Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 9 (3): 474-482, November 2023 e-ISSN: 2685-7332

Duration Effects of Shrimp Paste Storage on ALT of Mold Colonies: Variations by Brand and Pre-Standard Treatment

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Submitted July 24th 2023 and Accepted October 28th 2023

Abstract

Shrimp paste is a commonly employed condiment or flavoring in culinary preparations. As a result of the nutrients present in shrimp paste, fungi and other microbial contaminants are able to proliferate and develop on the paste. This study aims as follows: 1) to determine ALT measurements of mold colonies to assess the quality of two brands of pre-treated shrimp paste; and 2) to identify contaminating mold species present in steamed and unsteamed shrimp paste prior to storage. Descriptive quantitative and qualitative research methods were employed within the Biology Department of FMIPA UM's Microbiology Laboratory. A series of three samplings were conducted on treated shrimp paste brands A and B, one week apart between each sampling. A volume of 90 ml of 0.1% peptone water was used to dissolve 10 grams of shrimp paste, resulting in a dilution of 10-1; this process was repeated until the dilution reached 10-5. 0. The surface of PDA (Potato Dextrose Agar) medium was inoculated with 1 ml of suspension from each dilution. The medium was subsequently incubated at a temperature of 25oC for 7x24 hours. The findings of this study indicate the following: 1) At the 28th day of storage, both brands of shrimp paste that underwent treatment with steamed and unsteamed shrimp paste remained of acceptable quality for human consumption. 2) In steamed and unsteamed shrimp paste, thirteen species of mold contaminants were identified: *Chrysosporium corda*, *Cladosporium sphaerospermum*, *Penicillium frequentans*, *Penicillium chrysogenum*, *Rhizoctonia* sp_1., *Aspergillus candidus*, *Fusarium equeseti*, *Colletotrichum ti*, *Moniliella acetobutens*, and *Rhizoctonia* sp_3.

Keywords: *Shrimp paste, Brand, ALT, Contaminant mold, Storage time, Contaminant mold species*

https://doi.org/10.36987/jpbn.v9i3.4690

INTRODUCTION

Shrimp paste is a commonly utilized dietary supplement that serves as a flavoring or ingredient. Regionally distinctive shrimp paste products can be found in nearly every South Asian nation. Superior shrimp paste is distinguished by its flavor, agreeable fragrance, and reddish hue, among other qualities. The assessment of shrimp paste quality encompasses its color, appearance, fragrance, and the identification of insect, caterpillar, and parasite presence (Aristyan et al., 2014).

The production of shrimp paste, a traditional processed product, involves the application of fermentation techniques. Shrimp is one of the fundamental components of *Rebon* shrimp paste whose production value is stable (Ukhty et al., 2017). *Rebon* shrimp are a tiny species of shrimp (Gobel et al., 2016). Shrimp and fish are particularly susceptible to injury when they are not sold out. Fishermen incur losses as a consequence. As a result, a portion of the fish or shrimp caught by fishermen is refined into shrimp paste.

The presence of nutrients, which are the fundamental components in the production of shrimp paste, facilitates the growth and multiplication of a variety of microbial contaminants, including mold and bacteria. The focus of this study was fungal contaminants. Mold contamination of shrimp paste may result in a decline in its quality, rendering it unfit for human ingestion. Food product quality may alter or deteriorate throughout the storage procedure. The potential influence of storage duration on the Total Plate Number (ALT) of a mold colony arises from the fact that an extended storage period provides the mold with increased opportunities for growth and reproduction. Research result by Danarsi & Noer (2016) regarding the effect of storage on microbial growth in instant porridge showed an increase in the number of microbes during the product storage period of 0-4 weeks. The longer the storage time, the number of microbes also increases.

