Chapter 1: Introduction

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1.1 Introduction

The term fermentation is derived from the Latin word *fervere* meaning "to boil". It basically describes the appearance of the action of yeast on extracts of fruit or malted grain during the production of alcoholic beverages. It may be defined as any process for the production of a product by the mass culture of microorganism [1]. Indigenous people have been using microbes unknowingly for various purposes [2]. Fermentation is one of the oldest and most economic methods of preserving the quality and safety of foods. Moreover, fermented foods have further benefits of providing bio-nutrients and minerals and enhancement of flavour and aroma. The process also increases digestibility and exert health promoting benefits [3]. Fermentation may assist in the destruction or detoxification of certain undesirable compounds which may be present in raw foods. These are compounds such as phytates, polyphenols and tannins [4]. Fermented foods are encountered worldwide and their origin is due to their prolonged shelf life, reduced volume, shorter cooking times and superior nutritive value as compared to the non-fermented ingredients. The traditional way of carrying out fermentation at the household-scale is still followed using relatively simple processing facilities. These products often contain mixed microbial populations because of the lack of sterility and the use of natural fermentation [5].

Beer is an immutably microbial product where microbial activity is involved in every step of its production. The consumption of rice beer prepared from rice is a common practice among many tribal communities residing in the North-Eastern states of India and many of them have been preparing it since time immemorial [6,7]. It also plays an important role in the socio-cultural life of the tribal people as it is found to be associated with many occasions like merry making, ritual ceremonies, festivals, marriages and even death ceremonies [8]. The preparation and consumption of this type of liquor emerged mainly due to the climatic conditions and discovering the use of surrounding natural resources [9]. There are also reports of rice beer being used as a drug [10]. It works effective against insomnia, headache, body ache, inflammation of body parts, diarrhoea and urinary problems, expelling worms and as a treatment of cholera [11,12]. The process of manufacturing rice beer consists of saccharification of the rice starch by fungal enzymes followed by alcoholic fermentation by yeasts supplied by the starters. This process is unique and the product differs from commercial malt beer or wine. Even though the methodology of production has resemblance with malt beer, however, there is difference in the saccharification process of both. In malt beer, the enzymes for conversion of starch to sugars (α and β amylases) and proteases are produced during the germination process of the barley grains. Whereas, in case of rice beer, these enzymes are produced by fungus supplied externally. The moulds present in the starters produce the starch degrading enzymes viz., α-amylase and amyloglucosidase (glucoamylase) which result in liquefaction and saccharification of rice starch into dextrins and maltose, but mainly into glucose. The whole process of preparation involves saccharification of the starch present in steamed or boiled rice by fungal enzymes followed by alcoholic fermentation by yeasts. Yeasts are unicellular fungi that belong to different taxonomic groups. Yeasts of the *Saccharomyces sensustricto* species have been used for thousands years by mankind for fermenting beverages as they are able to convert sugar into ethanol and CO_2 via fermentation.

1.2 Review of Literature

1.2.1 Rice beer in general

The process of preparing alcoholic beverages and its consumption is one of the oldest activities of mankind [13]. The production of beer from rice is very common in the Asian countries and is known by different names such as *shaosingiju* and *lao-chao* of China, *sake* of Japan, *chongju* and *takju* in Korea, *tapuy* in Phillipines, *brembali* and *tape-ketan* in Indonesia, *khaomak* in Thailand, *rou nep than* in Vietnam and *tapai-pulul* in Malaysia [14].

In the Indian subcontinent, fermented food and beverages, prepared using local food crops and other biological resources have been going on since time immemorial and is a common practice even today [15]. The North-Eastern region of India is comprised of the cluster of eight states namely Assam, Meghalaya, Arunachal Pradesh, Mizoram, Nagaland, Tripura, Manipur and Sikkim. The region can be physiographically categorized into the Eastern Himalayas, Northeast hills (Patkai-Naga Hills and Lushai Hills) and the Brahmaputra and Barak Valley plains. Northeast India is characterized by a vast assortment of tribal communities, each of which belongs to the aboriginal race of the region. Approximately 225 tribes out of the 450 tribes of India reside in this region [16]. The people of these states have a very rich reserve of traditional knowledge owing to their livelihood in the hilly terrains. This area is inhabited largely by tribal people who make up 75% of the population of the region [17]. These people possess great knowledge of the environment and depend on the forests, plants and plant products for food and other purposes [18]. Learning about edible plants and processing and conservation of foods for consumption and medicinal purposes has been in the large part due to incremental and cumulative learning among these societies living in close connection with nature [19]. These people of Northeast India have a strong correlation with nature, which is revealed by the fermentation technologies practised and their expertise in assessment of the microbial benefits.

