor}} \) and \( A_{\text{blank}} \) represented the peak areas of hippuric acid generated in the sample and control groups, respectively.

2.4. Effect of different processing conditions on the activity of ACE-inhibitory peptide

2.4.1. Heating

The peptide were dissolved in distilled water at the concentration of 1 mg/mL (peptide concentration, the same below). Peptide solutions were incubated at different temperatures (25, 40, 60, 80 and 100°C) for 1 h. On the other hand, the
peptide were also incubated at various times (20, 40, 60, 120 and 180 min) at 100°C. All solutions were cooled to room temperature on ice after treatment, and then the activity of ACE-inhibitory peptide were measured as described before.

2.4.2. pH
The pH of peptide was adjusted to 3, 5, 7, 9, and 11 with 1 M HCl or 1 M NaOH and then maintained at room temperature for 1 h. The pH was then changed to 7 followed by the determination of peptide activity.

2.4.3. Food ingredients
The peptide was combined with 0, 2, 4, 6 and 8% (w/w) NaCl and glucose, respectively, maintained at 100°C for 15 min and then rapidly cooled to room temperature on ice. The activity determination of peptide was then performed.

2.5. In vitro gastrointestinal digestion
Resistance of the peptide against gastrointestinal proteases, pepsin, and trypsin was assessed essentially according to the method of You, Zhao, Regenstein, and Ren (2010) with modifications. Briefly, the peptide solutions were adjusted to pH 2.0 with 4 M HCl. The pepsin was added (E/S = 16,000 U/g) and then the mixture was incubated at 37°C for 2 h. Next, the pH was adjusted to 5.3 with 0.9 M NaHCO₃ and further neutralized to pH 7.0 with 1 M NaOH. To stop the reaction, the tubes were placed in boiling water for 10 min. Subsequently, the samples were cooled to room temperature on ice and centrifuged at 10,000 g for 10 min, 4°C. The supernatant was divided into two portions. One was lyophilized and stored at −20°C prior to analysis. The other was adjusted to pH 7.5 with 1 M NaOH and digested further by trypsin (E/S = 11,400 U/g) at 37°C for 2 h. After 2 h, the mixture was inactivated, centrifuged and lyophilized for further analysis.

2.6. Total and free amino acid analysis
The total amino acid profiles of peptide were determined according to the method described by Dong et al. (2008) with slight modifications. The lyophilized peptide powder was dissolved in 6 M HCl and hydrolyzed at 110°C for 23 h prior to derivatization with phenyl isothiocyanate. Two milliliter samples were filtered through a PES filter having a pore diameter of 0.22 μm. Twenty microliter of each was analyzed with a fully automated amino acid analyzer (L-8900; HITACHI, Tokyo, Japan).

The preparation of samples for free amino acid determination was based on the method reported by Aro Aro et al. (2010). The analysis of free amino acids was achieved by fully automated amino acid analyzer (L-8900; HITACHI, Tokyo, Japan). The filtrate (20 μL) were subjected to the analyzer and the absorbance was measured at 440 and 570 nm after reacting with ninhydrin at 135°C. Each FAA was quantified on the basis of the peak areas of the amino acids with those of external standards.

2.7. Surface hydrophobicity measurement
Surface hydrophobicity was determined using ANS as fluorescence probe following the method of Haskard and Ecy (1998) with slight modifications. Each sample was prepared with 0.1 M phosphate buffer (pH 7.0) to obtain an appropriate concentration. Afterwards, 2 mL of the sample with concentrations ranging from 0.1 to 1.0 mg/mL were mixed with 10 μL of ANS solution (8 mM in 0.1 M phosphate buffer, pH 7.0). The fluorescence intensity of each mixture was determined at an excitation and emission wavelength of 374 and 485 nm. The slope of the plot between protein concentration versus fluorescence intensity was valued as the index of surface hydrophobicity (Ho).

2.8. Statistical analysis
All tests were repeated in triplicate. The collected data were analyzed by one-way analysis of variance (ANOVA). Significant difference among mean values were identified by Duncan’s multiple range test at a 95% confidence level with the SAS 8.1 software. All the data were presented as mean ± SD.

