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FULL PAPER

Total peptide content and amino acid profile of fermented shrimp (*Litopenaeus vannamei*) sausage

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Abstract

Hypertension can be prevented by bioactive containing food such as shrimp. Food value can be increased through fermentation. During the process of fermentation, microbes and enzymes will break down proteins into peptides and amino acids, making the foods easier to digest in the body. This study aimed to determine the fermentation effect on shrimp sausage's peptide and amino acid content. An experimental study with a completely randomized design, involving the treatment of 4 levels of fermentation duration as control, was carried out three times. Fermentation was conducted spontaneously with a salt concentration of 1.2%, smoking at 50°C for 3 hrs, and a fermentation temperature of 35°C. The parameters measured were peptide content and amino acid profile using the O-phtaldehyde (OPA) and HPLC methods. The peptide content data statistical analysis used the One Way ANOVA and continued with the post hoc test. Amino acid profile data are further presented descriptively. There is an influence between the fermentation duration and the peptide content in the product ($p \leq 0.05$), and 15 types of amino acids were obtained in fermented shrimp sausage. According to this study, the third day of fermentation is the most efficient time for generating peptides and amino acids in shrimp sausage, with glutamate being the highest amount of amino acid produced throughout fermentation. Fermentation process and duration had effect on shrimp sausage's peptide and amino acid content.

1. Introduction

Cardiovascular disease, such as hypertension is one of the top 10 causes of death in the world (Citrakesumasari, 2009). Hypertension is a silent killer because the sufferer is unaware of the symptoms, with a systolic and diastolic blood pressure of 140 mmHg and 90 mmHg. According to data from the World Health Organization (WHO), 1.5 million people in Southeast Asia died from this disease (WHO, 2011). The 2018 Riskesdas data reported that 34.11% of the Indonesian population aged 18 years suffered from hypertension. This figure is higher than the rate in 2013, which was 25.8% of the population (Kemenkes RI, 2019). Hypertension can occur due to several factors, such as obesity, kidney disease, diabetes, stress, drug consumption, and food (diet) (The National High Blood Pressure Education Program, 2003; Cuffee *et al*., 2014; Whelton *et al*., 2018).

A protein intake study in patients found an administration effect on lowering blood pressure (Kusumastuty *et al*., 2016). This was related to the breakdown of protein into active peptides and amino acids in the digestive process, functioning as an anti-hypertension, antibacterial, antioxidant, antiobesity, antidiabetic, and anticoagulant (Yokoyama *et al*., 1992; Mendis *et al*., 2005; Rajapakse *et al*., 2005; Kim *et al*., 2007; Jo *et al*., 2008; Stensvåg *et al*., 2008; Liu *et al*., 2008; Ramadhan *et al*., 2018). The substances have direct or indirect functions in treating hypertension.

Fortunately, the protein source food is abundantly available in Indonesia, especially marine products, because most of the landmass is covered by water. Shrimp constitutes the greatest export commodity and has a fairly high level of consumption in Indonesian society, with the largest commodities being white, dongol, tiger, krosok, queen, and barong shrimp (Badan Pusat Statistik Indonesia, 2018; 2021). This protein source food contains bioactive peptides and amino acids that function as anti-hypertension (Hai-lun *et al*., 2006; Vasdev and Stuckless, 2010). Bioactive peptides can also

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be obtained by the fermentation process. During this process, enzymes and microbes will break down proteins to liberate amino acids and bioactive peptides (Yokoyama *et al*., 1992; Torino *et al*., 2013). Fermented sausage is processed meat with the addition of salt and microbial culture to increase the shelf life and nutritional value (Phadke *et al*., 2014). Previous studies found an increase in antioxidant activity in fermented shrimp sausage for 3 days of fermentation and a decrease in fatty acid content (Fitriana *et al*., 2021; Afifah *et al*., 2022). Another study found di- and tri-peptides and amino acids with anti-hypertension function as the largest constituent (Wang *et al*., 2008; Pongsetkul *et al*., 2017).

There has been no study on the fermentation time effect with peptide content and amino acid profile on fermented shrimp sausage products. Therefore, this study analyzed the total peptide and amino acid content of fermented shrimp sausage with different fermentation times.

