2. REVIEW OF LITERATURE

Alcoholic foods and beverages, in which starch hydrolysis and fermentation are accomplished by amylolytic moulds and yeasts, range from very primitive Thai rice wines to highly sophisticated Japanese saké, which itself developed from a primitive beverage (Steinkraus, 1983). A list of common traditional fermented beverages of Asia is shown in Table A.

Table A. Traditional fermented beverages of Asia prepared by using starters

Type	Substrate	Starter	Nature	Country
Brem	Rice	Ragi	Liquor	Indonesia
Krachae	Rice	Loogpang	Wine	Thailand
Lao-chao	Rice	Chiu-yueh	Alcoholic beverage	China
Pachwai	Rice	Bakhar	Rice beer	India
Tapuy	Rice	Bubod	Wine	The Philippines
Tapai	Rice	Jui-piang	Wine	Malaysia
Tapé	Rice, cassava, maize, millet	Ragi	Sweet-sour alcoholic paste	Indonesia
Saké	Rice	Koji	Distilled alcoholic drink	Japan
Yakju and Takju	Rice, wheat, barley, maize	Nuruk	Alcoholic beverages	Korea

2.1. STARTERS

In Asia three types of inoculum as starter are commercially produced to convert starchy materials to sugars and subsequently to alcohol and organic acids (Hesseltine *et al.*, 1988):

1. In koji, pure cultures of *Aspergillus oryzae* and *Aspergillus sojae* are used in combination. At the same time they produce amylases that convert starch to fermentable sugars, which are then used for the second stage yeast fermentation to make miso and shoyu, while proteases are formed to break down the soybean protein.
2. In a second type, whole-wheat flour with its associated flora is moistened and made into large compact cakes, which are incubated to select certain desirable organisms. The cakes, after a period of incubation, are used to inoculate large masses of starchy material, which is then fermented to produce alcohol. Cakes contain yeasts, *Rhizopus* and *Absidia.* This inoculum is used in the so-called Kao-liang process for making alcohol.
3. The third type of starter is a mixed culture of yeast, fungi and bacteria. This starter is in the form of flattened or round balls of various sizes, compact in texture, and dry. The starter is inoculated with some previous starter. This mixed flora is allowed to develop for a short time, then dried, and used to make either alcohol or fermented foods from starchy materials. The starters have a variety of names such as ragi in Indonesia, bubod in the Philippines, Chinese yeast and chiu-chu in China and Taiwan (Hesseltine, 1983), loogpang in Thailand (Steinkraus,

1983), nuruk in Korea (Park *et al.*, 1977), which are used as starters for a number of fermentations based on rice and cassava or other cereals in Asia.

Calmette (1892) was the first to report the presence of several wild yeast species accompanied by *Amylomyces, Mucor, Aspergillus* and 30 different bacteria in starters used in Indochina to produce alcohol.

2.1.1. Ragi

Ragi is a starter used in Indonesia in the form of flat cakes where rice is used as a substrate (Saono *et al.*, 1974). During production of ragi, mainly rice or millet or cassava or other starchy bases are milled, mixed with herbs and spices, roasted together, sieved, water added and starter (ragi) from previous batch is mixed and shaped into balls. These are incubated at 25-30° C for 72 h in humid environment. Balls are dried in the sun and used as inoculum for the various fermentations. Went and Prinsen-Geerligs (1896) found *Monilia javanicus* (= *Pichia anomala*) and *Saccharomyces cerevisiae* as principal yeasts in ragi. Dwidjoseputro and Wolf (1970) reported the yeasts *Candida parapsilosis, C. melinii, C. lactosa, Hansenula subpelliculosa, H. anomala* and *H. malanga* in ragi. Addition of spices to some ragi contributes other microorganisms or may inhibit the growth of undesirable microorganisms (Soedarsono, 1972). Saono *et al.* (1974) conducted studies on mycoflora of ragi and products fermented by ragi such as tape keté la, tapé ketan hitam, oncom hitam and oncom mérah from various places in West Java and reported that *Candida* spp was dominating among yeasts, *Mucor* spp and *Rhizopus* spp were dominating among

