Food Research 8 (2) : 361 - 370 (April 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Effect of *Pediococcus acidilactici* bioaugmentation on the quality improvement of milkfish (*Chanos chanos*) *Bekasam*

Suharto, S., Agustini, T.W. and *Amalia, U.

Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang, 50275, Indonesia

Article history:
Received: 21 September 2022
Received in revised form: 31 October 2022
Accepted: 6 December 2023
Available Online: 10 April 2024

Keywords:
Bekasam,
Bioaugmentation,
Fermentation,
Milkfish,
Starter,
Pediococcus acidilactici

DOI:
https://doi.org/10.26656/fr.2017.8(2).483

Abstract

Bekasam is a traditional Indonesian fermented food usually composed of freshwater fish, salt, steamed rice as a carbohydrate source, and garlic, prepared by spontaneous fermentation for approximately one week up to 1 month, which gives it a distinctive scent and flavor. Milkfish is commonly used as a main ingredient due to its popularity in certain areas and the many possible variations of *Bekasam*. This study investigated the effect of *Pediococcus acidilactici* bioaugmentation on the quality of *Bekasam*. During the 12 days of production, the spontaneous milkfish *Bekasam* (SMB) was compared to the milkfish *Bekasam* with the addition of *P. acidilactici* (PAMB). The starter significantly impacted the properties of *Bekasam*, giving it a slightly brown appearance as a results of Maillard reaction and increasing the protein content (P<0.05), indicating proteolytic activity during fermentation which directly affects the flavor and texture of the end product. The considerably increased protein content was a result of the lengthy fermentation period. Additionally, the fermentation starter resulted in a lower total plate count and increased growth of lactic acid bacteria than the non-starter (P<0.05) *Bekasam*. Furthermore, the total sugar concentration reduced as fermentation progressed, indicating that the starter and the length of the fermentation period play a role in enhancing *Bekasam's* quality and health-related properties.

1. Introduction

Fermented foods are highly popular these days (Narzary *et al.*, 2021), owing to their health advantages and variety. *Bekasam* is a traditional Indonesian fermented food usually composed of freshwater fish, salt, steamed rice as a carbohydrate source, and garlic, prepared by spontaneous fermentation for approximately one week up to 1 month, which gives it a distinctive scent and flavor (Ijong and Ohta, 1996; Desniar *et al.*, 2013). Spontaneous fermentation generally produces lactic acid bacteria (LAB) that have many health benefits (Melia *et al.*, 2018). Adding salt during fermentation is intended to extend the product's shelf life by suppressing the growth of spoilage bacteria (Bautista *et al.*, 2013), and adding rice to *Bekasam* acts as a carbohydrate source to promote the growth of LAB (Melia *et al.*, 2019; Wulandari *et al.*, 2020).

In Indonesia, *Bekasam* is produced traditionally (at a household scale) with many variations of fish and methods. Moreover, no standard can be referred to, unlike other popular Indonesian fermented products such as terasi (shrimp paste). Due to the lack of fermented milkfish products in Indonesia, this study used milkfish (*Chanos chanos*) as the main ingredient for *Bekasam.* Milkfish is a popular processed soft-boned milkfish product (Agustini *et al.*, 2010). Additionally, milkfish, also known as bandeng, is one of the prospective cultural fish in Indonesia, among the most popular fish in the country due to its special characteristics and flavor. It has a huge amount of omega-3 fatty acids, DHA, and other vital nutrients (Yap *et al.*, 2007; Putra *et al.*, 2021). *Bekasam* processing relies on spontaneous fermentation which opens it up to a risk of bacterial contamination (Santos, 1996; Tofalo *et al.*, 2016). Furthermore, spontaneous fermentation in unregulated environments may cause the finished product to be unstable or lose its distinctive flavor (Kim *et al.*, 2016; Hua *et al.*, 2020).

To maintain the quality of *Bekasam*, proper manufacturing is crucial and the safety of *Bekasam* for human consumption must be determined. Previous studies revealed that adjusting the fermentation duration and applying LAB can enhance the quality and health function of fermented foods (Okafor, 2009) by inhibiting both spoilage. Applying LAB may inhibit the growth of

*Corresponding author.
Email: *ulfah.amalia@live.undip.ac.id*

eISSN: 2550-2166 /
Published by Rynnye Lyan Resources

pathogenic bacteriocinogenic bacteria by, for example, applying *Staphylococcus xylosus* in salt-fermented anchovy (Mah and Hwang, 2009) or *Lactobacillus plantarum* in sauerkraut (Kalac *et al.,* 2000), silver carp sausage (Zhang *et al*., 2013), and som-fug (Thai fermented sausages) (Kongkiattikajorn, 2015). Lactic acid fermentation improves protein solubility and the availability of several minerals and limiting amino acids (Rollan *et al.,* 2019). Tannins (50 %), phytates (90 %), and oligosaccharides (90 %) are also decreased by this method (Samtiya *et al.*, 2020). As we know, tannins and phytates are anti-nutrients that reduce the digestibility of protein and minerals. Rice in *Bekasam* also acts as a carbohydrate source for microorganism activity during fermentation. It breaks down sugars in carbohydrates into lactic acid (Nath *et al.*, 2013; Khan *et al.*, 2017) which affects the distinctive flavor of *Bekasam*.

Recently, the importance of bacteriocinogenics, including *Pediococcus* strains, has been thoroughly established. Certain *Pediococcus* strains, such as *P. acidilactici*, have been recommended for use as probiotics (Bhagat *et al.*, 2020). Yan *et al*. (2019) reported that antimicrobials isolated from LAB, including *P. acidilactici*, have potential use against *Staphylococcus aureus* biofilm-related infections. Bacteriocin produced by *P. acidilactici* has potential as a food biopreservative (Melia *et al.*, 2019) and its application as a starter in *Bekasam* allows the product to be more durable and safer. The present study aimed to observe the quality of *Bekasam* based on varying starters and fermentation durations. A promising microbial with advantageous features might be employed as a functional starting culture in the production of fermented fish products.

2. Materials and methods

2.1 Materials

Raw whole milkfish (Figure 1) with a length of 25-30 cm and a weight of 250-300 g was purchased from a traditional wet market located in Semarang City. Milkfish were kept at a cold (-20°C) temperature in a sterile Styrofoam box for transport to the laboratory. Rice, and the condiments for *Bekasam* production, were purchased in a supermarket and stored at room temperature properly for later use.

