Final Report

of the R&D project

Quality Improvement of Traditional Method of Rice Beer Production by the Tribal People of North-East India

Submitted to

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Submitted by

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CONTENTS

1. INTRODUCTION

North East India is characterized by a diverse population of people with different ethnic background. Most of the people of this region are tribal and bear their own methods of fermenting food materials for the purpose of preservation and taste enhancement and they have been carrying these from time immemorial. All the fermented products are region specific and have their own unique substrates and preparation methods. Materials such as soybeans, bamboo shoots and locally available vegetables are commonly fermented by most of the tribes. The fermented alcoholic beverages prepared in this region are unique from the rest of the world in several aspects and bears deep attachment with the socio-cultural lives of the people. The starter cultures used and the utilization of indigenous microbes reflect the expertise of these people in customary microbiology. Microbes such as *Saccharomyces cerevisiae*, *Candida* spp, Lactic Acid Bacteria (LAB) and *Bacillus* spp. have been found to be abundant of common occurrence in these products. These products also serve as a source of economy to many of the rural people, who prepares them at home and market locally. Detailed studies on the nutritive and medicinal value of these products can provide valuable information would prove beneficial in the use of these products on a wider scale. Formulation of new techniques to increase their shelf life would help in the commercialization of these products.

The process of preparing alcoholic beverages and its consumption is one of the oldest activities of mankind. 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, *brem bali* and *tape-ketan* in Indonesia, *khaomak* in Thailand, *rou nep than* in Vietnam and *tapai pulul* in Malaysia. In case of the general type of beer, the main ingredients are yeasts to carry out the fermentation process, a cereal to serve as the source of fermentable carbohydrates, proteins, polypeptides, minerals, etc. and hops which imparts a bitter taste and its hop characteristics and has antimicrobial properties and water (liquor).

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. 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. The preparation and consumption of this type of liquor emerged mainly due to the climatic conditions and discovering the use of surrounding natural resources. There are also reports of rice beer being used as a drug. It works effective against insomnia, headache, body ache, inflammation of body parts, diarrhoea and urinary problems, expelling worms and as a treatment of cholera. All of the tribes prepare their indigenous alcoholic beverages at home using round to flattened solid ball-like mixed dough inocula or starter. 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. Various plants have been reported to be used in the preparation of rice beer starter cultures in North-East India by various authors. Some are *Albizia myriophylla* by the *Maiteis* in the state of Manipur, *Amomum aromaticum* by the *Jaintia* tribe of Meghalaya, *Plumbago zeylanica* , *Buddleja asiatica* , *Vernonia cinerea* and *Gingiber officinale* in the state of Sikkim, *Glycyrrhiza glabra* by the *Dimasas* in Assam, *Ananas comosus, Artocarpus heterophyllus, Calotropis gigantea, Capsicum frutescens* etc. by the Rabha tribe of Assam and sprouted rice grains by the *Angamis* in Nagaland.

In rice beer the process of manufacture consists of the saccharification of the starch present in steamed or boiled rice by fungal enzymes followed by alcoholic fermentation by yeasts supplied by traditional starters. These starters are usually in the form of dry powder or hard balls containing a mixed flora of both fungi and bacteria. However in some cases powdered sprouted rice is also used as starter. The final product contains various live microorganisms and common type are the yeasts *Saccharomycopsis, Saccharomyces, Pichia, Hansenula, Torulopsis* and *Candida*, the moulds *Mucor, Aspergillus* and *Rhizopus*, and bacteria of the type *Pediococcus, Lactobacillus, Micrococcus, Bacillus, Aerobacter* and *Leuconostoc*. During the fermentation process, a succession of microbes with a delicate balance between different kinds is observed along with changes in biochemical parameters especially in sugar contents. The highest organoleptic score has been reported to be obtained in between two and three day period of fermentation and the shelf stability of these products are short. The product is mildly alcoholic and is sweet flavoured. Many volatile compounds have been characterized as odour active compounds in rice beer and these are said to provide alcohol like, sweet, fruity, buttery and pungent aroma.