2. Materials and methods

This study is experimental research in food technology carried out at the Food Technology Science Laboratory, Nutritional Chemistry Laboratory, Faculty of Medicine, Universitas Diponegoro between 3-10 January 2022, and the Integrated Laboratory of Institute Pertanian Bogor between 13 September 2019.

2.1 Fermented shrimp sausage making

The ingredients used are headless shrimp (*Litopenaeus vannamei*), ice water, egg white, tapioca flour, corn oil, lime, sugar, salt, ginger, garlic, pepper, sausage casings and liquid smoke. Meanwhile, the tools are digital scales, a blender, an oven and an incubator.

About 62.3% headless shrimp is soaked in 2% lime juice and grind with 12% ice water and 1.2% salt. The sausage dough compaction is carried out by adding 7.5% tapioca flour, 9.3% egg white, 3.1% corn oil, 1.2% sugar, 0.6% garlic and 0.2% pepper. Subsequently, the dough is put in a sausage casing.

In the smoking stage, the shrimp sausage is soaked in a 5% liquid smoke solution, generated from 50 mL liquid smoke in 950 mL water, for 30 mins. The sausages are baked in the oven at 50°C for 3 hrs. After baking, they were divided into 4 groups on days 0, 1, 2, and 3, then incubated at 35°C. The sausages on day 0 was analyzed immediately for their contents.

2.2 Peptide content analysis

Analysis of peptide content was measured using spectrophotometric analysis and the O-pthaldehyde (OPA) method according to Church *et al*. (2010) with modifications by Budiari (2018). A 50 mM phosphate buffer pH 7 was prepared by mixing 50 mL of 50 mM $NaH_2PO_4.2H_2O$ and 50 mL of 50 mM $Na_2HPO_4.2H_2O$. The phosphate buffer mixture was then diluted with 100 mL of distilled water. A total of 50 mL of O-pthaldehyde (OPA) reagent was prepared by mixing 25 mL of 100 mM $NaBH_4$, 2.5 mL of 20% SDS, 40 mg of OPA dissolved in 1 mL of methanol, and 100 µL β-mercaptoethanol. Subsequently, the mixture was diluted to 50 mL using deionized water.

In the preparation step, 10 mg of the crushed sample was diluted to 10 mL in phosphate buffer by simultaneous addition in a test tube, then vortex until dissolved. About 100 µL was dissolved into 4 mL of OPA reagent and incubated at room temperature for 4 mins. The absorbance was measured using a UV-VIS spectrophotometer at 340 nm, and the standard for calculating peptide content was conducted through casein tryptone in phosphate buffer.

2.3 Amino acid content analysis

Amino acid analysis was performed by HPLC based on the in-house method. The sample containing 6 mg of protein was put into a screw tube with 6N HCl. The screw tube was fed with nitrogen gas for 0.5-1 min and was closed. The closed tube was heated in an oven at 110°C for 24 hrs. After heating, the solution was transferred to a screw tube into a rotary evaporator for drying. The dried sample was dissolved with 0.01N HCl to 10 Ml and filtered using millipore paper before adding potassium borate buffer pH 10.4 in a 1:1 ratio. OPA reagent solution was prepared with 25 mg dissolved in 2 mL of methanol. Subsequently, 0.020 mL of mercaptoethanol, 0.050 mL of 30% brij-30 solution, and 0.5 mL of 1M borate buffer pH 10.4 were added.

In amino acid analysis, 5 L of sample and 25 L of OPA reagent were put in a vial and allowed to stand for 1 min for complete derivatization. Meanwhile, 5 L of the solution was injected into an HPLC column with a Thermo Scientific ODS-2 column, a flow rate of 1 mL/ min, a fluorescence detector, buffer A formulating from a mixture of sodium acetate pH 6.5, sodium EDTA, methanol, tetrahydrofuran (THF), and HP water, and buffer B making from a combination of 95% methanol and HP water. The standard for calculating amino acids uses SIGMA's parent standard calculated using the formula:

$$\%\ \text{Amino Acid} = \frac{\text{sample area} \times C \times Fp \times BM}{\text{Standard area} \times \text{sample weight}} \times 100\%$$

Where C: Standard concentration of amino acids (µg/mL), Fp: Dilution factor, BM: the molecule's weight

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of each amino acid (g/mol)

2.4 Data analysis

The peptide content data were analyzed using statistical software. The normality test was carried out using the Shapiro Wilk test because the data was less than 50. Furthermore, the statistical analysis was continued using analysis of variance (ANOVA) and post hoc test using Duncan. The independent variable effect on the dependent will be considered significant when the p-value ≤ 0.05. Meanwhile, the amino acid profile data will be analyzed descriptively.