A strain of *P. acidilactici* F-11 was generously provided by the Research Center of Diponegoro University. Nutrient Agar/Broth (NA/B) and the Man-Rogosa-Sharpe (MRS) were purchased from Oxoid, LTG, England. All chemicals without description in this paper were obtained from Merck, Germany.

Experiments and analyses were conducted at the microbiology laboratory of Fisheries Products Technology, Universitas Diponegoro, Indonesia. The identification of microbes contained in *Bekasam* was performed using VITEK 2 at the Research Center of Universitas Diponegoro, Indonesia.

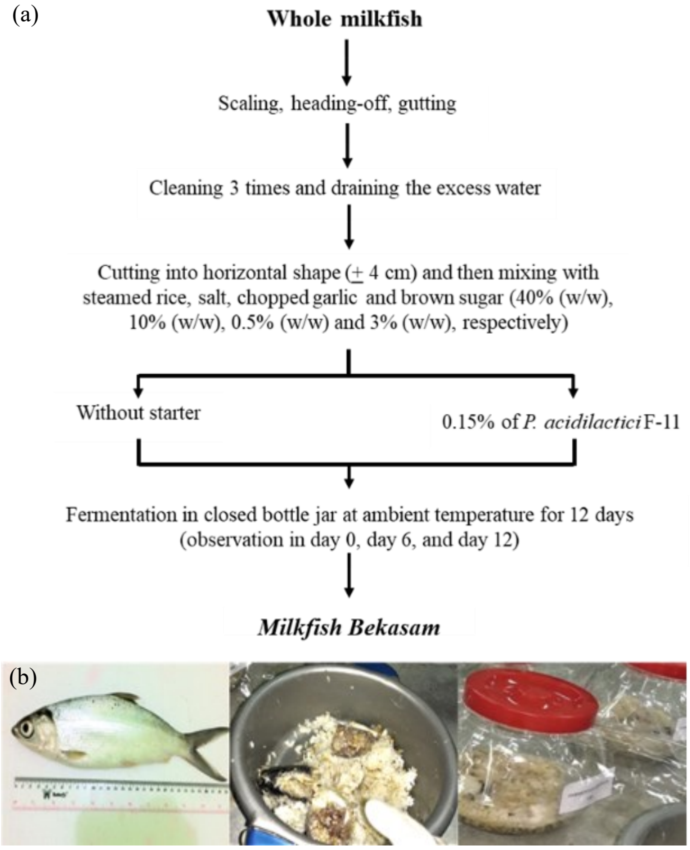

Figure 1. The appearance of the (a) flow chart of milkfish *Bekasam* production, (b) left to right: fresh milkfish, mixing of milkfish with steamed rice and other condiments, and the fermentation step in the manufacturing of Bekasam.

2.2 Starter cultivation

To activate the culture, 100 µL sample of *P. acidilactici* was homogenized by adding 9 mL of MRS broth medium and incubated for 24 hrs at 37°C to attain stationary phase.

2.3 Preparation of Bekasam

Figures 1(a) and 1(b) show that the preparation of *Bekasam* began with the descaling of 5 kg of the whole milkfish and removing the head, followed by gutting it and finally carefully rinsing it three times with enormous amounts of clean water and draining all excess water. The milkfish body, weighting 4.25 kg, was cut into horizontal shapes 4 cm in width, mixed with steamed rice, salt, finely chopped garlic, and crushed brown sugar, of about 40% (w/w), 10% (w/w), 0.5% (w/w), and 3% (w/w) respectively. Incubation at ambient temperature lasted for 12 d and the culture suspension of 6.375 mL, or 0.15% (v/w), was added to the fish mixed with the other ingredients. Each treated sample (PAMB:

Bekasam with *P. acidilactici* and SMB: *Bekasam* without starter) was made in triplicate, kept in tightly closed bottle jars, and fermented at ambient temperature for 12 days. Chemical and microbiological analyses were performed on day 0, 6 and 12. The fermentation process lasting 12 days refers to a similar fermented fish product from Thailand, namely Pala-raa*,* in which rice is used as an additional ingredient to improve the taste and the product is fermented for 7 d up to a year (Phithakpol, 1987). In this study, all samples were taken for sensory evaluation on day 12.

2.4 Microbiological investigation

2.4.1 Total plate count and lactic acid bacteria count

The microbiological investigation was performed on each sample to quantify the total plate count (TPC), and lactic acid bacteria (LAB) count. The TPC was estimated using plate count agar (PCA), while the LAB count was measured using MRS agar. This was per the protocols of the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM, 2021).

2.4.2 Identification of dominant microbes in milkfish Bekasam

The bacteria on *Bekasam* were identified using VITEK 2 (bioMe'rieux, Inc., France), as per protocol refers to a previous study conducted by Kerry *et al.* (2018). Organisms were identified by comparing them to known species-specific responses in the VITEK 2 database.

2.5 Chemical analysis

2.5.1 Acidity

Each sample's pH was measured with a digital pH meter (D-51 HORIBA, Japan) for a slurry consisting of compost and distilled water 1:10 (w/v), following calibration using pH 4 and pH 7 standard buffer solutions.

2.5.2 Protein content

The protein content was measured using an automatic Kjeldahl device (BUCHI Digest System K-437 and Distillation B-324, Switzerland), as per the AOAC protocol (AOAC, 2005). Briefly, the dried sample of *Bekasam* (1 g) was subjected to two processes: digestion and distillation. The sample was mixed with a Kjeldahl tablet and H_2SO_4 (10 mL, 99%). The resulting solution was distilled after adding NaOH, and distillate was collected in a flask with H_3BO_3 (4%) and mixed indicator. Finally, the mixture was titrated with 0.005 mol/L H_2SO_4 (0.005 mol/L). The percentage of nitrogen quantified was transformed into protein content by multiplying by a conversion factor of 6.25.

2.5.3 Salt content

Pearson's procedure (1973) was used to calculate the salt content. Approximately 1 g of *Bekasam* was precisely weighed and added with 50 mL of distilled water. Using 0.5 to 1 mL of 5% potassium chromate, the mixture was titrated with 0.1 N silver nitrate until the first appearance of a faint orange tint against the yellow color of the indicator. The following formula was used to compute the percentage of NaCl in the sample: 1 mL of 0.1 $AgNO_3$ equals 0.005845 g NaCl.

