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Protein Functional Groups Analysis of Smoked Vaname Shrimp (*Litopenaeus Vannamei*) with Various Cooking Methods

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A B S T R A C T

Shrimp is a raw material source of animal protein which is rich in amino acids. The types of amino acids commonly found in shrimp are glutamic acid, aspartic acid, arginine, lysine, leucine and alanine. The amino acid content in shrimp is different for each treatment given to the shrimp. The aim of this research is the characteristics of smoked white shrimp protein functional groups using several cooking methods and protein damage in smoked white shrimp products using several cooking methods based on protein functional groups. Testing for smoked white vaname shrimp quality standards refers to SNI 2728-2018. Chemical testing parameters were carried out using Fourier-Transform Infrared Spectrometer (FTIR), Protein and Amino Acid Profile testing. The parametric data results were analysed using One-Way ANOVA. The results showed that the protein value of smoked vaname shrimp with different cooking methods was significantly different ($p<0.05$). The results of the FTIR test for smoked vaname shrimp gave rise to spectrum peaks in the wave range 3300cm-1 – 3600cm-1 which indicated the presence of amide compounds (NH2). This is also supported by the distribution of spectrum peaks in the fingerprints zone at wavelengths of 500cm-1 – 1500cm-1. The absence of a spectrum peak in the spectrum range 1600cm-1 – 1690cm-1 indicates that secondary proteins have not been formed as markers of protein damage. The highest protein value was found in the control smoked white vaname shrimp at 38.84% and the smallest protein value was found at the oven method at 27.80%. The amino acid profile test shows higher values of glutamic acid, aspartic acid, arginine, glycine, alanine, leucine and lysine. The organoleptic values of all cooking methods were found to have varying results from the parameters of appearance, smell and texture in each cooking method treatment showing results that were not significantly different.

Keywords: Functional group, Vaname Shrimp, Liquid Smoked

1. Introduction

White shrimp (*Litopenaeus vannamei*) is a variety of shrimp that remains a strategic priority in efforts to meet national shrimp production goals. According to data from the Balai Besar Riset Sosial Ekonomi Kelautan dan Perikanan (2020), Indonesian vaname prawn output ranges between 10 and 50 tons per hectare per cycle.

Shrimp is a good source of high-quality animal protein. Shrimp is a good source of amino acids. Glutamic acid, aspartic acid, arginine, lysine, leucine, slysine, and alanine are the amino acids most typically present in prawns. The amino acid concentration of prawns varies depending on the treatment given to them. As a result, different treatments have varying effects on prawn amino acid buildup (Saputri & Febriyanti, 2019).

The large potential for shrimp production causes the need for shrimp product preservation technology that not only keeps the physical quality of the shrimp fresh, but also the nutritional content therein. Using liquid smoke on shrimp is considered quite effective for preserving shrimp. Apart from that, the use of liquid smoke also reduces the amount of protein in shrimp less than the use of boiling and frying methods (Swastawati et al., 2017).

Previous study has frequently investigated the application of liquid smoke as an antioxidant in a variety of products. The use of liquid smoke is known to preserve prawn products against oxidation due to the phenolic components in the smoke (Daniswara et al., 2019). Based on the foregoing, more research is needed into the usage of liquid smoke in prawn products in order to determine the protein functional groups in prawn products cooked using various ways. It is envisaged that using liquid smoke will sustain the activity of proteins that are quickly destroyed by oxidation.

The objective of this research is to identify the features of smoked vaname shrimp protein functional groups using various cooking ways, as well as protein damage in smoked white vaname shrimp products using various cooking methods based on protein functional groups.

2. Material and methods

2.1 Materials and tools

The material used is vaname prawn (L. *vannamei*) sourced from the Jati market in Semarang, Central Java. The liquid smoke used is of the La Fronthea brand. Scales, an electric oven, frying pan, a basin, an FTIR spectrophotometer, a steamer, a gas stove and a thermometer are utilized.