3. Results and discussion

3.1 Peptide content

Table 1 shows that there was a fermentation time effect on the fermented shrimp sausage peptide content ($p < 0.005$) and no significant difference between the 2nd and 3rd days. The lowest peptide content was in fermented shrimp sausage during day 0 (0.00 mg/mL), while the highest was on day 3 (230.35 mg/mL).

Shrimp is a protein source rich in active peptide compounds and amino acids (Zhao *et al*., 2011). This food contains bioactive compounds that function as anti-hypertension substances with Phe-Cys-Val-Leu-Arg-Pro, Ile-Phe-Val-Pro-Ala-Phe, Lys-Pro-Pro-Glu-Thr-Val, Tyr -Leu-Leu-Phe amino acid chains, and Ala-Phe-Leu as well as glutamate, cysteine, arginine, leucine, and taurine (Hai-lun *et al*., 2006; Vasdev and Stuckless, 2010; Wikanta *et al*., 2011).

Peptides with 2-20 amino acid chains are known as bioactive compounds. In this study, fermentation time was affected by the peptide content ($p < 0.005$) of fermented shrimp sausage. The peptide content increased with the length of fermentation. During fermentation, enzymes and microbes will break down complex compounds into simpler ones (Phadke *et al*., 2014). Previous studies on fermented shrimp sausage for 3 days also found that protein content decreased with fermentation time (Fitriana *et al*., 2021). This is consistent with the study on changes in trichloroethane acid (TCA) soluble peptides in Kapi (shrimp paste fermentation) with 10, 20, and 30 days of fermentation (Pongsetkul *et al*., 2017). In this study, fermentation on day 0 showed a peptide content of 0.00 mg/mL. Therefore, the equipment cannot read the small peptide content, and the highest on the 3rd day was 250.35 mg/ mL. However, there was no significant difference between the 3rd and 2nd days ($p > 0.05$).

The study of peptide content has not been able to show the chain. A previous study on fermented shrimp by Wang *et al*. (2008) found the presence of bioactive peptides with amino acid chains Asp-Pro, Gly-Thr-Gly, and Ser-Thr as the largest content with anti-hypertension function as an angiotensin converter enzyme inhibitor (ACE-i), and can inhibit angiotensin II formation. The body can directly absorb peptides with chains two and three by peptide transporter 1 (PEPT1) in the brush bolder of the small intestine (Daniel, 2004; Zachos, 2016).

3.2 Amino acid profile

Profile test was conducted on 8 essential and 7 non-essential amino acids in fermented shrimp sausage. Table 2 shows that days 0 and 1, there was no change in content, with the highest essential and non-essential amino acids being leucine (1.08% w/w) and glutamate (1.93% w/w). On the second day of fermentation, the amino acids increased to 21.54% (w/w), with the highest essential and non-essential amino acids being leucine (2.01% w/w) and glutamate (3.82%) w/w). On the 3rd day of fermentation, amino acids increased to 24.77% (w/w). The highest essential and non-essential amino acids in shrimp sausage fermentation on day 3 were lysine (2.41% w/w) and glutamate (4.38% w/w).

Amino acids are composed of proteins with many roles for the body. In this study, the content of shrimp sausage with fermentation time was 0 and 1 day (11.09% w/w). The absence of this change may occur because the protein has not been broken down into the simplest compounds, with the highest composition being glutamate (1.93% w/w). Furthermore, the content increased on day 2 (21.54% w/w) with the highest composition of glutamate (3.82% w/w). On the 3rd day of fermentation, the content increased to 24.77% w/w, while glycine and arginine decreased.

