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VACUUM PACKAGING AND COLD STORAGE FOR EXTENDING THE SHELF LIFE OF *Clarias* sp. MARINATED WITH NATURAL SEASONINGS

Aji Sukoco[1], Nurhayati[1,*], Ahmad Nafi'[1], Sony Suwasono[1], Muhammad Fathurrohman[1], Olivia Yofananda[2]

[1]Study Program of Agricultural Product Technology, Universitas Jember, Indonesia
[2]Department of Fisheries, Universitas Gadjah Mada, Indonesia

*Corresponding author's email: nurhayati.ftp@unej.ac.id

ABSTRACT

Catfish, scientifically named *Clarias sp.*, is one of the most appreciated fish species in Indonesia owing to its nutritional components, availability, and affordability. Prolonging the shelf life of fresh gutted catfish and serving it as a ready-to-cook product are necessary conditions to tackle the rapid biodeterioration of the gutted fish during storage and simultaneously offer consumer-friendliness. Therefore, this study investigated the use of packaging methods on the microbiological and physical properties of marinated catfish under different storage temperatures. A factorial randomized complete block design was implemented in this study, with factor A as a type of packaging consisting of A1 (vacuum-packaging) and A2 (air-packaging), and factor B as a storage temperature involving room (B1), chilling (B2), and freezing (B3) temperatures. Results showed that the combination of vacuum packaging and freezing (A1B3) exhibited the lowest microbial load (4.05 $\log_{10}$ CFU/g) of the marinated catfish after 18-day storage. This satisfied the Indonesian regulation of maximum microbial load (5.69 $\log_{10}$ CFU/g) in fresh fish under SNI 2729:2021. On the other hand, the highest microbial load (8.28 $\log_{10}$ CFU/g) was found in marinated catfish kept in air-packed at room temperature (A2B1) after 3-day storage. As a consequence, the reductions in color (lightness = 41.68) and texture (16.91 g/mm) properties of the marinated catfish were noticeable, while the pH increased to 7.54. The findings underscore that vacuum packaging at freezing temperatures can extend the shelf life of marinated catfish by 18 days.
Keywords: catfish, freezing, marination, packaging, vacuum

INTRODUCTION

Among the various food sources, fish have drawn significant interest because they offer a diverse range of cost-effectiveness, nutritional components, and taste preferences. *Clarias sp.*, commonly known as catfish, has been widely cultivated in tropical regions where challenging environments are well-known. Due to the special auxiliary breathing organs, catfish exhibit good adaptability to areas deficient in water quality, quantity, and oxygen concentration, as well as high salt and ammonia concentrations. An interesting fact is that catfish has been one of the most popular freshwater fish species globally over the past five years. In 2018, catfish ranked third with a world production of about 5.78 million tons, below the production of carps and cichlids. Following this, there were slight fluctuations until 2021, after which the catfish production increased markedly to around 6.62 million tons in 2022, a little more than the cichlid production [1]. In Indonesia, Kementerian Kelautan dan Perikanan Republik Indonesia (KKP) (2024) reported the top 10 inland captured species in the last five years, in which the catfish production fluctuated at approximately 18 to 36 thousand tons annually [2]. This indicates that catfish is one of the fastest-growing cultivated species, supporting the global food security goals.

However, fish are naturally perishable due to various causes. In common practices in traditional markets, catfish are often displayed outdoors on tables alongside other freshwater/saltwater products. This phenomenon leads to cross-contamination and fat oxidation, culminating in rapid fish spoilage, which eventually can pose a risk to human health. In this sense, the short shelf life of fresh catfish has been a critical marketing and distribution challenge. Therefore, proper handling processes that prevent microbial invasion and fat oxidation are necessary conditions to extend its shelf life and to provide consumer-friendliness.

Spices are valued for their preservative and sensorial effects in fish preservation due to their richness in active compounds. Turmeric, coriander seeds, ginger, cinnamon, rosemary, and garlic are common natural preservatives that can be utilized in commercial marinade ingredients [3]. Curcuminoids, zingibain, and allicin are examples of active compounds present in turmeric, ginger, and garlic, respectively. These active compounds play key roles in interfering with the extracellular and intracellular processes of the microbes to prevent or retard their growth. Also, active compounds promote proteolytic and antioxidative activities, thereby rendering the fish meat tender. On one hand, these natural preservatives enhance pleasant aromas owing to their strong volatile active compounds; on the other hand, these volatile compounds can deceive consumers into thinking microbial spoilage may not happen as early. It is important to note that despite marinades with natural preservatives being applied in fish preservation, their antimicrobial capacities are limited to several factors, such as microbial target, antimicrobial concentration, and dilution in the medium (e.g., water or salted water).

