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The Effects of Pineapple Fruit Extracts (*Ananas comosus*) on the Quality of Chemical and Microbiological-Rabbitfish (*Siganus spp.*) Sauce Products

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Abstract: The present work aims to study the effect of pineapple fruit extracts on the quality of Chemical and microbiological - rabbitfish sauce products. Utilizing pineapple extract can speed up the fermentation process because pineapple extract contains bromelain enzymes that will break down rabbitfish's protein, carbohydrate, and fat molecules into simpler molecules. The research method applied involved experimental laboratory procedures. This study used a completely randomized design. The variables observed were protein, water content, acidity (pH), total microbe, total lactic acid bacteria, *Escherichia coli*, *Staphylococcus*, and taste values. The pineapple extracts and fermentation length affected sauce product quality significantly. Pineapple extracts of 15% and 12-day fermentations produced the best sauce quality, consisting of 14.18% protein content, 73.55% moisture content, and a pH of 5.26. Besides, other parameters observed were total microbes 7.8 x 104 CFU/g, total lactic acid bacteria 1, 0 x 102 CFU/g, *E. coli*, and *Staphylococcus sp.* (negative), taste value was 3.46, but consumers did not like it so much. However, these results have met the Indonesian National Standards for the product and are safe for human consumption.

Keywords: Chemical; Fermentation; Microbiological; Pineapple fruit extracts; Rabbitfish

Introduction

Recently, fish sauce produced and sold in Indonesia brews anchovies and salt by adding various cooking spices in varying amounts and varieties. It can also use mackerel, squid, salmon, and rabbitfish. Rabbitfish is one of the economically important coral reef fish, containing nine essential and seven non-essential amino acids, which healthy-nutrition for human health (Wahyuningtyas et al., 2017). Aside, they describe eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), called Omega-3. It is one of the long-chain polyunsaturated fatty acids functional for human health. Rabbitfish can create long-chain polyunsaturated fatty acids (LC-PUFA) from PUFA precursors having a Carbon atom of 18 (Sun et al., 2020; You et al., 2017). It contains EPA and DHA 0.54% and 6.45%, respectively (Wahyuningtyas et al., 2017). The other studies also reported this type of fish contains high omega-3 fatty acids (EPA and DHA), which are adequate for maintaining heart health, brain, kidneys, and eyes and preventing osteoporosis, cancer, diabetes, and other dangerous diseases (Kosasih et al., 2020; Mohanty et al., 2016; Nabavi et al., 2015).

Rabbitfish catch in Lombok island (Indonesia) is abundant, but not good handling, so it quickly decomposes and damages, finally, a low price. Therefore, rabbitfish and various fishes need processing as soon as possible. More than 40% of about 50% of the total catch used classic methods in processing. The fish processing industry of Indonesia comprises mainly traditional fish products factories that are commonly operated by small to medium-scale processors to meet the domestic market. The main fish products of Indonesia are salted-dried fish, salted-boiled fish (pindang), fish paste, fermented fish (peda), fish sauce,

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smoked fish, etc. (Mulyani et al., 2007). Many fermented fish products in Indonesia provide different flavors that meet various consumer appetites unhappily available locally. In contrast to the nearest country, for example, Malaysia, the majority of the marine fish catch, about 63%, is eaten naturally (Huda, 2012). The remainders are iced at 1%, preserved at 11%, formed into the fish meal at 20%, and have 5% liability in different ways. The classification of preserved fish is 28 % comprised of dried products (salted, smoked), boiled (5%), fermented results (28%), and more outcomes of 39% such as a fish cookie and meatballs.

Fermentation is one of the traditional fish processing methods that can provide specific products. Enzymes have the most significant role in changing the texture and producing the flavors, while microorganisms aid has played a role in aroma and flavor (Varzakas, 2020; Wang et al., 2015). Individuals' consumption of fermented foods worldwide tends to increase until now (Varzakas, 2020). They play many roles, from preservation to food security, improved nutrition, and social well-being. Fermented foods provide many health benefits such as preventing chronic diseases, supporting immune and skin health, cholesterol control, as anti-oxidant, anti-microbial, anti-fungal, anti-inflammatory, anti-diabetic, and anti-atherosclerotic activity (Savary-Auzeloux et al., 2021; Varzakas, 2020).

