

Short Communication

Microbiological, histamine and 3-MCPD contents of Malaysian unprocessed 'budu'

*Rosma, A., Afiza, T. S., Wan Nadiah, W. A., Liong, M. T. and Gulam, R. R. A.

Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia,
11800 Minden, Penang, Malaysia

Abstract: Unprocessed 'budu' is a mixture of anchovies and salt that has been fermented for a period of time, and has not been heat-treated nor formulated with additional ingredients. This study analyzed Malaysian unprocessed 'budu' from 12 producers for microbiological, salt, protein, histamine and 3-MCPD contents. The results demonstrated that Malaysian unprocessed 'budu' were free from pathogenic *Coliform*, *E. coli*, *V. parahaemolyticus* and *V. cholerae* contaminations. Carcinogenic 3-MCPD was below detection level of 2 ppb for all 12 samples tested. However, 58% of the unprocessed 'budu' had histamine content greater than the hazardous levels of 50 mg/100 g sample.

Keywords: fermented fish, budu, fish sauce, histamine, 3-MCPD

Introduction

'Budu', a dark-brown-to-black condiment, is an indigenous fermented food produced and consumed mainly by people in the East Coast states of West Malaysia, namely Kelantan and Terengganu; and the Southern Provinces of Thailand. 'Budu' is a type of fish sauce formulated from fermented fish and salt mixture (unprocessed 'budu') added with tamarind, palm sugar, monosodium glutamate and flavoring compounds. The product is then filtered, heat-treated and bottled. Unprocessed 'budu' is obtained by fermenting *Stolephorus* spp., *Sardinella* spp. or *Decapterus macrosoma*, in the presence of high salt concentration in covered earthen containers under the sun for 6 to 12 months (Beddows, 1985, Klamkiao *et al.*, 2006). In other countries, fish sauce is known as 'patis' (Philippines), 'ngapi' (Burma), 'nam-pla' (Thailand), 'nuoc-mam' (Cambodia and Vietnam), 'ishiru' or 'shottsuru' (Japan) (Itoh *et al.*, 1993) and in Indonesia, it is called 'bakasang' (Ijong and Ohta, 1996). Lopetcharat *et al.* (2001) had extensively reviewed the fish sauce products and manufacturing.

There are 12 registered 'budu' producers in Kelantan and Terengganu, Malaysia (DOF, 2006), but there are also many unregistered backyard scale

producers scattered in both of these states. Besides providing cheap protein in the diet for those residing in rural areas (Brillantes, 1999), 'budu' is also used as a seasoning. Unprocessed 'budu' is presently prepared by traditional and usually unhygienic methods, paving the way for possible microbial contamination and evolution of food toxicants that can affect quality and pose public health hazards (Anihouvi *et al.*, 2006). Thus, the high quality, pathogen-free and non-hazardous unprocessed 'budu' is important to attain safe product and achieve an international standard.

Histamine is formed from the action of microbial histidine decarboxylase on histidine in fish by spoilage halophiles such as *Photobacterium phosphoreum*, *Photobacterium histaminum* sp. nov., *Enterobacteriaceae*, *Proteus morganii*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae* and *Hafnia alvei* (Tsai *et al.*, 2006). Although the ingestion of food containing small amounts of histamine has little effect on humans, histamine in an amount of more than 50 mg/100 g can bring about scombroid fish poisoning (Taylor, 1986). A concentration of 100 mg histamine/100 g product could cause clinical illness with symptoms such as flushing of facial and neck areas, feeling of intense heat, nausea, vomiting, diarrhea, hypotension,

*Corresponding author.

Email: : rosmah@usm.my

Tel: +604-6532118; Fax: +604-6573678

headache, palpitation, itching, swelling of the lips and burning sensation in the throat (FDA, 2001). The regulatory limits for histamine concentration in fish products vary in different countries. In the United States of America, 20 mg/100 g denotes a mishandling of fish, while 50 mg/100 g levels indicate a 'hazard action level'. In Germany and Sweden, 20 mg/100 g histamine in fish results in the rejection of a consignment (Infofish, 1987). The presence of histamine in 'budu' acts as an indicator of product deterioration and is a potential public health hazard.