Mold is a filamentous, multicellular, thread-shaped fungus that reproduces through spores and is typically aerobic (Hafsan, 2011). Mold can grow on foods that have low water content (Aris et al., 2021). Contaminant molds, which are pathogenic in nature, are frequently encountered in the atmosphere, affix to implements, insect bodies, and human skin. Their existence enables them to instigate diverse contaminations of food, beverages, and additional components. Additionally, contaminated mold can develop in areas or equipment where the sterilization process is inadequate (Suryani et al., 2020). Several variables impact the development of mold, including substrate, humidity, temperature, pH, oxygen, and water activity (Aw) (Lestiani & Pawenang, 2018).

Species of contaminant mold are capable of surviving and adapting to shrimp paste, where they can cause a variety of harm, including alterations in texture and color, foul odor, and alteration in flavor. A number of contaminant mold species are also capable of producing mycotoxins, which are hazardous to human health (Hastuti et al., 2011). Mold contaminants have the potential to induce a range of clinical complications, such as allergies, pregnancy disorders and poisoning (Artanti et al., 2019). Several types of mold that can be identified in samples of shrimp-based foods such as shrimp paste include: *Aspergillus niger*; *Aspergillus flavus*; *Penicillium sp.* (Handajani, 2006).

One of the communities that frequently incorporates shrimp paste into a variety of dishes is Indonesian society. Shrimp paste is typically stored in airtight containers to

prevent microbial contamination, particularly mold. There are a variety of community practices concerning the treatment of shrimp paste prior to storage, such as grilling, frying, steaming, or frying. This precaution is taken to safeguard the shrimp paste against microbial impurities, such as mildew, which could adhere to unprocessed shrimp paste. Additionally, this serves the purpose of evoking the unique aroma and flavor of the shrimp paste, thereby augmenting its palatability. In addition to its utility in cooking, shrimp paste should be evaluated regarding its expiration life.

In general, packaged shrimp paste does not specify a storage period; therefore, the majority of individuals store the paste until its flavor alters. Upon acquiring a rancid flavor, the shrimp paste is deemed unfit for human sustenance. According to Hastuti (2021), the microbiological quality of food, especially shrimp paste, is determined based on several aspects, one of which is based on the ALT of mold colonies with reference to the provisions of the Director General of POM, in particular the maximum limit for contamination of mold, seasonings and ready-to-use pasta codiments as determined in Indonesian Food and Drug Authority (BPOM) regulation no. 13 of 2019, is 2 x 10^3 cfu/g.

The results of research conducted by Nurcahyo et al., (2016) regarding determining the shelf life of shrimp paste demosntrated that the best results were treated at a temperature of 40^{o}C with aluminum foil packaging for 1 year 6 months 28 days, while at a temperature of 30^{o}C with aluminum foil packaging for 1 year 1 month 13 days.

Based on the background stated above, the objectives of the research are as follows: (1) determining the quality of 2 brands of shrimp paste based on the ALT of mold colonies on steamed and unsteamed shrimp paste before storage; (2) comparing the shelf life of two brands of shrimp paste steamed before storage and not steamed based on the ALT of mold colonies; (3) identifying contaminating mold species in steamed and unsteamed shrimp paste before storage.

METHOD

This research used quantitative and qualitative descriptive methods and was carried out in January–April 2023 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang. The tools used in this research are: analytical balance, beaker glass, test tube, measuring cup, stir bar, Erlenmeyer flask, petri dish, gas stove, autoclave, macropipette, micropipette, LAF, spirit lamp, inoculation needle, microscope, glass object, cover glass, glass pipe, petri dish, inoculation needle, and identification key book. The materials used in this research included PDA medium, shrimp paste, 0.1% peptone water, lactophenol solution, lactophenol cotton blue solution, tissue, cotton wool, and distilled water.