In addition to spices, packaging is indeed useful for preserving fish. Previous studies have noted the sensory, oxidative, and microbiological stabilities of fillets of Pacific yellowtail emperor fish [4], gray triggerfish [5], and snakehead fish [6] using vacuum packaging during an extended cold storage period. Moreover, marination in the acetic acid/salt-based solution [7,8], or in the mixtures of lemon juice, garlic, turmeric, pepper, and chili [9-10], improved oxidative and microbiological properties of vacuum-packed and air-packed trout, anchovy, and herring fillets under low storage temperature. Marination also gives a convenience aspect to consumers by providing ready-to-cook spice-marinated fish. Many previous studies have focused on the preservation of saltwater fish. Whereas, packaging and temperature roles for preservation of potential freshwater fish are not much explored. Therefore, the objective of this study was to investigate the effects of packaging types and storage temperatures on the microbiological and physical qualities of marinated catfish.

MATERIALS AND METHODS

Materials

Catfish were collected at 90 days old from a local farmer in Bangsalsari village, Jember. The fish were packed in a roll polyethilene plastic containing water for delivery to the Food and Agricultural Microbiology Laboratory, Universitas Jember. Upon arrival, they were gutted and cleaned for marination. Spices were purchased from local supermarkets in Jember.

Sample preparation

Marinade was prepared by grinding 150 g turmeric, 250 g garlic, 50 g ginger, 0.5 g candlenut, 0.5 g lime leaves, 1.5 g salt, 0.5 g granulated mushroom bouillon, 0.5 g white pepper powder, and 200 mL water. Approximately 4 kg of cleaned catfish was then marinated for 30 min in the fridge. Afterward, the marinated fish were packed in two different methods (vacuum-packaging and air-packaging). For both conditions, storage was carried out at different temperatures, those were room temperature at 27.5±2.5°C, chilling temperature at ±5°C, and freezing temperature at ±-8°C.

Sample analysis

Total plate count (TPC) was analyzed according to the protocol of SNI 2332.3 [11]. Texture, color, and pH were measured as described by Esteves *et al.* [5].

Experimental design

A randomized complete block design with triplicate measurements was used in this experimental design. Factor A was a type of packaging method which was applied by vacuum-packaging (A1) and air-packaging (A2). Factor B was a storage temperature, including room

(B1), chilling (B2), and freezing (B3) temperatures. Sample kept in a room temperature was analyzed on days 1, 2, and 3, while samples from chilling and freezing temperatures were measured on days 6, 12, and 18. Marinated fish without packaging and storage applications was analyzed on day 0, indicated as A0B0.

Data analysis
A quantitative descriptive analysis was conducted in this study, and Microsoft Excel 365 was used.

RESULTS AND DISCUSSION
Total plate count of marinated catfish
The TPC of the marinated catfish is presented in Table 1. The TPC of freshly pepared marinated catfish was 4.02 $\log_{10}$ CFU/g. According to SNI 2729:2021, this value falls below the maximum TPC of fresh fish, approximately 5.69 $\log_{10}$ CFU/g [12]. Either using vacuum or air packaging, the TPC values of marinated catfish kept at room temperature increased about 1.20-1.42 $\log_{10}$ CFU/g every day, reaching 7.59-8.28 $\log_{10}$ CFU/g on day 3 of storage. The daily increase of TPC was higher than that stored at a chilling temperature by as much as 0.88-1.02 $\log_{10}$ CFU/g, resulting in a lower TPC (6.41-7.10 $\log_{10}$ CFU/g) in a prolonged storage time. At the end of storage time, neither room nor chilling temperatures was capable of minimizing TPC value below the allowable safety limit. Our result showed a similar pattern but lower TPC values compared to a previous study of Esteves *et al.* [5]. They found that the total viable counts of vacuum- and air-packed gray triggerfish fillets were 6.8 and more than 10 $\log_{10}$ CFU/g on day 15 at a refrigeration temperature (4±1°C), respectively.

Table 1. Total plate count of marinated catfish at varying packaging types and storage temperatures.

