nutrición clínica y Dietética Hospitalaria

Artículo Original

Nutr Clín Diet Hosp. 2022; 42(4):73-78
DOI: 10.12873/424mentang

Shelf life and presence of pathogens in liquid-smoked Skipjack *pampis* packed in vacuum packaging (VP), modified atmosphere packaging (MAP), and stored at ambient temperature

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Recibido:1/septiembre/2022. Aceptado: 11/noviembre/2022.

ABSTRACT

Background: Skipjack *pampis*, a typical seafood dish from Manado, North Sulawesi, is made from smoked Skipjack *(Katsuwonus pelamis L.).* Our earlier research showed that adding liquid smoke (LS) to smoked skipjack considerably reduced the amount of benzo(a)pyrene in the fillet while also enhancing its sensory qualities.

Objective: The study tried to examine the shelf life and presence of pathogens in LS Skipjack *pampis* stored in modified atmosphere packaging (MAP) and vacuum packaging (VP) with 100% N_2 for 75 days at room temperature.

Methods: At 0, 3, 6, 9, 25, 50, and 75 days of storage, microbiological and organoleptic characteristics were noted. Samples were analyzed at 0, 3, 6, 9, 25, 50, and 75 days for water content, pH, total plate count (TPC), and pathogenic bacteria (coliform, *E. coli*, *Salmonella sp*, and vibrio). Sensory analysis such as appearance, flavor, taste, and texture has also been determined using the Triangle test.

Results: We found that the TPC of Skipjack *pampis* kept in a 7.8 x 10^3 CFU/g VP and 7.0 x10^4 CFU/g MAP were still good for 25 days but rose significantly at 50 d and 75 days in the sample which used the MAP method. However, the samples that were packed in VP were still acceptable after 50 days (6.9 x 10^4 CFU/g) and 75 days (9.9 x 10^4 CFU/g). The flavor, taste, and odor could still be tolerated in VP until 75 days of storage while in MAP, the products were just acceptable only 25 days of being kept. Pathogenic bacteria were not found in the analyzed samples.

Conclusion: The results showed that vacuum packaging was better than MAP for *Cakalang pampis.*

KEYWORDS

Skipjack *pampis*, liquid smoke, vacuum packaging, modified atmosphere packaging.

INTRODUCTION

Several seafood dishes, including smoked Skipjack *pampis*, are native to Manado. The product has recently become a popular dish among tourists and travelers. *Pampis* is frequently used as a topping, sandwich filling, or as a component of Manado porridge (*tinutuan*). The products are typically kept in supermarkets, retail stores, restaurants, and gift shops and are typically packaged in glass bottles, plastic pouches, and plastic containers. Fish has been preserved for generations by smoking, particularly in Manado, Indonesia. Traditional smoked fish has issues with high levels of benzo(a)pyrene, which is a carcinogenetic indication[1]. Additionally, traditional smoking raises health and environmental problems because of the polycyclic aromatic hydrocarbons (PAH) present in smoke and smoked foods, including benzo(a)pyrene, which has been identified as a possible carcinogenic agent[2]. Several investigations have reported the presence of PAH in several cured fish items, including smoked skipjack, grilled fish, and smoked *Sardinella aurita* and *Lates miloticus*[3,4].

The use of liquid smoke (LS) flavoring as a modern smoking therapy option has been researched[4-6]. To maximize antibacterial activities and histamine potential, LS solutions can be easily controlled and assessed for composition and consis-

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tency of application. Phenols and organic acids are the two primary components of liquid smoke that have been shown to have bactericidal and bacteriostatic effects[7]. The two primary substances often function as synergetic preservatives. Even so, other substances and physicochemical traits are likely involved in the growth suppression, as previously observed[8], as well as in the reaction of microorganisms to this stress, even if the antibacterial activities of the smoke are typically assigned to the phenol. The LS had an inhibitory effect on the pathogens such as *Escherichia coli*, *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Listeria monocytogenes*, according to Lingbeck et al., 2014[9]. Following a prior study, LS produced by the condensation of coconut shells has a reduced benzopyrene level of 0.25 ppb[6]. The results indicated that applying LS to skipjack fillet and halfbeak (*Hemirhamphus sp*) at various concentrations may improve product quality and reduce benzo(a)pyrene content; the highest results on sensory evaluation and PAH reduction were seen at an LS concentration of 0.8 percent.

