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The Effect of Coconut Shell Liquid Smoke Concentration on the Sensory Characteristics at Day 0 and Total Bacterial Count of Catfish (*Pangasius* sp.) Meatballs Substituted with Oyster Mushrooms During Storage

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A B S T R A C T

This study aimed to determine the effect of coconut shell liquid smoke concentration on the sensory characteristics at Day 0 and the total bacterial count during storage of catfish meatballs substituted with oyster mushrooms. A completely randomized design was used with treatments of 0.5%, 1%, 1.5%, and 2% (v/v) coconut shell liquid smoke. Sensory evaluation was conducted at Day 0 using a hedonic scale with 30 semi-trained panelists who evaluated color, aroma, taste, and texture. Sensory data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a 5% significance level. The treatment with the best sensory characteristics was selected using the De Garmo Effectiveness Index and subsequently evaluated for total bacterial count during 12 days of storage at 6°C and 30°C. The best treatment was 1.5% coconut shell liquid smoke, with sensory scores of 4.43 (somewhat like) for color, 4.90 (like) for aroma, 5.30 (like) for taste, and 4.83 (like) for texture. At 30°C, total bacterial count increased from a baseline of 3.70 log CFU/g to 7.30 log CFU/g on Day 4, remained at 7.30 log CFU/g on Day 8, and reached 8.10 log CFU/g on Day 12. At 6°C, total bacterial count increased from 3.70 log CFU/g to 4.42 log CFU/g, 4.67 log CFU/g, and 5.19 log CFU/g on Days 4, 8, and 12.

Contribution to Sustainable Development Goals (SDGs):

SDG 2: Zero Hunger

SDG 9: Industry, Innovation and Infrastructure

SDG 12: Responsible Consumption and Production

1. INTRODUCTION

1.1. Research Background

Fish meatballs are a type of diversified fishery product that offers high economic value and better nutritional content compared to beef and chicken meatballs. Fish meatballs are a processed fishery product containing at least 40% fish paste or surimi. The addition of oyster mushrooms to catfish meatballs provide an alternative meatballs product with high nutritional value while reducing reliance on meat products associated with cholesterol concerns [12].

The combination of oyster mushrooms and catfish results in a moisture content of 74.34 in the final meatball product, exceeding the Indonesian National Standard (SNI) maximum moisture content of 70% for fish meatballs. This relatively high moisture content can facilitate microbial growth and may reduce product shelf life. Alternative preservatives are needed to reduce reliance on conventional preservatives; one potential alternative is coconut shell liquid smoke. In this study, coconut shell liquid smoke was used as a boiling medium to help maintain meatball quality during storage [13].

Coconut shell liquid smoke contains phenolic compounds that contribute to smoke aroma and flavor and can function as antibacterial and antioxidant agents. Carbonyl compounds, such



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as aldehydes and ketones, contribute to flavor formation, while organic acids can lower pH and inhibit bacterial growth [5]

1.2. Literature Review

Fish meatballs are a food product made from minced fish meat, mixed with other ingredients, shaped into balls, and then boiled. The manufacturing process involves grinding the fish meat into a fine paste, then mixing it with starch or grains as well as spices to enhance the flavor. The addition of chemicals and other food additives is permitted only if authorized and does not pose a health hazard [15].

Catfish (*Pangasius* sp.) is one of the most readily available fishery commodities in Indonesia. Its high protein content makes it a good protein source in daily meals [5]. Per 100 grams of catfish meat, there are 70.3 grams of water, 17.3 grams of protein, 6.3 grams of fat, and 4.3 grams of carbohydrates. Protein is the most abundant chemical component in catfish. This protein contains amino acids such as glycine, lysine, arginine, threonine, leucine, glutamic acid, serine, and proline. Catfish also contains several minerals, namely calcium (110 mg/kg), phosphorus (551 mg/kg), iron (11.2 mg/kg), and zinc (4.3 mg/kg) [3]. Catfish has a high freshness level, minimal fishy odor, and relatively few bones and skin. Its white flesh makes it suitable as a raw material for various processed products [24]. With these characteristics, patin fish is considered suitable for processing into fish meatballs.

