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Natural preservation of Indian mackerel (*Rastrelliger kanagurta*) using bio-oil and chitosan-based edible coatings

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Abstract

Background: This study explores the initial application of chitosan combined with bio-oil to create an edible coating for preserving Indian mackerel (*Rastrelliger kanagurta*).

Methods: Chitosan was extracted from shrimp shells, and bio-oil was produced through the pyrolysis of coconut shells at 350 °C and subsequently purified using adsorption or distillation. Various formulations of the edible coating were created with chitosan concentrations of 0%, 2%, 4%, 6%, and 8%, combined with bio-oil concentrations of 3% and 5%. The Indian mackerel samples were immersed in the edible coating for 20 minutes to promote preservation. The quality and freshness of the Indian mackerel were assessed every 24 hours over a three-day storage period. GC-MS analysis confirmed the presence of phenolic chemicals and acetic acid in the bio-oil, both of which have antibacterial effects.

Results: The findings indicated that the optimal edible coating formulation contained 5% bio-oil and 8% chitosan, resulting in a total volatile base (TVB) of less than 30 mg N 100 g of sample and a total plate count (TPC) of less than 1×10^3 CFU/mL.

Conclusion: The use of chitosan and bio-oil obtained from coconut shells as an edible coating is efficient in preserving mackerel by inhibiting microbial growth and maintaining sensory quality. Pyrolysis, followed by adsorption purification or distillation, provides a natural and environmentally beneficial alternative to synthetic fish preservatives.

Keywords: Antibacterial agents, Bio-oil, Chitosan, Edible films, Pyrolysis

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Introduction

According to the Food and Agriculture Organization (FAO), Indonesia is the world's largest coconut producer, accounting for approximately 19 million tons per year, or 31% of global coconut production in 2016. A mature coconut contains 35% husk, 12% shell, 28% endosperm, and 25% water (1,2). While the edible components, such as coconut flesh and water, are widely utilized in the food and beverage industry, the coconut shell is often considered agricultural waste. However, this waste has significant potential for conversion into valuable products, including bio-oil obtained through the pyrolysis process (3,4).

Pyrolysis is a thermochemical decomposition process that occurs at high temperatures in an oxygen-free or low-oxygen environment. This process is one of the most efficient and environmentally friendly biomass conversion methods for creating biofuels and bio-based compounds (5,6). The breakdown of complex organic polymers into smaller molecular compounds generates three main products: biochar, syngas, and bio-oil. This oil contains a variety of oxygenated organic compounds, including acids, phenols, and ketones, which affect its physical, chemical, and sensory properties (7). The type of biomass used in pyrolysis significantly affects the composition and quality of the bio-oil produced. This is due to the variations in the lignin, cellulose, and hemicellulose content of coconut shells which in turn affect the number of chemical compounds produced. These changes affect the bio-oil's thermal stability, energy content, and potential applications. As a result, it offers a promising alternative to bio-based chemicals and fuels in sustainable energy systems.

Coconut shells are considered a primary raw material for bio-oil production, as their hemicellulose, cellulose, and lignin, content decomposes to yield antimicrobial compounds such as alcohols, phenols, aldehydes, carbonyls, ketones, and pyridines. Bio-oil is rich in phenolic compounds and acids that have both antibacterial and antioxidant properties, with phenolic compounds representing approximately 14.87% of its composition (8,9). These phenolic compounds can inhibit the growth of bacteria and fungi, making bio-oil an effective preservative. However, pyrolysis can also produce undesirable byproducts, including tar and

benzo[a]pyrene, which must be separated to yield a pure product. The concentration of benzo[a]pyrene in bio-oil can be reduced through distillation or adsorption using activated zeolite. In certain countries, such as Germany, the maximum allowable concentration of benzo[a]pyrene in products is limited to 1 ppb (10,11).

Bio-oil has a wide range of applications, including its use as a food preservative, particularly for Indian mackerel (*Rastrelliger kanagurta*). Fish possess a high moisture content, creating an ideal habitat for the growth and proliferation of spoilage bacteria and other microbes. This disease accelerates biochemical and microbiological degradation, rapidly reducing fish qualityby altering its texture, color, smell, and overall nutritional value (12,13). Moisture content, ambient temperature, and unhygienic environments can exacerbate the problem. Bio-oil-based preservatives derived from coconut shell pyrolysis can be used to enhance the shelf life of Indian mackerel safely. Indian mackerel remains fresh for only 15 hours at room temperature, and untreated fillets become unsafe to consume after 12 hours, exceeding the National Standardization of Indonesia SNI 2332.3-2015 limit of 5×10^5 CFU/g after 24 hours. This situation results in much waste, particularly during periods of high fish production. Natural preservation methods often prove inadequate due to the short shelf life caused by ongoing physiological processes (14). The difficulty of preserving fish, especially during high production periods, leads to significant waste. This study emphasizes the crucial need for efficient bio-oil-based preservation solutions to extend the shelf life of Indian mackerel while reducing food waste.

