

## Characteristics of Bakasang Fermented with Lactic Acid Bacteria-Mixed Culture

Frans Gruber IJONG and Yoshiyuki OHTA

*Faculty of Applied Biological Science, Hiroshima University,  
Kagamiyama 1-44, Higashi Hiroshima-shi 739, Japan*

Received April 23, 1995

**Abstract** The fermentation of bakasang, an Indonesian traditional fish sauce, was carried out using lactic acid bacteria (LAB) –mixed culture collected from fermented whole sardine (*Sardinella* sp.) at 37°C for 5 days. The fermentation characteristics and product quality of bakasang were determined. Bakasang produced from raw sardine gave more hydrolyzed products as compared to bakasang produced from cooked sardine. The addition of LAB-mixed culture into cooked sardine showed significant difference on chemical characteristics of the product as compared to the control. In general, the addition of LAB-mixed culture increased the concentration of amino acid such as aspartic acid, glutamic acid, glycine, alanine, valine and lysine. The sensory assessment showed the addition of LAB-mixed culture into bakasang influence significantly acceptability of flavor and color but not that of aroma.

**Key words:** bakasang, fermentation, LAB-mixed culture, sardines.

---

### INTRODUCTION

In Indonesia, fermented fish product is known under different names, such as *kecap ikan*, *terasi*, *pindang* (PLATT, 1978) and *bakasang* (IJONG and OHTA, 1995). Bakasang is a fermented fish sauce produced by fermenting small whole sardines (*Sardinella* sp., or *Stelophorus* sp.) and or the gut of big fish (*Katsuwonus pelamis*) as a by-product from smoking process. In the process, about 1.5 to 3.0 parts of salt is mixed to 5 parts of fish, packed into small bottles and placed in the kitchen near the fire place with the temperature ranges from 30 to 60°C. It is allowed to ferment for about 3 to 6 weeks. The fermentative action degrades the fish to form a concentrated salty mixture which possesses a characteristic aroma and flavor.

Bakasang is, like all others fermented fish sauce, commonly used as a condiment to add taste to bland rice dishes. It is popular in the Eastern part of Indonesia, especially in the North Celebes Island among the Manadonese people.

The presence of salt in the fermentation creates a favorable environment for growth of lactic acid bacteria (LAB) during fermentation and suppresses growth of underisable microorganisms during storage (FLEMING *et al.* 1987). According to PLATT (1978) the bacteria involved in the fish sauce fermentation are salt-tolerant (*Micrococcus*, *Staphylococcus* and *Bacillus*) and LAB (*Pediococcus* and *Lactobacillus*).

LAB fermentation is very important in improving flavor and quality of fermented meat

products have been reported by some investigators (DYETT *et al.* 1981; GIBBS, 1987; HUANG and LIN, 1993). LAB is also widely used as a starter culture for these products (DYETT *et al.* 1981). In our previous study on the microbiological aspects of traditional bakasang (unpublished data). LAB was the predominant microorganisms among the isolated strains. They belonged to *Lactobacillus* and *Streptococcus*. Their presence in the bakasang fermentation process may play an important role in improving upon the flavor and quality of the final product.

Many studies have been reported on the use of LAB in the fermentation of meat (GIBBS, 1987; HAMMES, 1990; HUANG and LIN, 1993). Essentially, however, no work has been reported on LAB-mixed culture in fish sauce processing. The objective of this study was to study the effect of the addition of LAB-mixed culture on: (1) the physico-chemical characteristics and, (2) the sensory characteristics of bakasang. In addition, the study was done to develop methods for using LAB in the production of bakasang.

## MATERIALS AND METHODS

### Preparation of bakasang

Laboratory bakasang samples were prepared from fresh small sardines (5 to 10 cm long) purchased from a local market. The sardines were washed, sorted and cut into small parts. It was then mixed with salt (100 g salt per 1,000 g fish) and LAB-mixed culture. The chopped sardines, salt and LAB-mixed culture were well mixed by glove covered hands. The samples were then packed in small glass bottles (180 ml), sealed with a cork plug and then incubated at 37°C for 40 days. This experiment was carried out using raw sardine (sample A) and cooked sardine (sample B). Bakasang samples not inoculated with LAB-mixed culture served as a control for both samples. Fig. 1 shows the general design of the experiment.

