Food Sci. Preserv., 31(2), 210-217 (2024)
pISSN: 3022-5477, eISSN: 3022-5485

Food Science and Preservation
https://doi.org/10.11002/fsp.2024.31.2.210

Research Article

Pre-cooking and liquid smoke affect the physicochemical and microbiological quality and polyhydroxy aromatic hydrocarbon (PAH) content in smoked skipjack tuna (*Katsuwonus pelamis* L.)

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Abstract This study aimed to compare the quality of smoked fish products processed with liquid smoke with and without pre-cooking when stored at room temperature (25℃) for 0, 2, 4, and 6 days. The observations included moisture content, water holding capacity, color, and total plate and mold counts. The treated samples were also analyzed for polyhydroxyaromatic hydrocarbons (PAHs) at day 0. The study found that the pre-cooked products had a lower moisture content (55.1%) than the control (59.9%) during storage. Additionally, the pre-cooked product had a higher water holding capacity than the control. The TPC value indicates that the pre-cooked product meets the Indonesian standard for smoked fish up to the 2nd day of storage, whereas the control product only meets the standard on day 0. However, the total mold of the control products meets the Indonesian standard until the 2nd day of storage, while the pre-cooked products only meet the standard on day 0. The pre-cooked product had a higher lightness value (L*) than the control. The analysis of polyhydroxy aromatic hydrocarbon (PAH) showed that the pre-cooking process did not affect the concentration of PAH.

Keywords skipjack tuna, liquid smoke, pre-cooking, quality, room temperature storage

Citation: Harikedua SD, Saragih ED, Mongi EL, Damongilala LJ, Salindeho N, Dien HA, Taher N, Lohoo HJ, Siby MS. Pre-cooking and liquid smoke affect the physicochemical and microbiological quality and polyhydroxy aromatic hydrocarbon (PAH) content in smoked skipjack tuna (*Katsuwonus pelamis* L.). Food Sci. Preserv., 31(2), 210-217 (2024)

Received: October 11, 2023
Revised: February 02, 2024
Accepted: February 14, 2024

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1. Introduction

Tuna (*Thunnus* sp.), skipjack (*Katsuwonus pelamis* L.), and mackerel (*Euthynnus affinis*) commodities contributed about 12.39%, worth USD 189.53 million, to the Indonesian fishery surplus in 2022 (KKP, 2022). Of these, some will be sold fresh and processed using modern and traditional methods. Data show that Indonesia's fisheries and aquaculture output is used for export and domestic consumption. The national fish consumption rate in Indonesia reached an average value of 56.48 kg/capita/year in 2023 and is targeted to be higher in 2024. That is why improving the quality of traditional fisheries and diversifying fishery products is an alternative way to achieve this.

People of North Sulawesi, Indonesia, utilize skipjack (*Katsuwonus pelamis* L.) in traditional processed forms of smoked fish, known as *cakalang fufu* (skipjack loin smoked). Smoking is one of the preservation methods that is used widely in the world. The processing of smoked fish includes preparation, washing, draining, and smoking. Currently, the smoking used to produce *cakalang fufu* is hot smoking for 5-7 hours using wood or coconut shells as raw material. Many researchers have conducted research on smoked skipjack products in North Sulawesi for the past decade: research on the quality of smoked skipjack with the use of modified closed-smoked house (Dotulong et al., 2018), the effect of coconut water addition as a natural additive on the quality of skipjack smoked (Wonggo et al., 2018;), effect of mangosteen peel extract on the quality of skipjack smoked (Alfianto et al., 2020; Gusu et al., 2021), and the quality of smoked skipjack presto (Anti et al., 2018; Wowiling et al., 2020). Several studies on the use of liquid smoke on skipjack also have been conducted by many researchers, i.e., the quality of skipjack smoked using different types of liquid smoke raw material (Hadinoto et al., 2016; Talib et al., 2020; Malambu et al., 2021), the proximate composition of skipjack smoked using liquid smoke (Bora and Gasong, 2021), and the effect of liquid smoke on lead metal (Pb) of smoked skipjack (Djalil et al., 2024). Generally, the use of traditional smoking and liquid smoke study indicate the variety of processing results in non-uniform product quality and shelf life.