This study focuses on Indian mackerel (*Rastrelliger sp.*) due to its substantial economic value in Indonesia, where it is regularly fished and consumed domestically, and much of it is exported. Preserving this fish, rich in omega-3 fatty acids and other critical elements, is essential for maintaining its nutritional value over an extended period. Its distinct physical characteristics, including fat content and texture, have a significant impact on its shelf life, making it a perfect choice for evaluating preservation methods. In Indonesia, traditional techniques such as high-temperature brine boiling (70–105 °C) are often used; however, these processes could eliminate important component including lysine. To address these difficulties, bio-oil obtained from the pyrolysis of coconut shells or corncobs has been proposed as an alternative preservative. Bio-oil-based preservation, which might be improved with chemicals that prevent microorganisms from developing, appears to be a promising strategy to extend the shelf life of Indian mackerel, reduce food waste, and help sustain this fish resource (15,16).

Developing an edible coating from bio-oil reinforced with chitosan is essential for preventing changes in nutritional and sensory qualities while enhancing the quality of bio-oil as a fish preservative. Chitosan contains positively charged amino ($-NH_2$) functional groups that can bind to negatively charged bacterial cell walls. Incorporating chitosan into pyrolysis-derived bio-oil results in excellent preservation conditions for meatballs, with 1.5% chitosan and 5% bio-oil preserving quality for up to 54 hours with a total plate count (TPC) of 4.91×10^4 CFU/g (17,18). Higher concentrations of chitosan and bio-oil result in lower TPC values. The amino groups in chitosan, when dissolved in an acid (such as the dilute acids found in bio-oil), become protonated to form cationic amino groups, disrupting the membranes of Gram-negative bacteria and inhibiting DNA replication (19). At a concentration of 3.5%, chitosan has been shown to preserve smoked salmon and exhibit antibacterial qualities by preventing bacterial growth for up to nine days (20). Given this background, further research is warranted to investigate the effects of integrating pyrolysis-derived bio-oil with chitosan as an edible coating for the natural preservation of Indian mackerel.

Materials and Methods

Materials

Coconut shells were obtained from the traditional market in Malang City, East Java, Indonesia. *Salmonella-Shigella* agar (SSA) nutrient agar (NA) media were purchased from HiMedia. All chemicals used in this study were sourced from Merck, including NaOH, PCA, boric acid, HCl, PP, and $K_2Cr_2O_7$, whereas Sigma-Aldrich provided the Folin-Ciocalteu reagent, Na_2CO_3, and Conway indicator. Boric acid and perchloric acid (PCA) were employed for chemical analyses. The Folin-Ciocalteu reagent and sodium carbonate (Na_2CO_3) were used to determine the phenolic content. Titration and TVB-N tests were conducted using hydrochloric acid (HCl), phenolphthalein (PP), the Conway indicator, and potassium dichromate ($K_2Cr_2O_7$) to evaluate the freshness of the fish throughout storage.

Pre-treatment process

The coconut shells were cleaned and cut into 4×4 cm segments. The moisture content of the coconut shells was reduced by drying them in an oven at 100 °C for one hour. The chemical composition of the coconut shells was analyzed to determine their potential as lignocellulosic biomass feedstock. The investigation demonstrated that the coconut shells had significant potential as a primary source of fiber, containing 26.4847% cellulose. Lignin, the primary component serving as a natural adhesive in the lignocellulose matrix, constituted 29.6241% of the total, whilst hemicellulose, which offers structural support, accounted for 11.7177%. The ash percentage was negligible at 0.0202%, indicating a minimal presence of inorganic materials. The moisture content was reported as 7.1924%, indicating a moderate degree of material humidity. These findings confirm that coconut shells are lignocellulose rich raw materials with considerable promise for diverse

biomass applications.

Additionally, chitosan derived from prawn shells was used in the development of the edible coating. Indian mackerel (*Rastrelliger kanagurta*) was chosen as the test specimen to evaluate the efficacy of the coating in fish preservation. Chitosan extraction was performed using 0.1 N NaOH, and distilled water was used for sample cleaning and solution formulation. Vaseline was utilized to reduce contamination during storage.

Bio-oil production process

Bio-oil was produced through the pyrolysis of 500 g of raw material at 350 °C for 120 minutes (21). The resulting bio-oil contained a significant amount of tar, which affected its clarity, so it required purification. We used two methods to purify and separate the bio-oil from impurities: distillation and adsorption. In the distillation process, 250 mL of the bio-oil sample was heated to temperatures over 100 °C using an electric heater. For the adsorption process, 80 g of activated zeolite was treated with 0.5 M H_2SO_4 at a volume of 1000 mL for three hours. The zeolite was subsequently washed with distilled water until a neutral pH was attained and then dried in an oven at 110 °C for 24 hours. The adsorption procedure was performed over 48 hours, utilizing an adsorbent-to-bio-oil ratio of 80 g to 200 mL. Following the procedure, pH (ASTM E70), density (ASTM D4052), viscosity (ASTM D445), total phenols (Folin-Ciocalteu method), and total acids (ASTM D664) were used to analyze the products. The chemicals in the bio-oil were analyzed using the Gas Chromatography-Mass Spectrometry (GC-MS) QP2010 Plus (Shimadzu, Japan).

