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Quality Improvement of Semi-Wet *Terasi* by Optimizing the Starter Culture Ratio of Controlled Fermentation

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ABSTRACT

***Terasi* is a traditional fermented shrimp paste used in Indonesian dish as condiments. Due to its affordability, the paste is widely consumed among the general population, and thus has a great impact in Indonesia. Currently, small-scale or home industry is common for *terasi* production, and natural fermentation process is the traditional method. Fermentation process is considered complete when desired aromatic odors are obtained. However, this makes the fermentation process subjective, because the decision is solely dependent on the producer. Additionally, natural fermentation poses a higher risk for contamination of microbial pathogens. As a result, the quality of the final product varies greatly from region to region. Therefore, it is necessary to improve the quality of *terasi* by means of controlled fermentation. Hence, the objective of the research is to optimize the controlled fermentation condition of *terasi* by determining the most optimal ratio of mixed starter culture. Optimal fermentation conditions were determined by analyzing the effect of the various starter inoculum on the inner microbial community, and results indicated that mixed culture of *Staphylococcus saprophyticus*, *Bacillus subtilis*, and *Lactobacillus murinus* with ratio of 2:1:2 was the most effective for suppressing the growth of unwanted microorganisms. The difference in the microbial composition also resulted to a change in the metabolite profile of *terasi*.**

1. Introduction

Fermented shrimp paste in Indonesia is specifically known as *terasi* and is commonly produced from planktonic shrimp *Schizopodes* spp. or *Mydes* spp. *Terasi* is served as a condiment, and the functional property of *terasi* is to provide salt and umami taste for the dish (Hajeb and Jinap 2012). The protein content of the shrimp paste can range from 25-66%, which is significantly higher than other fermented fishery products, and the salt content is between 6 to 38% (Tambaria 2016). This research focuses on *terasi* because it is an important condiment consumed by a wide range of population, and thus has a great impact in Indonesia.

Terasi is usually produced on small-scale, or in and home industry is common. Typically, natural fermentation is used, and thus the process can last anywhere from few weeks to several months (Aryanta 2000; Waryono 2001; Hajeb and Jipan 2012). The fermentation period varies based on the producer's decision, and the end of fermentation is determined based on the fermentation is considered "done" complete when desired aromatic odors are obtained. However, this makes the fermentation process subjective, because the decision is solely dependent on the producer. Because the fermentation process is not uniformed, the quality, such as nutrition content and aroma, of the final product varies greatly from region to region (Tambaria 2016). Additionally, natural fermentation poses higher risk for microbial pathogens (Capozzi *et al.* 2017). As a result, the quality of the final product from natural fermentation varies greatly from region to region because the fermentation process is not uniformed, the quality, such as nutrition content and aroma, of the final product varies greatly from region to region (Tambaria 2016). Therefore, it is necessary to improve the quality of *terasi* by using controlled fermentation. Quality improvement can yield safer products, and can also help expand the *terasi* industry by changing the production process from small-scale to large-scale.

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Starter culture for controlled fermentation of *terasi* has already been isolated in previous study. Starter culture includes a mixture of *Staphyloccocus saprophyticus, Bacillus subtilis*, and *Lactobacillus murinus*, and these were isolated from the natural fermentation of Cirebon *terasi* (Astuti *et al.* 2018). The ratio of these three dominant isolates has been optimized to shorten the fermentation period of dry *terasi*. In the market, the semi-wet form of the *terasi* is more commonly sold, but the ratio of the three dominant isolates has not been optimized. Optimization of the ratio is necessary for the semi-wet form because it contains more moisture content, which can lead to more microbial growth (Roos 2003). Therefore, the objective of the research is to improve the quality of semi-wet *terasi* by determining the most optimal ratio of fermentation starter culture with *Staphylococcus saprophyticus, Bacillus subtilis*, and *Lactobacillus murinus*. Most optimal conditions will be determined by analyzing the effect of the various inoculum ratios on the inner microbial community and the metabolome profile during the fermentation process.

2. Materials and Methods

2.1. Preparation of Starter Culture Inoculum for Fermentation

Glycerol stock of *S. saprophyticus, B. subtilis*, and *L. murinus* isolated directly from *terasi* in previous study was used. *S. saprophyticus* and *B. subtilis* were isolated on nutrient agar (NA) plate and cultivated in nutrient broth (NB) medium. *L. murinus* was isolated on pantothenic acid (PTT) plate and cultivated in PTT medium. For pre-pre-culturing, bacteria colony was suspended in 50 ml of liquid medium and cultured at room temperature for 24 hours at 125 rpm. For pre-culturing, bacteria were suspended in 50 ml of liquid medium with 12% salt concentration and cultured at room temperature for 24 hours at 125 rpm. For main culture, bacteria were suspended in 150 ml of liquid medium with 12% salt concentration and cultured at room temperature at 125 rpm until exponential phase.

2.2. Fermentation of *Terasi*

The semi-wet *terasi* was prepared for fermentation based on the production process in Cangkol, Cirebon, West Java. The shrimp was first grounded into fine pieces and dried. Then, 12% salt (w/w), 10% palm sugar (w/w), and 10% water (v/w) was added. To initiate fermentation, 10% (v/w) of inoculum was added to the shrimp mixtures with different ratio of starter culture, as indicated in Table 1, under an aseptic condition. Natural fermentation indicates shrimp mixture without inoculum culture. Then, the shrimp mixture was fermented in a plastic jar with aluminum-covered lid at room temperature for a period of 28-days. Three biological replicates were conducted for each fermentation conditions.

