

CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF BUDUK, A COMMERCIAL FISH SAUCE FROM WEST KALIMANTAN

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Abstract

Buduk is a fish sauce prepared by fermenting fresh small fish with various ingredients such as sugar, roasted rice, vinegar or wine. This study was aimed to assess the physicochemical and microbiological properties of commercial buduk in the Province of West Kalimantan. The Buduk had pH 5-5.5, moisture 52.35%, salt 1.96%, titrated acid 10.80%, free amino nitrogen 1.83 mmol/g, free fatty acid 17.96%, ethanol 0.95%, total of mesophilic bacteria 4.81 log CFU/g and lactic acid bacteria 2.09 CFU/g, but no pathogenic bacteria detected. Each commercial buduk showed different physicochemical properties probably caused by variation in recipe of each producer. The microbiological properties did not significantly different but the TMAB found more than the threshold value indicating each producer needs to improve hygienical buduk processing to improve the quality of buduk.

Keywords: anchovies, buduk, fermentation, fish sauce

Sifat Fisikokimia dan Mikrobiologi Buduk, Saus Ikan Komersial dari Kalimantan Barat

Abstrak

Buduk adalah saus ikan yang dibuat melalui fermentasi ikan-ikan kecil yang segar dengan bermacam-macam bahan tambahan seperti: gula, beras yang sudah disangrai, cuka atau arak. Studi ini bertujuan untuk menilai sifat fisikokimia dan mikrobiologi komersial buduk yang beredar di pasaran Provinsi Kalimantan Barat. Sifat fisikokimia dan mikrobiologi ditentukan. Komersial buduk di Provinsi Kalimantan Barat memiliki pH 5,5, kelembaban 52,35 %, garam 1,96 %, keasaman yang bisa dititrasi 10,80%, nitrogen amino bebas 1,83 mmol/g, ALB 17,96 %, etanol 0,95%, ALT 4,81 log CFU/g, BAL 2,09 log CFU dan tidak terdeteksi adanya bakteri patogen. Umumnya komersial buduk menunjukkan perbedaan signifikan sifat-sifat fisikokimia yang mungkin disebabkan oleh resep tiap produser yang digunakan berbeda. Sifat-sifat mikrobiologi komersial buduk menunjukkan tidak berbeda signifikan tetapi jumlah ALT yang melebihi nilai ambang batas mengindikasikan bahwa tiap produser memerlukan pengetahuan yang baik tentang cara-cara produksi yang higienis untuk meningkatkan kualitas buduk komersial.

Kata Kunci: buduk, fermentasi, ikan teri, saus ikan

INTRODUCTION

Buduk is a traditional and spontaneous fermentation food and categorized as a fish sauces which can be found in West Kalimantan. Buduk is prepared by fermenting fresh small fish with various ingredients such as sugar, roasted rice, vinegar, or wine. Buduk is usually

consumed with rice by the community of West Kalimantan. Buduk contributes significantly to the diet, particularly increasing protein intake. Fish sauce and fish paste is very common in South-East Asia and known with different names and the recipes as well as the processings. Beside buduk, other fish sauce

have been reported by some researchers such as budu from West Sumatera (Huda and Rosma, 2006) and Malaysia (Nadiah *et al.* 2014). The budu from West Sumatera is made from big size marine fish such as *Scomberomorus* sp. (Spanish Mackerel) or *Chorinemus* sp. (Huda and Rosma 2006). The Malaysian budu, a dark brown liquid, is made from anchovies (*Stolephorus* sp./bilis fish, *Sardinella* sp., *Decaterus macrosoma*) as the raw material. The anchovies were mixed with salt in the ratio 3:2 then fermented for 6-12 months (Ray and Montet 2015). However, the Malaysian budu and The Province of West Kalimantan of buduk are different in their production or product appearance. Rusip, the other fish sauce of Bangka Island (East Sumatera), Indonesia, uses anchovies (*Stolephorus* sp.) mixed with salt and palm sugar and kept for two weeks (Nadiah *et al.* 2014). Toeuktrey, a Cambodian fish sauce, uses freshwater fishes and is incubated for less than a month and several months (Chuon *et al.* 2014). Jeotgal, a fermented fish sauce of Korea, is made from sea animals (whole fish, fish roe, internal organs of fish and shellfish) and high salt (20-30% [w/w]) (Jung *et al.* 2018). Bakasang, a fermented fish sauce of Indonesia is made from small fish (sardine, *Engraulis japonicus*) (Ijong and Ohta 1996)

