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PHYSICOCHEMICAL AND MICROBIOLOGICAL PROFILES OF COMMERCIAL CINCALOK FROM WEST KALIMANTAN

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Abstract

Cincalok, a traditional fermented shrimp product, is prepared with mix small and fresh shrimp, salt, and sugar in a certain ratio incubated for 3-7 days. Different recipes of each the commercial cincalok products obtain different of quality and safety level. The aim of this study is to assess the quality and safety of the commercial cincalok products of West Kalimantan based on their physicochemical and microbiological properties. Seven commercial cincalok products collected from the traditional market of West Kalimantan were analyzed physicochemical (moisture content, pH, free amino nitrogen (FAN), titratable acidity, salt content, glucose content, sucrose content, ethanol content) and microbiological (total number of mesophilic aerobic bacteria (TMABs), total halotolerant bacteria (THBs), endospores bacteria, lactic acid bacteria (LABs), fungi, Enterobacteriaceae, *Bacillus cereus, Clostridium perfringens*, and *Staphylococcus aureus*) properties. The mean of moisture content, pH, free amino nitrogen (FAN), titratable acidity, salt content, glucose content, sucrose content, ethanol content for 7 samples was 67.59%, 5.16, 0.60 mM/g, 1.75%, 10.56%, 1.30%, 0.49% and 0.59%, respectively. The mean of TMABs, THBs, endospores bacteria, LABs, fungi, and Enterobacteriaceae for 7 samples was 1.19-5.18 log CFU/g and 1.76 log CFU/g, respectively, while *B. cereus, C. perfringens*, and *S. aureus* were not detected for all of the samples. The results showed high variation of physicochemical and microbiological properties of the products. Some of the products are safe to ate without cooked but the other still contain pathogenic bacteria such as some of the enterobacteriaceae strains.

Keyword: cincalok, fermented shrimp, microbiological profile, proximate, physicochemical profile,

Profil Fisikokmia dan Mikrobiologi Cincalok Komersial dari Kalimatan Barat

Abstrak

Cincalok merupakan suatu produk fermentasi udang yang bersifat tradisional yang dibuat dengan cara mencampurkan udang kecil yang segar, garam, dan gula dengan rasio tertentu dan diinkubasi selama 3-7 hari. Perbedaan resep tiap produk cincalok akan menghasilkan kualitas dan tingkat keamanan produk yang berbeda. Tujuan dari penelitian ini adalah untuk menentukan kualitas dan keamanan produk cincalok komersial dari Kalimantan Barat berdasarkan sifat fisikokimia dan mikrobiologisnya. Sebanyak tujuh produk cincalok komersial dikumpulkan dari pasar tradisional, Kalimantan Barat dan dianalisis secara fisikokimia (kadar kelembaban, pH, nitrogen amino bebas (NAB), keasaman titrasi, kadar garam, kadar glukosa, kadar sukrosa, kadar etanol) dan mikrobiologis (jumlah total bakteri aerobik mesofilik (TBAM), total bakteri halotolerant (TBH), bakteri endospora, bakteri asam laktat (BAL), jamur, Enterobacteriaceae, *Bacillus cereus, Clostridium perfringens, dan Staphylococcus aureus*). Nilai rata-rata dari kadar kelembaban, pH, NAB, keasaman titrasi, kadar garam, kadar glukosa, kadar sukrosa, kadar etanol dari 7 sampel adalah 67,59%, 5,16, 0,60 mM/g, 1,75%, 10,56%, 1,30%, 0,49% dan 0,59%, secara berturut-turut. Rata-rata TBAM, BHT, bakteri endospora, BAL, jamur, Enterobacteriaceae dari 7 sampel adalah 1,19-5,18 log CFU/g sedangkan *B. cereus, C. perfringens,* dan *S. aureus* tidak terdeteksi pada semua sampel. Hasil penelitian menunjukkan variasi sifat fisikokimia dan mikrobiologis yang tinggi pada cincalok komersial. Beberapa produk cincalok komersial relatif aman untuk dimakan secara langsung tetapi beberapa peroduk masih mengandung bakteri patogen seperti beberapa strain Enterobacteriaceae.