2.3. Conducting Time-Course Sampling

To monitor the fermentation process, 50 g of the sample is taken from the plastic jar under aseptic condition for each sampling point. During the 28-day fermentation period, a total of five sampling was carried out on day 0, 7, 14, 21, and 28. Samples for microbial community dynamics analysis was stored at 4°C, while samples used for metabolomics analysis was stored in -20°C to prevent changes in metabolites.

2.4. Microbial Dynamics Analysis using Three Different Agars

1 g of sampled *terasi* was homogenized in 9 ml of 0.1% peptone water to create a 10-fold dilution. Then, the sample was diluted further using 0.1 ml of the previous dilution fold with 0.9 ml of 0.1% peptone water. For each sample, 100 µl of the appropriate dilution of the *terasi* solution was plated on the NA plate, PTT plate, and eosin-methylene blue (EMB) plate. NA plate and EMB plate were incubated at 37°C for 24 hours, while PTT was incubated at 37°C for 48 hours. Then, the microbial community dynamics analysis was carried out by counting the bacteria plate, and the bacteria are microscopically identified.

2.5. Non-Targeted Metabolomics Analysis using GC/MS

Sample *terasi* were made into powder-form by lyophilizing and crushing, using Multi-bead shocker® (Yasui Kikai, Japan). Then, 1 ml of 80% methanol with ribitol (used as internal standard;

Table 1. Inoculum ratio of *S. saprophyticus, B. subtilis*, and *L. murinus*

	S. saprophyticus	*B. subtilis*	*L. murinus*
Natural	-	-	-
Terasi A	1	1	1
Terasi B	1	2	2
Terasi C	2	1	2

100 µl/ml) was added to 10 mg of powedered *terasi* sample. The samples were incubated in the shaker incubator at 1,200 rpm at 37°C for 20 minutes. After incubation, the samples were centrifuged at 10,000 rpm at 4°C for 10 minutes to pellet down the proteins and other big particles in the solution. Then, 200 µl of supernatant was concentrated using centrifugal concentrator at 150 rpm at 25°C for 90 minutes to remove the organic solvents, and lyophilized for 12 hours to remove the water content. Next, the samples were derivatized. For oxyimation reaction, 100 µl of 20 mg/ml of methoxyamine hydrochloride in pyridine neat was added to the sample and incubated in shaker incubator at 1,200 rpm at 30°C for 90 minutes. For silyation reaction, 50 µl of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was added and incubated in shaker incubator at 1,200 rpm at 37°C for 30 minutes. Gas chromatography/mass spectrometry (GC/MS) analysis was carried out using GCMS-QP2010 (Shimadzu, Japan) with InterCap® 5MS/NP (30 mm x 0.25 mm I.D., df.=0.25 µm; GL Sciences, Japan) for column. A total of 1 µl was injected.

2.6. Proximate Analysis

Proximate analysis was conducted by PT. Sarawanti Indo Genetech by referring to SNI 01-2891-1992. The analysis quantified the content of carbohydrate, protein, fat, water, and ash. For the purpose of this research, only one biological replicate was used.

3. Results

3.1. Changes in Microbial Community During *Terasi* Fermentation

Most optimum fermentation condition was determined based on the ratio of starter culture that suppressed the growth of indigenous microbes the most. NA plate was used to monitor nonfastidious microorganisms, PTT plate was used to monitor *Lactobacillus* species, and EMB plate was used to check for the presence of coliform bacteria.

3.1.1. Change in Microbial Community of Non-Fastidious Bacteria on NA Plate

For naturally fermented *terasi*, the raw material was not inoculated with any starter culture. The total microbial count decreased from 5.41 to 4.83 log CFU ml^{-1}, and the indigenous bacteria count decreased from 4.63 to 4.54 log CFU ml^{-1} (Table 2). The relative abundance of *S. saprophyticus* decreased until day 21 and increase slightly at the end, while the relative abundance of *B. subtilis* decreased until day 14, and increased again from day 14 to day 28 (Figure 1). On day 28, *B. subtilis* and *S. saprophyticus* together dominates 49.6% of the microbial population.

Table 2. Quantification of microorganisms from NA plate analysis. Percentage decrease of indigenous bacterria was calculated based on difference of CFU ml^{-1} count from Day 0 and Day 28. Average decrease rate of indigenous bacteria was calculated based on of CFU ml^{-1} count from Day 0 and Day 28 and divided by the fermentation period

	Natural		*Terasi* A		*Terasi* B		*Terasi* C	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Total bacteria (log CFU ml^{-1})	5.41	4.83	5.81	4.81	5.95	4.78	6.10	4.77
Total indigenous bacteria (log CFU ml^{-1})	4.63	4.54	5.43	4.58	5.92	4.23	6.07	4.40
Percentage decrease of indigenous bacteria (%)	82		86		98		98	
Average decrease rate of indigenous bacteria (log CFU ml^{-1}/day)	2.50		3.91		4.47		4.62	