Pre-cooking is a processing technique used to address issues related to the texture, shelf life and safety of fishery products. It involves a physical change in the fish meat due to alterations in its chemical content. The objective of pre-cooking is to process the fish, remove its body juice (fat/oil) to prevent rancidity, and eliminate pathogenic and histamine-forming bacteria (Nuraini, 2014). The aim of the pre-cooking process is to reduce moisture and fat content and make the fish meat more compact (Irianto and Giyatmi, 2015). There is limited literature discussing the effect of pre-cooking on fishery products. Yuliastri et al. (2015) studied the effect of pre-cooking on smoked catfish, while Kpoclou et al. (2021) studied the effect of pre-cooking on smoked shrimp. This study will use pre-cooking combined with liquid smoke and the findings will provide valuable insights into their potential to enhance the quality and shelf life of skipjack smoked fish. Additionally, understanding the physicochemical and microbiological changes during room-temperature storage will help establish proper storage conditions and guidelines for the commercial production of skipjack smoked fish.

2. Materials and methods

2.1. Materials

2.1.1. Food materials

The research raw material was fresh skipjack tuna (*Katsuwonus pelamis* L.) procured from the local market in Manado, North Sulawesi.

2.1.2. Chemical materials

The chemical used for polyhydroxy aromatic analysis (PAH) in gas chromatography/mass spectrometry analysis was analytical grade from Merck (Darmstadt, Germany). Plate count agar (PCA), Butterfield's phosphate buffer solution, and potato dextrose agar (PDA) from Merck, and chloramphenicol were used as media for microbiological analysis. Other materials used were grade A liquid smoke from coconut shells Lubna Co. (Semarang, Central Java, Indonesia) and PE (polyethylene) plastic for the packaging purchased from a local store.

2.2. Processing and storage conditions

Fresh skipjack fish weight was, on average, 550±42.7 g. The fish was put into PE plastic, put into a cool box with a ratio of ice and fish 1:2 brought to the laboratory. The skipjack was washed thoroughly and the gills, head, and viscera were removed. The cleaned fish was pre-cooked by steaming for 30 min. Steaming was done by using boiling water. The steamed fish was cooled for 15 min. The cooled fish was immersed in liquid smoke with a concentration of 5% for 20 min. The fish was then dried using an oven at 60℃-80℃ for 10 h. Fish is then cooled and packaged using PE plastic then stored at room temperature 25±2℃. The analysis was conducted every 2 days until 6 days of storage.

2.3. Measurement of quality characteristics

2.3.1. Color

The color was evaluated using CHN CS-10 to determine L* (lightness), a* (redness), and b* (yellowness). Before each use, the chromameter was calibrated using a white and black calibration plate. Smoked fish are placed in a clear container that is flat and not too thick. Then the chromameter was attached as close to the sample and illuminated. L*, a*, and b* readings. Five readings were averaged for each measurement.

2.3.2. Moisture content

Analysis of moisture content is carried out using the gravimetric method according to AOAC (2005). The moisture content is calculated as a weight percent by how many grams of sample weight with the difference in the weight of the sample that has not been evaporated with the sample that has been dried. Each sample was replicated twice. Three replicates were carried out for each of the samples analyzed.

2.3.3. Water holding capacity (WHC)

The water holding capacity was calculated as the percentage of water retained in the ground meat after centrifugation for 5 min, according to Rustad (1992). Fish samples were ground with a grinder and centrifuged for 5 min at 1,477 rpm (264 ×*g*). Five replications were performed for each sample.

2.3.4. Polycyclic aromatic hydrogen (PAH)

Using GC-MS, PAH was analyzed using the US EPA 8100 Method (EPA, 1984). The extraction of hydrocarbons from the samples was performed using a sonicator according to US SW-846 method 3550. A 10.00 g of each composite sample was extracted with a 50:50 mixture of acetone and methylene chloride, spiked with 1 mL of PAH internal standard and shaken thoroughly to ensure good mixing before an ultrasonic bath. A 2.00 μL sample of the extracts was injected into the GC port set to column conditions: HP-5 cross-linked PH-ME siloxane, length 30 m, I.D: 0.25 mm, thickness 1 μm with helium carrier gas set in spitless constant flow mode at 1.2 mL/min flow rate. Identifying and quantifying individual PAHs was based on an internal calibration standard containing known concentrations of the 16 PAHs (EPA-16). The specificity of the 16 PAHs sought in the samples was confirmed by transition ions (quantifier and qualifier), as shown by their retention times, which corresponded to their respective standards. The measured area ratios of the precursor to the quantifier ion were close to those of the standards. The results obtained were expressed as mg/kg concentration per analyte.

