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QUALITY AND MICROBIOLOGICAL CONDITIONS OF SMALL-SCALE SHRIMP PASTE PROCESSING IN CIREBON PRODUCTION CENTERS

Mutu dan Kondisi Mikrobiologis Pengolahan Terasi Udang Skala UKM di Sentra Produksi Cirebon

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ABSTRACT

Terasi is a fermented product made from fish or shrimp, that undergoes a salting process. The small-medium scale of shrimp paste industry are widespread in Indonesia, particularly in the Cirebon area. Traditional processing of shrimp paste involves natural or spontaneous fermentation processes, which involves microbes from the surrounding environment that can grow and contaminate the shrimp paste. This study aims to analyze the microbiological conditions and quality of traditional shrimp paste produced in Cirebon production centers based on SNI 2716: 2016 for shrimp paste. The survey method used purposive sampling based on specific criteria to determine sample points. The observation parameters tested included pathogenic bacteria *E. coli* (presumptive) using the petrifilm method (3M™), *Salmonella* sp. and *Vibrio parahaemolyticus* using the PCR method, total mold using the ALT (Total Plate Number) method, a_w, pH, and proximate. The overall data was analyzed using quantitative descriptive analysis. The results showed that *E. Coli* counts ranging from 6.00 x 10^2 to 2.10 x 10^4 CFU/g, thus not meeting SNI 2716: 2016 standard for shrimp paste. However, the final product did not show contamination by *Salmonella* sp, and *Vibrio parahaemolyticus*. The total mold contamination in the final shrimp paste product from processor A (Desa Jatimerta) and B (Desa Panjunan) ranged from 1.80 x 10^2 to 4.30 x 10^3 CFU/g. The chemical analysist result of the final products from both processor generally met the SNI 2716: 2016 standards, except for processor B's samples, where the water and ash content did not meet the standards.

Key words: Fermentation, Molecular Identification, Contamination, Pathogen, Shrimp Paste

ABSTRAK

Terasi merupakan suatu produk hasil fermentasi ikan atau udang yang mengalami perlakuan penggaraman. Industri terasi udang skala rumah tangga banyak tersebar di Indonesia salah satunya di daerah Cirebon. Proses pengolahan terasi yang diolah secara tradisional dalam tahap fermentasi terjadi proses fermentasi alami atau spontan yang melibatkan mikroba yang berasal

dari lingkungan sekitar yang dapat tumbuh dan mengontaminasi terasi. Penelitian ini bertujuan untuk menganalisis kondisi mikrobiologis dan mutu terasi udang tradisional yang di produksi di sentra produksi Cirebon berdasarkan SNI 2716: 2016 tentang terasi udang. Metode yang digunakan dalam penelitian ini yaitu metode survei dengan penentuan titik sampel diambil menggunakan metode *purposive sampling* berdasarkan kriteria tertentu. Parameter pengamatan yang diuji di antarannya bakteri patogen *E.coli* (presumptif) menggunakan metode petrifilm (3M™), *Salmonella* sp., dan *Vibrio parahaemolyticus* menggunakan metode PCR, total kapang menggunakan metode ALT (Angka Lempeng Total), a_w , pH, dan proksimat. Data keseluruhan dianalisis mengunakan analisis deskriptif kuantitatif. Hasil penelitian menunjukkan bahwa jumlah cemaran *E.coli* berkisar antara 6,00 x 10^2 - 2,10 x 10^4 CFU/g sehingga belum memenuhi standar SNI 2716: 2016 . Namun demikian, pada produk akhir tidak ditemukan cemaran bakteri *Salmonella* sp, dan *Vibrio parahaemolyticus* pada terasi. Adapun jumlah cemaran total kapang pada produk akhir terasi dari kedua pengolah berkisar antara 1,80 x 10^2 s.d. 4,60 x 10^3 CFU/g. Hasil analisa kimia pada produk akhir dari kedua pengolah secara umum telah memenuhi standar SNI 2716:2016 kecuali pada sampel pengolah B di mana kadar air dan abu belum memenuhi standar.

