

Effect of Time Length Fermentation to *Katsuobushi* Oxidation Rate As Fish Flavor Based

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Abstract. *Katsuobushi* or dried smoked skipjack had a distinctive flavor and widely used in traditional Japanese cuisine. This study aimed to evaluate the oxidation rate of *Katsuobushi* with different length fermentation. The processing treatment of the product were the differences of fish boiling time (30 min and 60 min) and the length of fermentation: 1 week, 2 weeks and 3 weeks. The glutamic acid content, the oxidation rate (thiobarbituric acid and peroxide value) and Total Plate Count of *katsuobushi* were analyzed statistically using analysis of varians. Significant differences were found among 3 weeks of fermentation compare to 1 weeks fermentation ($P < 0.05$). The conclusion of this study was *katsuobushi* with 60 min boiling and 3 weeks fermentation was potential to be developed become basic ingredients for the fish flavor.

Keywords: Fermentation, Fish flavor, *Katsuobushi*, Oxidation

1. Introduction

Skipjack is one of marine fishes consumed and produced in Indonesia, including in Central Java. The statistical data of Marine and Fisheries Bureau, Central Java province in last 5 years (2011-2016) noted the skipjack on the first position as the marine fish living in Central Java, Indonesian waters around Central Java, which potentially to be exploited and further processed. The potential value of skipjack is about 29,000 tons per year with the 114 % utility [1]. *Smoked fish* was one of traditional product as skipjack as a raw material, processed with conventional method using coconut shell, coconut husks, corn cobs, and firewood as fuel. Smoked fish products could be stored within 2-3 days at room temperature. The high amount of water content caused microbial growth rapidly and fish spoilage [2]. Therefore, its necessarily needed to create skipjack-based products which had a long-term storage. In Japan, there was a product made from dried smoked fish, known as *Katsuobushi*, with a variety of processing, among others, boiling, drying in the sun, smoking, and fermentation [2]. The specific taste and aroma of *katsuobushi* was to be suitabled for enhanced *umami* taste of many dishes. Fermentation processing on *katsuobushi* made



the presence of glutamic amino acid (umami taste producer) as resulted from fish protein degradation. The range of free glutamate in other savoury condiments ranges widely, but is comparable. It must be noted that the above condiments are added to foods during food preparation in a relatively small amount and they interact with the original food matrix, including the present glutamate and other free amino acids [3]. Numerous studies on the source of umami flavor were previously performed from various scientific [4]. In previous studies, the concentration of free glutamate found in 11 *belacan* samples ranged from 601 to 4207 mg/100 g [3], which is comparable to values reported previously [5, 6]. Research related to the processing of fish flavor have been reported, including by Giyatmi et. al. [7] stated that the fermentation conducted for 3 weeks was the optimum time to produce the most preferred of katsuobushi. The differences of smoking method affect the characteristic of smoked fish flavor. This study aimed to to evaluate the oxidation rate of Katsuobushi with different lenght fermentation

2. Methods

2.1. Preparation of skipjack samples

Raw materials used in this study were fresh skipjack weight in the range of 200-300 gram for each fish and obtained from TPI Tambak Lorok Semarang, Indonesia. All samples were cleaned and stored at $< 5^{\circ}\text{C}$ or chilled at -18°C until use.

2.2. Experimental method and apparatuses

The chemicals reagents and microbiological reagents tests were distilled water, potassium chromate, AgNO_3 , nutrient agar and others. The tools used in this study were cutting boards, pots, stoves, spinner, table drainer and digital scales. The equipments used for the analysis of the quality of katsuobushi were oven (Mettler), kjeltec system (Kjeltec 2300 Analyzer Unit; Foss Tecator AB), furnace (Mettler), soxhlet apparatus (Soxtec Avanti 2050 Auto System; Foss Tecator AB, Hoganas, Sweden), spectrophotometers (Prestige-21) and HPLC (Shimadzu RF-138). The procedure to make katsuobushi followed method proposed by Giyatmi et al., [7] with slight modifications in the boiling and fermentation process. The production of katsuobushi is in Fig. 1. After became katsuobushi, all samples were then wrapped in plastic and placed in used container plastic for 3 weeks at temperature room ($30-32^{\circ}\text{C}$).

