The Quantitative Test of Amino Acids in Smoked Sausage

Elysabeth Agnes Jaairi Coot Mau, Meilla Dwi Andrestian, Siti Mas'odah

Department of Nutrition, Polytechnic of Health-Ministry of Health-Banjarmasin
meilladwi74@gmail.com

Abstract. Ampatin smoked sausage made from tofu pulp flour and Pangasius sp. is a high-protein product innovation. Amino acids composition will determine the quality of protein, which is an important micronutrient for the human body. Smoking technique is a process of utilizing heat and smoke from burning wood to ripen food products. Antimicrobial, antioxidant and organoleptic quality of smoking products have been proven to be superior. This study aimed to observe the effect of smoking time on the amino acids quality of ampatin smoked sausage. The research was conducted in two stages. The first stage was a literature study to find ampatin sausage formulation and smoking time. The second stage was sausage making experiments. The amino acids content of both ampatin sausage samples showed that the time (30 minutes and 60 minutes) and smoking temperature (120ºC) did not cause protein denaturation. The ampatin sausage was able to meet SNI standards with a minimum protein content of 9%. Sample P1 with protein of 10.6 %w/w showed amino acids of 9.5 %w/w and sample P2 with protein of 11.39 %w/w showed amino acids of 9.4 %w/w. The statistical test results of Independent Sample T-tests obtained a p value of 0.910 > α (0.05) with the conclusion that there is no difference in amino acids between P1 and P2.

Keywords: Sausage, Smoking, Amino Acid.

1 Preliminary

Proteins consumed daily can be found in various forms and are almost found in every food item eaten. Protein has a very important function, namely as the main source of energy besides carbohydrates and fats, as a building and regulating substance, maintaining tissue and body cells. Protein must be fulfilled every day through the food we consume and also need to pay attention to the processing process so as not to damage the function of protein. Protein is a molecule that has a molecular weight of about five thousand to one million, so proteins are very easy to denature [1]. Some factors that cause protein denaturation, one of which is temperature [2]. Fish smoking can cause changes in color, appearance and consistency of meat that is compact and more attractive, but can cause a decrease or increase in the components of the nutritional components in the fish meat [3]. Hot smoking is a heating process that applies a temperature of 120ºC for 2-3 hours. Hot smoking time that is not as long as cold smoking will make the product still contain high water content. High water content is one of the factors

T. Triwiyanto et al. (eds.), *Proceedings of the 6th International Conference of Health Polytechnic Surabaya (ICoHPS 2023)*, Advances in Health Sciences Research 72,
https://doi.org/10.2991/978-94-6463-324-5_44

that can make food products vulnerable to damage because it can be a medium for the development of spoilage microorganisms. The raw material for making charcoal in South Kalimantan is widely produced in the Takisung Regency area using laban wood [4]. Burning of laban wood (Vitex pinnata) with a pyrolysis temperature of 300ºC for 1.5 hours produces several components in the form of total charcoal 29.3%, liquid smoke 42.55%, hemicellulose 11.47%, cellulose 48.18%, phenol 10.45 ± 0.20, pH 2.97 ± 0.01, total acids 14.04 ± 0.12, total carbonyl 15.43%, and antioxidants 84.26% higher than kandis, ubar, and coconut shell [5]. The benefits of the smoking process include preservation, bacteriostatic, antioxidative, and organoleptic effects [6]. The making of smoked sausage made from tofu pulp flour and Pangasius sp. aims to determine and analyze the difference in smoking time on amino acids of ampatin sausage.

2 Method

The experiment in this study is to make smoked sausage from tofu pulp and *Pangasius* sp. with the application of different smoking times at 120ºC. Sample P1 will be smoked for 30 minutes and sample P2 for 60 minutes. This research was conducted in 2 stages, which can be seen in Fig 1.

