Microbial Diversity and Proximate Composition of Tapai, A Sabah's Fermented Beverage

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ABSTRACT

Tapai is a well-known indigenous fermented alcoholic beverage among Kadazan-Dusun-Murut (KDM) ethnics during festive occasions and gatherings in East Malaysia. Unfortunately, very little research has been done on this beverage. The objective of this study was to identify functional microfloras involved in the production of tapai. Samples from local producers were obtained for microbiological and proximate analysis. The fermentation process was predominated by yeasts and lactic acid bacteria (LAB) with initial numbers (CFU/g) of 10^5 and 10^6, respectively, which gradually increased during the first 2 weeks fermentation but decreased thereafter. The yeasts were identified as *Sacchromyces cerevisiae*, *Candida krusei*, *C. pelliculosa*, *C. guillermondii*, *C. magnoliae* and *Rhodotorula glutinis*, whereas the LAB were *Lactobacillus brevis*, *L. plantarum*, *L. collinoides* and *Pediococcus* sp. Moulds and Enterobacteriaceae were only present during the first 2 days of fermentation. Acetic acid bacteria were not detected throughout the entire process. The pH of tapai declined slowly from 6.6 to 3.4 in 14 days, and then showed an increment to 4.0. On the other hand, titratable acidity (as % lactic acid) increased from 0.06 to 0.86 in 10 days, and then decreased to 0.82 at the end of the fermentation process. Alcohol was produced and the content can reach as high as 12.3% after 3 weeks fermentation. Proximate composition analysis showed that the moisture content in the end product was 61.8±6.1% whereas ash, protein, fat and crude fiber (of dried samples) were 0.50±0.1%, 8.7±0.1%, 0.29±0.01% and 0.56±0.03%, respectively.

Keywords: Microflora, Indigenous, Fermented beverage, Malaysia

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INTRODUCTION

Indigenous fermented foods have become a new interest and consequently provided new subjects for intellectual creation these few years. While traditionally produced food products may be of health concern to non traditional consumers due to the therapeutic properties of fermented food reported, advanced scientific knowledge on food fermentation and its microbial agent has increasingly revealed many beneficial effects which lead to new applications other than food preservation, safety and sensory appreciation. Many studies have been done on indigenous fermented foods from around the world (Tamang and Thapa, 2004; Sefa-Dedeh *et al.*, 2004; Mugula *et al.*, 2003; Muyanja *et al.*, 2002; Leisner *et al.*, 2001; Omafuvbe *et al.*, 2000; Wacher *et al.* 2000) and information on microbiological, biochemical and nutritional changes during fermentation is well documented. Moreover, products like Indonesian tempe and Oriental soy sauce are well known indigenous fermented foods that have industrialized and marketed globally years ago (Wood, 1994).

Tapai is a well-known indigenous fermented alcoholic beverage among the Kadazan-Dusun-Murut (KDM) ethnic group of Sabah during festive occasions and gatherings. Unlike tapai in other Southeast Asia countries like *tape ketan* (Indonesia), *tapai* (Peninsular Malaysia, Singapore and Brunei), *basi* (Philipines) and *Khao-mak* (Thailand)

(Campbell-Platt, 2000) which are prepared as a food, Sabah's tapai is prepared as an alcoholic beverage. It has an alcoholic aroma with combination of sweet-sour-bitter taste and sometimes sparkling feel. Tapai is made from glutinous rice with *sasad* as starter culture although rice, cassava, pineapples and maize can be used as substitute for glutinous rice in some part of Sabah. During preparation of tapai, glutinous rice is cleaned and washed before cooked. Cooked glutinous rice is then spread for cooling in an open surface ($\approx 30^{o}$C). Starter culture cakes are grinded into powder and approximately 1.0-1.5% (by weight) is sprinkled on the cooled glutinous rice followed by mixing thoroughly using a wooden scoop. The mixture is transferred into *tajau* (earthen jar) and left open for 1 day before the lid of *tajau* is sealed. Good quality and matured tapai undergoes 3 weeks fermentation. There are few ways to consume tapai. The most popular way is by drinking *hing* or *lihing* (wine must). Tapai can also be consumed as *kinomulok* (fermented glutinous rice after the wine must have been separated) and *linutau* (water extract from *kinomulok*). *Siopon* or *Sisopon* is a way of consumption where a thin bamboo straw is inserted into water added *kinomol* (fermented glutinous rice in tajau) for sipping tapai extracts. Other than that, *Montoku* and *talak* (distilled wine must) are famous alcoholics beverage among KDM.

