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Microflora and Chemical Assessment of an Indonesian Traditional Fermented Fish Sauce "Bakasang"

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Abstract The microflora and chemical properties of bakasang, a traditional fermented fish sauce which originated in North Celebes, Indonesia was investigated. Total aerobic and anaerobic, lactic acid bacteria (LAB), total coliform (TC), spore forming bacteria (SFB) and staphylococcal counts were determined and the isolate microorganisms from each media were identified. The coliform bacteria were not detected in all samples, but the total staphylococcal count was high in all samples; it ranged from 4.70 to 5.59 (log cfu/ml). *Staphylococcus* sp. and *Lactobacillus* sp., were the predominant bacteria isolated from traditional bakasang samples. Generally, traditionally-produced bakasang has a low pH and is relatively high in nutritive value. Glutamic acid, phenylalanine, and isoleucine were predominant amino acids.
Key words: bakasang, traditional fermented fish sauce, microflora.

INTRODUCTION

Fermented fish products constitute a major source of protein in the diet in many Southeast Asian countries including Indonesia. In Indonesia, fermented fish products are known under different names, such as, for example, *kecap ikan*, *pindang* (CAMPBELL-PLATT, 1987), *terasi* (CHAYOVAN *et al.* 1983) and *bakasang* (IJONG and OHTA, 1995).

Bakasang is a traditional fermented fish sauce produced by fermenting small whole sardines (*Sardinella* sp., or *Stelophorus* sp.) or the guts of big fish (*Katsuwonus pelamis*). In the process, approximately 1.5 to 3.0 parts salt is mixed with 5 parts fish, packed into small bottles, and placed at the kitchen near a fire place (temperatures ranging from 30 to 60°C) and allowed to ferment for about 3 to 6 weeks. The product has become closely integrated with the eating habits of Eastern Indonesian people, especially the Manadonese people (in the North Celebes Island). It is usually used as a flavoring agent for many dishes or mixed with red chilies, tomato, red onion, and garlic, then saüteed with coconut oil and eaten together with hot porridge mixtures of rice and vegetables called "tinutuan".

Fermented fish sauces are generally high in protein and amino acid compounds (CHAYOVAN *et al.* 1983; BRYAN, 1988). The major problems associated with traditional fermented fish products are the lack of quality control and microbiological aspects should be considered.

The microbiological aspect of many traditional fermented fish product had been re-

ported. Much information is available on Thai fish sauce, *nampla* (BRYAN, 1988), and Korean fermented fish products (SAND and CRISAN, 1974) but virtually no published information is available on bakasang.

The present study was performed to determine the total counts of microflora, and the types of microflora present in the traditional bakasang samples; also, the chemical aspects were carried out.

MATERIALS AND METHODS

Collection of sample

Five kinds of traditional bakasang samples designated A, B, C, D and E were obtained from the local markets and or supermarkets in Indonesia, in the North Celebes Island.

Chemical analysis

All samples were analyzed for moisture, salt, total lipid, and crude protein (total N×6.25) using standard methods (AOAC, 1975). The pH was determined using a pH meter (Model 240, Corning Co., USA). Amino acid composition was determined according to the modification method of HUGHES and FRUTIGER (1990) using a Hitachi HPLC model L-4000 (Tokyo, Japan). Amino acid standard solution Type H (Wako, Co., Osaka, Japan) was used as a calibrating factor.

Microbial analysis

A 25g sample was taken aseptically from the sample bottles and homogenized in 225ml of sterilized 0.9% NaCl solution. Serial dilutions of homogenates were made and total plate counts (TPC) for both aerobic and anaerobic microorganisms were determined using the pour plate method on nutrient agar (Nissui, Japan). Lactic acid bacteria (LAB) counts were determined using Rogosa agar (pH 6.5; Merck, Germany). Total coliform (TC) was determined using Deoxycholate agar (pH 7.1; Nissui, Japan). Total spore forming bacteria (SFB) was determined on nutrient agar (Nissui, Japan), serial dilutions of homogenate samples were boiled for 10min before plating. Total count of staphylococcus was determined using Mannitol Salt agar (pH 7.5; Nissui, Japan). All plates were incubated at 37°C for 48h (KATO *et al.* 1985).

Typical colonies from all plates were sub-cultured for purification and identification. They were cultured on slants of nutrient agar or Rogosa agar and stored at 4°C until used.

Identification of microorganisms was done using their morphological and biochemical properties (SNEATH *et al.* 1986).