Spontaneous fermentations utilize natural microorganisms contained in the raw material as a starter to trigger protease production during fermentation. The natural microorganisms help converting protein of fish flesh to simple peptides. The freshness of raw material contributes to the protein quality of fish sauce (Nadiah *et al.* 2014). Each raw material contains certain microorganisms causing a high variation of the buduk quality or inconsistency in the products, which can be assessed by chemical and microbiological properties. The buduk quality also depends on the freshness of anchovies, the recipe, and the condition during buduk processing. This study was aimed to determine the chemical and microbiological characteristics of commercial buduk from West Kalimantan.

MATERIALS AND METHODS

Materials

The chemical reagents for chemical analysis were formaldehyde (Merck), NaOH (Merck), phenolphthalein (Merck), K_2CrO_4 (Merck), $AgNO_3$ (Merck), NaCl (Merck), KI (Merck), H_2SO_4 (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 (Merck's 1.05404.0500), and McBride Listeria Agar Base/MLAB (Fluka 62355). The equipment used in this research were pH-Meter (Contech, India), balance (Ohaus, USA), Laminar flow (Sterimac, India), and oven (Vinci, France)

Sampling

Commercial buduk was bought randomly from various traditional market in West Kalimantan including: Pontianak, Singkawang, Mempawah, Sambas, and Kuburaya.

Methods

Chemical Analysis

Preparation of samples

Each sample was prepared by homogenization using a hand blender. The homogenate was analysed for their chemical and proximate compositions. The homogenate was stored at 4°C before use.

pH

One g of homogenate was diluted with nine mL of distilled water. Then, the mixture was stirred using a glass stirring rod. The pH of the mixture was measured using digital pH-meter.

Free amino nitrogen (FAN)

Free amino nitrogen (FAN) was analyzed based on the formol titration (Northrop 1926; Lee *et al.* 2016). Two grams of homogenated

sample were diluted with 18 mL of distilled water. After addition of 5 mL of 37% formaldehyde, the sample was titrated with 0.1 M NaOH until pH to be 8.5, which should be constant for 30 s. The FAN was measured as follow:

$$\text{FAN} = \frac{\text{M NaOH} \times \text{V NaOH}}{\text{Mass of sample (g)}}$$

Titrateable acidity

Titrateable acidity was determined based on acid-base titration. Five g of homogenated sample were centrifugated at 4.000 x g for 30 min to generate supernatant. Five mL of the supernatant were diluted with 10 mL of boiled water. After cold, the mixture was added with five drops of phenolphthalein indicator and subsequently titrated with NaOH 0.1 M. The titrateable acidity was measured as follow:

$$\text{Titrateable acidity} = \frac{\text{M NaOH} \times \text{V NaOH} \times 0.9}{\text{V sample}} \times 100\%$$

Ethanol content

Ethanol content was determined using reduction-oxidation titration. A small vial containing two g of sample was hung to a rubber stopper which used to cover a conical flask containing 10 ml of acid dichromate (0.01 M $\text{K}_2\text{Cr}_2\text{O}_4$ in H_2SO_4 5 M). After incubation at 30°C for 24 hours, the acid dichromate was added with two-three drops of KI 10% and titrated with $\text{Na}_2\text{S}_2\text{O}_7$ 0.03 M until yellow. This solution then added with two-three drops of amylum 1% and re-titrated with $\text{Na}_2\text{S}_2\text{O}_7$ 0.03 M until slightly blue. Percentage of ethanol was calculated as follow:

$$\% \text{ Ethanol} = \frac{\text{Mass of ethanol (g)}}{\text{Mass of sample (g)}} \times 100\%$$

