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FULL PAPER

Characteristics of chikuwa with the addition of liquid smoke as an antibacterial agent

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Abstract

Chikuwa is a surimi-based product that quickly deteriorates due to bacterial growth. Chikuwa has a high nutrient content from fish as its main ingredient. The high nutritional content leads to quality degradation and a shortened shelf life. The addition of liquid smoke to chikuwa can inhibit the growth of microorganisms, such as bacteria. This research aimed to investigate the effect of adding liquid smoke as an antibacterial agent to catfish and snapper surimi-based chikuwa. The results showed that the application of liquid smoke significantly affected the pH, total volatile base nitrogen, and total plate count of chikuwa compared to chikuwa without the addition of liquid smoke. However, the addition of liquid smoke did not affect the a_w value. The use of different types of fish significantly affected the results. This is due to the different nutrient contents in each fish. Chikuwa with and without the addition of liquid smoke resulted in good quality and consumable products as indicated by TVBN and TPC values has met the standards of 35 mg-N/100 g and 1×10^5 CFU/g, respectively.

1. Introduction

Chikuwa is a surimi-based product with seasoning shaped using bamboo rods and roasted at 130-180°C until its inner temperature reaches 75°C (Jia *et al*., 2018; Leviyani *et al*., 2019). Chikuwa belongs to a gel-based fishery product, where texture is an important parameter to determine the quality. Low-quality chikuwa is easily broken when chewed. As a result, fish meat used as raw materials affects the quality of chikuwa. The texture of catfish varies depending on the species (Cheng *et al.*, 2014).

Catfish (*Clarias* sp.) and red Snapper (*Lutjanus* sp.) are Indonesian fishery commodities whose production is expected to increase annually. They come from the broader Indonesian ocean that reaches 104,000 km (Tran *et al*., 2017; Colon, 2018). In Indonesia, catfish are easily cultured freshwater fish (Hastuti and Subandiyono, 2014). Catfish is mainly consumed by frying or grilling, and it can be processed into Kamaboko (Suryaningrum *et al*., 2015) and Abon (meat floss) (Sundari *et al*., 2017). Red Snapper is an export commodity and is mainly processed into fillets (Rucitra, 2019). Since there has been no research on catfish and red snapper processing into Chikuwa, the fish can be used as raw materials.

Catfish and red snapper have a high nutrient content. Catfish contains 52.45% moisture, 4.05% ash, 20.83% protein, 3.85% carbohydrate, and 13.86% lipids (Adeniyi *et al*., 2012). Red snapper contains 78.00% moisture, 1.46% ash, 20.45% protein, and 1.37% lipids (Nurnadia *et al*., 2011). High nutrient content can affect the shelf life of chikuwa. The deterioration is mainly caused by enzymatic activity and the growth of gram-negative microorganisms that leads to shorter shelf-life and a decrease in consumer acceptance (Masniyom, 2011). Therefore, it is necessary to have a food additive that can serve as an antibacterial agent to extend its shelf-life.

Liquid smoke results from condensed steam distillation products and contains chemical components that serve as antimicrobial properties and natural

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preservatives. Liquid smoke could be used as a food preservative because of the antimicrobial and antioxidant compounds such as aldehydes, carboxylic acids, and phenols. Liquid smoke is able to maintain the quality of food with its antioxidant and antimicrobial properties and gives the desired colour, flavour, and aroma (0; Lingbeck *et al*., 2014). Liquid smoke has been added to many foodstuffs, including pork sausages (Bhuyan *et al*., 2018), yellowfin tuna (Nithin *et al.,* 2015), and bacon (Soares *et al*., 2016). The novelty of this research is the application of liquid smoke in chikuwa. Therefore, this study investigated the effect of liquid smoke as an antibacterial agent on catfish and snapper surimi-based Chikuwa.