RESULTS AND DISCUSSION

Table 1 shows the moisture, pH, salt, total lipid and crude protein of the samples. The product was brown or yellow-brown in color. All samples were similar in moisture content and pH, except sample C which showed a relatively higher pH value. The salt contents varied significantly, ranging from 8% to 18%. Crude protein and lipid were also varied, ranging from 14% to 17% and 0.1% to 3.0%, respectively. The amino acid compositions of the traditionally-produced bakasang samples are shown in Table 2. The amino acid content was varied among the samples, however they had a higher value of phenylalanine, isoleucine, and glutamic acid. The results show that traditional bakasang products vary signifi-

Table 1. Moisture, pH, salt, total lipid and crude protein of the traditional bakasang*

	Samples**				
	A	B	C	D	E
Moisture (g/Kg)	668.9 ± 7.72	676.2 ± 13.5	689.6 ± 11.02	681.1 ± 12.6	663.0 ± 2.00
pH	5.90 ± 0.06	5.92 ± 0.05	6.30 ± 0.06	5.84 ± 0.02	5.95 ± 0.10
Salt (NaCl, g/Kg)	180.2 ± 13.5	130.4 ± 10.9	103.7 ± 4.37	84.2 ± 13.1	170.0 ± 3.50
Lipid (g/Kg)	30.0 ± 0.30	25.0 ± 0.30	10.0 ± 0.10	10.0 ± 0.10	14.1 ± 0.60
Crude protein (g/Kg)	174.4 ± 10.89	141.9 ± 7.79	143.6 ± 7.85	147.8 ± 10.31	140.0 ± 6.00

* Values represented are means ± standard deviations for $n \leq 3$ or 5.
** Various traditional sample products were purchased from markets in Indonesia-Manado.

Table 2. Amino acid contents (%) of the traditional bakasang*

	Samples**				
	A	B	C	D	E
Aspartic acid	8.72 ± 0.65	8.82 ± 0.62	2.15 ± 0.18	2.14 ± 0.22	4.30 ± 0.45
Glutamic acid	10.48 ± 0.61	11.40 ± 0.58	7.05 ± 0.07	6.95 ± 0.03	10.27 ± 1.76
Serine	9.01 ± 0.14	4.38 ± 0.06	9.79 ± 0.67	6.30 ± 0.48	4.07 ± 0.20
Glycine	2.22 ± 0.31	6.52 ± 0.33	2.04 ± 0.72	2.05 ± 0.04	2.49 ± 0.28
Histidine	0.71 ± 0.15	2.61 ± 0.74	0.00	3.95 ± 0.50	0.62 ± 0.03
Arginine	3.69 ± 0.01	4.07 ± 0.12	1.89 ± 0.17	1.46 ± 0.14	2.33 ± 0.29
Threonine	6.28 ± 0.17	5.63 ± 0.21	1.27 ± 0.02	8.45 ± 0.50	2.54 ± 0.24
Alanine	5.84 ± 0.20	4.86 ± 0.57	5.07 ± 0.14	3.67 ± 0.15	20.23 ± 2.36
Proline	2.90 ± 0.56	5.87 ± 0.34	10.96 ± 0.21	4.38 ± 0.16	0.16 ± 0.01
Tyrosine	4.54 ± 0.14	2.99 ± 0.25	4.30 ± 0.44	3.50 ± 0.15	1.96 ± 0.19
Valine	5.78 ± 0.25	4.52 ± 1.19	5.23 ± 0.39	2.85 ± 0.70	9.77 ± 0.64
Methionine	2.51 ± 0.06	2.90 ± 0.78	3.32 ± 0.63	2.79 ± 0.20	3.95 ± 0.69
Cysteine	4.15 ± 0.09	2.83 ± 0.66	2.43 ± 0.29	1.58 ± 0.48	4.61 ± 0.73
Isoleucine	8.18 ± 0.55	5.98 ± 0.39	5.05 ± 0.93	4.14 ± 0.53	13.33 ± 1.16
Leucine	4.11 ± 0.59	2.84 ± 0.18	6.70 ± 0.29	5.88 ± 0.19	3.59 ± 1.21
Phenylalanine	11.35 ± 0.27	20.30 ± 4.65	31.89 ± 0.30	37.07 ± 1.07	11.99 ± 0.23
Lysine	9.58 ± 0.21	3.46 ± 0.80	2.75 ± 0.30	2.87 ± 0.74	8.86 ± 1.63

Legends and conditions are the same as in Table 1.

cantly in their quality characteristics. This could be related to the method of the processing and the raw material used. The major problem associated with traditional products is the lack of standardization requirements for the finished product. Hence, the method of processing varies depending on the processors, as does the quality of the finished product (BERHIMPON *et al.* 1980). Amino acids are major contributors to the taste of seafood, and it is likely they would contribute to the taste of bakasang (IJONG and OHTA, 1995). Similar results have also been reported by JONES (1961) that the flavor of Thai fish sauce, *nampla*, could arise in part from glutamic acid, histidine, and proline.

