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Chemical Characteristic and Microbiological Safety of Commercial Fermented Ale-Ale from West Kalimantan, Indonesia

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Abstract: The aim of the present study was to examine the chemical and microbiological composition of commercial fermented ale-ale obtained from traditional market. Fermented ale-ale, Indonesian traditional fermented shellfish, is made by mixing ale-ale (*Meritrix meritrix*) meat with salt, porridge rice or angkak (*Monascus purpeveus*) in certain ratio, kept in an earthen pot and fermented for 7 days. There are 2 types of commercial fermented ale-ale (white and red) were analyzed for their chemical composition namely pH, moisture, ash, glucose, protein, carbohydrate, salt content, FAN, titratable acidity, ethanol content as well as microbiological composition namely total count of mesophilic aerobic bacteria, total halotolerant bacteria, fungi and lactic acid bacteria as well as pathogenic bacteria (bacterial endospores, *B. cereus*, endospore, *C. perfringens*, *S. aureus*, *Enterobacteriaceae* and *L. monocytogenes*). Chemical composition of both white and red fermented ale-ale showed that there were not different significantly ($p<0.05$) among the samples. pH, moisture content, protein content and fat content of *M. meritrix* meat was decrease significantly than that of both white and red fermented ale-ale, however ash and carbohydrate content of *M. meritrix* meat was increase significantly than that of both white and red fermented ale-ale. Glucose content of *M. meritrix* meat and both white and red fermented ale-ale that there were not different significantly ($p<0.05$) among the samples. Fermented ale-ale showed high total count mesophilic aerobic bacteria than total halotolerant bacteria and fungi. *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Enterobacteriaceae* and *Listeria monocytogenes* were not found from fermented ale-ale samples. Dominant fungus in fermented ale-ale is *Penicillium coryliphilum Dierckx* which can produce mycotoxin. Due to the presence of *P. coryliphilum Diercks*, fermented ale-ale was recommended cooked before served.

Key words: Fermented ale-ale, *Meritrix meritrix*, chemical composition, pathogenic bacteria, fungi

INTRODUCTION

Fermented ale-ale, a fermented shellfish product, is popular for people in West Kalimantan. It is used as a side dish weather cooked or uncooked before served. It is processed by natural fermented and mostly known as homemade product. Ale-ale is

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identified as *M. meritrix* included bivalvia class. Fermented ale-ale is originally produced from Ketapang Regency and it is made from *M. meritrix* meat. There are 2 types of fermented ale-ale based on its color: white and red. White fermented ale-ale is prepared by mixing ale-ale *M. meritrix* meat with salt and rice porridge in certain ratio, then pressed tightly in a plastic bottle. It is fermented for 7 days in room temperature before selling to domestic market. Processing of red fermented ale-ale is similar with white fermented ale-ale but rice porridge is replaced with angkak (*Monascus*-fermented rice). The ratio of raw materials differs for every product. The taste of product is slightly sour and salty.

Salt, rice porridge and angkak are used commonly as a traditional additive for preserving fish and meat. Salt may have a significant inhibitory effect on mesophilic spoilage flora (Gram, 1991). In addition, high salt environment may also decrease both an endogenous proteinase activities in raw materials and proteinase produced by certain bacteria (Siringan *et al*., 2006). Carbohydrates such as rice porridge play to decrease pH quickly by reducing the buffer capacity of fish (Owen and Mendoza, 1985). According to Ostergaard *et al*. (1998), addition of carbohydrates did not always ensure a rapid decrease in pH but depend on the bacteria involved. Angkak can give a function as a flavoring agent such as a flavoring agent, aromatic fragrance and vivid red color. In addition, pigment contained in angkak can be used as an antibacterial and antifungal agent that can limit growth pathogen microorganisms (Lin *et al*., 2008).