3. Results and discussion

3.1. Activity of ACE-inhibitory peptide derived from duck meat
In this study, the activity of ACE-inhibitory peptide (<3 kDa) derived from duck meat was shown in Figure 1. The activity of peptide increased gradually with the increasing of peptide concentration, and the IC₅₀ (the concentration of peptide required to reduce 50% of ACE activity) was determined as 0.62 mg/mL. Similarly, Chen, Wang, Zhong, Wu, and Xia (2012) reported that the ACE-inhibitory peptide (<3 kDa) derived from hydrolysate of grass carp exhibited highest activity with an IC₅₀ value of 0.308 mg/mL. Moreover, Norris, O’Keeffe, Poyarkov, and FitzGerald (2015) found that the ACE-inhibitory peptide (<3 kDa) from bovine α-casein hydrolysate showed more potent activity than other higher molecular weight fractions. Tian et al. (2017) also claimed that the ACE-inhibitory peptide (<3 kDa) obtained from yak skin Proteinase K hydrolysate had strongest activity with an IC₅₀ value of 1.119 mg/mL. It can be seen that the low-molecular weight ACE-inhibitory peptide (<3 kDa) could exhibit favorable activity.
3.2. Effect of different processing conditions on the activity of ACE-inhibitory peptide

3.2.1. Heating
Due to the necessity of heating treatment in food processing, it is meaningful to consider the effect of various heat treatment conditions on the activity of ACE-inhibitory peptide. The effect of different heating temperatures (from 25 to 100°C) on the activity of ACE-inhibitory peptide is shown in Figure 2(a). There was no significant difference in the activity of peptide under various temperatures, suggesting that the activity of ACE-inhibitory peptide have satisfactory resistance to heat processing. A similar trend was observed in the study of Hwang (2010), which discovered that the effect of various heating temperatures (20–100°C, 2 h) on the activity of tuna cooking juice-derived ACE-inhibitory peptide was not significant (P < 0.05). Moreover, the current result is also consistent with the study of stability of ACE-inhibitory peptide extracted from Jinhua ham (Zuo, Zhang, Xing, Zheng, & Zhou, 2017). The effect of heating time on the activity of ACE-inhibitory peptide is shown in Figure 2(b). With the increase of heating time (20–120 min), there was no significant loss of activity, while slightly decreased at 180 min (P < 0.05).

The above results may be ascribed to the good structural stability of ACE-inhibitory peptide. Unlike those proteins (> 50 KDa) with quaternary structure, low molecular weight peptide just can form secondary structures, which are important to the bioactivity of peptide. Extremely high temperature might affect the secondary structure of peptide and lead to the instability of peptide activity (Zhu, Zhang, Kang, Zhou, & Xu, 2014). Our study showed that the activity of ACE-inhibitory peptide from duck meat has great thermal stability.

3.2.2. pH
Each peptide has its ideal pH range, in which the structure and activity of peptide are relatively stable. The effect of pH on the activity of ACE-inhibitory peptide is shown in Figure 2(c). The activity of peptide first increased and then decreased.

![Figure 2](image_url)

**Figure 2.** Effect of different processing conditions on the activity of ACE-inhibitory peptide: (a) heating temperature, (b) heating time, (c) pH, (d) NaCl content, and (e) glucose content. Different letters indicate the significant different values (P < 0.05).

**Figura 2.** Efecto de diferentes condiciones de procesamiento en la actividad del péptido inhibidor de la ACE: (a) temperatura de calentamiento, (b) tiempo de calentamiento, (c) pH, (D) contenido de NaCl, y (c) contenido de glucosa. Las letras diferentes indican diferentes valores significativos (P < 0.05).
with pH varying from 3 to 11, and the highest activity of ACE-inhibitory peptide was attained at neutral condition (pH = 7). However, the activity of ACE-inhibitory peptide was greatly affected by alkaline condition. When pH was increased to 11, the ACE-inhibitory peptide just maintained 79% of its original activity (pH = 7).