Aryani (2016) reported the addition of the bromelain enzyme from pineapple juice before fermentation can shorten the process of making fish sauce. Marco et al. (2017) stated that food fermentation processes consisted of two categories: the primary metabolites and microorganisms involved: alcohol and carbon dioxide (yeast), acetic acid (Acetobacter), lactic acid (lactic acid bacteria (LAB) belonging to genera such as Leuconostoc, Lactobacillus, and Streptococcus), propionic acid, and ammonia and fatty acids (Bacillus, molds). A combination of low pH and organic acids (specially lactic acid) is the main preservation factor in fermented fish products. pH should be below 5–4.5 to inhibit pathogenic and spoilage bacteria (Owens et al., 1985). The bacterium that generally lives in the intestines of people and animals that can cause intestinal infection and lead to severe, potentially life-threatening complications is Escherichia coli (E. coli). E. coli O157 is a strain of bacteria that has become an important food and waterborne pathogen that causes diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome in humans via transmission occurs via the fecal-oral route after consumption of contaminated, undercooked liquids and foods (Ameer et al., 2018).

There are reasons for using bromelain from pineapple fruit in producing fish' sauce, mainly inexpensive-price, easy to get, very stable at high temperatures between 40 and 60 ^{o}C, good for health, and accelerates protein hydrolysis (Coêlho et al., 2014; de Lencastre Novaes et al., 2016; Dutta et al., 2013; Lee et al., 2016; Pavan et al., 2012; Rathnavelu et al., 2016). Ariansyach (2017) reported the utilization of fisheries production in Indonesia is eighty-five percent goes to the local market for sale, while the rest is exported mainly to Asian markets. Fishermen distribute fishery products from small-scale to local markets severally two for direct consumption or via processing, for example, fumigation and salting. Fishery products of medium to large-scale fisheries are generally for canning products, used as raw materials for processing into export products, boiled fish products, fish bait, and fortification products market for sale.

One of the efforts to get advanced and awaken its nutritional fill in a long time is manufacturing a sauce through a short fermentation process using pineapple fruit extracts that contain the enzyme bromelain. Besides, rabbitfish sauce has a more promising business opportunity than sauce widely circulating in the market because of the raw materials available, the high protein content, its delicious taste, and cheap, simple technology (Balakin et al., 2021). Another obstacle is difficulty getting soybeans as an imported commodity with a very high price is an obstacle alone. Based on the reasons previously described by researchers interested in researching the chemistry properties and microbial value of rabbitfish fish sauce from accelerated fermentation with the addition of the bromelain enzyme.

Method

Materials

The research used fresh rabbitfish (Figure 1), adult pineapple piece (Figure 2), sodium chloride, reagent, benzoic acid, and spices including Cinnamomum, ginger, bay leaf, lemongrass, galanga, saffron, cilantro, sodium acetate, K2CrO4, and AgNO3. Rabbitfish sauce is produced of rabbitfish meat extract fermented product using pineapple fruit extracts.

Figure 1. Rabbitfish

Figure 2. Pineapple

Experimental Design

The study was an experimental laboratory procedure carried out in the Sciences Education and Laboratories of Analytical Chemistry and Microbiology of Mataram University, Indonesia. The experimental design used is a completely randomized experimental design (CRD). There are two treatment factors: first is pineapple fruit extract with concentrations of 0%, 9%, 12%, and 15% (w/w). Secondly, fermentation times of 0, 4, 8, and 12 days respectively, with three times replication. Parameters observed were protein content, moisture content, pH, total microbial, microbial type, and organoleptic scores including taste, smell, color, and overall pleasure. Two stages to manufacturing rabbitfish sauce: (1) pineapple fruit extraction to find out crude bromelain enzymes; (2) hydrolyzing the meat of rabbitfish to produce a sauce. To extract mature pineapple fruit with a method modified (Ali, 2015; Dubey et al., 2012).

