

The effectiveness of salt, citrus and calcium propionate on microbiological and organoleptic qualities of smoked eel stored at room temperatures

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ABSTRACT

Introduction: Smoking preserves fish but yields products with short shelf life (2 days) prone to microbial spoilage. Salt, citrus, and calcium propionate may enhance preservation.

Aims: To evaluate the effectiveness of these additives on microbiological/organoleptic qualities of smoked eel stored at room temperature.

Materials and Methods: Three treatments: (A) 15% NaCl + 15% citrus immersion + heating; (B) Heating + 2.5% calcium propionate spray; (AB) Combination of A+B. Stored 0/3/6 days. Analyzed: water/protein content, TPC, mold, pathogens, sensory traits.

Results: AB treatment showed optimal results with water (50.9–55.4%), protein (13.75–16.8%), TPC (4.07–6.38 log CFU/g), mold (3.60–4.64 log CFU/g). Sensory scores (1–9 scale): appearance (7.0), flavor (8.0), taste (7.4), texture (7.2). Organoleptic analysis show that panelists like the smoked eel even for 6 days of storage, because of the aroma and flavor of smoked fish are still good and the color of products has not changed was golden brown color. No pathogens detected. Total number of bacterial colonies 4.07 to 6.38 cfu/g, total mold colony 3.60 to 4.64 cfu/g.

Conclusion: AB treatment extends shelf life to 6 days while maintaining quality.

KEYWORDS

Smoked ell, preservation, quality, microbiological, organoleptic

INTRODUCTION

Eel (*Anguilla spp*) is one of the popular consumption fish in some countries. In Japan smoked eel called unagi, and is believed to increase the stamina during the summer. Eel (*Anguilla spp*) has 19 species that spread throughout the world, and 7 (seven) of them are found in Indonesian waters^{1,2}. In Indonesia processing of Eel is still very limited, consumption or export fresh and frozen. Eel has a high nutritional content, especially vitamins A, E, B1, B2, B6, C, D and unsaturated fatty acids, as well as protein³⁻⁵. Omega 3 in eel especially EPA is 742mg/100g and DHA is (1,337 mg/100g) higher than EPA and DHA content in salmon⁶. Smoked eel has the opportunity to be developed as a food source because of their high in nutrients.

The problem that is often found in smoked fish processing was the short shelf life (2 days) at room temperature storage and easily deteriorated quality, especially microbiological quality^{7,8}. Food preservatives such as: Sodium chloride, sorbic acid, sodium benzoate, calcium propionate and citric acid are widely used in order to extend of shelf life and improve food quality. Salt (NaCl), citrus and calcium propionate have been applied in the preservation of smoked meat and other processed foods that can maintain shelf life and inhibit bacterial growth in smoked products⁹⁻¹¹. Therefore, this study was conducted to investigate the effectiveness of salt, citrus and calcium propionate on microbiological and organoleptic qualities of smoked eel (*Anquilla sp*) stored at room temperatures.

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Smoking food is one of the oldest food processing method where is still frequently used nowadays, to give a smoke flavor to meat and fish. In Indonesia, total smoked fish production was 92,52 MT¹², while smoked fish production of North Sulawesi province was 31,408 MT, or 34% of Indonesian smoked fish production¹³. Some regions in Indonesia have a specific smoked fish, for example: smoked Skipjack called *Cakalang fufu* in North Sulawesi and *Cakalang asar* in Malucas, smoked Milkfish called *Bandeng asap* in East Java, smoked Eel called *Sogili fufu* in South East Sulawesi, and smoked Catfish called *Lele selai* in West Sumatera. In North Sulawesi smoked fish can be categorized as exotic indogenous food. Two famous smoked fish in North Sulawesi i.e. *Cakalang fufu* or smoked Skipjack (*Katsuwonus pelamis* L.) processed by hot smoking (temperature 80-100°C for 3-4hr), and *Roa fufu* or smoked Halfbeak (*Hemiramphus far*) processed by semi-hot smoking (temperature 80°C for 1-2hr and than 50-60°C for 10-15hr).