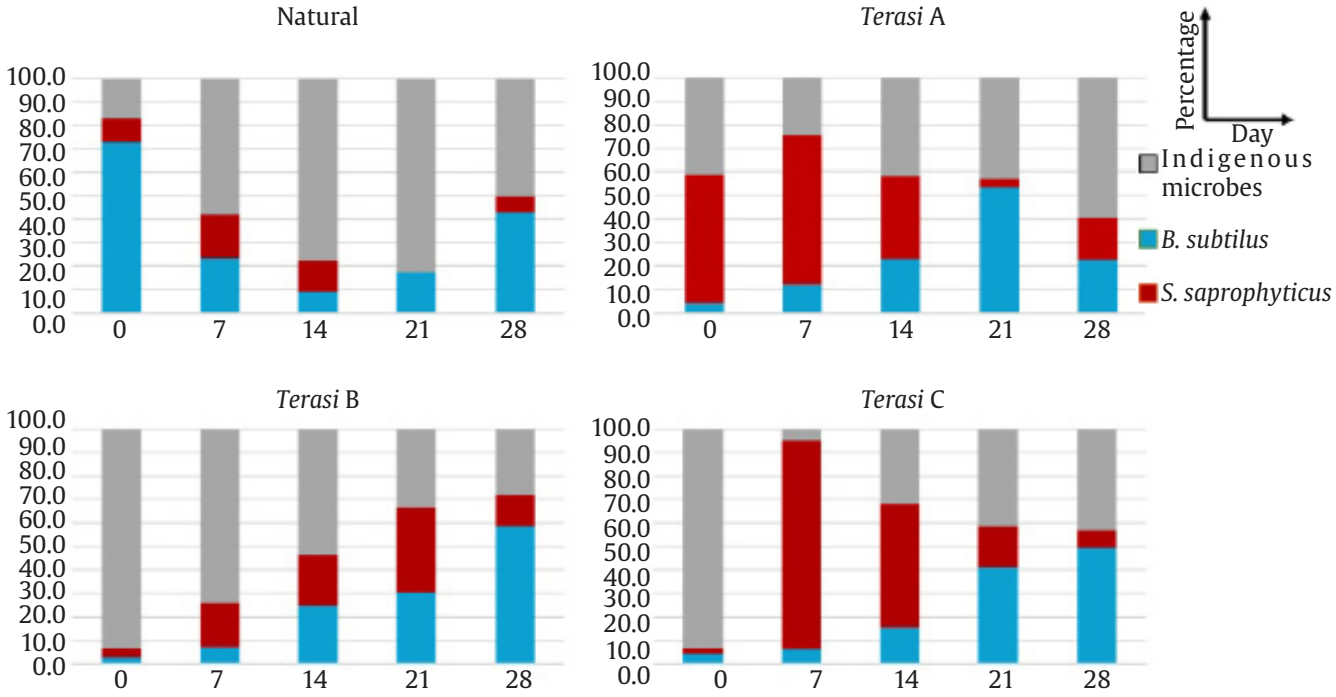


Figure 1. Relative abundance of microorganisms on NA plate. Gray indicates indigenous microbes, blue indicates *B. subtilis*, and red indicates *S. saprophyticus*. The y-axis is the percentage, with 100% as the maximum, and the x-axis indicates day 0, day 7, day 14, day 21, and day 28. Natural refers to fermentation without inoculum. Controlled fermentation was inoculated with *S. saprophyticus, B. subtilis*, and *L. murinus* in *Terasi* A with 1:1:1, *Terasi* B with 1:2:2, *Terasi* C with 2:1:2, respectively

For *terasi* A, the total bacterial count decreased from 5.81 to 4.81 log CFU ml^{-1}, and the indigenous bacteria count decreased from 5.43 to 4.58 log CFU ml^{-1} (Table 2). Overall, both quantitative bacteria count of *B. subtilius* and *S. saprophyticus* decreased throughout fermentation, and on day 28, *B. subtilis* and *S. saprophyticus* together dominates 40.2% of the microbial population (Figure 1).

For *terasi* B, the total bacterial count decreased from 5.95 to 4.78 log CFU ml^{-1}, and the indigenous bacteria count decreased from 5.92 to 4.23 log CFU ml^{-1} (Table 2). The quantitative count of *B. subtilis* was constant through fermentation while *S. saprophyticus* decreased. On day 28, *B. subtilis* and *S. saprophyticus* together dominates 72.2% of the microbial population (Figure 1).

For *terasi* C, the total bacterial count decreased from 6.10 to 4.77 log CFU ml^{-1}, and the indigenous bacteria count decreased from 6.07 to 4.40 log CFU ml^{-1} (Table 2). Overall, both quantitative bacteria count of *B. subtilius* and *S. saprophyticus* decreased throughout fermentation, and on day 28, *B. subtilis* and *S. saprophyticus* together dominates 56.8% of the microbial population.

3.1.2. Change in Mmicrobial Community of Lactic Acid Bacteria On PTT Plate

For naturally fermented *terasi*, the total bacterial count decreased from 5.29 to 4.48 log CFU ml^{-1}, and the indigenous bacteria count decreased from 5.25 to 4.09 log CFU ml^{-1} (Table 3). The relative abundance of indigenous bacteria decreased significantly in the last seven days, and on day 28, *L. murinus* makes up 58.8% of the population.

For *terasi* A, the total bacterial count decreased from 5.95 to 4.49 log CFU ml^{-1}, and the indigenous bacteria count decreased from 5.92 to 4.33 log CFU ml^{-1} (Table 3). At the end of fermentation, the indigenous population dominates the population, and *L. murinus* only makes up 30.5% of the population (Figure 2).