Storage time (day)	Microbial load ($\log_{10}$ CFU/g)						
	A0B0	A1B1	A2B1	A1B2	A2B2	A1B3	A2B3
D0	4.02 ± 0.18						
D1		4.97 ± 0.14	5.74 ± 0.32				
D2		6.17 ± 0.06	6.99 ± 0.17				
D3		7.59 ± 0.33	8.28 ± 0.00				
D6				4.51 ± 0.04	5.26 ± 0.31	4.13 ± 0.12	4.25 ± 0.20
D12				5.53 ± 0.06	6.18 ± 0.26	4.13 ± 0.13	4.25 ± 0.20
D18				6.41 ± 0.12	7.10 ± 0.29	4.05 ± 0.11	4.25 ± 0.20

Vacuum-packaging (A1) and air-packaging (A2).
Room (B1), chilling (B2), and freezing (B3) temperatures.
Values are presented as mean ± standard deviation (n = 3).

Vacuum packaging can limit the atmospheric oxygen that prevents the growth of microbes, in particular aerobic bacteria and fungi. Those catfish packed in a vacuum condition exhibited slightly lower TPC than the air-packed catfish. In addition to the packaging type, there may be the effect of the marinade, which can slow down the microbial growth in the treated catfish, while the previous study used unmarinated fish. Natural antimicrobial compounds from phenolic (flavonoids, tannins, phenolic acids, etc.) and non-phenolic (alkaloids, sterols, terpenes, etc.) groups in marinade can cause cell lysis and death due to the interaction with cell wall-constituting protein and the inhibition of enzyme activity [13-14]. It does make sense that the lower TPC was obtained in this study.

Furthermore, the TPC values of frozen marinated catfish complied with SNI 2729:2021 [12] until day 18 and remained stable at no more than 4.25 $\log_{10}$ CFU/g, whether using vaccum or air packaging. Our result is consistent with Maktabi *et al.* [9], who observed that the TPC of spice-marinated and unmarinated rainbow trout fillets stored at -18°C was in the range of 2.5-4.5 $\log_{10}$ CFU/g for 56 days of storage. Freezing appears to be the most suitable for retaining the microbial load. When fish are frozen at approximately -8°C, several mechanisms contribute to microbial inhibition. *i*) Physical impacts of ice formation. As water freezes, extracellular ice crystals first form outside the microbial cells. This process lowers the amount of unfrozen water surrounding the cells and creates an osmotic gradient that draws water out of the cells, leading to cellular dehydration. During rapid freezing, small intracellular ice crystals can also form inside the cells. These crystals can mechanically damage the cell membrane and cell wall, causing membrane rupture and loss of structural integrity. Consequently, the cells lose membrane fluidity and intracellular solutes leak out, resulting in cell injury or death. *ii*) Osmotic stress and solute concentration. The solutes, like salts and other marinade ingredients, react with the remaining unfrozen water. As a consequence, the water penetrates out of the cell due to hypertonic surroundings to cause cell dehydration. *iii*) Metabolic disruptions. Most spoilage bacteria heavily rely on temperature to carry out enzymatic and cellular activities. Their inactivation at frozen temperatures leads to the reduction of DNA replication and cell division, as well as nutrient transport across the membrane. This condition makes them enter a dormant state [15-16]. Therefore, the number of colonies counted in TPC analysis in A1B3 and A2B3 samples remained lower for a longer storage period. In this respect, plastic wrap can be used to cover the fish meat to avoid freezer burn. A proper thawing should also be applied to the frozen marinated fish; for example, thaw the fish using cold running water, in the microwave, or in the refrigerator to prevent the growth of unwanted microorganisms and ensure uneven cooking.

Texture of marinated catfish

Table 2 shows that the texture of marinated catfish decreased over time, regardless of the packaging and storage temperature applied. However, the most remarked change in the texture of marinated catfish was that stored at room temperature. The reduction of its texture was doubled, from 36-38 g/mm to 16-18 g/mm, and air-packed catfish had a lower texture. As the temperature of storage increased, it triggered chemical reactions that diminished the fish meat texture. Additionally, the characteristics of the texture of fish meat can be affected by protein and lipid contents [7]. A higher load of microbes in the A2B1 sample (Table 1) can expedite the denaturation of the muscle proteins due to spoilage bacterial actions, which promotes the loss of texture. On the other hand, progressive lipid oxidation likely happens during storage at room temperature, as reported by Qiu *et al.* [17]. Both primary (hydroperoxides) and secondary (ketones and aldehydes) oxidation products can react with proteins to accelerate the texture degradation. Owing to a higher oxygen availability in air packaging, this mechanism can be exacerbated.

