Profile of proximate levels and abundance of microbial contamination in hydrolyzed sauce of Razor Clams (*Solen* sp.) as food flavoring from The Coastal Madura

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Abstract. *Solen* sp. is one of marine organisms that not only abundant in the Madura strait, but also rich in nutrients making it a promising candidate for development into functional food products such as clam sauce. This study aims to determine the best formula and analyze the differences in physical characteristics, proximate levels, and abundance of microbial contamination in clam sauce. Four formulations were tested, each with varying proportions of clams (%), salt (%), and papain enzyme (%) during the hydrolysis process: F1 (90:2.5:7.5), F2 (90:5:5), F3 (90:7.5:2.5), and F4 (90:6:4). Based on the proximate composition and microbial contamination levels, formulation F4 was identified as the most suitable, meeting the standards set by SNI 01.4275:1996, SNI 4275:2022, and PerBPOM No. 13 of 2019. The proximate composition of F4 included protein ($3.58 \pm 0.27\%$), moisture ($76.77 \pm 0.19\%$), ash ($0.62 \pm 0.01\%$), fat ($0.43 \pm 0.13\%$), and carbohydrates ($0.06 \pm 0.00\%$). The microbial profile showed a Total Plate Count (TPC) of 0.286×10^4 CFU/g and Escherichia coli levels of <3 MPN/g. While microbial contamination levels were statistically similar across all formulations, significant differences ($p < 0.05$) were observed in most proximate parameters, with the exception of moisture and carbohydrate content. These findings support the potential of F4 as a standardized and safe formulation for the development of a novel razor clam-based sauce product.

1 Introduction

Clams are bivalve mollusks that represent an important fishery commodity, particularly in the waters of the Madura Strait [1], with high abundance observed in coastal areas of Bangkalan Regency. One species that is especially abundant and widely utilized by coastal communities in Madura is the razor clam (*Solen* sp.). The average density of this species in the Madura Strait has been reported at approximately 8-10 individuals per 25×25 cm transects area [2]. Commonly referred to as the bamboo clam, *Solen* sp. Is characterized by its small, elongated, and symmetrical shell with a smooth, glossy surface resembling a straight razor

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[3]. Razor clams are commonly consumed by local communities as a dietary protein source, based on the perception that they possess high nutritional value.

The nutritional composition of razor clams (*Solen* sp.) is highly diverse and holds significant potential to contribute to the adequate nutritional intake of the Madurese community. These clams contain a range of primary metabolites, including water, ash, protein, fat, and carbohydrates [4], as well as secondary metabolites such as alkaloids, steroids, and flavonoids [5]. Among these, the protein content is notably high, with razor clams from Madurese waters reported to contain $56.90 \pm 1.15\%$ protein [4]. Additionally, they are a source of saturated fatty acids-such as palmitic and stearic acids-as well as unsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [6]. The rich nutritional profile, combined with their umami flavor when incorporated into traditional Madurese dishes, contributes to the high consumer demand for razor clams in the region.

Despite the high nutritional value and consumer demand for razor clams, their economic value remains relatively low, as reflected in the modest market price. This low price is disproportionate to the labor-intensive harvesting process, given that razor clams are burrowing organisms that inhabit marine sediments. Thus, value-added processing and product innovation are essential to enhance the market value of razor clams in Madura. One such innovation is the development of razor clam sauce, a novel seasoning product that is currently not available commercially. Similar products made from other clam species include oyster sauce (*Magallana gigas*) [7], green mussel sauce (*Perna viridis*) [8], sweet clam sauce (*Gafrarium pectinatum*) [9], and blood clam sauce (*Anadara granosa*) [10]. Given its potential as a flavor enhancer in daily meals, particularly in traditional Madurese cuisine, razor clam sauce represents a promising avenue for product diversification and economic development.

The innovationt of razor clam sauce as a potential flavoring agent characteristic of Madurese cuisine necessitates scientific research to ensure the product's safety, quality, and acceptability at the market level. Clam-based sauces are typically associated with a strong umami flavor profile [11], which can be enhanced through the addition of table salt-an ingredient that is also abundantly produced in Madura [12]. The local availability of both key raw materials-razor clams and salt-underscores the feasibility of producing this value-added product within the region. The production of razor clam sauce involves a hydrolysis process that incorporates salt and enzymatic treatment, such as the use of papain. The incorporation of papain enzyme has been shown to help preserve the nutritional quality of the product, particularly its proximate composition [13]. Additionally, salt not only contributes to flavor enhancement but also facilitates the release and formation of peptides and amino acids during hydrolysis, thereby enriching the product's taste and nutritional profile [14].