All the tribal communities of Northeast India predominantly cultivate rice and its consumption in the form of fermented alcoholic beverage is a common traditional practice and many of them have been preparing it since time immemorial [6,7]. The preparation and consumption of this type of liquor emerged mainly due to the climatic conditions and discovering the use of surrounding natural resources [9]. The product is mildly alcoholic and is sweet flavoured [20]. This drink also plays an important role in the socio-cultural life of the tribal people as it is found to be associated with many occasions like merry making, ritual ceremonies, festivals, marriages and even death ceremonies [21]. This age old product which is generally consumed as a mild alcoholic beverage is considered to have certain medicinal properties and is being used by the tribal people as a traditional medicine for many ailments. There are also reports of rice beer being used as a drug [22] and it is said to be effective against insomnia, headache, body ache, inflammation of body parts, diarrhoea and urinary problems, expelling worms and as a treatment of cholera [11,12].

In North-East India rice beer is prepared using starter cultures in the form of dried cakes. Various parts of plants are used in the preparation of starter cultures, by different tribes depending on different geographical locations [10,12,22,23,24,25,26]. The starter cultures used in North-Eastern part of India in preparation of rice beer are similar to other Oriental starters such as *Ragi* of Indonesia, *Nuruk* of Korea, *Bubod* of the Philippines, *Loogpang* of Thailand, *Chiuyueh* of China and *Men* of Vietnam [27]. Various plant materials are used in the preparation of these starters. All of the tribes prepare their indigenous alcoholic beverages at home using round to flattened solid ball-like mixed dough inocula or starter [6,27] and these contain amylolytic and alcohol-producing yeasts, starch degrading moulds and lactic acid bacteria [28].

A large number of volatile and aromatic compounds have also been identified in rice beer by GC-MS methods and have been reported by several authors [29-33]. Some of the compounds reported are ethanol, *n*-propanol *iso*-butyl alcohol, *n*-butanol, *iso*-amyl alcohol, 2,3-butanediol, benzene ethanol, ethyl acetate, *iso*-butyl acetate, *iso*-Amyl acetate, ethyl pyruvate, ethyl lactate, acetone, diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), acetic acid, *iso*-butyric acid, furfural, cyclopentane, heptanes, n-octane, trans 2-octene, isobutyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-octen-3-ol, isobutyraldehyde, butanal, 2-methylbutyraldehyde, isovaleraldehyde, hexanal ketones, 2-butanone furans, 2-methylfuran, 3-methylfuran 2,5-dimethylfuran, 2-pentylfuran, isopropyl formate, ethylbenzene, propylbenzene, α-methylstyrene, benzaldehyde, phenylacetaldehyde, acetophenone, limonene, 1,8-cineole, dimethyl trisulfide, cyclohexyl, isothiocyanate etc.

1.2.2 Plants involved in rice beer starter preparation and their properties

The methodology of fermentation carried out by different tribes is almost the same, except that the difference comes from the different types of plant species used in starter culture preparation [34]. Some of the plants reported to be used in starter culture preparation along with powdered rice are *Veronia cinerea* Less and *Clerodendron viscosum* Vent in the state of Arunachal Pradesh [23], *Albizia myriophylla* Benth. by the *Maiteis* in the state of Manipur [22], *Amomum aromaticum* Roxb. by the *Jaintia* tribe of Meghalaya [12], *Artocarpus heterophyllus, Cinnamomum bejolghota, Costus speciosus, Desmodium pulchellum, Coffea bengalenses, Cyperus* sp., *Equisetum* sp., *Lygodium flexuosum, Melastoma malabathricum* and many others by the *Deori* tribe of Assam [24], *Plumbago zeylanica* L., *Buddleja asiatica* Lour, *Vernonia cinerea* Less and *Gingiber officinale* in the state of Sikkim [25], *Lygodium flaxuosum* Linn., *Leucas aspera* Spreng, *Cissampelos Pereira*, *Scoparia dulcis* Linn., *Cinamommum glanduliferum* Meissn. and *Piper betle* Linn. by the *Ahoms* of Assam [21], *Glycyrrhiza glabra* L. by the *Dimasas* in Assam [26] and sprouted rice grains by the *Angamis* in Nagaland [35].

Alcoholic beverage is prepared by many of the tribes residing in central India, and plays an important role in the social life of these people, as reported by Kumar and Rao [36]. The authors studied the methodology of preparation of *Handia* which is prepared from grains *Oryza* *sativa* L. in Surguja district of central India. The starter culture is known as *Ranu* or *Ranu goti* and is a mixture of rice flour and roots, barks, rhizome and leaves of several plant species. The plant species reported to be used in the preparation of *Ranu* are *Argyreia bella, Bombax ceiba, Buchanania lanzan, Casearia graveolens, Cassine glauca, Catunaregam spinosa, Cissampelos pareira, Crotalaria albida, Cryptolepis buchanani, Matura letal, Elphantopus scaber, Euphorbia prolifera, Hemidesmus indicus, Holarrhena pubescens, Knoxia sumatrensis, Pueraria tuberose, Scoparia dulcis, Senecio nudicaulis, Symplocos racemosa, Tylophora rotundifolia* and *Wattakaka volubilis.* Similar type of rice beer has also been reported by Ghosh and Das [7], who surveyed the process of rice beer production among the tribal inhabitants of tea gardens in Terai of West Bengal. The starter cake is known as *ranu dabai*, which is a mixture of rice flour with different parts if the plants *Coccinia grandis, Vernonia cinerea, Clerodendrum viscosum, Plumbago zeylanica, Stephania japonica, Stephania glabra, Oroxylum indicum, Mussaenda roxburghii, Scoparia dulcis, Rauvolfia serpentina, Artocarpus heterophyllus* and *Wattakaka volubilis.*