### Preparation of LAB-mixed culture

LAB-mixed culture was prepared by fermenting the mixture of sardine and salt for 5 days at 37°C. The fermented sardine was then filtered through a sieve (meshes=84). The filtrate containing LAB (log 6.5 cfu/ml) was used as a LAB-mixed culture for bakasang production.

### Physical analysis

The samples were thoroughly mixed by shaking the bottles. A 10 g of each sample was then taken and blended with 90 ml deionized water and the pH of the homogenized samples was measured with a pH meter (Model 240, Corning Co., USA).

### Chemical analysis

Total soluble protein of the filtrate was determined using standard methods (AOAC, 1975). Titrable acidity (as % w/w of lactic acid) was determined according to procedure suggested by NOUT *et al.* (1989). The extent of hydrolysis was determined using the formol titration (BEDDOWS *et al.* 1976). Thiobarbituric acid reactive substances (TBARS) were determined by the method of RAMANATHAN and DAS (1992). Composition of amino acids was determined using RP-HPLC method (HUGHES and FRUTIGER, 1990), HPLC (Model L-4000, Hitachi, Tokyo, Japan) was used. Amino acid composition of bakasang samples was calculated by calibrating with standard amino acids (Amino acid standard solu-

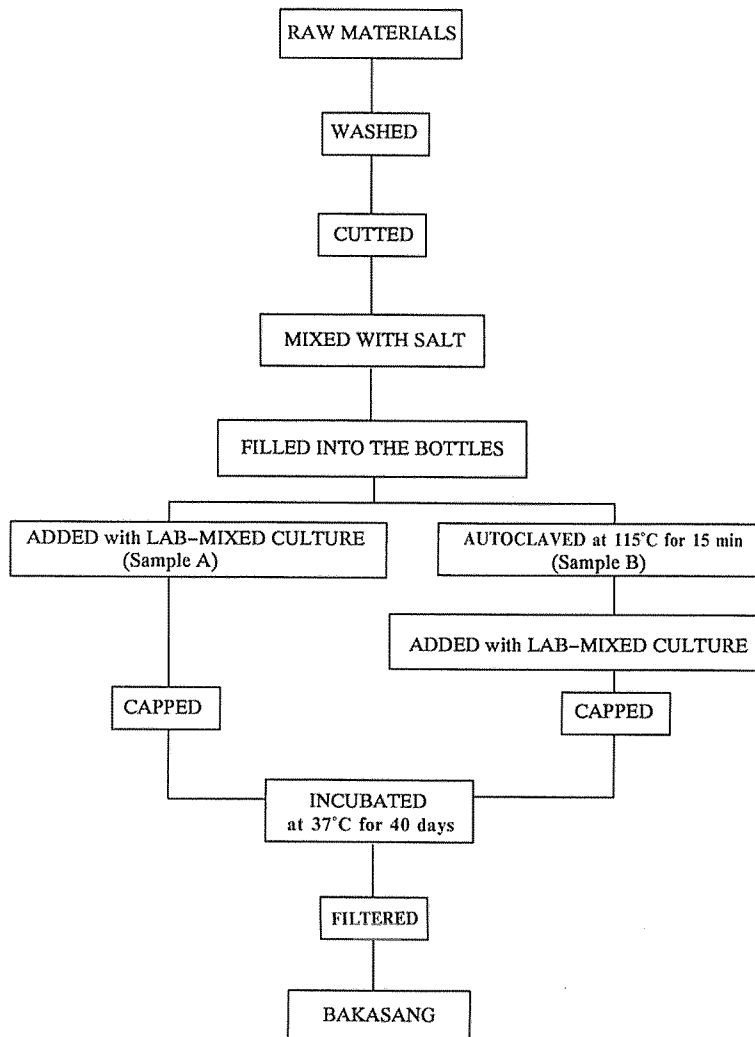


Fig. 1. Flow sheet of laboratory bakasang process.

tion Type H, Wako, Co., Osaka, Japan) .