2. OBJECTIVES

The following were the proposed objectives of the project work

1. Standardization of traditional method of rice beer preparation by the tribal people of Northeast India
2. Biochemical characterization of the plants used as starter material during fermentation in the traditional method
3. To characterize the rice beer both microbiologically and biochemically collected from different tribal belts of Northeast India
4. Replacement of rice grain with tapioca roots as raw material for the production of beer and its quality evaluation both microbiologically and biochemically

Accordingly, all the objectives of the project have been fulfilled in a systematic order. Some additional studies have also been done on the microbiological properties of the starter cakes used for fermentation, which is also being reported.

3. LITERATURE SURVEY

A. At regional level

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 depanding on different geographical locations (Tiwari and Mahanta, 2007, Singh and Singh, 2006, Samati and Begum, 2007, Deori *et al*., 2007, Tsuyoshi *et al*., 2005, Saikia *et al*., 2007, Chakrabarty *et al*., 2009). 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 (Tamang *et al*., 2007).

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 (Tiwari and Mahanta, 2007), *Albizia myriophylla* Benth. by the *Maiteis* in the state of Manipur (Singh and Singh, 2006), *Amomum aromaticum* Roxb. by the *Jaintia* tribe of Meghalaya (Samati and Begum, 2007), *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 (Deori *et al*., 2007), *Plumbago zeylanica* L., *Buddleja asiatica* Lour, *Vernonia cinerea* Less and *Gingiber officinale* in the state of Sikkim (Tsuyoshi *et al*., 2005), *Lygodium flaxuosum* Linn., *Leucas aspera* Spreng, *Cissampelos Pereira, Scoparia dulcis* Linn., *Cinamommum glanduliferum* Meissn. and *Piper betle* Linn. by the *Ahoms* of Assam (Saikia *et al*., 2007), *Glycyrrhiza glabra* L. by the *Dimasas* in Assam (Chakrabarty *et al*., 2009) and sprouted rice grains by the *Angamis* in Nagaland (Teramoto *et al*., 2002).

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 (Tamang *et al*., 2007).

Tamang and Sarkar (1995) 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.* (2005) 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.* (2007) 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 *marcha* 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.*, 2008. 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 as reported by Tamang and Thapa (2006). The

authors 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. During fermentation the temperature remained relatively constant between 28°C to 30°C, the pH decreased from 6.3 to 3.2 within the second day and slightly increased to 3.9 at the end. The titratable acidity expressed as percentage of lactic acid of sample increased from 0.01% to 0.20% till the fourth day and remained at a level of 0.17% till the end. The alcohol content increased from 0 % to 5.9 %. The reducing sugar contents increased from 0.01% to 12.6% by the third day, and declined until the end of fermentation. The total sugar contents decreased from 64.1% to 13.4%.

Thapa and Tamang (2004) 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 identifies 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. The authors 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.* (2002). 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.

B. At national level

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, 2007. 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 (2004), 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 roxburghiana, 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* (Rana *et al.*, 2004).

Kyalakond *et al.* (2006), screened different varieties of rice to test their suitability for rice beer production, using diastase α-amylase as an enzyme. After saccharification, the must was subjected to fermentation by inoculating the standard yeast strain *Saccharomyces cerevisiae* var ellipsoideus 101. The beer obtained was tested for residual reducing sugars, pH, protein, total soluble solids, ethanol, titrable acidity, colour and brightness and organoleptic evaluation by following twenty point scale. Based on the results, the authors concluded that Intan variety of rice (provided by Agricultural Research Station, Mugad) was most suitable for rice beer production among all the varieties studied.

Balam is a wheat based starter culture used in the fermentation of several beverages by the Bhotiya community of Uttaranchal Himalaya. 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 (Roy *et al.*, 2004; Das and Pandey, 2007). 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* (Das and Pandey, 2007).

Thakur *et al.* (2004) studied 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. *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 prepare rice beer known as *handia* by using the starter cake known as *bakhar. Bhakkar* 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* (Dhal *et al.*, 2010).