Since an advanced in packaging and storing technologies, smoking fish was developed to produce a high value added products to satisfy consumer's taste¹⁴. According to¹⁵ over the past few decades traditional smoking of food has been replaced by use of smoke flavorings such as liquid smoke. However¹⁶, stated that smoke flavorings did not contain the same compounds as natural smoke since liquid smoke is filtered to remove toxic and carcinogenic impurities¹⁷. stated that increasing phenol content in smoked meat could potentially enhance the smoky flavor of product. The problems of traditional smoked fish are: high contents of Polycyclic Aromatic Hydrocarbon (PAH) especially benzo(a)pyrene (BP), because of them have well-known genotoxic, mutagenic, and carcinogenic properties¹⁸. According to¹⁹ BP is an indicator of carcinogenetic and traditional smoked fish contained about 0.7-60ng/g (wb) especially in skin layer. Benzo(a)pyrene is high in traditional smoked fish where the fish usually smoked directly above the fire (hot smoking). Content of PAH in some of cured fish products are: 716.84mg/kg in smoked *Sardinella aurita* in Ghana²⁰; 7.46 to 18.79mg/kg in smoked *Lates niloticus* in Kenya²¹; and 0.56 to 1.46mg/kg in all boiled, grilled, and fried fish in Western Cape, South Africa²². No data of PAH content in commercially smoked fish available in Indonesia yet. However²³, studied Benzo(a)pyrene (BP) in skipjack smoked with paddy chaff and coconut shell liquid smoke found BP of 9.55 and 8.72ppm respectively. Beside PAH, traditional smoked fish also have low edible portion, no processing standard, flavor varied, difficulties in packaging, low performance, and short shelf life. Liquid smoke is an alternative, because easy to produce, use a simple equipment, can be found also in market, concentration can be controlled, quality of the product including flavor can be standardized, and smoked fish has high edible portion (100%). The aims of this study were to develop the best smoking method and the best liquid smoke concentration, based on PAH and sensory assessments.

MATERIALS AND METHODS

Materials

Fresh eel (*Anguilla sp*) has been found from hatchery at Lolak region Minahasa. The fish were put in cool box with fish: ice ratio = 1:2, and then transported by car for 30 minutes to laboratory. In laboratory, fish were washed and eviscerated, and fresh fillets (3 cm) were prepared. All treatments and procedures were done followed good manufacturing practices (GMP).

Treatments

Three different application methods of treatment were: A. fish fillets were dipped in 15% NaCl and 15% citric acid for 20 minuts, then heated at 70-80°C for 4hr; B. fresh fillet were heated at 70-80°C for 4hr and sprayed with 2.5% calcium propionate; C. fish fillets were dipped in 15% NaCl, 15% citric acid with the same way of treatment A, and then heated at 70-80°C for 4hr and sprayed with 2.5% calcium propionate. Smoked eel were stored for 0,3 and 6 days at room temperature.

Research procedure

Research procedure can be seen in Figure 1.

Samples analysis

Analysis have been done for water content²⁴, protein content²⁴ and sensory characteristics for appearance, flavour, taste, and texture. Hedonic assessment was used for sensory characteristics, and Triangle test has been used to assess the differences between treatments using 18 to 21 semi-trained panelists²⁵. Score sheet have been used for both of hedonic and triangle assessments.

Bacterial Isolation

Each Sample was weighed as much as 25 g, input into 225 ml of sterile 0.9% NaCl solution and shacked strongly, and then taken 1 ml suspension and input into 9 ml of sterile 0.9% NaCl solution, formed 10⁻² dilution, and then homogenized by shaking the tube, and then taken 1 ml from 10⁻² dilution input into 9 ml suspension formed a dilution rate of 10⁻³. The procedure be continued in the same way for further dilution necessary.

Furthermore, from each dilution was taken 1 ml suspension and transferred into the NA medium for TPC test with a pour plate method, and BSA medium for *Salmonella* with scratch method, APW-TCBS medium for *Vibrio parahaemolyticus* test, and lactose broth-EMBA medium for Coliforms and *E. Coli*, and each of medium using the Most Probable Number (MPN) method. All samples were labelled indicated sample type and dilution level. Petri dish of media NA and BSA, Hack tubes containing medium APW and lactose broth in a Hach tube with Durham, were kept in an incubator and incubated at 37 °C for 24 and 48 hours for observation.