2.3. Analysis of katsuobushi

The quality analysis of katsuobushi include the total number of bacteria [8], The content of glutamic acid [9], Thiobarbituric Acid Reactive Substance (TBARS) [10], and Peroxide Value (PV) [11]. And also the content of Free Fatty Acids (FFA) [11]: 2 mL 0.5N NaOH/methanol was added to 20 mg of fat, which was later saponified for 10 minutes at 105°C . It was examined after applying 2 mL boron trifluoride/methanol, and methylated. Then, 2-3 mL hexane (HPLC grade) and 2 mL saturated NaCl solution were added. The supernatant of the mixture used the separated funnel was analyzed by gas chromatography (Hewlett Packard 6890 series; Palo Alto, CA, USA). The column was set up with an HP-FFAP capillary column (25 m x 0.32 mm internal diameter, 0.5 μm film thickness); initial oven temperature of 130°C (1 minute), increased at $2.5^{\circ}\text{C}/\text{min}$ to a final temperature of 230°C (10 minutes); injector temperature 230°C , detector temperature 250°C ; helium carrier gas with a split ratio of 20 : 1, and flow rate of 1 mL/min.

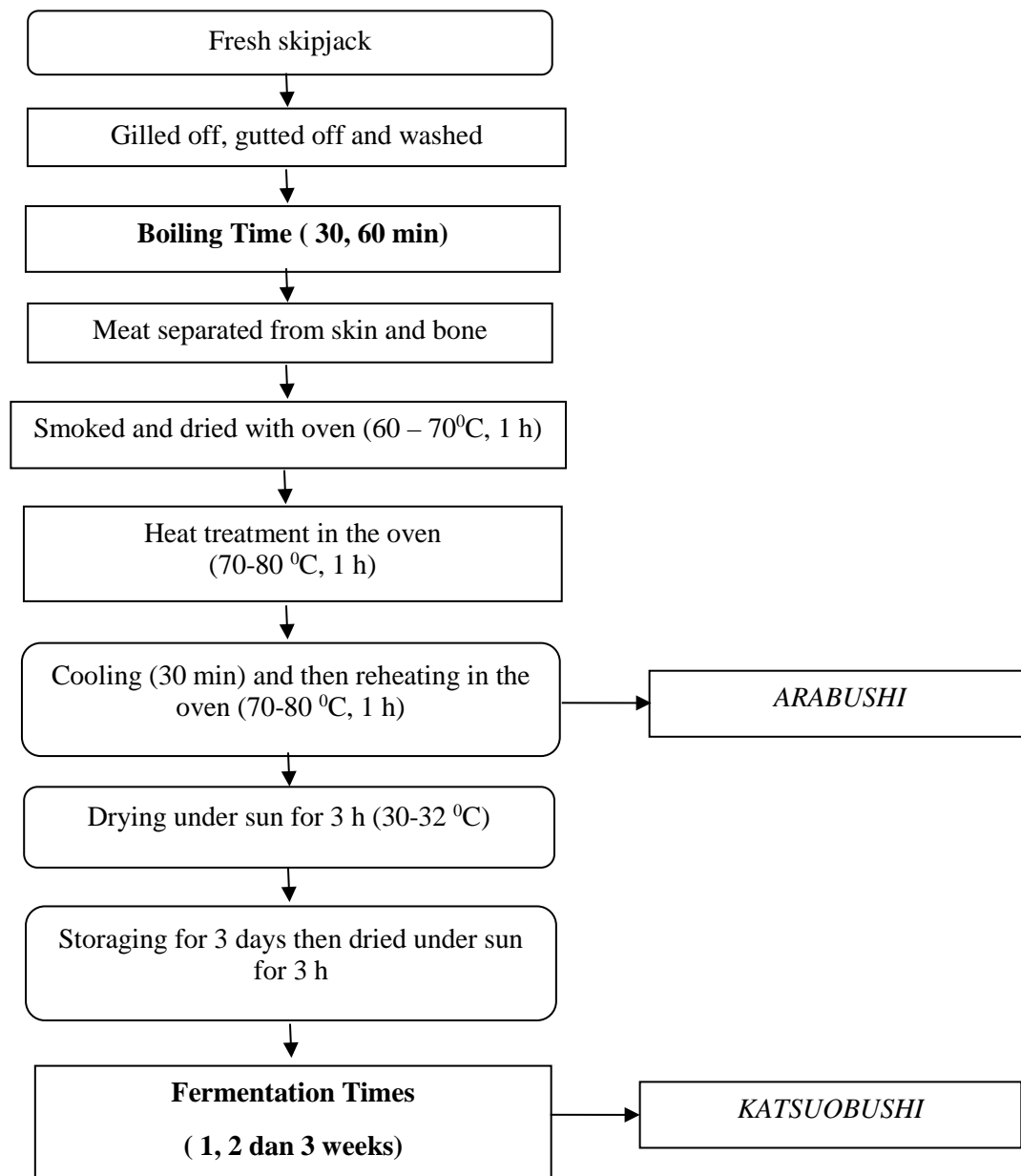


Figure 1. The flow chart of katsuobushi production

2.4. Data analysis

The contents of glutamic acid, TBA, PV, and the number of bacteria on katsuobushi were analyzed using analysis of variants