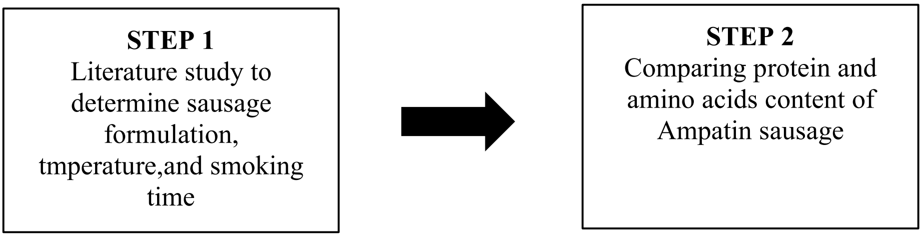

Fig. 1. Research stages.

The making of Ampatin sausage was carried out at the Food Technology Laboratory of the Nutrition Department of Banjarmasin Health Polytechnic and testing of protein and amino acids levels of Ampatin sausage was carried out at the IPB Testing and Certification Services Laboratory. The ingredients used in making Ampatin sausage are tofu pulp flour, fresh *Pangasius* sp., tapioca flour, salt, vegetable oil, powdered sugar, pepper, egg white, ice water, shallots, garlic and ginger. The tools for making tofu pulp are gauze, basin, spoon, pot, aluminum, sieve and oven, basin, knife, cutting board, chooper, spoon, sausage mold, rubber, pot, plate, scissors and smoking machine. The process of making tofu pulp flour can be seen in Fig 2.

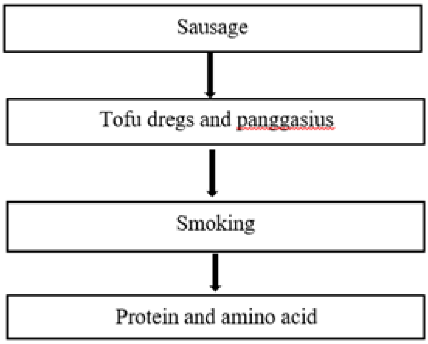

Fig. 2. Procedure for making tofu dregs flour.

The stages of the Ampatin sausage making process can be seen in Fig 3.

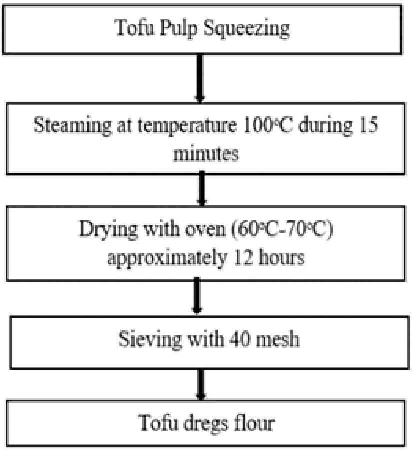

Fig. 3. Procedure for making smoked sausage.

The data obtained is primary data which includes data from chemical quality tests, namely protein and amino acids levels which are then entered into a table. Statistical testing was conducted to determine the effect of smoking time on the chemical quality of Ampatin sausage.

3 Result

The best formulation in making sausages with raw materials of tofu pulp flour and *Pangasius* sp. based on the results of literature studies shows that the best treatment is the addition of 10% of the net weight of *Pangasius* sp. [7]. The literature study also states that the best smoking time for sausage is 60 minutes [8].

3.1 Protein Content

The heating process can cause a denaturation process in proteins. Denaturation occurs when heat breaks down the walls of the meat and decreases the functional properties of the protein so that it can damage amino acids, this is what causes protein levels to decrease as the heating temperature increases. Protein content in Ampatin sausage can be seen in Table 1.

Table 1. Protein Content of Ampatin Smoked Sausage.

Protein Content (%w/w)	
P1	P2
10,6	11,39

The results showed that the average protein content of P2 Ampatin sausage (11.39%) was higher than P1 (10.6%) as shown in Table 1. The average protein content of the Ampatin sausage met the SNI standard (minimum 9%) and was not significantly different, indicating that the smoking time did not affect the protein content of the two samples.

3.2 Amino Acids Content

Amino acids composition will determine the quality of protein which is an important micronutrient for the human body [10]. Denaturation caused by heat will cause changes in the functional properties of proteins. The amino acids content of Ampatin smoked sausage can be seen in Table 2.

Table 2. Amino Acids Content of Ampatin Smoked Sausage.