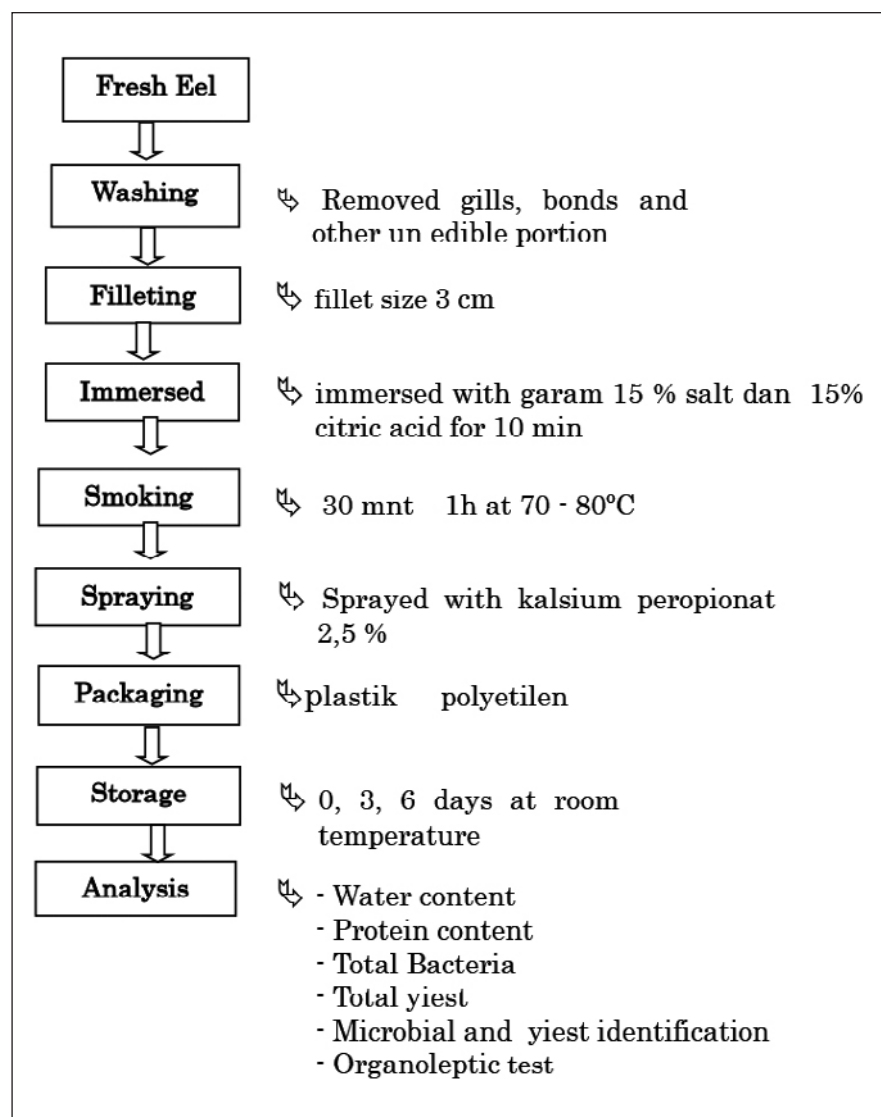


Figure 1. Scheme of research procedure

Statistical analysis

There are two factors implementing on this study, namely: application methods with three sub-factors i.e.: A, B, and AB; and storage time with three factors i.e.: C₁, C₂, C₃. Completely Randomize Design (CRD) Factorial 3x3 was designed. Replication of treatments was done twice. Analysis of Variance has been used to analyze the data, and continued by Least Significant Difference (LSD) test for a significant treatment²⁶.

RESULTS

Water content

Water contents of smoked Skipjack varied from 47.6% for the sample heated, dipped in 0.8% liquid smoke, and then heated again, to 60.6% for the fillet that was steamed, dipped in 0.6% liquid smoke, and then heated. Figure 2

showed that all steamed samples had high water content due to increased moisture after steaming and protein denaturation.

Analysis of variance showed that liquid smoke concentration and the interaction between the two treatments had no significant effect ($P \geq 0.05$) on water content, while the application method of liquid smoke had a highly significant effect ($P \leq 0.01$).