moulds. Kato *et al.* (1976) studied the properties of glucoamylase from ragi isolates of *Saccharomycopsis fibuligera*. Saono and Basuki (1978) reported thirteen species of *Candida* from ragi of Indonesia. Hadisepoetro *et al.* (1979) reported that population of yeast in three ragi was 5.6×10^6 to 1.4×10^7, bacteria was 3×10^4 to 1.8×10^5 and mould was 3.2×10^4 to 4×10^4. Ardhana and Fleet (1989) found only one yeast *Candida pelliculosa* and one mould *Amylomyces rouxii* in four samples of ragi. Yokotsuka (1991) reported the presence of mixed cultures in ragi mainly *Rhizopus* and *Mucor* among molds; other organisms such as *Amylomyces*, *Aspergillus*, *Fusarium*, *Candida*, *Saccharomyces*, *Hansenula*, *Endomycopsis* (= *Saccharomycopsis*). Ishimaru and Nakano (1960) isolated *Streptococcus faecalis*, *Lactobacillus plantarum* and *Pediococcus pentosaceus* in ragi in the range of 10^5 to 10^8 cfu/g. Hesseltine and Ray (1988) reported that most of the bacteria isolated from ragi belong to *Pediococcus pentosaceus* and *Streptococcus faecalis*, which may produce secondary products from the glucose formed by the amylolytic yeasts and moulds always found in the starters. Ardhana and Fleet (1989) reported the presence of bacteria in all four samples studied, which included *Bacillus coagulans*, *B. brevis*, *B. stearothermophilus* and an unidentified species of *Acetobacter* at the level of 10^3 to 10^4 cfu/g.

Saono *et al.* (1984) prepared ragi by using pure cultures of the selected molds and yeasts, *Amylomyces rouxii* strains AU-3 and CB-3, and *Saccharomyces cerevisae* strains RM-1and K-3 and pure culture of *Rhizopus formosaensis* and also prepared brem from this improved ragi. Elegado and Fujio (1993) isolated two polygalacturoniase producing strains

of *Rhizopus* spp from ragi and studied the enzyme stability in wide range of pH from 2-11 and tolerance at 50° C for 20 min. Uchimura *et al.* (1991) revealed that there is a higher variability rate of *Pediococcus pentosaceus* in older ragi than younger ones and the result suggested that rod-shaped bacteria cannot survive for a long time under dry conditions in ragi.

2.1.2. Bubod

Bubod is used as a starter in the Philippines (Tanimura *et al.*, 1977). Rice and ginger are powdered, and mixed thoroughly with enough water to have a consistency that permits rolling the material into a ball and flattening it. The discs are coated with 1-3 month old bubod and incubated in rice straw for 36 h at room temperature and sun-dried. Tanimura *et al.* (1977) found *Mucor, Rhizopus* and filamentous yeasts in bubod. Kozaki and Uchimura (1990) reported the presence of *Mucor circinelloides*, *M. grisecyanus*, *Rhizopus cohnii*, *Saccharomyces cerevisiae* and *Saccharomycopsis fibuligera* in bubod. Sanchez (1986) reported that the molds present in bubod ranged from 10^3 to 10^5 cfu/g, yeasts from 10^7 to 10^8 cfu/g, and lactic acid bacteria from 10^5 to 10^7 cfu/g. Hesseltine and Kurtzman (1990) reported that *Saccharomycopsis fibuligera* was dominant in bubod.

2.1.3. Koji

Koji is mould-culture and is prepared from steamed-cooked cereal. The substrate is usually rice, or sometimes steamed legume beans. The steamed substrate is spread on trays usually made of bamboo strips to depth of 5-7 cm which are stacked with gaps of about 10 cm in between to allow air circulation. It is followed by inoculation with 0.1 % mould spores, *tane-koji*