The extraction of mature pineapple fruit through the following stages: (1) to weigh and clean six pineapples well using water and squash being small pieces (pineapple extract); (2) homogenizing and filtering pineapple extract) using fine cloth called filtrates; (3) centrifuging the filtrates for 10 minutes with a velocity of 10,000 rpm called supernatant; (4) storing it in benzoic acid at 1.4 g concentration as an enzyme of bromelain. Next, producing rabbitfish sauce used methods of Mahrus et al. (2023) as follows: first, weeding and washing rabbitfish meat using water; (2) grounding it smoothly; (3) mixing it with 1% spices; (4) beating it in 15 minutes at temperature 90-95^{o}C; (5) mixture it by extracts of pineapple fruit at level 6, 8, 10, and 12% (w/w) respectively; (6) setting down every specimen or sample in a well-behaved shut bottle; (7) fermenting it in four, eight, and day-12; (8) heating it for 12 minutes at temperature ranged 90-95^{o}C; (9) sieving and going in for a vacuum flask, and the last step is rabbitfish sauce product.

The observed chemical parameters included pH value, protein content, and moisture determined using the AOAC method (Andersen et al., 2018). Organoleptic parameters include flavor, stain, odor, and overall pleasure using 20 semi-trained panelists. It used the hedonistic level of 9 points for calculating the level of food satisfactoriness, one is the least acceptable, and nine is the highest. Total microbes used the inoculation method (Guo et al., 2017), and the presence of microbes and their types were identified with the method used by Sandle (2015).

Statistical Analysis

Analyzing data used Analysis of Variance (ANOVA) by SPSS software (Ver. 21). Test of differences treatment means used the Least Significant Difference Test (LSD) test to measure a more conservative difference using multiple comparisons. Further analysis would compare the sauce product with the Indonesian National Standard of fish sauce for parameters. They consist of protein content, pH, and microbiology.

Result and Discussion

Result

Chemical characteristics

Fish sauce's chemical characteristics studied are pH value, protein content (%), and moisture content (%) in Table 1. As shown, the pH value, protein content, and moisture content are affected by the additions of pineapple extract and the fermenting period. They involve pH values ranging from 5.26 – 5.74, protein content 14.16 -17.12%, and moisture content 73.62-74.72%, respectively. It means that the additions of pineapple extract and the fermenting period have affected the sauce product quality. The same phenomenon also occurs in the microbe content and taste value.

Table 1. pH Value, Protein Content (%), and Moisture Content (%)

Sample	pH	Protein content	Moisture content
E1L1	5.74± 0.92	17.12± 0.12	74.71±2.27
E1L2	5.72± 0.90	16.83± 0.09	74.68±2.25
E1L3	5.68± 0.87	16.36± 0.08	74.45±2.26
E2L1	5.62± 0.85	15.82± 0.07	74.23±2.23
E2L2	5.56± 0.82	15.63± 0.07	74.10±2.22
E2L3	5.45± 0.79	15.12± 0.06	73.85±2.20
E3L1	5.41± 0.66	14.65± 0.05	73.71±2.17
E3L2	5.36± 0.62	14.32± 0.05	73.62±2.13
E3L3	5.26± 0.59	14.16± 0.04	73.55±2.10

Notes: E= Formulation of fish and pineapple extract (E1=9%, E2=12%, E3=15%); L= Fermentation times (L1=4 days, L2=8 days, L3=12 days).

Microbiology characteristics

The results of the chemical characteristic of rabbitfish sauce products are shown in Table 2. They consist of total microbes ranging from 1,0 x 102 – 4.4 x 103 CFU/g, total LAB 6.2 x 104 - 8.2 x 104 CFU/g, *E. coli*, and *Staphylococcus sp* (negative).

Table 2. Total Microbes (CFU/g), the Total for *Lactobacillus* (CFU/g), *E. coli*, *Staphylococcus sp*.