3-MCPD (3-monochloropropane-1,2-diol) has been shown to be a carcinogen. It is a chemical contaminant resulting from the reaction of chloride with lipids in food. 3-MCPD is not produced naturally during the fermentation of 'budu', but is a well-known contaminant of acid-hydrolyzed vegetable protein (acid-HVP) (JFSSG, 1999). The inclusion of acid-HVP as a savory ingredient to sauces or the use of acid hydrolysis in the production of sauces possibly leads to the contamination of the sauces with 3-MCPD (Wong *et al.*, 2006). Food Regulations had recommended that 3-MCPD level in foods is below 10 ppb (FAC, 1999).

There has yet to be any published data to our knowledge that study on the safety standard of unprocessed 'budu'. However, with the increasing safety consciousness of consumers today, we aim to establish quantitative standard for 'budu' for consumption which is pathogen-free and hygienic indicated by low histamine content and almost free-3MCPD.

Materials and Methods

Unprocessed 'budu' samples

Unprocessed 'budu' samples were collected from the point indicated in the flow process of 'budu' production (Figure 1). Twelve different samples were from 12 producers in Kelantan, Malaysia representing 100% of registered budu producers in Malaysia (DOF, 2006). Duplicate 1 L samples were collected from each producer in capped glass bottles. All the samples were collected on a single day and transported back to the laboratory within 6 hours at ambient temperature. The samples were kept frozen (-18°C) until needed for analysis to stop further fermentation. Samples were thawed for 2 h at ambient temperature of 25°C and analyzed for salt, crude protein, histamine, 3-MCPD and microbial loads. Sample collections from the same producers were repeated for 2 more-times while maintaining the same condition of sample transportation and storage prior to analyses.

Analyses

Salt and histamine

Salt, protein and histamine contents were determined using AOAC Official Methods (AOAC, 2000). The salt content was measured by the Volhard method (AOAC, 935.43). Protein was determined by multiplying N-content obtained according to Kjeldahl method (AOAC, 954.01) with 6.25. The presence of histamine was determined by the spectrophotometric method (AOAC, 957.07) using a UV-Vis spectrophotometer (Thermo, Spectronic Genesys 10UV, U.S.A) and the concentration was calculated from the histamine (Fluka, Switzerland) standard curve (0-3.0 µg/mL).

3-Monochloropropane-1,2-diol (3-MCPD)

Samples were sent to the DOPING Center of Universiti Sains Malaysia for 3-MCPD determination. The presence of 3-MCPD was determined according to Brereton *et al.* (2001) using a gas chromatography-mass spectrometry system with a detection limit of 2 ppb (Hewlett Packard GC-MSD HP6890, U.S.A). Deuterated 3-MCPD (Fluka, Switzerland) was used as an internal standard.

Microbial analysis

The detection and enumeration of *E. coli*, *Coliform*, *V. parahaemolyticus* and *V. cholerae* were carried out according to the Bacteriological Analytical Manual (FDA, 2000). All the agar were purchased from Merck (Darmstadt, Germany)

Statistical Analyses

Data analysis was carried out with SPSS Inc. software (version 15.0) (SPSS Inc., Chicago, U.S.A). One-way analysis of variance was used to determine the significance difference between means ($n=3$), with a significant level of $\alpha = 0.05$. Tukey's test was used to perform multiple comparisons between means. All data presented are mean values of triplicates, obtained from 3 batches of samples, unless stated otherwise.