2.2 Procedure

The research procedure employed in this study is based on that of (Agustin et al., 2012), with some adjustments. After being cleansed and washed, the prawns are soaked in liquid smoke at concentrations of 0% and 5% for three hours. The prawns are then steamed, fried and grilled. Steam the prawns in a steamer at 100°C for 15 minutes, or until they are cooked and pink. Fry the prawns in a frying pan at 140°C for 5 minutes, or until they turn reddish. Roast the prawns in an electric oven at 100°C for 2 hours, or until they turn red.

The shrimp products are subsequently vacuum-packed and transported to the Analysis Laboratory of the Department of Fisheries Product Technology at Diponegoro University for quality testing. Vacuum packing is a vacuum packaging technology that removes air from the package to increase shelf life. This vacuum packaging process involves inserting the product into plastic packaging and controlling the air with a vacuum packaging machine. This eliminates damage caused by oxidation, allowing the packaged product to last 3-5 times longer than products packaged using packaging. non-vacuum. Sampling was performed three times at different times.

2.3 Shrimp Organoleptic Test (Badan Standardisasi Nasional/Indonesian National Standards, 2020)

Organoleptic testing in accordance with Indonesian National Standards (INS) 2705:2020. The organoleptic test is a sensory test for frozen prawns. This organoleptic research was carried out by panelists at the Analysis Laboratory, Fisheries Product Technology Study Programme, Diponegoro University. According to Rahayu (1998), the trained panelists consisted of 5-6 people who had previously been educated to determine these sensory attributes. Panelists who have been somewhat trained can be chosen from a limited pool by first testing their sensitivity.

2.4 Protein ([AOAC] Association of Official Analytical Chemist, 2005)

The sample was weighed between 0.1 and 0.5 g and placed in a 100 mL Kjeldhal flask. The sample is destroyed (heated while boiling) until the solution turns clear green and the SO2 evaporates. The solution was allowed to cool before being transferred to a 50 ml flask and diluted with distilled water until it reached the tera mark. It was then placed in a distillation device and distilled with 5-10 ml of 30-33% NaOH. The distillate is collected in a solution of 10 ml of 3% boric acid and a few drops of indicator (0.1% bromcresol green solution and 29 0.1% methyl red solutions in 95% tatist, individually mixed with 10 ml of bromcresol green and 2 ml of methyl red). Titrate with 0.02 N HCl solution until the solution changes color to pink. The protein content in the ingredients is calculated using the formula:

$$\%Protein = \frac{(VA - VB)HCl \times N\ HCl \times 14{,}0007 \times 100\%}{w\ sample \times 1000}$$

Information :

VA : ml HCl for sample titration

VB : ml HCl for blank titration

N : normality of the standard HCl used

14.007 : atomic weight of Nitrogen

W sample : sample weight in grams

2.5 Fourier-Transform Infrared Spectrometer (FTIR)

The degree of deacetylation is measured via FTIR analysis. In this investigation, FTIR will detect the functional groups found in chitosan, specifically the NH, OH, and C-C functional groups. Chitin has the chemical formula CH and C=O. The results of FTIR detection are shown as functional group peaks at their corresponding wave numbers (Suptijah et al., 2011).

In the research, the Infrared Spectrophotometer used was Shimadzu IR for liquid samples, with the sample container type ATR-8200 H/8200 HA. The data obtained from the infrared spectrum results in the form of information on the statistical functional groups of a compound (Putri et al., 2019).