The significant decline of activity under alkaline condition could be explained by the following several aspects. One reason is the formation of peptide isomers in the racemization reaction occurred under alkaline condition, and it is known that differences in bioactivities existed between iso-

mers (Liu et al., 2017a). In addition, the higher pH will promote the possibility of deamination and lead to changes in structure and conformation of peptide and thereby the change of activity. Moreover, the side chains of peptides could be damaged under alkaline catalysis (Tsoubeli & Labuza, 1991). The results showed that the ACE-inhibitory peptide from duck meat is not suitable for placing at alkaline condition in food processing.

### 3.2.3. Food ingredients

NaCl and glucose are important ingredients in functional foods and processed food products. In such case, it is necessary to determine the effect of these two food ingredients on the activity of ACE-inhibitory peptide. As shown in Figure 2(d), there was no significant change in the activity of ACE-inhibitory peptide under different contents of NaCl (P > 0.05). It was reported that 6% NaCl could decrease the bioactivity of peptide through the reduction of solubility (Liu, Wan, Liu, Zou, & Liao, 2017b). The result in our study may be attributed to the good solubility of ACE-inhibitory peptide in NaCl solution. On the other hand, the Millard reaction between peptide and glucose could be a factor affecting the bioactivity of peptide via the role of generated substances, such as aldehydes and ketones (Nicolli, Anese, Parpinel, Franceschi, & Lerici, 1997). As shown in Figure 2(e), the effect of glucose on the activity of ACE-inhibitory peptide was not significant (P > 0.05).

Based on the above results, we concluded that the activity of duck meat-derived ACE-inhibitory peptide has favorable stability to food ingredients.

### 3.3. Effect of in vitro digestion on amino acid contents of peptide

As shown in Table 1, the amount of free amino acids before digestion accounted for 21.57% of the total amino acids. After 2 h of digestion with pepsin, the percentage of free amino acids increased to 24.10%. However, following 2 h of trypsin digestion, the content of free amino acids increased to 37.88%. This result may be explained that pepsin was just capable of hydrolyzing the peptides into smaller peptides. However, trypsin could further hydrolyze the peptides into larger amounts of free amino acids. It has been reported that low-molecular weight peptides absorbed better than whole proteins through the peptide transport system which plays a major role in digestion of peptide (Maebuchi et al., 2007). In this study, more than 60% of the final digests still presented in the form of peptide, which indicated the favorable absorption characteristic of the peptide.

Although the structure-activity correlation of ACE-inhibitory peptides has not been completed understood so far, Wijesekara and Kim (2010) discovered that these peptides actually share some common features. The C-terminal tripeptide sequence have been claimed to be significantly influenced factor to the activity of peptide by interacting with the subsites of ACE. Furthermore, peptides containing hydrophobic amino acids residues (Pro, Phe, Tyr, Leu, Ile, Val and Ala) at these positions are more potent ACE-inhibitory peptides. In final digest, it can be calculated that the percentage of hydrophobic amino acids included in peptides is about 53%, which may contribute to the activity of ACE-inhibitory peptide.

### 3.4. Effect of in vitro digestion on surface hydrophobicity of peptide

Surface hydrophobicity is an important index presenting the amount of hydrophobic groups on the surface of a protein contacting with the polar aqueous environment and has great effect on its functionality (Hu et al., 2013). Following the simulated gastrointestinal digestion, the change of

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Table 1. Changes in the free amino acids (FAA) content of peptides from duck meat after in vitro simulated digestion.