Oyster mushrooms (*Pleurotus* sp.) are a type of edible mushroom widely cultivated worldwide. Oyster mushrooms are often used to supplement the nutritional content of carbohydrate-rich staple foods. This is because oyster mushrooms contain a relatively high protein content, ranging from 15–20% of dry weight. Oyster mushrooms are a type of wood-decaying mushroom with a higher nutritional content compared to other wood-decaying mushrooms. Every 100 grams of dried oyster mushrooms contains 10.5–30.4% protein, 1.7–2.2% fat, 56.6% carbohydrates, as well as 0.20 mg of thiamine, 4.7–4.9 mg of riboflavin, 77.2 mg of niacin, and 314 mg of calcium. Oyster mushrooms also contain 18 types of essential amino acids required by the body [17].

Liquid smoke is obtained through the distillation or condensation of the combustion products of organic materials containing lignin, cellulose, hemicellulose, and other carbon compounds, either through direct or indirect combustion [9]. The production process involves three main stages: pyrolysis, condensation, and redistillation [18]. Pyrolysis is the thermal decomposition of organic materials with or without a limited amount of oxygen, breaking down hydrocarbon molecules into simpler molecules and producing three fractions: gas, liquid, and solid [22]. The solid particles in the smoke condense into a liquid, known as liquid smoke. This product is commonly used as an alternative to conventional smoking techniques [2]. The main components of liquid smoke include phenolic compounds, organic acids, and carbonyl compounds, which act as flavor enhancers and provide antibacterial and antifungal properties [10]. Raw materials commonly used in the production of liquid smoke include coconut shells, wood, oil palm trunks, sawdust, and various other types of biomass.

1.3. Research Objective

This study aimed to determine the effect of coconut shell liquid smoke concentration used as a boiling medium on the sensory characteristics of catfish meatballs substituted with

oyster mushrooms at Day 0 and on the total bacterial count of the best-sensory treatment during storage at refrigerated temperature (6°C) and room temperature (30°C).

2. MATERIALS AND METHODS

2.1 Material and Tools

The ingredients used in this study were catfish meat, oyster mushrooms, tapioca flour, egg whites, shallots, garlic, salt, sugar, pepper, oil, and ice cubes purchased at a nearby market, as well as coconut shell liquid smoke purchased from an online store. Analytical materials included Plate Count Agar (PCA) and vacuum packaging.

The equipment used in meatball processing included a Mitochiba CH-200 chopper, a stove, a boiling pot, a digital scale, a cutting board, a knife, and a spoon.

2.2 Experiment Design and Data Analysis

This study used a completely randomized design with coconut shell liquid smoke concentrations of 0.5%, 1%, 1.5%, and 2% (v/v). Sensory evaluation was conducted at Day 0 using a hedonic scale with 30 semi-trained panelists who evaluated color, aroma, taste, and texture. Sensory data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a 5% significance level. The treatment with the best sensory characteristics was selected using the De Garmo Effectiveness Index. The selected treatment was then analyzed for total bacterial count during 12 days of storage at 6°C and 30°C.

2.3 Implementation of Research

2.3.1 Meatballs processing

The meatball mixture was prepared using 560 g of catfish and 140 g of oyster mushrooms. The mixture was ground in a food processor with 25 g salt, 85 g ice cubes, and 3 g STPP. Additional ingredients (60 g tapioca flour, 15 g sugar, 78 g egg whites, 30 g shallots, and 4 g pepper) were then added and homogenized. The mixture was shaped into 3 cm diameter balls and boiled at 100°C for 15 minutes, until they floated. The cooked meatballs were drained and cooled to room temperature, then vacuum-packed for sensory evaluation and total bacterial count analysis.

2.4 Observation

2.4.1. Sensory Analysis

Sensory evaluation of the catfish and oyster mushroom meatballs was conducted at Day 0 using the hedonic scale scoring method with 30 semi-trained panelists. The observed parameters were color, aroma, taste, and texture. Each parameter was rated on a scale of 1 to 6.