Besides that, the active compounds in spices of marinade, such as zingibain, allicin, and curcuminoid, can enhance meat tenderization by interacting with myofibrillar proteins in different mechanisms. Zingibain is a cysteine protease derived from ginger, and it breaks down structural proteins, including actin and myosin (muscle fiber) and collagen (connective tissue), thus reducing shear force to increase tenderness [18]. Acidic components from lime leaves tenderize proteins, while allicin, which is primarily found in garlic, binds to endogenous proteases to modify myofibrillar protein to soften the texture [19]. Curcuminoids from turmeric possess antioxidant activity, protecting meat protein from degradation to maintain the meat texture [20]. While tenderizing the texture, the active compounds inhibit proteolytic microbes. In this context, texture of marinated catfish changes positively rather than through microbial invasion.

After storing at lower temperatures (chilling and freezing), the textures of marinated catfish were highly preserved. Even with an extended shelf life until day 18, minimum reductions in texture were observed at around 4-7 g/mm, still resulting in higher texture values (29-37 g/mm) than those kept at room temperature. However, air packaging indicated a lower texture. Xie *et al.* [21] demonstrated that the texture profiles of frozen (-20°C) air-packed Nile tilapia significantly decreased during 120-day storage, followed by a dramatic rise in lipid peroxidation. Ice crystallization and recrystallization can be attributed to these phenomena. Irregular and larger ice crystals are formed predominantly in the extracellular areas during slow freezing. It breaks the membranes of muscle cells, leading to an inability to retain water effectively. Temperature fluctuations of the freezer during storage can trigger recrystallization that produces bigger crystals, resulting in severe structural damage. The concentrated solutes in the unfrozen water phase further induce pro-oxidative reactions. Duun and Rustad [22] also confirmed that the myosin of vacuum-packed salmon fillets was substantially denatured at -1.4°C compared to those kept at -3.6°C. Our findings suggest that the texture of marinated catfish is better maintained under vacuum packaging and at freezing temperatures.

Table 2. Texture of marinated catfish at varying packaging types and storage temperatures.

Storage time (day)	Texture (g/mm)						
	A0B0	A1B1	A2B1	A1B2	A2B2	A1B3	A2B3
D0	42.36 ± 0.10						
D1		38.73 ± 0.68	36.11 ± 0.34				
D2		26.09 ± 0.65	23.84 ± 0.68				
D3		18.29 ± 0.39	16.91 ± 0.42				
D6				39.02 ± 0.56	36.44 ± 0.21	42.38 ± 0.6	42.40 ± 0.70
D12				39.42 ± 0.37	34.89 ± 0.27	39.76 ± 0.3	39.20 ± 0.24
D18				32.20 ± 0.42	29.71 ± 0.20	37.60 ± 0.3	36.27 ± 0.59

Vacuum-packaging (A1) and air-packaging (A2).
Room (B1), chilling (B2), and freezing (B3) temperatures.
Values are presented as mean ± standard deviation (n = 3).

Lightness and pH of marinated catfish

Beyond texture deterioration, discoloration of marinated catfish was also observed, as shown in Table 3. Fresh marinated catfish showed the highest lightness value (52.23). During initial storage, its lightness decreased markedly to 46.89 in the room-temperature sample packaged in air (A2B1). In contrast, reductions in initial lightness values of only 0.9-2.9 units were found in the other treatments, indicating relatively minor discoloration. At the end of storage, samples A1B1 and A2B1 showed the lowest lightness values regardless of whether vacuum or air packaging was used. Overall, marinated catfish stored under cold conditions maintained higher lightness values than those stored at room temperature.

The discoloration of marinated catfish during storage can be affected by oxidative and microbiological impacts in the fish muscle [23]. In air-packaged samples, oxygen promotes the formation of lipid oxidation products, which are generated from unsaturated fatty acids. Oxidative compounds can react with pigments and proteins, producing brownish discoloration, thereby lowering the lightness value. Denaturaion of muscle protein at higher temperatures also happens, and it alters natural pigments such as myoglobin and hemoglobin. Oxidation of these pigments forms darker compounds, such as metmyoglobin-like pigments, causing the fish surface to appear less bright. In a microbiological viewpoint, microbes proliferate intensively at room temperature, producing metabolites, enzymes, and degradation products that contribute to tissue breakdown and color deterioration.