Poor raw material quality, exploitation of time and temperature during boiling and storage, unhygienic tools and utensils, poor hand hygiene habits of food handlers, and recontamination are all potential drivers of microbial contamination[10]. Food preparation necessitated extensive contact with utensil surfaces, including those of the food handler's hands, skewers, spoons, blenders, preparation tables, containers, and grills. Many foodborne pathogens are known to be able to adhere to food contact surfaces, making it challenging to eliminate pathogens from the environment and utensils used in food processing[11]. In addition to assessing the entire food sanitation system used in the food operation, it has become standard practice to examine foods for microbiological indicator organisms such as Aerobic Plate Count, *Enterobacteriaceae* count, *Vibrio* count, and *Staphylococcus* count. To assess the likelihood that food workers are the source of infection, the *S. aureus* count was also employed. Although aerobic plate counts on fish and fishery products rarely signal risks to food safety, they occasionally can help determine quality, shelf life, and contamination after heat processing. *E. coli* and coliform are indicators that are used to assess the quality or safety of raw or processed food products, as well as sanitizing systems. Bacteria that produce toxins also cause diseases such as *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Salmonella typhi*.

Because there is such a keen interest in expanding the distribution of traditional items outside of market borders, increasing the shelf life of preserved fish products is a significant challenge for the seafood business. The use of high-quality raw materials, the creation of process improvements, the adoption of optimal storage conditions, and the use of packaging that is appropriate are the key methods for enhancing the quality and shelf life of food products. As opposed to storage in air, it is widely known that a modified atmosphere (MAP) with high CO_2 concentrations extends shelf life[12]. However, results comparing MAP and vacuum packaging (VP) are not always consistent[13]. High proportions of CO_2 and N_2 are frequently seen in modified atmospheres, along with low concentrations of O_2. Several spoilage bacteria cannot thrive when CO_2 levels are high and O_2 levels are low. Numerous writers have conducted studies on the quality of smoked fish[14,15]. To the best of our knowledge, however, there hasn't been any research done on the shelf life of Skipjack pampis packaged in both MAP and Vacuum packing. The purpose of this study was to determine how LS Skipjack *pampis* would stay fresh when packaged in either VP or MAP and stored at room temperature for 75 days. Monitoring of the microbiological data was specifically used to determine how the quality had degraded.

MATERIALS AND METHODS

Materials

At Manado's Bersehati fish market, first-grade fresh Skipjack (*Katsuwonus pelamis L.*) was purchased. The fish were placed in a cool box with a fish-to-ice ratio of 1: 2 before being driven to the lab for around 45 minutes. Fish were cleansed, eviscerated, and skin and bones were removed in the lab before making fresh fillets (15x5x3cm). LS was created by utilizing smoke condenser equipment[2] from the modification of previous equipment using coconut shells as the main fuel.

Treatments

Fresh fillets were dipped in a smoked solution containing LS at a concentration of 0.8 percent v/v for 20 minutes before being dried for 4 hours at 80–100°C in the oven. Fish flakes created from smoked fillets were divided into 1 kg portions and combined with salt, onion, and chili powder. Once the good flavor was detected, 100 g of chili powder, 100 g of onion, and the resulting mixture were cooked in oil. Then, two packaging techniques (VP and MAP) were used to package the brown pampis. Samples were kept at room temperature for 75 days.

Research Procedure

To ascertain the rate of quality decline, two replicates of vacuum bags and two samples of MAP were collected at 0, 3, 6, 9, 25, and 75 days. Three copies of each analysis were carried out.

Samples Analysis

Chemical analyses

Water content was analyzed according to AOAC, 2005[16] while pH value was analyzed using Adwa AD 1000 pH/mV pH meter.