To be considered suitable for national consumption, clam sauce products must comply with quality and safety standards established by regulatory bodies, including the Indonesian National Standard (SNI) of 1996 [15], the updated SNI of 2022 [16], and the Indonesian Food and Drug Authority Regulation (PerBPOM) No. 13 of 2019 [17]. In general, such products are expected to meet minimum nutritional adequacy requirements and be free from microbial contaminants, including Escherichia coli, coliforms, and exhibit acceptable levels in the Total Plate Count (TPC). The assessment of proximate composition and microbial load in hydrolyzed razor clam sauce is therefore essential, serving as both a baseline indicator and a determinant of product acceptability for consumers. This purpose of this study was to identify the optimal formulation of clam sauce and to evaluate the variations in its physical properties, proximate composition, and levels of microbial contamination.

2 Methodology

2.1 Materials

The tools used in this study were a 35°C incubator, porcelain cup, desiccator, oven, 0.331-inch mesh sieve, furnace, Whatman 41 filter paper, electric heater, condenser, soxhlet extractor, fat sleeve, blender, jar, 20ml diluent bottle, petri dish, test tube, analytical balance (0.0001g), microscope, pipette, bunsen, filter flask, autoclave, showcase, coolbox, basin, stainless steel pan, spatula, stove, stainless steel pan, sieve, frying pan thermometer, stainless steel spoon, hot plate, bent glass rod, measuring flask (1000ml, 100ml), erlenmeyer (250 ml, 100 ml), burette and support, volume pipette and pump, kedjhal flask, fume hood, standing pouch, aluminum foil, airtight glass jar. The materials used in this study were papain enzyme 200 mIU/L (merck Hunan Insen Biotec co.ltd), 87% NaCl consumption salt produced by multistage precipitation method, razor clams, Brown sugar, spices (garlic, galangal, bay leaves, lemongrass, coriander), carboxymethylcellulose (CMC), Aquades, Plate Count Agar, Nutrient Agar, Butterfields Phosphate Buffered Solution, Gram Stain, chromocult, Brilliant Green Lactose Bile (BGLB), Lauryl Tryptose Broth (LTB), EC Broth, Purple Carbohydrate Broth, Levine's Eosin Methylen Blue (L-EMB) agar, Tryptone, Tryptic Soy Agar, 70% Alcohol.

The sample hydrolysis process was carried out at the Salt House of Trunojoyo University, Madura. The making of razor clam sauce, chemical tests in the form of proximate products, physical tests, and tests of the abundance of microbial contamination were carried out at the Marine Biotechnology Laboratory of Trunojoyo University, Madura. The study was conducted for 3 months from August to October 2024.

2.2 Samples collection

Razor clam samples (*Solen* sp.) were collected from the coastal waters of Pesanggrahan Village, Kwanyar District, Bangkalan Regency, Madura, using a purposive sampling method. Specimens were obtained from local fishermen operating in the designated sampling area. Manual collection was conducted using a scoop, and the clams were placed into a single container. Upon collection, the clams were rinsed with running fresh water to remove adhering debris. Morphometric measurements-including shell length, shell width, and individual weight-were conducted on 200 specimens (**Figure 1**).

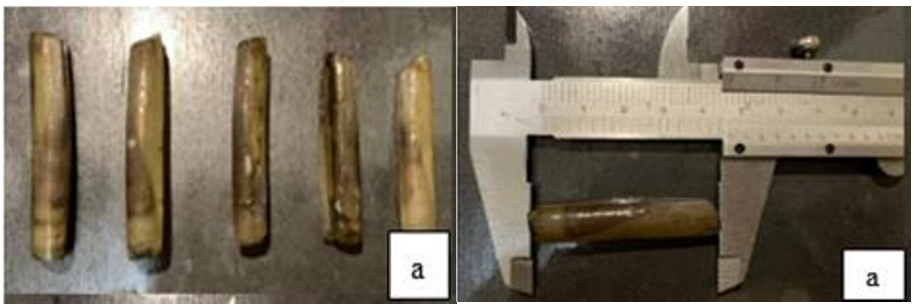

Figure 1. Sample of razor clams collected from the research location.