Amino Acids	P1 (%w/w)	P2 (%w/w)
Aspartic Acids	1.06*	1.05*
Threonin	0.45	0.45
Serine	0.41	0.40
Glutamate	1.68*	1.66*
Glycine	0.79	0.66
Alanine	0.67	0.62
Valine	0.53	0.53
Methionine	0.19	0.18
Ileucine	0.5	0.5
Leucine	0.82*	0.82*
Tyrosine	0.24	0.23
Phenylalanine	0.42	0.43
Histidine	0.33	0.36
Lysine	0.71	0.77
Arginine	0.73	0.73

The test results show that there are three highest types of amino acids, namely glutamate, aspartic acids,and leucine.

Table 3. Average amino acids content of Ampatin smoked sausage.

Average amino acids content (%w/w)	
P1	P2
10,6	11,39

The results showed that the average amino acids content of P1 Ampatin sausage (9.5%) was higher than P2 (9.4%) as shown in Table 3 and was not significantly different, indicating that smoking time did not cause protein denaturation in Ampatin smoked sausage.

4 Discussion

Proteins are macromolecules with nitrogen elements contained in food that are highly functional because they perform important functions such as providing structure to cells and regulating metabolic reactions [11]. The main factors driving a protein to fold into its native structure are numerous non-covalent inter-residue interactions, including hydrophobic effects, hydrogen bonds, van der Waals forces, and ionic bonds [11,12]. The most prevalent secondary structure is the right-handed spiral α-helix, in which a backbone amino group donates a hydrogen bond with another backbone carbonyl group and the sequence distance between these two groups is 3.6 amino acids on average. Another common secondary structure is the β strand, which exhibits an almost fully extended conformation [13].

Protein has long been known as a nutrient that can control appetite and satiety, hence most healthy diets often recommend high protein and fibre consumption. This is because protein and fibre can prevent weight regain after dieting, and in the case of obese people a diet high in protein and fibre can suppress overconsumption due to its association with satiety [14-17]. Consumption of protein higher than the requirement of 1.2 and 1.6 grams per day can lead to greater fat loss and still maintain fat mass. The advantages of a high-protein diet are that it promotes satiety for longer and can control blood sugar [14]. Given that the protein quantities within such studies are twice what Americans consume [18], Other research also confirms this fact, where one high-protein meal is considered enough to improve satiety, glycaemic control and body composition [19-22].

The most common alternative foods are plant proteins, artificial meat proteins, and insect proteins [23]. Nowadays, consumers are more concerned about sustainable consumption patterns that do not consume a lot of resources [24]. Attempts made to continue to develop sustainable products are developing research that protein can be obtained from other innovative products, to maintain the sustainability of the food system [25]. The key to providing a good source of nutrition in a short period of time is to utilise alternative foods, so alternative foods are expected to play an important role in the future [26]. Food alternatives will play an important role [27], Some of the reasons that support food innovation are that it provides convenience for anyone who consumes according to their needs, customisation and nutritional needs that have not been able to be met [28].

Ampatin sausage is one of the innovative products made by utilising tofu factory waste, namely tofu pulp which is processed into tofu pulp flour. to increase the nutritional content in this smoked sausage, catfish is added as another source of protein. The choice of catfish is because catfish is one type of fish that has a high protein content [29]. Protein in catfish has a complete composition and amount of essential amino acids [30].

The formulation of making Ampatin sausage is using tofu pulp flour as much as 10% of the net weight of catfish. The making of Ampatin sausage is divided into several stages, namely raw material preparation, sausage making material preparation, sausage processing and sausage smoking using a smoke machine. The first raw material preparation is the making of tofu pulp flour.

The initial stage after taking the tofu pulp from the factory, then the tofu pulp is squeezed using gauze to reduce the water content. The second stage is steaming the tofu pulp at 100ºC for 15 minutes. Steaming aims to maintain the quality of the tofu pulp until the drying process. The third stage is the drying process with an oven (60-70ºC) for approximately 12 hours, then grinding and sieving 40 mesh.