Table 1. Microbiological (TPC), pH and water content changes of liquid smoked Skipjack *pampis* stored in Ambient temperature, under Vacuum packaging (VP) and Modified atmosphere packaging (MAP)

Treatments	Analysis	Storage day						
		0	3	6	9	25	50	75
VP	TPC	6.7×10^1	1.07×10^2	2.1×10^2	2.6×10^3	8.8×10^3	6.9×10^4	9.9×10^4
	pH	6.20	6.51	5.75	5.48	4.76	5.89	5.82
	Water content	22.45	22.40	23.23	23.14	24.81	23.91	23.83
MAP	TPC	6.7×10^1	9.5×10^2	2.3×10^3	6.2×10^4	7.0×10^4	8.5×10^6	7.8×10^8
	pH	6.20	6.53	5.90	5.50	4.62	4.45	5.02
	Water content	24.45	22.53	23.40	23.51	22.12	23.56	23.80

Microbiological assessment

Samples were analyzed at 0, 3, 6, 9, 25, 50, and 75 days for total plate count (TPC)[17], pathogenic bacteria (coliform and *E. coli)* test[18], *Salmonella sp* test[19], and vibrio[20]. Each sample was weighed at least 25 g, added to 225 mL of sterile 0.9 percent NaCl solution, and vigorously shaken. Thereafter, 1 mL of the suspension was taken and added to 9 mL of the sterile 0.9 percent NaCl solution, forming a 10-2 dilution that was then homogenized by vigorously shaking the tube. Finally, 1 mL of the 10-2 dilution was added to 9 mL of formed suspension. If additional dilution is required, the process should be repeated in the same way. Additionally, 1 mL of the suspension from each dilution was transferred into the NA medium for the TPC test using the pour plate method, the BSA medium for the *Salmonella* test using the scratch method, the APW-TCBS medium for the *Vibrio parahaemolyticus* test, the lactose broth-EMBA medium for the coliforms and *E. coli* test, and the MPN method for each medium. Each sample was labeled with the sample type and degree of dilution. The media NA and BSA on a Petri dish, the medium APW in a Hack tube, and the lactose broth in a Hach tube with Durham were all incubated at 37 °C for 24 and 48 hours to allow for observation.

Sensory Analysis

Analysis has been done for sensory parameters of visual, taste, texture, and flavor. Hedonic assessment and triangle test were used for sensory characteristics and to assess the differences between LS *pampis* products stored under VP and MAP, using 18 to 21 semi-trained panelists. Both hedonic and triangular assessments have been conducted using score sheets. Panelists with VP and MAP were shown samples, and they were asked to rate the differences between the samples on a scale of 1 to 7. The panelists were instructed to base their selection on how well-rounded the sample was. As a result, the panelist's weighted average of the aforementioned sensory qualities must be used to determine the overall quality of the samples. The minimum requirement for sample acceptance was a score of 4. The panelists were also instructed to look for visual molds during the test, and the visual mold time (VMT) was calculated. As soon as visible mold was found on a product's surface, the sensory evaluation of the samples was halted.

Statistical Analysis

Two factors, the effect of VP (A1) and MAP (A2) on the shelf life of LS Skipjack pampis, with three sub-factors such as A1, A2, and storage time (B) at 0, 3, 6, 9, 25, 50, and 75 days were measured in this study. Data were analyzed using a Completely Randomized Design (CRD) with a designed factorial of 2x7. Treatments were duplicated twice. The data were analyzed using Analysis of Variance (ANOVA), and for significant treatment, the Least Significant Difference (LSD) test was performed.

RESULTS

Table 1 shows the microbiological, pH, and water contents changes of LS Skipjack *pampis* using VP and MAP during storage at room temperature. Water content data can be observed in Table 1. When kept at room temperature, the water content of *pampis* varied between 23.45 %-24.81,8% in VP, and 23.80%-24.45% in MAP. Table 1 stated that during the ambient temperature storage, the pH of LS Skipjack *pampis* slightly decreased, under both storage conditions. The pH range between 5.82-6.20 under VP storage and ranges between 5.02-6.20 under MAP storage. The lowest TPC for *pampis* stored in VP at room temperature was 6.7×10^1 (sample at 0 days) and the highest was 9.9×10^4 (sam-

ple at 75 days). According to TPC data, the lowest TPC for *pampis* stored in MAP was 6.7 x 10^1 (sample at 0 days) and the highest was 7.8 x 10^8 (sample at 75 days). In all scenarios, data stored in MAP has a greater TPC value than data stored in VP. The maximum TPC for fisheries products authorized under Indonesian National Standard (SNI) (30 ºC, 72 hours) is 5 × 10^5 CFU/g.

