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 *Saccharomyces cerevisiae*, *Candida krusei*, *C. pelliculosa*, *C. guillermondii*, *C. magnoliae* and *Rhodotorula glutinis*, whereas the LAB were *Lactobacillus brevis*, *L. plantarum*, *L. collinoides* and *Pedicoccus* 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

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 *tapa ketan* (Indonesia), *tapai* (Peninsular Malaysia, Singapore and Brunei), *basi* (Philippines) 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 ($≈ 30^\circ$C). Starter culture cakes are ground 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 alcohols 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 until 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. Crude fiber was determined using difference: 100-(%moisture + %protein + %fat + %ash + %fibre) (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
alcoholic beverages. C. sphaerica which was the anamorph state of K. lactis were isolated. K. lactis has been 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-sporoforming 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 tugwa (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 (Oywole and Odunfa, 1990). L. brevis often occur in fermenting plant material (Corsetti et al., 2001) and have been isolated from

![Figure 1: Growth of yeasts and moulds (●) and lactic acid bacteria (■) during fermentation of tapai for 20 days.](image)

<table>
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| Lactic acid bacteria     |                 |                  |      |      |      |      |      |      |      |
| Lactobacillus plantarum  | 76              | 1                | 2    | 9    | 10   | 6    | 7    | 7    | 10   |
| Lactobacillus brevis     | 32              | 0                | 0    | 0    | 5    | 3    | 1    | 6    | 2    |
| Lactobacillus paracasei subsp. paracasei | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pediococcus pentosaceus  | 14              | 10               | 4    | 0    | 0    | 0    | 0    | 0    | 0    |
| Lactococcus lactis subsp lactis | 6 | 2 | 4 | 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 15\textsuperscript{th} 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 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
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 availability 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