2.3.5. Total plate count

The total plate count was analyzed according to the Indonesian National Standard for Total Plate Count Analysis (SNI 2332.3:2015). Twenty-five grams of the sample were weighed and put in a sterile container. Two hundred and twenty-five mL of Butterfield's Phosphate Buffered solutions were added to the container and homogenized for 2 min. Ten mL of the above homogenate were then put into 90 mL of Butterfield's Phosphate Buffered Solution for a 10^{-2} dilution, etc. One (1) mL from each dilution was pipette and put into a sterile petri dish. 12 mL-15 mL of PCA was added to the petri dish containing the sample. Samples were mixed with PCA. Then, the petri dishes were incubated in an inverted position at 35±1℃ for 48 h.

2.3.6. Total mold count

The total mold was analyzed according to the Indonesian National Standard for Total Plate Count Analysis (SNI 2332.7:2009). Twenty-five grams of the sample were weighed and put in a sterile container. Two hundred and twenty-five mL of Butterfield's Phosphate Buffered solutions were added to the container and homogenized for 2 minutes. Ten mL of the above homogenate were then put into 90 mL of Butterfield's Phosphate Buffered Solution for a 10^{-2} dilution, etc. One mL from each dilution was pipette and put into a sterile petri dish. 12 mL-15 mL of PDA was added to the petri dish containing the sample. Samples were mixed with PDA. Then, petri dishes were incubated in an inverted position. Put in an incubator at 25±1℃ for 5 days.

2.4. Statistical analysis

The data was processed using Microsoft Excel 365. The results are presented as the average of measurements, expressed as mean and standard deviation. The effect of the storage and precooking study was analyzed using a two-way analysis of variance ($p<0.05$) with PAST software version 4.13. Statistical significance between groups was calculated using a t-test with evidence of $p<0.05$.

3. Results and discussion

3.1. Effect of processing conditions on skipjack smoked fish color

Color is produced by combining cold staining and the heat-induced Maillard reaction, which occurs when smoke and food components react chemically at elevated temperatures (Puke and Galurboda, 2020). Color is also associated with product quality, as discoloration can signify spoilage. The ANOVA test results indicate a significant effect of storage and treatment. Table 1 shows that the average L* value of the pre-cooking treatment (51.34) is higher than that of the control liquid smoked skipjack (47.25). This suggests that the

https://doi.org/10.11002/fsp.2024.31.2.210

Table 1. Color value changes of smoked skipjack during room temperature storage in control and pre-cooking samples

Treatment	Storage (days)	L*[1)]	a*[1)]	b*[1)]
Control[2)]	0	35.51±6.55^{b}	3.94±9.60^{b}	5.95±7.51^{a}
	2	33.7±18.83^{a}	1.31±1.40^{a}	6.31±3.69^{ab}
	4	50.31±2.15^{a}	-6.63±0.16^{c}	7.70±0.91^{c}
	6	70.05±1.96^{c}	-2.044±0.23^{c}	4.06±0.48^{a}
Pre-cooking[3)]	0	34.96±14.71^{b}	-4.95±1.80^{c}	6.44±1.30^{b}
	2	54.09±5.05^{a}	-1.13±0.67^{c}	7.14±1.95^{c}
	4	54.37±1.58^{a}	-1.28±0.56^{c}	11.86±0.12^{e}
	6	61.97±0.49^{c}	-4.51±0.42^{c}	9.52±0.14^{d}

[1)]All values are mean±SD (n=5). Different uppercase letters within each column indicate significant differences among samples (p〈0.05).
[2)]Fish treated with liquid smoke without pre-cooking.
[3)]Fish treated with liquid smoke and pre-cooked for 30 min.

pre-cooking treatment results in a lighter color for the skipjack tuna product compared to skipjack tuna without pre-cooking treatment. This is closely related to the value of water-holding capacity, where liquid smoked skipjack treated with pre-cooking has a high water-holding capacity value and a high L* value.