Protein content

Phenol contents varied from 1.98mg% for sample of fresh fillet dipped in 0.4% liquid smoke, and then heated, to 12.6% for sample that steamed, dipped in 0.8% liquid smoke and then heated. From Figure 2, it can be seen that fresh fillet that directly dipped in liquid smoke contained lower phenol contents, followed by fillet which heated before dipped, and the higher was fillet that steamed first before dipped and heated. It can be seen also that increasing of phenol content caused decreasing of pH.

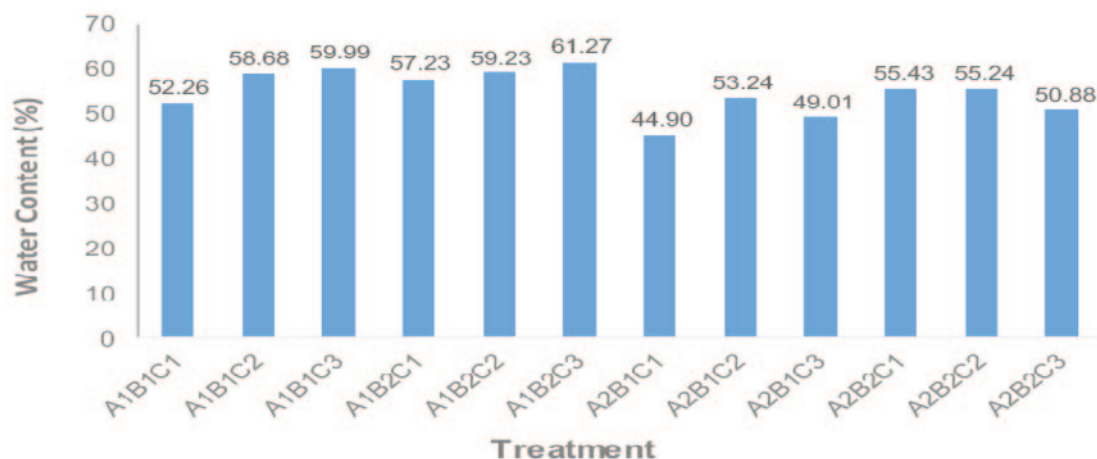
Microbiological Analysis

Total bacterial counts (TPC) varied from 4.07 to 6.47 log cfu/g across treatments and storage time. The combination treatment showed the lowest bacterial growth, with counts ranging from 4.07 log cfu/g at day 0 to 6.38 log cfu/g at day 6. Control samples showed higher bacterial counts (4.98-6.47 log cfu/g). Total mold counts ranged from 3.60 to 5.60 log cfu/g.