Kata Kunci: Fermentasi, Identifikasi Molekuler, Kontaminasi, Patogen, Terasi

INTRODUCTION

Shrimp is a fishery commodity with high demand. Based on data from the Indonesian Ministry of Maritime Affairs and Fisheries (KKP), shrimp is the largest export compared to other fishery products. In 2020, 239.28 million kg of shrimp were exported with a value of ± 2.04 billion US dollars (Krisandini, 2023). One of the shrimp species in Indonesia is rebon shrimp. Rebon shrimp is one of the abundant marine commodities (Gobel *et al*., 2016) This rebon shrimp is one of the fishery commodities that is smaller in size compared to other types of shrimp. This shrimp is called "rebon" by fishermen, especially in West Java because of its smaller size (Astawan, 2009). The protein content in 100 grams of rebon shrimp is quite high, namely 16.2% in fresh shrimp and 59.4% in dried rebon shrimp, then several other nutritional contents such as fat as much as 3.75% and carbohydrates as much as 3.33% (PERSAGI, 2009). Shrimp paste is usually used as a raw material for flavoring products such as shrimp paste and petis, or processed into dried shrimp paste which has a slightly salty taste, but people more often process shrimp paste into shrimp paste (Rusmiyati *et al*., 2022).

Shrimp paste is a fermented product of fish or shrimp that undergoes salting (Afrianto & Liviawaty, 2005). According to the National Standardization Agency (BSN) in SNI 2716.1: 2016 concerning Shrimp Paste, the term shrimp paste refers to processed fishery products using fresh, dried or mixed shrimp or other raw materials that undergo a fermentation process. The household-scale shrimp paste industry is widely spread in Indonesia, one of which is in the Cirebon area. Cirebon is still one of the largest centers for shrimp paste production in Indonesia because the Cirebon area is the main producer of shrimp paste which can be found in several rivers during high tide (Widyaningrum *et al*., 2022).

In general, the process of making shrimp paste on a home scale is no different from the general process of making shrimp paste which goes through several stages, namely shrimp sorting, shrimp washing, fermentation using salt, drying stage I, grinding stage I, drying stage II, grinding stage II, molding, drying stage III and packaging. The process of processing shrimp paste which is processed traditionally, the fermentation that occurs is in the form of natural or spontaneous fermentation, namely in the food ingredients there are microbes from the surrounding environment that can grow and contaminate the shrimp paste (Sajriawati, 2022).

Contamination of shrimp paste can be caused by a dirty environment, the use of unhealthy tools and less than hygienic sales conditions, so that the shrimp paste can be contaminated with bacteria. Yuhantaka (2018) proved that during the spontaneous fermentation process in traditional shrimp paste samples taken from Pakong Village, Madura, vibrio bacteria were found in 10 samples of the shrimp paste. Artika (2018) added that in shrimp paste samples from the Medan traditional central market, contamination of pathogenic bacteria was also found, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella disentryvical*, *Salmonella parathypi B/C*. The dominant bacteria found in the samples were Escherichia coli and Pseudomonas aeruginosa. The results of a study conducted by Kristanti (2015) also found eight isolates of the genus Aspergillus mold in shrimp paste. The same research also found that Fitria *et al*., (2023) found 11 positive samples of mold in shrimp paste in unbranded packaging.

Based on the above, this study needs to be conducted to analyze the microbiological conditions of shrimp paste as seen from biological parameters such as *Escherichia coli* bacteria, *Salmonella* sp., and *Vibrio parahaemolyticus* and mold along with the quality of shrimp paste as seen from the a_w, pH, and proximate parameters which are then compared to the quality standards of shrimp paste that have been set by the government based on SNI 2716:2016 concerning shrimp paste.

RESEARCH METHODS

Time and Place

Sampling was carried out at two household-scale shrimp paste processors who are traditional shrimp paste producers in Cirebon, namely processor A (Jatimerta Village) and processor B (Panjunan Village) in October 2023. Laboratory analysis was conducted from November 2023 to May 2024. Bacterial DNA extraction was conducted at the Laboratory of Fisheries and Marine Biotechnology, Faculty of Fisheries and Marine Sciences, Padjadjaran University. a_w (water activity) measurement and pH measurement were conducted at the Advanced Characterization Laboratory of BRIN Yogyakarta, and proximate analysis was conducted at the Nutrition Laboratory, Faculty of Animal Husbandry, Padjadjaran University.