For *terasi* B, the total bacterial count decreased from 5.98 to 4.44 log CFU ml^{-1}, and the indigenous bacteria count decreased from 5.97 to 4.01 log CFU ml^{-1} (Table 3). The relative abundance of *L. murinus*

Table 3. Quantification of microorganisms from PTT plate analysis. Percentage decrease of indigenous bacterria was calculated based on difference of CFU ml^{-1} count from Day 0 and Day 28. Average decrease rate of indigenous bacteria was calculated based on of CFU ml^{-1} count from Day 0 and Day 28 and divided by the fermentation period

	Natural		*Terasi* A		*Terasi* B		*Terasi* C	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Total bacteria (log CFU ml^{-1})	5.29	4.48	5.95	4.49	5.98	4.44	6.05	4.58
Total indigenous bacteria (log CFU ml^{-1})	5.25	4.09	5.92	4.33	5.97	4.01	6.04	4.11
Percentage decrease of indigenous bacteria (%)	93		97		99		99	
Average decrease rate of indigenous bacteria (log CFU ml^{-1}/day)	3.78		4.47		4.51		4.59	

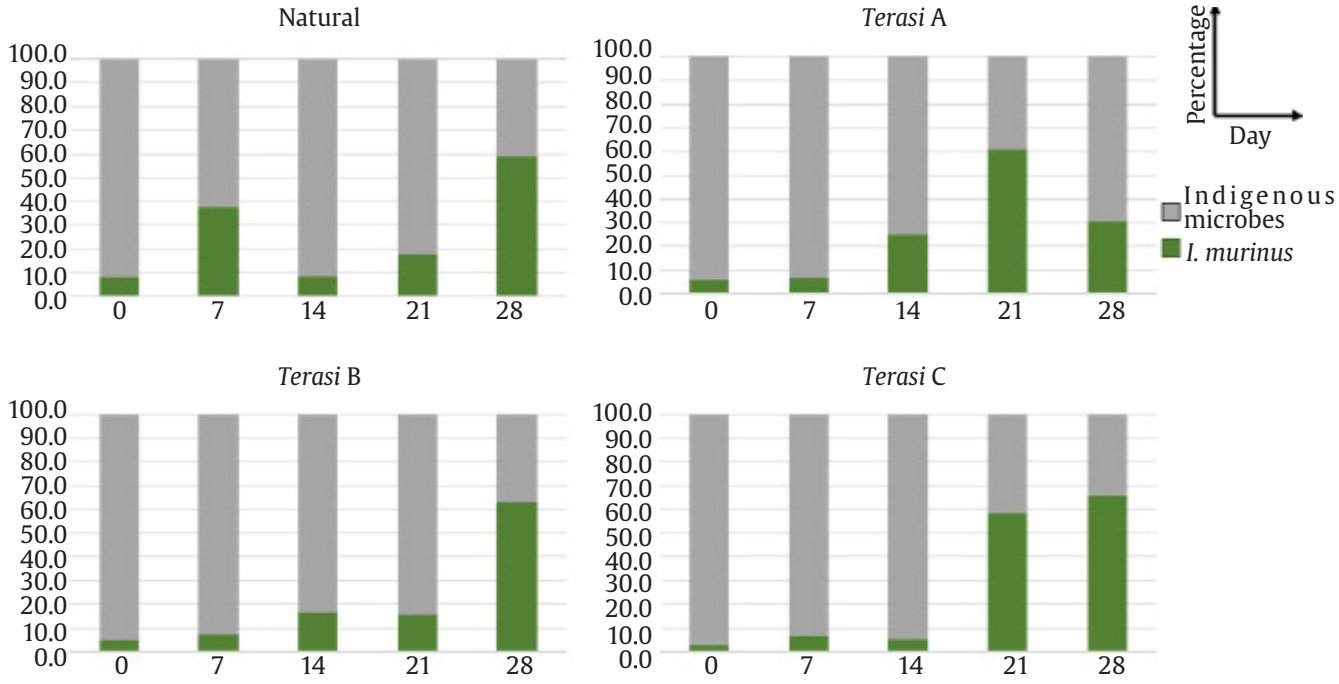


Figure 2. Relative abundance of microorganisms on PTT plate. Gray indicates indigenous microbes, and green indicates *L. murinus*. The y-axis is the percentage, with 100% as the maximum, and the x-axis indicates day 0, day 7, day 14, day 21, and day 28. Natural refers to fermentation without inoculum. Controlled fermentation was inoculated with *S. saprophyticus, B. subtilis,* and *L. murinus* in *Terasi* A with 1:1:1, *Terasi* B with 1:2:2, *Terasi* C with 2:1:2, respectively

is steady from day 0 to day 21, and increased greatly between day 21 to day 28 (Figure 2). On day 28, *L. murinus* makes up 63.1% of the population, even though the quantitative number of *L. murinus* decreased throughout fermentation.

For *terasi* C, the total bacterial count decreased from 6.05 to 4.58 log CFU ml^{-1}, and the indigenous bacteria count decreased from 6.04 to 4.11 log CFU ml^{-1} (Table 3). The relative abundance of *L. murinus* is steady from day 0 to day 14, and increased greatly between day 14 to day 21 (Figure 2). In the end, *L. murinus* dominates, and makes up 65.8% of the population, even though the quantitative number of *L. murinus* decreased throughout fermentation.

3.1.3. Checking for the Presence of Coliform Bacteria on EMB Plate

EMB plate was used to assess the presence of coliform bacteria. Coliform count is used as an indicator for the hygienic conditions in food production, and high coliform count can mean unsanitary conditions. In all *terasi* samples, fortunately, coliform bacteria were not detected throughout the 28 days of fermentation–this includes both the *terasi* that was fermented naturally, and the *terasi* that was fermented with starter cultures.