Kata kunci: cincalok, fermentasi udang, profil fisikokimia, profil mikrobiologi, proksimat

INTRODUCTION

Cincalok, a fermented shrimp, is made by spontaneous fermentation of fresh and small shrimp, salt and sugar with a certain ratio incubated in closed container for 3-7 days. Cincalok is a traditional side dish from West Kalimantan, Indonesia. In South Kalimantan, Indonesia, cincalok is also called ronto (Khairina *et al.* 2016[a]; Khairina *et al.* 2016[b]; Khairina *et al.* 2017; Soetikno *et al.* 2018). Beside in Indonesia, it can be found in Malaysia (known as cincaluk) and Philippine (known as Bagoong Alamang) (Hajar and Hamid 2013). There are 2 types of cincalok based on the taste which are salty and sweet. Salty cincalok is made by addition of higher salt than sweet cincalok. Small shrimp used varies particularly from family *Penaeidae* and *Sergestidae*. It is usually consumed directly as a side dish with or without cooked.

Spontaneous or natural fermentation is difficult to control quality and safety product. It depends on raw material, recipe, and environmental condition in production. Raw materials contaminated by harmful microorganisms will produce unsafe products. In addition, significant increase in the number of microflora can cause decay, giving rise to disease due to pathogenic microorganisms or toxins. Therefore, spontaneous fermented fish or seafood products generally show variety of chemical and microbiological composition and product quality such as: Myeolchi-aekjeot (Lee *et al.* 2016), kapi (Faithong and Benjakul 2014), jeotgal (Lee *et al.* 2016), jalo (Faithong *et al.* 2010) and koong-som (Faithong *et al.* 2010).

Various recipes of cincalok made in each traditional cincalok producers causes high variation quality of cincalok products. Some cincalok products probably have a good quality and safety of cincalok but some of them may show low quality and unsafe cincalok due to containing harmful microorganisms such as: *Escherichia coli, Bacillus cereus, Clostridium perfringens, Enterobacteriaceae, Staphylococcus aureus*, and *Listeria monocytogenes* (Feron 1997; Jansen 1997). Therefore, profile of physicochemical and microbiological cincalok is needed to get profile of cincalok quality in the traditional market of West Kalimantan. The aim of this study is to evaluate the physicochemical and microbiological profiles of cincalok from traditional market of different towns in West Kalimantan, Indonesia which was used to assess the quality and safety of the commercial cincalok products.

MATERIALS AND METHODS

Materials and Tools

All chemicals and microbiological reagents used in this research were analytical/ microbiological grade. The chemicals reagents were formaldehyde (Merck), NaOH (Merck), phenolphthalein (Merck), K_2CrO_4 (Merck), $AgNO_3$ (Merck), NaCl (Merck), KI (Merck), H2SO4 (Merck), $Na_2S_2O_3$ (Merck), HCl (Merck), $K_2Cr_2O_7$ (Merck), and Starch (Difco), The microbiological reagents used in this research were Plate Count Agar/PCA (Difco), Oxytetracycline Glucose-Yeast Extract Agar/ OGYE (Oxoid), de Man, Rogosa and Sharpe agar/MRSA (Scharlau 01-135), *Mannitol Egg Yolk Polymyxin agar*/MYPA (Oxoid), Tryptose Sulfite Cycloserine Agar/TSCA (Himedia), Violet Red Bile Glucose Agar/VRBGA (Scharlau 01-295), Mannitol Salt Phenol Red Agar/MSPRA (Mercks 1.05404.0500), and *McBride Listeria Agar Base*/MLAB (Fluka 62355). The equipments used in this research were pH-Meter (Contech, India), balance (Ohaus, USA), Laminar flow (Sterimac, India), and oven (Vinci, France)

Research Procedure

Sampling of cincalok

Seven of commercial cincalok products were purchased directly from traditional market or homemade in three regencies of West Kalimantan, Indonesia which were Sambas Regency, Pontianak Regency, and Kotamadya Pontianak.