Fish is an extremely perishable proteinaceous food due to the enzyme and the metabolism of spoilage microorganisms. It is also high on the list of food associated with outbreaks of foodborne disease (Huss, 1997). Fish and fish processed product may also be a vehicle for many pathogenic bacteria such as *Listeria monocytogenes*, *Staphyloccocus aureus*. *L. monocytogenes* and *S. aureus* were detected in fresh fish, smoked fish and salted fish in Iran (Basti *et al*., 2006). *Staphylococcus aureus* was also detected in fermented sardine (*Sardinella brasiliensis*) in Brazil (Oetterer *et al*., 2003).

Traditional fermented fish or seafood products are generally spontaneous fermentation using nature microorganisms and high variation recipes. Therefore, the products show variety of chemical and microbiological composition and product quality such as fermented fish sauce, bakasang (Ijong and Ohta, 1995a), fermented blue mussel meat (*Mytilus edulis*) (Jung *et al*., 2005), fermented oyster sauce (*Crassostrea gigas*) (Je *et al*., 2005) and fermented sea mussel meat, hoi dorng (Ostergaard *et al*., 1998). However chemical and microbiological composition of fermented ale-ale has not been reported. In this study, we present the chemical composition and microbiological aspect of fermented ale-ale obtained from traditional market.

MATERIALS AND METHODS

Materials

Fermented ale-ale was purchased from local markets in Pontianak, West Kalimantan, Indonesia namely Flamboyan and Serayu market on February, 24th 2007, 11th July 2007 and 1st August 2007 (Fig. 1). *Meritrix meritrix* meat was purchased from local market in Ketapang, Rangge Sentap, Delta Pawan District, Ketapang Regency, West Kalimantan, Indonesia on 2nd September 2007 and identified in Laboratory of Biology, Department of Biology, Mathematics and Natural Sciences Faculty, Tajungpura University, Indonesia (Fig. 2a, b).

Fig. 1: *Meritrix meritrix*

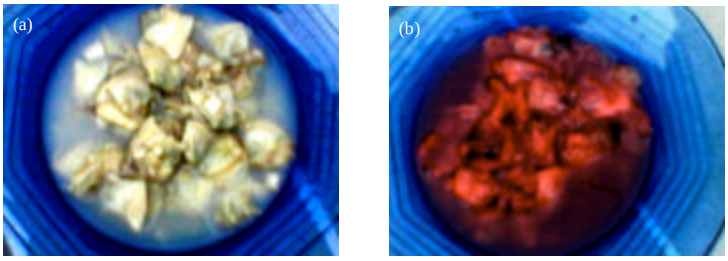

Fig. 2: Fermented ale-ale. (a) White fermented ale-ale and (b) Red fermented ale-ale

Methods

Preparation of Chemical and Proximate Samples

Each sample of 500 g fermented ale-ale or *M. meritrix* was blended and homogenized. Ten of homogenate sample will be used for chemical analysis.

Chemical Analysis

Proximate analyses were determined for both fermented ale-ale and *M. meritrix* meat. Carbohydrate content was estimated as 100 (% protein + % fat + % ash) (Akubor and Chukwu, 1999). Moisture content was determined using hot air oven method (AOAC, 1990). Protein content (total N×6.25) was done using the semimicro Kjedahl method (AOAC, 1990). Determination of fat content was determined using soxhlet method (AOAC, 1990). Ash content was determined using muffle furnace (AOAC, 1990). Salt content (NaCl) was determined using Mohr method (Han *et al*., 2001).

pH

Two grams of homogenate was diluted with 18 mL of distilled water and swirled to mix. pH of sample was measured from this mixture by using pH-meter digital.

Free Amino Nitrogen (FAN)

Free amino nitrogen was carried out as described by Han *et al*. (2001). Two grams of homogenate was suspended into 18 mL of distilled water and arranged pH to be 8.5. The suspension was added with 5 mL of 37% formaldehyde and let it for 2 min, then titrated with 0.1 M NaOH until pH to be 8.5 (pH should be constant for 30 sec). Free amino nitrogen was expressed as:

$$\text{Free amino nitrogen (mM g}^{-1}) = \frac{\text{M NaOH} \times \text{V NaOH}}{\text{Mass of sample (g)}}$$