Results and Discussion

Table 1 shows the salt, protein, histamine and 3-MCPD data of the 12 unprocessed 'budu' samples. The samples collected were of different ages ranging from 3 months to 12 months but mature enough to be harvested for further processing. In current practice, the maturity age of unprocessed 'budu' is determined solely by the producers' judgment and practices. These unprocessed 'budu' are subsequently sold to



Figure 1. Flow chart for production of Malaysian 'budu'. * indicates sample used in this study

Table 1. The salt, protein, histamine and 3-MCPD data of the unprocessed 'budu' samples

Sample	Fermentation period (month)	Salt * (% w/v)	Protein* (% w/v)	Histamine* (mg/100 g)	3-MCPD (ppb)
1	6	24.85 ± 0.39 ^{bc}	13.25 ± 0.42 ^{bc}	71.85 ± 0.49 ^h	<2
2	5	25.59 ± 0.11 ^c	13.03 ± 0.53 ^b	81.50 ± 0.14 ⁱ	<2
3	8	24.63 ± 0.08 ^b	9.92 ± 0.04 ^a	106.40 ± 0.42 ^k	<2
4	8	25.70 ± 0.06 ^c	12.95 ± 0.49 ^b	41.90 ± 0.42 ^d	<2
5	12	25.61 ± 0.02 ^c	24.88 ± 0.32 ^h	23.87 ± 0.31 ^b	<2
6	3	25.49 ± 0.01 ^{bc}	17.03 ± 0.01 ^d	26.86 ± 0.23 ^e	<2
7	3	25.69 ± 0.03 ^c	17.58 ± 0.04 ^e	22.21 ± 0.16 ^a	<2
8	12	25.66 ± 0.04 ^c	21.36 ± 0.07 ^f	23.65 ± 0.39 ^b	<2
9	8	21.50 ± 0.71 ^a	14.35 ± 0.21 ^c	57.15 ± 0.21 ^e	<2
10	8	25.59 ± 0.15 ^c	22.70 ± 0.28 ^g	102.25 ± 0.07 ^j	<2
11	5	25.62 ± 0.00 ^c	18.20 ± 0.00 ^{ef}	65.00 ± 0.14 ^g	<2
12	8	25.66 ± 0.00 ^c	13.80 ± 0.00 ^{bc}	63.40 ± 0.71 ^f	<2

* Results are expressed as means ± S.D; values are means of duplicates from three independent samples. Means in the same column followed by different lowercase letters are significantly different (p<0.05).

petty traders or further processed to bottled 'budu'. Budu is considered a unique food, produced in limited locations and consumed by a limited population mainly in the east coast of Malaysia. In this regard, a number of 12 producers was a practical decision (Holden *et al.*, 2002).

The protein concentrations of sample were between 9.92% and 24.88%. There was no correlation between sample age and protein level thus; a more standardized quality determination such as total crude protein content is suggested here. According to Malaysian Food Act 1983 and Food Regulations 1985 (Anon, 2007), fish sauce or 'budu' shall contain not less than 5% protein. However, a good grade fish sauce shall contain not less than 12.5% protein (Saisithi, 1994). With this concentration as standard marking level, 91.6% of the samples were of good quality unprocessed 'budu' and producers should refer to this protein level before harvesting their 'budu'.

The salt content of unprocessed 'budu' ranged between 21.50% and 25.70% (w/v) with a mean value of 25.10±0.10% (w/v). As a result of this high salt content, all the samples studied were free from *E. coli*, *Coliform*, *V. parahaemolyticus* and *V. cholerae* contamination. Due to a highly ionic environment in 'budu', various microbial endogenous enzymes are easily denatured and inactivated. Thus, the metabolic activities in pathogenic cells could not function or were completely halted. The high salt concentration also affects the type of micro flora and retards or

kills the pathogenic microbes during fermentation, which, in turn, affects the quality of the 'budu' (Lopetcharat *et al.*, 2001). The Malaysian Food Act 1983 and Regulations 1985 (Anon, 2007) have stated that salt in 'budu' must not be lower than 15%. A lower salt content may allow the growth of spoilage microorganisms (*Aspergillus chevalieri*, *Escherichia sp.*, *Serratia sp.*, *Pseudomonas sp.* and *Clostridium sp.*) and maggots that produce putrid odor and lower the fish sauce quality (Sidaway and Balasingam, 1971; Lee *et al.*, 1993; Lopetcharat *et al.*, 2001). On the other hand, a high salt concentration in 'budu' will act as natural preservation agent from pathogenic as well as spoilage microorganism.