and incubated at 23-25° C. The rise in temperature due to the growth of mould is kept within the range 35-45° C by stirring and turning koji top to bottom on trays at about 20 h and 40 h, normally fermented for 3 days, when mould mycelium spread throughout mass, and before sporulation (Lotong, 1985). The mould used is *Aspergillus oryzae*, which is used for starch saccharification in saké manufacture (Inoue *et al.*, 1992). Since koji is not cultivated in a closed system, koji is a mixture of several microorganisms. At an early stage of cultivation, yeast grows on steamed rice grain and after that, about 20 h after inoculation of seed koji, koji mold begins to grow. Koji usually contains 10^2/g saké yeast, 10^2 to 10^5 g film-foaming yeasts, 10^2 /g lactic acid bacteria, 10^4 to 10^6 /g micrococci, 10^7 /g bacilli, etc. Kodama and Yoshizawa (1977) studied the biochemical changes occurring in koji and found the increase of reducing sugar from 0.2 % to 21.4 %. Tanaka (1982) studied enzyme activity of steamed or unsteamed glutinous rice-koji inoculated with *Aspergillus oryzae* and *Rhizopus jawanicus* and found that α-amylase was 1527 U/g in *Aspergillus* and 100 U/g in *Rhizopus* in steamed rice koji, whereas 1255 U/g and 100 U/g in unsteamed rice koji, respectively.

2.1.4. Nuruk

Nuruk is the starter for preparing Korean alcoholic drink yakju and takju. Historically the substrate for nuruk was rice but presently it is wheat (Park *et al.*, 1977). Generally, nuruk is prepared by natural inoculation of molds, bacteria, and yeasts; however, it can be prepared by inoculation with *Aspergillus usamii*. Traditionally nuruk is prepared by moistening wheat

flour, kneaded and molded into a ball [0.8-1.6 kg (dry weight)] and fermented for 17 d at 30° C to 45° C, dried for 2 weeks and cured for 1-2 months at room temperature (Park *et al.*, 1977). Kim (1968) isolated *Aspergillus oryzae* (10^7 cfu/g), *A. niger* (10^7 cfu/g), *Rhizopus* sp (10^6 cfu/g), anaerobic bacteria (10^7 cfu/g), aerobic bacteria (10^6 to 10^7 cfu/g) and yeasts (10^5 cfu/g) from nuruk.

2.1.5. Chiu-yueh

Chiu-yueh or peh-yueh is the Chinese starter for lao-chao, fermented rice product of China. It is a gray-white ball containing yeasts and fungi grown on rice flour which is closely related to Indonesian ragi. Wei and Jong (1983) isolated yeasts and moulds from chiu-yüeh and tested the ability of these microorganisms to convert steamed glutinous rice into a good quality lao-chao.

2.1.6. Chou or Chu

It is moulded cooked cereal which acts like, malt, as rich source of enzymes with substrate in various fermentations (Campbell-Platt, 1987). Chu is ball, cake or brick (20 × 22 × 4.5 cm) shaped and made from moistened raw rice, wheat, sorghum or barley flour. The principal amylolytic enzyme producers of chu are *Rhizopus* and *Mucor* (Yokotsuka, 1991). The microorganisms of wheat chu were found to be *Rhizopus japonicus, R. hangchon, R. chinensis, Absidia, Mucor, Monilia, Aspergillus, Lactobacillus* and *Acetobacter* (Otani, 1973; Iizuka, 1979).

2.1.7. Loogpang

Loogpang is the starter commonly used in Thailand to prepare alcoholic drink and vinegar (Vachanavinich *et al.*, 1994). In loogpang, organisms are grown on bran (Steinkraus, 1983). The main ingredient of this starter is rice flour with the addition of different type of spices and microorganisms. The microorganisms are originated from the inoculum or surrounding place of preparation of previous batch (Vachanavinich *et al.*, 1994). Pichyangkura and Kulprecha (1977) found that the molds *Amylomyces*, *Rhizopus*, *Aspergillus*, *Mucor*, and *Absidia* in loogpang. Dhamcharee (1982) showed that the molds present in loogpang from different places in Thailand were *Rhizopus*, *Mucor*, *Amylomyces*, *Penicillium*, and *Aspergillus*, and the main yeast genera were *Endomycopsis* (= *Saccharomycopsis*), *Hansenula*, and *Saccharomyces*. Sukhumavasi *et al.* (1975) isolated a strain of *Endomycopsis* (= *Saccharomycopsis*) *fibuligera* from loogpang with high glucoamylase activity. Uchimura *et al.* (1991) reported the presence of *Saccharomycopsis fibuligera* and *Pediococcus* sp. in loogpang.