Shrimp paste samples were taken three times on brand A and B shrimp paste that had been treated, with a time interval of one week. 10 grams of shrimp paste were taken and ground, and then the sample was put into an Erlenmeyer flask containing 90 ml of 0.1% peptone water to obtain a dilution level of 10^{-1}. The suspension was diluted to obtain suspensions with dilution levels of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. 1 ml of the suspension from each dilution was inoculated into PDA medium at a temperature of 250 °C for 3 x 24 hours. If the mold has not grown, then continue incubation for 5 to 24 hours. After 7x24

hours, the ALT of mold colonies is calculated based on the dilution level using the (Fardiaz, 1992) formula:

$$\text{ALT mold colony} = \text{number of mold colonies per cup x } \frac{1}{dilution\ rate}$$

If the mold colony ALT results obtained have reached the maximum Indonesian Food and Drug Authority (BPOM) limit, the shelf life will then be compared between steamed and unsteamed shrimp paste. Based on the results of the examination, it is expected that a better method of treating shrimp paste before use will be found, between steamed and unsteamed shrimp paste before storing, so that it can be stored longer in good condition.

Molds that grow on PDA medium are isolated to obtain pure mold cultures. In addition, this aims to facilitate the process of describing and identifying contaminant mold species. Observations of both colony morphology and microscopic characteristics were carried out if the growth of mycelium, hyphae, conidiophores and conidia spores growing on the edge of the cover glass was visible. Identification of contaminating molds in shrimp paste was carried out on each isolated mold isolate, both on unsteamed and steamed shrimp paste samples. Identification is carried out based on the results of the description of the colony's morphological and microscopic characteristics, then referred to the mold identification key book.

RESULTS AND DISCUSSION

The results obtained in this study are data on the ALT values of mold colonies in samples of shrimp paste brands A and B that were treated with steaming and not steaming before being stored based on the length of storage time, namely; 0 days, 7 days, 14 days, 21 days, and 28 days. The ALT value of the mold colony that was obtained was then calculated as the average value of three replications which can be seen in the image below.

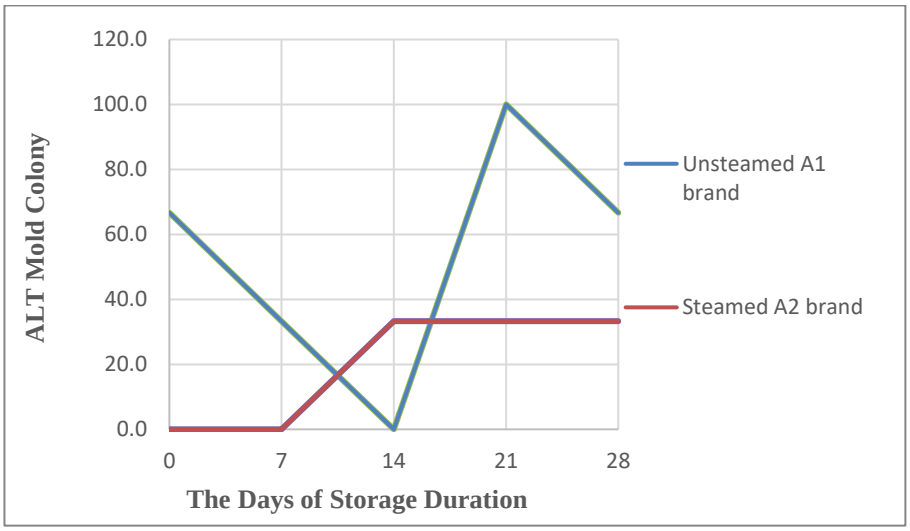

Figure 1. Graph of the average ALT of mold colonies for the length of storage duration of shrimp paste brand A