Sensory analysis

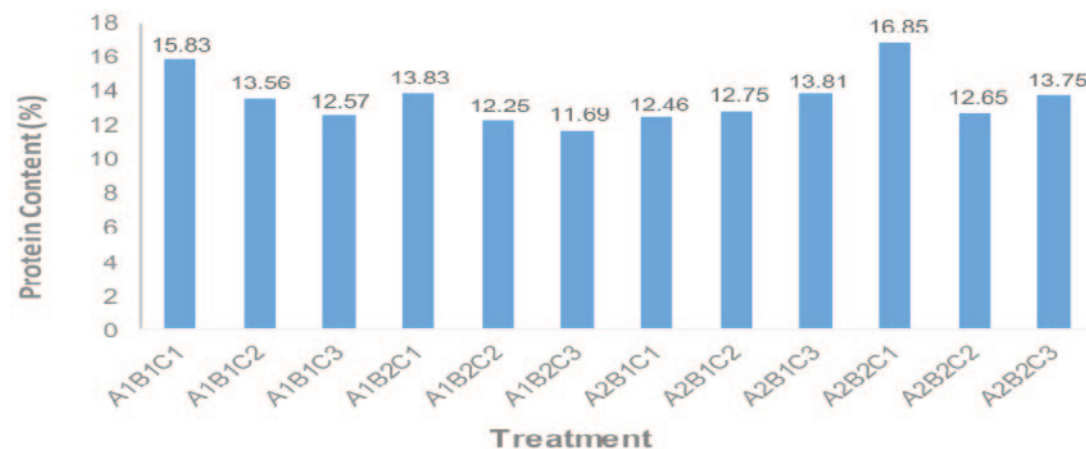
Appearance

The scores of appearances can be seen in Figure 4. The lowest score was in sample that steamed, dipped in 0.6% liquid smoke and then heated. The highest score was in sample that heated, dipped in 0.8% liquid smoke and then heated. Analysis of variance shown that only application methods of liquid smoke has a significant effect ($P \leq 0.05$) on appearance, while all other treatments including interaction did not give any significant effect ($P \geq 0.05$). For all concentrations of liquid smoke, fillet that steamed first before dipped, trend to have a low appearance. The best appearance was sample that heated before dipped in 0.8% liquid smoke, and then heated.



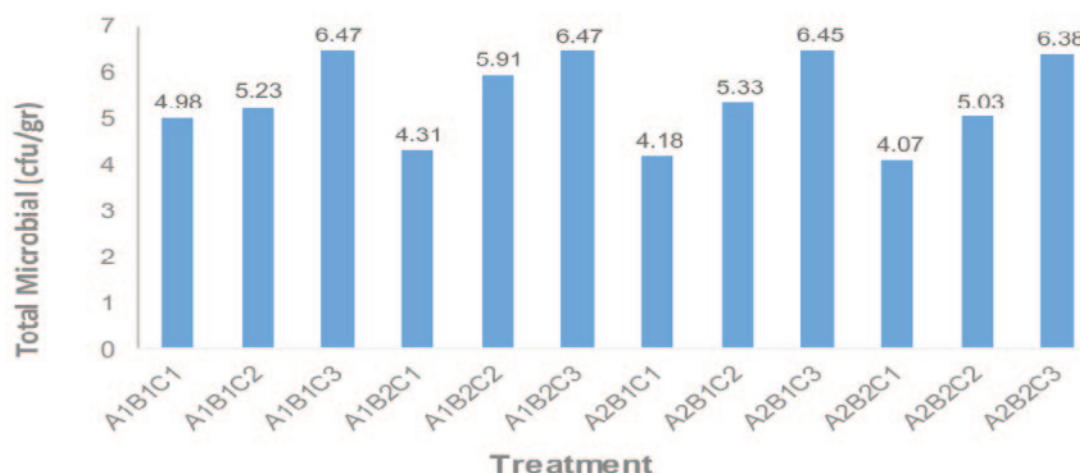
A1B1C1: control at 0 day; A1B1C2: control at 3 day; A1B1C3: control at 6 day; A1B2C1: sprayed with 2,5% calcium propionat at 0 day; A1B2C2: sprayed with 2,5% calcium A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day propionat at 3 day; A1B2C3: sprayed with 2,5% calcium propionat at 6 day; A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day; A2B2C1: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 0 day; A2B2C2: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 3 day; A2B2C3: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at at 6 day.

Figure 2. Water content of smoked Eel with different treatments.



A1B1C1: control at 0 day; A1B1C2: control at 3 day; A1B1C3: control at 6 day; A1B2C1: sprayed with 2,5% calcium propionat at 0 day; A1B2C2: sprayed with 2,5% calcium A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day propionat at 3 day; A1B2C3: sprayed with 2,5% calcium propionat at 6 day; A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day; A2B2C1: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 0 day; A2B2C2: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 3 day; A2B2C3: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at at 6 day.

Figure 3. Protein content (%) of smoked Eel with different treatments.



A1B1C1: control at 0 day; A1B1C2: control at 3 day; A1B1C3: control at 6 day; A1B2C1: sprayed with 2,5% calcium propionat at 0 day; A1B2C2: sprayed with 2,5% calcium A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day propionat at 3 day; A1B2C3: sprayed with 2,5% calcium propionat at 6 day; A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day; A2B2C1: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 0 day; A2B2C2: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 3 day; A2B2C3: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at at 6 day.

Figure 4. Protein content (%) of smoked Eel with different treatments.

Flavor

The scores of flavors can be seen also in Figure 4. It can be seen that the scores of flavors in generally higher than the scores of appearance. The higher score of flavor was for sample that heated first, then dipped in 0.8% liquid smoke and then heated, followed by samples that steamed, dipped in 0.8% liquid smoke and then heated. However, all samples shown satisfy values of flavor. Analysis of variance showed that all treatments including interaction did not give any significant effect ($P \geq 0.05$). From Figure 4 can be seen that the value of flavor were the highest among the sensory parameters assessed.