2.1.8. Bakhar

Bakhar is a starter culture used to make pachwai, rice wine in eastern part of India and contains *Rhizopus* sp., *Mucor* sp., and at least one species of yeast (Hutchinson and Ram-Ayyar, 1925). Ginger and other plant materials are dried, ground and added to rice flour. Water is added to make a thick paste and a small round cake of 1.0-1.5 cm in diameter are formed and inoculated with powdered cakes from previous batches. The cakes are then wrapped in leaves, allowed to ferment for 3 d and then sun-dried

(Hutchinson and Ram Ayyar, 1925). Ray (1906) reported the presence of *Saccharomyces* sp. in bakhar.

2.1.9. Marcha

Murcha (correctly spelled as marcha) is a ball-like starter, used to ferment starchy materials into fermented beverage in Nepal, Bhutan and the Darjeeling hills and Sikkim in India (Tamang and Sarkar, 1995). Kobayashi *et al.* (1961) reported *Rhizopus oryzae, Mucor praini* and *Absidia lichtheimi* in marcha samples collected from Sikkim. Hesseltine *et al.* (1988) isolated *Mucor* and *Rhizopus* spp. in marcha. Tamang and Sarkar (1995) identified the microorganism of marcha of the Darjeeling Hills and Sikkim as *Pediococcus pentosaceus, Saccharomycopsis fibuligera, Pichia anomala, Mucor circinelloides,* and *Rhizopus chinensis.* Batra and Miller (1974) reported *Hansenula anomala* var. *schneggii* (= *Pichia anomala)* in marcha. In Bhutan, marcha is called chang-poo, in which the dominating microorganism belonged to the genus *Saccharomycopsis,* moulds were *Penicillium* sp. and *Aspergillus* sp. (Uchimura *et al.*, 1990).

2.1.10. Mana

Mana is a granular type of starter culture prepared from steamed rice or wheat particularly in Nepal (Karki, 1994). Mana contains *Aspergillus oryzae,* mucoraceae, yeast and lactic acid bacteria (Nikkuni *et al.*, 1996).

2.2. ALCOHOLIC BEVERAGES PRODUCED BY STARTER

2.2.1. Tapé

Tapé is a sweet-sour paste with an alcoholic flavour, prepared from glutinous rice or cassava or other cereals by using starter ragi in Indonesia (Ko, 1972). During preparation of tapé, glutinous rice is washed, soaked, steamed until it is well cooked and sticky, cooled to room temperature on a woven bamboo tray, sprinkled with powdered ragi, packed in small banana leaves and fermented for 2 to 3 d at room temperature and soft juicy mass of tapé is produced. With continued incubation more liquid is produced in the product (Saono *et al.*, 1977). There are various substrates used to prepare tapé, they are cassava (tapé ketala), glutinous rice (tapé ketan), corn (tapé jagung), and millet (tapé cantel). Ko (1972) noted the fall of pH in tapé ketan from ~ 6.0 to 3.5, generally remaining at pH 4.0. Ethanol content ranged from 3 % v/v (Tanuwidjaja, 1972) to as high as 8.5 % v/v (Cronk *et al.*, 1977). A combination of *Aspergillus rouxii* and *Endomycopsis* (= *Saccharomycopsis*) *burtonii* reduced total solids by 50 % in 192 h at 30° C, which raised the crude protein in tapé ketan by 16.5 % on a dry basis (Cronk *et al.*, 1977, 1979). Cronk *et al.* (1977) also found that rice lipids were hydrolysed during tapé ketan fermentation. Ardhana and Fleet (1989) studied the tapé ketan fermentation using ragi as starter showing the importance of bacteria in the overall fermentation along with mould and yeast. Suprianto *et al.* (1989) while studying on tapé production by inoculating ragi found that the inoculation of *Streptococcus* in a mixed culture of *Rhizopus* and *Saccharomycopsis* produced a higher level of

aroma. He also found that liquefaction was not caused by amylases of *Saccharomycopsis* even though it produced high activity of ∝-amylase.