Where:
M = Molarity
V = Volume

Titratable Acidity

Titratable acidity was determined according to Han *et al.* (2001). A 150 g of homogenated sample was centrifugated at 3.200x g for 2 h. Ten milliliter of supernatant was diluted with 20 mL of distilled water then boiled for 1 min then the mixture was cooled into ambient temperature. It was added with 5 drops of phenolpthlein indicator and titrated with NaOH 0.1 M until slightly pink formed. Titratable acidity was calculated as a lactic acid concentration. Percentage of titratable acidity was computed by using the follow expression:

$$\text{Titrable acidity (b/v) (\%)} = \frac{\text{M NaOH} \times \text{V NaOH} \times 0{,}900}{\text{V sampel}} \times 100\%$$

Where:
M = Molarity
V = Volume (mL)

Ethanol Content

Ethanol was determined according to the method described in http//www.outreach.canterbury.ac.nz./chemistry/ethanol.html. A conical flask containing 10 mL of acid dichromate solution (0.01 M $K_2Cr_2O_4$ in H_2SO_4 5 M) was sealed with this rubber stopper before stored at 30°C. The small glass vial containing 2 g of homogenate was hung on a glass hook which was held in a rubber stopper. After 24 h, the rubber stopper was removed from a conical flask. Acid dichromate solution was added with 1 mL of KI 10% and titrated with $Na_2S_2O_7$ 0.03 M. When yellow formed, this solution was added with 1 mL of amylum 1% and titrated again with 0,03 M $Na_2S_2O_7$ until slightly blue formed. Determination ethanol content was conducted three times for each sample.

The relationship between the moles of sodium thiosulfate and the moles of ethanol as follow:

- As 1 mol of $S_2O_3^{2-}$ is equivalent to 6 mol of $Cr_2O_7^{2-}$
- And 2 mol of $Cr_2O_7^{2-}$ is equivalent to 3 mol of C_2H_5OH
- Then 1 mol of $S_2O_3^{2-}$ is equivalent to 0.25 mol of C_2H_5OH

Percentage of ethanol was calculated based on the relationship between the mole of sodium thiosulfate and the moles of ethanol then % ethanol was calculated as follow:

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

Glucose Content

Glucose content was determined based on Samogyi-Nelson method (Jacobs, 1973). Homogenated ale-ale of 0.5 mL was diluted with 1.5 mL of distilled water, added with 2 mL of

0,1 M Ba $(OH)_2$ and 0.2 mL of 0.1 M $ZnSO_4$, swirled to mix. The mixture was centrifugated at 5,000x g for 10 min. One milliliter of produced supernatant was added with 1 mL of Nelson reagent and boiled for 15 min and let the mixture reaching into the room temperature. The mixture was added into arsenomolibdad reagent and diluted with 10 mL of distilled water before measured at 510 nm using UV-Vis spectrophotometer for its absorbance.

Microbiological Analyses

Preparation of Microbiological Sample

Twenty grams of each fermented ale-ale homogenate samples were suspended into 180 mL of peptone saline (0.1% neutral peptone, 2.5% NaCl). The suspension was diluted into 10^{-2}, or 10^{-3} for microorganism determination in the sample.

Total Count of Mesophilic Aerobic Bacteria (TMAB)

The TMAB was determined by using pour plates methods in Plate Count Agar (PCA) supplemented with 25 g L^{-1} of NaCl. The plates were incubated in room temperature for 2-3 days. All colonies were counted for Colony Forming Units (CFU) per gram wet weight of sample. The test was carried out in triplicate.

Total Count of Halotolerant Bacteria (THB)

The THB was enumerated using pour plates methods in PCA supplemented with 100 g L^{-1} of NaCl and 175 g L^{-1} of NaCl. The plates were incubated at room temperature for 7 days. All colonies were counted for Colony Forming Units (CFU) per gram wet weight of sample. The test was carried out in triplicate.

Bacterial Endospores

Sample for determination of bacterial endospores was prepared by heating 1 mL of homogenate at 80°C for 10 min. Furthermore 10 μL of homogenate was enumerated in pour plates of PCA supplemented with 25 g L^{-1} of NaCl and incubated at room temperature for 2-3 days. All colonies were counted for Colony Forming Units (CFU) per gram wet weight of sample. The test was carried out in triplicate.