Edible coating preparation

The edible coating was produced by combining food-grade chitosan powder with bio-oil and distilled water. First, a bio-oil solution was made by mixing bio-oil at 3% and 5% concentrations with distilled water. Chitosan was subsequently added to the solution at concentrations of 0%, 2%, 4%, 6%, and 8% by mass per 100 mL of bio-oil solution. The mixture was agitated with a magnetic stirrer at 50 °C for 90 minutes until a homogenous solution was obtained. To preserve Indian mackerel, the cleaned flesh was immersed in a solution containing 5% bio-oil and 8% chitosan for 20 minutes. The efficacy of this preservation method was evaluated against sodium benzoate, a synthetic preservative utilized as a positive control (+), while untreated mackerel functioned as a negative control (-). A 1 g sample of the mackerel surface was collected for examination following the coating process. The studies performed on the preserved Indian mackerel encompassed total volatile base (TVB) as per ISO 2171-2007 and colony counts in accordance with ISO 4833-1:2013.

Results

Catalytic pyrolysis of coconut shells is a common method for producing bio-oil. However, the bio-oil produced from this technique is not entirely pure as it retains deleterious substances such as tar, which are influenced by the operational parameters of the pyrolysis process. Consequently, food products cannot directly employ bio-oil without a purification step that diminishes or eradicates these detrimental constituents. Various cleaning techniques, including distillation and adsorption utilizing activated zeolite, have been used to enhance bio-oil quality. The quality of the final product is significantly affected by the selection of an effective purification process. The product can be evaluated based on its pH, density, viscosity, total acid content, yield, color, aroma, and the chemical composition of the bio-oil.

Table 1 presents the quality analysis of bio-oil derived from coconut shells, prior to and after purification. This research evaluates the effectiveness of several bio-oil purification techniques by comparing the characteristics of the purified bio-oil with the Indonesian National Standard (SNI) number 8985:2021 for bio-oil quality, set forth by the National Standardization Agency (BSN) in 2021. This study provides significant insights into how purification enhances the quality of bio-oil, demonstrating its suitability for many industrial applications, including its potential as a bio-based chemical feedstock.

In order to determine the primary chemical components of the bio-oil following the purification procedure, the compound composition was examined using gas chromatography-mass spectroscopy (GC-MS) based on their molecular weight and volatility. This provides precise information regarding the composition of bio-oil. Post-distillation GC-MS analysis (Figure 1) demonstrates that distillation efficiently eliminates many high-molecular-weight compounds while enhancing the content of phenolic and carbonyl compounds, which possess antibacterial properties and contribute to preservation. Post-distillation GC-MS with activated zeolite (Figure 2) indicates that this technique is more effective in eliminating undesirable constituents and adsorbing non-polar compounds. In contrast to distillation, adsorption significantly improves the stability of beneficial components in bio-oil, thus enhancing product quality and sustainability.

Table 1. Analysis results of bio-oil from coconut shells

Parameter	Purification			SNI 8985:2021
	Before	After distillation	After adsorption	
pH	3.4	2.3	1.52	1.5–2.75
Density (g/mL)	1.0229	1.0118	1.0222	> 1.005
Viscosity (cP)	1.4851	1.3281	1.4273	-
Total acid (%)	14.2590	11.8556	13.0102	8–15
Yield (%)	22	46	37	-
Color	Sepia-brown	Olive-yellow	Brown-red	Brown yellow
Aroma	Very pungent	Pungent	Pungent	-

The results of the gas chromatography-mass spectrometry (GC-MS) test on bio-oil are shown in Table 2, which demonstrates how the component composition changed after the purification process. Table 2 presents comprehensive data on the principal chemicals discovered in bio-oil and their corresponding relative abundance (area) post-purification.

Chitosan was incorporated into bio-oil obtained from coconut shells to examine its effect on the quality of the bio-oil. The variables observed included pH,