3.2. Change in Metabolites During Fermentation of *Terasi*

From non-targeted GC/MS analysis, a total of 94 metabolites were annotated, as listed in Table 4, and non-targeted analysis was carried out to characterize the change in metabolites during fermentation. The most abundant group of compounds includes sugars, amino acids and peptides, fatty acids, and organic acids.

For all fermentation conditions, the samples from day 0, day 7, day 14, day 21, and day 28 clustered separately in the score plot (Figure 3a, c, e, g). This indicates that the metabolites changed during the fermentation from day 0 to day 28. In the loading plot for naturally fermented *terasi*, there are more metabolites on the positive axis along PC2, which corresponds to day 21 and day 28 samples (Figure 3b). For *terasi* A and B, most of the detected metabolites, including amino acids sugar compounds, organic acids, and fatty acids, accumulates towards the positive axis of PC1. This indicates that the detected metabolites are produced towards the end of fermentation (Figure 3d, f). For *terasi* C, the metabolites gradually accumulate from day 14 until day 28 (Figure 3h). This could indicates that polymers break down into monomers during the earlier period of fermentation.

Table 4. List of 94 metabolites annotated from terasi using GC/MS

Sugar	Amino acids and peptides		Fatty acids	Heterocyclic compounds
Arabitol	2-aminoadipic acid	Leucine	Behenic acid	2-hydroxypyridine
Fructose	2-aminobutyric acid	Lysine	Elaidic acid	Guanine
Galactoseamine	5-oxoproline	Methionine sulfoxide	Icosanoic acid	Hypoxanthine
Gluconic acid	Alanine	Methionine	Lauric acid	Nicotinic acid
Glucosamine	Asparagine	N-Acetyl valine	Myristic acid	Thymine
Glyceric acid	Aspartic acid	Ornithine	Oleic acid	Uracil
Glycerol	Glutamic acid	Phenylalanine	Palmitoleic acid	Uric acid
Lyxose	Glutamine	Proline	Pentadecanoic acid	Urocanate
Maltose	Glycine	Pyroglutamic acid	Plamitic acid	Xanthine
Maltotriose	Glycine-glycine	Sarcosine	Steraic acid	
Mannitol	Histidine	Serine		
Panose	Homocysteine	Threonine		
Psicose	Homoserine	Tryptophan		
Ribose	Iminodiacetate	Tyrosine		
Sorbose	Isoleucine	Valine		
Sucrose				
Tagatose				
Threonic acid				
Trehalose				
Turanose				
Xylitol				
Xylonic acid				

Nucleosides and nucleotides	Amines	Organic acids	Others
2-deoxyinosine	1,3-propanediamine	2-hydroxybutyrate	3-phenyllactic acid
Guanosine	2-aminoethanol	Fumaric acid	4-hydroxybenzoic acid
Inosine	Cadaverine	Glycolic acid	Inositol
Uridine	n-Butylamine	Hypotaurine	Quinic acid
	n-Propylamine	Lactic acid	
	Putrescine	Malic acid	
		Taurine	
		Urea	

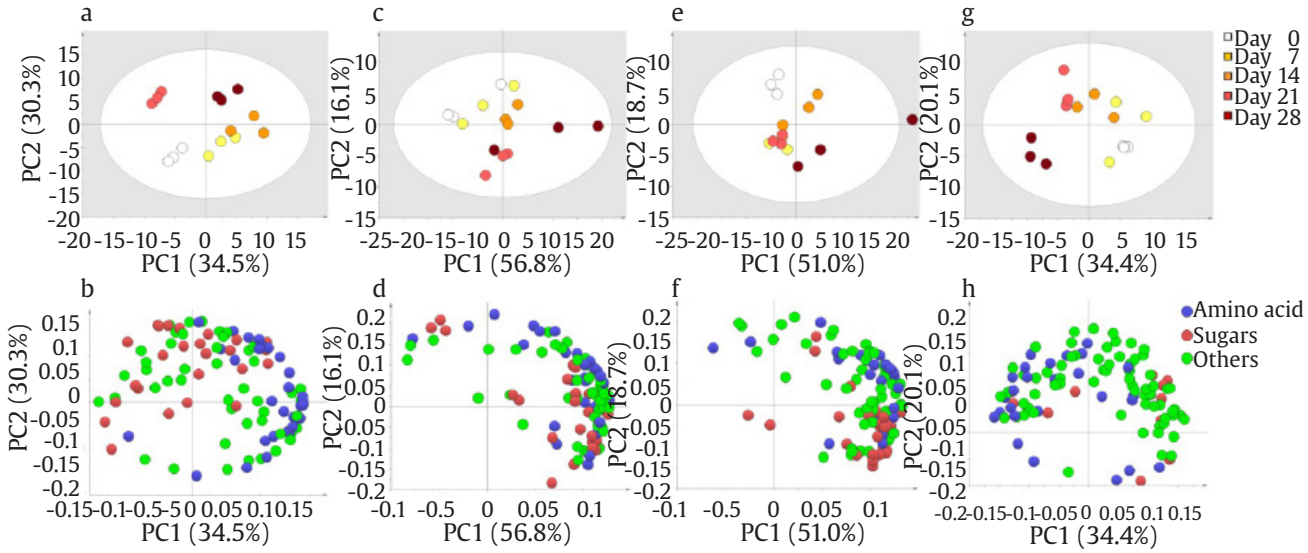


Figure 3. PCA score plot and loading plot for naturally fermented *terasi* (a, b), *terasi* A (c, d), *terasi* B (e, f), and *terasi* C (g, h), respectively. In the score plot, white is day 0, yellow is day 7, orange is day 14, red is day 21, and dark red is day 28. In the loading plot, amino acids are colored in purple, sugar compounds are colored in red, and other metabolites are colored in green. (Scaling: auto, transformation: none, replicate: n = 3, ellipse: Hotelling's T2 (95%)).