Lactic Acid Bacteria (LAB)

The LAB was enumerated in pour-plates of de Man, Rogosa and Sharpe medium (MRS, (Scharlau 01-135) supplemented with 15 g L^{-1} of NaCl and incubated at room temperature for 3 days. Determination of positive LAB was confirmed according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The positive colonies were counted as Colony Forming Units (CFU) per gram wet weight of samples. All tests were carried out in triplicate.

Bacillus cereus

Bacillus cereus was enumerated selectively on spread-plates of Mannitol Egg Yolk Polymyxin (MYP) with supplemented NaCl 15 g L^{-1} and incubated at room temperature for 3 days. Determination of positive *B. cereus* was confirmed according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The positive colonies were counted as Colony Forming Units (CFU) per gram wet weight of samples. All tests were carried out in triplicate.

Clostridium perfringens

Clostridium perfringens was enumerated selectively in pour-plates of Tryptose Sulfite Cycloserine Agar (Merck 1.11972.0500) supplemented with 25 g L^{-1} of NaCl and incubated

at room temperature for 18-24 h under anaerobic conditions. Determination of positive *C. perfringens* was confirmed according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The positive colonies were counted as Colony Forming Units (CFU) per gram wet weight of samples. All tests were carried out in triplicate.

Enterobacteriaceae

Enterobacteriaceae was enumerated selectively in pour-plates of Violet Red Bile Glucose Agar (Scharlau 01-295) with supplemented NaCl 15 g L^{-1} and incubated at room temperature for 24-36 h. Determination of positive Enterobacteriaceae was confirmed according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The positive colonies were counted as colony forming units (CFU) per gram wet weight of samples. All tests were carried out in triplicate.

Staphylococcus aureus

Staphylococcus aureus was enumerated selectively in pour-plates of Mannitol Salt Phenol Red Agar (Mercks 1.05404.0500) with supplemented NaCl 15 g L^{-1} and incubated at room temperature for 72 h. Determination of positive *S. aureus* was confirmed according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The positive colonies were counted as Colony Forming Units (CFU) per gram wet weight of samples. All tests were carried out in triplicate.

Listeria monocytogenes

Listeria monocytogenes enumerated selectively in pour-plates of McBride Listeria Agar Base (Fluka 62355) with supplemented NaCl 15 g L^{-1} and incubated at room temperature for 24-36 h. Determination of positive *L. monocytogenes* was confirmed according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The positive colonies were counted as Colony Forming Units (CFU) per gram wet weight of samples. All tests were carried out in triplicate.

Fungi (Yeast and Molds)

Fungi (Yeast and molds) were enumerated in pour plates of modified Oxytetracycline Glucose-Yeast Extract Agar (OGYE) (yeast extract 5 g L^{-1}, dextrose 12 g L^{-1}, dan agar-agar 15 g L^{-1}) supplemented with 0% NaCl, 1% NaCl and 2% NaCl. The plates were incubated at room temperature for 5 days. The colonies were counted as Colony Forming Units (CFU) per gram wet weight of samples. All tests were carried out in triplicate.

Statistical Analysis

All data are expressed as Mean±SD Statistical analyses were performed using one-way ANOVA followed by *post hoc* comparison of mean (Duncan's multiple range test) using SPSS 17 Programme. In all cases $p<0.05$ was taken as the significant level.

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of both white and red fermented ale-ale as well as *M. meritrix* meat, especially pH, moisture, ash, glucose, protein, carbohydrate, salt content, FAN, titratable acidity, ethanol content was presented in Table 1. The pH of both white and red