Starter material known as *keem* is also reported to be used in the preparation of alcoholic beverage known as *soor* (from rice or fruits) in the Tons Valley of Garhwal Himalaya. In this, barley flour is mixed with either the leaves, roots, bark, bulbils, fig or whole plant of the species *Artemisia roxburghiana, Berberis lyceum, Boerhaavia diffusa, Cajanus scarabeoides, Callicarpa macrophylla, cannabis sativa, Carissa opaca, Cassia tora, Cinnamomum tamala, Cissampelos pariera, Cocculus hirsutus, Colebrookia oppositifolia, Cymbopogon martini, Datura stramonium, Dicliptera rozburghiana, Dioscorea bulbifera, Euphorbia royleana, Ficus benghalensis, Ficus semicordata, Geranium nepalensis, Ichnocarpus frutescens, Indigofera linifolia, Leucas lanata, Melia azedarach, Parthenocissus semicordata, Physalis minima, Pinus roxburghii, Punica granatum, Rhus parviflora, Roylea cinerea, Rubus niveus, Sapindus mukorossi, Skimmia anquetila, Syzgium cumini, Vitex negundo, Woodfordia fruticosa* and *Zanthoxylum armatum* [9]. The Bhotiya community of Uttaranchal Himalaya prepares *Balam* which is s a wheat based starter culture used in the fermentation of several beverages. The plant species used in its preparation are *Chinnamomum zeylanicum, Amomum subulatum, Piper longum*, and *Ficus religiosa*. Some amount of old starter culture is also added to the mixture [9,37]. The pH, moisture content and ash content in samples of *balam* were recorded as 6.6, 9.4 % and 2.4 % respectively. The starch, fat and protein content were found to be 2.5 %, 2.0 % and

1.47 mg/gm respectively. Microbiological examination of the samples has revealed the yeast species present as *Saccharomycopsis fibuligera, Kluyveromyces maxianus* and *Saccharomyces* spp. The bacteria isolated were considered to be close to the genus *Bacillus* [37].

The rice beer known as *chhang/lugri* and the traditional inoculums known as *phab* prepared in the tribal belt of Lahaul and Spiti of Himachal Pradesh were studied by Thakur et al. [38]. *Saccharomyces cerevisiae*, *Bacillus* spp and Actinomycetes were isolated from samples of *phab.* Whereas *Saccharomyces cerevisiae*, *Candida cacoi*, *Leuconostoc* sp. and *Lactobacillus* sp. were isolated from samples of *chhang* and *lugri.* The tribal people of Orissa also prepare rice beer known as *handia* by using the starter cake known as *bakhar.* Bhakar is a mixture of powdered rice with different parts of the plants *Cissampelos pareira, Diospyros melanoxylon, Lygodium flexuosum, Orthosiphon rubucundus, Ruellia tuberose* and *Terminalia alata* [39].

The naturally occurring phytochemicals in plants like alkaloids, tannins, flavonoids, steroid, terpenoid, phenol-saccharide conjugate and other phenolic compounds have various bioactive properties [40] and have various antimicrobial and antioxidant activities, both in *in vitro* and *in vivo* conditions. In recent years, there has been renewed interest in plant derived natural bioactive compounds which might confer health benefits and help in preventing the infections caused by pathogenic microorganisms. Polyphenols are a wide range of biological molecules that can reduce the susceptibility of biological molecules to oxidants and their chemical structure determines their physiological actions, including the antioxidant activity [41,42]. They are the secondary metabolites produced by higher plants and they have potential health benefits on human health, mainly as antioxidants, antiallergic, antiinflammatory, anticancer, antihypertensive, and antimicrobial agents [43]. The impact of bioactive compounds derived from plants in preventing or controlling pathogenic microbes is enormous. Many reports are available on the use of plant derived phenolics in the control of human pathogenesis [44-48].

Free radicals and reactive oxygen species (ROS) are formed continuously as normal by-products of oxygen metabolism during mitochondrial oxidative phosphorylation in living organisms and results in cell death and tissue [49]. It correlates with diseases like cancer, coronary heart diseases, diabetes, neurodegenerative diseases, neural disorders, atherosclerosis, inflammatory injury and aging [50]. Antioxidants can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [51]. Antioxidants in the form of phenolic acids and flavonoids derived from plants have gained much attention for potential use in food, cosmetic and pharmaceutical products [52]. The phenolic compounds in plants have antioxidant properties and can act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating [53]. The antioxidants are of two types, viz. primary or chain breaking type which involves the addition of trace amount to inhibit oxidation of the substrate and the secondary or preventive antioxidant which retards the rate of oxidation through the removal of the substrate or singlet oxygen quenching [54].