Tools and materials

The tools used in the study were water bath, petri dish, clear plastic, autoclave, *microtube*, microtype, incubator, micropipette, beaker glass, fortex stirrer, magnetic stirrer, bunsen, freezer, scales, measuring pipette, laminar, Erlenmeyer flask, falcon bottle, a_w meter , pH meter, scott bottle, centrifuge, 3M petrifilm, cotton swab, DNA extraction kit, gel electrophoresis, plastic cup, desiccator, oven, thimble, fat-free cotton, Soxhlet micro tube, Kjehdahl flask, furnace, gloves and microwave.

The materials used in the study were raw material samples before fermentation, after fermentation and the final product of shrimp paste, Butterfield Phosphate Water (BPW), Simultaneous Enrichment Broth (SEB), alcohol, TE Buffer, Gram+ Buffer, GB Buffer, Ethanol absolute, W1 Buffer, Wash Buffer, Elution Buffer, Agarose Gel, Petroleum Benzene, Kjehdahl Salt, H_2SO_4, NaOH, Zn Granules, HCL, pp Indicator and Aquadest.

Field Sampling

a. Solid Sample

The samples consisted of shrimp paste raw materials before fermentation, raw materials after fermentation and the final shrimp paste product. Each sample was taken as much as 25 grams using *latex gloves*, then put into clear plastic. The samples consisted of 12 samples, namely: P1.R (Processor 1 Dry Rebon Chunks), P1.TB1 (Processor 1 Wet Shrimp Paste 1),

P1.TB2 (Processor 1 Wet Shrimp Paste 2), P1.TK1 (Processor 1 Dry Shrimp Paste 1), P1.TK2 (Processor 1 Dry Shrimp Paste 2), P1.TK3 (Processor 1 Dry Shrimp Paste 3), P2. U (Processor 2 Fresh Shrimp), P2. T1 (Processor 2 Pounded 1), P2. T2 (Processor 2 Pounded 2), P2. TB1 (Processor 2 Wet Shrimp Paste 1), P2. TB2 (Processor 2 Wet Shrimp Paste 2)., and P2. TB3 (Processor 2 Wet Shrimp Paste 3). Samples were taken in 3 repetitions.

b. Environmental Samples

The sample consists of 10 samples, namely P1.S1 (Processor of 1 Swab Tray), P1.S2 (Processor of 1 Swab Basket), P1.S3 (Processor of 1 Swab Drying Place), P1.S4 (Processor of 1 Swab Scale), P1.A1 (Processor of 1 Swab Washing Water), P2.S1 (Processor of 2 Swab Shrimp Trays), P2.S2 (Processor of 2 Swab Mortars), P2.S3 (Processor of 2 Swab Pounded Trays), P2.S4 (Processor of 2 Swab Pestle), and P2.A1 (Processor of 2 Swab Washing Water).

Observation Parameters

The parameters observed in this study were biological and chemical parameters. Microbiological parameters included analysis of pathogenic microbes *E. coli, Salmonella* sp., *Vibrio parahaemolyticus* and total mold. Chemical parameters included water activity (a_w), pH, and proximate (water content, ash content, fat content, protein content).

Data analysis

The data were analyzed using descriptive-quantitative analysis. The resulting data will be presented in the form of a table of the average number of *E. coli bacteria* and a table of proximate analysis results. based on SNI 2716:2016, table of multiplex PCR results, conventional on *Salmonella* sp bacteria, and *Vibrio parahaemolyticus*) referring to Triwibowo *et al.* (2020), table of average results of total mold analysis based on SNI 2332.7:2015, water activity analysis graph *(a_w)*, and pH analysis graph.

RESULTS

E. coli Test Results for Environmental Samples and Solid Samples

E. coli bacteria on solid environmental swab samples was carried out using the 3M™ petrifilm method. The dilution process used Butterfield's Phosphate Water (BPW) media with a dilution of up to 10^{-2} which was incubated for ± 48 hours at 35ºC. The results table is in Table 1 and Table 2.