Table 2. Composition of bio-oil based on GC-MS analysis

No.	Compound	Concentration after purification (%)	
		By distillation	By adsorption
1	Phenol	23.68	24.66
2	Furfural	17.14	13.34
3	Phenol, 2-methoxy-, mequinol	9.94	8.62
4	2-Amino-1,3-propanediol, 1,2-ethanediol, monoformate, acetic acid	8.62	11.67
5	2-Pentanone, 4-hydroxy-4-methyl-	8.46	5.94
6	Propanoic acid	7.97	7.96
7	2-Methoxy-5-methylPhenol	2.85	2.89
8	Phenol, 4-ethyl-2-methoxy-, 5-isopropyl-3,3-dimethyl-2- methylene-2,3-dihydrofuran	1.97	2.2
9	Phenol, 2-methyl-	1.69	1.65
10	2-Furancarboxaldehyde, 5-methyl-	1.05	1.07
11	p-Cresol	1.02	1.6
12	2-Cyclopenten-1-one, 2-methyl-	0.91	0.69
13	Ethanone, 1-(2-furanyl)-	0.9	1.84
14	Butanoic acid	0.83	1.07
15	Cyclopentanone	0.73	0.81
16	Phenol, 2,5-dimethyl-	0.53	0.53
17	2-Cyclopenten-1-one, 2,3-dimethyl-	0.5	0.63
18	Phenol, 4-ethyl-, phenol, 3-ethyl-	0.5	0.99
19	2-Cyclopenten-1-one, 2-methyl-	0.34	0.26
20	Phenol, 2-methoxy-3-methyl-, 2-methoxy-5-methylPhenol, creosol	0.29	0.24
21	Propanal	0.27	0.55
22	2-Cyclopenten-1-one, 2,3-dimethyl-	0.24	0.21
23	4-Hepten-3-one, 4-methyl-, 4-hexen-3-one, 4,5- dimethyl-, 4-ethyl-2-hydroxycyclopent-2-en-1-one	0.24	0.25
24	2-Butanone, 1-(acetyloxy)-, propanoic acid, 1-methylpropyl ester	0.22	0.32
25	Phenol, 2-methoxy-4-propyl-	0.18	0.23
26	Spirohexan-4-one, 5,5-dimethyl bicyclo[3.3.1]nonane, cyclohexanone, 3-ethenyl-	0.16	0.15
27	Phenol, 2,3-dimethyl-	0.16	0.16
28	Other	8.61	9.48
Total		**100**	**100**

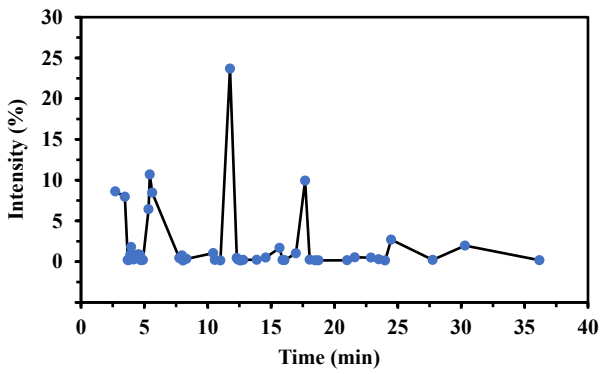

Figure 1. GC-MS analysis results of bio-oil after distillation

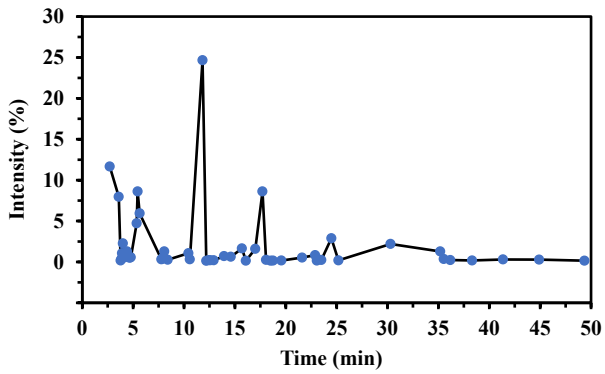

Figure 2. GC-MS analysis results of bio-oil after adsorption

density, viscosity, total acidity, and functional groups. These are crucial for evaluating the physical and chemical characteristics of the produced edible coating. Consequently, these properties influence the coating's effectiveness as a preservative agent. The results presented in Table 3 are crucial for understanding the effects of chitosan on the characteristics of bio-oil and its potential application in food preservation. A prior investigation into sustainable packaging materials revealed that varying concentrations of chitosan (0%, 2%, 4%, 6%, 8%, and 10%) incorporated into biofoam derived from tofu pulp starch, corn cobs, and sugarcane base significantly influenced the material's chemical and physical properties. These findings emphasize the importance of adjusting chitosan concentrations for specific material properties, hence supporting the technique of the present study (22).

Enzymatic and microbial activity in fish tissue leads to the formation of total volatile base (TVB), a compound resulting from protein degradationThe oxidation processes during storage contribute to increased TVB levels, which inversely correlate with fish freshness. An increase in TVB levels serves as a key indicator of fish quality deterioration, necessitating effective handling strategies to maintain freshness until it reaches the consumers. In this study, a coating technique utilizing bio-oil and an edible coating was applied to slow the increase in TVB levels and extend fish freshness. The effectiveness of this method was evaluated by measuring TVB levels after treatment (Table 4) to assess its impact on fish quality stability during storage.

Various methods of preservation were applied to Indian mackerel to evaluate their effects on bacterial growth, using sterile salt agar (SSA) and nutrient agar (NA) media. The determined treatments included edible coating, sodium benzoate, and both positive and negative controls. Edible coating and sodium benzoate were applied without additional media (NA) and with SSA medium. The positive and negative controls were assessed with and without preservatives, and with and without SSA medium. Table 5 presents the results of these investigations, demonstrating the impact of each treatment on bacterial proliferation in fish during storage.