Plant phenolics frequently occur in conjugation with glycosides and are usually located in the cell vacuolar structures, and solvent extractions are the most commonly used procedures to extract and liberate those [55]. The yield of the liquid-solid extraction is significantly influenced by the type of solvents with varying polarities, solvent proportion, extraction time, temperature, pH, liquid/solid ratio and particle size, as well as on the chemical composition and physical characteristics of the samples [56]. The efficiency of extraction is basically a function of process conditions and role of each factor in the mass transfer process may either be positive or negative. Each solvent system may show different behaviour towards each material [57].

1.2.3 Microbes involved in rice beer fermentation

In Asian countries, beer prepared from rice using traditional solid-state starters is a popular traditional alcoholic beverage [25]. These are highly nutritious and functional due to the content of various proteins, sugars, vitamins, bioactive compounds and various organic acids [58]. They are also a gluten free alternative to the conventional barley beer the consumption of which is not safe for gluten-sensitive coeliac patients [59]. These beers are produced differently than the conventional beers made from barley or wine made from grapes. The pH is acidic as the organic acids such as lactic acid, citric and tartaric acids and acetoin, which also impars a sour taste and a pleasant flavor. Selective plant parts are used in the preparation of these rive beer starters and diverse groups of endophytic organisms of plant origin serves as the functional microbes for mixed culture and multistage fermentation of rice [60].

The appearance of amylolytic fungus in beer starters have been reported earlier, like *Mucor*, *Rhizopus* and *Aspergillus* in takju of Korea [61], *Mucor circinelloides*, *Rhizopus* chinensis and *Saccharomycopsis fibuligera* in marcha of Nepal [62] and *Mucor circinelloides*, *Rhizopus chinensis* and *Rhizopus stolonifer* in marcha of Darjeeling, Sikkim and other parts of

Northeast India [63]. Also in 39 samples of nuruk (Korea), 174 filamentous fungal strains were isolated among which 6 genera (*Lichtheimia, Aspergillus, Rhizopus, Rhizomucor, Mucor* and *Syncephalastrum*) and 17 fungal species were identified. Molecular methods to identify fungus include analysis of polymorphism in DNA region that encodes the ribosomal RNA genes (5S, 5.8S, 18S and 26S) and the non-coding ITS (internal transcribed spacers) and IGS (intergenic spacer) [13]. For sequencing fungus, the ITS region is the most commonly used target because of sensitive detection by PCR as multiple copies of this ribosomal gene are present in all organisms, and also it is the optimal target for developing specific PCR primers since both highly conserved and variable regions are present in it [64].

Yeasts are unicellular fungi that belong to different taxonomic groups. Yeasts of the Saccharomyces sensustricto species have been used for thousands years by mankind for fermenting beverages as they are able to convert sugar into ethanol and CO_2 via fermentation [65]. The *Saccharomyces sensustricto* species complex contains *S. cerevisiae* (used in wine, bread, ale and weiss beer and sake fermentations), *Saccharomyces bayanus* (used in wine and cider fermentations) and *Saccharomyces pastorianus* (used in lager beer fermentation) [66]. The strains of brewing yeasts employed in the production of beers worldwide are classified into the categories based on flocculation behaviour, viz., top fermenting (ale yeast) and bottom fermenting (lager yeast), thereby categorizing two main classes of beer types (ales and lagers). Ale yeasts, which are basically *Saccharomyces cerevisiae* strains, are genetically more diverse, ferments at higher temperatures (18–24 ºC) and in traditional open fermenters rise to the surface of the vessel. They produce beer with vanilla, clove, spicy and nutmeg flavours due to the presence of the POF gene (PAD1). Lager yeasts are more genetically conserved, ferments at lower temperatures (8–14 ºC) and has tendency not to rise to the surface under any set of fermentation conditions. They have passed through iterations of *Saccharomyces carlsbergensis* and *Saccharomyces cerevisiae* lager type to the currently accepted name, *S. pastorianus*. Based on chromosomal fingerprints, the lager yeasts are further divided into the Carlsberg and Tuborg types. Phenotypic characteristics such as colony morphology, microscopic appearance, fermentation characteristics, growth at 37 ºC, utilization of melibiose and the presence of the POF gene can help to distinguish these yeast types [66,67].

There have been reports of yeast have been identified from rice beer or starter cultures. A strain of yeast isolated from zutho, a local rice beer from Kohima district in Nagaland, India has been identified as *Saccharomyces cerevisiae* and was found to be suitable to be used as a brewing yeast [35]. Yeasts such as *Saccharomycopsis fibuligera*, *Pichia anomala*, *Saccharomyces cerevisiae* and *Candida glabrata* have been identified in bhaati jaanr, a traditional fermented rice beverage of the Eastern Himalayas. It was also seen that *Saccharomycopsis fibuligera* was more dominant on the second day of fermentation than that of other yeasts [63]. The species of yeasts isolated from murcha (amylolytic starter) used to prepare jaanr (rice beer) in Sikkim, India have been identified as *Saccharomyces bayanus*, *Saccharomycopsis fibuligera*, *Saccharomycopsis capsularis*, *Candida glabrata*, *Pichia anomala*, and *Pichia burtonii* [25]. The yeast *Saccharomyces cerevisiae* have been identified and characterized from nuruk (starter) used to produce the Korean traditional rice wines yakju and takju [61].