#### Sensory evaluation

The sauce obtained after 40 days fermentation was evaluated for flavor, aroma, color and overall acceptability by 30-member panel. Ages of panelist ranged from 20 to 48 and the majority of the panelist were males (70%). For the flavor tests, about 1 ml of each sample, in teaspoons, were given to the panelist one after the other. Panelists were asked to rinse their mouth with water between samples. For the assessment of aroma, each sample was put into the small screw-tubes (13×100 mm) and panelist sniffed the samples. The color was judged by visual observation. About 10 ml of sauce was presented in uniform glass test tubes (15×160 mm). All samples were coded using quarter-numbers and were evaluated in random order. Evaluation was carried out using a 7-point Hedonic rating scale where 7 was equivalent to “accepted extremely” and 1 to “not accepted ex-

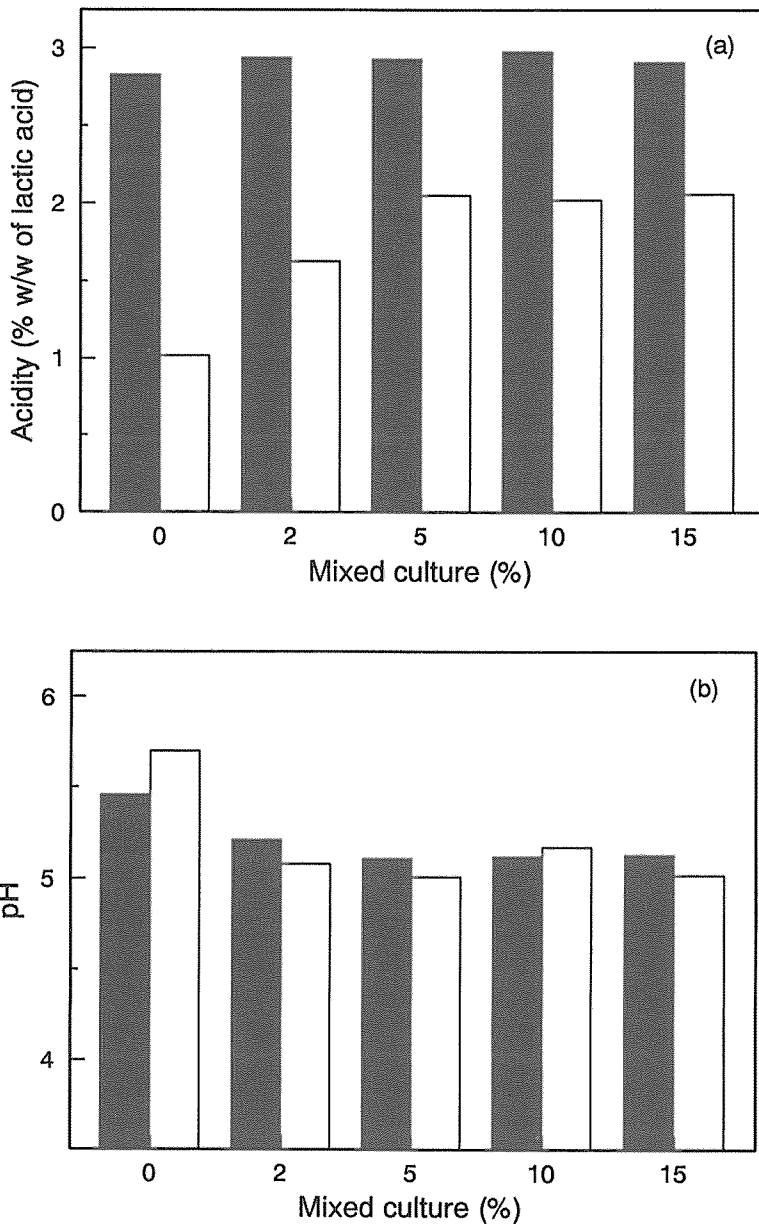


Fig. 2. Acidity (a) and pH (b) of the LAB-mixed culture added bakasang. (■) raw sardine; (□) cooked sardine.

tremely". Acceptability scores were analyzed using the mean of the observations and analysis of variance. Test of significance was at 0.05 levels of probability (LARMON, 1973).