2.5.4 Total reducing sugar

The total reducing sugar of *Bekasam* was evaluated with the DNS (3,5-dinitrosalicylic acid), as per Kokkinomagoulos *et al.* (2020).

2.5.5 Water activity

Water activity (A_w) of *Bekasam* was measured using an A_w-meter (Model 5803, Germany) and according to the producer's protocols.

2.6 Hedonic evaluation

The hedonic evaluation of *Bekasam* was done on a hedonic scale by using a score sheet for twenty-five semi -trained panels comprised of ten male and fifteen female students of the Fisheries Product Technology Department, Diponegoro University. Before the review, each *Bekasam* was given a brief description. Samples were then assigned a random letter. The methods of hedonic evaluation were observing the samples to assess appearance, aroma, taste, texture, and overall acceptance. The appearance was carefully observed by displaying the samples in a well-lit area. For a detailed aroma assessment, samples were placed about 5 cm directly below the nose and the aroma was inhaled deeply 3–5 times. The taste and texture were assessed after samples were deep fried at 180°C for 5 mins before consumption. They were then given a score of 1-9, with 1 meaning dislike, 5 meaning moderate like, and 9 meaning like.

2.7 Statistical analysis

All experiment data were analyzed with GraphPad Prism (version 9.4.0) (GraphPad Software, San Diego, California, USA), except for the sensory evaluation, whose data were analyzed with SPSS Statistics (version 22.0) (IBM SPSS Inc., Chicago, Illinois, USA).

3. Results and discussion

3.1 Microbiological properties of milkfish Bekasam

3.1.1 Total plate count and lactic acid bacteria count

Figure 2 shows that the TPC and LAB count of SMB

https://doi.org/10.26656/fr.2017.8(2).483

and PAMB were increased with fermentation time progressed (P<0.05). With spontaneous fermentation, the TPC and LAB count was 6.77-8.45 and 4.51-7.31 log CFU/g, respectively. The *P. acidilactici* starter in PAMB significantly decreased the TPC by 6.3% down to 6.45-7.64 log CFU/g and, surprisingly, increased the LAB count by 10.3% up to 5.51-7.59 log CFU/g. This indicates that during fermentation, as compared with SMB, starter addition in allows LAB to outgrow other microbial population. For example, after 12 days fermentation, the TPC of PAMB was 1.5 times lower than that SMB. This contradicts Desniar *et al.* (2012) who found that differences in salt concentration in common carp *Bekasam* had a significant impact on LAB count, but did not affect the TPC. The TPC of SMB in this study was lower than the TPC of Joruk, as per a similar type of *Bekasam* from small fish by Koesoemawardani *et al.* (2021).

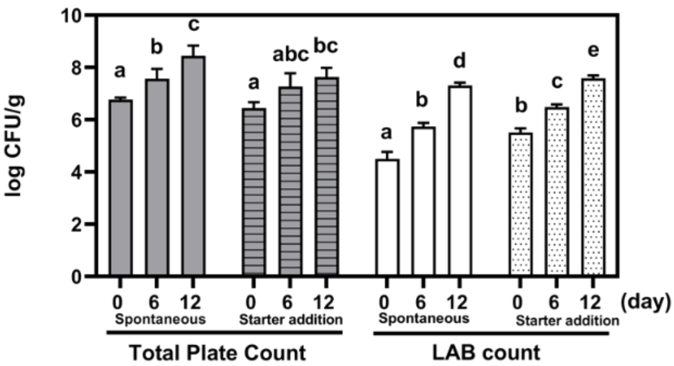

Figure 2. The total plate count and lactic acid bacteria count of SMB and PAMB. The error bars represent the standard deviation. Bars with different notations are statistically significantly different (P<0.05) analyzed using the Tukey–Multiple comparison test.

The LAB count in PAMB increased as a result that *P. acidilactici* producing secondary metabolites in the form of organic acids, which inhibit the growth of bacteria, spoilages, and pathogens. Rice and palm sugar in Bekasam production also influenced the LAB count, as those additional carbon sources created an optimal habitat for the growth of LAB. As previous studies reported, *Pediococcus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, and *Streptococcus* are the LAB most commonly used as a starter and have the potential to produce bacteriocin (an active protein). They have a bactericidal effect, especially against gram-positive bacteria, by disrupting membrane transport. As a result, energy production and protein or nucleic acid biosynthesis are inhibited (Albano *et al.*, 2009; Nieto-Lozano *et al.*, 2010; Hladikova *et al.,* 2012; Bintsis *et al.,* 2018).

This study also indicated that 12 days fermentation of both SMB and PAMB allows the end product to meet the standard for human consumption, owing to the presence of LAB. Liu *et al.* (2011) confirmed that LAB, and its contained complex nutritional requirements which include amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids, and carbohydrates, are generally recognized as safe for human consumption (Vodnar *et al.*, 2011).

3.1.2 Microbial identification by VITEK 2

Bacillus spp. was discovered to be the most dominant microorganism associated with milkfish *Bekasam* (MB) in this study based on VITEK 2 system identification. The existence of *Bacillus* spp. possibly stems from the main raw material, the milkfish, being obtained from aquaculture ponds. *Bacillus* spp. are very common bacterial genera found in soil, and its enzymes may help marine species assimilate nutrients from an unusual source (Soto, 2017; Saxena *et al.*, 2019). Vinderola *et al.* (2019) reported that *Bacillus* spp. belongs to genera isolated from traditional fermented food, characterized and further regulated under a probiotic mechanism. Along with *Lactobacilli* and *Bifidobacteria, Bacillus* spp. falls under the status of Qualified Presumption of Safety (QPS) or GRAS by the European Food Safety Authority (EFSA).

As a result of the salt concentration in *Bekasam* being relatively high (10%), the gram-positive sporulating bacteria in *Bekasam* do not exhibit toxigenic activity, indicating halotolerance. There was no indication in this study that rice in MB production caused the growth of *Bacillus cereus,* one of *Bacillus* spp.'s species with pathogenicity. These findings are unsurprising after the acidity and water activity results were shown to be 5.4-5.9 pH and 0.77-0.84 A_w. In such an environment, *B. cereus* is unlikely to grow. As reported by Jaquette *et al.* (1998), Mols *et al.* (2009) and Bottone (2010), boiled rice is a good growth medium for *B. cereus*, which grows best in low-oxygen environments and vegetative cells grow when water activity is 0.912-0.950.