| Amino acid | Total amino acids (mg/mL) | Free amino acids (mg/mL) | Free amino acids after pepsin digestion (mg/mL) | Free amino acids after trypsin digestion (mg/mL) |
|------------|---------------------------|--------------------------|-----------------------------------------------|-----------------------------------------------|
| Asp        | 2.760 ± 0.176             | 0.358 ± 0.016<sup>a</sup> | 0.431 ± 0.028<sup>b</sup> | 0.545 ± 0.108<sup>*</sup> |
| Thr        | 1.646 ± 0.081             | 0.272 ± 0.014<sup>a</sup> | 0.342 ± 0.028<sup>b</sup> | 0.462 ± 0.017<sup>*</sup> |
| Ser        | 1.396 ± 0.082             | 0.130 ± 0.006<sup>a</sup> | 0.157 ± 0.015<sup>b</sup> | 0.217 ± 0.007<sup>*</sup> |
| Glu        | 5.320 ± 0.238             | 1.264 ± 0.083<sup>a</sup> | 1.403 ± 0.072<sup>b</sup> | 1.591 ± 0.077<sup>*</sup> |
| Gly        | 1.597 ± 0.076             | 0.277 ± 0.007<sup>a</sup> | 0.312 ± 0.017<sup>b</sup> | 0.366 ± 0.031<sup>*</sup> |
| Ala        | 2.773 ± 0.124             | 0.648 ± 0.008<sup>a</sup> | 0.676 ± 0.046<sup>b</sup> | 0.807 ± 0.057<sup>*</sup> |
| Cys        | 0.231 ± 0.012             | 0.058 ± 0.005<sup>a</sup> | 0.067 ± 0.003<sup>b</sup> | 0.127 ± 0.008<sup>*</sup> |
| Val        | 1.775 ± 0.090             | 0.240 ± 0.004<sup>a</sup> | 0.262 ± 0.016<sup>b</sup> | 0.358 ± 0.027<sup>*</sup> |
| Met        | 1.139 ± 0.055             | 0.195 ± 0.016<sup>a</sup> | 0.212 ± 0.024<sup>b</sup> | 0.354 ± 0.010<sup>*</sup> |
| Ile        | 1.560 ± 0.073             | 0.119 ± 0.005<sup>a</sup> | 0.133 ± 0.007<sup>b</sup> | 0.199 ± 0.016<sup>*</sup> |
| Leu        | 3.296 ± 0.159             | 0.459 ± 0.011<sup>a</sup> | 0.488 ± 0.037<sup>b</sup> | 1.119 ± 0.057<sup>*</sup> |
| Tyr        | 1.173 ± 0.057             | 0.347 ± 0.004<sup>a</sup> | 0.406 ± 0.014<sup>b</sup> | 1.043 ± 0.076<sup>*</sup> |
| Phe        | 1.482 ± 0.079             | 1.417 ± 0.021<sup>a</sup> | 1.637 ± 0.096<sup>b</sup> | 2.376 ± 0.169<sup>*</sup> |
| Lys        | 2.570 ± 0.133             | 0.348 ± 0.021<sup>a</sup> | 0.371 ± 0.011<sup>b</sup> | 0.942 ± 0.069<sup>*</sup> |
| His        | 1.190 ± 0.059             | 0.183 ± 0.009<sup>a</sup> | 0.195 ± 0.008<sup>b</sup> | 0.251 ± 0.016<sup>*</sup> |
| Arg        | 1.798 ± 0.087             | 0.463 ± 0.029<sup>a</sup> | 0.495 ± 0.017<sup>b</sup> | 1.261 ± 0.096<sup>*</sup> |
| Pro        | 0.801 ± 0.030             | 0.232 ± 0.009<sup>a</sup> | 0.246 ± 0.033<sup>b</sup> | 0.295 ± 0.020<sup>*</sup> |
| Total      | 32.507 ± 1.612            | 7.011 ± 1.052<sup>a</sup> | 7.835 ± 0.459<sup>b</sup> | 12.315 ± 0.860<sup>*</sup> |

Mean values ± standard deviations (n = 3). Different letters in the same row indicate significant differences at P < 0.05.