## RESULTS AND DISCUSSION

The total acid and pH values of the samples are shown in Fig. 2. The pH (Fig. 2a) of the control was about 5.46 for bakasang made using raw sardine and 5.70 for bakasang made using cooked sardine. The bakasang prepared with LAB-mixed culture showed a low

pH value compared to the control. In spite of that, statistic analysis showed that the addition of LAB-mixed culture within 2% to 15% did not significantly ( $p < 0.05$ ) influence the pH.

Fig. 2b shows the acidity of the bakasang samples. Sample A has a high content of total acid than sample B. Addition of LAB-mixed culture did not significantly ( $p < 0.05$ ) influence the total acid contents of sample A, but significantly influence that of sample B ( $p < 0.05$ ). The addition of LAB-mixed culture up to 5% increased the total acid value of sample B. The LAB could produce various acids as end products of metabolism, to

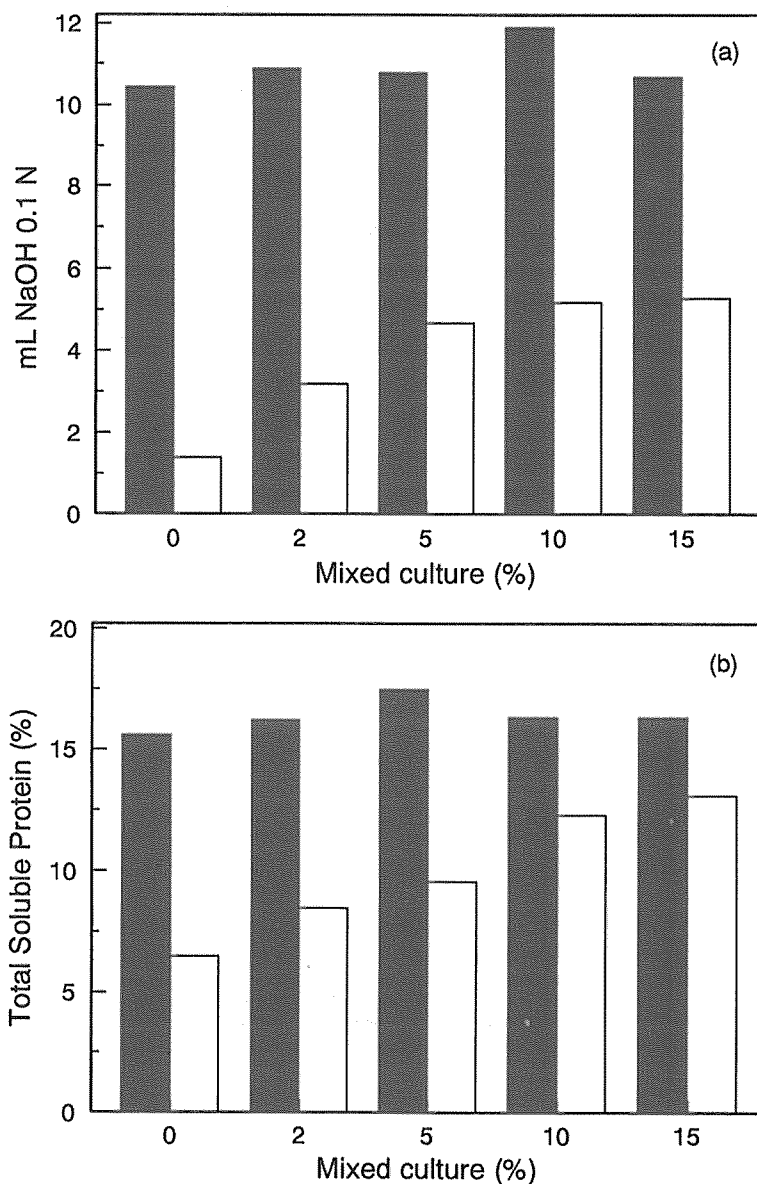


Fig. 3. Formol titration (a) and soluble protein (b) of the LAB-mixed culture added bakasang. Legends and conditions are same as in Fig. 2.

decrease pH of the bakasang, which increased acidity. These results agreed with the findings made by GUILLOU *et al.* (1972) .