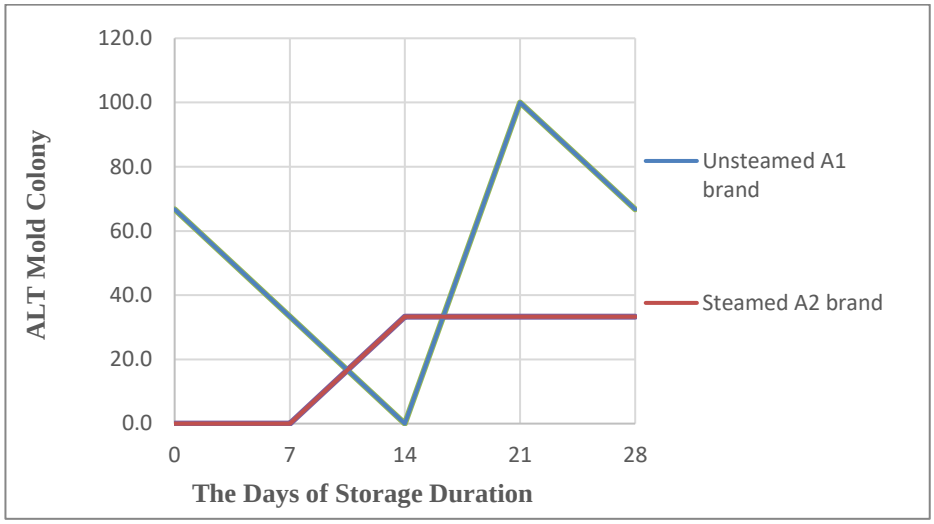

Figure 2. Graph of the average ALT of mold colonies for the length of storage time for brand B shrimp paste

The average ALT value of mold colonies in unsteamed (A1) and steamed (A2) brand A shrimp paste samples at 0 days of storage was 0.6 x 10^2 cfu/gram and 0 cfu/gram, while the average ALT value of mold colonies in branded shrimp paste samples B that is not steamed (B1) and steamed (B2) at 0 days of storage is 0.6 x 10^2 cfu/gram and 0 cfu/gram. Shrimp paste samples from the two brands showed differences in the mean ALT value of mold colonies on storage day 0. On brand A and B shrimp paste which was steamed before storage, no mold growth was seen, whereas on brand A and B shrimp paste which were not steamed before storage, mold growth was seen on the 0^{th} day of storage. This is in line with the statement by Putri et al. (2018) stating that Mold spores die at a heating temperature of 60^{o}C within 5-10 minutes, except for some mold species which are heat resistant.

The average ALT value of mold colonies in brand A samples that were not steamed (A1) and steamed (A2) at 28 days of storage was 0.6 x 10^2 cfu/gram and 0.3 x 10^2 cfu/gram. The average ALT value of mold colonies in brand B shrimp paste samples that were not steamed (B1) and steamed (B2) at 28 days of storage was 0.3 x 10^2 cfu/gram and 0.3 x 10^2 cfu/gram. Shrimp paste samples from brands A and B that were steamed before being stored showed an increase in the average ALT value of mold colonies as the storage time for brand A and brand B shrimp paste samples increased. The ALT value of mold colonies increased with the storage time of 28 days in Figure 1 and in Figure 2. Shrimp paste brands A and B that were not steamed for a storage period of 28 days did not show an increase in ALT of mold colonies. The ALT of mold colonies does not increase or decreases over the long storage period due to the way the mold adapts to the changing environment. A changed environment can cause the availability of nutrients to decrease, so that the need for energy to be used for the synthesis process in various cells in the mold also decreases (Nasution et al., 2011). Mold in shrimp paste degrades complex compounds into simpler compounds. The protein in shrimp paste is simplified into amino acids, and the fat is simplified into fatty acids and glycerol. These simple compounds can

be absorbed by mold as nutrients, so that the mold can grow and reproduce to form colonies in shrimp paste (Nurholipah & Ayun, 2021; Sakti et al., 2016).

Based on the isolation and description of mold through observation of colony morphology and microscopic characteristics, 13 mold isolates were found. Contaminating molds can grow and reproduce on shrimp paste that is steamed or not steamed before storage. Based on the results of the research that has been carried out, results have been obtained regarding the types of contaminant molds that can contaminate steamed or unsteamed shrimp paste before storage. The results of this study indicate that the mold species in steamed shrimp paste are generally not much different from unsteamed shrimp paste. This proves that the contaminant mold species are able to adapt to steam treatment. Data on the presence of mold on brand A shrimp paste can be seen in Table 1, and data on the presence of mold on brand B shrimp paste can be seen in Table 2.