In general, most of the essential amino acids increased or were constant during fermentation (Figure 4). Aspartic acid is the onlye (?) amino acid that significantly increase in all four fermentation conditions. In addition, in *terasi* A, glutamic acid, lysine, serine, tyrosine, and valine also significantly increased. Interestingly, in *terasi* C, glycine histidine, lysine, phenylalanine, proline, serine, threonine, and valine significantly decreased. On the other hand, asparagine, glutamine, and methionine decreased in all four fermentation conditions (Figure 5).

Similar to amino acids, sugars also showed an increase towards the end of fermentation (Figure 6). The three most abundant sugar in *terasi* samples were glucose, glycerol, and fructose. Glucose significantly decreased in *terasi* C, while glucose increased in other fermentation conditions. Glycerol and fructose increased in all fermentation conditions.

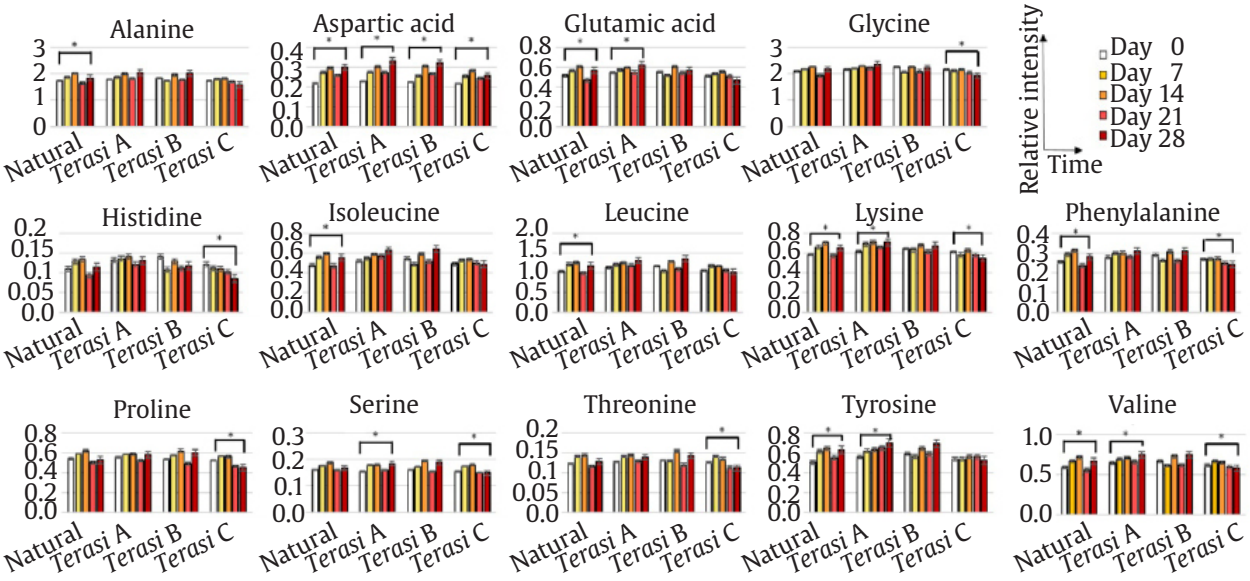
3.3. Proximate Analysis of *Terasi* During Fermentation

In the final product, *terasi* C has the lowest carbohydrate content, which indicates that more amount of carbohydrate was broken down into sugars. *Terasi* A has the lowest protein content, while *terasi* B and *terasi* C contain more protein content than the naturally fermented *terasi*. *Terasi* A also has the lowest fat content, but *terasi* B and *terasi* C also have lower fat content than the naturally fermented *terasi*. *Terasi* B has the lowest moisture content, while *terasi* C has the highest moisture content. The moisture content can be related to the soft texture of *terasi* C.

4. Discussion

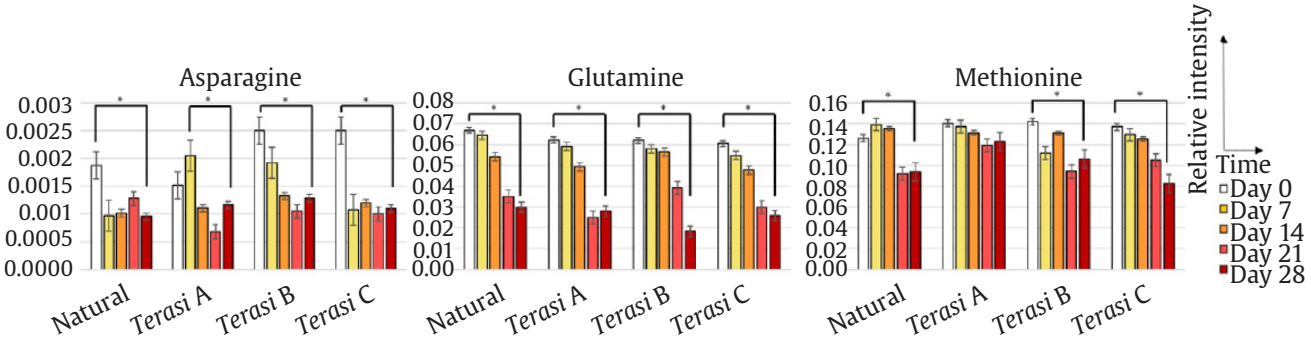
Optimum fermentation condition was determined based on the ratio of starter culture that suppressed the growth of indigenous microbes the most. The EMB plate analysis indicated that all *terasi* product was safe because coliform bacteria were no detected. From NA plate and PTT plate microbial dynamics analysis, *terasi* A, *terasi* B, and *terasi* C, showed a higher percentage decrease of indigenous bacteria compared to naturally fermented *terasi* (Table 2 and 3). This indicates that inoculating the raw material with starter culture is effective in suppressing the growth of indigenous bacteria. However, among the three controlled fermentation conditions, *terasi* C was the most optimum because *terasi* C had the fastest decrease rate of indigenous microbes (Table 2 and 3). To conclude, fermentation condition for *terasi* C, which was inoculated with a ratio of 2:1:2 of *S. saprophyticus, B. subtilis*, and *L. murinus*, respectively, was the most optimal ratio.