Table 1: Chemical composition of ale-ale and fermented ale-ale

Sample	pH	Moisture (% b/b)	Ash (% b/b)	Glucose (% b/v)	Protein (% b/b)	Fat (% b/b)
Meat ale-ale (n = 3)	7.17±0.12^{a}	86.89±0.27^{a}	1.79±0.22^{a}	0.038±0.52^{a}	33.95±0.81^{a}	0.42±0.14^{a}
White fermented ale-ale (n = 3)	5.19±0.99^{*b}	60.16±13.09^{*b}	14.68±0.58^{b}	0.004±0.0018^{*a}	8.10±2.30^{b}	0.18±0.02^{b}
Red fermented ale-ale (n=3)	5.06±1.45^{b}	60.10±9.02^{b}	20.16±0.68^{c}	0.035±0.01^{a}	5.16±5.47^{b}	0.06±0.07^{b}

Sample	Carbohydrate (% b/b)	Free amino nitrogen (mM g^{-1})	Titratable acidity (% b/v)	Salt (% b/v)	Ethanol (% b/b)
Meat ale-ale (n = 3)	64.25±0.67^{a}	nd	nd	nd	nd
White fermented ale-ale (n = 3)	77.21±2.71^{b}	0.72±0.190^{*a}	4.58±0.77^{*a}	1.55±0.11^{*a}	0.048±0.02^{*a}
Red fermented ale-ale (n = 3)	74.86±5.78^{b}	0.62±0.014^{*a}	4.17±0.99^{*a}	1.58±0.05^{*a}	0.054±0.02^{*a}

Mean values±Standard deviation in the same vertical column with different superscript letter are significantly different (*p<0.05) n = 7, nd : Not determined

fermented ale-ale products (pH = 5) decreased significantly than that of *M. meritrix* meat (pH = 7) due to bacteria and yeast produced organic acid (Ennoauli *et al*., 2006; Thapa and Pal, 2007). The enzyme contained *M. meritrix* meat will be autolysis to produce organic acids (Oetterer *et al*., 2003). The pH of white fermented ale-ale was not different from that of red fermented ale-ale although there are differences in carbohydrate sources. The pH of fermented fish products should be below 5-4.5 in order to inhabit pathogenic microorganism (Owen and Mendoza, 1985).

Moisture content is important to growth microorganism in fermentation. Reduction of moisture content will inhibit the microorganism growth. However, the moisture content is not always a certain indicator of the susceptibility of a product to undergo microbial spoilage. Fermentation of ale-ale meat leads to reduction of the moisture content from 86 to 60% (Table 1). Reduction of moisture content was also exhibited in other fermentation products such as anchovies of sardine (*Sardinella brasiliensis*) from 74.27% fall to 52% (Oetterer *et al*., 2003). Moisture content of fish sauce from 7 countries of Southeast and East Asia (Park *et al*., 2001) was higher than that of both white and red fermented ale-ale namely ranged from 61 to 79%.

Ash content generally increases in fermentation due to addition of both salt and carbohydrate. Ash content of fermented ale-ale increased significantly than that of *M. meritrix* meat (Table 1). The increase of ash content also found in anchovies of sardine (*S. brasiliensis*) (Oetterer *et al*., 2003).

The protein content of both white and red fermented ale-ale (ranged from 5-8%) was lower than that of *M. meritrix* ale-ale (34%) (Table 1). This decrease of protein content was also showed in barley-based probiotic fermented food mixture (BCGT food mixture) (Shindhu and Khetarpaul, 2004), Ogiri from fermented African locust beans, as well as ugba from African oil bean seeds, ogiri from caster seeds (Sanni *et al*., 2000). The reduction in protein content is attributed from accelerating rate in protein catabolism by microorganisms in fermentation. The result of protein catabolism is ammonia. The rate increase of protein catabolism was influenced by salt content in fermentation (Hjalmarson *et al*., 2007). The protein content of white-fermented ale-ale (8.1% equivalent with 1.30% of total nitrogen) was higher than that of red fermented ale-ale (5.1% equivalent with 0.82% of total nitrogen). Both white and red fermented ale-ale were considered low quality product due to its protein content. Fish sauce total nitrogen below 1.5% (equivalent with 9.38% of protein content) is classified as a low quality product in Thailand (Thai Industrial Standard, 1983).