Valores medios ± desviaciones estándar (n = 3). Las letras diferentes en la misma fila indican diferencias significativas en P < 0.05.
surface hydrophobicity is shown in Figure 3. After 2 h digestion with pepsin, the surface hydrophobicity of the sample was significantly increased. However, further digestion with trypsin lead to the decrease of surface hydrophobicity ($P < 0.05$). Protein degradation could be responsible for the exposure of hydrophobic groups (Pacifici, Kono, & Davies, 1993). In the present study, there was no significant changes of the content of free amino acid residues after pepsin digestion in contrast to the control ($P > 0.05$). However, the contents of free amino acids significantly increased to 12.315 mg/mL after trypsin digestion ($P < 0.05$) (Table 1). It indicates that pepsin may just split the peptides into smaller peptide fragments, thereby internal hydrophobic groups were exposed to the environment. While trypsin further hydrolyzed these fragments and produced more free amino acids leading to the decrease of surface hydrophobicity.

Figure 3. Changes in surface hydrophobicity of peptide from duck meat following pepsin-trypsin digestion. Different letters indicate the significant different values ($P < 0.05$).

Figure 4. Effect of pepsin–trypsin digestion on the activity of ACE-inhibitory peptide. Different letters indicate the significant different values ($P < 0.05$).

3.5. Effect of in vitro digestion on the activity of ace-inhibitory peptide

As shown in Figure 4, the activity of ACE-inhibitory peptide before digestion was $48.01 \pm 1.25\%$. After digestion with pepsin, the activity significantly increased to $65.29 \pm 1.37\%$ ($P < 0.05$). However, further digestion with trypsin lead to a significant decrease of activity ($P < 0.05$), but it still higher than control group (before simulated digestion).

The newly formed ACE-inhibitory peptides may be responsible for the increase of activity after pepsin digestion. It has been reported that two novel ACE-inhibitory peptide sequences such as MEVFVP and VSQLTR have been identified from flounder fish pepsin hydrolysate (Ko et al., 2016). Terashima et al. (2010) first identified ACE-inhibitory peptides MNVKHWPWMK and VTVNPYKWLP from the pepsin hydrolysate of chicken leg meat. For another, the decrease of activity with further trypsin digestion might be explained as following: the existed ACE-inhibitory peptides were further hydrolyzed and more free amino acids were released (Table 1), thus result in the decrease of activity of ACE-inhibitory peptide. Furthermore, the change of activity during simulated digestion is consistent with the variation of surface hydrophobicity, supporting the previously described idea that the activity of ACE-inhibitory peptide is related to the included hydrophobic amino acids.

4. Conclusions

The current research provided important theoretical basis for the application of ACE-inhibitory peptide in functional food industry. According to the result, the activity of ACE-inhibitory peptide from duck meat was stable against heat treatment and food ingredients. However, the alkaline conditions were unfavorable for the activity of ACE-inhibitory peptide. The simulated gastrointestinal digestion showed that more free amino acids were released after trypsin digestion than pepsin. The surface of hydrophobicity significantly increased after pepsin digestion and then decreased under digestion of trypsin. The activity of ACE-inhibitory peptide increased after hydrolysis by pepsin and decreased after trypsin treatment, but the final activity of peptide was stronger than before in vitro digestion. This study suggested that the duck meat-derived ACE-inhibitory peptide basically retains or even enhances their activity under different conditions, and thus could be a promising source of antihypertensive substance to improve human health.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Aro Aro, J. M., Nyam-Osor, P., Tsuji, K., Shimada, K. I., Fukushima, M., & Sekikawa, M. (2010). The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. Food Chemistry, 119, 279–285.

Balti, R., Bougatef, A., Sila, A., Guillochon, D., Duhlster, P., & Nedjar-Arroume, N. (2015). Nine novel angiotensin I-converting enzyme (ACE) inhibitory
peptides from cuttlefish (Sepia officinalis) muscle protein hydrolysates and antihypertensive effect of the potent active peptide in spontaneously hypertensive rats. Food Chemistry, 170, 519–525.

Bao, Z., & Chi, Y. (2016). In vitro and in vivo assessment of angiotensin-converting enzyme (ACE) inhibitory activity of fermented soybean milk by lactobacillus casei strains. Current Microbiology, 73, 214–219.