2.2.2. Brem

Indonesian brem are of three types, (i) *brem madiun*, which is yellowish-white in colour, sweet-sour in flavour and is prepared in blocks of 0.5 × 5 to 7 cm; (ii) *brem wonogiri*, which is sweet flavoured, very soluble, white, and thin circular blocks of 5 cm diameter; and (iii) *brem bali*, which is a famous alcoholic liquor produced in Bali. All the three brems are made from the liquid portions of tapé ketan. The filtrate of tapé ketan is boiled down, poured onto table covered with banana leaves or polythylene sheeting, and left to cool to ambient temperature over 8-12 h (*brem madiun*) or sun dried for 1 day to produce *brem wonogiri* (Campbell-Platt, 1987). Keeping the liquid portion of tapé ketan for 7 months, during which time solids precipitate leaving a clarified brem, known as *brem bali*. It is decanted and bottled (Basuki, 1977). Saono *et al.* (1984) produced brem wine by using improved ragi and observed that this wine had a grape aroma and more desirable than commercial one. Winarno (1986) reported that the alcohol content of brem wine to be 6.13 %.

2.2.3. Tapai

Tapai is the Malaysian fermented food-beverage. It is consumed as a desert but in East Malaysia it is the rice wine with lighter colour and less sweetness (Merican and Yeoh, 1989). Tapai is produced by adding pulverized ragi or jui-piang to washed, cooked and cooled glutinous rice on

a woven bamboo tray, covered with cloth and incubated at room temperature (25-26° C) for 3 days, stirring the fermenting mash at least once a day to keep the surface moist. After 1 week, wine or brandy is added to the mash as a preservative and allowed to ferment for an additional 25 days and wine is collected by immersing a strainer-like collection vessel into the mash (Wong and Jackson, 1977). There are two types of tapai, namely, tapai pulut (made by fermented glutinous rice) and tapai ubi (made from tapioca or cassava) (Merican and Yeoh, 1989). *Candida* spp, *Saccharomycopsis fibuligera*, *Amylomyces rouxii*, *Mucor circinelloides*, *M. javanicus*, *Hansenula* spp, *Rhizopus oryzae*, and *R. chinensis* have been found to be present in tapai (Wang and Hesseltine, 1970; Ko, 1972). According to Cronk *et al.* (1977) the protein content of rice is doubled to about 16% after fermentation as a result of losses of total solids and synthesis of proteins by the microorganisms. Merican and Norrijah (1985) showed that the organisms necessary to produce a good tapai pulut consist of a mixture of *Amylomyces rouxii, Endomycopsis* (= *Saccharomycopsis*) *fibuligera* and *Hansenula anomala*, and for a good quality tapai ubi the essential microorganisms are *Amylomyces rouxii,* and *Endomycopsis* (= *Saccharomycopsis*) *fibuligera.*

2.2.4. Tapuy

Tapuy is a popular rice wine of the Ifugao race of the Philippines, which is sweet though acidic and aromatic (Tanimura *et al.*, 1977). Tapuy is prepared by washing, cooking and cooling glutinous rice, placed in a clay pot and pulverized bubod is sprinkled over it. The pot is covered with

cheesecloth and incubated in a cool place for 2-3 d (Tanimura *et al.*, 1977). Uyenco and Gacutan (1977) isolated *Endomycopsis* (= *Saccharomycopsis*) *fibuligera, Rhodotorula glutinis, Debaromyces hansenii, Candida parapsilosis* and *Trichosporon fennicum,* homofermentative and heterofermentative lactic acid bacteria, including some species of *Leuconostoc.* Tanimura *et al.* (1977) studied biochemical changes in tapuy and found that reducing sugar ranged from 4.1 to 5.2 %; pH from 3.3 to 4.9; total acidity from 6.55 to 22.49 ml of 0.1(N) NaOH/100 ml; and ethanol from 13.5 to 19.1 %.