2.3 Sample yield

The yield of the mussel samples was determined by comparing the weight of the whole, unboiled mussels with shells intact (**Figure 2a**) to the weight of the boiled mussel meat after

separation from the shells and internal organs (**Figure 2b**). The obtained mussel meat was then homogenized using a blender and subsequently subjected to the hydrolysis process.

Figure 2. Samples of razor clam: a) The razor clams have been peeled and boiled, b) The razor clams have been mashed and the digestive organs removed.

The following is the calculation of the yield of the razor clam sample:

$$\text{Extract yield} = \frac{\textit{the weight of boiled clam meat without digestive organs}}{\textit{weight of clam meat before boiling}} \quad (1)$$

2.4 Hydrolysis process of razor clam meat

The method used for the hydrolysis process of the razor clam samples was carried out using the conventional method [14] using the salt house room of Trunojoyo University, Madura. The formula used in the hydrolysis of clams is 4 with a ratio of the composition of clam meat (%): salt (%): Papain enzyme (%) namely F1 (90:2.5:7.5), F2 (90:5:5): F3 (90:7.5:2.5), and F4 (90:6:4). The hydrolysis process begins by smoothing the razor clam meat that has been separated from its digestive organs using a blender (cosmos Smart Blenz Blender CB 802). The smooth clam meat is then added with 200 mIU/L papain enzyme (merck Hunan Insen Biotec co.ltd) and 87% NaCl salt. Samples that had been mixed with enzymes and salt were put into a closed and airtight jar, and stored in a Salt House using a temperature of 50^{o}C for 12 days.

2.5 Making hydrolyzed razor clam sauce

The making of clam sauce is done in 3 stages. The first stage, namely the mixing stage, is carried out by mixing 50 mL of the hydrolyzed clam meat sample with 200 mL of drinking water. The mixed sample is then cooked at a temperature of 80oC for 10 minutes. This cooking stage is used to stop the hydrolysis process in the clam meat. The second stage, namely the spice addition stage, is carried out by adding spices with a percentage composition of each spice as much as bay leaves (1.3%), lemongrass (1.3%), coriander (0.5%), garlic (1.2%), shallots (1.4%), and palm sugar (10%) then cook again for 10 minutes at a temperature of 70-80 in the hydrolysis mixture. The sample that has reached the cooking time is then filtered using a filter to separate the large spices and the filtrate (filtering I). The 3rd stage, namely the provision of thickening agents, is carried out by adding filtrate with CMC (Carboxymethyl Cellulose) food grade category (0.6%) then homogenized using a 1500 mAh electric hand mixer for 10 minutes at a temperature of 70-80 then filtered again (filtering II). The comparison of the composition of the ingredients used in making clam sauce products is made with a percentage of 20.4% clam meat: 0.6% CMC, 5% spices, and 74% boiled water.

2.6 Analysis proximate

Proximate analysis was carried out on all designed product and control. Proximate testing consists of water content using the SNI 2354.2:2015 method [18], ash content using SNI 2354.1:2010 [19], fat content using SNI 01-2354.3:2006 [20], protein content using the formol titration method [21], and carbohydrate content using the AOAC 2005 method [22].

2.7 Analysis of abundance of microbial contamination

Microbial abundance analysis was conducted on the 4 formulas of clam hydrolysate sauce and control. The control used was commercial oyster sauce. Analysis of Total Plate Count abundance of bacteria used the SNI 2332.3-2015 method [23], while *E. coli* bacteria tests used SNI 2332.1.2015 [24].

2.8 Data analysis

The collected data were presented in tables and graphs and interpreted with support from relevant previous studies. To evaluate the effects of different concentrations of table salt and papain enzyme on the physical characteristics, proximate composition, and microbial contamination levels of razor clam sauce, a one-way analysis of variance (ANOVA) was conducted at a significance level of 0.05. When significant differences were detected, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to determine which treatment groups exhibited statistically significant differences.

3 Results and discussions

3.1 Morphometrics and yield of razor clams

The morphometrics of the razor clams were observed to describe the size of the biota and estimate the yield that would be obtained if replicating the razor clam sauce product. The measurement results showed that the length, width, and weight of the razor clams collected in Bangkalan waters as the main raw material in making clam sauce were 28.41±17.83 mm long, 8.26±1.23 mm wide, 1.83±1.55 g per individual, and 6.60±1.24 mm thick umbo. The morphometric size of the razor clams found in Bangkalan waters is similar to the size of the razor clams obtained in Pamekasan waters. The morphometrics of the razor clams in Pamekasan range from 20-50 mm long with a weight of 0.8-3 g, for the width and thickness of the umbo around 6-9 mm [4]. The similar range of sizes of the clam samples is thought to be caused by the same conditions and sources of nutrients between Bangkalan and Pamekasan waters.