Physicochemical analysis

Moisture was determined according to AOAC (1990), pH value was determined using pH-digital meter. Free Amino Nitrogen (FAN) determined following the formol titration method (Northrop 1926; Lee *et al.* 2016). Salt content was determined according to Mohr titration (Han *et al.* 2001). Sucrose and glucose contents were determined

following Luff-Schoorl method (Tortajada 2015). Titratable acidity expressed as lactic acid was determined following acid-base titration with NaOH as a reagent standard and phenolpthlein as an indicator (Selli and Kelebek 2015).

Ethanol content determined by redox titration with potassium dichromate (Zoecklein *et al.* 1999). Two gram of homogenated sample in small glass vial was put into a conical flask containing 10 mL of potassium dichromate solution (0.01 M $K_2Cr_2O_4$ in 5 M H_2SO_4) and sealed with this rubber stopper for 24 hours. The potassium dichromate solution in conical flask was diluted with 100 mL of distilled water then added with 1 mL of KI 10% and titrated with $Na_2S_2O_7$ 0.03 M until yellow formed. This solution was added with 1 mL of amylum 1% and titrated again with 0.03 M $Na_2S_2O_7$ until the blue colour disappear. Percentage of ethanol was calculated based on the relationship between the mole of sodium thiosulfate and the moles of ethanol.

Microbiological analysis

Suspension sample for bacterial and fungal analysis was prepared by mixture 20 g of cincalok samples into 180 mL of saline peptone (0.1% neutral peptone, 2.5% NaCl). A 200 µL of suspension sample was inoculated on various media (PCA, modified OGYE, MRSA, MYPA, TSCA, VRBGA, MSPRA, MLAB) and incubation time depend on bacterial and fungal analysis. Medium PCA supplemented with 25 g/L of NaCl and incubation time for 2-3 days at 37°C was used to count total count of mesophilic aerobic bacteria (TMABs). PCA supplemented with 100 g/L of NaCl and 175 g/L of NaCl and incubated at 30°C for 7 days was used to count of total count of halotolerant bacteria 10 (THBs 17.5) and 17.5 (THBs 17.5), respectively. Modified OGYE medium supplemented with various NaCl concentration (0%, 1%, and 2%) at room temperature for 5 days was used to count fungal total (yeasts and molds). Determination of bacterial endospore total was prepared by heating of 1 mL of the suspension sample at 80°C for 10 minutes and enumerated in PCA supplemented with 25 g/L of NaCl then incubated at room temperature for 2-3 days. A 200 µL of the suspension sample was inoculated in pour-plates of MRSA supplemented with 15 g/L of NaCl and incubated at room temperature for 3 days. This procedure was used to count LAB. All colonies were counted for colony forming units (CFU) per gram wet weight of sample.

The suspension sample was enumerated selectively on spread-plates of MYPA supplemented with NaCl 15 g/L and incubated at room temperature for 3 days which was used to count the total of *B. cereus. C. perfringens* was inoculated selectively in TSCA supplemented with 25 g/L of NaCl and incubated at room temperature for 18-24 hour under anaerobic conditions. Enterobacteriaceae was counted using medium VRBGA supplemented with NaCl 15 g/L and incubated at room temperature for 2-3 days. The suspension sample was enumerated selectively in pour-plates of MSPRA supplemented with NaCl 15 g/L and incubated at room temperature for 72 hours for total of *S. aureus. L. monocytogenes* enumerated selectively in pour-plates of MLAB supplemented with NaCl 15 g/L and incubated at room temperature for 24-36 hours. All colonies were counted for colony forming units (CFU) per gram wet weight of sample. Determination of positive *B. cereus*, *C. perfringens*, *L. monocytogenes* and *S. aureus* was confirmed according to Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994).