Discussion

Coconut shell was selected as the primary raw material for bio-oil production due to its high lignin content. The degradation of lignin releases phenols that enhance flavor and safeguard bio-oil from oxidation. The cellulose (26.48%) and hemicellulose (11.72%) composition of the shell produces organic acids, including acetic acid, which have antibacterial characteristics. Additionally, lignin (29.62%) enhances the antioxidant properties of the bio-oil. The little ash content (0.02%) indicates a scarcity of inorganic minerals. Conversely, the moderate moisture content of 7.19% is crucial, as excessive moisture reduces the quality of bio-oil. Combustion of coconut

Table 3. Results of edible coating analysis

Edible coating			Parameter			
Purification by	**Bio-oil concentration**	**Chitosan concentration**	**pH**	**Density (g/mL)**	**Viscosity (Cst)**	**Total acid (%)**
Distillation	3%	0	2.22	1.0000	0.0011	3.2323
		2%	3.75	1.0052	0.0312	1.8277
		4%	3.92	1.0111	0.1142	0.6128
		6%	3.97	1.0120	0.9859	0.8178
		8%	4.04	1.0135	4.9532	1.2285
	5%	0	2.12	1.0010	0.0011	1.3145
		2%	3.54	1.0052	0.1282	0.6092
		4%	3.90	1.0094	0.0715	1.2235
		6%	3.90	1.0139	0.2932	1.1266
		8%	3.95	1.0168	2.1768	0.6317
Adsorption	3%	0	2.18	1.0021	0.0011	2.6317
		2%	3.64	1.0036	0.0471	1.1152
		4%	3.77	1.0094	0.0941	2.6509
		6%	3.85	1.0124	2.9693	1.6372
		8%	3.89	1.0130	2.9693	1.6372
	5%	0	2.08	1.0026	0.0011	2.8357
		2%	3.61	1.0021	0.0812	2.6317
		4%	3.62	1.0038	1.7984	1.6223
		6%	3.90	1.0129	1.0597	1.2277
		8%	3.78	1.0200	3.1218	0.6182

Table 4. Freshness test results for Indian mackerel using the TVB method

Edible coating			TVB (mg N/100 g)			
Purification by	**Bio-oil concentration**	**Chitosan concentration**	**0 hours**	**24 hours**	**48 hours**	**72 hours**
Distillation	3%	0	1.8209	3.9612	5.4067	9.2446
		2%	1.6808	3.3897	5.6028	7.6198
		4%	1.7649	2.9695	4.2021	5.3227
		6%	1.7929	3.3617	5.5188	5.8829
		8%	1.6808	4.4822	5.0425	7.3733
	5%	0	1.6808	4.2021	8.0400	9.5248
		2%	1.7929	3.7819	5.6028	7.6478
		4%	1.6808	3.2496	4.7624	6.7234
		6%	1.6808	3.3617	4.5663	7.2836
		8%	1.8629	3.5858	5.0425	7.1296
Adsorption	3%	0	1.6808	3.3617	5.6924	6.7234
		2%	1.7649	3.2496	4.2021	5.7429
		4%	1.6808	3.6138	5.0425	6.4432
		6%	1.8489	4.0060	6.2191	7.0035
		8%	1.7929	2.8014	4.4542	5.6028
	5%	0	1.7929	3.4177	7.2220	9.1466
		2%	1.8489	3.3897	6.8914	7.8159
		4%	1.5212	2.8854	5.7877	6.8746
		6%	1.5968	2.9415	4.8576	6.1183
		8%	1.5688	3.0115	4.0620	4.9865
Sodium benzoate, control (+)			12.0460	20.9265	22.3832	30.0310
Without treatment, control (−)			27.4537	31.8463	33.3367	35.9196

Table 5. TPC test results for Indian mackerel preservation

Preservative type	Medium type	Colony count (CFU/mL)			
		Day 0	**Day 1**	**Day 2**	**Day 3**
Edible coating	NA	1×10^3	1×10^3	1×10^3	1×10^3
	SSA	1×10^3	1×10^3	1×10^3	1×10^3
Sodium benzoate, control (+)	NA	1×10^3	1×10^3	4.22×10^5	6.78×10^5
	SSA	1×10^3	1×10^3	5×10^5	7×10^5
Without, control (−)	NA	1×10^3	1×10^3	1.49×10^6	2.08×10^6
	SSA	1×10^3	1×10^3	7×10^5	3×10^6

shell produces significant compounds, including phenol (23.68–24.66%) and acetic acid (8.62–11.67%). These findings demonstrate that coconut shell can be utilized to produce bio-oil with antibacterial and antioxidant properties (23).

Effects of different purification methods on bio-oil quality

The bio-oil produced from pyrolysis is not entirely pure and may contain harmful compounds influenced by processing conditions, making it unsuitable for direct application in food products (24). High-quality bio-oil can be obtained through purification processes that reduce or eliminate these harmful compounds. Common purification methods include distillation and adsorption using activated zeolite (25,26). The chosen purification method significantly affects the parameters of the resulting bio-oil, as shown in Table 1. The pH of the pyrolysis-derived bio-oil generally decreases after purification, falling from an initial value of 3.4 to 2.3 after distillation and to 1.52 after adsorption. This reduction is attributed to the evaporation of organic acids during purification, leading to increased concentrations of acetic acid and phenols. A higher total phenol concentration in the bio-oil is associated with a lower pH, indicating improved quality. The findings suggest that coconut shell-derived bio-oil is acidic, which influences its organoleptic properties and antimicrobial effectiveness as a preservative. The acidity of bio-oil is crucial to its preservation quality, as lower pH levels hinder the survival and growth of bacteria and microbes that could compromise the preservation process, thereby extending the shelf life of food products (27).

