# ISOLATION AND IDENTIFICATION OF MICROFLORA INVOLVED IN TAPAI, A SABAH'S FERMENTED ALCOHOLIC BEVERAGE

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**SCHOOL OF FOOD SCIENCE AND NUTRITION**
**UNIVERSITI MALAYSIA SABAH**
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## ABSTRACT

## ISOLATION AND IDENTIFICATION OF MICROFLORA INVOLVED IN TAPAI, A SABAH'S FERMENTED ALCOHOLIC BEVERAGE

Tapai is an indigenous fermented alcoholic beverage of Kadazan, Dusun and Murut ethnics in Sabah, yet little research has been done on this beverage. The objective of this study was to identify the microflora involved in tapai fermentation as well as to determine the physicochemical changes and proximate changes during the fermentation. Freshly prepared samples were obtained and kept at similar conditions through fermentation before withdrawn for microbiological and chemical analysis at each interval times. The alcohol content, pH, titratable acidity (as %lactic acid) and total soluble solids of the tapai were 12.3±0.97%, 4.0±0.17, 0.82±0.03% and 14°Brix, respectively at the end process of tapai fermentation. The moisture, ash, protein, crude fiber and carbohydrate content increased significantly ($p<0.05$) during fermentation whereas during 12 months of storage, only protein content showed significant changes ($p>0.05$). Aerobic mesophilies, yeast and lactic acid bacteria count increased in the early stage of fermentation but decreased thereafter until the end of the fermentation process. The yeasts were the only flora detected after 12 months of storage. Mould and Enterobacteriaceae were not detected on the 4$^{th}$ day of the fermentation and thereafter. A total of 102 yeasts were isolated, consisting of *Candida utilis, Candida krusei, Candida famata, Candida pelliculosa, Candida glabrata*, *Candida sphaerica* biotype 1, *Saccharomyces cerevisiae* biotype 1, *Saccharomyces cerevisiae* biotype 2, *Rhodotorula mucilaginosa* biotype 2, *Rhodotorula glutinis* and *Cryptococcus laurentii.* The mould identified was *Mucor circinelloides.* There were 116 lactic acid bacteria isolated, including *Pediococcus pentosaceus* biotype 1, *Lactobacillus plantarum* biotype 1, *Lactobacillus paracasei* ssp. *paracasei* biotype 3 and *Lactobacillus brevis* biotype 3. Only *C. utilis* and the unidentified mould showed amylolytic and proteolytic properties. The fermentation process of tapai was found initiated by non-*Saccharomyces* yeast and *P. pentosaceus* biotype 1, then replaced by *Saccharomyces* yeasts and *Lactobacillus* spp. in the later stage of fermentation. It is envisaged that identification of roles and potentials of the different isolates in controlled fermentation of tapai would help the production of starter culture that produce superior quality tapai. Additional studies using polyphasic approaches could be carried out for analysis of microbial dynamics in tapai fermentation.

**ABSTRAK**

*Tapai adalah sejenis minuman beralkohol terfermentasi orang Kadazan, Dusun dan Murut di Sabah, namun, kajian mengenainya masih terhad. Objektif kajian ini adalah untuk mengenalpasti mikroflora yang terlibat dalam fermentasi tapai serta menentukan perubahan fisikokimia dan proksimat semasa fermentasi. Sampel diperolehi dan disimpan pada keadaan serupa dengan pengeluar sepanjang fermentasi dan analisis mikrobiologi dan kimia dilakukan pada setiap selang masa tertentu. Kandungan alkohol, pH, keasidan dan pepejal terlarut tapai, masing-masing adalah 12.3±0.97%, 4.0±0.17, 0.82±0.03% dan 14°Brix pada akhir fermentasi. Kandungan kelembapan, abu, protein, serat kasar dan karbohidrat meningkat dengan signifikan ($p<0.05$) semasa fermentasi, manakala hanya kandungan protein sahaja yang menunjukkan perubahan signifikan ($p>0.05$) semasa penyimpanan selama 12 bulan. Kiraan mesofilis aerobik, yis dan bakteria asid laktik meningkat pada peringkat awal fermentasi tetapi semakin menurun hingga proses akhir fermentasi. Yis adalah satu-satunya flora yang dikesan selepas 12 bulan penyimpanan. Kulat dan Enterobacteriaceae tidak dapat dikesan selepas 4 hari fermentasi. Sejumlah 102 yis dipencilkan, termasuklah Candida utilis, Candida krusei, Candida famata, Candida pelliculosa, Candida glabrata, Candida sphaerica biotype 1, Saccharomyces cerevisiae biotype 1, Saccharomyces cerevisiae biotype 2, Rhodotorula mucilaginosa biotype 2, Rhodotorula glutinis dan Cryptococcus laurentii. Kulat yang dikenalpasti adalah Mucor circinelloides. Sementara itu, sejumlah 116 bakteria asid laktik telah dipencilkan, termasuklah Pediococcus pentosaceus biotype 1, Lactobacillus plantarum biotype 1, Lactobacillus paracasei ssp. paracasei biotype 3 dan Lactobacillus brevis biotype 3. Hanya C. utilis dan kulat yang tidak dikenalpasti menunjukkan ciri-ciri amilolitik dan proteolitik. Fermentasi tapai didapati dimulakan oleh yis bukan Saccharomyces dan P. pentosaceus biotype 1, yang kemudiannya diganti oleh Saccharomyces dan Lactobacillus spp. pada peringkat akhir fermentasi. Pengenalpastian peranan dan potensi pelbagai pencilan dalam fermentasi secara terkawal membantu menentukan kultur pemula yang menghasilkan tapai yang berkualiti. Kajian lanjutan menggunakan pendekatan polifasik perlu dilakukan untuk analisis dinamik mikrobial dalam pemprosesan tapai.*

