



Enhancing Milkfish Smoking with Mangrove Leaf Liquid Smoke Powder: Chemical and Microbiological Insights

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ABSTRACT

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antioxidant properties, food preservation, gas chromatography-mass spectrometry (GC-MS), liquid smoke, mangrove leaf, microbiological analysis, proximate analysis, sustainable processing

This study aims to develop the use of liquid smoke powder from mangrove leaf for smoking milkfish (*Chanos chanos*) using an electric oven. The experimental research method was through the manufacture of liquid smoke powder from mangrove leaf and the application of liquid smoke powder (with a concentration of 2.5%, 5%, 7.5% and 10%), on boneless smoked milkfish, and chemical analysis of liquid smoke powder with GC-MS, SEM analysis of liquid smoke powder, proximate analysis of smoked milkfish, Total Volatile Base (TVB) analysis, microbiological test (Total Plate Count/TPC) and organoleptic test. The results indicated that soaking milkfish in 5% liquid smoke was optimal, achieving appearance, aroma, taste, and texture scores of 7.8, 8.2, 7.75, and 8.25, respectively. Proximate analysis showed water content of 35.42%, protein 32.83%, fat 6.40%, and ash 5.12%. Microbiological tests revealed a Total Plate Count (TPC) of 1.3×10^3 CFU/g and a Total Volatile Base (TVB) of 31 mgN/100g. GC-MS analysis of the liquid smoke flour detected acid groups (Butanoic acid, phosphorothioic acid, etc.) and phenol groups (1,2-ethanediol, 1,2-diphenyl- and 1,2-diphenylethane-1,2-diol, etc.).

1. INTRODUCTION

Milkfish is a fishery product that has high nutrition and rich in omega 3, 6, and 9 fatty acids, which are very beneficial for the body [1]. Omega 3, 6, and 9 are unsaturated fatty acids that easily undergo fat oxidation when processed at high temperatures, which can reduce the functional properties of the fatty acid. Application of corncob liquid smoke can maintain textural quality and inhibit fatty acid oxidation in smoked milkfish [2] and milkfish also contains the amino acid histidine 1.37%, glutamic acid 10.82% and lysine 1.62% [3].

Milkfish meat in 100 g contains 129 kcal of energy, 20 g of protein, 4.8 g of fat, 150 mg of phosphorus, 20 mg of calcium, 2 mg of iron, 150 IU of vitamin A, and 0.05 mg of vitamin B1. Based on the nutritional composition, milkfish is classified as a high-protein and low-fat fish [4]. The Omega-3 ($\omega 3$) content in milkfish is 14.2%, exceeding the $\omega 3$ content in salmon (2.6%), tuna (0.2%), and sardines-mackerel (3.9%). This substantial protein level is essential for the growth and development of children, as protein plays a critical role in building and repairing tissues, producing enzymes and hormones, and supporting overall bodily functions [1].

The processing of fishery products in Indonesia is generally still traditional, especially fish smoking which uses a hot smoking system. Products resulting from hot smoking have weaknesses, such as: the presence of the carcinogenic compound benzo[a]pyrene; the product is not uniform; This

method can cause air pollution, and product quality does not meet Indonesia's national standards for smoked fish (SNI). One modern smoking method that can be used to improve product quality is by using liquid smoke [5]. Smoked meat products can be made industrially, the smoking procedure is generally carried out in an automated smokehouse. In an industrial combustion type generator, wood chips or sawdust are automatically fed into an electrically heated grate or plate furnace, which produces smoke more efficiently under controlled conditions. Furthermore, charcoal particles can settle as the smoke passes through the pipe system and minimize the formation of various unhealthy compounds from meat products, especially polycyclic aromatic hydrocarbons (PAHs) [6].

Smoking can still affect the shelf life of food because smoke components can inhibit the growth of some microorganisms. Smoking under uncontrolled technological conditions, which is characteristic of traditional smoking processes, produces high levels of polycyclic aromatic hydrocarbons (PAHs). To simplify the problems related to the diversity and variability of PAH content, benzo(a)pyrene (BaP) has been accepted as an indicator of PAH content in food products. The European Commission has set maximum levels of PAHs in certain foodstuffs through Commission Regulation No. 1881/2006. The maximum level of benzo(a)pyrene ($5 \mu\text{g kg}^{-1}$) is set for smoked meat and smoked fish products. GC-MS analysis that BaP levels in traditional smoked fish range from 0.8 to 13.9

$\mu\text{g kg}^{-1}$ [7].

Liquid smoke contains natural antioxidants such as phenol, aldehydes, carboxyl acids which are effective in inhibiting oxidation reactions. The use of liquid smoke in the processing industry has advantages compared to traditional fumigation including easy application, fast smoking process, produces better smoked products than traditional smoke and does not contain carcinogenic PAHs [8].

Liquid smoke through pyrolysis followed by smoke condensation. Liquid smoke can also be produced from biomass from agricultural and forestry residues, and any plant material containing cellulose, hemicellulose and lignin, the three main compounds that make up liquid smoke. Liquid smoke has been produced from coconut shells. Liquid smoke is used to replace traditional wood smoking methods in fish processing in developed countries. processing. The tar extracted from the smoke is recycled as fuel back into the process [9]. Liquid smoke is a more modern method and is produced by condensing wood smoke formed from the controlled, oxygen-poor pyrolysis of sawdust or wood chips. Liquid smoke is considered a healthier smoking method, reducing the processing time and weight loss of traditional combustion smoking, and eliminating nitrogen oxides and PAH levels in smoked meat products, thus providing most of the desired color and flavor of conventional smoking, creating new technological possibilities [10].