3. Results and Discussion

Katsuobushi Oxidation Rate

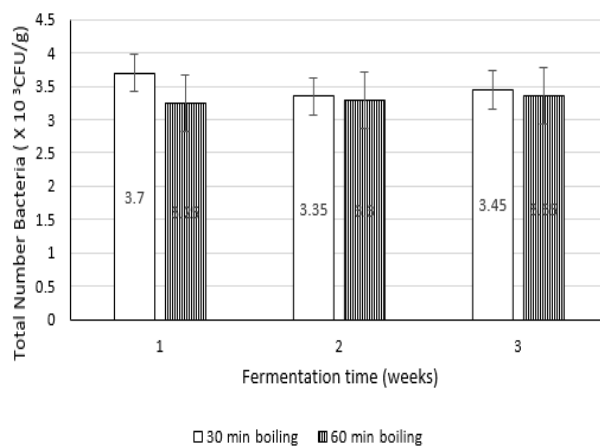


Figure 2. The total number of bacteria (cfu/g) on katsuobushi

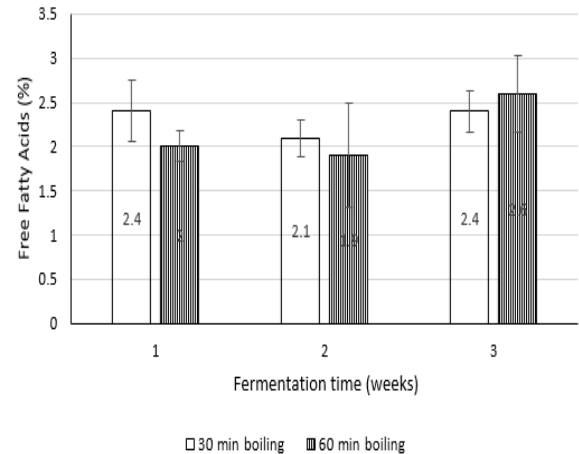


Figure 3. The Free Fatty Acid of katsuobushi

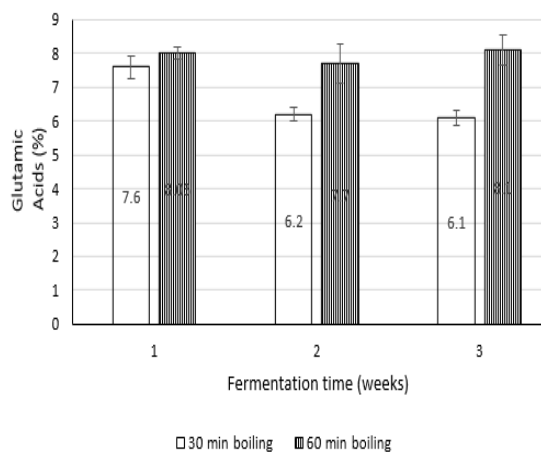


Figure 4. Glutamic acids content (%) of katsuobushi

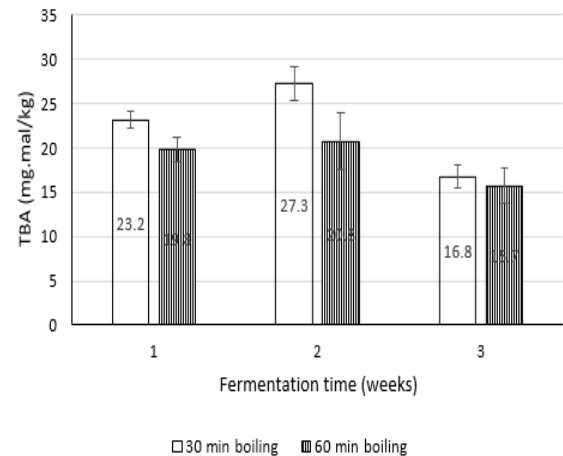


Figure 5. TBARS (mg.mal/kg) of katsuobushi

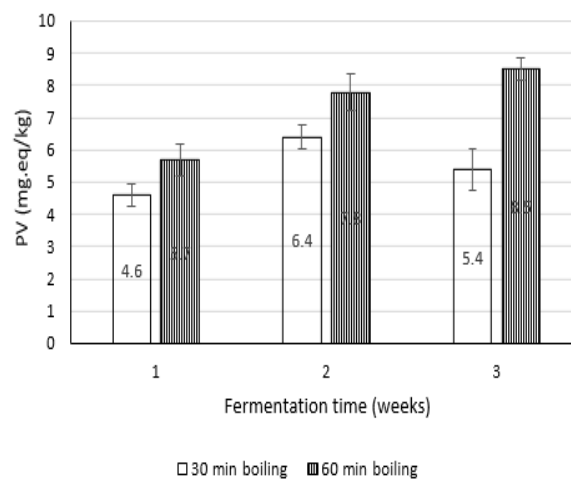


Figure 6. Peroxide value (mg.eq/kg) contained in katsuobushi

3.1. Total plate count (TPC) contents of katsuobushi

Fig. 2 shown that TPC contents of katsuobushi boiled for 60 minutes was lower than katsuobushi boiled for 30 minutes, during storage at room temperature ($28 \pm 1^\circ\text{C}$). The total microorganism growth of katsuobushi with 30 min boiling, after 1 week fermentation was 3.7×10^5 CFU/g. After 3 weeks, there was decreased in microbial growth, giving 3.45×10^5 CFU/g. This would indicate that nutrition contained at katsuobushi was decreased, and there was competition between microorganism contained in katsuobushi. Meanwhile, katsuobushi with 60 min boiling shown the normal trend compared with katsuobushi with 30 min boiling, whereas trend of TPC tend to increase until 3 weeks fermentation. This trend indicated that the boiling process combined with the liquid smoke from coconut shells were able to suppress microbial growth. Zuraida et al. [12] reported that liquid smoke contained bioactive compounds such as phenols, carbonyls and organic acids. Therefore, the coconut shell liquid smoke has the potential in increasing the shelf life of proteinaceous food products [12, 13].

3.2. Free fatty acids of katsuobushi

Based on Fig. 3, we showed that the values of free fatty acids (FFA) of katsuobushi for both treatment were fluctuative after 1 until 3 weeks of fermentation. It could be done because skipjack as one of marine fish contained more quantity of unsaturated fatty acid compared with saturated fatty acids. The component of long-chain unsaturated fatty acids, such as linoleic and linolenic acids, directly deriving from skipjack as a raw material of katsuobushi and also from microbial metabolism. This case was importance because their oxidative degradation may lead to the formation of a characteristic aging flavor [14].