Besides the *Pediococci* genus from the starter, *Staphylococcus* spp. was also found in MB, which indicates a diverse microorganism present in the product. Spore-forming bacteria in fermented fish products alongside the presence of LAB have been found by (Reynisson *et al.*, 2012). The results of this study were consistent with studies where *Staphylococci* and *Bacilli* were the dominant types of bacteria recovered from Lanhouin (Anihouvi *et al.*, 2007) and cassava's fermented fish product (Gassem, 2019). Furthermore, Bhutia *et al.* (2021) recently reported that *Bacillus* and *Staphylococcus* were the abundant genus identified in Sikkim, India's traditional fermented fish product.

https://doi.org/10.26656/fr.2017.8(2).483

3.2 Chemical characteristic of milkfish Bekasam

3.2.1 Acidity

Figure 3(a) shows the acidity of SMB and PAMB. The pH values were no different on each observation during the 12 days fermentation (P>0.05). The pH value of SMB decreased from 5.99 to 5.68 and 5.37 on days 0, 6 and 12, respectively. On the other hand, the pH of PAMB dropped from 5.93 to 5.67 and 5.41 on day 0, 6 and 12, respectively. Only after the fermentation process had progressed, did the pH values start to significantly (P<0.05) divert. This indicates that the addition of the starter did not have a large impact on the acidity of MB. However, the fermentation duration did affect the acidity. The pH during fermentation degraded because microorganisms in *Bekasam* utilized the rice as a carbohydrate source and metabolized it into lactic acid. Both SMB and PAMB have considerable acidifying activity. In other words, the growth of LAB in *Bekasam* inhibits the activity of spoilage and pathogenic bacteria. LAB can survive in low-pH environments and produce organic acids that can damage bacterial cell membranes. Similar studies of fermented sausages reported that a low pH may prevent harmful and spoilage microorganisms and assure the safety of the product (Hwanhlem *et al.*, 2011; Juturu and Wu, 2016; Rzepkowska *et al.*, 2017). The pH ranges of *Bekasam* in this study were lower than those reported by Gassem (2019) for Hout-Kasef, a traditional salted fermented fish product from Saudi Arabia, which ranged from 6.18 to 6.50. Even though there is no standard, pH is a quality factor of fermented fish products, and a fermented fish product with a pH of 6.5 or higher is regarded as of poor quality (Anihouvi *et al.*, 2012). The pH indicated the sufficient quality of *Bekasam* as a fermented fish product.

3.2.2 Protein content

The protein contents are presented in Figure 3(b). The protein content of SMB significantly (P<0.05) increased with the length of fermentation, rising from 22.1% on day 0 to 27.4% and 34.1% on days 6 and 12, respectively. This is in line with the increase of LAB both in SMB and PAMB, as seen in Figure 2. Microbes are referred to as single cell protein (SCP) or natural protein concentrations (Kurbanoglu, 2001). The protein content of PAMB was lower than that SMB, as on days 0, 6, and 12, it was 20.1%, 24.7% and 30.3%, respectively. During the fermentation, microorganisms, including *P. acidilactici*, produced protease enzymes that break down proteins into polypeptides and amino acids and utilized the small protein compounds to grow. The