All samples were free of pathogens, proving that no processing-related human, material, or equipment contamination occurred. Triangle tests have been performed on look, flavor, taste, and texture to see how VP products differ from MAP products and how each treatment differs. The findings revealed a significant ($p \leq 0.01$) difference between the LS *pampis* kept in VP and the MAP one in terms of appearance. Up to 50 and 75 days of storage, Skipjack *pampis* preserved in VP have a different flavor from those smoked in MAP.

DISCUSSIONS

Five microbiological and two chemical parameters, including water content, pH, and organoleptic characteristics, as well as total plate count, total coliform, *Escherichia coli*, *Salmonella*, *Vibrio*, and other bacteria, were measured in the current study. The water content of the *pampis* was quite low, and it's likely that their water activity (Aw) was similarly low, which inhibited bacteria from growing effectively during storage at room temperature, according to TPC data. Although it may thrive in the pH range of 4.8-11.0, *Vibrio parahaemolyticus* prefers a growing environment of 7.8 to 8.6, while *E. coli* and *Vibrio parahaemolyticus* can flourish in the pH range of 6.0 to 8.1[21]. In the study by Lingbeck et al. (2014), in the first few weeks of fermentation, the pH value was shown to have fallen[9]. That means that no fermentation process took place when the items were held at room temperature.

According to the findings, *pampis* kept in VP remained viable for 75 days (9.9 x 10^4 CFU/g) but only for 25 days (2 x 104 CFU/g) in MAP. However, it is advised that *pampis* packed in MAP only remain fresh for 25 days when stored at room temperature. For 75 days of storage, pampis *held* under VP had a longer shelf life than MAP. MPC, APC, and TPC are measures of the number of bacteria present in food or on surfaces that come into contact with it. Although they cannot identify specific infections, they can be a sign that the facility's sanitation procedures are subpar. The total plate count is the listing of mesophilic, aerobic organisms that thrive at temperatures between 20 and 45 ºC. This count, which takes into account both pathogens and non-pathogens, is used to gauge how hygienic the food being prepared is. This media for microbiological development is not picky. Although aerobic plate counts on fish and fishery products rarely signal risks to food safety, they occasionally can help determine quality, shelf life, and contamination after heat processing.

Indicator microorganisms are utilized in food systems for several tasks, such as assessing the quality or safety of unprocessed or processed food products and confirming the efficacy of microbial control techniques like sanitizing systems.

Total coliform is ideally a class of bacteria that is ubiquitous and, for the most part, not harmful to human health. These bacteria are a sign that potentially more dangerous species could be present, but they are not naturally present in groundwater. Within the broader coliform group, the subgroups fecal coliform and *E. coli* are primarily found in the excrement of warm-blooded animals. The presence of *E. coli* implies that feces have been present in the water, which poses an urgent risk to human health. *E. coli* is not wanted in ready-to-eat food since it is a sign of unsanitary circumstances that allowed for contamination or insufficient heat treatment. Since it is ideal to not find E. coli, the Most Probable Number test limit of 3 per gram has been established as the acceptable threshold for this bacterium. Levels above 100 per gram are undesirable and signify a high level of contamination that may have introduced pathogens or allowed any pathogens that were already in the food before processing to survive. Ehuwa et al., 2021 pointed out that the lower number of coliforms can be used to demonstrate the success of safety measures used during processing and handling[22]. Only seafood is relevant to pathogen microorganisms, such as *Vibrio parahaemolyticus*. However, levels from grams in raw seafood would indicate inadequate temperature controls since harvesting and should be taken into consideration. High levels of *V. parahaemolyticus* in cooked seafood indicate that the food has been inadequately cooked or cross-contaminated after cooking with subsequent time or temperature abuse and should prompt an investigation of the food handling controls used by the food[23]. Concerning strains that are Kanagawa-positive, *V. parahaemolyticus* is at a potentially dangerous level. *V. parahaemolyticus* levels in cases of food poisoning (relates to Kanagawa-positive strains). Salmonella is a zoonotic agent that is commonplace at the temperature of 37.5 ºC, and the pH range of 6-8, but at a temperature of 56 ºC and a dry state, it will perish. *S. typhimurium* is one of the numerous strains of *Salmonella* that can result in foodborne illness. *Salmonella* is to blame for more than 50,000 cases of food poisoning each year in the USA. The number of *Salmonella* cells per gram can result in salmonellosis, or between 107 and 109 cells. Olgunoğlu in 2012 remark that *Salmonella* contamination has often resulted in extremely high levels of infections that are directly damaging to human health[24]. *E. coli* presence in the samples also suggested recent fecal pollution of human origin[25].