2.6 Amino Acid Profile Test ([AOAC] Association of Official Analytical Chemist, 2005)

Amino acid analysis consists of 4 stages, namely: the protein hydrolysate production stage, the drying stage, the derivatization stage, and the injection stage and amino acid analysis. The hydrolysate sample was dried using a rotary evaporator for 15-30 minutes. The dried sample was added with 5 mL of 0.01 N HCl then filtered using Millipore filter paper. The derivatization stage is by adding 30 µL of derivatization solution to the dried sample. The derivatization solution consists of a potassium borate buffer solution with a sample of 1:1 then mixed with an Ophthaldialdehyde (OPA) solution with a ratio of 5:1 to the sample, then the mixture is filtered using Whatman filter paper. 5 µl of the filtered solution was injected into the HPLC. Wait until the separation of all amino acids is complete. The time required is around 25 minutes. Calculation of the concentration of amino acids in the material is carried out by making a standard chromatogram using standard amino acids. The amino acid content in the ingredients can be calculated using the formula:

$$\%Amino\ acid = \frac{Sample\ area \times Fp \times\ C\ \times BM \times\ 100\%}{Standard\ area \times\ Sampel\ weight\ (g)}$$

Information :

Fp = *Dilution factor*

C = *Standard concentration of amino acids (µg/mL)*

BM = *Molecular weight of some amino acids g/mol.*

3. Result and Discussions

3.1 Characterization of Smoked Vaname Shrimp (L. vannamei) Functional Groups with Various Cooking Methods

An Infrared Spectrophotometer was used to characterize the functional groups of smoked vaname prawns cooked in various ways, allowing the functional groups included in smoked vaname prawns to be identified. Figure 1, 2, 3 and 4 displays the infrared spectrophotometer results for smoked white vaname prawns. The infrared spectrophotometer will identify the presence of CC, CH, CO, NH, and OH functional groups, which are found in protein molecules. In their investigation, Suptijah et al., (2011) found that FTIR detects functional groups such as NH, OH, C-C, CH, and C=O. The findings of FTIR detection are shown as peaks and valleys of functional groups at the wave number of each functional group.