Table 1. Presence of Mold on Unsteamed (A1) and Steamed (A2) Brand A Shrimp Shrimp During Storage Time

Isolate Code	Name of Species	Found on-	
		Unsteamed	Steamed
1	*Chrysosporium corda*	√	-
2	*Cladosprorium sphaerospermum* Penz.	√	√
3	*Penicillium frequentans* Westling.	√	√
4	*Penicillium chrysogenum* Thom.	√	√
5	*Rhizoctonia* sp 1	√	-
6	*Aspergillus candidus* Link.	-	√
7	*Rhizoctonia* sp 2	-	√
8	*Beltrania* sp.	-	√
9	*Sclerotium*	-	-
10	*Fusarium equiseti* (Corda) Sacc.	-	√
11	*Colletrotichum ti* B.S. Weir & P.R. Johnst	√	√
12	*Moniliella acetobutens* Stolk & Dakin	-	-
13	*Rhizoctonia* sp *3*	√	-
	Total	7	8

Table 2. Data on the Presence of Mold on Unsteamed (B1) and Steamed (B2) Brand B Shrimp Shrimp During Storage Time

Isolate Code	Name of Species	Found on-	
		Unsteamed	Unsteamed
1	*Chrysosporium corda*	√	-
2	*Cladosprorium sphaerospermum* Penz.	√	√
3	*Penicillium frequentans* Westling.	√	√
4	*Penicillium chrysogenum* Thom.	√	√
5	*Rhizoctonia* sp *1*	-	-
6	*Aspergillus candidus* Link.	-	√
7	*Rhizoctonia* sp *2*	√	√
8	*Beltrania* sp.	-	-
9	*Sclerotium 1*	√	√
10	*Fusarium equiseti* (Corda) Sacc.	-	√
11	*Colletrotichum ti* B.S. Weir & P.R. Johnst	-	√
12	*Moniliella acetobutens* Stolk & Dakin	√	-

13	*Rhizoctonia* sp *3*	√	√
	Total	8	9

From the ANOVA results, it was found that there was no effect of storage time on the ALT value of mold colonies. This occurs because mold growth is not rapid, so the ALT increase in mold colonies is no different. This can happen because the growth of mold on shrimp paste, both brands A and B, is not correct, so the increase in the number of molds can be different based on the length of storage time. The storage times on days 0, 7, 14, 21, and 28 produced almost the same ALT of mold colonies, so they were not significantly different. Similar research has succeeded in isolating and identifying molds belonging to the genus *Aspergillus* and *Penicillium* as molds that can contaminate shrimp paste (Fitria et al., 2023). The results of research conducted by researchers also show the presence of mold species belonging to the genus *Aspergillus* and *Penicillium*, namely *P. frequentans*, *P. chrysogenum*, and *Aspergillus candidus* in both steamed and unsteamed shrimp paste. This proves that treatment before storing shrimp paste has no effect on the diversity of contaminating mold species.