2. Materials and methods

2.1 Materials

Catfish (*Clarias* sp.) and Snapper (*Lutjanus* sp.) were purchased from a local market in Semarang, Central Java, Indonesia. The distance between the local market and the laboratory is approximately 45 minutes. Both fish were brought to the laboratory in a Styrofoam icebox to maintain cold temperatures. After that, both fish were cleaned, filed, and the meat was separated from the gills, skin, and stomach. Then, the fish meat was grounded, and the prepared seasoning was added. Coconut shell liquid smoke was purchased from PT. Asap Cair Multiguna in Semarang, Central Java, Indonesia, and chemical reagents were purchased from PT. Bratachem, Semarang, Central Java, Indonesia.

2.2 Chikuwa preparation

The chikuwa was made according to Bhatkar *et al*. (2002). Fish fillets were washed and cleaned with running water and then crushed with a meat grinder. Minced fish were then put into a food processor to be mixed with seasonings, such as sugar, salt, potato starch, and ice cubes. Liquid smoke was added with 3% catfish (CLA) and snapper (CKA) weight. Homogeneous dough and 1 to 2 tablespoons of water were added. It was kneaded until homogeneity was achieved. The dough was moulded using a cutting board or placemat that had a flat surface. Then, the mixture was neatly rolled on the bamboo. The dough was flat-rolled using bamboo, and its edges were trimmed until it was neat. The chikuwa roasting process was carried out using an electric stove for 20 mins by rotating the bamboo several times. The roasting could be evenly distributed and produced an even brown colour. Ripe chikuwa was released from bamboo. Chikuwa was then packed airtight using polypropylene plastic and stored at -18°C for 24 hrs. The sample was then analyzed in the laboratory. The processing time from sample preparation to chikuwa fish product preparation was approximately 6 hrs. This study was divided into 4 groups: CL: chikuwa from Catfish (*Clarias* sp.), CK: chikuwa from Snapper (*Lutjanus* sp.), CLA: chikuwa from Catfish (*Clarias* sp.) with liquid smoke, and CKA: chikuwa from Snapper (*Lutjanus* sp.) with liquid smoke.

2.3 a_w assay

The a_w content was tested by putting the sample in a specialized tube and inserting it into an aw metre (Benchtop Water Activity Metre, Aqualab 4TE). The aw metre was calibrated by adding $BaCl_2.H_2O$ to the sample container. The a_w meter was closed and left for 3 mins until the aw scale was recorded at 0.9. Then, the a_w metre was opened, and the sample area was cleaned. After cleaning, the sample was inserted and recorded again after 5 mins. The scale was read by checking the temperature scale for the correction factor (Bhuyan *et al*., 2018).

2.4 pH assay

The pH measurement was done by weighing a sample that was cut into pieces as small as 10 g, smoothed on a mortar, and homogenized with 20 mL of distilled water for 1 min. The solvent was poured in a 10 mL beaker glass, and the pH was measured using a pH metre. The device was first calibrated with a pH buffer solvent 7 before use (Bhuyan *et al*., 2018).

2.5 Total volatile base nitrogen assay

The total volatile base nitrogen (TVBN) analysis was performed following the methods reported by National Standardization Agency for Indonesia number 2354.8: 2009. TVBN testing was carried out by weighing a sample as heavy as 10±0.1 g. Then, 90 mL of 6% perchloric acid was added. Samples were homogenized for 2 mins. The sample was filtered using coarse filter paper, and the extract (filtrate) was obtained. Up to 50 mL of extract was inserted into the distillation tube. Then, a few drops of the phenolphthalein indicator and a few drops of antifoaming silicon were added. The distillation tube was attached to a steam distillation apparatus, and 10 mL of 20% NaOH was added. An Erlenmeyer container containing 100 mL H_3BO_4 3% and 3–5 drops of the Tashiro indicator was prepared. Then, steamed distillation was carried out for approximately 10 min to obtain 100 mL of distillate. Therefore, the final volume was less than 200 mL of a green solvent. The blank solvent was distilled by replacing the sample extract with 50 mL of 6% PCA, further processing the sample. The sample distillates and blanks were titrated using 0.02 N HCl solvents. A purple reformation marked the endpoint of the titration. The TVBN content could be calculated using the formula (National Standardization