Bonezi, M., Loizzo, M. R., Statti, G. A., Michel, S., Tillequin, F., & Menichini, F. (2010). The synthesis and angiotensin converting enzyme (ACE) inhibitory activity of chalcones and their pyrazole derivatives. Bioorganic & Medicinal Chemistry Letters, 20, 1990–1993.

Chen, J., Wang, Y., Zhong, Q., Wu, Y., & Xia, W. (2012). Purification and characterization of a novel angiotensin-I converting enzyme (ACE) inhibitory peptide derived from enzymatic hydrolysate of grass carp protein. Peptides, 33, 52–58.

Dong, S., Zeng, M., Wang, D., Liu, Z., Zhao, Y., & Yang, H. (2008). Antioxidant and biochemical properties of protein hydrolysates prepared from silver carp (Hypophthalmichthys molitrix). Food Chemistry, 107, 1485–1493.

Haskard, C. A., & Ecy, L. C. (1998). Hydrophobicity of bovine serum albumin and ovalbumin determined using uncharged (PRODON) and anionic (ANS-) fluorescent probes. Journal of Agricultural & Food Chemistry, 46, 2671–2677.

Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. Food Hydrocolloids, 30, 647–653.

Hwang, J. S. (2010). Impact of processing on stability of angiotensin I-converting enzyme (ACE) inhibitory peptides obtained from tuna cooking juice. Food Research International, 43, 902–906.

Jang, A., & Lee, M. (2005). Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. Meat Science, 69, 653–661.

Jr, S. L., Kahn, J. R., & Shumway, N. P. (1956). The preparation and function of the hypertensin-converting enzyme. Journal of Clinical Investigation, 35, 295–299.

Khan, M. A., Ali, S., Yang, H., Kamboh, A. A., Ahmad, Z., Tume, R. K., & Zhou, G. (2019). Improvement of color, texture and food safety of ready-to-eat high pressure-heat treated duck breast. Food Chemistry, 277, 646–654.

Ko, J. Y., Kang, N., Lee, J. H., Kim, J. S., Kim, W. S., Park, S. J., … Jeon, Y. J. (2016). Angiotensin I-converting enzyme inhibitory peptides from an enzymatic hydrolysate of flounder liver (Paralichthys olivaceus) muscle as a potent anti-hypertensive agent. Process Biochemistry, 51, 535–541.

Lau, C. C., Abdullah, N., Shuib, A. S., & Aminudin, N. (2014). Novel angiotensin I-converting enzyme inhibitory peptides derived from edible mushroom Agaricus bisporus (J.E. Lange) Imbach identified by LC-MS/MS. Food Chemistry, 148, 396–401.

Liu, D., Chen, X., Huang, J., Zhou, X., Huang, M., & Zhou, G. (2017a). Stability of antioxidant peptides from duck meat after post-mortem ageing. International Journal of Food Science & Technology, 52, 2513–2521.

Liu, Y., Yan, S., Liu, J., Zou, Y., & Liao, S. (2017b). Antioxidant activity and stability study of peptides from enzymatically hydrolyzed male silkmoth. Journal of Food Processing and Preservation, 41, e13081.

Maebuchi, M., Samoto, M., Kohno, M., Ito, R., Koikeda, T., Hirotsuka, M., & Liu, Y., Wan, S., Liu, J., Zou, Y., & Liao, S. (2017). Optimization of hydrolysate generation of bioactive peptides from meat industry waste proteins: Identification of hydrolysates from hand-dried veal (Bos taurus) meat. Bioorganic & Medicinal Chemistry Letters, 27, 516–522.

Maebuchi, M., Samoto, M., Kohno, M., Ito, R., Koikeda, T., Hirotsuka, M., & Liu, Y., Wan, S., Liu, J., Zou, Y., & Liao, S. (2017). Optimization of hydrolysate generation of bioactive peptides from meat industry waste proteins: Identification of hydrolysates from hand-dried veal (Bos taurus) meat. Bioorganic & Medicinal Chemistry Letters, 27, 516–522.