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| % | percent |
| %T | percent transmittance |
| < | less than |
| > | more than |
| ≈ | approximately |
| CFU/g | colony forming unit per gram |
| $cm^2$ | square centimetre |
| g | gram |
| h | hours |
| kDa | kilo Dalton |
| M | Molar |
| min | minutes |
| ml | millilitre |
| $^oC$ | degree Celsius |
| v/v | volume per volume |
| w/v | weight per volume |
| e.g. | *Latin* exempli gratia (for example) |

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# CHAPTER 1

## INTRODUCTION

Fermentation of food is the oldest form of food preservation as well as being a precursor of modern biotechnology. It is a process dependent on the biological activity of microorganisms for a range of metabolites that can suppress the growth and survival of undesirable microflora in foodstuff (Paul-Ross *et al.*, 2002). If the products became ill smelling, or off-flavoured, consumers will try to avoid them and the foods are described as spoiled. If the microbial products pleasantly flavoured, have attractive aromas and texture, and are non-toxic, the consumer accepts them and they will designated as fermented foods (Steinkraus, 1996).

The fermentation processes were artisinal in nature and obviously, there could have been no appreciation of the role of microorganisms. In most cases, the methodologies and knowledge associated with the manufacturing of these fermented products were handed down from generation to generations in relatively small quantities that were distributed in or around the immediate areas. However, the blossoming of microbiology as a science at the 19$^{th}$ century has resulted the biological basis of fermentation being understood for the first time. Thus, the essential role of bacteria, yeasts and moulds in the generation of fermented food came to understanding and this ultimately led to the isolation of starter culture that could be produced on large scale to supply factories involved in manufacturing of fermented foods with more control and efficiency. These developments parallel significant technological advances in the handling and processing of milk which has resulted in dairy fermentation being among the most sophisticated and best researched of the food fermentation to this day. Research on lactic acid bacteria, which have a dominant role in the production of many fermented foods has continued to advance at a very impressive rate through the 20$^{th}$ century (Caplice & Fitzgerald, 1999).

Though, in developing countries, food fermentation today is still generally practiced as a household or cottage technology rather than through scientific principle, with very few operations carried out at an industrial level. Despite the developed world can claim to have elevated the production of some fermented foods to a large industrial and technologically sophisticated level (Rolle & Satin, 2002). In fact, many of these cottage-type fermented products are now considered to be of a premium type because they retain flavour and aroma characteristics that many would claim have all but disappeared in the 'factory' manufactured products. However, little information is available often deals with the identification and perhaps preliminary characteristic of the primary microflora in the finished fermented products (Caplice & Fitzgerald, 1999). Microbiological and biochemical aspects of a number of these processes are complicated and not fully understood. Physical aspects of the processes such as temperature, relative humidity and level of agitation and aeration are often poorly controlled, and production techniques are not standardized. In general, processes are low efficiency, and result in low yields products with variable quality. Moreover poor hygienic practices and improper handling during post fermentation processing and at the point of sale, render fermented products susceptible to contamination (Adams & Mitchell, 2002). The shelf life of a number of indigenous fermented products are limited by the unavailability of appropriate technologies for post fermentation processing treatments that terminate the bioprocess and consequently extend the shelf life of the fermented products. Furthermore, inadequate packaging of fermented products limits both the shelf life and competitiveness of these products in the markets (Rolle & Satin, 2002).



Meanwhile, it appears to be inevitable and even ironical that fermented products become more popular and as demands grow, the only way in which the expanding market can be satisfied is to upscale the manufacturing process where the use of starter cultures becomes almost essential (Caplice & Fitzgerald, 2002). Modern, large-scale production of fermented foods and beverages is dependent almost entirely on the use of defined strain starters, which have replaced the undefined strain mixtures traditionally used for the manufacture of these products. This switch to defined strains has meant that both culture performance and product quality and consistency have been dramatically improved, while it has also meant that a smaller

number of strains are intensively used and relied upon by the food and beverage industries (Paul-Ross *et al*., 2002).