The first stage of the process of making Ampatin sausage is cleaning *Pangasius* sp. weeding the fish (removing the head, tail, skin, and entrails), washing it with running water, filleting the *Pangasius* sp., and weighing it. The second stage is weighing all the spices used, namely tapioca flour, salt, vegetable oil, refined sugar, pepper, egg white, ice water, shallots, garlic, and ginger. The third stage is to grind the catfish and add ice water and salt. After that, 10% tofu pulp flour was added. Next, the sausage dough is put into a sleeve and steamed for 15 minutes. The fourth stage is smoking the sausages in a smoking machine.

Heating from the smoking process is one of the cooking processes that can be used on food ingredients. Heating in the smoking process comes from burning wood which will then produce the chemical components of smoke. The chemical components of smoke are highly dependent on the type of wood used. The wood used for smoking Ampatin sausage is halaban wood which has a high hemicellulose content. The chemical content of smoke is what will produce the advantages of smoked products including preservation effects, bacteriostatic effects, and effects on sensory. the advantage of smoked products is that they have a distinctive flavour that cannot be added by other food flavours.

The application of temperature and time in sausage making did not cause protein damage. The parameter used to see protein damage is the amino acid content in Ampatin sausage.

This denaturation process can be seen from the total amino acids contained in it. Heating will cause chemical changes in amino acid residues, which can cause changes in the structure, digestibility and functional properties of proteins and this depends on the processing process used [31]. The test results of amino acid content in both samples were not significantly different, which means that the protein did not undergo excessive denaturation.

Glutamic acid is a charged amino acid (polar) together with aspartic acid and belongs to the class of non-essential amino acids. Glutamate levels in Ampatin sausage did not differ significantly between P1 (0.68%w/w) and P2 (0.66%w/w). Glutamate acid plays a role in providing umami flavor if the concentration in the food product is above the taste threshold [32]. Arginine-type amino acids will impart a salty flavor [33]. Glycine and alanine can also give a sweet taste to aquatic foods [32].

Amino acid metabolism plays an important role in various physiological functions and not only for internal organ development or skeletal muscle gain. [35]. Methionine is an important amino acid for protein synthesis and has antioxidant functions, acting as a precursor of cysteine and glutathione in the transsulfuration pathway [36] and through methionine sulfoxide reduction action [37]. Elevated arginine levels have been shown to alleviate oxidative stress [38], stimulate protein synthesis through activation of mTOR pathway [39], improve antioxidant capacity, and humoral and cell-mediated immunity [40, 41]. The results of amino acids testing of Ampatin sausage can be concluded that both samples of Ampatin sausage do not experience significant protein denaturation because they have amino acids levels that are not significantly different. This proves that the smoking time of 30 minutes and 60 minutes at 120ºC does not make the protein denature the protein.

5 Conclusion

The results showed that the smoking time of 30 and 60 minutes at 120ºC did not make the protein in the ampatin sausage undergo a denaturation process because it was seen from the amino acid levels of the two samples not much different. However, the average amino acids content of P1 Ampatin sausage (9.5%) was higher than P2 (9.4%). Smoking for 60 minutes was the best time in making Ampatin smoked sausage. The highest amino acids contained in Ampatin sausage were glutamate, aspartic acids, and leucine. Further development of Ampatin sausage is expected to use other smoking techniques to get the best smoking method to produce Ampatin smoked sausage products.