Table 1. Rating Scale Criteria

Rating	Description
1	Strongly dislike
2	Dislike
3	Somewhat dislike
4	Somewhat like
5	Like
6	Strongly like

2.4.2. Total Bacterial Count Analysis

Catfish meatballs substituted with oyster mushrooms and exhibiting the best sensory characteristics were vacuum-packed for total bacterial analysis during 12 days of storage. The meatballs were stored at refrigerated temperature (6°C) and room temperature (30°C), and observations were conducted at 4 day intervals throughout the 12 day storage period.

Total bacterial count was determined according to SNI 2332.3:2015. Samples were collected aseptically, and 25 g of meatball sample was placed in a sterile bag. Butterfield's phosphate-buffered diluent (225 mL) was added and the sample was homogenized for 2 min to obtain a 10^{-1} dilution. A 10 mL aliquot was transferred into 90 mL of the same buffer to obtain 10^{-2} , followed by serial tenfold dilutions (10^{-3} , 10^{-4} , 10^{-5} , and further as required), with each dilution mixed at least 25 times. One milliliter from each selected dilution was transferred into sterile Petri dishes in duplicate, followed by addition of 12–15 mL Plate Count Agar (PCA) at approximately 45°C. The plates were gently mixed, allowed to solidify, inverted, and incubated at $35 \pm 1^\circ\text{C}$ for 48 ± 2 h for mesophilic microorganisms. Colonies were counted on countable plates and reported as CFU/g; the result was expressed as $\log_{10}$ CFU/g using the colony count and corresponding dilution factor.

3. RESULT AND DISCUSSION

3.1. Sensory Analysis of Meatballs

Table 2. Average Sensory Scores

	Concentration of Liquid Coconut Shell Smoke (%v/v)			
	0.5	1	1.5	2
Color	4.37 ^a ±1.07	4.37 ^a ±0.96	4.43 ^a ±0.97	4.30 ^a ±1.18
Aroma	4.50 ^{ab} ±0.82	4.67 ^a ±0.80	4.90 ^a ±0.92	4.47 ^b ±0.82
Taste	4.73 ^b ±0.78	4.93 ^a ±0.83	5.30 ^a ±0.60	4.90 ^{ab} ±0.84
Texture	4.93 ^a ±0.87	4.97 ^a ±0.89	4.83 ^a ±0.99	4.90 ^a ±1.06

Note: Mean values followed by different letters within the same sensory parameter indicate statistically significant differences ($p \leq 0.05$). Means sharing the same letter are not significantly different. Hedonic scale: 1 = strongly dislike, 2 = dislike, 3 = somewhat dislike, 4 = somewhat like, 5 = like, and 6 = strongly like

The results in Table 2 show that there was no significant difference among coconut shell liquid smoke concentrations in the acceptability of color ($p > 0.05$), as indicated by the identical superscript letter “a” for all treatments. The relatively small differences among concentrations (0.5%, 1%, 1.5%, and 2%) were not sufficient to produce a statistically detectable change in color. This finding is consistent with [10], who reported no significant color differences in tuna fish balls treated with 0–3% liquid smoke, and [16], who found no significant effect of coconut shell liquid smoke at 1.33–3.33% on fish meatball color.

Table 2 shows a significant effect of coconut shell liquid smoke concentration on aroma preference. The 1.0% and 1.5% treatments (both superscript “a”) did not differ significantly from each other, whereas the 2% treatment (superscript “b”) received a significantly lower aroma score than the 1.0% and 1.5% treatments. The 0.5% treatment (superscript “ab”) was not significantly different from either group. The higher preference at 1.0–1.5% may be associated with the formation of desirable smoke-related volatile compounds, whereas excessive smoke

concentration may produce an aroma that is too strong for panelists to prefer.