Sample	Total microbes	Total LAB	*Coliform/E. coli*	*Staphylococcus sp.*
E1L1	$4.4 \times 10^3 \pm 0.78$	$6.2 \times 10^4 \pm 0.82$	Negatives	Negatives
E1L2	$4.0 \times 10^3 \pm 0.75$	$6.5 \times 10^4 \pm 0.85$	Negatives	Negatives
E1L3	$3.7 \times 10^3 \pm 0.71$	$6.7 \times 10^4 \pm 0.87$	Negatives	Negatives
E2L1	$3.7 \times 10^3 \pm 0.68$	$7.0 \times 10^4 \pm 0.90$	Negatives	Negatives
E2L2	$3.4 \times 10^3 \pm 0.58$	$7.5 \times 10^4 \pm 0.92$	Negatives	Negatives
E2L3	$3.1 \times 10^3 \pm 0.52$	$7.8 \times 10^4 \pm 0.95$	Negatives	Negatives
E3L1	$2.8 \times 10^2 \pm 0.48$	$7.8 \times 10^4 \pm 0.98$	Negatives	Negatives
E3L2	$2.2 \times 10^2 \pm 0.27$	$8.0 \times 10^4 \pm 1.27$	Negatives	Negatives
E3L3	$1.0 \times 10^2 \pm 0.25$	$8.2 \times 10^4 \pm 1.62$	Negatives	Negatives

Notes: E= Formulation of fish and pineapple extract (E1=9%, E2=12%, E3=15%); L= Fermentation times (L1=4 days, L2=8 days, L3=12 days).

Microbial contamination is very important to analyze in food products because it has been the primary cause of the decay of food, food poisoning, and infection. Microbial poisoning of foodstuff generally took place because of fermentation, storage, transfer, and handling before utilization. Parameters of microbiological characteristics observed are total microbes, total lactic acid bacteria, Coliform/*Escherichia coli*, and *Staphylococcus sp*.

Organoleptic values

The sensory value of rabbitfish sauce is one main factor investigated. It affects the odorants and their correlation to product quality. The parameters observed are taste, color, aroma, and acceptance (Table 3). Sensory value data obtained are: taste value ranged from 2.24 - 3.63, color 3.09 – 4.42, aroma 2.15 – 2.96, and acceptance 2.32 -2.56, respectively.

Table 3. The Sensory Value of Rabbitfish Sauce Observed are Taste, Color, Aroma, and Acceptance

Sample	Taste	Color	Aroma	Acceptance
E1L1	3.63 ± 0.75	4.42 ± 0.83	2.52 ± 0.53	2.47 ± 0.58
E1L2	2.32± 0.71	3.35± 0.72	2.94± 0.58	2.56± 0.61
E1L3	2.98± 0.65	3.43± 0.75	2.96± 0.57	2.48± 0.58
E2L1	3.57± 0.73	4.16± 0.81	2.81± 0.49	2.44± 0.55
E2L2	2.24± 0.61	3.09± 0.68	2.85± 0.52	2.40± 0.52
E2L3	2.26± 0.61	3.85± 0.79	2.91± 0.55	2.38± 0.49
E3L1	2.28± 0.62	3.43± 0.75	2.15±0.37	2.35± 0.43
E3L2	3.36± 0.75	4.37± 0.80	2.78± 0.43	2.34± 0.41
E3L3	3.46± 0.72	3.85± 0.79	2.87± 0.53	2.32± 0.41

Notes: E= Formulation of fish and pineapple extract (E1=9%, E2=12%, E3=15%); L= Fermentation times (L1=4 days, L2=8 days, L3=12 days).

Statistical analysis

Data presented as mean ± standard deviation of three measurements. Analyzing data used Analysis of Variance (ANOVA) by SPSS software (Ver. 21) followed by Tukey's multiple comparison tests to measure a more conservative difference using composite comparisons. Further analysis would compare the sauce product with the Indonesian National Standard of fish sauce for parameters. They involve protein content, pH, microbiology, taste value, color, aroma, and acceptance.

According to a test of multivariate analysis of variance, the additions of pineapple extract and ferment period have affected sauce product quality significantly ($p \leq 0.05$). Furthermore, the results of the LSD examination of Table 4, nearly samples have affected the distinction means between whole protein content, moisture content, pH, total microbes, and total LAB, except for *Escherichia coli* and *Staphylococcus sp*. (negative). The same is true of organoleptic values in Table 5 showed pineapple extract and fermentation times had affected to signify distinction in the middle of taste value, color, aroma, and acceptance significantly ($p \leq 0.05$).