Determination of 3-MCPD on all 12 unprocessed 'budu' samples showed values of less 2 ppb. The unprocessed 'budu' samples in this study had been produced naturally whereby no acid hydrolysis was performed during fermentation and no addition of acid-HVP was made to the 'budu'. There has been no scientific report on the occurrence of 3-MCPD in fish sauce or 'budu' but a Consumer Association had reported that a fish sauce available in the market contained as much as 213 ppb 3-MCPD (Mohamed Idris, 2007), more than twenty times the permitted level of 10 ppb. Moreover, out of 421 samples of soy and oyster sauce determined for 3-MCPD level, 45 samples (10.7%) had 3-MCPD levels of over 20 ppb (Wong *et al.*, 2006). Therefore, 3-MCPD level should be determined in all protein-rich sauces namely soy, oyster and fish sauces.

The results showed a significant ($p < 0.05$) variation of histamine levels in the samples, ranging from 22.21 mg/100 g to 106.40 mg/100 g sample. A total of 58% of all unprocessed 'budu' samples analyzed had more than 50 mg/100 g histamine, regarded as "hazard action level". A high level of histamine indicates poor handling and processing of fish products, which results in a high degree of contamination. Histamine was formed both in the raw material and during fermentation (Brillantes *et al.*, 2006). Delay in mixing the fish with salt will result in higher histamine content (Lopetcharat *et al.*, 2001). Histamine formation however, can be easily controlled by implementing hygienic practices. Immediate icing of the fish after catching and keeping them in chilled condition until delivery to the processing plant could have restricted or minimized the growth of histamine-forming bacteria. Several authors (Baladarati *et al.*, 1980) have reported that there was little or no formation of histamine when temperature of fish was below 10 °C. The immediate mixing with salt upon arrival at factory while the fish was still at low temperatures may have helped to stop bacterial processes (Voskresensky, 1965). At present, 'budu' producers mix the fish with salt on board collection vessels or upon arrival at factory without chilled-storage of the fish.

Conclusion

Unprocessed 'budu' samples of Kelantan, Malaysia contained high protein concentration up to 24.88%. High salt content had prevented unprocessed 'budu' of from *E. coli*, *Coliform*, *V. parahaemolyticus* and *V. cholerae* contaminations. Samples were almost free from carcinogenic 3-MCPD but 58% samples required hazard control action as the histamine contents were more than 50 mg/100 g.

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References

- Anihouvi, V.B., Ayernor, G.S., Hounhouigan, J.D. and Dawson, E.S. 2006. Quality characteristics of lanhouin: traditionally processed fermented fish product in the republic of Benin, *African Journal of Food, Agriculture, Nutrition and Development* 6 (1).
- Anon. 2007. Food Act and Regulations, Act 281 and all amendments up to August 2007, MDC Publisher Sdn. Bhd, Kuala Lumpur, Malaysia.
- AOAC. 2000. Official Methods of Analysis, 17th edition, AOAC International, Gaithersburg, M.D.
- Baladarati, G., Fornari, M.B., Spotti, E. and Incerti, I. 1980. Effect of Temperature on Histamine Formation in Fish Rich in Histidine. *Con Industry* 55: 114-122.
- Beddows, C.G. 1985. Fermented fish and fish products in *Wood, B.J.B. (Ed.), Microbiology of fermented foods*. Elsevier Applied Science Publishers, Vol 2, 1-39, London, U.K.
- Brereton, P., Kelly, J., Crews, C., Honour, S., Wood, R. and Davies, A. 2001. Determination of 3-Chloro-1,2-Propanediol in foods and food ingredients by gas chromatography with mass spectrometric detection: collaborative study. *Journal of AOAC International* xx: 455-465.
- Brillantes, S. 1999. Histamine in fish sauce: health and safety consideration. *INFOFISH International* 4: 51-56.
- Brillantes, S., Paknoi, S. and Totakien, A. 2002. Histamine Formation in Fish Sauce Production. *Journal of Food Science*, 67 (6): 2090-2094.
- Department of Fisheries, Malaysia. 2006. Direktori industri perikanan syarikat/kilang/pembekal dan pemproses hasil-hasil perikanan. Accessed on 23 March 2008 from <http://www.dof.gov.my/v>.
- Food and Drug Administration. 2000. Bacteriological Analytical Manual, 7th ed., AOAC International, Washington, D.C.
- Food and Drug Administration. 2001. Processing parameters needed to control pathogens in cold smoked fish potential hazards in cold-smoked fish: Biogenic amines. U.S. FDA, Center for food safety and applied nutrition.