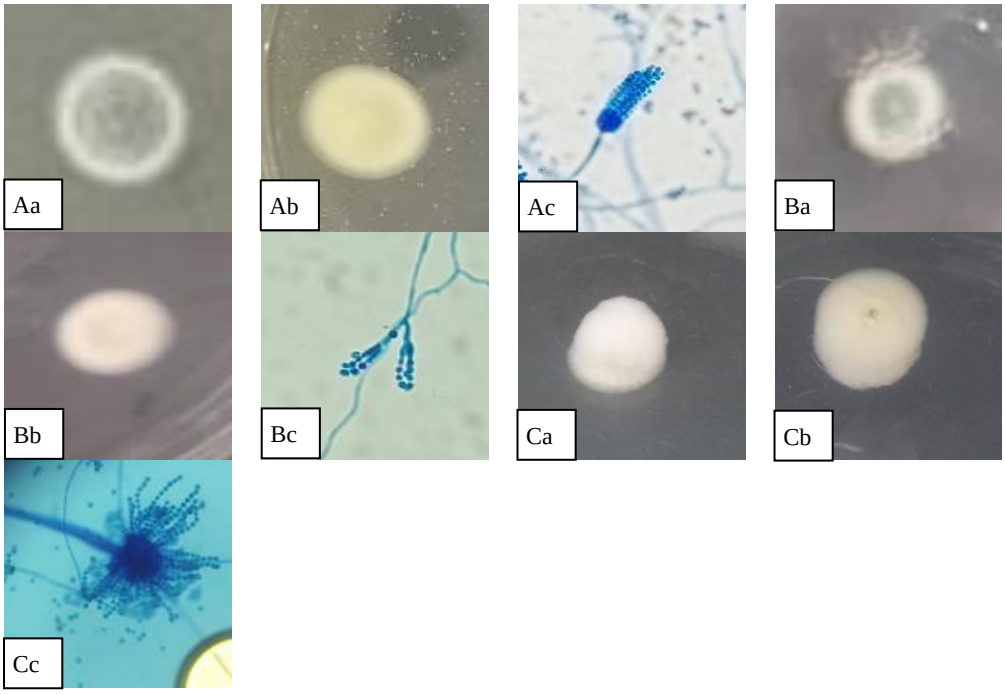

Figure 3. Aa) Upper surface of *P. frequentans*; Ab) Lower surface of *P. frequentans*; Ac) Microscopic *P. frequentans*; Ba) Upper surface of *P. chrysogenum*; Bb) lower surface of *P. chrysogenum*; Bc) Microscopic *P. chrysogenum*; Ca) Upper surface of *A. candidus*; Cb) lower surface of *A. candidus*; Cc) Microscopic *A. Candidus*

The mold species found in the shrimp paste samples belonged to the xerophilic mold group. This can happen if the shrimp paste processing process is carried out in an unhygienic way, the materials used in the manufacturing process, or the tools for making the shrimp paste are not clean. According to Wiryosoendjojo et al. (2019), Xerophilic mold is mold that can grow and survive in dry conditions. This mold can survive even

though it has gone through a steaming process (100°C) and air humidity during the storage period that exceeds the optimal humidity for growth, namely; 77.32%. Xerophilic mold can grow and reproduce at 70% humidity.

Xerophilic molds can produce solutes such as glycerol to create optimal osmotic pressure for reproduction and growth. Dry external conditions can be sensed by the osmosensor membrane, then *xerophilic* mold produces glycerol as a compatible solute to balance internal and external osmotic pressure (Sahay, 2022).

CONCLUSION

Based on the findings of the study, it can be inferred that brand A and brand B shrimp paste, which underwent steaming prior to storage, maintained a satisfactory quality and remained fit for human consumption even after 28 days. Based on the ALT of mold colonies on shrimp paste on the 28th day of storage, this result does not exceed the BPOM-mandated maximum limit for mold contamination. Thirteen species of contaminating mold were effectively isolated and identified in brand A and brand B shrimp paste that had been steamed and unsteamed prior to storage. The species were essentially identical. This may be due to the mold species' comparable adaptability to brand A and brand B shrimp paste, which are respectively subjected to steaming and non-steaming prior to storage.

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How To Cite This Article, with *APA style* :

Syafitri, N.L., Hastuti, U.S., & Prabaningtyas, S. (2023). Duration Effects of Shrimp Paste Storage on ALT of Mold Colonies: Variations by Brand and Pre-Standard Treatment. *Jurnal Pembelajaran dan Biologi Nukleus*, 9(3), 474-482. https://doi.org/10.36987/jpbn.v9i3.4690