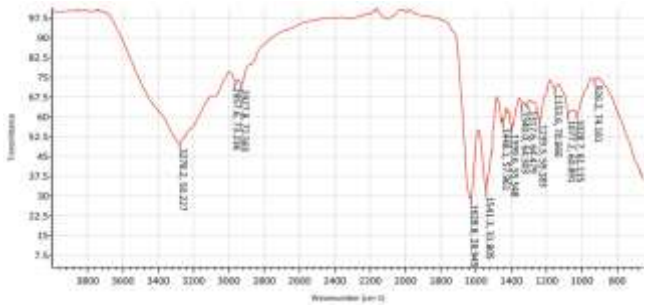

Fig. 1 – control fresh vaname shrimp

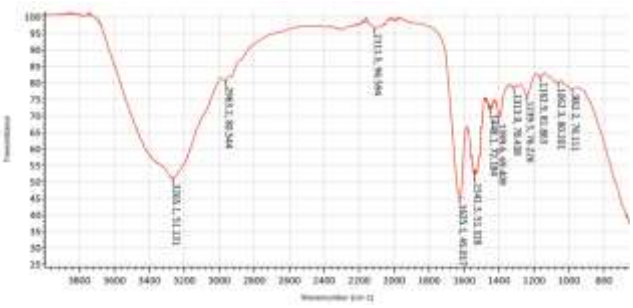

Fig. 2 – steamed vaname shrimp

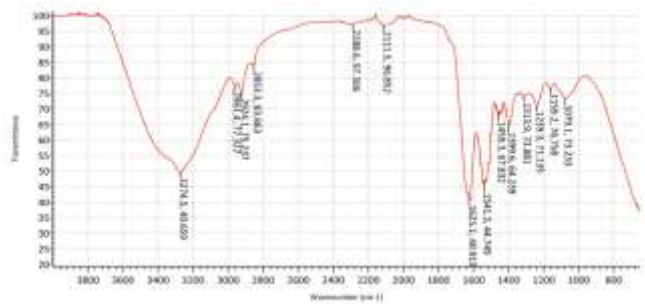

Fig. 3 – fried vaname shrimp

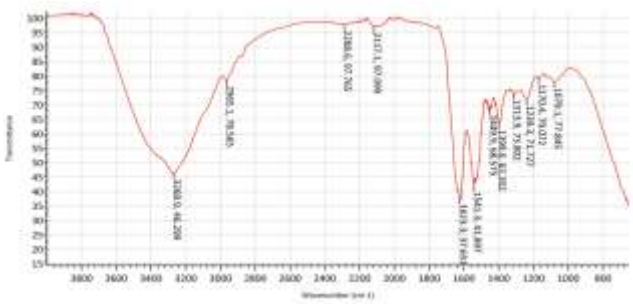

Fig. 4 – oven grilled vaname shrimp

The FTIR spectrum revealed no significant variations between the cooking process treatments applied to smoked prawns. There are 12 to 14 major peaks, which indicate the presence of specific functional categories. Protein compounds' structure is distinguished by the presence of NH_2 or amine compounds as components. The NH_2 compound is known to have an IR spectrum ranging from 3300cm^{-1} to 3600 cm^{-1}. Dong et al., (2023) found that the structure of CT-N is nearly identical to that of CT-C. Thus, the 3249 cm^{-1} range is thought to be the result of N-H structural stretching.

Functional group analysis of smoked prawns prepared using various cooking methods reveals the presence of amino acid compounds that comprise protein, as evidenced by the development of several indications of amino acid structures. The spectrum of smoked prawns prepared using various cooking

methods ranges from 3265 cm^{-1} to 3278 cm^{-1}, indicating the presence of NH, OH, and NH_2 groups. According to Knidri et al., (2017), the FTIR spectrum ranges from 3290 cm^{-1} to 3357 cm^{-1}, indicating the existence of N-H, O-H, and NH_2 molecules.

The presence of an NH_2 group on the long carbon chain distinguishes amino acid molecules found in proteins. The NH_2 group, often known as the amide group, will appear in the number spectrum from 3290 cm^{-1} to 3600 cm^{-1}. Protein damage is shown by the development of secondary amide structures, which indicate denaturation of the protein. The formation of secondary structures indicates protein degradation, according to Pramono et al., (2018). Protein secondary structure is commonly found as an amide group with a wavelength range of 1600cm^{-1} to 1690cm^{-1}. Based on this theory, it is known that the protein in smoked vaname shrimp using various cooking methods is still in good condition even though it experiences changes in the primary structure of the protein.

Spectrum peaks in the range 1550cm^{-1} – 1610cm^{-1} indicate the presence of carbonyl compounds formed. Carbonyl compounds can be formed from oxidation reactions that occur in smoked white vaname shrimp. According to Swastawati et al., (2019), carbonyl compounds contained in smoking products are produced from fat oxidation reactions which can react with amino acid compounds derived from protein. This can cause a decrease in the benefits of protein or what is known as "protein utilization", especially the essential amino acid lysine.

3.2 Protein Content of Smoked Vaname Shrimp (L. vannamei) with Various Cooking Methods

Protein tests in smoked white vaname prawn items yielded variable results depending on the cooking process. Figure 5 shows the protein value of smoked vaname prawns. Fresh or control prawns had the highest protein value, 38.84%. Meanwhile, smoked prawns cooked in the oven or on the grill had the lowest protein content, at 27.80%. This demonstrates that the cooking procedure decreases or degrades the protein value of smoked white vaname prawns. According to Kurniawati & Ranowati, (2018), protein damage can occur as a result of product effects such as heating, the addition of acids, bases, and salts, and mechanical agitation.

The frying method for smoked vaname prawns produced a protein content of 36.27%, which was higher than that of other cooking methods. The water content of smoked vaname prawns is instantly reduced when they are fried. Reducing the water content of prawn products is thought to induce a continued drop in protein value. Utami et al., (2016) found that low water content impacts the amount of protein in fried items. This causes the protein content to be higher when evaluated than when using other methods. Cooking also causes the protein composition of the product to fluctuate.