C. At International Level

A mixed starter inoculums called *murcha* is used in the production of local alcoholic beverages in India, Tibet, Nepal, Bhutan etc. (Tsuyoshi *et al.*, 2005). Tiwari *et al.* (2007) studied the lactose assimilating yeast from Nepalese *murcha* to elucidate the lactose assimilation by indigenous of yeast in Nepal. 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, 1988. 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.

Sayuki *et al.*, 1996 reported the effect of microflora of mana which acts as a starter culture on brewing of raksi (distilled liquor in Nepal). 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*.

Later on, Shrestha *et al.* (2002) 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.

Limtong *et al.* (2002) studied the diversity of yeast in loog-pangkao-mag (starter for alcoholic sweetened rice) and loog-pang-lao (starter for rice wine) used in Thailand. 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, T. delbrueckii* and *Trichosporon 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).

In one of the studies carried out by Menz *et al.* (2010) 80 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.

Sujaya *et al.* (2001) identified lactic acid bacteria present in *rage tape,* which is a traditional dry starter of Balinese rice wine, on the basis of 16S rDNA sequencing. The species identified were *Pediococcus pentosaceus, Enterococcus faecium, Lactobacillus curvatus, Weissella confuse* and *W. Paramesenteriodes.*

A large number of volatile and aromatic compounds have been identified in rice beer by GC-MS methods and have been reported by several authors (Luo *et al.*, 2008; Yoshizaki *et al.*, 2010; Chuenchomrat *et al.*, 2008; Mo *et al.*, 2009; Isogai *et al.*, 2005). 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.

4. INSTRUMENTS PROCURED

A. Deep Freezer

As per the purchase order of Tezpur University No. TU/11-15/PUR/FPT/2010/4912-A dated 30.09.2010, the Deep Freezer (ultra low temperature) (Fig 1) of make New Brunswick Scientific (Model U410) was supplied by the firm Eppendorf India, Chennai, and installation was done successfully on 28.01.2011.

Fig 1: Deep freezer

B. Refrigerated Incubator Shaker

As per the purchase order of Tezpur University No. TU/11-15/PUR/FPT/2010/4917-A dated 30.09.2010, the Refrigerated Incubator Shaker make New Brunswick Scientific (Model E24R) (Fig 2) was supplied by the firm Eppendorf India, Chennai, and installation was done successfully on 28.01.2011.

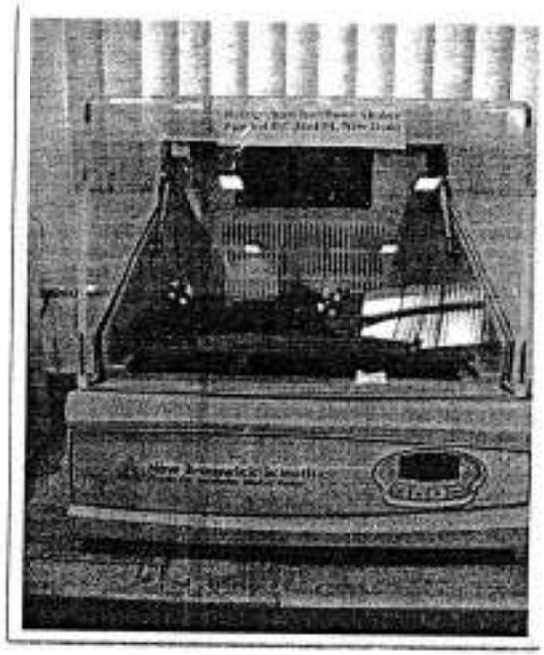


Fig 2: Refrigerated incubator shaker

C. Electronic Balance

As per the purchase order of Tezpur University TU/11-15/PUR/FPT/2010/4913-A dated 30.09.2010, the Electronic Balance of make Sartorius (Model CPA 225D) (Fig 3) was supplied by the firm North East Enterprise, Guwahati, and installation was done successfully on 17.03.2011.