Salt content

Salt content was determined based on Mohr method. Five mL of supernatant (titrateable acidity procedure) were diluted with 45 mL of aquadest. Two drops of 10% of K_2CrO_4 was added and the solution then was titrated with 0.1 AgNO_3 M until white precipitation formed. Salt content was calculated as follow:

$$\% \text{ Salt} = \frac{\text{M AgNO}_3 \times \text{V AgNO}_3 \times 0.0584}{\text{V sample}} \times 100\%$$

Free fatty acid (FFA)

Five hundreds milligram of homogenated sample was mixed with 5 mL of ethanol (95%) then heated at 70°C. Two drops of phenolphthalein was added into the solution. The sample was titrated with NaOH 0.1 M until pale pink colour. The FFA was reckoned using formula as follow:

$$\% \text{ FFA} = \frac{\text{M NaOH} \times \text{V NaOH} \times 282}{\text{Mass of sample (g)} \times 10} \times 100\%$$

Microbiological analysis

Five grams of each buduk sample were suspended in 45 mL of saline buffer (0.9% of NaCl solution). The suspension sample was used for microbiological analysis.

Total of Mesophilic Aerobic Bacteria (TMAB) was determined using a serial dilution of the suspension sample (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) inoculated on plate count agar (PCA) medium and incubated at 37 °C. After 48 hours, the mounted of TMAB was calculated by following formula = the number of colony x dillution factor. Each colony appeared in the medium surface was counted as colony forming unit (CFU) of TMAB.

Five microliters of the suspension sample was inoculated into Mannitol Egg Yolk Polymyxin Agar (MYPA) supplemented with 15 g/L of NaCl, Violet Red Bile Dextrose Agar (VRBD) supplemented with NaCl 15 g/L and Mannitol Salt Agar (MSA) supplemented with 15 g/L of NaCl and incubated at 37 °C for 3 days. A positive colony of *Bacillus cereus*, *Enterobacteriaceae*, and *Staphylococcus aureus* were identified with a pink colony on MYPA, a pink colony on VRBG, and golden yellow colony on MSA. Each positive colony was further confirmed using morphological and biochemical tests based on Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994). *Clostridium perfringens* and *Listeria monocytogenes* were detected by inoculating the suspension sample (5 µL) in selective media Tryptose Sulfite Cycloserin Agar (TSCA) enriched with NaCl 15 g/L

and Listeria Salt Agar (LSA) enriched with 15 g/L of NaCl, respectively. After incubation at 37 °C for 24 hours, a black and yellow colony were predicted as *C. perfringens* and *L. monocytogenes*, respectively then further confirmed based on Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994).

Five microlitres of the suspension sample was inoculated into MRS agar supplemented with CaCO₃ 1% and incubated anaerobically at 30 °C for three days. A clear zone around colonies was suspected as Lactic Acid Bacteria (LABs). The LABs was confirmed using morphological and biochemical tests according to Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994).

The sample was inoculated into Potatoes Dextrose Agar (PDA) medium enriched with 15 g/L of NaCl and incubated at 30 °C. After five days, each fungal colony was counted as colony forming units (CFU) per gram wet weight of samples.

Statistical analysis

All of the experimental data were grouped into blocks based on a randomized complete block design approach. All of the data were presented as mean ± standard deviation, and the variance between the group was analyzed using an LSD with 95 % (p<0.05) confidence level. The IBM® Statistics 23 was used for the data analysis.

RESULTS AND DISCUSSION

Sampling

Buduk is a favorite meal in Province of West Kalimantan, particularly people who live

along the coast (Figure 1a). We focused on collecting samples from municipality/regency close with the coast or the islands which are probably to be the primary producers of the buduk in West Kalimantan such as Pontianak, Singkawang, Pontianak, Sambas, and Kuburaya.

The raw material of buduk is small fish with low selling price such as white or black anchovies (*Stolephorus indicus*), seluang fish (*Rasbora dusonensis*), and small other fish. However, *Stolephorus indicus* is the common raw material to make buduk in West Kalimantan particularly Temajo Island dan Kabung Island (Figure 1b). The other materials in buduk production are salt, sugar, roasted rice or cooked rice, vinegar or wine. Different raw materials is used in the buduk production affect the quality of the product mainly taste, flavour, aroma and nutrition.