Liquid smoke powder is liquid smoke that is converted into powder and will provide ease of mobilization and storage. At this time, it is necessary to develop an application of liquid smoke powder as a flavor enhancer and food preservative. The purpose of this study was to determine the effect of adding liquid smoke powder on the taste and shelf life of the food sample used, which was sponge cake. The method used to observe shelf life is ESS (Extended Storage Studies) [11]. Adding smoke powder to goat's milk for producing Domiatti cheese can reduce saturated fatty acids and increase unsaturated fatty acids, improving the quality of the cheese. The amount of short and medium chain fatty acids is lower while long chain fatty acids are higher in smoked cheese compared to the control. Essential fatty acids, linoleic acid (C18: 2) are higher in smoked cheese [12].

Mangroves (*Avicennia marina*) contain hemicellulose, cellulose and lignin with respective percentages of 19.78%; 32.46% and 25.34%. The decomposition of the three chemical elements will function as a food preservative. The decomposition of hemicellulose leads to the formation of furfural, furan and their derivatives along with a long series of carboxyl acids. The results of pyrolysis of cellulose compounds in wood produce acids, phenols, ketons and carbonyls. The decomposition of the lignin fraction consists of phenolic and phenolic ether compounds such as guaiacol (2-methoxyphenol), syringol (1,6 dimethoxyphenol), and its homologs and derivatives [13].

The aim of this research is to develop the use of mangrove leaf waste in the form of liquid smoked powder which is applied to smoking milkfish using an electric oven to produce quality smoked milkfish (*Chanos chanos*) from a microbiological and chemical perspective.

2. METHODOLOGY

The experimental research method was through the manufacture of liquid smoke powder from mangrove leaf and

the application of liquid smoke powder (with a concentration of 2.5%, 5%, 7.5% and 10%), on boneless smoked milkfish, and chemical analysis of liquid smoke powder with GC-MS, SEM analysis of liquid smoke powder, proximate analysis of smoked milkfish, Total Volatile Base (TVB) analysis, microbiological test (Total Plate Count/TPC) and organoleptic test.

2.1 Sample

The sample used in the research was mangrove leaf that made to liquid smoke at the workshop of Tribuana Tunggal Dewi University Malang, while boneless milkfish will be processed by smoking with a size of 350-400 grams/head purchased from the Sidoarjo Milkfish.

2.2 Production of liquid smoke with some modification

Small dry mangrove leaves are cut into 10cm-15cm sizes, put into the pyrolysator (Figure 1) as much as 5 kg and then closed tightly after that heated to a temperature of ± 200 - 250°C until the leaf burn out for 100 minutes. The aerosol smoke that is generated will enter the distillatory, resulting in the production of grade 3 liquid smoke. This liquid smoke is collected in a bottle and subsequently stored for seven days to precipitate the tar. Subsequently, the liquid smoke is separated from the tar to produce grade 2 liquid smoke. The tar is removed through a purification process that involves distillation with absorbent zeolite at a temperature of 105°C to 150°C until the liquid smoke ceases to drip into the reservoir's glass. This liquid smoke is referred to as liquid smoke grade #1 [14, 15]. Specifications of liquid smoke maker: 1. Pyrolyzer with stainless steel metal material consisting of a pyrolyzer tube ($\text{Ø } 50 \text{ cm h } 70 \text{ cm}$) and a smoke pipe and a condenser tube filled with water (there is a coil pipe through which smoke becomes liquid smoke); 2. Distiller for cleaning dirty liquid smoke.



Figure 1. Pyrolysator is a tool that produces liquid smoke

2.3 Making liquid smoke powder of mangrove leaf

The yellowish brown liquid smoke is put into a glass beaker or plastic container and maltodextrin and alginate flour are added with a ratio of 1:1 then stirred until the mixture is even then the mixture is placed on a 2-3 cm thick stainless steel tray then put in a vacuum dryer. The drying temperature is set at 50°C and the drying time is 5-8 hours (every hour is controlled). The condition have higher energy efficiency, and

a processing environment lacking oxygen with a tool pressure of 70 mmHg and required for reproducibility 2 times a day. After that the tray is removed from the vacuum dryer then cooled for a moment and the flakes are ground to produce smoke powder [16]. For more details, refer to Figure 2.