The density of pure bio-oil obtained through both distillation and adsorption processes corresponds to the SNI 8985:2021 standard for crude lignocellulose, which stipulates a minimum density of 1.005 g/mL. Distillation produces bio-oil with a density of 1.0118 g/mL, which is inferior to bio-oil purified through adsorption (1.0222 g/mL). The pyrolysis temperature affects the density of bio-oil, which exceeds that of water. Elevated temperatures typically result in an increased concentration of organic substances, including organic acids, phenols, and

carbonyls, all of which possess densities greater than that of water. The different parts of bio-oil, which separate into groups with varying water content and densities, along with its high viscosity, complicate the assessment of specific parameters, which can lead to inconsistent results. Consequently, pre-treatment processes such as dilution, separation, or filtration may be essential to ensure measurement accuracy. The processing parameters and pre-treatments utilized substantially affect the physicochemical composition of bio-oil. Density, a critical parameter, is typically determined using a digital density meter (ASTM D4052) or a pycnometer (ASTM D369). The density of bio-oil typically ranges from 0.95 to 1.23 kg/dm^3, showing how important accurate density measurements are for describing bio- oil quality (28,29).

The temperature conditions of the distillation process (90–100 °C) are higher than those of the adsorption process. This indicates that bio-oil obtained through distillation has a lower viscosity (1.3281 cP) compared to bio-oil produced through adsorption (1.4273 cP). Elevated temperatures promote molecular dispersion, hence reducing viscosity. Glass capillaries (ASTM D 445 or EN ISO 3104) are often used to measure viscosity, and the temperature is maintained below 50 °C to keep volatile compounds from evaporating. At 40 °C, bio-oil has a kinematic viscosity between 20 and 125 mm^2/s. Bio-oil produced through pyrolysis exhibits higher viscosity, corrosivity, and oxygen content compared to fossil fuels. Research on bio-oil derived from sugarcane indicates that viscosity remains stable regardless of variations in oxygen concentrations. The results indicate that processing factors influence bio-oil viscosity and that precise characterization requires standardized measurements. Understanding changes in viscosity is important for making bio-oil more useful, keeping its fuel properties stable, and making it more likely to be used as an alternative energy source (29,28).

The total acid content is a crucial chemical characteristic that determines the quality of the produced bio-oil. This study indicates that the total acid content of the bio-oil varies from 11.86% to 14.3%, aligning with the 8–15% range stipulated by SNI 8985:2021. Adsorption produces bio-oil with a greater total acid content compared to distillation. Due to the increased volatility of the compounds involved, distillation is typically more effective in reducing overall acid levels. Conversely, adsorption may not eliminate acidic chemicals, contingent upon the characteristics and capacity of the employed adsorbent. This is consistent with previous studies indicating that the combination of oxidized bio-oil with methanol and n-butanol reduces the total acid content from 61.4% to 10.1% and 12.3%, respectively. The esterification process is more effective with the removal of water, indicating that distillation is superior to adsorption in eliminating acidic compounds. In esterification without the elimination of water, carboxylic acids are not effectively transformed into esters (30).

The bio-oil yield from distillation was 46%, exceeding the 37% yield achieved using the adsorption method. The operating conditions significantly influence the bio-oil yield. Elevated temperatures typically accelerate reactions and facilitate the more rapid decomposition of feedstock relative to the heating rate. A rapid heating rate facilitates the production of volatile products, increases pressure, and reduces the duration of product residence in the reactor. This increases the yield, as the organic components of the source material decompose more rapidly. Similarly, the application of alkaline catalysts in hydrothermal liquefaction (HTL) highlights the significance of optimal working conditions. Alkaline catalysts, including Na_2CO_3, NaOH, K_2CO_3, and KOH, exhibit improved catalytic effectiveness, augmenting bio-oil yields by promoting the hydrolytic depolymerisation of biomacromolecules, including cellulose, hemicellulose, and lignin. These catalysts enhance decomposition efficiency, leading to higher bio-oil yields under optimized conditions, while non-alkaline catalysts such as H_3PO_4 and $FeCl_3$ produce lower yields due to carbonization or dehydration, underscoring the critical impact of reaction conditions on bio-oil production optimization (31).

The purified bio-oil had a yellow-brown color, which was more transparent than the dark brown-black tint of pyrolysis-derived bio-oil. The darker hue of pyrolysis bio-oil is likely due to the presence of carbonyl compounds and tar, which are generally black, poisonous, and composed of high-molecular-weight molecules. The refined bio-oil exhibited colors ranging from olive yellow to brown-red, consistent with the Food and Agriculture Organization (FAO) quality requirements that delineate a color range from yellow to pale brown-red. Distillation at around 100 °C efficiently removes dark-hued carbonyl compounds, but the adsorption method tends to capture darkening agents, including benzo[a]pyrene and tar, within the zeolite adsorbent's pores. The presence of solvents and catalysts yields organic liquid products exhibiting colors from light brown to light yellow. The catalyst retains its activity across three cycles, ensuring uniform product distribution and retaining the color of the organic liquid (32).