The morphometric dimensions of the razor clam can indirectly affect the total yield to be obtained. The total percentage of the yield of the razor clam meat obtained in this study was 37.83%. The results of weighing the weight of the razor clam before being cleaned were 3000 g and decreased to 1135 g after the process of removing the digestive organs and the boiling process. The low percentage value of the yield of the razor clam sample is thought to be caused by the type of clam, the separation of the shell from the meat, the boiling process, and the removal of the digestive organs of the clam which caused the weight of the clam to decrease drastically. In addition to the boiling process, other factors that affect the yield value of the clam are the thickness of the shell wall [13] and the length of the digestive organs of the clam [25]. The longer the storage at room temperature, the more the quality of the shellfish decreases [26].

3.2 Characteristics of razor clams sauce

The razor clam sauce was produced using four different formulations alongside a control. Visual observations revealed notable differences in color appearance across the hydrolysates. At the initial stage of fermentation (day 0), when clam meat was mixed with salt and papain enzyme, all formulations exhibited a beige coloration (**Figure 3a**), corresponding to the natural color of fresh clam tissue. After a 12-day hydrolysis period, a distinct shift in coloration was observed among the formulations. Formulations F2, F3, and F4 developed a characteristic blackish-brown hue, whereas F1 retained a predominantly grayish tone (**Figure 3b**).

The atypical color observed in F1 is attributed to its lower salt concentration, which may have hindered effective fermentation and hydrolysis, potentially leading to protein degradation and the development of an unpleasant, putrid odor. These findings suggest that salt concentration plays a crucial role in facilitating the enzymatic breakdown and preservation of proteins during hydrolysis. The observed color darkening across other formulations is likely a result of natural pigment oxidation, a process influenced by factors such as temperature, duration, and pH during fermentation [13].

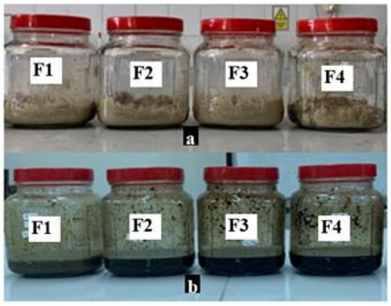

Figure 3 Asiatic hard razor clam hydrolysis; (a) day 1, (b) day 12.

According to physical appearance, the results of perfectly fermented mussel meat fermentation is characterized by consistency from solid to semi-liquid, indicating an increase in water content within the sample. The study also demonstrated the variation in enzymes concentration significantly influenced the rate of hydrolysis, with higher papain concentration accelerating the breakdown of mussle tissue. Additionally, the controlled addition of salt appeared to mitigate spoilage, suggesting its role in enhancing microbial stability during fermentation. The greater concentration of papain enzyme to the sample contributed to a more rapid degradation of protein structure as a result facilitating moisture release. These results are similar with previous research, which reported that the higher concentration of papain enzyme promoted increased water content in tutut soy sauce products, notably owing to significant protein hydrolysis [14].

Figure 4. Product visualization of razor clam sauce all formulas and controls (Fs 1 is Formula *Solen* 1).

The hydrolysis sample of the clam that is formed can directly affect the quality and visual of the razor clam sauce product (**Figure 4**). Damage to the hydrolysis sample of the razor clam formula 1 has an impact on the visual degradation to the clam sauce product. The main cause of the unsuitability of formula 1 as a clam sauce formula is due to the incomplete hydrolysis process of the clam meat which is lead to the deterioration of raw material quality. This incomplete hydrolysis process was further evidenced by unpleasant odor from formula 1, indicative of protein degradation and spoilage.

3.3 Proximat value in razor clams sauce

Proximate value is an initial test to trace the Nutritional Adequacy Rate in a product. Some parameters tested proximately include water content, ash content, fat content, protein content and carbohydrate content [27]. Proximate value analysis needs to be tested on clam sauce and tofu clam products to determine the water content, ash content, fat content, protein content and carbohydrate content in these products. The results of proximate testing on clam sauce products are listed in **Table 1**.