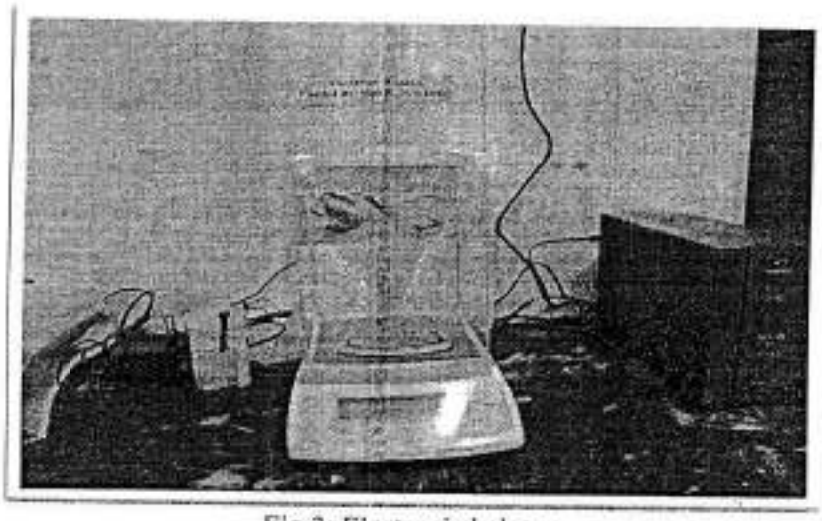


Fig 3: Electronic balance

D. Hot Air Oven

As per the purchase order of Tezpur University TU/11-15/PUR/FPT/2010/4914-A dated 30.09.2010, the Hot Air Oven of make HMG India (Fig 4) was supplied by the firm North East Enterprise, Guwahati, and installation was done successfully on 17.03.2011.

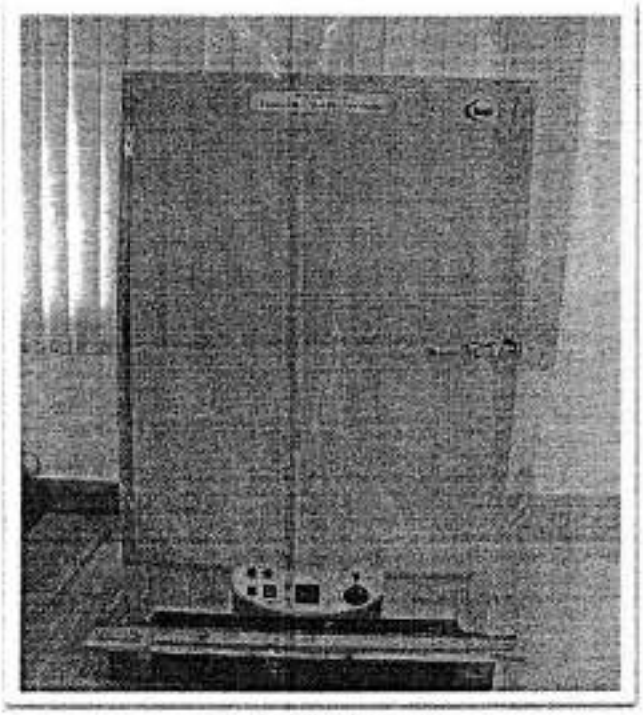


Fig 4: Hot air oven

E. Laminar Air Flow Workstation

As per the purchase order of Tezpur University TU/11-15/PUR/FPT/2010/4915-A dated 30.09.2010, the Laminar Air Flow of make Microfield (Model HHL 4) (Fig 5) was supplied by the firm North East Enterprise, Guwahati, and installation was done successfully on 17.03.2011.