Table 3. Lightness of marinated catfish at varying packaging types and storage temperatures.

Storage time (day)	Lightness						
	A0B0	A1B1	A2B1	A1B2	A2B2	A1B3	A2B3
D0	52.23 ± 0.08						
D1		51.28 ± 0.32	46.89 ± 0.19				
D2		49.60 ± 1.00	45.23 ± 0.78				
D3		42.74 ± 0.96	41.68 ± 2.57				
D6				50.84 ± 0.81	49.38 ± 1.26	51.32 ± 0.8[illegible]	50.87 ± 0.28
D12				47.24 ± 0.67	45.17 ± 0.74	50.02 ± 0.8[illegible]	50.09 ± 0.79
D18				45.42 ± 0.33	43.98 ± 0.71	50.37 ± 0.5[illegible]	49.44 ± 0.13

Vacuum-packaging (A1) and air-packaging (A2).
Room (B1), chilling (B2), and freezing (B3) temperatures.
Values are presented as mean ± standard deviation (n = 3).

Furthermore, the pH of marinated catfish increased over time as microbial populations increased (Table 4). This increase can be associated with the reduction of trimethylamine oxide into trimethylamine, a basic volatile compound responsible for fishy odors in spoiled fish. Cathepsins, endogenous enzymes in fish muscle, together with microbial enzymes, can contribute to this reduction. pH is closely related to the color alteration owing to the conditions of protein structure, water distribution, and light scattering [23]. Higher pH causes muscle proteins to carry more net negative charges, which increases electrostatic repulsion between protein filaments. The isoelectric point (pI) of muscle proteins is approximately 5.0-5.5. The muscle structure becomes more open and retains more water within the myofibrils. Because less water accumulates between fibers, there are fewer interfaces to scatter light. As a result, more light penetrates deeply into the muscle rather than being reflected back to the surface, making the fish mean appear darker. It can be seen in samples A1B1, A2B1, A1B2, and A2B2. Conversely, lower pH reduces the net charge on proteins, especially when approaching the pI of muscle proteins. Protein filaments shrink and expel water into extracellular areas. These spaces create many interfaces between water, proteins, and air that strongly scatter incoming light. Greater surface reflectance causes the fish meat to appear lighter. This is indicated by samples A1B3 and A2B3.

Due to the marination process, the lightness values of stored catfish observed in this study (41-50) were lower than those reported by Esteves *et al.* [5], where lightness values remained above 52.50 during storage. Spices or other seasoning ingredients in the marinade may directly contribute to darker surface coloration. Many spices contain natural pigments that can alter the appearance of fish meat. For instance, turmeric contains curcumin, which is responsible for the yellow color, and lime leaves can impart a greenish color.

Table 4. pH of marinated catfish at varying packaging types and storage temperatures.

Storage time (day)	pH						
	A0B0	A1B1	A2B1	A1B2	A2B2	A1B3	A2B3
D0	6.00 ± 0.07						
D1		6.33 ± 0.03	6.91 ± 0.02				
D2		6.91 ± 0.04	7.32 ± 0.02				
D3		7.32 ± 0.04	7.54 ± 0.08				
D6				6.16 ± 0.04	6.58 ± 0.02	6.20 ± 0.03	6.23 ± 0.03
D12				6.42 ± 0.02	6.97 ± 0.03	6.13 ± 0.03	6.20 ± 0.07
D18				6.91 ± 0.07	7.37 ± 0.03	6.21 ± 0.02	6.34 ± 0.02

Vacuum-packaging (A1) and air-packaging (A2).
Room (B1), chilling (B2), and freezing (B3) temperatures.

Values are presented as mean ± standard deviation (n = 3).

CONCLUSION

During storage, the TPC, texture, lightness, and pH of marinated catfish deteriorated to varying degrees. Storage at room temperature caused more pronounced deterioration in these parameters, which can be due to accelerated microbial growth. Conversely, chilling and freezing temperatures effectively halted microbial proliferation, resulting in lower TPC values and better preservation of texture and color properties. Similar protective effects were observed in vacuum-packed catfish, where limited oxygen availability slowed down the quality deterioration during storage. For the preservation of marinated catfish, vacuum packaging and freezing temperatures are recommended. Further research should investigate the protein and lipid contents, oxidative stability, and enzymatic activity of both fresh and stored marinated catfish.

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