Table 1. *E. coli* Test Results for Environmental Samples

Sample Code	Colony Count (CFU/g)
P1.S1 (A)	> 2.5 x 10^3
P1.S2 (A)	5 x 10^3
P1.S3 (A)	> 2.50 x 10^4
P1.S4 (A)	> 2.50 x 10^4
P1.A1 (A)	> 2.50 x 10^4
P2.S1 (B)	> 2.50 x 10^4
P2.S2 (B)	15 x 10^3
P2.S3 (B)	5 x 10^3
P2.S4 (B)	10.6 x 10^3
P2.A1 (B)	> 2.50 x 10^4

Table 2. Results of *E. coli Test* on Solid Samples

Sample Code	Number of Colonies
P1.R (A)	5×10^{-2}
P1.TB1 (A)	3×10^{-2}
P1.TB2 (A)	5.5×10^{-2}
P1.TK1 (A)	6×10^{-2}
P 1.TK2 (A)	1.34×10^{-4}
P1.TK3 (A)	2×10^{-3}
P2.U (B)	5×10^{-3}
P2.T1 (B)	1.50×10^{-4}
P2.T2 (B)	2.00×10^{-4}
P2.TB1 (B)	2.10×10^{-4}
P2.TB2 (B)	3.5×10^{-3}
P2.TB3 (B)	5×10^{-3}

Duplex PCR Results *Salmonella* sp. and *Vibrio parahaemolyticus*

PCR identification of *Salmonella* sp. and *Vibrio parahaemolyticus* bacteria was carried out on 10 environmental samples and 12 solid samples obtained through the DNA extraction stage, Duplex PCR with the target band InvA (*Salmonella* spp.), and ToxR (*V. parahaemolyticus*), electrophoresis and then visualization of the electrophoresis results. The primers used in the PCR process can be seen in Table 3 and the PCR test results can be seen in Figure 1 and Figure 2.

Table 3. Primer pairs for conventional duplex PCR analysis targeting the *InvA gene* (*Salmonella* spp), *ToxR gene* (*V. parahaemolyticus*) and *internal control* 16S rDNA

Target gene	Primary pair	Language sequence	PCR Product Size (bp)
InvA	*InvA* F	5-TAT CGC CAC GTT CGG GC A-3'	275
	InvA R	5'-TCG CAC CGT CAA AGG AAC C-3'	
ToxR	*ToxR* F	5'-GTC TTC TGA CGC AAT CGT TG-3'	368
	ToxR R	5'- ATA CGA GTG GTT GCT GTC ATG-3'	
16S	16S F	5'-CCT ACG GGA GGC AGC AGT-3'	475
	16S R	5'-CGT TTA CGG CGT GGA CTA C-3'	

Source: Triwibowo *et al.*, (2020)

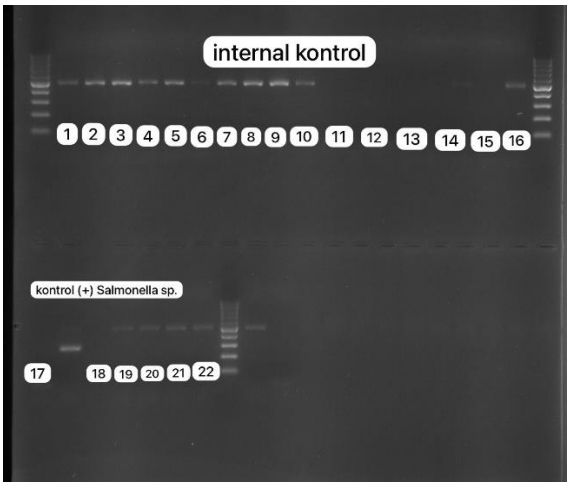

Figure 1. Results of *Salmonella* sp. Duplex PCR using control (+) *Salmonella* sp.