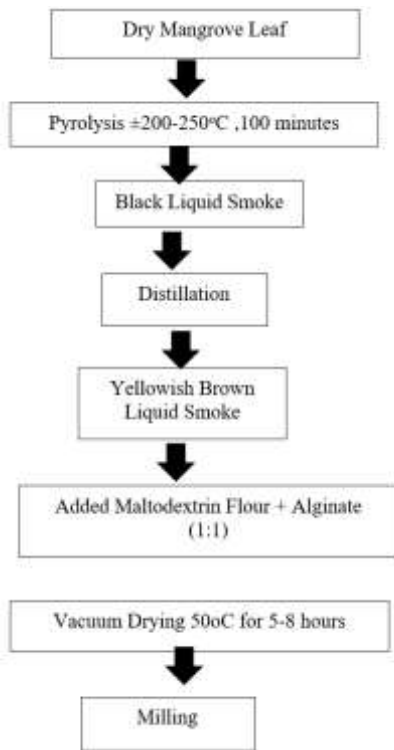


Figure 2. Flow chart for making smoked powder

2.4 Gas chromatography-mass spectrometry analysis liquid smoke powder of mangrove leaf with modification technology

Gas chromatography-mass spectrometry can be used to detect various compounds present in samples, including flavonoids, polyphenols, terpenes, and other volatile components [17]. Liquid smoke powder is extracted using ethanol and hexane solvents. A total of 1.5 g of liquid smoke was added to 3 mL of ethanol and 45 mL of hexane in the Ultra-Turrax disperser for 45 minutes at a speed of 6500 rpm. Then filtered under vacuum and washed successively using 15 mL of hexane and 20 mL of ethanol.

Furthermore, the sample solution was separated from the solvent using a rotary evaporator with a temperature of 40°C. The residue is then diluted with acetone. The fraction generated in the second extraction was combined with the fraction generated in the first extraction, then concentrated by nitrogen gas blowing until the remaining volume was roughly 1 mL. Shimadzu's GC-MS-QP2010S then picked up these findings. Using ionizing type EI (Electron Impact) 70 eV, injector temperature 290°C, detector temperature 280°C, column type Rxt-5MS (95% dimethyl polysiloxane; 5% diphenyl), with a long column 30 meters, column temperature 70°C to 230°C with a temperature rise of 5%.

2.5 Scanning electron microscopy (SEM) with modification technology

Comprising liquid smoke powder, the samples were cross-sectioned and set on slabs using adhesive tape. Using a

Scanning Electron Microscope (SEM) at 10.0 kV (Hitachi S-3400, Krefeld, Germany), the shape of the specimens was investigated following dry to a critical point and gold coating. An optical microscope equipped with an objective micrometer at 400× magnification assessed capsule dimensions. Staining with iodine allowed one to evaluate the cell dispersion inside the arrowroot matrix under light microscopy. With a rupture distance of 1.0 mm, a 35 mm diameter cylindrical aluminum probe was compressed in mode at 0.1 mm/s and the peak force recorded in grams. Every bead underwent three replicate tests for every treatment [18].

2.6 Production of smoked milkfish with liquid smoked powder

Boneless milkfish, gutted, washed with water and silenced ± 15 minutes. Furthermore, the milkfish is soaked in a 1% salt mixture with liquid smoke powder which has been dissolved with distilled water or water with a concentration of 2.5%, 5%, 7.5% and 10%, for 30 minutes then silenced ± 15 minutes. Next, the milkfish is placed on an aluminum tray and put in the Electric oven at 130°C for 90-120 minutes. For more details, refer to Figure 3.



Figure 3. Milkfish smoked from liquid smoke powder with oven technology

2.7 Data analysis

2.7.1 Yield value of liquid smoke and liquid smoke powder (%)

$$Yield = \frac{Output}{input} \times 100\% \quad (1)$$

where, Output is the amount of production produced (kg or gr or L or ml); Input is the amount for one-time raw materials production (kg or gr or L or ml).

2.7.2 Total plate count

After diluting five grams of smoked fish meat with 45 milliliters of 0.85% NaCl, successive 1:10 dilutions were performed to achieve a concentration of 10⁻⁷ grams per milliliter. Approximately 15 ml of Plate Count Agar (PCA; Himedia) was added to one milliliter of each diluted aliquot and poured into Petri dishes. Once the agar had solidified, all Petri plates were inverted and incubated at 37°C for 48 hours. Colony-forming units were counted according to ISO guidelines for microbiological techniques [19, 20].

$$N = \frac{\sum c}{(n_1 + 0.1n_2)d} \quad (2)$$

where, $\sum c$: amount of whole bacteria colony in all dishes

which contain 30 - 300 colonies; n_1 : amount of Petri dish which counted on the first dilution; n_2 : amount of Petri dish which counted on the later dilution; d : factor of the first dilution.

2.7.3 Proximate test

Moisture content: Drying 5 gm of fish sample in an oven at 105°C until a constant weight according to A.O.A.C. technique helped to ascertain moisture content [21].

The crude protein content: Macro Kjeldahl's technique measured total nitrogen content [22]. By multiplying the nitrogen concentration by the factor 6.25, the protein content was computed and stated as % protein [23].

The crude fat: Using Soxhlet equipment as defined by A.O.A.C., total crude fat content of dried processed fish was calculated as a weight loss after 16 hrs of extraction with petroleum ether (40-60°C) [21].

The ash content: Using a muffle-oven at 550°C for 24 hours as advised in A.O.A.C., the ash content was calculated [21].

2.7.4 Determination of TVB

Smoked fish meat was weighed 100 g and combined with 600 mL of 5% trichloroacetic acid. To get clear extract, the mixture was next centrifuged for one hour at 3000 x g. Five milliliters of the extract were pipetted into the Markhan device with five milliliters of 2 M NaOH added. Steam distilled this into 15 mL of normal 0.01 M HCl with 0.1 mL rosolic indicator. The surplus acid was then titrated in the receiving flask using normal 0.01 M NaOH to produce a pale pink end point following distillation. Five mL trichloroacetic acid was used in a procedural blank without a sample and titrated as before. Computation of TVB's concentration in mg/100 g sample yielded follows:

$$\text{TVB} \left(\frac{\text{mg}}{100 \text{ g sample}} \right) = \frac{(M)(V_b - V_s)(14)(300 + W)}{5} \quad (3)$$

where, V_b = mL NaOH used in blank titration; M = molarity of NaOH standard solution; W = water content of sample in g/100 g; V_s = mL NaOH used in sample titration.