The physicochemical profile of the buduk was significantly different (p<0.05) for all of the sample except the ethanol content (Table 1). The moisture of buduk (52.35±5.975%) was lower than that of budu (67%), rusip (65%), cincalok (67%), or ale-ale (60%) (Nadiyah *et al.* 2014; Nofiani and Ardiningsih 2018; Nofiani *et al.* 2010). It is thought that salt content and fermentation time affect the moisture in fermentation.

Chemical analysis

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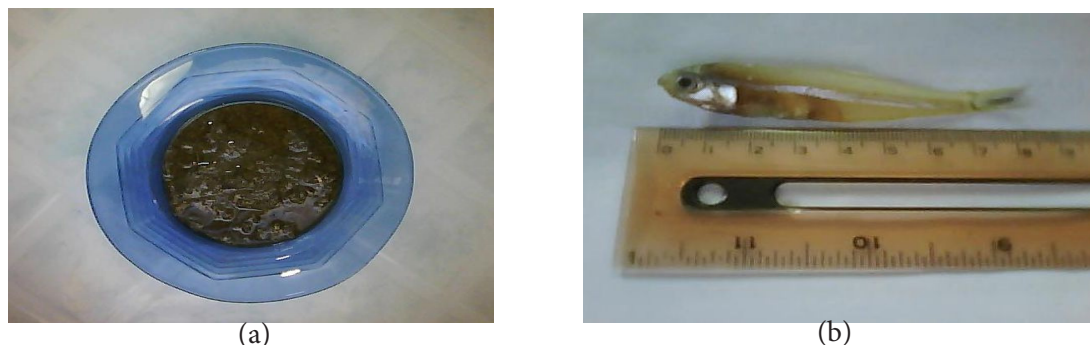


Figure 1 (a) Sample of commercial buduk (b) *Stolephorus indicus* as the primary material to make Buduk

Table 1 Chemical properties of the buduk

Location of producer	pH	Moisture (% w/v)	Salt (% w/v)	Titration acidity (% w/v)	FAN (mmol/g)	Ethanol (% w/w)	FFA (% w/w as oleic)
Pontianak	5.67 ± 0.53 ^a	55.05 ± 2.87 ^a	2.04 ± 0.09 ^a	11.26 ± 0.30 ^a	2.16 ± 0.04 ^a	0.93 ± 0.03 ^a	17.96 ± 0.52 ^a
Singkawang	5.42 ± 0.05 ^{ac}	54.66 ± 2.43 ^a	1.61 ± 0.42 ^{bc}	9.89 ± 0.09 ^b	1.56 ± 0.48 ^b	0.95 ± 0.05 ^a	21.71 ± 4.34 ^b
Sambas	5.27 ± 0.07 ^{bc}	48.49 ± 6.81 ^b	2.03 ± 0.12 ^{ad}	10.45 ± 0.32 ^c	1.93 ± 0.11 ^a	0.96 ± 0.02 ^a	21.37 ± 2.86 ^b
Kuburaya	5.98 ± 0.11 ^{ac}	52.66 ± 5.03 ^a	2.20 ± 0.01 ^{ae}	13.37 ± 0.04 ^d	1.53 ± 0.15 ^b	0.96 ± 0.03 ^a	20.96 ± 0.49 ^{ab}
Mempawah	5.76 ± 0.46 ^{acd}	52.97 ± 8.11 ^a	2.00 ± 0.25 ^{af}	10.48 ± 0.64 ^{ce}	1.58 ± 0.25 ^b	0.92 ± 0.08 ^a	21.41 ± 0.59 ^b
Average	5.55±0.38	52.35±5.97	1.96±0.28	10.80±1.04	1.83±0.33	0.95±0.05	20.72±2.76

Information: The different letter in the same column assigns significantly different ($P < 0.05$). $n = 11$. $r = 3$

cincalok (67%), or ale-ale (60%) (Nadiyah *et al.* 2014; Nofiani and Ardiningsih 2018; Nofiani *et al.* 2010). It is thought that salt content and fermentation time affect the moisture in fermentation.

pH value is used to determine proton dissociated from organic acids during fermentation such as acetic acid or formic acid. Organic acids are produced as a result of lactic acid bacterial activities. The proton of the organic acids causes decrease in pH of fermentation products, giving an effect toward the microorganism profile of fermentation. Therefore, pH can be used as an indicator to determine the quality of the fermentation product.