Taste

The scores of taste can be seen in Figure 4. The lowest score was for sample that heated, dipped in 0.4% liquid smoke then heated again, while the highest score was in sample that heated, dipped in 0.8% liquid smoke and then heated, and followed by samples that steamed, dipped in 0.8% liquid smoke and then heated. Analysis of variance shown that an only application method of liquid smoke has no significant effect ($P \geq 0.05$) on taste, but smoke concentrations gave a highly significant effect ($P \leq 0.01$), and interaction gave a significant effect ($P \leq 0.05$). For all concentrations of liquid smoke, sample which steamed first before dipped, and also

heated first before dipped, trend to have a high taste. The same as appearance, the best taste was for sample that heated before dipped in 0.8% liquid smoke and then heated.

Texture

The scores of textures can be seen in Figure 4. The same as appearance, the score of texture little bit lower than score of flavor and taste. However, all sensory characteristics (appearance, flavor, taste, and texture) showed that panelist like the all assessed samples. The lowest score was in sample that heated and then dipped in 0.4% liquid smoke then heated, while the highest score was in sample that steamed, then dipped in 0.8% liquid smoke and then heated, followed by samples that steamed, dipped in 0.4% and then heated. Samples that heated, dipped in 0.8% liquid smoke and then heated also have a high score. Analysis of variance shown that liquid smoke concentration did not give significant effect ($P \geq 0.05$), while application methods of liquid smoke has a significant effect ($P \leq 0.05$) on texture.

Triangle test

Triangle test have been done for appearance, flavor, taste, and texture, to find the difference between new products compared to traditional (conventional) product, and the dif-

ferences between each treatments. The results shown that for appearance, sample that was heated first, dipped in 0.6% liquid smoke and then heated again, had a highly significant ($P \leq 0.01$) different and better compare to the conventional smoked skipjack and other treatments. The sample has a yellowish-brown color and bright. Sample which heated, then dipped in 0.8% liquid smoke, and then heated also had a highly significant ($P \leq 0.01$) different with the conventional smoked skipjack and other treatments, although this sample had a little bit dark brown color.

For flavor, sample heated first and followed by dipped in 0.8% liquid smoke and then heated again, has a different and better flavor compared to the conventional smoked skipjack and the other treatments. The flavor recognized as a real smoked skipjack flavor and more preferred by panelist. Taste also has a same result as flavor. Sample heated and followed by dipped in 0.8% liquid smoke and then heated, has a different and better taste compared to the conventional smoked skipjack and the other treatments. The sample taste was more delicious compare to the conventional smoked skipjack and the other treatments. Texture also has a same result; the sample heated first, then dipped in 0.8% liquid smoke and then heated again, has a good texture, more dry and solid. The picture of conventional (traditional) smoked skipjack and smoked skipjack heated first, and dipped in 0.8% liquid smoke and then heated again, can be seen in Figure 5.