Fat content of *M. meritrix* meat decreased significantly than that of both white and red fermented ale-ale (Table 1). The decrease probably resulted from high activities of microorganism as well as enzymatic activities in muscle. The result from these activities

produced specific aroma of fermented ale-ale. The characteristic aroma of the food may come from the reaction of fatty acid with some other component of the fermenting mash to form esters (Sanni *et al*., 2000).

Carbohydrate content of both white and red fermented ale-ale was higher significantly than that of *M. meritrix* meat (Table 1). High carbohydrate content of both white and red fermented ale-ale might be caused by addition of angkak and plain white rice in fermentation. Catabolism of carbohydrate is resulted from microorganism activities. One of catabolism products were detected as reducing sugar namely glucose. Glucose content of fermented ale-ale did not differ from that of *M. meritrix* meat (Table 1). It is not clear about the relationship between the relative constant of glucose content in both white and red fermented ale-ale Possibly glucose content was directly processed into other products. During the first 12 h of chickpea fermentation, population of bacilli increased and caused high activities of amylase, α-galactosidase, invertase and cellulase activities which resulted in an increase of glucose content (Hatzikamari *et al*., 2007). Subsequently, glucose content decreased due to its utilization as carbon source by increasing population clostridia.

Titratable acidity of white fermented ale-ale did not differ significantly ($p<0.05$) from that of red fermented ale-ale (Table 1). Titratable acidity was counted as lactic acid content. Lactic acid was produced by lactic acid bacteria or enzyme from the raw material. Lactic acid bacteria convert glucose into lactic acid in fermentation (Vuyst, 2000). Different source of carbohydrate from both white and red fermented ale-ale did not affect titratable acidity value.

The FAN is measured to determine individual amino acid, small peptides and ammonia ion. FAN can be used to be a good index for yeast growth (Lekkas *et al*., 2007) and as an indicator autolysis and microbial degradation of the fish muscle (Ijong and Ohta, 1995b) as well as an indicator for increase of pH values (Han *et al*., 2001). FAN content of white fermented did not differ significantly from that of red fermented ale-ale. FAN content of both fermented ale-ale was between 0.62 and 0.72 mM g^{-1} (Table 1).

Salt content of both white and red fermented ale-ale is much lower than that of other fermented fish or shellfish. Salt content of bakasang, fermented fish sauce, varied from 8% to 18% (Ijong and Ohta, 1995a). Salt content of both fermented blue mussel (*M. edulis*) (Jung *et al*., 2005) and anchovies of sardine (*Sardinella brasiliensis*) (Oetterer *et al*., 2003) was 20%. Although, both white and red have low salt content (1.5%), they do not contain pathogenic bacteria.

Ethanol content of both white and red fermented ale-ale was low and did not different from each other (Table 2). Ethanol is one of end-metabolite products by lactic acid bacteria. Based on end-metabolites of glucose catabolism, there are two types of fermentation by lactic acid bacteria namely homofermentative and heterofermentative. Homofermentative fermentation is a glucose catabolism of lactic acid bacteria to produce lactic acid. Homofermentative fermentation is a glucose catabolism of lactic acid bacteria to produce

Table 2: The microbiological composition of fermented ale-ale

	Total of colony (Log_{10} cfu g^{-1})						
Sample	TMAB	THB 10% (7 days)	THB 10% (14 days)	THB 17,5% (7 days)	THB 17,5% (14 days)	Fungi 0%	Fungi 1%
White fermented ale-ale (n=7)	8.48±0.41^a	4.88±0.26^a	4.49±0.76^a	3.83±2.23^a	4.86±0.49^a	2.88±0.74^a	2.64±0.72^a
Red fermented ale-ale (n=3)	8.95±0.10^a	4.75±0.33^a	4.09±0.35^a	2.65±2.00^a	4.64±0.23^a	2.42±0.17^a	2.58±0.38^a

	Total of colony (Log_{10} cfu g^{-1})							
Sample	Fungi 2%	BAL	Bacterial endospores	*B. cereus*	*C. perfringens*	*S. aureus*	*Enterobacter*	*L.monocytogenes*
White fermented ale-ale (n=7)	2.67±0.71^a	-	-	-	-	-	-	-
Red fermented ale-ale (n=3)	1.63±1.58^a	-	-	-	-	-	-	-

Mean values±Standard deviation in the same horizontal row with different superscript letter are significantly different ($p<0.05$), -: Not detected

lactic acid, carbon dioxide and ethanol as showed in shrimp waste ensilation without starter (Shirai *et al*., 2001). Since both white and red fermented ale-ale produced lactic acid and ethanol, it was included as heterofermentative fermentation.