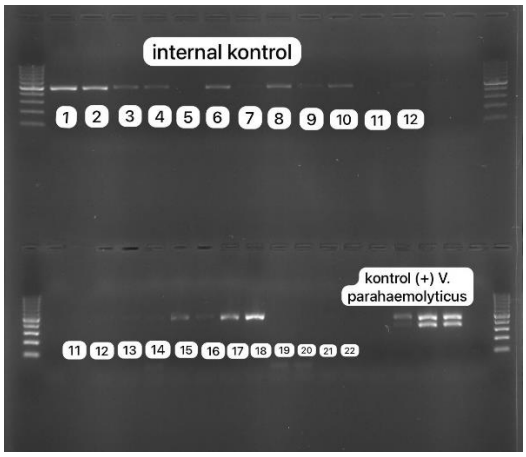

Figure 2. Results of *Vibrio parahaemolyticus Duplex PCR* using control (+) *Vibrio parahaemolyticus*

Information:
Hole 1 code P1.R (Terasi Chunks), Hole 2 P1.TB1 (Terasi Wet 1), Hole 3 P1.TB2 (Terasi Wet 2), Hole 4 P1.TK1 (Terasi Dry 1), Hole 5 P1.TK2 (Terasi Dry 2), Hole 6 P1.TK3 (Terasi Dry 3), Hole 7 P2.U (Rebon Shrimp), Hole 8 P2.T2 (Pound Shrimp 1), Hole 9 P2.T2 (Pound Shrimp 2), Hole 10 P2.TB1 (Terasi Wet 1), Hole 11, P2.TB2 (Terasi Wet 2), Hole 12 P2.TB3 (Terasi Wet 3), Hole 13 P1.S1 (Swab Tray), Hole 14 P1.S2 (Basket Swab), Hole 15 P1.S3 (Drying Swab), Hole 16 P1.S4 (Weighing Swab), Hole 17 P1.A1 (Washing water), Hole 18 P2.S1 (Shrimp Tray), Hole 19 P2.S2 (Mortar), Hole 20 P2.S3 (Pounded Tray), Hole 21 P2.S4 (Pestle), Hole 22 P2.A1 (Washing water).

Total Mold Test Results

Total mold analysis was conducted at 3 points, namely raw materials before fermentation, after fermentation and the final product of shrimp paste using the spread plate method whose procedure refers to SNI 2332.7: 2015 with a dilution of 10-2 and was carried out in duplicate at each dilution. The test results are in Table 3.

Table 3. Results of Total Mold Test of Solid Samples

Sample Code	Calculation results (CFU/gr)
P1.R (A)	3.7×10^{-3}
P1.TB1 (A)	4.6×10^{-3}
P1.TB2 (A)	3.1×10^{-3}
P1.TK1(A)	4.3×10^{-3}
P1.TK2 (A)	5.6×10^{-2}
P1.TK3 (A)	1.8×10^{-2}
P2.U (B)	4.1×10^{-2}
P2.T1 (B)	1.6×10^{-2}
P2.T2 (B)	9.9×10^{-2}
P2.TB1 (B)	1.13×10^{-3}
P2.TB2 (B)	7.1×10^{-2}
P2.TB3 (B)	1.36×10^{-3}

pH Test Results

Total mold analysis was conducted at 3 points, namely raw materials before fermentation, after fermentation and the final product of shrimp paste using the spread plate method whose procedure refers to SNI 2332.7: 2015 with a dilution of 10-2 and was carried out in duplicate at each dilution. The test results are in Table 3.

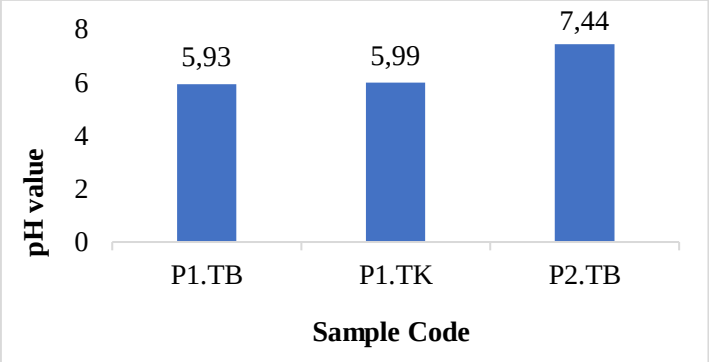

Figure 3. Graph of the results of measuring the pH of shrimp paste at Processor A and Processor B.