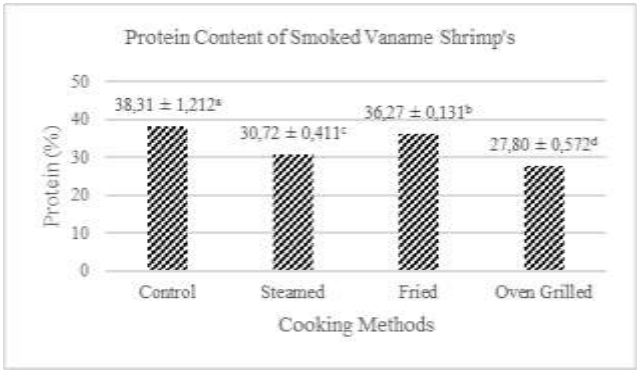

Fig. 5 – Protein Content of Smoked Vaname Shrimp's

The roasting/oven process results in a protein value that is 27.80% lower than other procedures. This is because to the uniformity of temperature and the relatively long time it takes to grill smoked white vaname prawns. Temperature and time consistency are elements that contribute to protein degradation. This is consistent with Hadi et al., (2022), who claimed that using high temperatures for long periods of time leads the protein content in products to become more sensitive to harm. Heating in the food preparation process induces protein denaturation in food, causing protein levels to drop.

Gao et al., (2016) explained in their study that variations in the ability and texture of water to contain protein affect the protein-water interaction. Heating causes denaturation and aggregation of proteins because it shrinks the filament and collagen lattice, exposing hydrophobic aliphatic side chains in the protein. This enables intra- and inter-protein interactions to result in a more compact protein structure.

The amount of protein contained in prawns is also controlled by the meal given to them. Feed with a high protein concentration will boost the protein content of prawns. According to AlFaris et al., (2022), prawn protein content is heavily influenced by their feeding behavior. This will have an impact on the essential amino acid composition as well. However, cooking prawns at a temperature of 130ºC for 10 minutes has been shown to harm their protein content.

The difference in protein content between cooking techniques for smoked white vaname prawn items is caused by the protein being hydrolyzed to form a hydrolysate product. Aside from that, some proteins form residues during the cooking process, such as being dissolved in water when steaming or in oil when frying. According to Yuniarti et al., (2021), the discrepancy in protein content is produced by the protein contained in the product being hydrolyzed into a hydrolysate state, with a portion of it found in non-hydrolyzed residue. Thus, the crude protein test identifies all of the amine groups in the protein. Therefore, additional testing is required to determine the particular value of prawn protein content, such as evaluating the amino acid profile.

Significant differences were found between control shrimp and smoked shrimp treated with liquid smoke. This is because the use of liquid smoke can reduce the protein in smoked shrimp. Based on research by Swastawati, Riyadi, et al., (2022), it was shown that treatment with liquid smoke on blood cockles showed a decrease in protein content. This is because the acid content in liquid smoke can cause damage to proteins. Proteins are related to metals, but their stability can be reduced in acidic conditions.

3.3 Amino Acid Profile of Smoked Vaname Shrimp (L. vannamei) with Various Cooking Methods

Testing revealed 15 different types of amino acids in smoked white vaname shrimp. From (Table 1) shows the amino acid profile analysis results for smoked vaname prawns prepared using various cooking methods. Using different cooking procedures produced diverse findings in amino acid profile testing. This is due to the fact that both methods of cooking involve heating. The heating induces an increase in the hydrolysis of amino acids. According to Adawyah et al., (2020), study demonstrates that particular amino acids increase during processing at specific periods and temperatures. The rise in amino acids is caused by the cooking process, which deaminates amino acids. This is also supported by Jacoeb & Wisnu Cakti, (2008), cooking processes such as boiling will have an influence on the amino acids of a material.

When compared to other cooking processes, the frying method produces the most amino acids. This is because the frying process requires a higher temperature than other cooking methods. In comparison to steaming and roasting, frying takes less time. According to Utami et al., (2016), the frying process significantly increases amino acid levels. Although each cooking method has an increasing influence on amino acids, the extent of the increase differs. This is caused by affecting elements such as the temperature utilized, heating time, and the heat transfer medium.