Microbiological Analysis

The result microbiological analysis of both white and red fermented ale-ale showed that fermented ale-ale contained TMAB, THB, fungi and no lactic acid bacteria (Table 2). The TMAB showed the high population microorganism from 8.4 to 9.1 Log cfu g^{-1}. This high value was probably due to the low salt content. The TMAB of Lanhouin obtained from cassava fish (*Pseudotolithus* sp.) and king fish (*Scomberomorus tritor*) was also high namely 6.0±0.6 log and 7.0±0.8 log cfu g^{-1}, respectively (Anihouvi *et al*., 2006).

Total count of halotolerant bacteria in PCA medium containing 10% NaCl and 17,5% NaCl varied from 3.7 to 5.4 log cfu g^{-1}. Halophilic bacteria count (Log cfu g^{-1}) of lanhouin produced from cassava fish and king varied from 4.5 to 5.5 and 5.4 to 6.3, respectively (Anihouvi *et al*., 2006).

The LAB has been playing an important role in fermented food namely flavor and texture formation, as well as the inhibition of spoilage and pathogenic microorganism growth. The LAB in this research was counted as Lactobacilli. All samples were not detected for Lactobacilli, although the titratable acidity counted as the lactic acid was detected with ranged from 3.5 to 6.0. The lactic acid is possibly produced by other genus of lactic acid bacteria or Lactobacilli may have died shortly before the examination because of high titrable acidity in samples. Lactobacilli can live in percent of acidity below 2.5% and it was gradually died off was in milk followed by acid-tolerant yeast and molds establish (Potter and Kiss, 1995).

Fungus dominant in fermented ale-ale is *Penicillium coryliphilum Dierckx* (Watanabe, 1994). *Penicillium* sp. was also detected in other food fermentation such as a Spanish variety of fermented meat sausage, chorizo de Cantimpalos, containing *Penicillium commune* and *Penicillium olsonii* (Lopez-Diaz *et al*., 2001). *Penicillium coryliphilum Dierck* produced mycotoxin causing liver failure. Mycotoxin will be damage at temperature above 30°C. Although, there has been no report toxicity case or illness caused by consuming directly fermented ale-ale, it is suggested to cook fermented ale-ale before it is consumed.

Pathogenic bacteria namely bacterial endospores, *B. cereus*, endospore, *C. perfringens*, *S. aureus*, *Enterobacteriaceae* and *L. monocytogenes* were not detected in all fermented ale-ale (Table 2). It indicate that chemical composition of fermented ale-ale especially pH and titratable acidity can inhibit successfully the growth of pathogenic bacteria in ale-ale fermentation. Ahamed and Mahendrakar (1996) reported there was no coliforms, *E. coli*, Enterococci, Salmonella, *C. perfringens* and Staphylococci found in the fermented fish viscera from 48 h at 37 °C and 72 h at 26°C. Eventhough salt content of fermented ale-ale was low (1.5%), those pathogenic bacteria were not found in fermented ale-ale. Pathogenic bacteria can not survive in the presence of ethanol, lactic acid and pH combination. The growth of *L. monocytogenes* was inhibited by 0.4% lactic acid with pH of 4.5 in tryptic soy broth with supplemented yeast extract (Conner *et al*., 1990). In addition, Hu *et al*. (2007) reported that the pH was the main hurdle for the growth of *Enterobacteriaceae*.

CONCLUSION

Chemical and microbiological composition of both white and red fermented ale-ale was similar except ash content. All of fermented ale-ale in this research showed low quality

product based on its protein content. Due to the presence of *P. coryliphilum Diercks*, fermented ale-ale was recommended to be cooked before served.

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