The redness (a*) value tends to decrease to negative during storage, indicating that the fish is more greenish. The b* value increased during the storage process until day 4 and decreased on day 6 in the control and pre-cooking treatments. This indicates that smoked skipjack has a characteristic yellowish color, but the value changes along with the biochemical changes of the meat during storage. The results showed that both treatments experienced decreased redness and changes in yellowness during storage. This agrees with the study on smoked salmon fillets, which demonstrated that the color of smoked fillets from all treatments decreased redness and yellowness over time (Chan et al., 2020).

3.2. Effect of storage and processing conditions on skipjack smoked fish moisture content

Fig. 1 shows the average moisture content of liquid smoked skipjack tuna during storage with and without pre-cooking. The results indicate a significant difference in moisture content between the two treatments and storage time. Smoked fish that underwent pre-cooking had lower moisture content than those that did not. This suggests that

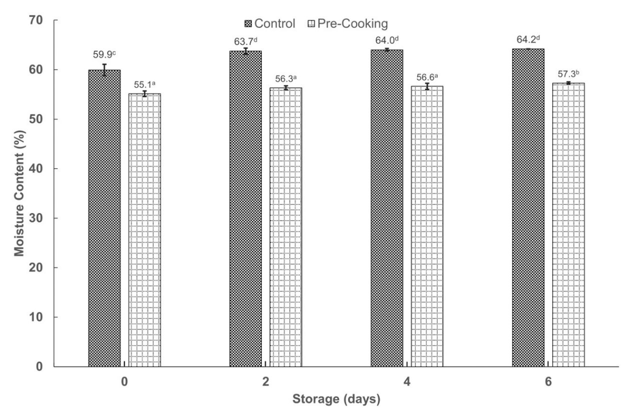

Fig. 1. Moisture content of liquid smoked tuna during storage in control and pre-cooking samples. All values are mean±SD (n=3). Different superscript letters on the bars represent significant differences by Tukey's Honest Significance Difference Test at p〈0.05.

using steam as a heat transfer medium reduces the moisture content of the fish. Steaming fish meat for one hour at 90-95℃ reduces fat and moisture content (Karo, 2017). This, in turn, affects the aroma of the fish meat as the lower the moisture content, the more liquid smoke content will be absorbed into it. The moisture content value of smoked skipjack from both samples tends to increase from 0 to 6 days of storage. The moisture content of smoked fish may be affected by temperature, duration of soaking and drying, and storage time after processing. According to the Indonesian National Standard (SNI 2725:2013), the maximum allowable moisture content for hot-smoked fish is 60%. The control smoked skipjack product meets the Indonesian standard only on the production day, while the pre-cooking treatment remains compliant for up to 6 days.

3.3. Effect of storage and processing conditions on skipjack smoked fish water holding capacity (WHC)

Fig. 2 shows the average water holding capacity (WHC) of liquid smoked skipjack during storage under control and pre-cooking conditions. The results indicate a significant difference between the treatments but no significant difference during storage. The pre-cooking treatment can improve the fish meat's ability to retain water during the heating and drying after being soaked in a liquid smoke solution. Maintaining WHC is crucial for preserving fish quality. Decreasing WHC can negatively impact appearance and surface texture, ultimately reducing the sensory quality of the food (Chan et al., 2020).

3.4. Effect of storage and processing conditions on skipjack smoked fish polycyclic aromatic hydrocarbon (PAH)

The concentrations of the 16 EPA target PAHs (mg/kg) in the liquid smoke skipjack samples are shown in Table 2. Only the very low molecular weight PAH naphthalene was detected in both samples at 0.03 mg/kg. The concentrations of the other PAHs were below <0.01 mg/kg. The benzo[a]pyrene, the concern of the Indonesian National Standard for Smoked Fish, was detected at low concentration. It is indicated that using liquid smoke may reduce the concentration of benzo[a]pyrene; however, the pre-cooking process did not affect the benzo[a]pyrene concentration. In addition, the benzo[a]pyrene in both samples (<10 μg/kg) was probably higher than the maximum background limit of 5 μg/kg set by the Indonesian National Standard of Smoked Fish. This could be because the samples may contain lower levels of PAHs. However, the detection limit of the instruments cannot indicate the exact concentration. Therefore, we can only suggest that liquid smoke should be lower than 5% when processing smoked fish.