Fig. 3 shows the formol titration and total soluble protein of the bakasang samples.

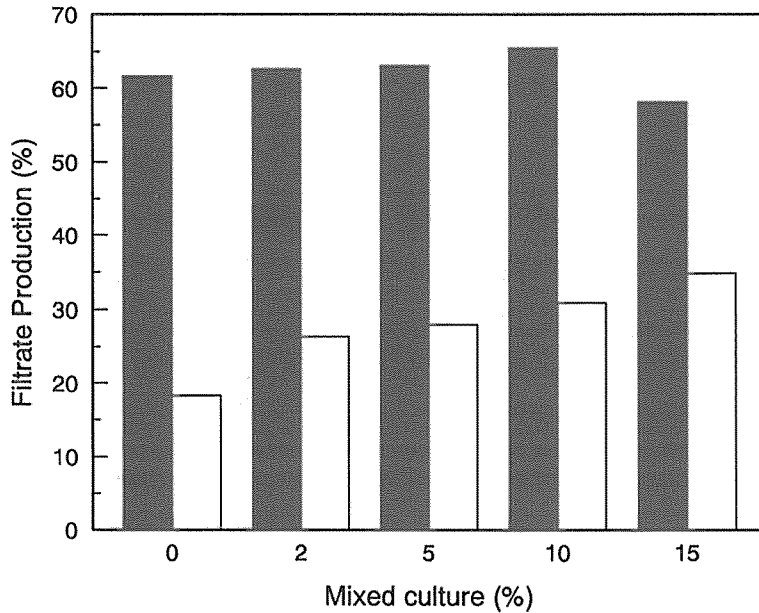


Fig. 4. Sauce production of the LAB-mixed culture bakasang.  
Legends and conditions are same as in Fig. 2.

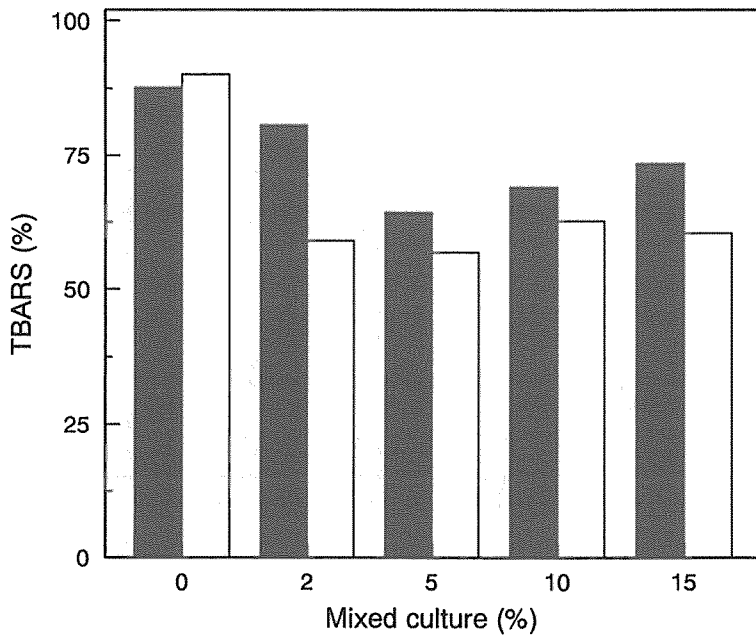


Fig. 5. Rancidity of the LAB-mixed culture bakasang.  
Legends and conditions are same as in Fig. 2.

The LAB-mixed culture added into sample A did not give significant ( $p < 0.05$ ) effect on the hydrolyzing rate. However, addition of that (2 to 5%) did significantly ( $p < 0.05$ ) effect on sample B (Fig. 3a.). Similar pattern was observed in the sauce production (Fig. 4). This result suggests that the LAB and their enzymes are responsible for biochemical changes which occur during bakasang fermentation. PLATT (1978) reported that the LAB has an important role in proteolysis during fermentation of the foods.