Test Results a $_w$

Water activity (a $_w$) testing was carried out using an a $_w$ meter (Aqualab 4TE, Singapore). pH measurements were carried out on 3 samples taken based on the type of shrimp paste, namely wet shrimp paste and dry shrimp paste. The measurement results can be seen in Figure 4.

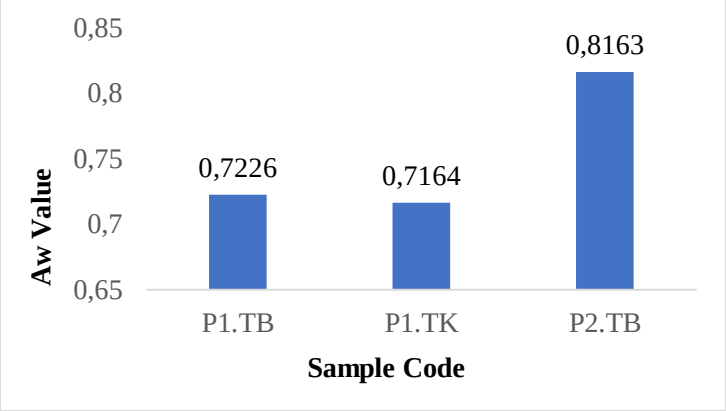

Figure 4. Graph of the Results of Measurement of the Value of a $_w$ on Shrimp Paste from Processor A and Processor B

Proximate Analysis Results

The proximate analysis conducted in this study were water content, fat content, ash content and protein content. This proximate analysis was only conducted on the final shrimp paste product, namely on sample P2.TK (processor a) and sample P6. TB (processor b). The results of the proximate analysis on the final shrimp paste product can be seen in Table 4.

Table 4. Results of Proximate Analysis of Terasi Final Products

No	Sample	Water (%)	Ash (%)	Protein (%)	Fat (%)
1	P1TK	28.65	20.38	47.16	4.53
2	P2TB	48.68	28.01	40.87	9.93

DISCUSSION

Based on the results of the presumptive *E. coli* test using petrifilm on 10 environmental swab samples and 12 solid samples, it was shown that in the shrimp paste processing unit A (Panjunan Village) and shrimp paste B (Jatimerta Village), *E. coli* bacteria were found growing on the equipment and environment where the shrimp paste was made, so that it could contaminate the shrimp paste products produced. The results of the *E. coli* bacteria test showed that the bacteria growing in the samples exceeded the threshold for *E. coli* microbial contamination that was safe for consumption because based on SNI 2716: 2016 concerning shrimp paste, the limit for *E. coli* microbial contamination that was still safe for consumption was <3 CFU/g. The large number of *E. coli* bacteria found in the samples was caused by the processors not paying enough attention to the cleanliness of the shrimp paste making equipment and the storage of the equipment. This is in accordance with the statement of Lee *et al*. (2014), the process of making fermented foods using raw materials and equipment that are not sterile can cause the growth of pathogenic bacteria.

The PCR identification results showed that in the shrimp paste of processor A and processor B there was no contamination of *Salmonella* sp. and *Vibrio parahaemolyticus* bacteria. This is in accordance with the SNI 2716:2016 standard that *Salmonella* sp. bacteria are not allowed in shrimp paste. The BPOM Agency (2019) also stated about the maximum limit of microbial contamination in food, that food (raw materials and processed products) must not contain *Salmonella* sp. *Vibrio parahaemolyticus* grows optimally in water environments in river estuaries and on the coast, so this bacteria is rarely found in processed fish products,

especially shrimp paste products, but is often found to infect fresh fish and kill farmed fish larvae (Jawetz *et al*., 2007).