There are several potential health or nutritional benefits from some species of LAB which includes improved nutritional value of foods, control of certain types of cancer and intestinal pathogens, control of serum cholesterol levels and improved lactose utilization. In order to utilize a specific benefit from LAB is necessary to consider not only the wide variation among species but also that among the strains [68]. LAB are common components of probiotics, due to the fact that they are 'generally regarded as safe' as they have long been used in the manufacture of dairy foods and are desirable members of the intestinal rnicroflora [69]. When probiotics are added to any food product, they should be able to survive in the product and become active when entering the consumer's gastrointestinal tract [70]. Hence a study of various physiological and functional propertied of LAB strains is essential to be carried out before they can be applied for any probiotic purpose.

In East-Asia, lactic acid fermentation has been applied for various purposes such as to preserve perishable vegetables, prepare acidic dishes, to ensure the quality of rice wines, and to make plant based beverages [71]. LAB are Gram-positive and usually catalase-negative bacteria which comprise a wide range of genera and the most important ones are Lactobacillus, *Lactococcus, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Weissella*, *Carnobiacterium*, *Tetragenococcus*, *Oenococcus* and *Vagococcus* [72,73]. With over 60 species, Lactobacillus is a heterogeneous genus (ymol% G + C content ranging from 33 to 55) about one-third of which are strictly heterofermentative [73]. LAB has long been safely applied in the production of fermented foods and beverages. They occupy a central role in traditional starter cultures and are known to enhance the shelf life, and improve microbial safety, texture, and sensory profile of the fermented product, mainly through the production of organic acids, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes. They have been industrially used in the production of antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, useful enzymes, or nutraceuticals, or as probiotics [74].

Much of the works on starter cakes have been done on *marcha* and *hamei*. *Marcha* and *hamei* are mixed dough inocula used as starters for preparation of various indigenous alcoholic beverages in the North-Eastern states of Sikkim and Manipur respectively [27]. Tamang and Sarkar [62] studied various characteristics of *marcha* cakes and found them to be mild acidic (pH 5.2) with 13% w/w moisture and 0.7% w/w ash (dry weight basis). Microbiological examination of the samples revealed the predominant species to be *Pediococcus pentosaceus*, *Saccharomycopsis fibuligera, Pichia anomala, Mucor circinelloides* and *Rhizopus chinensis.* Amylolytic activity was exhibited by the moulds *M. circinelloides* and *R. chinensis* and the yeast *S. fibuligera*. Tsuyoshi et al. [25] isolated 22 strains of yeast from sample of *marcha* collected from different regions of Sikkim. By phylogenetic and phenotypic study, they were identified as *Saccharomyces bayanus, Candida glabrata, Pichia anomala, Saccharomycopsis fibuligera, Saccharomycopsis capsularis and Pichia burtonii.* Out of these, *S. fibuligera, S. capsularis and P.burtonii* had shown amylolytic activity. Whereas, ethanol production was exhibited by *S. bayanus, C. glabrata* and *P. anomala.*

In another work, Tamang et al. [27] isolated the lactic acid bacteria (LAB) from *hamei* and *marcha* and identified them based on phenotypic and genotypic characteristics and also studied some of their technological properties. They reported the average population of LAB in *hamei* to be 6.9 log CFU/g and *marcha* to be 7.1 log CFU/g. The isolates from *hamei* were identified as *Lactobacillus plantarum* and that from *marcha* as *Lactobacillus brevis.* Whereas, *Pediococcus pentosaceus* was found in both the type of samples. All the strains of LAB isolated from *hamei* showed strong antimicrobial activity against *Listeria innocua*, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus,* whereas in m*archa* only one strain of *P. pentosaceus* showed strong inhibition zones. The strains of *Pediococcus* isolated from *hamei* were found to produce bacteriocin against *Listeria innocua* and *Listeria monocytogenes*. The molecular identification of yeast species associated with *hamei* has been reported by Jeyaram et al. [6]. Yeasts were found in the range of 8-9 log CFU/ g and moulds in 5-7 log

CFU/g. They carried analysis of the restriction digestion pattern generated from PCR amplified internal transcribed spacer region along with 5.8S rRNA gene. The restriction analysis was carried out with three endonucleases (*Hae* III, *Cfo* I and *Hinf* I). Based on ITS-RFLP profile nine different groups were identified as *Saccharomyces cerevisiae, Pichia anomala, Trichosporon sp., Candida tropicalis, Pichia guilliermondi, Candida parapsilosis, Torulaspora delbrueckii, Pichia fabianii* and *Candida montana*. The most frequent yeast species were found to be *S. cerevisiae* (32.5%), *P. anomala* (41.7%) and *Trichosporon* spp (8%).