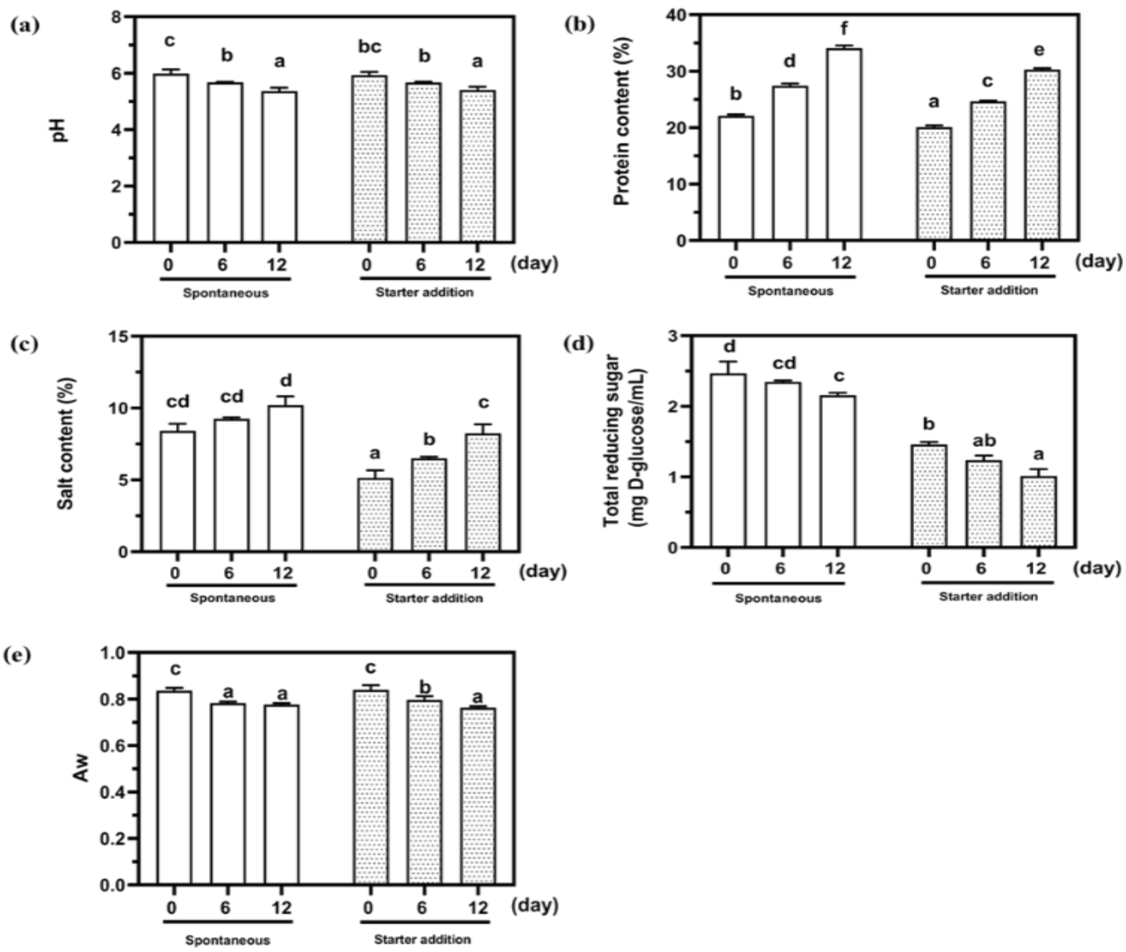

Figure 3. The chemical properties of milkfish Bekasam: (a) pH, (b) protein content, (c) salt content, (d) total reducing sugar, and (e) water activity. The error bars represent the standard deviation. Bars with different notations are statistically significantly different (P<0.05) analyzed using the Tukey–Multiple comparison test.

https://doi.org/10.26656/fr.2017.8(2).483

results are in line with Luan *et al.* (2021), who reported that starters accelerated the protein breakdown of inoculated sausages. The protein content in SMB on day 6 was 27.43±0.28%, higher than the protein content of rabbit meat *Bekasam* without a starter after fermenting for 7 days, which, according to Wulandari *et al.* (2020), had a protein content of 26.86±0.01%. The raw material and method of *Bekasam* production, therefore, had an impact on the protein content.

3.2.3 Salt content

Figure 3(c) presents the salinity of the *Bekasam* products. As the histogram shows, the *P. acidilactici* started had an effect ($P<0.05$) on *Bekasam* with sharply increasing salt contents ranging from 5.15 to 8.24%. However, the results were lower than the salt overall content of SMB, which ranged from 8.42 to 10.20%. The salinity was lower than in salt-fermented fish products of Thailand and Laos that on average contained 12.98% (Marui *et al.* 2015), and in salt-fermented fish with an average of 15.19% (Gassem, 2019). The salinity of PAMB was lower than that of SMB . This reduction is a result of increased LAB activity during fermentation in breaking down macronutrients such as proteins and carbohydrates into smaller compounds. The salinity is therefore closely related to the abundance of LAB, which in PAMB increased as fermentation progressed, as shown in Figure 2. Both *Bekasam* qualities based on the ranges of salinity were sufficient. These findings are novel and distinct from prior studies, which found that LAB strains can grow across a wide salinity range of 8.3 –8.6% (Marui *et al.*, 2015).

3.2.4 Total reducing sugar

The total reducing sugar (TRS) in both the SMB and PAMB were significantly ($P<0.05$) decreased as fermentation progressed. However, the decline of TRS in PAMB was sharper than SMB. As shown in Figure 3(d), the TRS in SMB and PAMB on day 12 of fermentation was 2.16±0.04 and 1.01±0.09 mg D-glucose/mL respectively. These results indicate a significant difference in TRS degradation after the starter addition. Specifically, TRS in SMB dropped by 12.57% down from the initial 2.47 mg D-glucose/ml. In PAMB, the drop was 30.59% down from the initial 1.46 mg D-glucose/mL. During fermentation, microorganisms in *Bekasam* hydrolyze carbohydrates into simpler molecules. The overall remaining sugar in the final product in this study was expected to be low due to the extended fermentation period. Previous studies reported that the glucose generated during fermentation is a preferred substrate for bacteria fermenting the food, which might explain why overall carbohydrate levels decreased after 24 hrs of fermentation (Osman, 2011).

3.2.5 Water activity

Figure 3(e) shows the water activity of both SMB and PAMB were sharply declined as fermentation progressed ($P<0.05$). However, mostly, no significant differences were observed during fermentation in SMB and PAMB, whereas SMB with 6 d fermentation was significantly different with PAMB with 12 days fermentation. The initial A_w of both *Bekasam* ranged from 0.83±0.01 to 0.84±0.02, while the final A_w after 12 days fermentation ranged from 0.76±0.01 to 0.78±0.01 ($P<0.05$). Confirming these findings, Lee *et al.* (2016) found that the final A_w of salted fermented fish with *Bacillus polymyxa* starter was 0.75. Water activity affects microorganism activity due to the presence of water as a medium for bacterial growth. Petrus *et al.* (2013) reported that the A_w of Wadi Betok (traditional fermented fish from South Kalimantan, Indonesia) with the same salt concentration (10 % added to the product) was higher (0.89±0.01%) than in this study. The A_w results revealed that the *Bekasam* acts as an intermediate moisture product.

3.3 Physical appearance and hedonic evaluation

The physical appearance of the final *Bekasam* product can be seen in Figure 4. It shows that a long fermentation duration (12 days) tends to produce a darker color, especially for SMB. The opposite occurred for the PAMB, where neither the fermentation duration of 6 or 12 d seemed to affect the appearance.

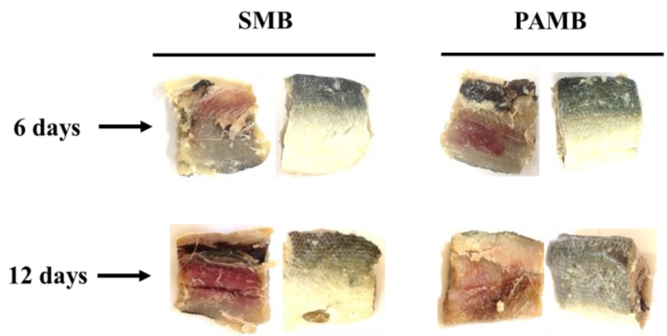

Figure 4. The physical appearance of milkfish *Bekasam*. SMB: spontaneous milkfish *Bekasam,* PAMB: milkfish Bekasam with *P. acidilactici* starter.