Agency for Indonesia, 2009):

$$TVBN\ \left(\frac{mg}{100g}\right) = ((V_c - V_b) \times N \times 14.007 \times 100)/W \quad (1)$$

Where, V_c is the volume of HCl solvent at sample titration, V_b is the volume of HCl solvent on blank titration, N is the normality of HCl solvent, W is the sample weight (g), and 14.007 is the atomic weight of nitrogen.

2.6 Antibacterial assay

An antibacterial assay was performed according to National Standardization Agency for Indonesia number 2332.3:2015. The total plate count (TPC) using the pour plate method was performed. Samples preparation was carried out by adding 50 g samples and 450 mL of butterfield's phosphate buffered solution, then homogenized for 2 mins. Tenfold serial dilution was performed until the 10^{-5}. An aliquot (1 mL) from each dilution was then transferred into an empty petri dish. Molten agar was then poured into the inoculated petri dish and swirled following a figure-eight pattern. Once the agar solidified, the petri dishes were incubated at 37°C for 48 hrs. The number of colonies was calculated using a colony counter (National Standardization Agency for Indonesia, 2015).

3. Results and discussion

3.1 a_w content

The a_w indicates the amount of free water in food that microbes can use for growth. If the water content of the material is reduced, microbial growth slows. Park (2008) reported that water in food is found in various forms, such as free water and bound water. Free water can help the damage to foodstuffs, such as chemical, enzymatic, and microbiological elements.

The a_w value between the chikuwa in the treatment and control groups ranged from 0.899 to 0.900 and was not significantly different ($p>0.05$) (Figure 1). This result indicated that liquid smoke did not affect the a_w. Several studies have suggested that a_w in common carp surimi ranges between 0.96-0.98, piramutaba surimi 0.98, and anchovy kamaboko 0.92 (Galvao *et al*., 2012; Ramos *et al*., 2012; Liu *et al*., 2014). A food product with an a_w of 0.9–1 is a water-rich food that allows for bacterial growth (Ramos *et al*., 2012).

The a_w content of foodstuffs is closely related to bacterial growth. The results of a_w measurements were used to develop products, control product quality, and as essential criteria for evaluating product safety. Higher a_w content in foodstuffs is caused by several factors, such as the type and quality of raw materials and the processing. Bacteria have the minimum, optimum, and maximum a_w conditions for growth (Abbas *et al*., 2009). Foodstuffs with an a_w content of 0.85 could be safe to reduce the growth rate of pathogenic bacteria. As a moist food, Chikuwa had an a_w level greater than 0.85 and was categorized as a high a_w product, but it was safe if other chemical factors, such as pH, were adequately considered.

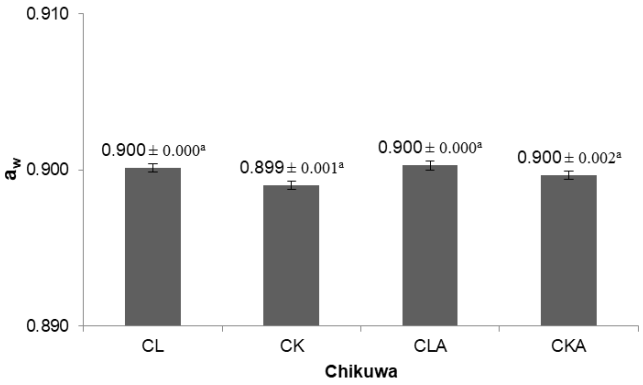

Figure 1. The water activity of chikuwa from a different group. Values are expressed as mean ± standard deviation. Values with different superscript are significantly different ($P<0.05$). CL: chikuwa from Catfish (*Clarias* sp.), CK: chikuwa from Snapper (*Lutjanus* sp.), CLA: chikuwa from Catfish (*Clarias* sp.) with liquid smoke, and CKA: chikuwa from Snapper (*Lutjanus* sp.) with liquid smoke.