References

1. Zaldy R dan Lusi A, 2020. Modifikasi metode analisis daya hambat terhadap proses denaturasi protein yang diinduksi oleh panas. CHEESA, Vol. 3(2).
2. Rusli, Arham, et al. 2022. Bimtek diversifikasi olahan ikan bandeng menjadi fish burger bagi kelompok usaha produktif di Kab. Pangkajene dan Kepulauan." Prosiding Seminar Nasional Politeknik Pertanian Negeri Pangkajene Kepulauan. Vol (3).
3. Setyastuti, Aryanti Indah, et al. 2021. Karakteristik kualitas ikan tongkol (Euthynnus affinis) asap dengan asap cair bonggol jagung selama penyimpanan beku." Akuatika Indonesia Vol. 6(2).
4. Abidin Z., Jauhari A., Afriza M. H. 2018. Kajian potensi dan pengembangan pengusahaan arang kayu di desa ranggang luarkecamatan takisung kabupaten tanah laut provinsi Kalimantan Selatan. Jurnal Hutan Tropis Vol. 6(2).
5. Juwita S, Bustari H, Tjipto L, 2015. Karakteristik kimia asap cair hasil pirolisis beberapa jenis kayu. Fakultas Perikanan dan Ilmu Kelautan, Universitas Riau.
6. Frontea S. 2018. Teknologi Pengasapan Ikan Tradisional. Penerbit: Intimedia.
7. Suryani, 2018. Pengaruh proporsi tepung terigu dan tepung ampas tahu terhadap kandungan protein dan serat serta daya terima biskuit program makanan tambahan anak sekolah (PMT-AS). Jurnal Kedokteran dan Kesehatan Vol. 14(1).
8. Nisa, 2016. Pengaruh lama pengasapan dan lama fermentasi terhadap sosis fermentasi ikan lele (Clarias gariepinus). Jurnal Pangan dan Agroindustri Vol. 4(1) p.367-376.
9. Yuniarti D. W., Sulistiyati T. D., Suprayitno E. 2013. Pengaruh suhu pengeringan vakum terhadap kualitas serbuk albumin ikan gabus (Ophiocephalus striatus). THPi student journal Vol. 1(1).
10. Pratama I, Rostini I, Rochima E. 2018. Profil asam amino, asam lemak, dan komponen volatil ikan gurami segar (Osphronemus gourami) dan kukus. JPHPI Vol. 21(2).
11. Branden C, Tooze J. Introduction to protein structure. 2nd ed. New York: Garland Science; 1998.
12. Finkelstein AV, Ptitsyn OB. Protein physics: a course of lectures. 2nd ed. Amsterdam: Elsevier; 2016.
13. B. Huang, L. Kong, C. Wang, F. Ju, Q. Zhang, J. Zhu, T. Gong, H. Zhang, C. Yu, W-M. Zheng, D. Bu, Protein Structure Prediction: Challenges, Advances, and the Shift of Research Paradigms, Genomics, Proteomics & Bioinformatics (2023).
14. Leidy HJ, Clifton PM, Astrup A, Wycherley TP, Westerterp-Plantenga MS, Luscombe-Marsh ND, Woods SC, Mattes RD. The role of protein in weight loss and maintenance. Am J Clin Nutr 2015;101(6):1320S–9S.
15. Dhillon J, Craig BA, Leidy HJ, Amankwaah AF, Osei-Boadi Anguah K, Jacobs A, Jones BL, Jones JB, Keeler CL, Keller C, et al. The effects of increased protein intake on fullness: a meta-analysis and its limitations. J Acad Nutr Diet 2016;116(6):968–83.
16. Clark MJ, Slavin JL. The effect of fiber on satiety and food intake: a systematic review. J Am Coll Nutr 2013;32(3):200–11.