Table 1 – Amino Acid Profile Content of Smoked Vaname Shrimp's

No.	Parameter	Amino Acid Profile (mg/g)			
		fresh	steamed	fried	oven grilled
1	L-Alanine**	11.03	12.04	14.74	11.65
2	L-Arginine*	12.04	13.31	20.71	14.07
3	L-Aspartic acid**	10.82	14.71	20.00	14.99
4	Glycine**	10.09	10.75	17.40	12.63
5	L-Glutamic acid**	21.37	28.44	32.68	26.10
6	L-Histidine*	2.94	2.69	4.03	2.96
7	L-Isoleucine*	5.33	5.03	3.40	3.60
8	L-Leucine*	12.56	14.29	13.77	11.76
9	L-Lysine*	8.65	12.46	12.34	10.69
10	L-Valine*	5.49	5.86	5.05	4.63
11	L-Phenylalanine*	10.35	9.53	10.03	8.16
12	L-Proline**	4.93	8.82	12.40	9.18
13	L-Serine**	6.00	7.72	9.40	7.23
14	L-Threonine*	4.92	5.33	5.23	4.54
15	L-Tyrosine**	7.55	8.05	8.50	6.45
total		134.10	159.03	189.70	148.65

Information : *essential amino acids

**non-essential amino acids

According to test results, the amino acids *Alanine, Arginine, Aspartic Acid, Glycine, Glutamine Acid, Leucine*, and *Lysine* are present in considerable amounts in smoked prawn items prepared using various cooking methods. According to Ngginak et al., (2013), the amino acids commonly found in prawns are *glutamic acid*, *aspartate*, *arginine*, *lysine*, *leucine*, *glycine*, and *alanine*.

In the smoked prawn amino acid profile test, glutamic acid produced the highest values. Where glutamic acid contributes to shrimp's characteristic flavor. The savory or umami flavor associated with shrimp products is due to the glutamic acid found in shrimp. The frying cooking process also yields the highest concentration of glutamic acid seen in smoked prawns. According to Karomah et al., (2021), glutamic acid present in protein in its free form contributes to the umami or savory taste of food. Heating is one method of converting protein-associated glutamate into free glutamate.

When compared to other cooking processes, oven roasting has the lowest amino acid numbers. This is achievable because high temperatures are employed for a significantly longer period of time than other cooking processes. Temperature and cooking time, according to Xia et al., (2021), are characteristics that influence the physical quality and chemical composition of food items. However, the temperature difference has little effect. On the other side, cooking time influences amino acid hydrolysis.

Table 1 shows the average amino acid values.2. demonstrates that each cooking process increases the amino acid value of smoked vanname prawn products. However, the temperature and cooking time used have an impact on the growth in value. This is consistent with Utami's et al., (2016) findings, which showed that all types of amino acids rose during the cooking process. The rise in amino acid levels also vary according to the different processing temperatures employed.

Apart from that, the use of liquid smoke also showed an increase in amino acid values in smoked white vaname shrimp compared to control samples. This is because the phenol compounds in liquid smoke are thought to increase protein digestibility, thereby increasing the amino acid value of smoked vaname shrimp. According to Swastawati, Wahidah, et al., (2022) stated that the use of liquid smoke in smoked barracuda products increases the amino acid value. The increase in amino acids is caused by the acetic acid content in liquid smoke. The acidic nature of liquid smoke can donate H+ ions so that it can cause amino acids to be at the isoelectric point and take amphoteric form (zwitter ions). Amino acids at this point can release hydrogen bonds to form secondary, tertiary and quaternary structures. So as a result of breaking down the complex structure, a primary structure of amino acids is produced which is bound by peptide bonds and increases the value of the amino acid.