Drying an initial weight of fish sample at 77°C in an oven to constant weight helped one to find the water content (W) of the sample. This temperature limits the evaporation of volatile components and helps to completely dry the material [23].

2.7.5 Organoleptic tests (Modification test)

Organoleptic tests were carried out using 20 panelists and two types of tests, namely scoring tests and hedonic tests. In this study, an organoleptic test was carried out with a hedonic test or level of liking with a score of 1 = extremely dislike, 2 = very dislike, 3 = dislike, 4 = somewhat dislike, 5 = neutral, 6 = somewhat like, 7 = like, 8 = like very much, 9 = extremely like [24].

3. RESULTS AND DISCUSSION

3.1 Characteristic liquid smoke and liquid smoke powder of mangrove leaf

The results of the analysis show that liquid smoke and liquid smoke powder have pH and yield values. For more details, refer to Table 1.

Table 1. Characteristics of liquid smoke and liquid smoke powder of mangrove leaf

Description	Characteristic			
	Water Content (%)	Visual/ Subjective Color	pH	Yield (%)
Liquid Smoke	18	Yellowish-brown	4.12	25.52
Liquid Smoke Powder	5.45	Brownish yellow	5.15	11.45

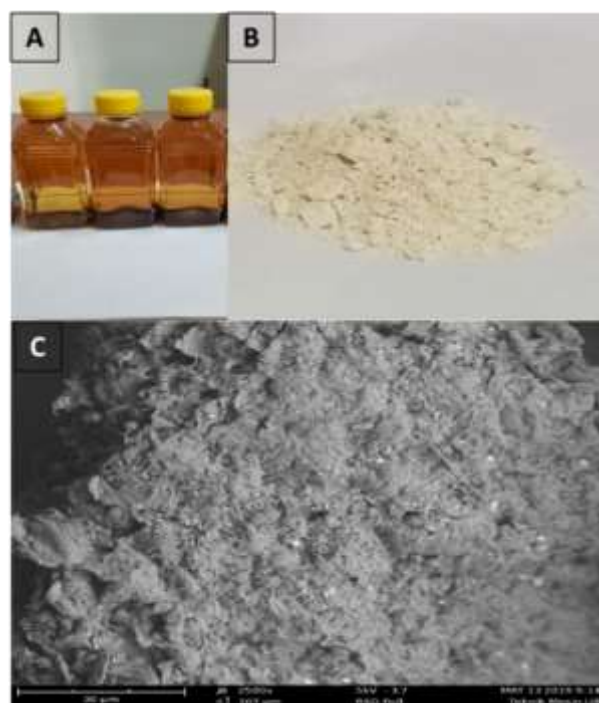


Figure 4. (A) Liquid smoke of mangrove leaf; (B) Liquid smoke powder of mangrove leaf; (C) SEM of Liquid smoke powder of mangrove leaf

Figure 4 shows that the liquid smoke product from the distillation results will be yellowish brown in Figure 4(A) and the liquid smoke powder will be yellowish white from vacuum drying in Figure 4(B), while Figure 4(C) shows the scanning electron microscopy (SEM) test (the structure of the smoke powder is compact and has no air cavities).

3.2 Chemical composition of liquid smoke powder of mangrove leaf

The results of the GS-MS analysis identified the components of liquid smoke from mangrove leaf and the chemical components of the substances contained were known (Figure 5, Table 2).

The phenol group in liquid smoke powder functions as a preservative to prevent or kill the performance of microorganisms that destroy food [25]. Meanwhile, acid compounds come from pyrolysis of cellulose. Phenolic and acid compounds function as antimicrobials, antioxidants, flavor and color builders [26]. Apart from that, acids and phenols function as antibacterials and antioxidants with a distinctive aroma [27]. Based on the data in Table 2 it can be seen that there is no PAH content, namely benzalpyrine, in grade 1 liquid smoke, this may be influenced by the lack of standards for PAH compounds at the time of testing. Apart

from that, the findings of GC-MS analysis of liquid smoke show no PAH content; this is impacted by the temperature during the pyrolysis process, where is PAH compound will occur at temperatures > 500°C [28]. This finding underscores

the critical role of temperature control in pyrolysis processes to prevent the generation of PAHs, which are harmful compounds associated with mutagenicity and carcinogenicity.