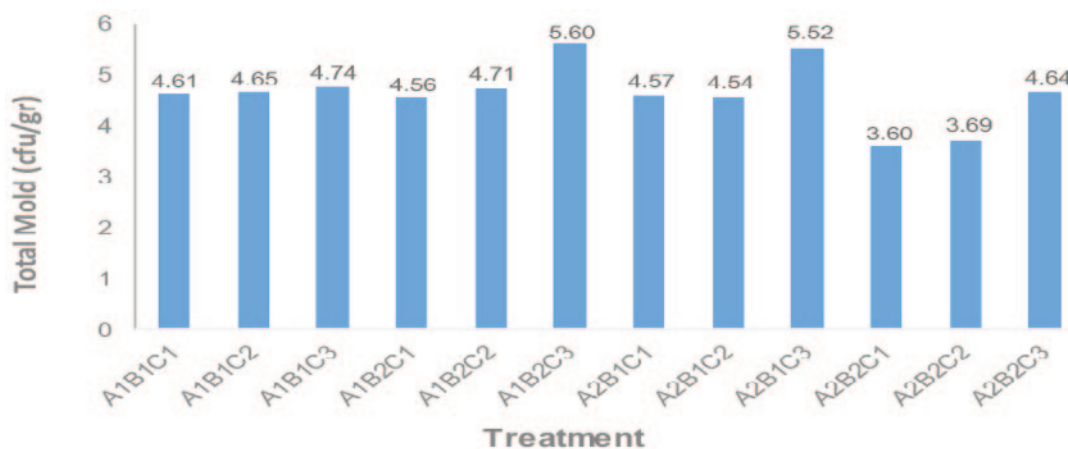
DISCUSSIONS

Water content

The variation in water content across treatments highlights the impact of processing methods, particularly steaming, which increased moisture retention due to protein denaturation. Except for the sample that was steamed before dipping, the range of water contents was similar to traditional local products. Water contents of traditional smoked fish products varied between 46–59%^{27,28}. Traditional methods aligned closely with non-steamed samples, suggesting that liquid smoke application can replicate conventional smoking while maintaining product stability^{27,28}. The significant effect of application method ($P \leq 0.01$) supports the role of protein denaturation in water retention. This condition was influenced by increased water-holding capacity (WHC) in treatments B2 and B3 due to protein denaturation, a phenomenon also observed by²⁹ in their work with smoked fresh and frozen fish.

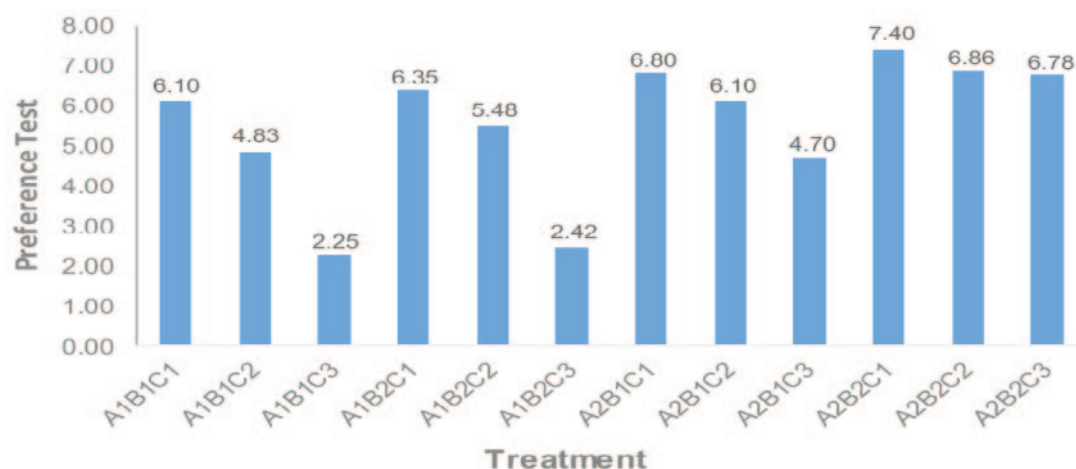
Protein content

Phenol content differences reflect smoke penetration efficiency, with steaming enhancing absorption. Phenol is representative of all smoke components that exist in smoked fish. Increased phenol content, followed also by increased in other smoke components²⁸. The correlation between phenol levels and pH reduction aligns with²⁸, confirming phenol as a key



A1B1C1: control at 0 day; A1B1C2: control at 3 day; A1B1C3: control at 6 day; A1B2C1: sprayed with 2,5% calcium propionat at 0 day; A1B2C2: sprayed with 2,5% calcium A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day propionat at 3 day; A1B2C3: sprayed with 2,5% calcium propionat at 6 day; A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day; A2B2C1: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 0 day; A2B2C2: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 3 day; A2B2C3: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at at 6 day.

Figure 5. Total mold of smoked Eel with different treatments.



A1B1C1: control at 0 day; A1B1C2: control at 3 day; A1B1C3: control at 6 day; A1B2C1: sprayed with 2,5% calcium propionat at 0 day; A1B2C2: sprayed with 2,5% calcium A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day propionat at 3 day; A1B2C3: sprayed with 2,5% calcium propionat at 6 day; A2B1C1: immersed in 15% NaCl + 15% citric acid at 0 day; A2B1C2: immersed in 15% NaCl + 15% citric acid at 3 day; A2B1C3: immersed in 15% NaCl + 15% citric acid onat at 6 day; A2B2C1: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 0 day; A2B2C2: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at 3 day; A2B2C3: immersed in 15% NaCl + 15% citric acid and sprayed with 2,5% calcium propionate at at 6 day.