Additionally, indigenous fermented food has become a new interest and consequently provided new subjects for intellectual creation in these few years. While traditionally produced food products may be of health concern to non traditional consumers, 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 around the world recently (Thapa & Tamang, 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.

Unfortunately, most of the fermented products in Sabah also undergo spontaneous fermentation, such as *nonsom* (fermented fresh water fish) under household conditions with relatively simple equipments. Spontaneous fermentation typically results from the competitive activities of a variety of contaminating microorganisms. Those best adapted to the food substrates and to technical control parameters, eventually dominate the process. The metabolites (e.g. organic acid) inhibitory to other contaminating microbes (e.g. Enterobacteriaceae) may provide an additional advantage during fermentation. However, spontaneous fermentation process takes a relative long time, with high risk of failure. It is neither predictable nor controllable (Hozapfel, 2002) and often empirically applied without a comprehensive understanding of the underlying principles of the fermentation process and the requirements for ensuring quality and safety. Such an approach presents a major pitfall, as it may lead to unsafe products depending on the process, environmental condition and the condition of the raw materials (Motarjemi, 2002).

Tapai is a well-known indigenous fermented alcoholic beverage among the Kadazan, Dusun and Murut 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 rice, glutinous rice, cassava or sometimes pineapples and maize in some parts of Sabah using a starter cake known as *sasad* (Kadazandusun language) or *ragi tapai* (Malay language). The ingredients used to prepare *sasad* are rice flour with addition of spices and proportion from plants. Yet, usage of the two latter ingredients mentioned in preparing *sasad* is closely guarded trade secret as every producer has their own recipe or methodology handed down from their ancestors with very little adaptations. There is no control of the incubation condition in preparing these traditionally made *sasad* and the success of the fermentation is influenced by the weather as it is exposed to the atmosphere and contaminated by various microorganisms of the environment during fermentation (Merican *et al*., 1984). During preparation of tapai, glutinous rice was cleaned and washed before cooked. Cooked glutinous rice was then spread for cooling in an open surface (≈30°C). The previous batch of *sasad* was ground into powder and approximately 1.0-1.5% (by weight) was sprinkled on the cooled glutinous rice followed by mixing thoroughly using a wooden scoop. The mixture was 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 (Appendix A). 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 (Chiang *et al*., 2006).



While fermented food are treasured as a major dietary constituent and has became more and more popular among health conscious consumers, researches and developments of fermented food in Sabah is still in its infancy. The documentation of indigenous fermented foods in Sabah has not yet been carried out, as the information of these foods is extremely rare. At present, there is neither scientific knowledge of the process involved nor appropriate biological inoculants and process controls for tapai fermentation. Studies should be designated to investigate the technological constraints

of Sabah's indigenous fermented foods so as to upgrade fermentation technology as well as to improve the status of food industry in Sabah. Thus, this study was carried out to isolate and identify the microflora involved in the traditional tapai fermentation. The specific objectives of this research were:

i. To isolate, identify and characterize microflora responsible for tapai fermentation.

ii. To investigate hydrolytic properties (amylolytic, proteolytic, cellulolytic & pectinolytic) of the isolates.

iii. To study physicochemical changes and proximate composition as well as alcohol content of tapai during fermentation.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 CLASSIFICATION OF FOOD FERMENTATION

Food fermentation can be classified in a number of ways by different authors (Table 2.1) (Steinkraus, 1997) but the classifications were found not always distinctively differentiate fermented products as the lines between them were ambiguous. Therefore, the classification of food fermentation by Steinkraus (1996) that based on chemical, physical and nutritive changes in the fermented products (Table 2.2) was found to be practical as the classification characteristically distinguished various fermented foods around the world and was found to be useful way as the microorganisms that may be involved are predictable according to the category proposed.



The simplest classification of commercially useful food fermentations may only be either solid-state or submerged culture fermentation. Solid-state fermentation (SSF) is defined as the fermentation involving solids in absence (or near absence) of free water, although capillary water may be present whereas submerged fermentations (SmF) may use a dissolved substrate like sugar solution, or a solid substrate, suspended in a large amount of water to form slurry (Pandey *et al.*, 2000). In addition, both solid state and submerged fermentations may each be subdivided into oxygen-requiring aerobic produces and anaerobic process that must be conducted in the absence of oxygen. Examples of aerobic fermentations include submerged-culture citric acid production by *Aspergillus niger* and solid-state koji fermentation. Fermented meat products such as bologna sausage, dry sausage, pepperoni and salami are produced by solid sate anaerobic fermentation. A submerged-culture anaerobic fermentation occurs in yoghurt making (Christi, 2000).