Hedonic evaluations were used to produce the *Bekasam* and Figure 5 displays the results. The panelists preferred the PAMB for 12 days fermentation (sample code: D), giving it an overall score of 7.72 and 8.68 and 9 for aroma and flavor, respectively. This suggests that the addition of *P. acidilactici* and the longer fermentation period led to increased enzyme activity by LAB in *Bekasam*, which in turn will have impacted the aroma and flavor of *Bekasam*. Previous studies reported that LAB contributes to the aroma, texture, and nutritional value of fermented foods and are employed as

https://doi.org/10.26656/fr.2017.8(2).483

supplementary cultures (Hasan *et al.,* 2014; Kennedy, 2016; Sharma *et al.*, 2020). However, panelists gave sample D low scores of 4.52 and 4.68 for texture and appearance respectively, which means that panelists moderately liked sample D. The lowest score of *Bekasam's* appearance in the hedonic evaluation was given to sample C (3.72), followed by sample D (4.68), B (6.92) and A (7.4).

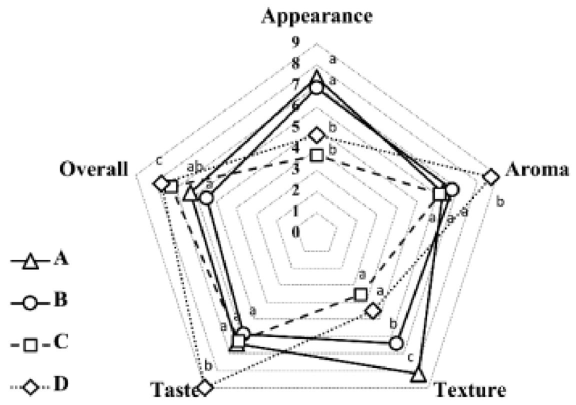

Figure 5. The hedonic test of milkfish Bekasam. A: SMB, 6 days fermentation, B: SMB, 12 days fermentation, C: PAMB, 6 days fermentation, D: PAMB, 12 days fermentation.

4. Conclusion

Pediococcus acidilactici has good potential in bioaugmentation for the production of milkfish *Bekasam*, to improve the quality of the final product in terms of food safety, as demonstrated by the enhancement by lactic acid bacteria, compared to milkfish *Bekasam* without the starter. This study revealed the improvement in the physicochemical quality of milkfish *Bekasam*, which panelists preferred PAMB over than the *Bekasam* without starter. In addition, the longer fermentation demonstrate the production of high lactic acid bacteria which allows an increased enzymatic activity on *Bekasam* products. Identifying microbes in milkfish *Bekasam* would help promote the *Bekasam* as a nutritious seafood fermented product, particularly in Southeast Asia.

Conflict of interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Indonesia, for Research Grant No. 1501-28/ UN7.5.10/LT.

References

Agustini, T.W., Susilowati, I., Subagyo and Setyati, W.A. (2010). Will soft–boned milkfish a traditional food product from Semarang city, Indonesia breakthrough the global market? *Journal of Coastal Development,* 14(1), 81-90.

Albano, H., Pinho, C., Leite, D., Barbosa, J., Silva, J., Carneiro, L., Magalhaes, R., Hogg, T. and Teixeira, P. (2009). Evaluatin of a Bacteriocin-Producing Strain of *Pediococcus acidilactici* as a Biopreservative for "Alheira", a Fermented Meat Sausage. *Food Control,* 20(8), 764-770. https://doi.org/10.1016/j.foodcont.2008.09.021.

Anihouvi, V.B., Sakyi-Dawson, E., Ayernor, G.S. and Hounhouigan, J.D. (2007). (Microbiological changes in naturally fermented cassava fish (*Pseudotolithus* sp.) for lanhouin production. *International Journal of Food Microbiology,* 116(2), 287-291. https://doi.org/10.1016/j.ijfoodmicro.2006.12.009.

Anihouvi, V.B., Kindossi, J.M. and Hounhouigan, J.D. (2012). Processing and quality characteristics of some major fermented fish products from Africa: A critical review. *International Research Journal of Biological Science,* 1(7), 72-84.

AOAC. (2005). Official Methods of Analysis of International, 18th ed. Gaithersburg, USA: AOAC International,

BAM (Bacteriological Analytical Manual). (2021). Chapter 3: *Aerobic Plate Count*. Retrieved from BAM FDA website: http://www.cfsan.fda.gov/~ebam/bam-5.html

Barbosa, J., Borges, J., Amorim, M., Pereira, M.J., Oliveira, A., Pintado, M.E. and Teixeira, P. (2015). Comparison of spray drying, freeze drying and convective hot air drying for the production of probiotic orange powder. *Journal of Functional Foods*, 17, 340-351. https://doi.org/10.1016/j.jff.2015.06.001

Bautista, G.J., Rantsiou, K., Garrido-Fern´andez, A., Cocolin, L. and Arroyo-L´opez, F.N. (2013). Salt reduction in vegetable fermentation: reality or desire. *Journal of Food Science,* 78(8), R1095-R1100. https://doi.org/10.1111/1750-3841.12170.

Bhagat, D., Raina, N., Kumar, A., Katoch, M., Khajuria, Y., Slathia, P.S. and Sharma, P. (2020). Probiotic properties of a phytase producing *Pediococcus acidilactici* strain SMVDUDB2 isolated from traditional fermented cheese product, Kalarei. *Scientific Report,* 20, 1926. https://doi.org/10.1038/s41598-020-58676-2.

Bintsis, T. (2018). Review: Lactic acid bacteria as starter cultures: An update in their metabolism and genetics.

https://doi.org/10.26656/fr.2017.8(2).483

AIMS Microbiology, 4(4), 665-684. https://doi.org/10.3934/microbiol.2018.4.665.

Bottone, E. (2010). *Bacillus cereus*, a volatile human pathogen. *Clinical Microbiology Review*, 23(2), 382-398. https://doi.org/10.1128/CMR.00073-09.

Bhutia, M.O., Thapa, N., Shangpliang, H.N.J. and Tamang, J.P. (2021). High-throughput sequence analysis of bacterial communities and their predictive functionalities in traditionally preserved fish products of Sikkim, India. *Food Research International*, 143, 109885. https://doi.org/10.1016/j.foodres.2020.109885.

Desniar, R.I., Setyaningsih, I. and Sumardi, R.S. (2012). Chemical and microbiological parameter changes and isolation of acid-producing bacteria during fermentation process of common carp (*Cyprinus carpio*) *Bekasam*. *Indonesia Fisheries Process Journal,* 15*,* 232-239.

Desniar, R.I., Suwanto, A. and Mubarik, N.R. (2013). Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (*Bekasam*) and their antimicrobial activity against pathogenic bacteria. *Emirates Journal of Food Agriculture,* 25, 489-494. https://doi.org/10.9755/ejfa.v25i6.12478.