2.2.5. Lao-chao

Lao-chao is a popular Chinese fermented food with a sweet taste and mild alcoholic flavour with a fruity aroma, made from rice by using chiu-yueh or peh-yueh as starters (Wang and Hesseltine, 1970). It is served as a dessert and is also a traditional diet for new mothers who believe that it helps them regain their strength (Wei and Jong, 1983). The process of manufacture is identical to Indonesian tapé keatan. Wei and Jong (1983) studied the flora of lao-chao and found *Rhizopus, Amylomyces, Torulopsis,* and *Hansenula.* Pure culture fermentation method of lao-chao was developed by Wang and Hesseltine (1970) and showed that a good fermented rice was made when a mold, *Rhizopus chinensis* NRRL 3671, and a yeast, *Endomycopsis* (= *Saccharomycopsis*) sp. NRRL Y7067, used as inocula instead of a commercial starter.

2.2.6. Yakju and Takju

Yakju and Takju are Korean alcoholic beverages, made from rice by using nuruk (Park *et al.*, 1977). The lower or diluted concentration of yakju is known as takju. During yakju preparation, steamed and cooled rice is mixed with nuruk and fermented and liquid pressed from the fermenting mass is filtered under pressure, aged and bottled (Park *et al.*, 1977). Microbial studies of yakju revealed the presence of many yeasts, *Bacillus* spp. and *Lactobacillus* sp. and *Leuconostoc* spp. (Shin and Cho, 1970; Kim, 1970; Kim and Lee, 1970; Lee and Rhee, 1970). Kim and Lee (1970) reported that *Saccharomyces cerevisiae* is the most important organism in alcohol production while *Hansenula* spp. play an important role in flavour development. Kim (1963) and Chung (1967) found various organic acids formation during yakju (takju) fermentation and recorded a total acidity reaching 0.44 to 0.62 % as lactic acid. Kim and Choi (1970 a & b) while studying nutritional changes observed the increase of thiamine during first two days and riboflavin decrease for first 3 d and increase markedly during the following 2 d.

2.2.7. Ewhaju

Ewhaju is a traditional wine prepared from rice by using nuruk in Korea (Kim and Kim, 1993). They suggested that brewed ewhaju could be remained with high quality for long period without any addition of preservatives/heat treatment, also found out that stored ewhaju contained high amylases activity which might contribute to digestion.

2.2.8. Krachae or Nam-khaao

Krachae or nam-khaao or sato is the Thai rice wine, prepared from Thai rice using loogpang as starter (Vachanavinich *et al.*, 1994). Lotong (1985) noted that moulds play an important role at the initial stage of fermentation for cleavage of sugar polymers present in rice to substantial sugars which can be used for substrates for simultaneous fermentation by yeast and lactic acid bacteria. Ko (1982) also noted that moulds produce sugars from starch, and subsequently yeast converts sugars to alcohol. Lim (1991) reported that lactic acid bacteria play a role in formation of flavour and taste in krachae.

2.2.9. Khao-maak

Khao-maak is a fermented glutinous rice beverage prepared by using loogpang, common in Thailand (Phittankpol *et al.*, 1995). It is semi-solid, juicy, white coloured, with sweet taste and slightly alcoholic. It is eaten as a dessert. If the fermentation is prolonged to 10 d, rice wine (krachae or nam-khaao) is made, if prolonged to 17 d, vinegar is made (Phittankpol *et al.*, 1995).

2.2.10. Basi

Basi is a traditional alcoholic beverage of the Philippines made by fermenting boiled, freshly extracted sugarcane juice with a mixture of yeast, bacteria and moulds or with organisms found in 'samac' (*Macharanga tanarius* or *M. gradifolia* Linn.) leaves, bark, or fruit (Tanimura *et al.*, 1978). Kozaki (1976) reported that the dominant organisms in basi are *Saccharomyces*, *Saccharomycopsis* and lactic acid bacteria. Sanchez and Kozaki (1984) developed an improved method for

preparation basi (sugarcane wine) which was more acceptable in all the sensory attributes than the traditionally prepared basi.