The starter cultures are effective for suppressing the growth of the indigenous microbes because



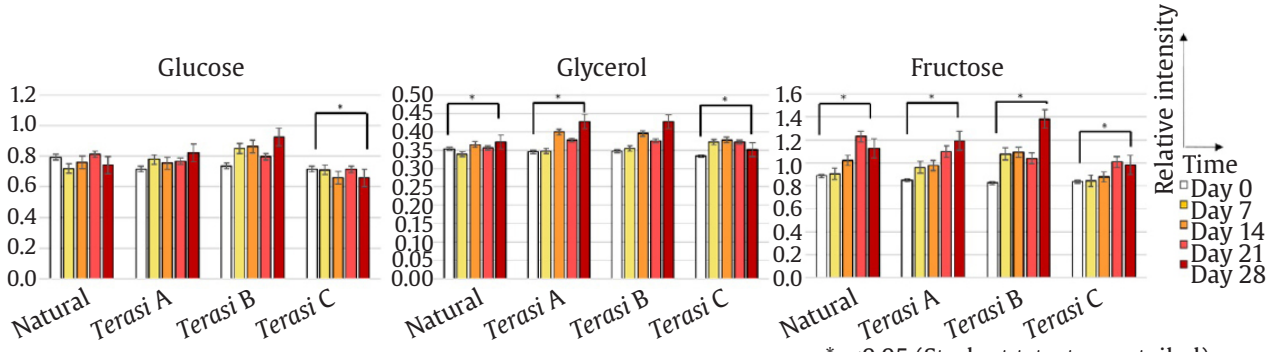
*p<0.05 (Student t-test, one-tailed)

Figure 4. Univariate analysis for essential amino acids that increased or stayed constant throughout fermentation. The bar graph shows the relative intensity, and white is day 0, yellow is day 7, orange is day 14, red is day 21, and dark red is day 28



*p<0.05 (Student t-test, one-tailed)

Figure 5. Univariate analysis for essential amino acids that decreased throughout fermentation. The bar graph shows the relative intensity, and white is day 0, yellow is day 7, orange is day 14, red is day 21, and dark red is day 28



*p<0.05 (Student t-test, one-tailed)

Figure 6. Univariate analysis for essential amino acids that decreased throughout fermentation. The bar graph shows the relative intensity, and white is day 0, yellow is day 7, orange is day 14, red is day 21, and dark red is day 28

all three bacteria have antimicrobial activities. For instance, *S. saprophyticus* produces enzyme that is capable of suppressing the growth of gram-positive and gram-negative bacteria (Hovelius and Mardh 1984). Furthermore, *B. subtilis* is known for producing many types of antibiotics, and have been found to produce antimicrobial peptides during fermentation of foods (Stein 2005; Cheng *et al.* 2017). The antimicrobial activity of *B. subtilis* can account for the effectiveness of using *B. subtilis* as a starter. The lactic acid bacteria, *L. murinus*, produces extracellular organic acid during fermentation that can suppresses the growth of microbes (Rhee *et al.* 2011). Additionally, *L. murinus* are known to produce bacteriocin, which inhibits the growth of both gram-positive and gram-negative bacteria (Elayaraja 2014). Taken altogether, the antibacterial effect of all three starters had a role in suppressing the growth of other bacteria.

For metabolomics analysis, the concept of non-targeted analysis was to identify the metabolites in the samples and provide a non-biased, global view of the sample. From the PCA loading plot, *terasi* A and *terasi* B show similar trend. where the metabolite accumulates greatly towards day 28 of fermentation (Figure 3d and f). However, for *terasi* C, the metabolites gradually accumulate from day 14 until day 28 (Figure 3h). In other words, *terasi* C shows completely different trends from *terasi* A and *terasi* B, and this indicates that *terasi* C has a different metabolite profile compared to the naturally fermented *terasi*, *terasi* A, and *terasi* B. The difference could possibly be accounted by the extracellular activity of the starter cultures. *S. saprophyticus* have surface-associated proteins with extracellular lipase activity, whereas *B. subtilius* produce extracellular protease that breaks down proteins into amino acids (Sakinc *et al.* 2005; Mok *et al.* 2019).