Gassem, M.A. (2019). Microbiological and chemical quality of a traditional salted-fermented fish (Hout-Kasef) product of Jazan Region, Saudi Arabia. *Saudi Journal of Biological Sciences,* 26(1), 137–140. https://doi.org/10.1016/j.sjbs.2017.04.003

Hasan, M.N., Sultan, M.Z. and Mar-E-Um, M. (2014). Significance of fermented food in nutrition and food science. Journal of Scientific Research, 6(2), 373-386. https://doi.org/10.3329/jsr.v6i2.16530.

Hladikova, Z., Smetankov, J., Greif, G. and Greifov, M. (2012). Antimicrobial activity of selected lactic acid cocci and production of organic acids. *Acta Chimica Slovaca*, 5(1), 80-85. https://doi.org/10.2478/v10188-012-0013-3.

Hua, Q., Gao, P., Xu, Y., Xia, W., Suna, Y. and Jiang, Q. (2020). Effect of commercial starter cultures on the quality characteristics of fermented fish-chili paste. *LWT - Food Science and Technology*, 122, 109016. https://doi.org/10.1016/j.lwt.2020.109016.

Hwanhlem, N., Buradaleng, S., Wattanachant, S., Benjakul, S., Tani, A. and Maneerat, S. (2011). Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (*Plasom*) and production of Plasom from selected strains. *Food Control*, 22(3-4), 401-407. https://doi.org/10.1016/j.foodcont.2010.09.010.

Ijong, F.G. and Ohta, Y. (1996). Physicochemical and microbiological changes associated with bakasang processing—a traditional Indonesian fermented fish sauce. *Journal of the Science of Food and Agriculture*, 71(1), 69-74. https://doi.org/10.1002/(SICI)1097-0010(199605)71:1%3c69::AID-JSFA549%3e3.0.CO;2-W

Jaquette, C.B. and Beuchat, L.R. (1998). Survival and growth of psychrotrophic *Bacillus cereus* in dry and reconstituted infant rice cereal. *Journal of Food Protection,* 61(12), 1629-1635. https://doi.org/10.4315/0362-028x-61.12.1629

Juturu, V. and Wu, J.C. (2016). Microbial production of lactic acid: The latest development. *Critical Reviews in Biotechnology,* 36(6), 967-977. https://doi.org/10.3109/07388551.2015.1066305.

Kalac, P., Spicka, J., Krizek, M. and Pelikanova, T. (2000). The effect of lactic acid bacteria inoculants on biogenic amines formation in sauerkraut. *Food Chemistry,* 70(3)*,* 355-359. https://doi.org/10.1016/S0308-8146(00)00103-5

Kennedy, D.O. (2016). B vitamins and the brain: Mechanisms, dose and efficacy—A review. *Nutrients,* 8(2), 68. https://doi.org/10.3390/nu8020068.

Kerry, R.G., Patra, J.K., Gouda, S., Park, Y., Shin, H. and Das, G. (2018). Benefaction of probiotics for human health: A review. *Journal of Food Drug Analysis,* 26(3), 927-939. https://doi.org/10.1016/j.jfda.2018.01.002.

Khan, I., Qayyum, S., Maqbool, F., Rehman, M., Hayat, A. and Farooqui, M.S. (2017). Microbial organic acids production, biosynthetic mechanism and applications. *Mini review Indian Journal of Geo Marine Sciences*, 46, 2165-2174.

Kim, B.M., Park, J.H., Kim, D.S., Kim, Y.M., Jun, J.Y., Jeong, I.H., Nam S.Y. and Chi, Y.M. (2016). Effects of rice koji inoculated with *Aspergillus luchuensis* on the biochemical and sensory properties of a sailfin sandfish (*Arctoscopus japonicus*) fish sauce. *International Journal of Food Science and Technology,* 51(8), 1888-1899. https://doi.org/10.1111/ijfs.13162.

Kokkinomagoulos, E., Nikolaou, A., Kourkoutas, Y. and Kandylis, P. (2020). Evaluation of yeast strains for pomegranate alcoholic beverage production: Effect on physicochemical characteristics, antioxidant activity and aroma compounds. *Microorganisms,* 8 (10), 1583. https://doi.org/10.3390/microorganisms8101583.

Kongkiattikajorn, J. (2015). Potential of starter culture to reduce biogenic amines accumulation in som-fug, a Thai traditional fermented fish sausage. *Journal of Ethnic Foods,* 2(4), 186-194. https://doi.org/10.1016/

j.jef.2015.11.005.

Kurbanoglu, E.B. (2001). Production of single-cell protein from ram horn hydrolysate. *Turkish Journal of Biology,* 25(4), 371-377. https://journals.tubitak.gov.tr/biology/vol25/iss4/2.

Koesoemawardani, D., Afifah, L.U., Herdiana, N., Suharyono, A.S., Fadhallah, E.G. and Ali, M. (2021). Microbiological, physical and chemical properties of joruk (fermented fish product) with different levels of salt concentration. *Biodiversitas,* 22(1), 132-136. https://doi.org/10.13057/biodiv/d220118.

Lee, Y.C., Kung, H.F., Huang, C.Y., Huang, T.C. and Tsai, Y.H. (2016). Reduction of histamine and biogenic amines during salted fish fermentation by *Bacillus polymyxa* as a starter culture. *Journal of Food and Drug Analysis,* 24(1), 157-163. https://doi.org/10.1016/j.jfda.2015.02.002

Liu, S., Han, Y. and Zhou, Z. (2011). Lactic acid bacteria in traditional fermented Chinese foods. *Food Research International,* 44(3), 643-651. https://doi.org/10.1016/j.foodres.2010.12.034

Luan, X., Feng, M. and Sun, J. (2021). Effect of *Lactobacillus plantarum* on antioxidant activity in fermented sausage. *Food Research International*, 144, 110351. https://doi.org/10.1016/j.foodres.2021.110351.

Mah, J.H. and Hwang, H.J. (2009). Inhibition of biogenic amine formation in a salted and fermented anchovy by *Staphylococcus xylosus* as a protective culture. *Food Control,* 20(9), 796-801. https://doi.org/10.1016/j.foodcont.2008.10.005.