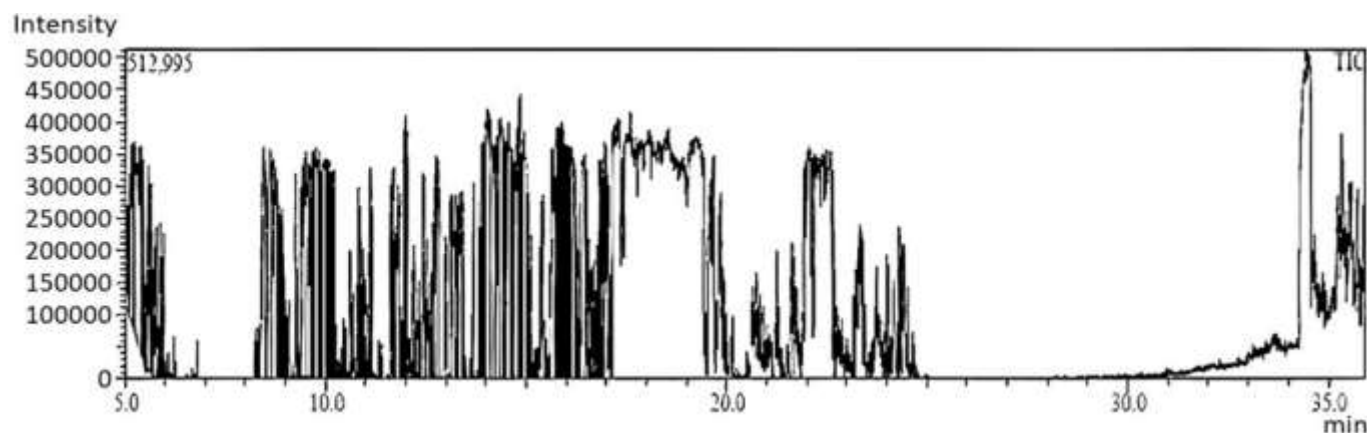


Figure 5. GC-MS chromatogram of chemical components of liquid smoked powder

Table 2. Results of GC-MS analysis of liquid smoke powder of mangrove leaf

Formula	Component
	Acid and Their Derivatives
C ₁₁ H ₁₂ O ₃	butanoic acid, 3-oxo-, phenylmethyl ester, benzyl 3-oxobutanoate and nacetoacetic acid, benzyl ester and benzyl acetoacetate and benzyl acetylacetate
C ₉ H ₁₁ C ₁₃ NO ₃	phosphorothioic acid, o,o-diethyl o-(3,5,6-trichloro-2-pyridinyl) ester and o,o-diethyl o-(3,5,6-trichloro-2-pyridinyl) thiophosphate and 2,3,5-trichloro-(diethyloxy thioxo phosphono) pyridine and 2-pyridinol, 3,5,6-trichloro-, o-ester with o,o-diethyl phosphorothioate and Acetic acid (CAS): Ethylic acid and Ethanoic acid
CH ₃ COOH	Acetic acid (CAS): Ethylic acid and Ethanoic acid
	Phenol and Their Derivatives
	1,2-ethanediol, 1,2-diphenyl- and 1,2-diphenylethane-1,2-diol and .alpha.,.alpha.'-bi[benzyl alcohol] and, 1,2-diphenyl- and 1,2-diphenyl-1,2-ethanediol and
C ₁₄ H ₁₄ O ₂	Phenol, 3,4-dimethyl- (CAS), 3,4-Xylenol, 3,4-Dimethylphenol and Phenol,4-methyl- p-cresol, p-Toluol, 4-Cresol and 60 Phenol, 3-ethyl- CAS, m-ethylphenol, 1-Hydroxy-3-ethylbenzene and Phenol, 2-methoxy-4-methyl-CAS, p-Creosol
	Other Components
C ₉ H ₁₁ NO ₂ PS	benzeneacetamide, .alpha.-hydroxy-n-methyl- and 2-hydroxy-n-methyl-2-phenylacetamide and 2-hydroxy-n-methyl-2-phenylacetamide and 2-hydroxy-n-methyl-2-phenylacetamide and 2-hydroxy-n-methyl-2-phenylacetamide and benzeneacetamide, alpha-hydroxy-n-methyl- and mandelamide, n-methyl- and n-methyl-mandelic acid amide and n-methyl mandelamide

In the analysis of liquid smoke powder of mangrove leaves, no PAH was detected. There are 15 PAHs as genotoxic carcinogens, namely benz[a]anthracene, benzo[b]fluoranthene,

benzo[j]fluoranthene, benzo[k]-fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, chrysene, cyclopenta[c,d]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene, and 5-methylkrylene. However, for benzo[g,h,i]perylene, clear evidence was found for genotoxicity but not for carcinogenic effects.

3.3 Studies of chemical, microbiological and organoleptic of smoked milkfish

Figure 3 shows boneless milkfish, which was added with liquid smoke powder and then placed in an electric oven at a temperature of 130°C for 90-120 minutes.

The results of chemical, mycobiological and organoleptic analysis of milkfish smoked with liquid smoke powder from mangrove leaf can be seen in the Table 3.

Table 3. Average of chemical and microbiology values

Parameter	Concentration Average of Liquid Smoke Powder of Mangrove Leaf			
	2.5%	5%	7.5%	10%
Water Content (%)	46.7	35.42	44.37	50.72
Protein (%)	29.8	32.83	27.15	28.4
Lipid (%)	16.37	16.4	16.47	19.91
Ash (%)	3.54	5.12	3.53	3.37
aW	0.8	0.81	0.77	0.67
TPC (CPU/g)	2.0 × 10 ⁴	1.3 × 10 ³	1.6 × 10 ²	4.0 × 10 ¹
TVB (mgN/100g)	29	31	30	28

3.3.1 Water content

Water is the largest content in fish and water is also a means for microorganisms to grow, so the smoking process aims to remove the water content in fish and is expected to extend the shelf life of smoked fish [29-31], content is also a very important characteristic of food, because water can affect the appearance, texture and taste of food [30]. The water content of ingredients has a close relationship with the durability of food ingredients. During processing, water in food is often removed or reduced by evaporation or thickening and drying.