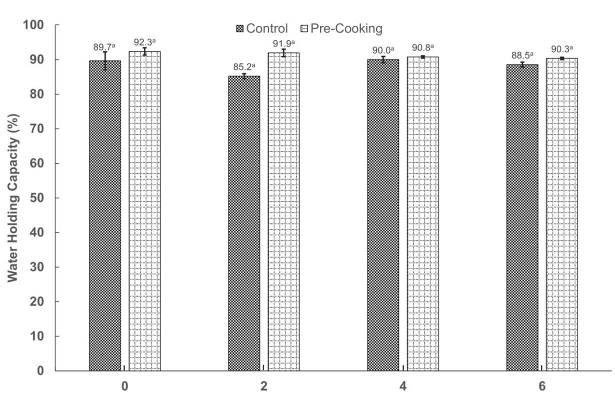

Fig. 2. **Water holding capacity (WHC) of liquid smoked skipjack tuna during storage in control and pre-cooking samples.** All values are mean±SD (n=3). Different superscript letters on the bars represent significant differences by Tukey's Honest Significance Difference Test at $p<0.05$.

3.5. Changes in microbial load during storage

3.5.1. Total plate count (TPC)

The smoked skipjack without pre-cooking and stored for 6 days had the highest average TPC value, 9.8×10^8 or log 8.99 CFU/g (Table 3). Meanwhile, the lowest TPC average value was observed in the control and with pre-cooking treatment on day 0. The research data indicated that the TPC value increased during product storage at room temperature, due to the decreased quality of smoked fish products. Samples that meet the quality requirements for smoked fish, based on the Indonesian Standard of Maximum Limit of Microbial Contamination in Food (SNI 2725:1:2009), are those treated without pre-cooking and stored for 0 days (0 CFU/g), as well as those treated with pre-cooking and stored for 0 (0 CFU/g) and 2 days (3.6×10^5 CFU/g), where the maximum limit of TPC value is 5.0×10^5 CFU/g. The high and low microbial count is strongly influenced by the quality of raw materials, processing, and storage. The growth of bacteria slows down as the temperature of the fish decreases. The high humidity in the plastic packaging is caused by the high moisture content of the product, which leads to rapid microbial growth. Moisture content is a crucial factor in determining the quality of smoked fish. The shelf life of smoked fish is significantly affected by its moisture content, as it provides a medium for microbial growth.

3.5.2. Total mold count

The control sample and the 30-minute pre-cooking sample without storage had the lowest mold values, while the highest mold value was found in the control smoked fish sample

https://doi.org/10.11002/fsp.2024.31.2.210

Table 2. Polyhydroxy aromatic hydrocarbons (PAH) contents of liquid smoked skipjack with different treatments

No	Parameter	Samples (mg/kg)	
		Control[1)]	Pre-cooking[2)]
1	Naphtalene	0.03	0.03
2	Acenaphtilene	$<$0.01	$<$0.01
3	Acenaphtene	$<$0.01	$<$0.01
4	Fluorene	$<$0.01	$<$0.01
5	Phenanthene	$<$0.01	$<$0.01
6	Antrachene	$<$0.01	$<$0.01
7	Fluoranthene	$<$0.01	$<$0.01
8	Pyrene	$<$0.01	$<$0.01
9	Benz(a)Anthracene	$<$0.01	$<$0.01
10	Chrysene	$<$0.01	$<$0.01
11	Benzo[a]pyrene	$<$0.01	$<$0.01
12	Benzo[b]fluoranthene	$<$0.01	$<$0.01
13	Benzo[k]fluoranthene	$<$0.01	$<$0.01
14	Indeno[1,2,3-cd] pyrene	$<$0.01	$<$0.01
15	Dibenz [a,h] anthracene	$<$0.01	$<$0.01
16	Benzo[ghi]perylene	$<$0.01	$<$0.01
Total % PAH		0.03	0.03

[1)]Fish treated with liquid smoke without pre-cooking.
[2)]Fish treated with liquid smoke and pre-cooked for 30 min.