Statistical Analysis

All data are expressed as mean ± standard deviation from 7 samples collected from three regencies of West Kalimantan, Indonesia (n=7, r=3). Statistical analyses were performed using one-way ANOVA followed by *post hoc* comparison of mean (Tukey test) using SPSS 23 Program (Kokoska 2015).

RESULTS AND DISCUSSION

Chemical Composition

Moisture content on food products is a pivotal indicator in food quality, preservation and resistance to deterioration. High moisture content can increase deterioration product so decrease shelf-life product. In this study, most moisture content of commercial cincalok of West Kalimantan was around 67.59±4.18%

(Table 1). It is lower than ronto and Koong som which are 69-82% (Khairina *et al.* 2016a) and 73.35-80.71% (Faithong *et al.* 2010), respectively. Koong som, fermented small shrimp from Thailand is made by mixing small shrimp with salt and sugar concentrated of palm. Cincalok can be seen in Figure 1.

The pH values of fermentation products are an important and simple way to evaluate the product quality particularly related to deterioration attribute. It exhibits a positive correlation with titratable acidity. In this study, the titratable acidity is calculated as lactic acid. In addition to lactic acid, the other organic acids that contributed to decrease the pH value are acetic acid and formic acid. The pH values and the titratable acidity showed a relative similar value for each the products of West Kalimantan (Table 1). The pH value of the products is higher than Koong som (3.71-3.89) (Faithong *et al.* 2010). The ideal pH value of fermented fish products is less than 5-4.5 (Owen and Mendoza 1985) giving a positive indicator for safety products. However, some pathogenic bacteria can possibly live in the pH range.

Organic compounds production (e.g. lactic acid) is affected by microorganism species. The LABs responsible in production of lactic acid showed high contribution in pH value. Fermentation using the LABs as starter usually shows lower pH value compared with spontaneous fermentation. Som-fug prepared by LABs starters at 4 and 6 log CFU/g decreases pH value to be 4.6 within 36 h and 24 h of fermentation, respectively (Riebroy *et al.* 2008). A carbohydrate as one of substrates in fermentation also contributes to decrease pH value. For instance, the cooked rice usually is added to som-fug to stimulate growth of the LABs. As a results, it can increase the lactic acid production of the son-fug (Saisithi *et al.* 1986). All of the commercial cincalok products containing both of the low sucrose and glucose concentrations was not enough to stimulate the LABs growth (Table 1). In addition, rate of pH decrease is affected by different buffering capacities of muscle proteins in fish or shrimp (Riebroy *et al.* 2007).

Salt is main and common ingredient in fermented fish. It can be used to inhibit and kill certain microorganisms (Frazier and Westhoff, 1988). High concentration salt can increase osmotic pressure in microorganism cells and induce plasmolysis. In addition, salt can ionize to produce chlorine ion which is dangerous for microorganisms. Salt also can reduce the solubility of oxygen and disrupt the action of proteolytic enzymes. The products showed high variation of salt concentration (Table 1). It was also detected on ronto which is 13.6-17.46% (Khairina *et al.* 2016[a]). However, the salt of the products was higher than koom song (2.74-3.81%) (Faithong *et al.* 2010).

FAN is parameter to determine concentration of amino acid and small peptides which can be utilized for indicator of protein degradation. FAN of the commercial cincalok products (0.6 mM/g or 8.4 mg/g) is higher than that of the shrimp sauce prepared for the shrimp processing byproduct (Kim *et al.* 2003). This result showed that the

Table 1 Physicochemical composition of commercial cincalok from various trational market in West Kalimantan
(Tabel 1 Komposisi kimia cincalok komersial dari berbagai pasar tradisional di Kalimantan Barat)

Parameter/ *Parameter*	Nilai/ *Value*
Moisture content/ Kadar Air (%)	67.59±4.18
pH/ pH	5.16±0.69
Free amino nitrogen/ nitrogen amino bebas (mM/g)	0.60±0.30
Titratable acidity/ keasaman titrasi (%)	1.75±0.56
Salt/ kadar garam (%)	10.56±3.20
Glucose/ kadar glukosa (%)	1.30±0.41
Sucrose/ kadar sukrosa (%)	0.49±0.27
Ethanol/ kadar etanol (%)	0.59±0.38

Gambar 1 Cincalok asal Kalimantan Barat
(*Figure 1 Cincalok from West Kalimantan*)

protease activity on the fermentation of the product was high. The high salt content of the products (Table 1) did not seem to inhibit protease activity.