3.2 pH

pH is a vital parameter in d determining the quality of a product because a decrease in the quality of fresh fish can affect the pH content (Susanto *et al*., 2011). A pH test was performed to determine the acidity or basicity of a sample. Based on the data (Figure 2), the pH of chikuwa CL and CK was neutral. This result indicated the freshness of the raw materials and corresponded to the a_w value. The addition of liquid smoke decreased the pH, as shown by the pH values of CLA and CKA of 5.67 and 5.47, respectively. The addition of liquid smoke gave a sour attribute to the sample. Prasetyo *et al*. (2015) reported that adding rice husk liquid smoke to milkfish decreased the pH to approximately 5.5-5.6. The fish meat absorbed the acid compound from the liquid smoke and led to a decrease in pH. High and low pH contents are influenced by the smoking duration, prolonged smoking, and smoking elements and acids are more absorbed and attached to the product (Tnuwo *et al.,* 2019).

The organic acid in liquid smoke is dominated by acetic acid (Montazeri *et al*., 2013; Budaraga *et al*., 2017). Kailaku *et al.* (2017) reported that the pH of coconut shell liquid smoke is 2.79. The decreased pH in chikuwa is also caused by water loss due to the reaction of polyphenols and carbonyl in liquid smoke with protein content in fish meat (Swastawati *et al*., 2013). The pH of

the CLA was higher than the CKA. This was due to protein content differences in both fish.

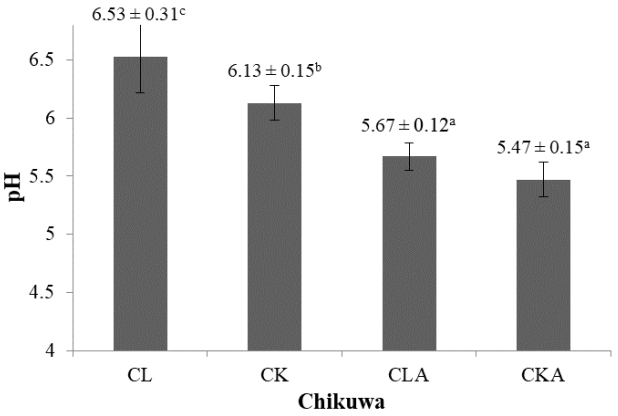

Figure 2. pH of chikuwa from different groups. Values are expressed as mean ± standard deviation. Values with different superscript are significantly different (P<0.05). CL: chikuwa from Catfish (*Clarias* sp.), CK: chikuwa from Snapper (*Lutjanus* sp.), CLA: chikuwa from Catfish (*Clarias* sp.) with liquid smoke, and CKA: chikuwa from Snapper (*Lutjanus* sp.) with liquid smoke

Processed fish meat with a high pH was commonly caused by essential compounds such as ammonia, trimethylamine, and other volatile compounds, which can reduce the organoleptic content of the product. An increase in pH is caused by spoilage bacteria that produce proteolytic enzymes. This enzyme can break down proteins into ammonia (NH_3), trimethylamine, and volatile components, thus increasing the pH content (Goulas and Kontominas, 2005). According to Montazeri *et al.* (2013), preservation with liquid smoke results in a pH of approximately 2.3-5.7 or even lower or acid, which may inhibit the growth of microorganisms. Some commercial liquid smoke has a pH of 3–4, where pH ≤ 4 can inhibit the growth of bacteria and fungi (Chemie, 2003; Swastawati *et al*., 2014).