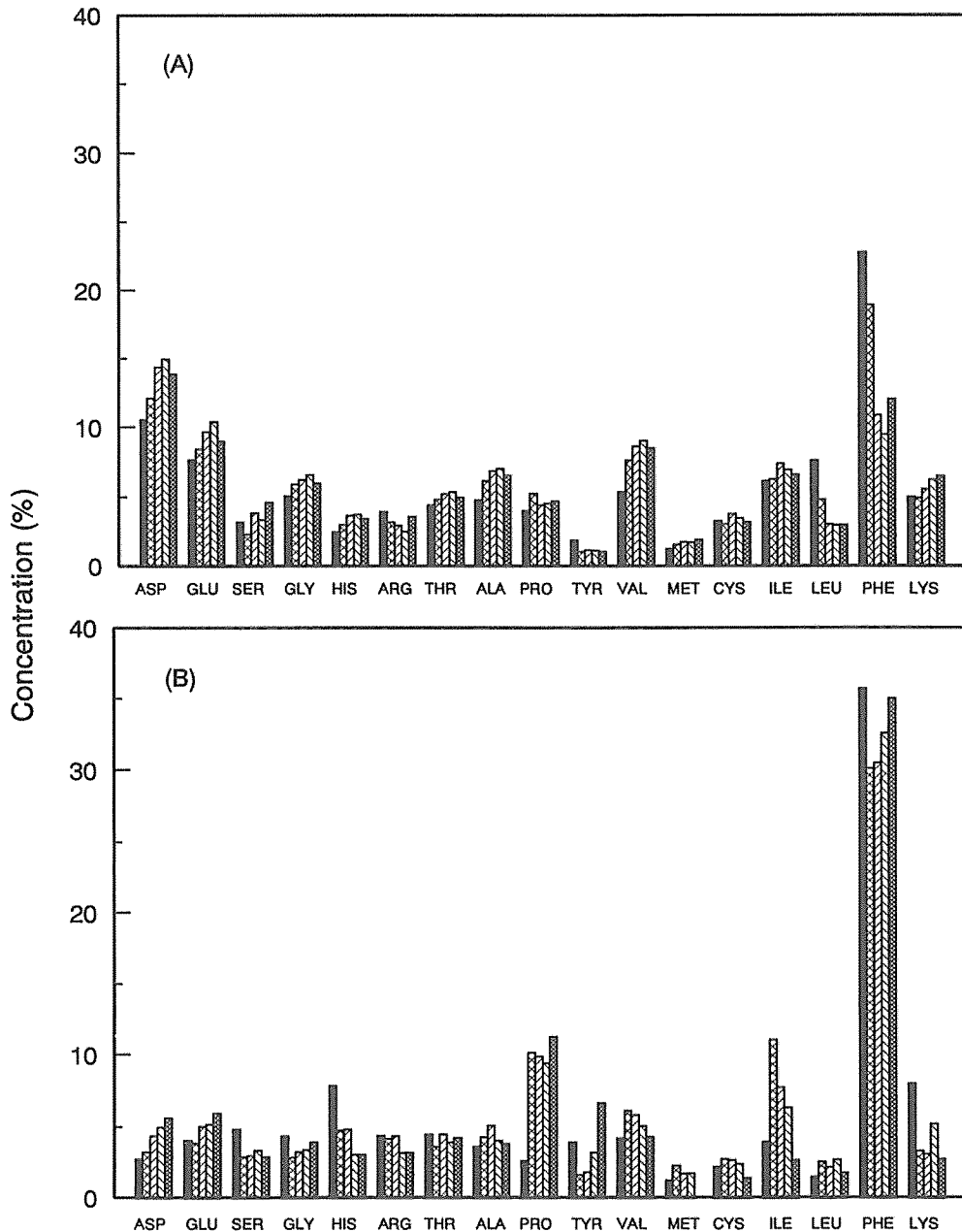


Fig. 6. Amino acid of LAB-mixed culture added bakasang. (A) raw sardine; (B) cooked sardine; (■) control; (▨) LAB2%; (▩) LAB5%; (▮) LAB10%; (▯) LAB15%.