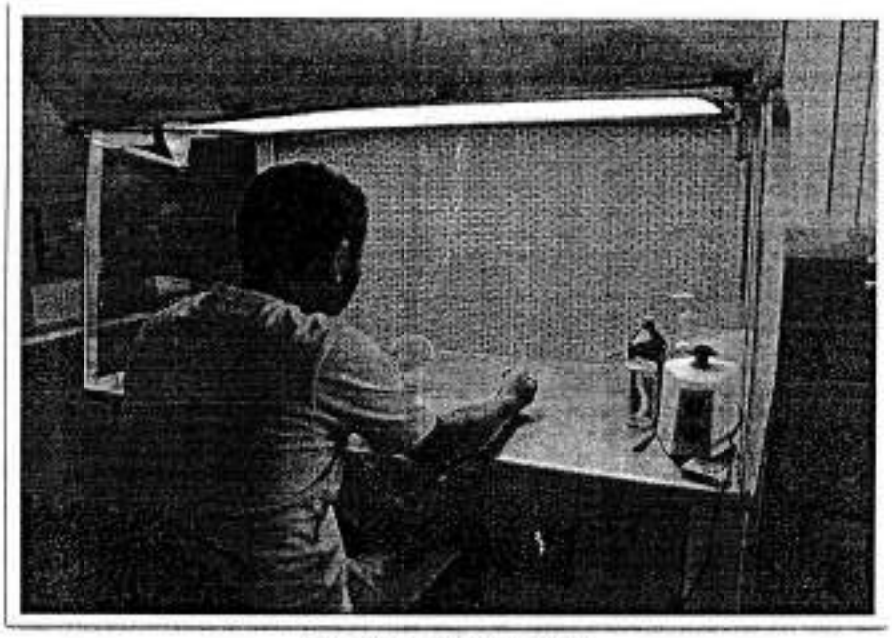


Fig 5: Laminar air flow

F. HPLC System

As per the purchase order of Tezpur University TU/11-15/PUR/FPT/2010/5058-A dated 07.10.2010, the HPLC system of make Dionex (Model Ultimate 3000) (Fig 6) was supplied by the firm Dionex Softron Gmbh., Germany, and installation was done successfully on 07.03.2011.

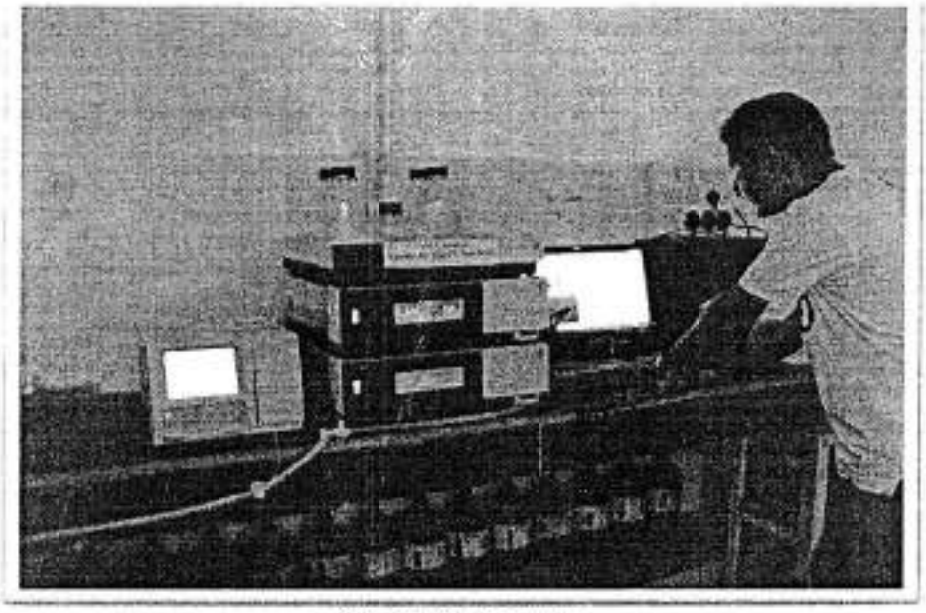


Fig 6: HPLC system

G. Fluorescent Microscope

As per the purchase order of Tezpur University TU/11-15/PUR/FPT/2011/833-A dated 30.05.11, the Trinocular Microscope of make Leica (Model DM 3000) (Fig 7) was supplied by the firm M/s Leica Mikrosysteme Vertrieb GmbH, Germany and installation was done successfully on 20.12.2011.