Marui, J., Boulom, S., Panthavee, W., Momma, M., Kusumoto, K.I., Nakahara, K. and Saito, M. (2015). Culture-independent bacterial community analysis of the salty-fermented fish paste products of Thailand and Laos. *Bioscience of Microbiota, Food and Health*, 34(2), 45-52. https://doi.org/10.12938/bmfh.2014-018.

Melia, S., Suryanto, D. and Yurnaliza. (2018). Antimicrobial activity of lactic acid bacteria isolated from bekasam against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Salmonella* sp. *IOP Conference Series: Earth and Environmental Science*, 130, 012011. https://doi.org/10.1088/1755-1315/130/1/012011.

Melia, S., Purwati, E., Kurnia, Y.F. and Pratama, D.R. (2019). Antimicrobial potential of *Pediococcus acidilactici* from *Bekasam*, fermentation of sepat rawa fish (*Tricopodus trichopterus*) from Banyuasin, South Sumatra, Indonesia. *Biodiversitas,* 20(12), 3532-3538. https://doi.org/10.13057/biodiv/d201210.

Mols, M., Pier, I., Zwietering, M.H. and Abee, T. (2009). The impact of oxygen availability on stress survival and radical formation of *Bacillus cereus. International Journal of Food Microbiology*, 135 (15), 303-311. https://doi.org/10.1016/j.ijfoodmicro.2009.09.002.

Narzary, Y., Das, S., Goyal, A.K., Lam, S.S., Sarma, H. and Sharma, D. (2021). Fermented fish products in South and Southeast Asian cuisine: indigenous technology processes, nutrient composition and cultural significance. *Journal of Ethnic Food*, 8, 33. https://doi.org/10.1186/s42779-021-00109-0.

Nath, S., Chowdhury, S., Sarkara, S. and Dora, K.C. (2013). Lactic acid bacteria – A potential biopreservative in sea food industry. *International Journal of Advanced Research,* 1(6), 471-475.

Nieto-Lozano, J.C., Reguera-Useros, J.I., Pel Minayo, G., Gutierrez-Fernaez-Martínez, M.C., Sacristandez, A.J. and Hardisson de la Torre, A. (2010). The effect of the pediocin Pa-1 produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. *Food Control,* 21(5), 679-685. https://doi.org/10.1016/j.foodcont.2009.10.007.

Okafor, N. (2009). Fermented foods and their processing. In Biotechnology-Volume VIII: Fundamentals in Biotechnology, Vol. 8, p. 19. Oxford, United Kingdom: Eolss Publishers.

Osman, M.A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural Sciences*, 10(1), 1-6. https://doi.org/10.1016/j.jssas.2010.06.001

Pearson, D. (1973). Flesh Foods - Meat and Fish - assessment of freshness. In Laboratory Techniques in Food Analysis, p. 166-212. London: Butterworth-Heinemann

Petrus, Purnomo, H., Suprayitno, E. and Hardoko. (2013). Physicochemical characteristics, sensory acceptability and microbial quality of Wadi Betok a traditional fermented fish from South Kalimantan, Indonesia. *International Food Research Journal,* 20 (5), 933-939.

Phithakpol, B. (1987). *Plaa-raa*: traditional Thai fermented fish. Proceedings of Conference of Foods and Their Processing in Asia, p, 182-188. Tokyo, Japan: NODAI Research Institute, Tokyo University of Agriculture.

Putra, D.F., Muhsinah, M. and Arisa, I.I. (2021). The substitution of soybean meal by fermented tofu dregs in the milkfish (*Chanos chanos)* diet. *IOP Conference Series: Earth and Environmental*

Science, 674, 012102. https: //doi.org/10.1088/1755-1315/674/1/012102.

Reynisson, E., þór Marteinsson, V., Jónsdóttir, R., Magnússon, S.H. and Hreggvidsson, G.O. (2012). Bacterial succession during curing process of a skate (*Dipturus batis*) and isolation of novel strains. *Journal of Applied Microbiology*, 113(2), 329-338. https://doi.org/10.1111/j.1365-2672.2012.05349.x

Rollan, G.C., Gerez, C.L. and LeBlanc, J.G. (2019). Lactic fermentation as a strategy to improve the nutritional and functional values of pseudocereals. *Frontier in Nutrition*, 6, 98. https://doi.org/10.3389/fnut.2019.00098

Rzepkowska, A., Zielińska, D., Ołdak, A. and Kołożyn-Krajewska, D. (2017). Organic whey as a source of Lactobacillus strains with selected technological and antimicrobial properties. *International Journal of Food Science and Technology,* 52(9), 1983-1994. https://doi.org/10.1111/ijfs.13471

Samtiya, M., Aluko, R.E. and Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: An overview. *Food Production Processing and Nutrition*, 2, 6. https://doi.org/10.1186/s43014-020-0020-5

Santos, M.H.S. (1996). Biogenic amines: their importance in foods. *International Journal of Food Microbiology,* 29(2-3), 213-231. https://doi.org/10.1016/0168-1605(95)00032-1

Saxena, A.K., Kumar, M., Chakdar, H., Anuroopa, N. and Bagyaraj, D.J. (2019). *Bacillus* species in soil as a natural resource for plant health and nutrition. *Journal of Applied Microbiology,* 128(6), 1583-1594. https://doi.org/10.1111/jam.14506.

Sharma, R., Garg, P., Kumar, P., Bhatia, S.K. and Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. *Fermentation,* 6(4), 106. https://doi.org/10.3390/fermentation6040106.

Soto, J.O. (2017). Chapter two - *Bacillus* probiotic enzymes: external auxiliary apparatus to avoid digestive deficiencies, water pollution, diseases and economic problems in marine cultivated animals. *Advances in Food and Nutrition Research*, 80, 15-35. https://doi.org/10.1016/bs.afnr.2016.11.001.

Tofalo, R., Perpetuini, G., Schirone, M. and Suzzi, G. (2016). Biogenic amines: toxicology and health effect. *Encyclopedia of Food and Health,* 424-429. https://doi.org/10.1016/B978-0-12-384947-2.00071-4.

Vinderola, G., Ouwehand, A.C., Salminen, S. and von Wright, A. (2019). Microbiological and functional aspect 5th: Lactic Acid Bacteria. USA: Taylor and Francis Group, CRC Press.