Unfortunately at present, there is no adequate information on the spectrum of microorganisms associated with tapai fermentation in Sabah as

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information on indigenous fermented food is extremely rare although this knowledge is essential for the development of this product with improved quality for commercial production and marketing. Thus, the objective of this research was to identify functional microfloras involved in the microbiological, biochemical and nutritional changes that took place during tapai production.

MATERIALS AND METHODS

Enumeration, isolation and identification

Freshly inoculated glutinous rice in *tajau* were purchased from a local tapai producer in Kampung Karanaan, Tambunan, Sabah and transported to the Food Microbiology Laboratory of the School of Food Science and Nutrition at University Malaysia Sabah for immediate microbial analysis. Twenty five grams of samples were homogenized with 225 ml of Quarter Strength Ringer's Solution (Merck, Germany) in a Bag Mixer (Interscience, France) for 30 min and serially diluted in the same diluent in duplicate. During tapai fermentation, the same amount of sample was collected from the *tajau* for microbial analysis every 2 successive days for 20 days. Yeast and mould counts were determined using Dichloran Rose Bengal Choramphenicol (DRBC) Agar (Merck, Germany) (Ardhana & Fleet, 2003) and incubated at 25°C for 3 days. Lactic acid bacteria (LAB) were enumerated in M17 Agar (Merck, Germany) aerobically and on de Man Rogosa and Sharpe (MRS) Agar (Merck, Germany) anaerobically (Oxoid Carbon Dioxide System BR 39, England) at 30°C for 2 days (Mugula *et al*., 2003). Acetic acid bacteria were enumerated using Glucose Yeast Extract Calcium Carbonate Agar (glucose 5%, yeast extract 1%, calcium carbonate 3% and agar 2%) and incubated at 30°C for 6 days (Du Toit and Lambrechts, 2002). Enterobacteriaceae and aerobic mesophilic count was determined using Violet Red Bile Glucose Agar and Plate Count Agar (Merck, Germany) (Thapa and Tamang, 2004) at 37°C for 2 days, respectively.

About 10-15 colonies were selected randomly from the plates. Purity of the isolates was checked by streaking again on fresh agar plates of the isolation medium. Yeasts and LAB isolates were stored at Potato Dextrose Agar (Merck, Germany) and MRS Agar slants, respectively. Yeasts were identified according to their cell morphology, physiological, carbon sources assimilation and fermentation patterns described by Kurtzman and Fell (1998) supplemented with API 20C AUX test strips (BioMérieux, France). LAB were identified according to cell and colony morphology, Gram and catalase reactions, gas production from glucose and fermentation pattern in API 50 CH and API 50 CHL Medium (BioMérieux, France).

Analysis of proximate composition

Ten grams sample of fermented glutinous rice was blended with 20 ml of distilled water in a homogenizer for 30 seconds and the pH of the slurry was determined by digital pH meter calibrated with standard buffer solutions (Merck, Germany). Titratable acidity (expressed as percent lactic acid) of tapai was determined by titrating the filtrates, a well blended 10 g sample in 90 ml distilled water with 0.1 N sodium hydroxide to end point using phenolphthalein as indicator. Alcohol content was determined by blending 10 g sample with 90 ml distilled water in the homogenizer described above using distillation method.

The moisture content was determined by drying samples in oven for overnight at 70°C to constant weight. Ash content was determined by heating the dried end product in furnace at 550°C overnight till the difference between two successive weighing was not more than 0.1%. Protein and fat contents were determined using Kjeldahl distillation method and Soxhlet method on dried sample respectively. For crude fiber content, 2 g sample was boiled for 30 minutes in 12.5% sulfuric acid, filtered and washed with hot distilled water untill no longer acidic before boiling in 12.5% sodium hydroxide and the same procedure repeated except it was filtered through a Gooch crucible and washed till no longer alkaline. Crucible with its content was dried at 100°C, transferred into the incinerator oven and heated at 550°C overnight till the difference between two successive weighing was not more than 0.1%. Crude fiber content was estimated by calculating percentage of weight loss in the sample. Carbohydrate content of sample was estimated by difference: 100-(%moisture + %protein + %fat + %ash + %fiber) (Nitisewojo, 1995). The determinations were done in triplicates and the mean values recorded.