Bhatti jaanr is a fermented rice beverage and prepared in the rural areas of Darjeeling, Sikkim and other parts of Northeast India. Tamang and Thapa [63] prepared *bhatti jaanr* by using *marcha* and studied the fermentation dynamics. The filamentous moulds *Mucor circinelloides, Rhizopus chinensis* and *Rhizopus stolonifer* were isolated during the initial stage of fermentation. Their population was found to decrease during fermentation and disappeared after the fifth day. The yeasts *Saccharomycopsis fibuligera*, *Pichia anomala, Saccharomyces cerevisiae* and *Candida glabrata* were isolated and it was found that the population of yeasts increased from 5 log CFU/g to 8 log CFU/g after two days and decreased to a level of 5 log CFU/g after ten days. It was also seen that *S. fibuligera* was more dominant on the second day than that of other yeasts. The LAB isolated were identified as *Pediococcus pentosaceus* and *Lactobacillus bifermentans* and their population increased until the second day of fermentation and then declined slowly. Thapa and Tamang [20] examined 40 samples of *kodo ko jaanr* which is a popular fermented finger millet beverage in the Eastern Himalayan regions and is prepared using *marcha* as the starter. The total count of aerobes was 7.4 log CFU/g, yeasts ranged from 6.3 to 7.4 log CFU/g and LAB counts ranged from 4.1 to 6.5 log CFU/g. Phenotypic characterization led to the identification of the yeast species as *Pichia anomala, Saccharomyces cerevisiae, Candida glabrata* and *Saccharomycopsis fibuligera.* Out of the LAB isolates, the cocci-tetrads were identified as *Pediococcus pentosaceus* and the rods were identified as *Lactobacillus bifermentans.* The pH, acidity (percentage of lactic acid) and alcohol content in the products ranged from 3.7 – 4.5, 0.23 – 0.5% and 1.8 – 8.7% respectively. There was also found an increase in the content of manganese, iron and phosphorus in the product than the raw material. Characterization of *zutho* (an alcoholic beverage prepared from rice in the state of Nagaland) has been done by Teramoto et al. [35]. They isolated a strain of yeast from *zutho* and concluded that it closely resembled *Saccharomyces cerevisiae*. The fungus *Rhizopus* spp was found in the starter sprouted rice grains, but it was not isolated from the sample of *zutho.* The collected sample was found to have alcohol content, pH and acidity of 5.0%, 3.6 and 5.1 respectively.

Lactose assimilating yeast from Nepalese *murcha* to elucidate the lactose assimilation by indigenous of yeast in Nepal was studied by Tiwari et al. [23]. In this study, 31 strains were isolated from 8 murcha samples from different localities of Nepal. They found that around 22.58% of isolates possessed lactose assimilating activity, which are almost of *Bullera* spp. All the lactose positive strains were able to assimilate glucose, sucrose and maltose. Around 57.14% of the lactose assimilating isolates were also able to assimilate galactose. Sixty-nine strains of bacteria were isolated from *murcha* and *ragi* (amylase starters) belonging to Java, Bali, and Nepal by Hesseltine and Ray [75]. Most of them belonged to *Pediococcus*, probably *P. pentosaceus*, and to *Streptococcus faecalis*. None of the isolates were found to utilize starch directly unless yeasts and moulds were added at the same time. They concluded that these bacteria may be involved in the production of certain secondary products from the glucose formed by the amylolytic yeasts and moulds.

The effect of microflora of mana which acts as a starter culture on brewing of raksi (distilled liquor in Nepal) have been reported by Nikkuni et al. [76]. In this study, they found that a rice koji mana sample from Nepal contained 6.1 log CFU/g of mucorales, 7.5 log CFU/g of aspergilli and 5.04 log CFU/g of lactic acid bacteria and less than 3 log CFU/g of yeast. They also found that the sequence of partial 18S rRNA gene of the isolate was identical to those of *Aspergillus oryzae* and *Aspergillus flavus.* Shrestha et al. [77] also reported the microbial population of *murcha* and *poko* (rice based fermented food) samples from Nepal. They found that lactic acid bacteria and yeast were dominant at 5.6 log to 9 log CFU/g range while fungi were present at 5.3 log to 7 log CFU/g. Coliforms (2 to 5.1 log CFU/g*), E. coli* (3 log CFU/g), and. *S. aureus* (2 log CFU /g) were present in some of the *murcha* starters. *Saccharomyces cerevisiae*, *Candida versatilis*, *Lactobacillus* spp, *Pediococcus* spp and *Rhizopus* spp were identified from the *poko* sample. The pH, acidity, reducing sugar, total sugar, and alcohol after 2 and 3 days of fermentation was found in the range of 3.2-3.0, 1.1-1.3 (% lactic acid), 14.4-15.6 (%),14.6-18.2 (%) and 1-1.6 (%) respectively.