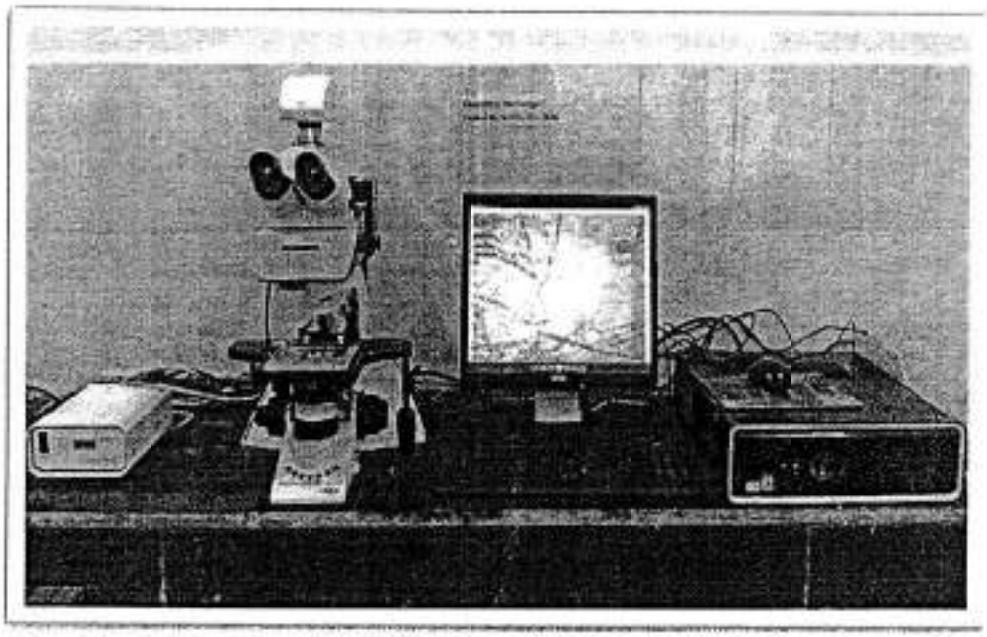


Fig 7: Fluorescent Microscope

H. Fermentor

As per the purchase order of Tezpur University TU/11-15/PUR/FPT/2011/831-A dated 30.05.2011, the Fermentor of make New Brunswick Scientific (Bioflo 115) (Fig 8) was supplied by the firm M/s Eppendorf India Limited, Chennai, and installation was done successfully on 03.02.2011.

Fig 8: Fermentor

I. Autoclave

As per the purchase order of Tezpur University TU/11-15/PUR/FPT/2011/832-A dated 30.05.11, the Autoclave of make HMG India (Fig 9) was supplied by the firm M/s North East Enterprise, Guwahati and installation was done successfully on 21.10.2011.

Fig 9: Autoclave

5. MATERIALS AND METHODS

A. Collection of Samples

A field survey was carried out in the villages and rural areas of the states of Assam, Nagaland, Arunachal Pradesh, Maghalaya and Sikkim for five months (September, 2010 to January, 2011). The areas were selected based on the information available upon the prevalence of traditional methods of rice beer preparation. Information was collected from the producers predominantly involved in the process of making rice beer. The women in all the communities visited were mostly involved and they were inquired about their practices for preparation such as making of starter cakes along with plants and their parts added, fermentation procedure, duration and uses of the beverage. Samples of starter cakes and rice beer were collected in 500 ml sterile containers, marked according to the place of collection, brought to the laboratory and stored at 4^{0}C. Some of the nearby fields and forests were visited along with local help and the available plant samples were collected and stored in plastic bags and sealed.