Table 4. LSD Test of Multiple Comparisons for Chemical and Microbiological Variables

Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
pH	E1	E2	.17000*	.05617	.023	.0325	.3075
		E3	.37000*	.05617	.001	.2325	.5075
	E2	E1	-.17000*	.05617	.023	-.3075	-.0325
		E3	.20000*	.05617	.012	.0625	.3375
	E3	E1	-.37000*	.05617	.001	-.5075	-.2325
		E2	-.20000*	.05617	.012	-.3375	-.0625
Protein_content	E1	E2	1.24667*	.27510	.004	.5735	1.9198
		E3	2.39333*	.27510	.000	1.7202	3.0665
	E2	E1	-1.24667*	.27510	.004	-1.9198	-.5735
		E3	1.14667*	.27510	.006	.4735	1.8198
	E3	E1	-2.39333*	.27510	.000	-3.0665	-1.7202

Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Moisture_content	E1	E2	-1.14667*	.27510	.006	-1.8198	-.4735
		E2	.55333*	.11923	.004	.2616	.8451
		E3	.98667*	.11923	.000	.6949	1.2784
	E2	E1	-.55333*	.11923	.004	-.8451	-.2616
		E3	.43333*	.11923	.011	.1416	.7251
	E3	E1	-.98667*	.11923	.000	-1.2784	-.6949
		E2	-.43333*	.11923	.011	-.7251	-.1416
Total_microbes	E1	E2	633.333	483.812	.238	-550.51	1817.18
		E3	2033.333*	483.812	.006	849.49	3217.18
	E2	E1	-633.333	483.812	.238	-1817.18	550.51
		E3	1400.000*	483.812	.028	216.15	2583.85
	E3	E1	-2033.333*	483.812	.006	-3217.18	-849.49
		E2	-1400.000*	483.812	.028	-2583.85	-216.15
Total_LAB	E1	E2	-966.667*	243.432	.007	-1562.32	-371.01
		E3	-1533.333*	243.432	.001	-2128.99	-937.68
	E2	E1	966.667*	243.432	.007	371.01	1562.32
		E3	-566.667	243.432	.059	-1162.32	28.99
	E3	E1	1533.333*	243.432	.001	937.68	2128.99
		E2	566.667	243.432	.059	-28.99	1162.32

*. The mean difference is significant at the 0.05 level.

Table 5. LSD Test of Multiple Comparisons for Organoleptic Values

Dependent Variable	(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Taste	E1	E2	.28667	.56531	.630	-1.0966	1.6699
		E3	-.05667	.56531	.923	-1.4399	1.3266
	E2	E1	-.28667	.56531	.630	-1.6699	1.0966
		E3	-.34333	.56531	.566	-1.7266	1.0399
	E3	E1	.05667	.56531	.923	-1.3266	1.4399
		E2	.34333	.56531	.566	-1.0399	1.7266
Color	E1	E2	.03333	.44223	.942	-1.0488	1.1154
		E3	-.15000	.44223	.746	-1.2321	.9321
	E2	E1	-.03333	.44223	.942	-1.1154	1.0488
		E3	-.18333	.44223	.693	-1.2654	.8988
	E3	E1	.15000	.44223	.746	-.9321	1.2321
		E2	.18333	.44223	.693	-.8988	1.2654
Aroma	E1	E2	-.05000	.22019	.828	-.5888	.4888
		E3	.20667	.22019	.384	-.3321	.7454
	E2	E1	.05000	.22019	.828	-.4888	.5888
		E3	.25667	.22019	.288	-.2821	.7954
	E3	E1	-.20667	.22019	.384	-.7454	.3321
		E2	-.25667	.22019	.288	-.7954	.2821
Acceptence	E1	E2	.09667*	.02828	.014	.0275	.1659
		E3	.16667*	.02828	.001	.0975	.2359
	E2	E1	-.09667*	.02828	.014	-.1659	-.0275
		E3	.07000*	.02828	.048	.0008	.1392
	E3	E1	-.16667*	.02828	.001	-.2359	-.0975
		E2	-.07000*	.02828	.048	-.1392	-.0008

*. The mean difference is significant at the 0.05 level.