Overall, the proximate content that had a significant difference was the ash, fat, and protein content, except water content and carbohydrates. Additionally, the water content in all formulas did not differ significantly, presumably because the volume of water used in each formula was the same. Water content is a proximat indicator that plays a role in estimating the level of durability of fishery products [28]. Indirectly, water content is an important parameter to ensure the quality and shelf life of products made from razor clams [29]. The water contained in food products affects the physical characteristics of the product, especially the texture, appearance, and taste of the product [30]. The water content contained in all formulas ranges from 76.77±0.19%-86.63±0.72%. High water content in materials and products can reduce the percentage of other proximate content [4], conversely, lower water content in products and materials can increase the content of other proximate contents proportionally [31].

Table 1. Proximate content of razor clam sauce products and its comparison with quality standards for oyster sauce.

Parameter (%)	Formulation of razor clams sauce				Clam sauce quality standards[@]
	F1	F2	F3	F4	
Water	86.63±0.72^{a}	87.58±2.27^{a}	83.61±0.11^{a}	76.77±0.19^{a}	*
Ash	0.13±0.03^{a}	0.19±0.08^{a}	0.44±0.01^{bc}	0.62±0.01^{c}	*
Fat	0.62±0.10^{b}	0.21±0.02^{a}	0.66±0.12^{b}	0.43±0.13^{ab}	Min 3
Protein	0^{a}	1.40±0.75^{b}	2.58±0.17^{cd}	3.58±0.27^{d}	*
Carbohidrate	0.08±0.00^{a}	0.10±0.02^{a}	0.09±0.00^{a}	0.06±0.00^{a}	*

Note: Different letters in the same raw indicate a significant difference ($p<0.05$) based on Tukey HSD, [@]The quality standard for clam sauce is oyster sauce based on SNI 4275:2022, *not specified

Formula 4 demonstrated the highest protein content among all formulas, with a value of 3.58±0.27%, and exhibited the lowest water content at 76.77±0.19%. In addition, this formula also has an ash content of 0.62±0.01%, a fat content of 0.43 ± 0.13% and a carbohydrate content of 0.06 ± 0.00%. Formula 4 is the only one formula that meets the minimum proximate content requirements for oyster sauce according to SNI 01.4275:1996 and SNI 4275:2022.

On the other hand, Formula 1 showed the lowest performance for the proximate content for a clam sauce formula, that is included in the category of unfit for consumption because this formula produces damaged visuals, off-odor and does not contain any protein at all. Protein is a series of essential amino acids with peptide bonds used to build and repair tissue cells in living organisms [32]. The results of proximate analysis showed that Formula 1 had a water content of 86.63±0.72%, ash content of 0.13±0.03%, fat content of 0.62±0.10%, protein content of 0%, and carbohydrate content of 0.08±0.00%.

3.4 Abundance of microbial contamination in razor clams sauce

The abundance of contaminating microorganisms in the clam sauce product was carried out to ensure the product was fit for consumption. The abundance of microbial contamination did not differ significantly in all formulas. The parameters of the contaminating bacteria tested were the total plate count of bacteria to determine the number of bacterial colonies in the razor clam sauce and the abundance of *E. coli* bacteria in the clam sauce. The results of the identification and calculation of the abundance of microorganisms showed that the highest bacterial abundance was found in formula 1, which was 796 colonies, causing the total plate count of bacteria in this formula to be included in the category of not being able to be counted (**Table 2**). The high abundance of bacteria in formula 1 owing to the lowest proportion of salt rather than the other formula, trigerring incomplete hydrolysis process in the formula and caused spoilage. The lowest number of bacterial colonies was found in Formula 2, which was 214 colonies and the total plate count of bacteria was $0.214x10^3$ MPN/g. The Total Plate Count of bacteria is generally used to determine the number of microbes that develop into large colonies or small colonies on plate count agar media [33].

Table 2. Abundance of microbial contamination in razor clams sauce.