The compounds present in the purified bio-oil were analyzed using gas chromatography-mass spectrometry (GC-MS), revealing a predominant presence of phenols, furfural, carboxylic acids, acetic acid, and creosol, as detailed in Table 2 obtained from Figure 1 and Figure 2. The concentration of phenols in the bio-oil obtained through distillation was greater than that found in the adsorption process. An increase in phenol content corresponds with a decrease in pH, resulting in a more acidic bio-oil. The high phenol content in coconut shell bio-oil suggests its potential as an effective preservative and as a compound that inhibits lipid oxidation (33,34). Furfural is generated

from the pyrolysis of hemicellulose at temperatures between 200 and 260 °C. The acetic acid in the bio-oil is an organic acid formed from the decomposition of cellulose and hemicellulose in wood (35). Creosol exhibits stronger antibacterial properties than phenol. Analysis results confirmed that the purified bio-oil was free from benzo[a]pyrene and polycyclic aromatic hydrocarbons (PAHs), which are known carcinogens, making it safe for use as a natural preservative (36).

Effects of chitosan addition on edible coating

The edible coating, made from bio-oil and chitosan, serves as a natural and efficient preservative that improves food safety, quality, and shelf life while reducing adverse effects on health and the environment. Chitosan is a biodegradable polymer that comes from chitin. It is widely used in food coatings, as it is antibacterial, biocompatible, and biodegradable. Its antibacterial properties compromise bacterial membranes, helping food preservation (37). The concentrations of bio-oil and chitosan affect the physical properties of the edible coating, including its pH. According to Table 3, the optimal pH level for the edible coating was 4.04, achieved by mixing 3% distilled bio-oil with 8% chitosan. On the other hand, the edible coating with 5% bio-oil that had not been mixed with chitosan had the lowest pH of 2.08. The pH of the edible coating was relatively insensitive to changes in bio-oil concentration, whereas variations in chitosan concentration significantly affected pH levels. The pH of the edible coating increased with higher chitosan concentration. The increase is attributed to the neutral pH of distilled water and the intrinsically neutral characteristics of chitosan (pH 7–9), indicating that the incorporation of chitosan affects the pH of the edible coating (38).

The highest density of the edible coating was observed at a concentration of 3% distilled bio-oil combined with 8% chitosan, measuring 1.0135 g/mL. In contrast, the lowest density was recorded in the 3% distilled bio-oil with no chitosan added, which is 1 g/mL. The addition of chitosan to 5% bio-oil does not significantly differ from its addition to 3% bio-oil. The concentration of bio-oil does not notably affect the density of the edible coating, whereas the concentration of chitosan is directly proportional to the density. Since chitosan has a higher specific gravity than the solvent, its inclusion directly influences the density of the edible coating (18).

The highest viscosity of the edible coating was recorded at 4.9532 cSt for 3% distilled bio-oil combined with 8% chitosan. Conversely, the lowest viscosity was measured at 0.0011 cSt for 3% distilled bio-oil with no chitosan added. The viscosity of the edible coating increased with higher concentrations of chitosan due to its high positive charge, which generates repulsive forces. This caused the chitosan polymer, initially in a coiled form, to unfold into a straight chain, thereby increasing the viscosity of the solution (39). The highest total acid content found in the edible coating was 3.2323%, which occurred in 3% distilled bio-oil with no added chitosan. In contrast, the lowest total acid content was 0.6128%, observed in 3% distilled bio-oil combined with 4% chitosan. The concentrations of both bio-oil and chitosan did not significantly impact the total acid value of the edible coating. Instead, factors including the type and composition of the raw materials, along with the initial pH of the edible coating, played a more critical role in determining total acid levels. The inclusion of chitosan decreased the total acid content, as chitosan is neutral, and raised the pH of the bio-oil.

Effects of edible coating on total volatile base (TVB) in Indian mackerel (Rastrelliger kanagurta)