RESULTS AND DISCUSSION

Microorganisms

Tapai fermentation was predominated by yeast and LAB as they were present from day 0 to day 20. Yeasts and moulds grew to 8.0 log CFU/g on the 4th day of fermentation from 5.1 log CFU/g but the population of yeast population declined gradually thereafter until 6.1 log CFU/g. However, moulds were undetected on the 5th day onwards (Figure. 1). The population of LAB with 6.1 log CFU/g initially became 1.0 log CFU/g greater on the 4th day and decreased to 5.1 log CFU/g after 20 days. Acetic acid bacteria was not detected in all tapai samples. Enterobacteriaceae numbers declined from 4.6 log CFU/g till an undetectable number within 4 days whereas aerobic mesophilies increased from 6.6 log CFU/g to 8.4 log CFU/g in 2 days and decreased to 5.3 log CFU/g at the end fermentation process.

Saccharomyces cerevisiae, *Candida krusei*, *C. pelliculosa*, *C. glabrata*, *C. utilis, C. sphaerica, C. magnoliae, Rhodotorula mucilaginosa, R. glutinis* and *Cryptococcus laurentii* were identified from among the yeasts isolated (Table 1). *S. cerevisiae* occurred in the highest number in tapai and was present at all stages of the fermentation. It has been isolated frequently from acidic fermentation of plant materials such as sourdough (Gobetti *et al*., 1994) and *ogi* (Nago *et al*., 1998). Thus, it
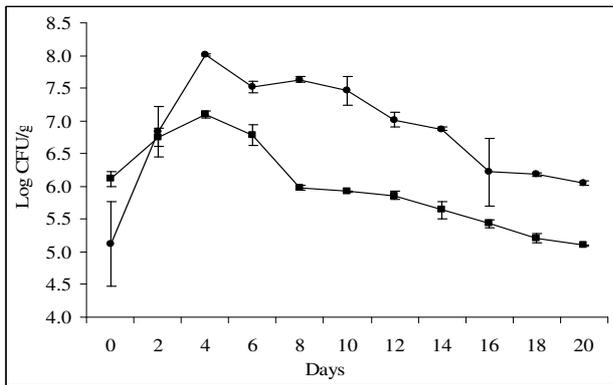
Figure 1: Growth of yeasts and moulds (●) and lactic acid bacteria (■) during fermentation of tapai for 20 days.

has been suggested as playing a primary role in alcohol fermentation (Tsuyoshi *et al.*, 2005). *C. krusei*, *C. pelliculosa* and *C. glabrata* were previously isolated from *fufu* (fermented cassava roots) (Oyewole, 2001), *togwa* (fermented sorghum, millet or finger millet) (Mugula *et al.*, 2003) and *kodo ko jaanr* (alcoholic fermented finger millet beverage) (Thapa and Tamang, 2004), respectively and *C. krusei* has been associated with flavor and aroma of the end product of *fufu* (Oyewole, 2001). *Saccharomyces* and *Candida* species were reported capable of proliferating at low pH in porridge (Akinrele, 1970; Nout *et al.*, 1989). *C. utilis* is known to produce a significant amount of ethyl acetate from biomass such as diluted sugar and ethanol. Ethyl acetate is recognised as an important flavour compound in wine and other grape-derived alcoholic beverages. Such non-*Saccharomyces* yeast might contribute to flavour and aroma in the

alcoholic beverages. *C. sphaerica* which was the anamorph state of *K. lactis* were isolated. *K. lactis* has found to be present in fermented dairy products such as cheese (Fadda *et al.*, 2001) and is currently used for industrial applications for years as a source of β-galactosidase (Bolotin-Fukuhara *et al.*, 2000). Unsurprisingly *C. magnoliae* was food spoilage yeast and it present may be due to contamination of the starter cake or utensil used during preparation as well as *R. mucilaginosa* and *R. glutinis* which were previously referred to as the species of common air contaminants or natural contaminants in cheese before they were stored (Viljoen and Greyling, 1995). Isolates not identified are due to inadequacy of the identification system used in the study and further investigation should be carried out. Though mould was only present in the early stage of fermentation, it was suspected to play a role in the degradation of glutinous rice into simpler substrate molecule to be utilized by yeast and LAB.