Regarding the sensory analysis, the panelists appreciated the flavor more since they recognized it as being a true smoked Skipjack flavor. Taste and flavor produce the same outcome. According to sensory analysis, sample taste was ac-

ceptable until 75 days of storage, in contrast, sample storage in MAP was acceptable just until 25 days. Products were rejected by a panelist at 50 days and 75 days of storage in MAP, which may indirectly imply the product's shelf-life. The quality of the finished product depends on the raw material, the location and time of the capture, whether it is fresh or frozen, and the storage conditions. These factors impact not only the microbiological parameters but also the physical and chemical changes in the fish. Pre-treatment techniques (using salt or acids) can enhance the texture of the fish and produce better-tasting smoked salmon. The final product undergoes several sensory and textural changes as a result of fish processing techniques such as heat treatment, the use of liquid smoke or wood chips, or adjustments to the smoking conditions. The quality of the smoked fish might be impacted by any of these factors[26].

CONCLUSION

In conclusion, *pampis* stored in MAP is still good for 25 days ($7.0x10^4$ CFU/g) and 75 days (TPC $9.9x10^4$ CFU/g) when it is stored in VP. Even though no pathogenic microorganisms were discovered in the samples for either type of preservation, this investigation indicated that vacuum packaging outperformed MAP in terms of the shelf life of LS Skipjack *pampis*.

ACKNOWLEDGMENT

This study was supported by funding from the Indonesian Ministry of Research, Technology, and Higher Education's Directorate General of Strengthening Research and Development.