The pH value in fermentation products can be increased by extending the fermentation time, as a result of, for example, ammonia production (Anggo *et al.*, 2015). The average pH of the commercial buduk was 5.55 ± 0.38 (Table 1). Some of the fermented fish from West Kalimantan have similar pH value such as fermented ale-ale and cincalok (Nofiani *et al.* 2010; Nofiani and Ardiningsih 2018). The pH of the fish paste produced from the Province of West Java was higher than that of the buduk which is 6.42 (Anggo *et al.* 2015). Traditional bakasang shows pH value from 5.95 to 6.55 (Ijong and Ohta 1996). The pH of budu (5.17) and rusip (4.98) from Malaysia is lower than that of buduk (Nadiyah *et al.* 2014).

Titration Acidity (TA) was also measured in this study, to determine the total concentration of free proton and undissociated acids, which can react with a strong base. In this study, high TA values was not followed by low pH values. Therefore, the pH and TA

values did not show a positive correlation (Table 1). For example, the TA of Pontianak is 11.26, higher than that of Mempawah, but the pH of Pontianak was lower than that of Mempawah (Table 1). The TA of buduk was in average $10.80 \pm 1.04\%$ w/v (Table 1). This value was probably produced as a result of LAB activities that convert carbohydrate to lactic acid, propionic bacteria, butyric acid, and acetic acid (Elferink *et al.* 2001; Pessione 2012).

Salt is pivotal ingredient to adjust microorganism profile involved in fermentation. For example, salt with a certain concentration can inhibit the growth of some spoilage and pathogenic bacteria. In addition, salt contributes to decreasing water activity of substrate by increasing substrate osmosis pressure which reduces water from the substrate. As a result, the only specific microorganism can ferment the substrate. High salt concentration can inhibit proteolytic enzymatic activity contained in substrate and LAB activity. However, the salt value of buduk was categorized as low salt, which did not likely inhibit the LAB growth. The salt value did not significantly different among each district except for Singkawang. This salt value is similar to that of fermented ale-ale but lower than that of other fermented food such as cincalok, Bangka buduk (17-30%) and Malaysian budu (21.50-25.70 % (w/v) (Rosma *et al.* 2009; Nofiani and Ardiningsih 2018; El Sheikha and Montet 2015).

The free amino nitrogen (FAN) in the fermentation is used to determine the degradation level of protein by microorganism proteolytic activities, which are counted as

Table 2. Microbiological Properties of Commercial Buduk

Microorganism	Total Log CFU/g of Block Samples				
	Pontianak	Singkawang	Sambas	Kuburaya	Mempawah
TMABs	5.06 ± 0.32 ^a	4.99 ± 0.39 ^a	5.03 ± 0.55 ^a	4.34±0.23 ^a	4.51 ± 0.15 ^a
LABs	2.00 ± 2.83 ^a	2.06 ± 2.9 ^{1a}	1.00 ± 1.73 ^a	4.08±0.01 ^a	1.85±2.62 ^a
Fungi	n.d.	n.d.	n.d.	n.d.	n.d.
Pathogenic Bacteria	n.d.	n.d.	n.d.	n.d.	n.d.

Information: Pathogenic bacteria: *B. cereus*, *C.perfringens*, *Enterobacteriaceae*, *S. aureus*, and *L. monocytogenes*.