Eighty four percent strains of LAB were non-sporeforming rods (Table 1). They were tentatively identified as *Lactobacillus plantarum*, *L. brevis* and *L. paracasei* subsp. *paracasei* but only *L. plantarum* and *L. brevis* showed predomination in the tapai fermentation process and were isolated from at stages of fermentation. *L. plantarum* have been isolated from several indigenous fermented foods including *togwa* (Mugula *et al.*, 2003), *tempoyak* (fermented durian fruit pulp) (Leisner *et* al., 2001) and *kule nato* (fermented milk) (Mathara, 2004). It has been recognized as the dominant organism at the end of several natural lactic acid fermentations (Brauman *et al.*, 1996; Kunene *et al.*, 2000), probably due to its acid tolerance (Fleming and McFeters, 1981) and superior ability to utilize the substrates (Oyewole and Odunfa, 1990). *L. brevis* often occur in fermenting plant material (Corsetti *et al.*, 2001) and have been isolated from

Table 1: Numbers of identified of yeast and lactic acid bacteria isolated from tapai fermentation

Species identified	No. of isolates	Days of isolation										
		0	2	4	6	8	10	12	14	16	18	20
Yeast												
Sacchromyces cerevisiae	50	0	1	4	4	2	3	5	7	5	10	8
Candida krusei	5	4	0	0	0	1	0	0	0	0	0	0
Candida pelliculosa	18	0	5	3	1	2	3	1	2	0	0	1
Candida glabrata	1	0	1	0	0	0	0	0	0	0	0	0
Candida utilis	15	6	1	1	3	0	1	3	0	0	0	0
Candida sphaerica	2	0	0	0	0	0	2	0	0	0	0	0
Candida. magnoliae	2	0	0	1	0	0	0	0	0	1	0	0
Rhodotorula mucilaginosa	1	0	0	1	0	0	0	0	0	0	0	0
Rhodotorula glutinis	4	0	0	0	0	2	0	1	1	0	0	0
Cryptococcus laurentii	1	0	0	0	0	1	0	0	0	0	0	0
Unidentified	3	0	0	0	0	0	1	0	0	1	0	1
Lactic acid bacteria												
Lactobacillus plantarum	76	1	2	9	10	6	7	7	10	9	7	8
Lactobacillus brevis	32	0	0	1	5	3	1	6	2	3	7	4
Lactobacillus paracasei subsp. *paracasei*	4	2	0	0	0	0	2	0	0	0	0	0
Pediococcus pentosaceus	14	10	4	0	0	0	0	0	0	0	0	0
Lactococcus lactis subsp *lactis*	6	2	4	0	0	0	0	0	0	0	0	0

fermented foods like *kenkey* (fermented maize dough) (Halm *et al.*, 1993), *mawe* (fermented maize dough) (Hounhouigan *et al.*, 1993), and agbelima (fermented cassava dough) (Kofi *et al.*, 1996). *L. paracasei* spp. *paracasei* has been reported to occur in boiled rice used to prepare *som fak*, a Thai fermented fish (Paludan-Müller *et al.*, 1999). Sixteen percent of the LAB isolates were cocci and identified as *Pediococcus pentosaceus* and *Lactococcus lactis* subsp. *lactis.* Contrary to the report which showed domination of *P. pentosaceus* in the latter stage of corn dough fermentation (Nche *et al.*, 1994), *P. pentosaceus* was only present in the early stages of fermentation, thus it seems that tapai fermentation is initiated by *P. pentosaceus* but finally dominated by *L. plantarum* as in the fermentation of *mesu* (fermented bamboo shoot) (Tamang and Sarkar, 1996). Similar to *P. pentosaceus*, *Lactococcus lactis* subsp. *lactis* were also only present in the early stages of fermentation correspond to the report by Muyanja *et al.* (2002) on *bushera* due to its inability to grow at lower pH as the tapai fermentation proceed.

A co-metabolism between yeasts and lactic acid bacteria has been suggested, whereby the bacteria provide the acid environment, which selects for the growth of yeasts and, the yeasts provide vitamins and other growth factors to the bacteria (Gobbetti *et al.*, 1994; Steinkraus, 1996). Yeasts have also been reported to make a useful contribution to the improvement of flavour and acceptability of fermented cereal gruels (Banigo *et al.*, 1974; Odunfa and Adeyele, 1985). Tapai is a safe fermented product to consume as no enterobacteriaceae were found at the end of the fermentation process, probably due to its low pH, elevated titratable acidity and high alcohol content. Their disappearance may also be due to the presence of other antimicrobial compounds. The absence of acetic acid bacteria in tapai could be due to the sealed earthen jar which provide unsuitable growth condition as they as strictly aerobic bacteria.