The diversity of yeast in loog-pangkao-mag (starter for alcoholic sweetened rice) and loog-pang-lao (starter for rice wine) used in Thailand was studied by Limtong et al. [78]. The yeast species present in both types of samples were *Saccharomycopsis fibuligera, Pichia anomala*, *Issatchenkia orientalis* , *P. burtonii* , *P. fabianii* , *Candida rhagii* , *C. glabrata*, *Torulaspora globosa, P. mexicana* and one isolate each of *P. heimii, Rhodotorula philyla*, *Saccharomyces cerevisiae*, *Trichosporon delbrueckii* and *T. asahii.* Strain *S. fibuligera* was the single yeast species found in 60.53 % samples of loog-pang-kao-mag and 36.84% samples of loog-pang-lao. Strains of *S. fibuligera* revealed strong amylolytic activity and produce low ethyl alcohol (2 % v/v from 18 % glucose at 48 hours). Whereas, other yeast species showed low amylolytic activity but high or moderately high in alcohol fermenting ability (as high as 6.03% v/v). Lactic acid bacteria present in *rage tape,* which is a traditional dry starter of Balinese rice wine, were identified on the basis of 16S rDNA sequencing by Sujaya et al. [79]. The species identified were *Pediococcus pentosaceus, Enterococcus faecium, Lactobacillus curvatus, Weissella confuse* and *W. paramesenteriodes.* In one of the studies carried out by Menz et al. [80]. Eighty numbers of microbrewed beers were screened from 19 breweries for lactic acid bacteria. Almost 30 % of them contained culturable lactic acid bacteria, and many had lactic acid levels well above the flavour threshold. The pH values ranged from 3.64 to 4.61, with a mean of 4.16 and the ethanol levels ranged from 2.90 to 11.0 % (v/v). RAPD-PCR revealed the strain as *Lactobacillus brevis,* which was found to be the most frequently isolated species. All isolates were capable of spoiling beer and contained putative hop resistance genes.

In East-Asia, lactic acid fermentation has been applied for various purposes such as to preserve perishable vegetables, prepare acidic dishes, to ensure the quality of rice wines, and to make plant based beverages. LAB are Gram-positive and usually catalase-negative bacteria which comprise a wide range of genera and the most important ones are *Lactobacillus*, *Lactococcus, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Weissella*, *Carnobacterium*, *Tetragenococcus*, *Oenococcus* and *Vagococcus* [81]. A variety of region specific fermented foods and beverages are traditionally produced and consumed, and even locally marketed in North-East India. Different types of substrates and fermenting organisms are being employed for the production of these ethnic products and the process employed also varies from place to place.

1.2.4 Preparation of beer from alternative sources and sensory evaluation

Fermentation is an age old tradition and is carried out in many parts of the world including India and extensively across Northeast India. Some of the commonly fermented food materials in Northeast India are soy beans, leafy vegetables, rice, fish and bamboo shoots. In the state of Assam, the starch rich food crops such as rice, banana and cassava are cultivated extensively. The culinary or plantains are a rich source of nutrients and plantain (*Musa* ABB) is the only culinary banana found in the entire Northeastern region of India. These are traditionally grown for cooking purposes as a part of staple diet or for processing of more durable products such as flour that can be stored for later use. Cassava is an important root crop in Africa, Asia, South Africa and India, providing energy for about 500 million people. The root is composed almost entirely of carbohydrates which can be used as important food source [82]. The fermentation of rice, plantain and cassava has been carried out traditionally, both as a form of food preservation technology and to produce beer. The fermented product is preferred because of their pleasant taste, texture and colour. Among these, the production and consumption of rice beer is very common. In the process of preparation of rice beer, flattened solid ball-like mixed dough inocula or starter cakes are used and these contain various amylolytic and alcohol-producing yeasts, starch degrading moulds and lactic acid bacteria. The rice beer prepared in the Northeastern states of India are also a potential source of nutrition, owing to their various biochemical compositions such as carbohydrates, amino acids, organic acids and aromatic compounds. However, till today no scientific studies have been in record to optimize the fermentation process of rice beer produced in this region in order to improve its consumption or to obtain processed products.

Various compounds such as organic acids, sugars, polyphenols and carbonyl compounds have impacts on the sensory profiles of beer and these can be evaluated by sensory evaluation [83]. Sensory evaluation may be defined as scientific means of perception of characteristics of food materials by the use of human senses. In this technique human judges are used to measure the sensory characteristics of food such as colour, smell, taste and mouth feel and these are obtained through subjective evaluation [84]. The sensory criterion for judging the quality food provides important and useful information about the characteristics of food to the food industry and food scientists [85]. Proper sensory evaluation helps the consumers to get the best product and also helps the manufacturers to ensure that their product will be successful commercially [86].