Total volatile base (TVB) measures the breakdown of proteins and oxidative reactions in fish muscle tissue caused by bacterial and enzymatic activity, which ultimately determines the freshness of the fish. An increase in TVB is associated with the activity of proteolytic enzymes produced by bacteria, which break down proteins into simpler nitrogen compounds and convert trimethylamine oxide into trimethylamine (40). A higher TVB value is inversely related to fish freshness. While freshness cannot be improved, it can be preserved. One effective preservation method is the application of an edible coating. The edible coating composed of a mixture of bio-oil and chitosan was applied to Indian mackerel (*Rastrelliger kanagurta*), and the resulting TVB values are presented in Table 4. The TVB values indicate the freshness of the fish, categorized into four criteria: 1) TVB < 10 mg N/100 g (very fresh), 2) TVB = 10–20 mg N/100 g (fresh), 3) TVB between 20–30 mg N/100 g (safe for consumption), and 4) TVB > 30 mg N/100 g (spoiled fish) (41). The results indicated that Indian mackerel preserved with the edible coating for 72 hours (3 days) remained in the very fresh category, with the lowest TVB value of 4.9865 mg N/100 g observed for the edible coating using adsorbed bio-oil at a concentration of 5% and an addition of 8% chitosan. The TVB values for Indian mackerel preserved with the edible coating on day 3 were lower than those of the control (+) group that used sodium benzoate as a preservative, which maintained freshness for only up to 72 hours. This indicated that preservation using the edible coating is significantly more effective than using sodium benzoate. Indian mackerel without any preservation, as shown by the control (−) group, lasted only 24 hours before being categorized as spoiled and unfit for consumption. These coatings demonstrate antimicrobial capabilities, effectively inhibiting *E. coli* and *S. typhimurium*, thereby serving as a cost-effective and sustainable alternative to conventional approaches such as vacuum packaging (42). In addition, edible coatings made from biopolymers, including proteins and polysaccharides, reduce the need for synthetic additives and plastic packaging,

which is consistent with global goals for sustainability (43). Research on Indian mackerel treated with bio-oil and chitosan substantiates that edible coatings surpass sodium benzoate in preserving freshness, highlighting their viability as an environmentally sustainable food preservation method (44).

The edible coating that produced the lowest TVB values was then used for microbiological quality testing using the total plate count (TPC) method. Two types of media were utilized for the TPC test: selective media (*Salmonella-Shigella* agar, SSA) and a general medium (nutrient agar, NA). The results of the TPC test for Indian mackerel preserved with the edible coating using 5% bio-oil and 8% chitosan are presented in Table 5. The results from the total plate count (TPC) test revealed that Indian mackerel samples preserved with the edible coating maintained an average colony count of 1×10^3 CFU/mL on both nutrient agar (NA) and *Salmonella-Shigella* agar (SSA) throughout the testing period. Bacterial counts for Indian mackerel preserved with sodium benzoate increased significantly by days three and four on both media types. Indian mackerel samples without any preservation showed the highest bacterial counts on days 3 and 4. The maximum allowable total plate count for all fish and seafood, including mollusks and echinoderms, is 5×10^5 CFU/mL (45). The microbiological quality testing demonstrated that Indian mackerel preserved with the edible coating and sodium benzoate after three days of storage met the safety standards for consumption, while unpreserved Indian mackerel was classified as unfit for consumption. A similar trend was noted in chikuwa, where samples subjected to an edible coating, specifically the T4 formulation, maintained freshness for as long as 168 hours. Microbiological studies showed that the total plate count (TPC) and most probable number (MPN) of *E. coli* in samples treated with T4 stayed within the acceptable limits set by the Indonesian National Standards (SNI) for up to 120 hours. The TPC values did not exceed 4.72×10^4 CFU/g, and the *E. coli* levels were 460 MPN/g, indicating that the edible coating inhibits the growth of microbes and extends shelf life (43).

This study highlights the significant potential of an edible coating consisting of bio-oil and chitosan in reducing food waste and enhancing sustainable food storage methods. The findings indicate that this method of preserving Indian mackerel significantly extends its shelf life, maintaining freshness for up to 72 hours, compared to traditional preservatives such as sodium benzoate. This method employs bio-oil from coconut shells, a renewable biomass that encourages waste reduction and the utilization of sustainable resources. In addition, using natural ingredients, including bio-oil and chitosan, this bio-oil fits with the growing need for methods of preserving food that are safe, good for the environment, and break down naturally. Consistent with previous research, this edible covering demonstrates efficacy in suppressing microbial proliferation and maintaining food quality. Future research could investigate other biomass sources for making bio-oil, including agricultural waste and other byproducts, to make the process last longer. Putting this coating on other foods that spoil quickly, including fruits, vegetables, or seafood, could make this environmentally friendly way of preserving food even more useful. This would enhance the sustainability of the food chain and mitigate the environmental impact of food processing and packaging (44,46,47).

Conclusion

Coconut shell was selected as the feedstock for bio-oil due to its high lignin concentration, which generates phenols that serve as antioxidants and flavor enhancers, together with its cellulose and hemicellulose, which make organic acids and carbonyls. The distillation and adsorption of bio-oil using zeolite increased its phenol concentration, reduced its pH, and enhanced its antibacterial efficacy. Distillation was significantly more efficient in lowering total acid concentrations and generating bio-oil with enhanced yield and coloration. The bio-oil and chitosan-based edible coating improved food safety and prolonged shelf life, surpassing sodium benzoate in the preservation of Indian mackerel, as indicated by decreased TVB values and lower bacterial colony counts, thereby meeting consumption standards for up to three days. This environmentally sustainable preservation technology mitigates food waste and fosters sustainable practices in food processing.

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Competing interests

The authors declare that they have no conflict of interest regarding the publication of the present article.

Ethical issues

Not applicable.

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