Proximate composition

The pH of inoculated glutinous rice was 6.6 initially and it decreased rapidly and stabled steadily at 3.4 on the 15th day. It increased to 4.0 at the end of fermentation. This may be due to the increased of titratable acidity (expressed as percent of lactic acid) from 0.06 to 0.86% in 10 days, and decreased to 0.82% in the end of tapai fermentation (Figure 2). The correlation between acidity and pH is believed to be associated with both yeasts and LAB as LAB were well known for production of acids especially lactic whereas some yeasts were previously reported to produce acid in alcohol fermentation to make a positive contribution to the products' flavour (Fleet, 2003). At the same time, low pH and high acidity also eliminated enteropathogen, coliforms and spoilage organisms in this product.

Meanwhile, its alcohol content increased day by day and was as high as 12.3% (v/v) after 20 days fermentation (Figure 3). Yeasts may produce alcohol; however, *Lactobacillus* species have also been reported to produce
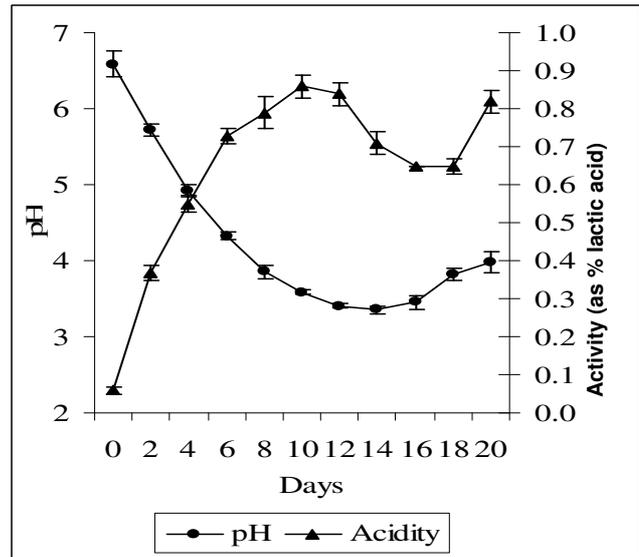

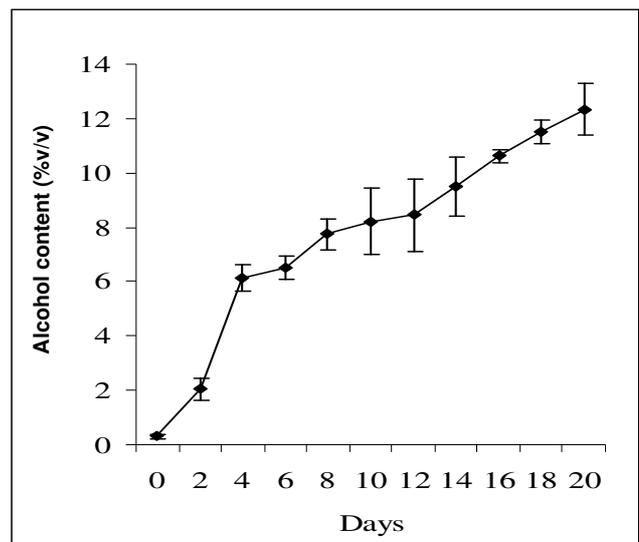**Figure 2**: Changes of pH and acidity during tapai fermentation

Figure 3: Alcohol content in tapai during fermentation.

ethanol. Alcohols produced play a role in helping to extract flavour components from fermenting substrates. Yeasts and LAB also produce other volatile compounds such as malty flavored 2-methyl-propanal. The flavors contributed by yeasts and LAB yield unique fermented products appreciated by consumers which were much different from the unfermented substrate.

The moisture and ash contents in the end product of tapai fermentation were 61.8±6.1% and 0.2±0.041% respectively. Crude protein, crude fat and crude fiber (of dried samples) were 3.3±0.04%, 0.1±0.004% and

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0.2±0.01%, respectively. The estimated carbohydrate content in tapai was 34.3±0.04%. Because of its high calorie, tapai were not only consumed in festive occasions and gatherings, but also by ailing persons and post natal women to regain strength. Tapai were also consumed as a family staple food by some ethnic groups in rural areas.