3.3 Total volatile base nitrogen

A TVBN test was one of the testing parameters to determine the level of freshness of fish. The TVBN test was carried out by measuring the number of nitrogenous bases formed in fish due to the bacterial metabolism of protein. According to Chudasama *et al*. (2018), high protein content can cause protein degradation or contamination of proteins, peptides, and amino acids found in fish bodies. This degradation process was caused by the activity of bacteria, which can convert proteins into volatile bases such as ammonia, diethylamine, and trimethylamine.

The results indicated that the addition of liquid smoke decreased the TVBN value in both treatment samples (CLA and CKA) compared to the control sample (CL and CK) (Figure 3). The results were similar to Achmadi *et al*. (2013), who applied liquid smoke to pomfret fish, resulting in a lower TVBN than the control sample. Hadanu and Apituley (2013) reported that liquid smoke contains phenols, organic acids, aldehydes, and ketones that work as antibacterial agents. The main chemical compounds in the smoke include formic acid, acetic acid, butyric acid, caprylate, vanillate, syringic acid, methoxyphenyl, furfural glyoxal metals, methanol, ethanol, octanol, acetaldehyde, diacetyl, acetone, and 3.4 benzopyrenes. These chemical compounds can play a bacteriostatic, bactericidal role and inhibit fat oxidation. These compounds also stick to meat and provide a preservative effect (Budaraga *et al*., 2017; Janairo and Amalin, 2018). Therefore, the application of liquid smoke inhibited the growth of bacteria in chikuwa and produced a lower TVBN value than the control sample.

The use of liquid smoke in tuna can inhibit bacterial growth due to phenol and acid contents in liquid smoke, which damage the bacterial cell membrane. A lower value of TVBN indicates this compared to tuna without liquid smoke (Lasindrang, 2017). Furthermore, the chemical components of liquid smoke, including organic acids, carboxylic acids and some ingredients, cause acidic conditions. Acidic conditions inhibit bacterial and fungal growth.

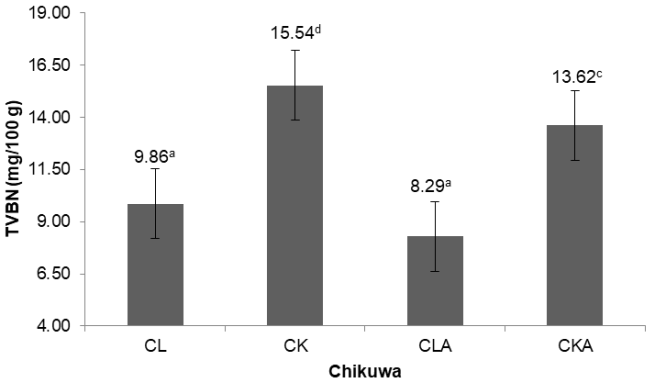

Figure 3. TVBN Value of Chikuwa. Values with different superscript are significantly different (P<0.05). CL: chikuwa from Catfish (*Clarias* sp.), CK: chikuwa from Snapper (*Lutjanus* sp.), CLA: chikuwa from Catfish (*Clarias* sp.) with liquid smoke, and CKA: chikuwa from Snapper (*Lutjanus* sp.) with liquid smoke.

A static test showed a significant difference (p <0.05) in the TVBN values for CLA and CKA. The TVBN values in CLA were lower than those in CKA, amounting to 8.29 mg-N/100 g and 13.62 mg-N/100 g, respectively. This difference was due to the different protein contents in the fish. The protein content in catfish was 15.86% (Mahboob *et al*., 2019), whereas, for snapper, it was 21.46%. The production of TVBN is due to protein breakdown by microbes (Kumar *et al*., 2014). Additionally, both chikuwa with the treatment and control had TVBN values below the standard for consumption (35 mg-N/100 g). TVBN values in fishery

products is 35 mg-N/100 g (European Commission, 2008).