This decrease in amino acids was similar to the study by Kleekayai *et al*. (2016) on Kapi Ta Dam and Kapi Ta Deang products which were fermented for 0, 2, 4, and 6 months. It found that glycine in Kapi Ta Deang decreased in the 4th month. Furthermore, arginine also

Table 1. Analysis result of peptide content of fermented shrimp sausage.

Component	Result of sample peptide content (mg/mL)			
	Day 0	Day 1	Day 2	Day 3
Peptide	0.00 ± 0.00^{a}	57.86 ± 9.42^{b}	231.37 ± 39.49^{c}	250.35 ± 23.77^{c}

Values are presented as mean±SD, n = 24. Values with different superscripts are statistically significantly different with Duncan's test ($p\leq0.05$.

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decreased in Kapi Ta Dam products in the 2nd to the 6th month and Kapi Ta Deang in the 4th month (Kleekayai *et al*., 2016). The decrease could occur due to microbes in shrimp sausage using these amino acids as an energy source (Neis *et al*., 2015; Pongsetkul *et al*., 2017). This is in agreement with previous studies, where there was an increase in lactic acid bacteria in fermented shrimp sausage with 3 days of fermentation (Afifah *et al*., 2022). The highest amino acid content in shrimp sausage fermentation occurred on the 3rd day, composing glutamate (4.38% w/w), Lysine (2.41% w/w), Aspartic Acid (2.32% w/w), Leucine (2.3% w/w), Alanine (2.14% w/w). This concurred with a study by Kleekayai *et al*. (2016) on Kapi Ta Dam and Kapi Ta Deang. Glutamate was reported as the largest amino acid, followed by lysine, leucine, aspartic acid, and arginine (Kleekayai *et al*., 2016).

Table 2. Amino acid profile of fermented shrimp sausage.

Parameter (%w/w)	Sample			
	Day 0	Day1	Day 2	Day3
Essential amino acids				
Histidine (His)	0.32	0.32	0.67	0.72
Isoleucine (Ile)	0.59	0.59	1.12	1.32
Leucine (Leu)	1.08	1.08	2.01	2.3
Lysine (Lys)	1.02	1.02	1.9	2.41
Methionine (Met)	0.28	0.28	0.55	0.70
Penylalanine (Phe)	0.55	0.55	1.07	1.25
Threonine (Thr)	0.45	0.45	0.84	0.98
Valine (Val)	0.59	0.59	1.15	1.35
Non-essential amino acids				
Aspartic Acid (Asp)	1.08	1.08	1.99	2.32
Serine (ser)	0.42	0.42	0.73	0.90
Glutamate (Glu)	1.93	1.93	3.82	4.38
Glycine (Gly)	0.88	0.88	1.82	1.74
Alanine (Ala)	0.78	0.78	1.7	2.14
Tyrosine (Tyr)	0.33	0.33	0.59	0.75
Arginine (Arg)	0.80	0.80	1.57	1.51
Total amino acids	11.09	11.09	21.54	24.77

Glutamate and leucine, classified as the main amino acids, have a role in reducing hypertension. A study found that glutamate has a role in arginine formation, which is a nitric oxide (NO) precursor. Furthermore, glutamate, cysteine, and glycine form the glutathione chain (GSH) to reduce oxidative stress (Vasdev and Stuckless, 2010). The glutamate content as an antioxidant is in agreement with previous studies regarding the activity of fermented shrimp sausage to increase with the fermentation duration for 3 days (Afifah *et al*., 2022). Moreover, glutamate and leucine can reduce body fat, improve insulin resistance, and increase muscle mass (Vasdev and Stuckless, 2010). Leucine is an essential amino acid, and its daily metabolism requirement could reach 7-12 g (Vasdev and Stuckless, 2010).

4. Conclusion

This study proves that there is a fermentation time effect on the fermented shrimp sausage peptide content. The highest content of peptides and amino acids occurred on day 3, with glutamate as the largest. Further study with different periods to see differences during fermentation is expected, as well as improving the quality of fermented shrimp sausages in terms of ingredients and the microbes used. The structure of the peptides in fermented shrimp sausage should also be analyzed. The recommended consumption of fermented shrimp sausage products is 300-500 g daily.

Conflict of interest

The authors declare no conflict of interest.

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