According to Table 3, it can be concluded that the higher

the concentration of liquid smoke, the lower the water content contained in the product [32]. Apart from being influenced by liquid smoke, the low water content in liquid smoked milkfish may also be influenced by soaking in salt solution, temperature and length of oven. The longer the oven time, the water content will continue to decrease. Apart from liquid smoke, soaking in salt solution and the oven process are also factors that influence the low water content in the product. This is in accordance with the research [33], the content of liquid smoke is able to bind free water in the product during processing.

Claims that elements during the smoking process affect the water content value: smoking temperature, ambient humidity, kind and condition of fuel and the concentration used [34]. This is consistent with the claim of study [35], evaporation from the product resulting from the effect of air temperature and humidity in the surrounding environment causes the drop in water content. The amount of phenol absorbed into fish flesh can influence the acceleration of water evaporation in fish flesh and smoking parameters and fish size, as observed in smoked Atlantic salmon [36]. The quality characteristics of fish, such as skin and flesh coloration, can also be impacted by dietary components [37].

3.3.2 Protein content

Apart from serving as fuel for the body, protein is a food component that is rather vital since it is also a building and regulating agent. Proteins in the human body, especially in tissue cells, act as cell membrane materials, can form connective tissue such as collagen and elastin, and form inert proteins such as hair and nails. Besides that, proteins can work as enzymes, act as plasma (album), form antibodies, form complexes with other molecules [38].

Determination of protein levels according to study [32], carried out using the Kjeldahl method. Basically, it can be divided into three stages, namely the destruction, distillation and titration process. In the digestion process the sample is heated with concentrated H_2SO_4 so that it breaks down into its elements. To make the process faster, Na_2SO_4 , $CuSO_4$ and selenium catalysts are used. The digestion process is complete when the solution is clear or colorless.

The distillation stage is that ammonium sulfate is broken down into ammonia by adding NaOH until alkaline and heated. The ammonia formed is collected in concentrated H_3BO_3 which has been given BCG and methyl red indicators. The amount of H_3BO_3 that reacts with ammonia can be determined by titrating it using 0.02 M HCl. The end of the titration is marked by a change in the color of the solution from dark blue to pink. Blank treatment was carried out to determine the nitrogen originating from the reagent used.

3.3.3 Fat content

Fat is a source of energy for the body. The energy produced by fat is 2.25 times greater than carbohydrates and protein. One gram of fat provides 9 calories while carbohydrates and protein only have 4 calories. Triglycerides are the main form of fat, both in the human body and in food. Chemically, triglycerides consist of 3 fatty acids attached to glycerol via ester bonds [32].

The Soxhlet method consistently records the highest oil yield, making it suitable for industrial oil extraction purposes [33]. The Soxhlet extraction method is carried out by weighing 2 g of sample which has been ground, dried, and wrapped in filter paper. The sample was placed in a Soxhlet extraction tube, installed in a Soxhlet apparatus with hexane as the

solvent for 4 hours, and the resulting hexane containing the fat extract was transferred to an Erlenmeyer flask for further processing by distillation. The extraction results were dried in an oven at $100^\circ C$ until constant weight. Residue weight is expressed as fat weight. Observation data and data analysis of smoked milkfish fat content with different concentrations can be seen in Table 3.

3.3.4 Ash content

The ash content of a material describes the amount of minerals that are burned into substances that can evaporate. The greater the ash content of a food ingredient, the higher the minerals contained in the food ingredient [34]. Observation data and data analysis of smoked milkfish ash content with different concentrations can be seen in Table 3.

Mineral elements are known as organic substances or ash content. In the combustion process, organic materials are burned but inorganic substances are not, therefore it is called ash. Until now, it is known that there are 14 different types of mineral elements needed by humans for good health and growth. In the body, minerals will combine with organic substances, some of which are in the form of free ions. In the body, mineral elements function as building and regulating substances [31].

3.4 Organoleptic test

Organoleptic characterization tests were carried out to determine the panelists' acceptance of smoked milkfish using liquid smoke powder with different concentrations. In organoleptic testing, a very important role is the sensitivity of human senses such as sight, smell with the nose and taste with the tongue in assessing a product. In this research, organoleptic tests were carried out using 20 panelists and two types of tests, namely scoring tests and hedonic tests. In this study, an organoleptic test was carried out with a hedonic test or level of liking with a score of 1 = extremely dislike, 2 = very dislike, 3 = dislike, 4 = somewhat dislike, 5 = neutral, 6 = somewhat like, 7 = like, 8 = like very much, 9 = extremely like.

Discussion of organoleptic tests as follows:

3.4.1 Appearance scoring

Appearance is a very important parameter in evaluating a product. The appearance scoring test uses the sense of sight and the information received directly gives an attractive or unattractive impression of the product being presented. Things to pay attention to in the appearance scoring test include the color, shape and brightness of the product itself.