Discussion

The results in Table 1 look that pineapple extract and the fermenting period have a principal role in producing rabbitfish sauce. It is like studying before that fermentation long has a dominant role in an enzyme for metabolizing substrate in achieving a constant reaction (Nout, 2014; Raveendran et al., 2018; Robinson, 2015; Sharma et al., 2020). The role of the bromelain enzyme has very important to run hydrolysis of fish substrate-like protein, contributing the most to flavor formation and the changes in texture in fermented fish products (Arshad et al., 2014). Hou et al. (2022) stated that the production process speeds and finally decreases protein content. Next, the increasing bromelain enzyme can melt

and break bonds-peptide in manufacturing sauce. The other researches previously similar studies reported by the researcher as follows: Koffi-Nevry et al. (2011), levels for moisture and protein on the fish fermentation namely 71.25% and 26.81%, respectively. It also reported that moisture content was 77.3%, and 18.2% protein on eggs of Thunnus albacares in the most valuable fish food products in the most valuable fish food products. This research has been appropriate when viewed from the Indonesian National Standard for a fish food product. pH ranged from five to six and the protein content was at least 5%, respectively. It reported a pH value of 5.26 and protein levels of 14.16%. According to similar research reported by Mohamed et al. (2012), rabbitfish sauce produces a content of 14.16% protein and an acidity of 5.26. It means that all microbes causing disease will not contaminate the sauce.

The total number of microbes in Table 2, fish sauce with the addition of pineapple extracts and fermentation time, tends to decrease. This study was almost equivalent to previous studies (Sim et al., 2015). The decreasing pH value can increase the number of lactic acid bacteria in acidic conditions. Besides, the length of fermentation had a significant effect on the total microbes that grew, because microorganisms also contribute to the fermentation process and serve in the smell and taste shaping (Beddows, 1998). Preliminary other research found that the products of optimum fish sauce with the number of microbes at least on the product with 7% NaCl, one day of hydrolysis, and 6% pineapple juice containing bromelain enzymes (Prasetyo et al., 2012). Nofiani et al. (2010) reported a higher total count of mesophilic aerobic bacteria than halotolerant bacteria and fungi on fermented ale-ale (traditional' fermented food). They recommended that should cook it before being served.

An analysis of microbial contamination in rabbitfish sauce products included *Staphylococcus sp.* and *E. coli* negative. It means the microbial of *E. coli* and *Staphylococcus sp*. did not contaminate the rabbitfish sauce product by the Indonesian National Standard for a fish sauce like microbial contamination is <3. Producing sauce is successful because of the hygienic handling of making soy sauce. For example, the study used washing water and clean equipment. Unhygienic handlings such as using dirty washing water and dirty equipment will cause bacterial contamination (Adanech et al., 2018). Both types of bacteria are pathogenic bacteria that cause various infections in humans. Staphylococcus causes diseases ranging from minor skin infections to food poisoning, so it is necessary to know its presence in a food product (Goja et al., 2013). *E. coli* can cause various diseases ranging from diarrhea, and vomiting to death (Ameer et al., 2018; Leboffe et al., 2021).

Data in Table 3 showed the addition of pineapple fruit extrication and decomposition length makes the sauce darker. Flavor, smell, and oral excitement usually would generate disclosure of individual perceptions later consume byproduct. Consequently, digit choices scarcely are adequate to check out of it. Some factors that influenced numerical choice are panelists, materials, methods, and fermentation length (Gutiérrez-Salomón et al., 2014; Mohamed et al., 2012; Rodrigues et al., 2017).

Other research reported that colors and aroma properties affect consumers' perceptions (Rodrigues et al., 2017; Samborska et al., 2019). It means that the customer dislikes rabbitfish sauce having fermentation longer. It also will produce more acidic sauce products. Therefore, it is thought this caused the panelist don't like it so much. Besides that, appearance and physical quality influence customer appreciation. If we compare this study to the quality of Thai and Malaysian fish sauce (called Budu) reported by Mohamed et al. (2012), this sauce was of overall quality lower. A food product can evoke a consumer's mood, so it will impact the explanation of options for food products available (Delime et al., 2020). There is a good suggestion for collecting emotional responses in the future. The goal is to make food products preferred by consumers, unlike these results didn't like it so much by consumers.