Table 3 shows the total count of microflora from the samples. Total aerobic and anaerobic counts were similar for all the samples, except sample B which had low anaerobic counts. Total LAB counts ranged from 4.80 to 6.15 (log cfu/ml). Sample A showed higher

Table 3. Total count of microflora (log cfu/mL) of the traditional bakasang*

	Samples**				
	A	B	C	D	E
TPC:					
aerobic	5.72 ± 0.24	6.08 ± 0.14	5.44 ± 0.16	5.03 ± 0.33	4.18 ± 0.10
anaerobic	3.38 ± 0.85	1.96 ± 0.07	3.84 ± 0.42	3.27 ± 0.09	3.00 ± 0.25
LAB	6.15 ± 0.30	4.95 ± 1.12	4.99 ± 0.50	4.97 ± 0.32	4.80 ± 0.30
Coliform	nd	nd	nd	nd	nd
SFB	nd	nd	2.08 ± 0.24	5.01 ± 0.06	nd
Staphylococcal	5.38 ± 0.39	5.59 ± 0.15	5.34 ± 0.60	4.72 ± 0.67	4.70 ± 0.38

Legends and conditions are the same as in Table 1.
nd: not detected

Table 4. Microflora isolated from traditional bakasang*

Samples**	Genera/species
A	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus* sp., and *Lactobacillus* sp.
B	*Staphylococcus aureus*, *Staphylococcus* sp., *Micrococcus* sp., and *Streptococcus* sp.,
C	*Staphylococcus aureus*, *Staphylococcus* sp., *Streptococcus* sp., *Lactobacillus* sp., and *Clostridium* sp.
D	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus* sp., *Streptococcus* sp., *Lactobacillus* sp. and *Clostridium* sp., and *Bacillus* sp.
E	*Staphylococcus aureus*, *Staphylococcus* sp., *Micrococcus* sp., and *Streptococcus* sp.

Legends and conditions are the same as in Table 1.

LAB counts than all others. There were no coliform bacteria detected in all the samples. SFB was not detected in samples A, B, and E, but it was detected in samples C and D. Total staphylococcal count was high in all samples. It ranged from 4.70 to 5.59 (log cfu/ml).

Table 4 shows the bacteria isolated from traditional products. Six genera of bacteria belonging to *Staphylococcus*, *Lactobacillus*, *Micrococcus*, *Streptococcus*, *Clostridium*, and *Bacillus* were identified. *Staphylococcus* sp., and *Lactobacillus* sp., were the predominantly isolated microorganisms. Similar results have also been reported by several workers (BRYAN, 1988; SANDS and CRISAN, 1974; BUCKLE *et al.* 1978). According to CAMPBELL-PLATT (1987) the microorganisms usually involved in the traditional fermented fish sauce are salt tolerant bacteria and LAB. The presence of LAB in the fermentation of bakasang could contribute to the lowering pH and prevented the growth of undesirable organisms such as coliform or pathogenic bacteria (HAYES, 1995; VARNAM and EVANS, 1991). Salt also acts as bacteriostatic for most of the bacteria. The use of salt in fermentation is to prevent the growth of spoilage bacteria, including the pathogenic.

The presence of *Clostridium* sp., in samples C and D corresponds with the total SFB as reported in Table 2. This organism has been reported to contaminate *pindang*, a cooked and fermented fish product of Indonesia, and *izushi*, Japanese sour-rice made from raw

fish, boiled rice and fresh vegetables (CAMPBELL-PLATT, 1978).

Microbiological safety hazards in fermented fish are represented by *Staphylococcus aureus* and *Clostridium botulinum* (FRAZIER and WESTHOFF, 1988; HAYES, 1995). The presence of *Staphylococcus aureus* and *Clostridium* sp., in traditional samples seem to be attributable to the contamination of raw materials used during processing. Both microorganisms could be associated with salt (SAISITHI *et al.* 1966), or the raw fish (VARNAM and EVANS, 1991: CHANG *et al.* 1992) used in the processing. However this kind of contamination can be prevented during processing if the processing is applied under hygienic conditions.

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インドネシアの伝統的魚醤「バカサン」の微生物的及び化学的評価

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インドネシアの北セレベス地方で造られている，伝統的魚醤の微生物相と化学的性質について検討した。好気性菌，嫌気性菌，乳酸菌，大腸菌群，胞子形成菌，ブドウ球菌についてそれぞれの菌数を調べた。そして，分離菌株について同定を行った。その結果，大腸菌群は何れの試料からも見い出せなかった。しかし，ブドウ球菌は全ての試料から多く見い出された。その菌数は 4.70～5.69 (log cfu/ml) であった。また，主として，*Staphylococcus* 属及び *Lactobacillus* 属の細菌が存在していた。一般に伝統的な方法で造られたバカサンの pH は低く，グルタミン酸，フェニールアラニン，イソロイシンなどのアミノ酸が多く存在していた。

キーワード：菌相，伝統的魚醤，バカサン