The result of this study indicated that tapai contains a variety of yeasts and LAB. Various flavor compounds were believed present in tapai making it a favorite alcoholic beverage by Sabah peoples. Thus, controlled fermentation should be done to assess contribution of yeasts and LAB on flavor and aroma of this traditional alcoholic fermented beverage. There is a need for investigation into the selection of the most suitable strain for better controlled tapai fermentation. Starter cultures development is important for the potential small-scale commercial production of tapai and for improvement of its acceptability, microbiological stability and hygiene safety. Detail avaibility of nutrient values which included minerals and vitamins in tapai should be carried out. Strains isolated from tapai could be screened for their properties of exo- and endocellular enzymatic activities as well as potential probiotic and nutraceutical properties for application in improvement of human health.

REFERENCES

Akinrele, I.A. (1970). Fermentation studies on maize during the preparation of a traditional African starch-cake food. *Journal of Science and Food Agricutural.* **21: 619– 625**.

Ardhana, M.M. and Fleet, G.H. (2003). The microbial ecology of cocoa bean fermentation in Indonesia. *International Journal of Food Microbiology* **86: 87-99**.

Banigo, E.O.I., deMan, J.M. and Duitschaever, C.L., (1974). Utilization of high-lysine corn for the manufacture of *ogi* using a new improved processing system. *Cereal Chemistry* **51: 559– 572**.

Bolotin-Fukuhara, M., Toffano-Nioche, C., Artiguenave, F., Duchateau-Nguyen, G., Lemaire, M., Marmeisse, R., Montrocher, R., Robert, C., Termier, M., Wincker, P. and Wésolowski-Louvel, M. (2000). Genomic Exploration of the Hemiascomycetous Yeasts: 11. *Kluyveromyces lactis. FEBS Letters* **487: 66-70**.

Brauman, A., Keleke, S., Malonga, M., Miambi, E. and Ampe, F. (1996). Microbiological and biochemical characterization of cassava retting, a traditional lactic acid fermentation for foo-foo (cassava flour) production. *Applied Environmental Microbiology* **62: 2854– 2858**.

Campbell-Platt, G. (2000). Fermented Foods. *In.* Encyclopedia of Food Microbiology. Robinson, R.K., Batt, C.A. and Patel, P.D. (eds.). London: Academic Press. **p.736-773**.

Corsetti, A., Lavermicocca, P., Morea, M., Baruzzi, F., Tosti, N. and Gobbetti, M. (2001). Phenotypic and molecular identification and clustering of lactic acid bacteria and yeast from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of southern Italy. *International Journal of Food Microbiology* **64: 95– 104**.

Du Toit, W.J. and Lambrechts, M.G. (2002). The enumeration and identification of acetic acid bacteria from South African red wine fermentations. *International Journal of Food Microbiology* **74: 57-64**.

Fadda, M.E., Cosentino, S., Deplano, M. and Palmas, F. (2001). Yeast populations in Sardinian feta cheese. *International Journal of Food Microbiology* **69: 153-156**.

Fleet, G.H. (2003). Yeast interactions and wine flavor. *International Journal of Food Microbiology* **86: 11-22**.

Fleming, H.P. and McFeters, R.F. (1981). Use of microbial cultures: vegetable products. *Food Technology* **35: 84**.

Gobbetti, M., Corsetti, A. and Rossi, J. (1994). The sourdough microflora. Interactions between lactic acid bacteria and yeasts: metabolism of carbohydrates. *Applied Microbiology Biotechnology* **41: 456– 460**.

Halm, M., Lillie, A., Sørensen, A. and Jakobsen, M. (1993). Microbiological and aromatic characteristics of fermented maize dough from kenkey production in Ghana. *International Journal of Food Microbiology* **19: 135– 143**.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H. and Rombouts, F.M. (1993). Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of *mawe*, a fermented maize dough from Benin. *International Journal of Food Microbiology* **18: 279– 287**.

Kofi, W., Amoa-Awua, A. and Appoch, F.E. (1996). Lactic acid fermentation of cassava dough into agbelima. *International Journal of Food Microbiology* **31: 87– 98**.

Kunene, N.F., Geornaras, I., von Holy, A. and Hastings, J.W. (2000). Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. *Applied Environmental Microbiology* **66: 1084–1092**.

Kurtzman, C.P. and Fell, J.W. (1998). *The Yeast, a taxonomy study.* 4th edition. Amsterdam: Elsevier Science Publisher.