Information	Abundance of microbial in razor clams sauce			
	F1	**F2**	**F3**	**F4**
N (coloni)	796^a	214^b	239^b	286^b
TPC (Coloni/g)	can't be counted*	$0.214x10^3$	$0.239x10^3$	$0.286x10^3$
E.coli (MPN/g)	<3.0	<3.0	<3.0	<3.0

Note: Different letter marks on the same line indicate significant differences, N: Number of product colonies (colonies/mL or colonies/g), TPC: Total plate count (colonies/g)

The abundance of contamination bacteria that must be considered next is *E. coli* bacteria. E. coli bacteria is gram-negative bacteria and belong to the class of contamination bacteria that are difficult to inhibit [34] because these bacteria have systemic ability [35], and good adaptability to impure antibacterial compounds, for example seagrass extract [36]. This bacterium has the characteristic of being able to produce gas in durham tubes [37] and the color of the bacterial colonies is blue with a round shape when grown in Cromocult media [38]. The results of the abundance of *E. coli* bacteria abundance in all formulas of <3.0 (MPN/g). This indicates that the sausage of a razor clam is suitable for consumption because according to PerBPOM Regulation No. 13 of 2019 the delay of the limit of microbial contamination in food is <3 MPN/g. The data also informs that shellfish sauce is safe for consumption by consumers because one of the infections caused by food products containing *E. coli* above the quality standard threshold can cause stomach cramps, diarrhea, vomiting, and urinary tract infections of the human body [39].

3.5 Comparison of the best razor clam sauce with other clam sauce

Based on the results of the proximate test (**Table 1**) and the abundance of microbial contaminants (**Table 2**), the best formula for razor clam sauce is Formula 4. In general, Formula 4 has the highest protein content compared to other formulas at 3.58±0.27%. The average protein content of formula 4 meets the SNI standard SNI 4275: 2022 [16]. In addition, the abundance of microbial contaminants in this formula is still included in the category below the required standard [17]. Overall, Razor clam sauce is suitable and safe for consumption.

The proximate content, especially the protein content of the razor clam sauce is higher compared to commercial oyster sauce circulating in Indonesia (**Table 3**). However, the protein content of the razor clam sauce is lower than the tofu clam sauce (*Maretrix* sp) at 3.81±0.03% and the green clam sauce (*Perna viridis*) at 7.97%. The low protein content in razor clam sauce compared to tofu clam sauce and green clam sauce due to several factors, for example, clam eating habits [40] and the abundance of nutrients in the clam's living environment, age, and fishing season [41]. The test results also showed that the commercial oyster sauce product as a control formula did not meet the protein content standards determined by SNI 4275:2022, namely 1.36±0.15%. Due to in commercial products, based on the nutritional value information listed on the product, are dominated by salt in the form of 19% sodium, carbohydrates, and sugar. Commercial oyster sauce generally uses a small amount of oyster shell extract, namely 12.8% (stated on the packaging composition) so this indirectly causes the low protein value contained in the product.

Table 3. Comparison of the formulation of the best razor clam sauce with other sauce.

Parameter	Razor clam sauce	Oyster Sauce[c]	Tofu clam sauce [8]	Green mussel sauce [9]	Quality standards
Water (%)	76.77±0.19	51.44±0.44	80.90±0.44	84.44	*
Ash (%)	0.62±0.01	14.3±0.87	3.96±0.05	1.51	*
Fat (%)	0.43±0.13	0.90±0.01	1.57±0.18	1.67	*
Protein (%)	3.58±0.27	1.36±0.15	3.81±0.03	7.97	Min 3.0
Carbohidrate	0.06±0.00	31.93±0.52	9.74±0.69	*	*
TPC (Coloni/g)	$0{,}286x10^3$	$0.19x10^4$	$0.33x10^4$	*	Max 10^4
E.coli (MPN/g)	<3.0	<3.0	<3.0	*	<3

Note: * not identified, [c] Control from commercial sauce

4 Conclusion

The conclusion of this study is formula 4 with the composition of the proportion of razor clam meat (*Solen* sp): consumption salt with 87% NaCl content: pure papain enzyme, namely 90%:6%:4%. Hydrosilated meat samples in 4 formulas significantly caused no significant difference in the levels and carbohydrate content in clam sauce, but caused significant differences in the ash content, fat content, and protein content contained in the clam sauce. The proximate value of the razor clam sauce formula 4 is a protein content of 3.58±0.27%, water content of 76.77±0.19%, ash 0.62±0.01%, fat 0.43 ± 0.13%, carbohydrate 0.06±0.00%. The razor clam sauce formula 4 is recommended as the best formula because it has the highest protein value compared to other formulas and meets the minimum protein content requirements required by SNI 01-4275-1996 and SNI 4275:2022. The abundance of contaminant bacteria in formula 4 products include a Total Plate Count of $0.286x10^3$ and *E. coli* <3.0 MPN/g which is still below the quality threshold for clam sauce based on the regulation of the Food and Drug Supervisory Agency no. 19 of 2019 concerning food categories.

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