The interaction between the levels of enzyme and long ferment influenced rabbitfish sauce quality significantly (Table 4 and Table 5). It means that the additions of pineapple extract and the fermenting period have affected sauce product quality significantly ($p \leq 0.05$). This way showed reciprocal action give effects on the grade of the rabbitfish dressing results. Pineapple extracts of 15% produced the best quality rabbitfish sauce during fermentation times of 12 days or more word that the excellent value for rabbitfish sauce outcome occurred on extractions levels of pineapple fruit 15% with length ferment 12-day (Sample E3L3). They consisted of 14.16% protein content, 73.55% moisture content, a pH of 5.26, total microbes 1, 0 x 102 CFU/g, total lactic acid bacteria (LAB) 8.2 x 104 CFU/g, E. coli, and Staphylococcus sp. (negative), taste value was 3.46, color 3.85, aroma 2.87, and acceptance 2.32, respectively. The results mostly have met the Indonesian National Standards for the fish sauce product and are safe for human consumption.

Aryani (2016) reported the best research results obtained in the additional volume of 40 % pineapple juice with the percentage of protein obtained at 8,9301 % and a pH value of 5. In contrast to the newest study results reported by Faidah et al. (2021), the best physicochemical characteristics of betook fish sauce with the addition of pineapple waste extract of as much as 14% obtained protein content of 14.00%, as well as public acceptance. Some parameters such as water

levels, peptides, and lipids are the key to manufacturing high grades for rabbitfish sauce results (Delime et al., 2020; Lynch et al., 2019; Mahrus et al., 2023; Sandle, 2015).

In contrast to the research results reported by Isnawati et al. (2014), the best treatment was the addition of 20% volume of pineapple juice produced fish sauce with the criteria: brown yellow in the color of fish sauce; specific fish sauce in odor; delicious and salty in flavor; and protein content 8.63%, salt content 8.04%, pH 5.59, crude fiber content 6.05% and volume of liquid fish sauce 12.29%. It means that there not be an exact concentration of pineapple juice extract that is certain to produce the best quality fish sauce. It depends on the technique used, the fermentation, the quality of the pineapple fruit, and so on. These results emphasized on principal roles of enzyme immobilization and a proper nutritional environment in producing the final best products. For example, food products are free from microbe-pathogens and have a sensory-pleasure standard for consumers.

Varzakas (2020) stated that microorganisms are involved in food fermentation activities to have a high diversity. One of these microorganisms involved in fermented foods is lactic acid bacteria, which has a strong antibacterial effect due to producing bacteriocins (Agriopoulou et al., 2020). Some traditional African fermented products have well-known uses in human health to help safeguard chronic diseases from general gut health, support immunity, and protect skin health, cholesterol control, and lactose intolerance (Varzakas, 2020).

Besides that, Chapman et al. (2018) stated that the multi-scale implementation of enzyme immobilization is the key to increased product yield at maximum market profitability and minimum logistical burden on the environment and user. In this relationship, Al-Sa'ady et al. (2016) also reported that Bromelain extracted from pineapple fruit showed a maximum enzyme activity at pH 7 and 30 min of incubation with casein as substrate. Next, Demain et al. (2017) stated that other important parts of industrial production are a proper nutritional environment for the organism to grow and produce its products and the anti-avoidance of effects of a factors number.

Fermented foods can also have enhanced nutritional and functional properties due to the transformation of substrates and the formation of bioactive or bioavailable end-products. Many fermented foods also contain living microorganisms similar to probiotics genetically thought it has a crucial role in the fermentation process. According to limited clinical studies in fermented foods, at least their evidence is that this food provides health benefits beyond the starting food materials. For these reasons, Marco et al. (2017) stated fermented foods and beverages had value-added initially valued because of their improved shelf life, safety, and organoleptic properties.

Conclusion

In conclusion, adding pineapple fruit extracts significantly affects the quality of chemical and microbiological rabbitfish sauce. The pineapple extracts of 15% and 12-day fermentation produced the best rabbitfish sauce quality in chemical and microbiological. They consisted of 14.18% protein content, 73.55% moisture content, pH of 5.26, total microbes 7.8 x 104 CFU/g, total lactic acid bacteria 1, 0 x 102 CFU/g, *E. coli*, and *Staphylococcus sp* (negative), taste value was 3.46, but consumers did not like it so much. However, these results have met the Indonesian National Standards for the product and are safe for human consumption.

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