REFERENCES

1. Erhunmwunse N, Tongo I, Enuneku A, Ainerua M, Lawrence IE. Levels of Benzo(a)pyrene (BaP) in Smoked and Barbecued Fish within Benin Metropolis. Conference Proceeding: 7th African Toxicology Symposium. 2015;7:113-4.
2. Berhimpon S, Montolalu RI, Dien HA, Mentang F. Studies on exotic and non-carcinogenic smoked fish, in order to increase economic value of product. Report of the Competitive Grant (MP3EI). Indonesia: Directorate General of Higher Education. 2013.
3. Essumang DK, Dodoo DK, Adjei JK. Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish products. Journal of Food Composition and Analysis. 2012;27(2):128-138.
4. Swastawati F, Darmanto YS, Sya'rani L, Kuswanto KR, Taylor KDA. Quality Characteristics of smoked skipjack (Katsuwonus pelamis) using different liquid smoke. Internasional Journal of Biochemistry and Bioinformatics. 2014;4(2):94-99.
5. Kang J, Tang S, Liu RH, Wiedmann M, Boor KJ, Bergholz TM, Wang S. Effect of curing method and freeze-thawing on subsequent growth of Listeria monocytogenes on cold-smoked Salmon. Journal of Food Protection. 2012;75(9):1619-1626.
6. Berhimpon S, Montolalu RI, Dien HA, Mentang F, Meko AUI. Concentration and application methods of liquid smoke for exotic smoked Skipjack (*Katsuwonus pelamis* L.). International Food Research Journal. 2018;25(5):1864-1869.
7. Saloko S, Darmadji P, Bambang S, and Yudi P. Antioxidative and Antimicrobial Activities of Liquid Smoke Nanocapsules using Chitosan and Maltodextrin and Its Appication on Tuna Fish Preservation. Food Bioscience. 2014;7:71–79.
8. Løvdal T. The Microbiology of Cold Smoked Salmon. Food Control. 2015;54:360-373.
9. Lingbeck JM, Cordero P, O'Bryan CA, Johnson MG, Ricke SC, Crandall PG. Functionality of liquid smoke as an all-natural antimicrobial in food preservation. Meat Science. 2014;97(2):197–206.
10. Anihouvi D, Kpoclou YE, Massih M, Iko AH, Assogba M, Covo M, Scippo ML, Hounhouigan D, Anihouvi V, Mahillon J. Microbiological characteristics of smoked and smoked–dried fish processed in Benin. Food Science & Nutrition. 2019;7.
11. Consuelo L, Jos A, Lcia FH, Eder A, Joele R. Microbiological contamination of surfaces in fish industry. African Journal of Microbiology Research. 2014;8:425-431.
12. Babic J, Milijasevic M, Đorđević V. Modified atmosphere packaging of fish – an impact on shelf life. IOP Conference Series: Earth and Environmental Science. 2019;333:012028.
13. Mireles DC, Oliveira A. Modified Atmosphere Systems and Shelf Life Extension of Fish and Fishery Products. Foods. 2016;5:48.
14. Sulieman, AM, Mustafa W, Shommo S. Assessment of the Quality of Smoked Fish Obtained From White Nile River. Project: Upgrading Fish Industry in Sudan, Focussing in Al Deueim Area, White Nile. 2018;20-25.
15. Adeyeye S, Oyewole O, Adewale O, Omemu A. Quality and safety of traditional smoked fish from Lagos State, Nigeria. International Journal of Aquaculture. 2015;5:1-9.
16. AOAC. 2005. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Washington DC.
17. BSN. Badan Standarisasi Nasional (National Standarization Board). 2006. SNI 01-2323.1-2006. Microbiology assesment part 3: Determination of total plate count in fishery product. BSN Indonesia. Jakarta.
18. BSN. Badan Standarisasi Nasional (National Standarization Board). 2006. SNI 01-2323.1-2006. Microbiology assessment part 1: Determination of coliform and Escherichia coli in fishery product. BSN Indonesia. Jakarta.
19. BSN. Badan Standarisasi Nasional (National Standarization Board). 2006. SNI 01-2323.1-2006. Microbiology assesment part 2: Determination of Salmonella in fishery product. BSN Indonesia. Jakarta.
20. SNI (Standard Nasional Indonesia) 2008. Assessment method of microbiological contamination in meat, egg, and milk, and its products. BSN. Jakarta.

21. Soto-Rodriguez S, Lozano-Olvera R, Palacios-Gonzalez D, Bolan-Mejía M, Aguilar-Rendon K. Characterization and growth conditions of Vibrio parahaemolyticus strains with different virulence degrees that cause acute hepatopancreatic necrosis disease in Litopenaeus vannamei. Journal of the World Aquaculture Society. 2019;50:1-14.

22. Ehuwa O, Jaiswal AK, Jaiswal S. Salmonella, Food Safety and Food Handling Practices. Foods. 2021;10(5):907.

23. Hara-Kudo Y, Kumagai S. Impact of seafood regulations for Vibrio parahaemolyticus infection and verification by analyses of seafood contamination and infection. Epidemiology and Infection. 2014;142(11):2237-2247.

24. Olgunoğlu İA. Salmonella in Fish and Fishery Products. In: Salmonella - A Dangerous Foodborne Pathogen. London: IntechOpen; 2012.

25. Khan FM, Gupta R. Escherichia coli (E. coli) as an Indicator of Fecal Contamination in Groundwater: A Review. In: Jeon, HY. (eds) Sustainable Development of Water and Environment. ICSDWE 2020. Environmental Science and Engineering. Springer, Cham.

26. Puke S, Galoburda R. Factors affecting smoked fish quality: a review. 132-139. 2020.