Based on the results in Table 4, it can be concluded that the organoleptic test results scoring the appearance were the highest in the treatment (5%) with an average value of 7.8 ± 1.05 and the organoleptic test results scoring the appearance were the lowest in the treatment (2.5%) with an average value -average of 6.8 ± 1.23 . Based on the results of the Kruskal Wallis test, a significant value of 0.000 was obtained with the conclusion that the use of liquid smoke powder with different concentrations in the appearance parameters gave significantly different results. Variations in liquid smoke powder concentration affect the phenol content and acid content in the product.

Apart from that, phenol and acid compounds also influence color, color is produced by the interaction between carbonyl compounds and amino acid groups on the surface of the substance [35]. The color of smoking is generally golden

yellow to dark brown, depending on the type of wood used [29]. According to study [30], smoking using softwood produces a darker colored product, because it contains more resin than hardwood. The resin in softwood ranges from 2.0 – 3.5, while in hardwood it ranges from 1.8 – 3.0.

Table 4. Average of organoleptic value

Parameter	Treatment	Average	Category
Appearance	2.50%	6.8 ± 1.23	Somewhat like
	5.00%	7.8 ± 1.05	Like
	7.50%	7.15 ± 1.34	Like
	10%	7.35 ± 1.72	Like
	2.50%	7.15 ± 1.30	Like
Aroma	5.00%	8.2 ± 1.32	Like very much
	7.50%	6.45 ± 1.43	Somewhat like
	10%	6.6 ± 1.87	Somewhat like
Flavor	2.50%	6.05 ± 1.82	Somewhat like
	5.00%	7.75 ± 1.40	Somewhat like
	7.50%	6.8 ± 1.88	Neutral
	10%	7.2 ± 2.09	Like
	2.50%	7.2 ± 1.36	Somewhat like
Texture	5.00%	8.25 ± 1.06	Like very much
	7.50%	7.2 ± 1.65	Like
	10%	7.7 ± 1.52	Like

3.4.2 Aroma scoring

Aroma is an indicator of the product quality variable which has the greatest influence on consumers in making purchasing decisions [37]. Aroma is more influenced by the sense of smell. In general, the odors that can be received by the nose and brain are mostly a mixture of odors including fragrant, sour, rancid and burnt.

Based on the results in Table 4, it can be concluded that the highest aroma scoring organoleptic test results were found in the treatment (5%) with an average value of 8.2 ± 1.32 and the lowest aroma scoring organoleptic test results were found in the treatment (2.5%) with an average value of 7.15 ± 1.30 based on the results of the Kruskal Wallis test, a significant value of 0.013 was obtained with the conclusion that the use of liquid smoke with different concentrations on aroma parameters gave significantly different results. This is affected by the concentration difference that determines the phenol content of the product; so, phenolic chemicals provide scent to the smoking process.

The substances that dominate the formation of aroma or odor are the smoke components attached to the product. Phenol is the main compound that forms the distinctive smoke aroma. The aroma in question is the aroma of the smoke in the product and the odor quality criteria for smoked fish is a smoke odor that is soft to quite sharp, not rancid, without foreign odors, without a sour odor, and without a musty odor [38].

According to study [38], fish that has just undergone the smoking process has a soft to quite sharp smoke aroma, not rancid, without a bad smell, without a foul smell, without a foreign smell, without a musty or sour smell due to the concentration and type of liquid smoke powder used. Food that is still good has a distinctive smell from the food and will of course be more stimulating to eat. If the smell is different or distorted, the food is considered to have started to rot [39].

3.4.3 Taste scoring

Taste scoring is a panelist's assessment ability to determine the taste of a product. This taste scoring test involves the taste

buds, namely the tongue. According to study [40], taste is influenced by several factors, namely chemical compounds, temperature, concentration and interactions with other taste components. The results of the hedonic organoleptic test of taste scoring in the main research can be seen in Table 4.

Based on the results in Table 4, it can be concluded that the organoleptic test results for taste scoring were highest in the treatment (5%) with an average value of 7.75 ± 1.40 and the lowest organoleptic test results were in the treatment (2.5%) with an average value of 6.05 ± 1.82 . Based on the results of the Kruskal Wallis test, a significant value of 0.367 was obtained with the conclusion that the use of liquid smoke powder with different concentrations in the taste scoring parameters showed no difference. This may be influenced by the use of the same salt concentration in each treatment, namely 5%. The purpose of soaking in a salt solution is not only to add flavor but also as a preservative [41]. Apart from adding salt, the phenol content also plays an important role in absorbing the taste [42].

Taste plays an important role in product selection because even though the nutritional content is good, if the taste is not acceptable to consumers, then the target is to increase. Furthermore, the smoke components, such as 1,2-ethanediol, 1,2-diphenyl- and 1,2-diphenylethane-1,2-diol, are predominant in smoke and contribute significantly to the flavor profile of smoked products [43]. The process of smoking is not only a traditional method for food preservation but also serves to enhance the color, aroma, flavor, and appearance of smoked food products [44]. The use of liquid smoke has been explored as a safer alternative to traditional smoking methods, as it can provide additional flavor, color, act as antioxidants, and exhibit bacteriostatic properties [45].

3.4.4 Texture scoring

Texture is a factor that influences the taste of a processed food or product. Texture is very important in consumer acceptance of a product. Texture can include soft, chewy or hard depending on how consumers like or accept a product. The results of the organoleptic test for texture scoring in the main research can be seen in Table 4.