The addition of LAB-mixed culture into sample A did not give a linear effect in increasing the soluble protein. However, LAB-mixed culture supplemented into sample B increased significantly ( $p < 0.01$ ) soluble protein, parallel to increasing amounts of LAB-mixed culture added. From the result observed here, it is indicated that supplementation of bakasang with LAB-mixed culture could increase the protein level of the product. This was similar to results reported by HUANG and LIN (1993). LUCKE (1985) also reported that the use of LAB in processing of food would increase the quality of the product.

The addition of LAB-mixed culture into bakasang processes was also potent to inhibit the extent of rancidity (Fig. 5). The TBARS value was higher in the control than in the LAB-mixed culture added samples regardless of the raw material used (raw and or cooked sardine). Addition of LAB-mixed culture about 2% (w/w, final concentration) did effectively inhibit the development of TBARS content compared to the control for both of sample A and sample B. During fermentation LAB probably produces antioxidants which inhibit the oxidation of the lipids in the fermenting mixture, although sardine lipid is said to be highly susceptible to oxidation (CHANG *et al.* 1992). Cooking also seems to lead to substantial increases in TBARS value of the sauce. This is in agreement with findings made by several workers elsewhere (RAMANATHAN and DAS, 1992; SIU and DRAPER, 1978).

In general, the addition of LAB-mixed culture increased the concentration of amino acid such as aspartic acid, glutamic acid, glycine, alanine, valine and lysine for sample A and aspartic acid, glutamic acid and proline for sample B (Fig. 6). The high concentration of phenylalanine in the both controls of sample A and sample B might be attributable to the characteristics of raw material used. The results suggest that inoculating bakasang with LAB-mixed culture increased ( $p < 0.01$ ) certain amino acid content, such as aspartic acid, glutamic acid, proline and valine. These amino acids could contribute to the flavor of bakasang. It have been reported by JONES (1961) that the flavor of Thai fish sauce is thought to arise partly from glutamic acid, histidine and proline.

Table 1. Mean Scores<sup>a</sup> for sensory characteristics of the bakasang

Samples	Flavor	Aroma	Color
SAMPLE A:			
Control	5.14±0.65	5.88±0.65	5.36±0.68
2% LAB	6.71±0.26	5.63±0.47	6.50±0.42
5% LAB	6.21±0.26	5.38±0.47	6.62±0.56
10% LAB	6.29±0.34	5.13±0.35	5.85±0.46
15% LAB	5.86±0.35	5.00±0.57	6.00±0.51
SAMPLE B:			
Control	4.14±0.37	4.62±0.58	4.50±0.35
2% LAB	5.58±0.29	5.37±0.51	5.42±0.45
5% LAB	5.57±0.31	6.13±0.47	6.30±0.40
10% LAB	5.71±0.29	5.13±0.56	6.64±0.44
15% LAB	5.36±0.27	5.06±0.69	6.85±0.47

<sup>a</sup>n=30 ; 7-point scale where 4=rather not accepted and 6=accepted.



The results of sensory assessment are shown in Table 1. The addition of LAB-mixed culture did significantly influence acceptance of flavor of sample A, but did not for aroma and color. Slightly different from sample A, for sample B, the addition of LAB-mixed culture did significantly influence acceptance of color but did not for flavor and aroma. Sensory evaluation showed that in the terms of overall quality, addition of LAB-mixed culture into sample A had a poor relationship ( $r = -0.53$ ) with quality improvement. However, there was a high relationship ( $r = 0.83$ ) in sample B. This suggests that, LAB-mixed culture added into bakasang processes could improve upon the sensory characteristics of the product.

## CONCLUSION

Use of LAB-mixed culture in processing of bakasang would improve quality of final product. Sensory evaluation showed that addition of up to 5% LAB-mixed culture is quite acceptable. The use of LAB-mixed culture in bakasang processing also could contribute the standard quality of the final product steadily.