Leisner, J.J., Vancanneyt, M., Rusul, G., Pot, B. Lefebvre, K., Fresi, A. and Tee, L.T. (2001). Identification of lactic acid bacteria constituting the predominating microflora in an acid-fermented condiment (tempoyak) popular in Malaysia. *International Journal of Food Microbiology* **63: 147-157**.

Mathara, J.M., Schillinger, U., Kutima, P.M., Mbugua, S.K. and Holzapfel, W.H. (2004). Isolation, identification and characterization of the dominant microorganisms of *kule nato*: the Maasai traditional fermented milk in Kenya. *International Journal of Food Microbiology* **94: 269-278**.

Mugula, J.K., Nnko, S.A.M., Narvhus, J.A. and Sorhaug, T. (2003). Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food.

International Journal of Food Microbiology **80: 187-199.**

Muyanja, C.M.B.K., Narvhus, J.A., Treimo, J. and Langsrud, T. (2002). Isolation, characterization and identification of lactic acid bacteria from bushera: a Uganda traditional fermented beverage. *International Journal of Food Microbiology* **80: 201-210.**

Nago, C.M., Hounhouigan, D.J., Akissoe, N., Zanou, E. and Mestres, C. (1998). Characterization of the Beninese traditional ogi, a fermented maize slurry: physiological and microbiological aspects. *International Journal of Food Science Technology* **33: 307–315.**

Nche, P.F., Odamtten, G.T., Nout, M.J.R. and Rombout, F.M. (1994). Dry milling and accelerated fermentation of maize for industrialproduction of kenkey, a Ghanaian cereal food. *Journal Cereal Science* **20: 294– 298.**

Nitisewojo, P. (1995). *Prinsip Analisis Makanan*. Bangi: Penerbit UKM.

Nout, M.J.R., Rombouts, F.M. and Havelaar, A., (1989). Effect of natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. *International Journal of Food Microbiology* **8: 351–361.**

Odunfa, S.A. and Adeyele, S. (1985). Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *Journal of Cereal Science* **3: 173–180.**

Omafuvbe, B.O., Shonukan, O.O. and Abiose, S.H. (2000). Microbiological and biochemical changes in traditional fermentation of soybean for 'soy-daddawa'-Nigerian food condiment. *Food Microbiology* **17: 469-474.**

Oyewole, O.B. (2001). Characteristics and significance of yeast' involvement in cassava fermentation for 'fufu' production. *International Journal of Food Microbiology* **65: 213-218.**

Oyewole, O.B., (1997). Lactic fermented foods in Africa and their benefits. *Food Control* **8: 289–297.**

Oyewole, O.B. and Odunfa, S.A., (1990). Characterisation and distribution of lactic acid bacteria in cassava fermentation during fufu production. *Journal of Applied Bacteriology* **68: 145–152.**

Paludan-Muller, C., Huss, H.H. and Gram, L. (1999). Characterisation of lactic bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. *International Journal of Food Microbiology* **46: 219– 229.**

Sefa-Dedeh, S., Cornelius, B. Amoa-Awua, W. Sakyi-Dawson, E. and Afoakwa, E.O. (2004). The microflora of fermented nixtamalized corn. *International Journal of Food Microbiology* **96: 97-102.**

Steinkraus, K.H., (1996). *Handbook of Indigenous Fermented Foods*. 2nd ed. New York: Marcel Dekker.

Tamang, J.P. and Sarkar, P.K. (1996). Microbiology of mesu, a traditional fermented bamboo shoot product. *International Journal of Food Microbiology* **29: 49-58.**

Thapa, S. and Tamang, J.P. (2004). Product characterization of kodo ko jaanr: fermented finger millet beverage of the Himalayas. *Food Microbiology* **21: 617-622.**

Tsuyoshi, N., Fudou, R., Yamanaka, S., Kozaki, M., Tamang, N., Thapa., S. and Tamang, J.P. (2005). Identification of yeast isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation. *International Journal of Food Microbiology* **99: 135-146.**

Viljoen, B.C. and Greyling, T. (1995).Yeasts associated with Cheddar and Gouda making. *International Journal of Food Microbiology.* **28: 79 –88.**

Wacher, C., Canas, A., Barzana, E. Lappe, P., Ulloa, M. and Owens, J.D. (2000). Microbiology of Indian and Mestizo pozol fermentations. *Food Microbiology* **17: 251-256.**

Wood, B.J.B. (1994). Technology transfer and indigenous fermented foods. *Food Research International* **27: 269-280.**