Based on the results in Table 4, it can be concluded that the highest texture scoring organoleptic test results were in the treatment (5%) with an average value of 8.25 ± 1.06 and the lowest scoring organoleptic test results were in the treatment (2.5%) with an average value -mean 7.2 ± 1.36 .

Based on the results of the Kruskal Wallis test, a significant value of 0.004 was obtained, with the conclusion that the use of liquid smoke with different concentrations in the texture scoring parameters showed that there was a difference. This may be influenced by the addition of concentration which will have an influence on the water content of the product. This is in accordance with research [46], where the level of acceptability of food products based on texture is greatly influenced by water content.

According to study [47], the factor that influences the texture of smoked products is the smoking temperature. At a high smoking temperature, protein will coagulate more quickly, resulting in a more compact meat texture. The difference in the texture value of smoked fish is thought to be due to differences in water content, where the higher the water content of smoked fish, the lower the texture value. The water content in wood also provides variations in smoke composition [48].

3.5 Environmental impact of using mangrove leaf liquid smoke powder in the fish smoking process

The use of traditional smoking methods involving direct burning of wood or other organic materials often results in significant emissions of pollutants, including carcinogenic compounds such as benzo[a]pyrene and various polycyclic aromatic hydrocarbons (PAHs) [49]. These emissions not only have the potential to harm human health but also contribute to air pollution that is detrimental to the environment. Additionally, large-scale use of wood as fuel can contribute to deforestation, which has a negative impact on forest ecosystems and environmental sustainability.

In contrast, the fumigation method with liquid smoke from mangrove leaf developed in this research offers a more environmentally friendly solution. Liquid smoke is produced through a controlled pyrolysis process, which reduces emissions of harmful pollutants. During the pyrolysis process, the temperature is well controlled to prevent the formation of PAH compounds which often occurs at high temperatures in direct combustion methods [50]. This allows the production of liquid smoke that is free from carcinogenic compounds, so that the final product is safer to consume and has a lower environmental impact.

The use of liquid smoke powder from mangrove leaf offers antibacterial properties due to the presence of phenol, which can aid in preserving and enhancing the flavor of food products [51]. Moreover, the antibacterial properties of liquid smoke, attributed to components like phenol, can aid in preserving fish products and extending their shelf life, further supporting sustainable practices in the fish processing industry. Additionally, liquid smoke powder has been utilized as a natural preservative in various food products, showcasing its potential to extend shelf life and maintain product quality [52]. The safety, quality, and nutritional aspects of smoked food products, such as milkfish, can be improved through the use of liquid smoke, addressing concerns related to traditional smoking methods.

Furthermore, this liquid smoke powder method has the potential to reduce the carbon footprint in the fish processing industry. In traditional smoking methods, the use of large amounts of wood fuel results in significant CO₂ emissions. On the other hand, the process of making and using liquid smoke requires less energy and produces less CO₂ emissions, especially if the pyrolysis process uses renewable energy [53]. Thus, this liquid smoke method can contribute to reducing the overall carbon footprint, supporting global sustainability goals.

Using mangrove leaf as raw material for liquid smoke powder also provides additional ecological benefits. Mangrove leaf serve as a renewable source of biomass, unlike wood, which can lead to deforestation [54]. Additionally, mangroves play an important role in mitigating climate change due to their ability to absorb carbon from the atmosphere. The ecological benefits of mangroves extend to their ability to improve soil properties, enhance water permeability, and reduce soluble salts, highlighting their importance in maintaining ecosystem balance [55]. By utilizing mangrove leaf that may have previously been unused or even considered waste, this research not only offers a cleaner fumigation solution, but also supports efforts to preserve the mangrove ecosystem.

By considering all these aspects, it can be concluded that the innovative approach of utilizing liquid smoke from mangrove leaf through controlled pyrolysis not only improves the quality

of smoking products in terms of safety and taste, but also offers significant environmental benefits compared to traditional smoking methods. This innovation has the potential to become a model for more sustainable industrial practices, reducing negative impacts on the environment, and supporting global efforts to mitigate climate change.

4. CONCLUSIONS

The results of this research indicate that the use of 5% liquid smoke originating from mangrove leaf in the process of smoking milkfish produces optimal organoleptic results. Specifically, the appearance, aroma, taste, and texture scores were 7.8 ± 1.05 , 8.2 ± 1.32 , 7.75 ± 1.40 , and 8.25 ± 1.06 , respectively. These results indicate a high level of acceptance of smoked milkfish. Proximate analysis of smoked milkfish showed water content of 35.42%, protein content of 32.83%, fat content of 6.40%, and ash content of 5.12%. Water activity (a_w) was measured at 0.80, and microbiological analysis showed a Total Plate Count (TPC) of 1.3×10^3 CFU/g and a Total Volatile Base (TVB) content of 31 mgN/100 g.

GC-MS analysis identified several important components in liquid smoke powder from mangrove leaf, including acids such as 3-oxo-phenylmethyl ester butanoic acid, etc. and phenol derivatives such as 12-ethanadiol and diphenyl, etc. The results of this study can be developed on a commercial scale by producing smoked powder from agricultural waste (coconut fiber, coconut shell, coffee skin, leaves, etc) using a spray dryer. Smoked flour will be applied in food as a natural preservative, flavor in sausages, etc.

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