The sameletter in the same row assigns no significantly different (P<0.05). n=11. r=3

amino acids, small peptides, and ammonium ion. High levels of FAN contribute to the rise of pH. The FAN of buduk in this study was in average of 1.83±0.33 mmol/g (Table 1) which is higher than the FAN of fermented ale-ale and cincalok from the Province of West Kalimantan (Nofiani *et al.* 2010; Nofiani and Ardiningsih 2018).

Buduk in this study contained ethanol with ranged from 0.92 to 0.96 % (b/b), which did not significantly different among samples (Tabel 1). The ethanol was produced by the LAB and the TMAB activities. The LABs are able to convert pulp substrate into ethanol, lactic acid, and acetic acid (Anal 2019). Ethanol and organic acids contribute to developing specific taste and flavor attributes (Chiang and Ismail 2006; Ayad *et al.* 2001; Ines and Falco 2019).

Free fatty acids (FFA) are product of lipase and phospholipase activities (Gandemer 2002). It can be used to develop flavor compounds of fermented products and as indicators of product quality such as spoilage and rancidity (Xu *et al.* 2019). The FFA of buduk showed range from 17.96% to 21.41% (Table 1).

This suspect colony showed Gram-positive with coccus form using Gram staining. The suspect colony also positive to ferment glucose, lactose, maltose, and sucrose, but not mannitol. The suspect colony possessed catalase and oxidase-negative and motile. On the basis of the morphological and biochemical tests, the suspect colony was identified as *Streptococcus* sp. *Streptococcus* sp. has been used to ferment milk to produce kefir and *Streptococcus thermophilus* was applied to produce yoghurt (García *et al.* 2019).

Microbiological analysis

Microorganisms are pivotal to improve the flavor and taste. Microorganisms such as lactic acid bacteria (LABs), *Micrococcus*, and yeast are involved in food fermentation (Wu *et al.* 2015). The buduk samples in this study contained TMAB and LABs but no fungi and pathogenic bacteria (Table 2).

The TMAB value can be used to describe the product quality, contamination, expiry date, and hygienic status but no relation to product safety. The TMABs of all samples were higher than the threshold value (<1 Log CFU/g allowed by Indonesian National Standard (SNI)7388:2009 (Table 2).

LABs are desirable bacteria in fish fermentation, particularly to inhibit microbial spoilage by competing them, developing toxins, enhance flavor and reduce pathogenic microorganisms (Rhee *et al.* 2011; Glatman *et al.* 2000; Sedanza *et al.* 2016; Xu *et al.* 2019). The positive colony LABs were identified by a clear zone around colony (Figure 2). A clear zone around the colony was isolated then identified based on Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994). The LAB and TMAB collony members of all buduk analyzed similar (Table 1).

CONCLUSION

The commercial buduk samples in this study showed a significant difference in the chemical properties except for ethanol content. However, all of the commercial buduk samples exhibited an insignificant difference in the microbiological properties. As a result, the

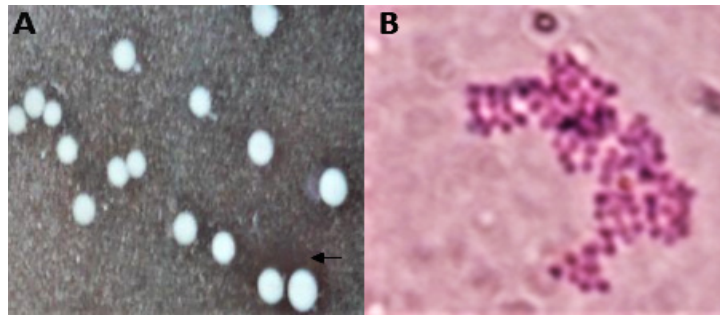


Figure 2. A. clear zone around LAB colonies (arrow) on MRS medium supplemented with CaCO_3 ; B. The LAB takes the form of a Gram-positive cocci

quality of commercial buduk in the market of West Kalimantan Province is different for each producer. The fungi and pathogenic bacteria were not detected in the commercial buduk, but the TMABs exceeds the value threshold permitted by Indonesian National Standard. On the basis of TMAB value, each producer needs to improve knowledge on the hygiene to increase the buduk quality.

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