3.4 Organoleptic Value of Smoked Vaname Shrimp (L. vannamei) with Various Cooking Methods

Based on Table 2, the organoleptic values of all cooking methods were found to vary. Appearance, odor and texture parameters for each cooking method treatment showed results that were not significantly different. Differences in cooking methods did not show any differences in the appearance, smell and texture of smoked vaname shrimp. This is because the shrimp used are still fresh so that when the cooking treatment is carried out it does not have a significant effect on the shrimp. According to Herliany et al., (2013), shrimp that have experienced a decline in quality will experience changes in terms of color, where shrimp that have deteriorated in quality have a duller color than shrimp that are still fresh. Apart from that, the texture of quality crawfish is no longer compact and dense but is more easily crushed and soft.

Table 2 – Organoleptic Value of Smoked Vaname Shrimp

Cooking Methods	Organoleptic Parameter			
	appearance	**aroma**	**texture**	**confidence interval**
Control	8,2±0,52	8,4±0,33	8,2±0,52	$8{,}199 \leq \mu \leq 8{,}341$
Steamed	8,2±0,58	8,2±0,61	8±0,62	$8{,}046 \leq \mu \leq 8{,}214$
Fried	8,4±0,59	8,4±0,70	8,4±0,69	$8{,}330 \leq \mu \leq 8{,}471$
Oven Grilled	8,2±0,70	8±0,61	8,4±0,68	$8{,}102 \leq \mu \leq 8{,}298$

Information:

- Data are the average results of 10 panelists ± standard deviation.

The treatment of various cooking methods showed that the value of the smell of smoked vaname shrimp was still acceptable to the panelists. This is because the vaname shrimp used are still fresh and have not experienced a decline in quality. Moreover, the quality deterioration process is caused by protein-destroying bacteria which will cause a bad smell to appear. This is in accordance with research conducted Ndahawali, (2016) which states that protein damage in shrimp can cause a bad smell. Protein damage in shrimp can be caused by the activity of microorganisms which cause protein denaturation. *Bacillus*, *Flavobacterium*, *Lactobacillus*, *Micrococcus*, *Sarcina*, *Staphylococcus*, *Alcalineus*, and *Proteus* are microbes that are often found to damage shrimp products.

There was no significant difference in the texture of smoked white vaname shrimp before and after cooking treatment. This is because the shrimp used are intact and good. Physical damage to shrimp usually occurs due to enzymatic activity that occurs after the shrimp dies. According to Sipahutar et al., (2019) damage due to enzymatic reactions in shrimp causes the texture of the shrimp to no longer be compact and dense. This occurs in the post rigor phase in shrimp, which is marked by the shrimp's body softening. The deterioration phase of shrimp quality consists of four stages, namely pre-rigor, rigor mortis, post rigor and rot.

The provision of liquid smoke to vaname shrimp products is also considered to play a role in providing changes from an organoleptic perspective. According to Swastawati et al., (2013), the reaction of carbonyls from smoke with protein and fat compounds in fishery products will affect the appearance, smell and texture of the product. Smoke has a role in influencing organoleptic due to the reaction of acids, phenols and other contents in smoke to fats, proteins and carbohydrates.

Conclusions

The conclusions obtained from research on Smoked Vaname Shrimp (L. *vannamei*) Protein Function Group Analysis with Various Cooking Methods are the functional groups of the smoked vaname prawn protein produce spectrum peaks, indicating the presence of amino acid molecules that comprise the protein. The spectra peak distribution begins around 3300 cm^{-1} - 3600 cm^{-1}, indicating the presence of amide chemicals (NH_2). This is further corroborated by the distribution of spectral peaks in the fingerprints zone at wavelengths between 500cm^{-1} and 1500cm^{-1}. Secondary protein molecules occur when proteins are damaged. Secondary proteins will show in the wavelength region of 1600cm^{-1} to 1690cm^{-1}. This demonstrates that the protein quality in smoked vaname prawns is still in good condition since no spectrum peaks were identified in this range.

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