Table 3. Total plate count of liquid smoked skipjack during room temperature storage in control and pre-cooking samples

Treatment	Total plate count (CFU/g) (log X)[1)]			
	Storage (days)			
	0	2	4	6
Control[2)]	0^{a}	5.87 ± 4.10^{b}	8.56 ± 5.32^{d}	8.99 ± 3.90^{e}
Pre-cooking[3)]	0^{a}	5.55 ± 3.40^{b}	7.04 ± 5.75^{c}	8.83 ± 8.32^{e}

[1)]All values are mean±SD (n=3). Different uppercase letters indicate significant differences between samples ($p<0.05$).
[2)]Fish treated with liquid smoke without pre-cooking.
[3)]Fish treated with liquid smoke and pre-cooked for 30 min.

stored on day 6, which was 1.7×10^{6} CFU/g or log 6.23 CFU/g (Table 4). The research data indicates that the average mold value increased from day 0 to day 6. Samples of skipjack that were smoked without pre-cooking on the second day still meet the requirements of the Indonesia National Standard of Smoked Fish (SNI 2725:2013) regarding mold in smoked fish, which is a maximum of 1×10^{2} CFU/g. However, samples that underwent a 30-minute pre-cooking treatment no longer meet the requirements of SNI 2725: 2013. Additionally, all treatments on day 4 and day 6 of storage have mold values that do not meet the requirements of SNI 2725:2013. Mold growth is generally influenced by the substrate, moisture content, pH, and chemical compounds in the environment. Additionally, mold growth in food is affected by the moisture content of the product. This is because the moisture content significantly impacts the growth

Table 4. Total mold count of liquid smoked skipjack during room temperature storage in control and pre-cooking samples

Treatment	Total Mold Count (CFU/g) (Log X)[1)]			
	Storage (days)			
	0	2	4	6
Control[2)]	0[a]	1.70±0.00[a]	4.65±3.96[b]	6.23±5.16[e]
Pre-cooking[3)]	0[a]	2.81±1.60[a]	4.93±5.50[c]	5.83±4.62[d]

[1)]All values are mean±SD (n=3). Different uppercase letters indicate significant differences between samples (p<0.05).
[2)]Fish treated with liquid smoke without pre-cooking.
[3)]Fish treated with liquid smoke and pre-cooked for 30 min.

rate of microorganisms and the rate of chemical/biochemical reactions that can cause food damage. Mold is a microbe that is resistant to heat and dry conditions. These selective conditions allow mold to grow well (Wattimena, 2020). Microbial activity can be influenced by storage; the longer the product is stored, the more the number of bacteria increases.

4. Conclusions

Pre-cooking for 30 minutes produced products with moisture content in compliance with the Indonesian National Standard of Smoked Fish for up to 6 days of storage. However, the total plate count and mold value of liquid smoked skipjack were only by the Indonesian Standard of Maximum Limit of Microbial Contamination in Food up to day 2 and 0 storage at room temperature, respectively. The results of WHC and color measurements show that if the WHC value is high, the resulting color (L*) will be lighter, and vice versa. If the WHC value is low, the color will be darker. The GC-MS analysis of PAHs showed that pre-cooking did not affect the concentration of PAHs in smoked fish. Further study should be conducted to determine the appropriate concentration of liquid smoke and pre-cooking time that can be used to produce high-quality smoked skipjack.

Funding

This work was financially supported by the Research and Community Service Agency of Sam Ratulangi University through Basic Research Scheme 2023 No. 996/UN12.13/LT/2023 for Silvana Dinaintang Harikedua.

Acknowledgements

None.

Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Harikedua SD, Mongi EL, Damongilala LJ. Methodology: Mongi EL, Damongilala LJ, Siby MS. Formal analysis: Saragih ED, Siby MS. Validation: Dien HA, Salindeho N, Taher N, Lohoo HJ. Writing - original draft: Saragih ED, Mongi EL, Harikedua SD. Writing - review & editing: Harikedua SD, Damongilala LJ.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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https://doi.org/10.11002/fsp.2024.31.2.210

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