## REFERENCE

- AOAC, 1975, Official Methods of Analysis of the Association of Official Analytical Chemists, 12th edn., Washington DC, USA.
- BEDDOWS, C. G., ISMAIL, M. and STEINKRAUS, K. H. 1976, The use of bromelain in hydrolysis of mackerel and the investigation of fermented fish aroma. *J. Food Technol.*, 11, 379-388.
- CHANG, C. M., OSHIMA, T. and KOIZUMI, C. 1992, Changes in composition of lipids, free amino acids and organic acids in rice-bran-fermented sardine (*Etremeus teres*) during processing and subsequent storage. *J. Sci. Food Agric.*, 59, 521-528.
- DYETT, E. J., HUGHES, R. B. and JONES, C. R. V. 1981, Meat and meat products. Applied Sci. Publisher, London and New Jersey.
- FLEMING, H. P., MCFEETERS, R. F. and THOMPSON, R. L. 1987, Effects of sodium chloride concentration on firmness and retention of cucumbers fermented and stored with calcium chloride. *J. Food Sci.*, 52, 653-657.
- GIBBS, P. A. 1987, Novel use for lactic acid fermentation in food fermentation. *J. Appl. Bacteriol. Sump. Suppl.*, 51S-58S.
- GUILLOU, A. A., FLOROS, J. D. and COUSIN, M. A. 1992, Calcium chloride and potassium sorbate reduce sodium chloride used during natural cucumber fermentation and storage. *J. Food Sci.*, 57, 1364-1368.
- HAMMES, W. P. 1990, Bacterial starter cultures in food production. *Food Biotechnol.*, 4, 383-397.
- HUGHES, G. J. and FRUTIGER, S. 1990, Amino Acid Analysis: protocols, possibilities and pretension, In Laboratory Methodology in Biochemistry. Amino acid analysis and protein sequencing (Fini, C., Floridi, A., Finelli, V. N. and Wittman-Liebold, B., eds) CRC Press Inc. Florida.
- HUANG, C. C. and LIN, C. W. 1993, Drying temperature and time affect quality of Chinese-style sausage inoculated with lactic acid bacteria. *J. Food Sci.*, 58, 249-252.
- IJONG, F. G. and OHTA, Y. 1995, Amino acid composition of bakasang, a traditional

- fermented fish sauce from Indonesia. *Lebensmittel-Wissenschaft und-Technologie*, 28, 236-237.
- JONES, N. 1961, Fish flavors. *Proceedings Flavor Chemistry Symposium*, p. 61. Campbell Soup Company, Camden. NJ.
- LARMON, E. 1973, Methods for Sensory Evaluation of Food. Department of Agriculture, Canada.
- LUCKE, F. K. 1985, The microbiology of fermented meat. *J. Sci. Food Agric.*, 36, 1342-1343.
- NOUT, M. J. R., ROMBOUTS, F. M. and HAVELAAR, A. 1989, Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. *Int. J. Food Microbiol.*, 84, 351-361.
- PLATT, C. G. 1987, Fermented Foods of The World. A Dictionary and Guide, Butterworths, London. 291 p.
- RAMANATHAN, L. and DAS, N. P. 1992, studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. *J. Agric. Food Chem.*, 40, 17-21.
- SIU, G. M. and DRAPER, H. H. 1978, A survey of the malonaldehyde content of retail meat and fish. *J. Food Sci.*, 43, 1147-1149.

## 乳酸菌混合培養物によって製造されたインドネシア の伝統的魚醬バカサンの性質

Frans G. Ijong · 太田 欽幸

広島大学生物生産学部, 東広島 739

インドネシアの伝統的魚醬バカサンを乳酸菌の混合培養物を用いて製造した。原料であるイワシ (*Sardinella* sp.) を生のままと蒸煮して用いた。生の原料の方が蒸煮物に比して加水分解の度合いが進んだ。乳酸菌混合培養物を加えることによって蒸煮原料の方に生の原料に比べて化学成分に変化があった。概して、乳酸菌を加えるとアスパラギン酸, グルタミン酸, グリシン, アラニン, バリン及びピリジンの量が増加した。官能的にも乳酸菌を加えた方が、風味, 色に好影響を与えたが、香りについては変化がなかった。

キーワード: 魚醬, バカサン, 乳酸菌