- Food Advisory Committee. 1999. Recent developments on 3-MCPD in food and food ingredients. *FAC Press Release* 5/99.
- Holden, J.M., Bhagwat, S.A. and Patterson, K.Y. 2002. Development of a multi-nutrient data quality evaluation system. *Journal of Food Composition and Analysis* 15: 339-348.
- Ijong, F. G. and Ohta, Y. 1996. Physicochemical and Microbiological Changes Associated with Bakasang Processing – A Traditional Indonesian Fermented Fish Sauce. *Journal of the Science of Food and Agriculture* 71: 69-74.
- Infofish. 1987. Infofish Marketing Digest No. 2/87, pp 38-41.
- Itoh, H., Tachi, H. and Kikuchi, S. 1993. In *Fish Fermentation Technology* (Lee, C.H., Steinkrauss, K.H. and Alan Reilly, P.J., eds.). United Nations University Press, Tokyo, 177.
- Joint MAFF/DH Food Safety and Standard Group, JFSSG. 1999. Survey of 3-monochloropropane-1,2-diol (3-MCPD) in soy sauce and similar products. *Food Surveillance Information Sheet* No. 187.
- Joint MAFF/DH Food Safety and Standards Group, JFSSG. 1999. Survey of 3-Monochloropropane-1, 2-diol (3-MCPD) in acid hydrolyzed vegetable protein. *Food surveillance information sheet* No. 181
- Joint MAFF/DH Food Safety and Standards Group, JFSSG. 2000. Survey of 3-Monochloropropane-1,2-diol (3-MCPD) in foods.
- Klomklao, S., Benjakul, S., Visessanguan, W., Kishimura, H. and Simpson, B.K. 2006. Effects of the addition of spleen of skipjack tuna (*Katsuwonus pelamis*) on the liquefaction and characteristics of fish sauce made from sardine (*Sardinella gibbosa*). *Food Chemistry* 98 (3): 440-452.
- Lopetcharat, K., Yeung, J. C., Park, J. W. and Daeschel, M. A. 2001. Fish sauce products and manufacturing: A review. *Food Reviews International* 17(1): 65–88.
- Mohamed Idris. 2007. Test all sauces for cancer-causing agent. *New Straits Times* (29 January 2007).
- Saisithi, P. 1994. Traditional fermented fish: Fish sauce production. In *Fisheries Processing Biotechnology Application* (A.M. Martin, ed.) pp 111-131. Chapman and Hall, London
- Sidaway, E.P. and Balasingam, M. 1971. Fish processing industry in west Malaysia Technical study of Malaysian Food Technology Research and Development Centre, Kuala Lumpur.
- Taylor, S.L. 1986. Histamine food poisoning: toxicology and clinical aspects. *CRC Critical Review Toxicological* 17: 91-128.
- Tsai, Y.H., Lin, C.Y., Chien, L.T., Lee, T.M., Wei, C.I. and Hwang, D.F. 2006. Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. *Food Chemistry* 98: 64-70.
- Voskresensky, N.A. 1965. Salting of Herring. In: Borgstrom, G. (ed) *Fish as Food III*. Academic Press, New York. Pp 107-131.
- Wong, K.O., Cheong, Y.H. and Seah, H.L. 2006. 3-Monochloropropane-1, 2-diol (3-MCPD) in soy and oyster sauce: Occurrence and dietary intake assessment. *Food Control* 17: 408-418.