Figure 6. Preference test of smoked Eel with different treatments.

smoke component. Traditional methods yielded lower phenol levels than liquid smoke treatments, suggesting intensified flavor profiles in the latter. Phenol contents of traditional hot smoked skipjack of North Sulawesi, Indonesia smoked directly above fire for 3 h were 5.1-9.1mg%^{28,30} found phenol content of the cold smoked salmon for 24h were 184.21±21.75mg GAE/100g. Traditional smoked catfish in Riau, Indonesia smoked with different firewoods have phenol content range of 0.048±0.002 – 0.060±0.003%³¹.

Microbiological Quality and Safety

Microbiological results demonstrate the preservative effect of combined treatments, with reduced bacterial and mold counts compared to controls. The inhibition of spoilage organisms underscores the antimicrobial properties of liquid smoke, consistent with findings in similar studies.

Sensory quality analysis

Sensory analysis revealed that heating before liquid smoke application optimized appearance, flavor, and texture, with 0.8% concentration yielding the highest scores. The comprehensive sensory evaluation demonstrated that flavor scores were generally higher than appearance scores across all treatments, with flavor values being the highest among all sensory

parameters assessed. This probably because of panelists could recognize that the flavor of smoked skipjack that smoked with liquid smoke was better than flavor of conventional smoked skipjack.

The application method of liquid smoke showed varying effects on different sensory attributes. For appearance, only the application method had a significant effect ($P \leq 0.05$), while for taste, smoke concentration showed a highly significant effect ($P \leq 0.01$) and interaction showed a significant effect ($P \leq 0.05$). Interestingly, for texture evaluation, liquid smoke concentration showed no significant effect ($P \geq 0.05$), while application methods demonstrated a significant effect ($P \leq 0.05$). Despite variations in individual parameter scores, all sensory characteristics (appearance, flavor, taste, and texture) indicated that researchers favorably received all assessed samples.

The result also similar to that found by¹⁴ worked with cold smoked mackerel. Furthermore they stated that sample of smoked mackerel at 28°C and the sample of liquid smoked salmon resulted with an equal preference level, followed by sample of cold smoked at 22°C.

The triangle test results provided additional validation of sensory preferences. A study distinguished liquid-smoked products from conventional ones, favoring their enhanced

sensory attributes¹⁴. The triangle test confirmed these preferences, particularly for samples treated with 0.8% liquid smoke, which exhibited superior color, flavor, and texture. The sample that was heated first, dipped in 0.6% liquid smoke and then heated again showed a yellowish-brown color and bright appearance, demonstrating highly significant differences ($P \leq 0.01$) compared to conventional smoked skipjack. Similarly, the 0.8% liquid smoke treatment produced samples with enhanced flavor profiles that were recognized as authentic smoked skipjack flavor and were more preferred, while also providing superior taste quality that was more delicious compared to conventional products.

According to³², components of smoke will penetrate to middle layer during storage, and the dark brown color in surface will change to more light, which explains the visual improvements observed in the liquid smoke treatments¹⁴. worked with cold smoked mackerel at 28°C, found that liquid smoked salmon had a same preference level compared with cold smoked mackerel, supporting the effectiveness of liquid smoke application in producing high-quality smoked fish products.

Overall assesment

Overall, liquid smoke application, especially with pre-heating, improves product quality while maintaining traditional characteristics, offering a viable alternative to conventional smoking methods. The study demonstrates that liquid smoke treatment can effectively replicate and potentially enhance the quality attributes of traditionally smoked skipjack while providing better control over the smoking process.

CONCLUSION

The best application method of liquid smoke for fresh Skipjack fillet was fresh fillet heated at 70-80°C for 4hr, dipped in 0.8% smoked liquid for 20 mins, and then heated again at 70-80°C for 4hr. The second best was fresh fillet steamed for 30 mins, dipped in 0.8% smoked liquid for 20 mins, and then heated at 70-80°C for 4hr.

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