Katsuobushi consist of many compounds (volatil during fermentation, and non-volatile) that affect katsuobushi flavor, many of these aroma compound are synthesized by bacteria, other derive directly from the raw materials. The combination of raw material condition, presence of bacteria, and degree of aeration would influenced medium-chain fatty acids such as hexanoic, octanoic and decanoic acid [15]. And then, as the composition of these acids increases, it will produce off flavors.

3.3. Glutamic acid contents of katsuobushi

Based on Fig. 4, we can seen that the differences of boiling time gave effect on katsuobushi glutamic acid content ($p < 0.05$). This is presumably because the protein was degraded into several amino acids, especially the generation of umami taste, glutamic amino acid caused by boiling process on the production of katsuobushi. This result is in line with research conducted by Mc Gee [16] and Daniel et.al. [17], explored katsuobushi as products that can be used as an umami flavor associated manufacturing process.

3.4. Thiobarbituric acid reactive substances (TBARS) of katsuobushi

The malondialdehyde (MDA) contents in katsuobushi samples are shown in Fig.5. TBARS values represent the content of secondary lipid oxidation products, mainly aldehydes and carbonyls of hydrocarbons, that contribute to off-flavors and flavors in meat [18]. As the period fermentation, the TBARS values are increased significantly ($P < 0.05$) from 1 week toward to 2 weeks fermentation, but then decrease after 3 weeks fermentation. This case done in both boiling treatment of katsuobushi. According to Ozer and Sariçoban [19], even though MDA is a secondary product of lipid oxidation, this does not unavoidably mean that TBARS value continues to increase throughout the storage. Also, this trend was probably caused by losses in the oxidation products formed, particularly the volatile counterparts. MDA and other short-chain carbon products of lipid oxidation are not stable and decompose to organic alcohols and acids, which are not detected by the TBARS test [20]. The decrease in the

TBARS values after 3 weeks fermentation is thought to be the result of MDA reactions with proteins [21].

3.5. Peroxide values of katsuobushi

The concentration of peroxide katsuobushi determined by hydroperoxides. Differences in heat process can be caused by differences in fish texture as a based raw for prepared katsuobushi. In this study, katsuobushi with 60 min boiling more easy to oxidized. As well as done with the katsuobushi with 30 min boiling that reached a peak at 2 weeks fermentation and decrease after 3 weeks fermentation. This case in line with previous study done by Lee et al. [22], who reported that peroxides value of cooked pork patties increases and thereafter decrease with the time of storage. Juntachote et al. [23] and Teets and Were [24] stated that peroxides are very reactive and may actually decrease during the storage of lipid-containing foods. This result is in line with the research conducted by Yusnaini et.al. [25] and Hwang et al. [26] which stated that heat temperatures caused high levels of oxidation. In addition, boiling and fermentation times affected the content of katsuobushi peroxide value ($p < 0.05$) (Fig. 6). Skipjack as red fleshed fish species had a high heme pigment especially myoglobin. Heme pigment is a source of Fe which is an indicator of heating and oxidation catalysts. Myoglobin will react with peroxide of polyunsaturated fatty acids fish. The average of katsuobushi PV with 60 min of boiling time was higher than katsuobushi PV boiled in 30 min. Fe^{2+} concentration increased dramatically during boiling so that resulted in at oxidation in fish. The increase of peroxide value supports the view that the formation of new hydroperoxides is occurring at a faster rate than the degradation of hydroperoxides into secondary oxidation products during the first part of refrigerated storage. As the lipid oxidation progresses, the decomposition of hydroperoxides into secondary products increases at a higher rate as compared to the formation of new hydroperoxides, resulting in decreased peroxide value [24].

4. Conclusion

In conclusion, this study showed that the longer time of boiling fish raw material and time of fermentation so the higher contents of glutamic acid, PV and TBA would rised. The free fatty acids was also rised, thus katsuobushi more easy to oxidized. So, for further research, it is necessary to conduct a study to make katsuobushi as the basic ingredient of making fish flavor more protected so as not easily oxidized.

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