1.2.5 Phenolic esters and anti-inflammatory compounds

The phenolic esters are widespread in natural products partially soluble in water and have useful natural antioxidant properties [87]. They have bioactive roles, e.g., NF-kB inhibition by caffeic acid phenylethyl ester (CAPE) which is obtained from honeybee propolis, HIV-1 reverse transcriptase inhibition by the EGCG mimic and hydroxytyrosol gallate. These compounds are unsophisticated structurally; however their synthesis is typically complicated, as the groups need to be protected in order to obtain improved chemoselectivity, or employment of harsh conditions or excess use of one of the reactants [88,89]. They are also used in medicine synthesis as important intermediates [88]. The esterfication of phenolic acid catalysed by an immobilized lipase from *Candida antarctica* and Novozyme 435 have been obtained with yields ranging from 3 to 98 % [87]. Phenoic acid esters have been obtained in $KHCO_3$/alkyl halide/DMF reaction system by chemoselective esterification of phenolic acids [88]. Also, the selective esterification of r-hydroxycarboxylic acids can be obtained by boric acid catalysis, thereby preventing significant esterification with other carboxylic acids [90]. Esterification yields are linked to the electronic distribution of phenolic acids which affects the reactivity of the carboxylic function and also the carbon chain length of the alcohols [87].

In mammalian cells, cyclooxygenase (COX) is a key enzyme in the biosynthesis of prostanoids from arachidonate. COX exists in at least two isoforms viz. COX-1 and COX-2 [91]. COX-2 is an inducible isoform of COX which is induced by inflammatory stimuli and cytokines and hence its selective inhibition results in anti-inflammatory actions [91,92]. Prostaglandins (PGs) are arachidonic acid metabolites produced by the action of cyclooxygenase enzyme and COX-2-derived PGs have pro-inflammatory properties [93]. Selective inhibitors of COX-2 activity are safer for the gastrointestinal mucosa than the inhibitors of both COX isoforms due to sparing action on in that they spare mucosal prostaglandin synthesis and COX-1 [91,93,94]. COX activity is inhibited by non steroidal anti-inflammatory drugs (NSAIDs) by excluding access for arachidonic acid into the enzyme channel [93]. Several reports are available related to *in silico* studies on inhibition on COX-2 enzyme by bioactive compounds like gallic acid structural analogues derived from *Emblica officinalis* [95], piperine [96], natural compounds found in *Myrica nagi* [97], newly synthesized diaryltriazole derivatives [98], etc.

1.3. Objectives

Based on these survey of literature done and local surveillance, this research work was planned in which the studies were done upon the starter culture used for fermentation and also on the rice beer prepared in Assam. This product which is prepared domestically even today is said to bear some health benefits apart from providing intoxication. The health benefits may be attributed to the various plant species used in the starter cultures and also on the probiotic nature of the product. Since no work has been reporter so far as regards the detailed examination of the starter cultures prepared in Assam, the study in this field was undertaken with the objective of revealing some interesting facts about this form of starter and its ingredients. Further, there is no established report regarding the microflora of the starters and rice beer from Assam and so the identification of these organisms might help in establishing the type of microflora generally present. Moreover, evaluation of the functional properties of the components of rice beer might give a better understanding of its health benefits. Hence, the following objectives were chosen for carrying out this research work.

1. Biochemical and microbiological characterization of rice beer and starter cultures produced in different regions of Assam

- Documentation of the methodologies followed by different tribes in the preparation of rice beer
- Biochemical analysis of the collected rice beer samples
- Studies on the effect of the microbial starters cakes on some quality attributes of rice beer

2. Evaluation of the antioxidative and antimicrobial properties of plants used in rice beer starter culture preparation

- Phytochemical constituents and antimicrobial activity of leaves of *Artocarpus heterophyllus* Lam., *Cyclosorus extensa* (Blume) Ching, *Oldenlandia corymbosa* L. and *Alpinia. malaccensis* (Burm. f.) Roscoe

- *In vitro* antioxidant activity of polyphenols purified from leaves of *Artocarpus heterophyllus* Lam., *Cyclosorus extensa* (Blume) Ching, *Oldenlandia corymbosa* L. and *Alpinia malaccensis* (Burm. f.) Roscoe
- Storage study of rice beer under accelerated temperature condition by incorporation of bioflavonoids from *Artocarpus heterophyllus* and *Cyclosorus extensa* leaves
- Optimization of the extraction of phenolic compounds from *Cyclosorus extensa* with solvents of varying polarities

3. Identification of fungal and lactic acid bacteria isolates from rice beer and starter cultures and evaluation of their functional properties

- Studies on amylolytic properties of fungal strains
- Studies on physiological properties of yeast strains
- Studies on functional properties of lactic acid bacteria

4. Laboratory scale optimization of rice beer making process and sensory evaluation of the products

- Optimization of the fermentation parameters for the preparation of rice beer
- Fuzzy logic assisted sensory evaluation of five different types of beer prepared using combination of three different substrates under optimized conditions

5. Studies on anti-inflammatory role of a newly synthesized ester in *in silico*, *in vitro* and *in vivo* models

- Synthesis of novel ester from compounds present in rive beer and *in silico* studies related its anti-inflammatory role
- Test for cytotoxicity of the compound in animal cell lines and studies on its antioxidative and antiinflammatory activity in animal models

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