3.4 Antibacterial activity

Foodstuffs that contain protein, such as chikuwa made from fish, are easily damaged by bacteria from the environment. The growth of microorganisms in this product can be caused by unwanted physical and chemical changes that are inappropriate for consumption because they cause health problems. Therefore, it was essential to determine the number of bacteria in foodstuffs to check their feasibility for consumption (Mailoa *et al*., 2017).

The application of liquid smoke in chikuwa decreased the TPC value compared to the control chikuwa (Figure 4). TPC values in CLA and CKA were lower than those of CL and CK. This indicated that liquid smoke acted as an antibacterial agent. The TPC value was below the maximum consumption level of 5×10^5 CFU/g. This result was better than that of Handayani *et al.* (2019) added sugarcane liquid smoke to tilapia dumplings, resulting in a TPC value of 5×10^3 CFU/g. The use of liquid smoke encapsulated with maltodextrin showed a small difference in the TPC value of Nile tilapia that received 0, 1 and 1.5% of liquid smoke during cold preservation. The TPC value was 2.867, 2.700, and 2.307, respectively. It was indicated that a higher liquid smoke concentration exhibited a higher antimicrobial level. The phenol content and pH contribute to antimicrobial effects in this product (Ariestya *et al*., 2016) .

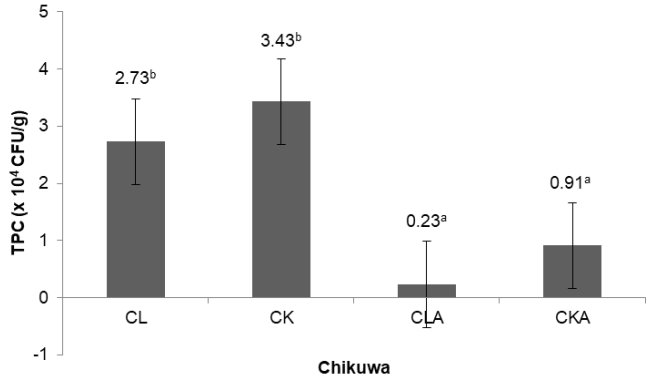

Figure 4. TPC Value of Chikuwa from different groups. Values with different superscript are significantly different (P<0.05). CL: chikuwa from Catfish (*Clarias* sp.), CK: chikuwa from Snapper (*Lutjanus* sp.), CLA: chikuwa from Catfish (*Clarias* sp.) with liquid smoke, and CKA: chikuwa from Snapper (*Lutjanus* sp.) with liquid smoke.

Liquid smoke is a potent antibacterial agent that can inhibit the growth of pathogenic bacteria such as *Escherichia coli* and *Salmonella enterica* serovar Typhi (Dien *et al*., 2019). Coconut shell liquid smoke effectively inhibits the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in fish meatballs. The addition of coconut shell liquid smoke on fish meatballs can increase the shelf life of fish meatballs from 16 to 32 hrs (Zuraida *et al.,* 2011) .

The TPC value was correlated with TVBN, and the addition of liquid smoke produced lower TPC and TVBN values in CLA and CKA than in CL and CK. TVBN was a product of bacterial spoilage metabolism, while TPC showed the number of bacteria. Based on the data, the addition of liquid smoke can inhibit the activity of bacteria in chikuwa. Liquid smoke has various functional properties, such as producing the desired flavour. Another function is preservation because of the content of phenol and acid compounds that act as antioxidants and antimicrobials (Toledo, 2008; Riyadi, 2019).

4. Conclusion

The application of liquid smoke can inhibit the growth of bacteria in chikuwa. This result was indicated by a decrease in pH, TVBN, and TPC in chikuwa based on catfish and snapper compared to the control chikuwa. However, the addition of liquid smoke did not affect the value of a_w. The nutritional content of each fish caused the difference in results between catfish and snapper chikuwa. The results of this study indicate that liquid smoke can act as an antibacterial agent in chikuwa products.

Conflict of interest

The authors declare no conflict of interest.

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