The results of the total mold analysis showed that all 12 samples were contaminated by mold. The largest number of molds was found in the fermented sample of processor A with an average number of colonies of 3.85 x 10^3 CFU/g. The growth of mold on shrimp paste products can be caused by the fermentation process still ongoing during the storage process. Shrimp paste that is stored for too long in high humidity environmental conditions can cause mold to grow and reproduce more. Processor A in Jatimerta Village stores the final shrimp paste product in a closed sack container and then stores it for 1 year, while shrimp paste processor B in Panjunan Village stores the shrimp paste in a large bucket container and then closes it and stores it for 2 years. This causes mold to grow, so that the shrimp paste processed by processors A and B is not safe for direct consumption. Fitria *et al*. (2023), in their research found 11 positive samples of mold in shrimp paste in unbranded packaging sold at the Samarinda City Traditional Market.

The results of pH measurements showed that shrimp paste in processor A and processor B had a fluctuating pH based on the length of fermentation time. Based on the results of interviews with processor A, the finished wet and dry shrimp paste was stored in a closed container for 1 year, while in processor B the wet shrimp paste was stored for 2 years, so the sample in processor B had a higher pH due to the longer shelf life factor. The fermentation process that occurs when shrimp paste is processed in processor A is carried out 3 times, while in processor B the fermentation is carried out 2 times. This is in accordance with the statement of Isdaryanti *et al.* (2022), that the difference in pH conditions in shrimp paste is caused by differences in raw materials, salt content, and fermentation time. The fermentation process if allowed to continue will result in an increase in pH and the formation of ammonia, resulting in shrimp paste easily rotting (Henny *et al.*, 2022).

The results of the a_w value measurement showed that the best a_w value was in the sample of dry shrimp paste processor A, because it had a lower value than the other samples. The difference in a_w value in shrimp paste processor A and processor B was due to the difference in salt content added to the shrimp paste and the water content of the final product. Processor A added 350 g of salt in 1 kg of shrimp paste, while processor B added 150 g of salt in 1 kg of shrimp paste, so the a_w value of shrimp paste processor A was higher because the salt content added was less. This is in accordance with the research of Rahmayati *et al.* (2014), that shrimp paste with the highest aw was shrimp paste with a salt concentration of 2%, which was 0.78, while shrimp paste with a salt concentration of 8.5% and 15% had a_w values of 0.77 and 0.74. This shows that the use of salt can affect the a_w value of a food ingredient. According to Albarracin *et al*. (2011), salt is added to food ingredients to increase water adsorption so that the a_w value of the food ingredients decreases.

The results of the analysis of water content in shrimp paste showed that the water content in shrimp paste from processor A still met the SNI 2716: 2016 standard, which is a maximum of 35% for the type of dry solid block shrimp paste, while the water content in shrimp paste from processor B did not meet the SNI 2716: 2016 standard, which is a maximum of 45% for the type of paste shrimp paste. The results of the analysis of the ash content in shrimp paste from processor A and processor B did not meet the SNI 2716: 2016 standard, which is a maximum ash content limit of 1.5%. The results of the analysis showed that the protein content in shrimp paste from processor A and processor B had met the SNI 2716: 2016 standard which was set at a minimum of 15%. The results of the analysis of fat content in the final products of shrimp paste from processor A and processor B showed that shrimp paste from processor A had a fat content of 4.53%, while shrimp paste from processor B had a fat content of 9.93%. The difference in fat content in shrimp paste products from the two processors can be caused by the difference in the

length of the fermentation process. The length of fermentation time after the final product is carried out continuously will increase the lipase enzyme produced by lactic acid bacteria so that more fat is degraded into fatty acids (Islami *et al*., 2022).

CONCLUSION

Based on the results of research that has been conducted on environmental samples during the production process in two different processors, it shows that *E. coli* bacterial contamination in environmental samples is quite high, especially in the water used for the production process. The high *E. coli contamination* in the environment causes high *E. coli contamination* in terasi products. The total amount of mold contamination in the final terasi products from the two processors ranges from 1.80×10^{2} to 4.60×10^{3} CFU/g concluded that terasi is not safe for consumption. Terasi pH value highest is in sample P2. Wet Terasi 7.44%. The value of a_w shrimp paste The best result in sample P 1. Dry Terasi 0.7164. The results of chemical analysis on the final products from both processors generally met the SNI 2716:2016 standard except in the processor B sample where the water and ash content did not meet the standard.

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