2.2.11. Saké

Saké is a Japanese rice wine, which is clear, pale yellow, containing 15 to 20 % alcohol. Polished rice is washed, steeped in water and steamed for 30-60 min, then cooled. Mixed with moulded rice koji and yeast moto, and water to form main mass moromi. Main fermentation in open tanks in cool conditions, starting at about 10° C, increasing to about 15° C. After 3 weeks' fermentation, filtered to give fresh saké, which settled, re-filtered, pasteurized and blended and diluted with water before bottling (Yoshizawa and Ishikawa, 1989). Murakami (1972) has reported the first organisms developed in the mash under traditional fermentation conditions to be the nitrate-reducing microorganism such as *Pseudomonas, Achromobacter, Flavobacterium, or Micrococcus* spp. These are followed, or possibly accompanied by *Leuconostoc mesenteroides* var *saké* and *Lactobacillus saké* followed by yeasts fermentation (Kodama and Yoshizawa, 1977). The highly refined saké brewed by the most skillful brewers using very highly polished rice at low temperatures of 9 to 11° C for 25 to 30 days is known as gonjoshu (Kodama and Yoshizawa, 1977). Changes in the various substances (starch, proteins, minerals, ether extractable lipids, and moisture) in rice grains with changes in milling ratio was studied by Yoshizawa and Ishikawa (1974) for saké making. Yoshizawa (1966) and Koizumi (1968) found the presence of n-propanol, i-butanol, i-amylalcohol, and their acetic acid esters, together with the ethyl esters of butyric acid,

and myristic acids in saké in lagre amount. Yoshizawa and Ishikawa (1989) noted that the temperature of the mash has a great influence on the quality of the saké production. Most lactic acid bacteria that spoil saké are homofermentative rods and are more tolerant to ethanol and acid than non-spoilers. *Lactobacillus casei* and *Lactobacillus leichmanii* are more tolerant (Inoue *et al.*, 1992).

2.2.12. Ruhi

Ruhi is an Indian drink primarily produced by the tribal people in Nagaland and in the eastern hill regions (Dahiya and Prabhu, 1977). It is a strong alcoholic beverage made by boiled rice fermentation. Boiled rice is spread on a mat, cooled and mixed with starter and nosan leaves, put into a cone shaped bamboo basket and fermented for 24 h. An earthenware pot is placed under the cone to collect the liquefied rice as it ferments. The juice is collected and transferred to new boiled rice about 3-4 time in succession. The total liquid collected becomes the first quality ruhi (Dahiya and Prabhu, 1977). They reported the ethanol content ranging from 12 to 14 % v/v, pH about 4.0, reducing sugars 2.5 % with total sugar of 3.0 % w/v from ruhi.

2.2.13. Madhu

Madhu is a alcoholic drink of Nagaland and eastern hill regions of India. It is a low-alcohol containing rice wine where soaked rice is the substrate. Raw rice is soaked for 2 h in cold water, drained and ground to a paste, slurry prepared with hot water to which cold water (15 L for 10 kg rice) and starter is added and fermented for 2-3 d (winter) and 1 d (summer). This

drink is used as an early morning meal in which salt is added to taste. *Mucor* and *Rhizopus* along with yeast and lactic acid bacteria were found in madhu fermentation (Dahiya and Prabhu, 1977).

2.2.14. Pachwai

Pachwai is a rice beer, prepared by adding powdered bakhar to steamed rice and fermented for 24 h after which the whole mass is transferred to an earthenware jar, water is added and fermentation continued. The beer is ready to drink in 1 or 2 d when it develops a characteristic alcoholic flavour (Hutchinson and Ram-Ayyar, 1925; Batra and Millner, 1976).

2.2.15. Millet beverage

In old literatures of Darjeeling and Sikkim, there are mentions of fermented millet beverages of the Darjeeling hills and Sikkim (Hooker, 1854; Gorer, 1938). The fermented beverage is also known as chang by the Sikkimese (Risley, 1928) and chi by the Lepcha (Gorer, 1938). Similar product called chhang has been reported from Ladakh region by Bhatia *et al.*, (1977). Thumba (correctly spelled toongbaa), the fermented beverage common in Darjeeling, Sikkim and Nepal has been reported by Hesseltine (1965, 1979) and Batra and Millner (1976).