Ethanol is a common product of fermentation which is produced by carbohydrate degradation from microorganisms. Low concentration of the ethanol was detected on these products (Table 1) which may contribute to inhibit unexpected bacteria and to develop unique taste on fermentation products (Chiang *et al.* 2006; Kim *et al.* 2003).

Microbiological Analysis

Microorganisms play a pivotal role in fermentation particularly in protein degradation and development of flavor and aroma (Majumdar *et al.* 2016). However, some microorganisms in fermentation probably responsible in deterioration and quality of food fermentation. The TMABs, TBHs 10, TBHs 17.5, and fungi are pivotal to evaluate food quality but it is a poor indicator for product safety. The TMABs can be used to measure sanitary quality, organoleptic acceptability, food processor information regarding raw materials, processing and storage condition, and handling product (Morton 2001). The TMABs of the products were lower than threshold, log 5 CFU/g based on regulation from Indonesian Ministry of Health for food fermentation (DEPKES 1991). The TMABs were identified based on Bergey's Manual Determinative (Holt *et al.* 1990) showed that bacteria were *Rothia dentocariosa* and *Enterobacter* sp. In addition, the dominant fungi of the products were *Candida tropicalis*. *Candida* sp. is reported involved in most fermentation products such as: *Candida kefyr, Candida intermedia, Candida versatilis, Candida zeylanoide*s (Bourdichon *et al.* 2012).

Bacteria used to evaluate safety of the products were endospore bacteria, Enterobacteriaceae, *B. cereus*, *C. perfringens*, dan *S. aureus* which may show high probability to associate with the raw material. The products did not contain *B. cereus*, *C. perfringens*, and *S. aureus* (Table 2). Some of the products (3 of 7 samples) contained Enterobactericeae which may cause the risk of opportunistic infection for consumers with low immune system. Enterobacteriaceae also showed the potential to produce endotoxin which can cause typhoid fever and parathypus, salmonellosis and dysentery (Belitz and Grosch 1987). However, the products are safer if cooked before being eaten.

CONCLUSION

The physicochemical and microbiological of the products showed different quality and safety level. Some of them showed good quality and safety but the other still contain pathogenic bacteria such as some of Enterobacteriaceae strains. The high variation of physicochemical and microbiological level showed that the products did not have quality and safety standards. Therefore, the consumers should be suggested to eat the cooked products. In addition, the product quality of West Kalimantan should be improved particularly the processing step and the recipe to obtain the best quality and safety of the products. It can be consumed directly or without cooked.

Table 2 Microbiological profile of commercial Cincalok various traditional market in West Kalimantan
(Tabel 2 Profil mikrobiologi Cincalok komersial dari berbagai pasar tradisional di Kalimantan Barat)

Parameter/ *Parameter*	Value/ *Nilai*
Total number of mesophilic aerobic bacteria (TMAB)/ *Total bakteri aerobik* mesofilik (TBAM)	4.66±0.60
Endospore bacteria/ *Bakteri endospora*	1.20±2.19
Total bacteri halotolerant TBH10/ *Total bakteri halotoleran TBH10*	4.61±0.49
TBH 17.5/ *TBH 17,5*	4.54±0.35
Lactic acid bacteria (BAL) /*Bakteri asam laktat* (BAL)	1.19±2.07
Fungi/ *Jamur*	5.18±0.40
B cereus	ND
C. perfringens	ND
Enterobacteriaceae	1.76±2.20
S. aureus	ND

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