17. Rebello C, Greenway FL, Dhurandhar NV. Functional foods to promote weight loss and satiety. Curr Opin Clin Nutr Metab Care 2014;17(6):596–604.
18. US Department of Agriculture, Agricultural Research Service. Energy intakes: percentages of energy from protein, carbohydrate, fat, and alcohol, by gender and age, What We Eat in America, NHANES 2017–2018. Beltsville, Maryland: USDA, Agricultural Research Service;2020.
19. Gwin JA, Leidy HJ. A review of the evidence surrounding the effects of breakfast consumption on mechanisms of weight management. Adv Nutr 2018;9(6):717–25.
20. Paddon-Jones D, Leidy H. Dietary protein and muscle in older persons. Curr Opin Clin Nutr Metab Care 2014;17(1):5–11.
21. Loenneke JP, Loprinzi PD, Murphy CH, Phillips SM. Per meal dose and frequency of protein consumption is associated with lean mass and muscle performance. Clin Nutr 2016;35(6):1506–11.
22. Leidy HJ, Gwin JA, Roenfeldt CA, Zino AZ, Shafer RS. Evaluating the intervention-based evidence surrounding the causal role of breakfast on markers of weight management, with specific focus on breakfast composition and size. Adv Nutr 2016;7(3):563S–75S.
23. C.A. G´omez-Luciano, L.K. de Aguiar, F. Vriesekoop, B. Urbano, Consumers' willingness to purchase three alternatives to meat proteins in the United Kingdom,Spain, Brazil and the Dominican Republic, Food Qual. Prefer. 78 (2019), 103732.
24. T.M. Rausch, C.S. Kopplin, Bridge the gap: consumers' purchase intention and behavior regarding sustainable clothing, J. Clean. Prod. 278 (2021), 123882.
25. A. Green, C. Blattmann, C. Chen, A. Mathys, The role of alternative proteins and future foods in sustainable and contextually-adapted flexitarian diets, Trends Food Sci. Technol. 124 (2022) 250–258.
26. A.B. Suwardi, N.Z. Indriaty, Z. Navia, Nutritional evaluation of some wild edible tuberous plants as an alternative foods, Innovare J Food Sci 6 (2) (2018) 9–12.
27. M. Al Mijan, B.O. Lim, Diets, functional foods, and nutraceuticals as alternative therapies for inflammatory bowel disease: present status and future trends,World J. Gastroenterol. 24 (25) (2018) 2673.
28. H. Seo, J. Hwang, Analysis of decisive elements in the purchase of alternative foods using bivariate probit model, Sustainability 14 (7) (2022) 3822.
29. Roziana, Fitriani, Marlina Y. 2020. Pengaruh pemberian mi basah ikan patin terhadap intake energy, protein, dan berat badan siswa SD di Pekanbaru. Journal of nutrition college Vol. 9(4).
30. Prameswari, G. N. 2018. Promo gizi terhadap sikap gemar makan ikan pada anak usia sekolah. Jurnal heal education Vol. 3(1).
31. Deng Y, Luo Y, Wang Y, Zhao Y. 2014. Effect of different drying methods on the myosin-structure, amino acid composition, protein digestibility and volatile profile of squid fillets. Food Chemistry. 171: 168-176.
32. Kawai M, Uneyama H, Miyano H. 2009. Taste-active components in foods, with concentrationon umami compounds. Journal of Health Science. 55: 667-673.
33. Zhao CJ, Scheber A, Ganzle MG. 2016. Formation of taste-active amino acids, amino acid-derivatives and peptides in food fermentations. Food Research International. 89: 39-47.
34. Florini, J., D. Ewton, and S. Coolican. 1996. Growth hormone and the insulin-like growth factor system in myogenesis. Endocr. Rev.17:481–517.
35. Castro, F., S. Su, H. Choi, E. Koo, and W. Kim. 2019a. L-Arginine supplementation enhances growth performance, lean muscle, and bone density but not fat in broiler chickens. Poult. Sci. 98:1716–1722.

36. Swennen, Q., P. A. Geraert, Y. Mercier, N. Everaert, A. Stinckens, H. Willemsen, Y. Li, E. Decuypere, and J. Buyse. 2011. Effects of dietary protein content and 2-hydroxy-4-methylthiobutanoic acid or DL-methionine supplementation on performance and oxidative status of broiler chickens. Br. J. Nutr. 106:1845–1854.
37. Luo, S., and R. Levine. 2009. Methionine in proteins defends against oxidative stress. FASEB J 23:464–472.
38. Atakisi, O., E. Atakisi, and A. Kart. 2009. Effects of dietary zinc and l-arginine supplementation on total antioxidants capacity, lipid peroxidation, nitric oxide, egg weight, and blood biochemical val-ues in Japanese quails. Biol. Trace Elem. Res. 132:136–143.
39. Bauchart-Thevret, C., L. Cui, G. Wu, and D. G. Burrin. 2010. Arginine induced stimulation of protein synthesis and survival in ipec-j2 cells is mediated by mTOR but not nitric oxide. Am. J. Physiol. Endocrinol. Metab. 299:E899–E909.
40. Tayade, C., T. Jaiswal, S. Mishra, and M. Koti. 2006. L-Arginine stimulates immune response in chickens immunized with intermediate plus strainof infections bursal disease vaccine. Vaccine 24:552–560.
41. Munir, K., M. Muneer, E. Masaoud, A. Tiwari, A. Mahmud, R. Chaudhry, and A. Rashid. 2009. Dietary arginine stimulates humoral and cell-mediated immunity in chickens vaccinated and challenged against hydropericardium syndrome virus. Poult. Sci. 88:1629–1638.