Table 2 shows a significant effect of coconut shell liquid smoke concentration on taste preference. The 1.0% and 1.5% treatments (both superscript “a”) were not significantly different from each other, whereas the 0.5% treatment (superscript “b”) had a significantly lower taste score. The 2% treatment (superscript “ab”) was not significantly different from either the 0.5% or 1.0–1.5% treatments. A combination of phenolic compounds such as guaiacol and 4-methylguaiacol, along with carbonyl compounds, produced a flavor profile that was increasingly preferred by the panelists at liquid smoke concentrations of 0–1.5%. However, at 2%, the score decreased because excess phenolic compounds tend to cause a bitter taste and an astringent sensation [15]. This is consistent with a study [23] stating that a 1% liquid smoke concentration in milkfish meatballs yielded the highest hedonic score, whereas higher concentrations were less preferred due to an overly strong smoky flavor and astringent character.

The results in Table 2 show no significant differences in texture acceptability among coconut shell liquid smoke concentrations, as all treatments share the superscript “a” ($p > 0.05$). This is because liquid smoke does not contain compounds that directly interact with the product's gel matrix. The texture of the meatballs is determined by the gel structure of patin fish myofibrillar proteins, the gelatinization of tapioca starch, and the polysaccharides from oyster mushrooms that form during heating [1]. This is consistent with a study [10] stating that adding liquid smoke to the boiling water did not result in significant differences among treatments regarding texture parameters. A study [16] also found that the concentration of coconut shell liquid smoke had no significant effect on the texture of fish meatballs.

3.2. Analysis of the Optimal Decision

Decision analysis provides the basis for selecting the best treatment based on observational and analytical results. The De Garmo Effectiveness Index method determines the best treatment by calculating the average relationship between weight values and treatment values; the treatment with the highest value is considered best [21]. The best results were obtained by adding 1.5% coconut shell liquid smoke, which yielded the following organoleptic characteristics: color 4.43 (somewhat liked), aroma 4.90 (liked), taste 5.30 (liked), and texture 4.83 (liked).

3.3. Analysis of Total Bacterial Count Meatballs

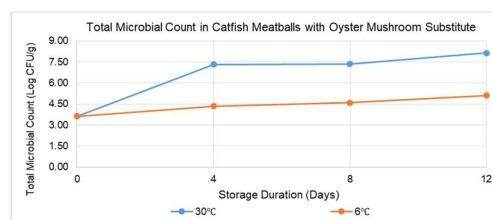


Figure 1. Total bacterial Count in catfish meatballs with oyster mushroom substitution

The results shown in Figure 1 indicate that storage at 30°C caused total bacterial counts to increase by 3.7 Log Cycle on day 4, 0.04 Log Cycle on day 8, and 0.79 Log Cycle on day 12. This increase occurs because room temperature provides optimal conditions for mesophilic bacteria, which generally dominate processed meat and fish products. At this temperature, microbial

metabolism is highly active, resulting in a short lag phase and rapid increases in bacterial colonies during storage [14]. This is consistent with a study [7] stating that meatballs stored at room temperature experience a rapid increase in total bacteria, resulting in a relatively short shelf life. High water activity and the availability of protein as a nutrient substrate accelerate this rapid growth.

Figure 1 shows that at 6°C, the total bacterial count increased more slowly: 0.72 log cycles on day 4, 0.25 log cycles on day 8, and 0.52 log cycles on day 12. Low temperatures slow intracellular enzyme activity, thereby significantly reducing the rate of cell division. Nevertheless, some psychrotrophic bacterial groups still survived and grew under these conditions, so the total bacterial count continued to increase throughout the storage period [21]. This is consistent with a study [20] on the application of coconut shell liquid smoke to frozen-stored smoked fish, which showed that low temperatures significantly slowed microbiological changes because microorganisms' metabolic activity decreased to very low levels.

4. CONCLUSION

The concentration of coconut shell liquid smoke had a significant effect on the organoleptic parameters of taste and aroma but did not have a significant effect on the organoleptic parameters of color and texture. The 1.5% coconut shell liquid smoke treatment was the best treatment, producing catfish meatballs with oyster mushroom substitution that were most preferred by the panelists, with color scores of 4.43 (somewhat liked), aroma 4.90 (liked), taste 5.30 (liked), and texture 4.83 (liked). Coconut shell liquid smoke concentration was found to inhibit increases in total bacteria, although it did not completely stop microbial growth during storage.

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