During the fermentation of *terasi*, most of the essential amino acids increased with the exception of asparagine, glutamine, and methionine. Previously, it has been reported that aspartic acid and glutamic acid are involved in the biosynthesis of antibiotic molecule production in *B. subtilis* (Majumdar and Bose 1958). Therefore, one possibility for the decreased in asparagine and glutamine is that the compounds are converted to aspartic acid and glutamic acid, respectively, and the converted structures are used for biosynthesis of antibiotic molecules. More specifically, aspartic acid and glutamic acid could be involved in the biosynthesis of antibiotic iturin (Besson *et al.* 1990).

From proximate analysis of the final product, the moisture content is between 28.48-32.19%, protein content is between 25.56-26.84%, and carbohydrate content is between 24.24-28.05% (Table 5). The moisture content and protein content follows the Indonesian National Standard (SNI) from 2016, and is also in agreement with previous studies (Aryanta 2000). However, the carbohydrate content is slightly higher than the SNI, which states between 12-20%. Compared to other fermented shrimp products in Southeast Asia, the moisture content is relatively low, protein content is comparable, and the carbohydrate content is relatively high (Kim *et al.* 2014; Pongsetkul *et al.* 2014).

In conclusion, the optimized fermentation starter culture ratio of 2:1:2 of *S. saprophyticus, B. subtilis,* and *L. murinus*, respectively, can be used to suppress the growth of unwanted microorganisms. The optimized ratio eliminates the indigenous microbes at a faster rate than natural fermentation, and can lead to improved quality of the final product.

Table 5. Proximate test results for terasi during fermentation (n = 1)

Sample	Carbohydrate (%)	Protein (%)	Fat (%)	Water (%)	Ash (%)
		Day 0			
Natural	26.02	25.68	2.41	30.97	14.92
		Day 7			
Natural	26.79	26.60	1.78	30.18	14.65
Terasi A	26.28	26.45	1.54	30.96	14.77
Terasi B	24.59	26.65	2.10	30.12	16.54
Terasi C	24.74	26.97	2.52	30.44	15.33
		Day 28			
Natural	28.05	25.79	2.07	29.07	15.02
Terasi A	27.92	25.56	0.98	30.22	15.32
Terasi B	26.06	27.68	1.70	28.48	16.08
Terasi C	24.24	26.84	1.41	32.19	15.32

References

Aryanta WR. 2000. Traditional fermented foods in Indonesia. *Japanese Journal of Lactic Acid Bacteria* 10:90-102.

Astuti *et al.* 2018. Physiological profiling and microorganism community analysis of Cirebon shrimp paste fermentation "*Terasi*" using BIOLOGTM EcoPlate. *Microbiology Indonesia* 12:15-22.

Besson *et al.* 1990. Studies on the biosynthesis of iturin, an antibiotic of *Bacills subtilis*, and a lipopeptide containing β-hydroxy fatty acids. *Biochim Biophys Acta* 1036:101-106.

Capozzi *et al.* 2017. Spontaneous food fermentations and potential risksfor human health. *Fermentation* 3:49-68.

Cheng AC *et al.* 2017. Isolation and characterization of antimicrobial peptides derived from *Bacillus subtilis* E20-fermented soybean meal and its use for preventing Vibrio infection in shrimp aquaculture. *Fish Shellfish Immunol* 67:270-279.

Elayaraja *et al.* 2014. Production, purification, and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. *Asian Pac J Trop Biomed* 4:305-311.

Hajeb P, Jinap S. 2012. Fermented shrimp products as source of Umami in Southeast Asia. *J Nutr Food Sci* 10:1-5.

Hovelius B, Mardh PA. 1984. *Staphylococcus saprophyticus* as a common cause of urinary tract infection. *Rev Infect Dis* 6:328-337.

Majumdar SK, Bose SK. 1958. Utilization of amino acids by *Bacillus subtilis* during growth and antibiotic production. *Biochimica et Biophysica Acta* 29:509-513.

Mok *et al.* 2019. A metabolomics approach to understand the solid-state fermentation of okara using *Bacillus subtilis* WX-17 for enhanced nutritional profile. *AMB Express* 9:60-72.

Pongsetkul *et al.* 2014. Chemical composition and physical properties of salted shrimp paste (Kapi) produced in Thailand. *Internatinal Aquatic Research* 6:155-166.

Rhee *et al.* 2011. Importance of lactic acid bacteria in Asian fermented foods. *Microbial Cell Factories* 10:5-18.

Roos YH. 2003. *Water activity-effect on food stability. In: Encyclopedia of Food Sciences and Nutrition*. London: Elsevier. pp. 6094-6101.

Sakinc *et al.* 2005. The surface-associated protein of *Staphylococcus saprophyticus* is a lipase. *Infect Immun* 73:6419-6428.

Sandle T. 2019. *Selection and Application of Culture Media. In: Biocontamination Control for Pharmaceuticals and Healthcare*. Elstree: Elsevier. pp. 103-123.

[SNI] Standar Nasional Indonesia. 2016. Terasi Udang. Badan Standardisasi Nasional, ICS 67.120.30. SNI 2716:2016.

Stein T. 2005. *Bacillus subtilis* antibiotics: structure, syntheses, and specific functions. *Molecular Microbiology* 56:845-857.

Tambaria MDA. 2016. Considering the Nutrional Value of *Terasi* and Incorporating into the Indonesian Diet. International Indonesia Forum.

United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20-2002. United States Pharmacopeial Convention, Inc., Rockville, Md.

Waryono T. 2001. The History and Social Value Characterisitcs of Kampung Laud Segara Anakan Cilacap Community. *In: Indonesia German Cooperation in Marine Science and Geoscience Action plan Meeting*. Purwokerto: Marine Study Center University of Indonesia.