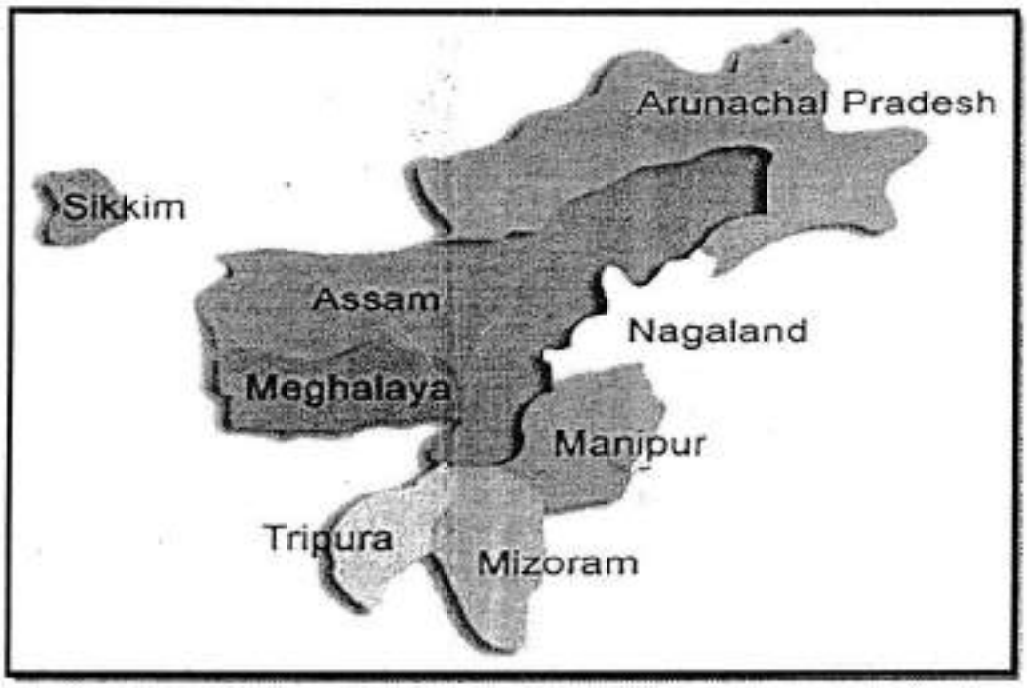


Fig 10: Map of North-East India

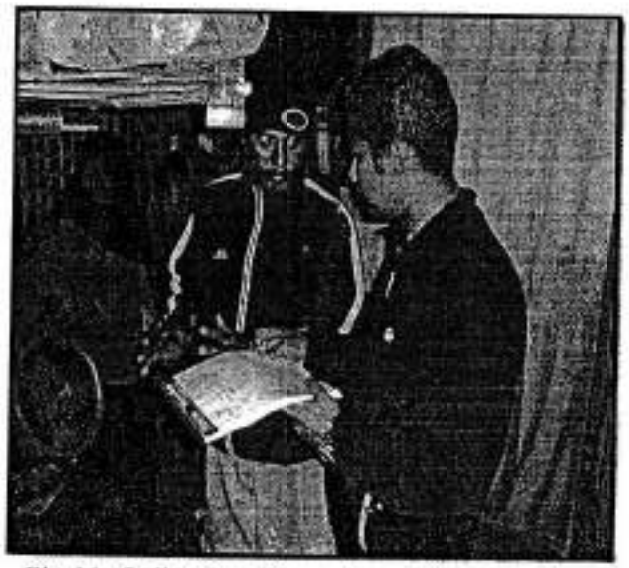


Fig 11: Collection of samples and enquiry about preparation technique in the state of Nagaland

B. Identification of the collected plant samples

The plant samples were dried and made into herbarium as per the guidelines given by Anderson, L. C. 1999.Further identification of the collected plant species, the plant samples and herbariums were done by Department of Agronomy, Assam Agricultural University, Jorhat, Assam and Department of Botany, Darrang College, Tezpur, Assam.

C. Microbial count of the samples

The microbial count of the samples was started immediately after the samples were brought to the laboratory. Different selective growth media were used for the enumeration of different groups of microbes present in the rice beer samples. The plating was done according to the method described by Brown, 2005. All the samples were serial diluted on 0.86% NaCl solution and plated on the specific media by pour plate or spread plate methods. Plate Count Agar (M091, Himedia, India) was used for enumeration of general bacteria at 37^0C. Potato Dextrose Agar (M1368, Himedia, India) supplemented with tartaric acid and Rose Bengal Chloramphenicol Agar (M640, Himedia, India) were used for the isolation of yeasts and moulds respectively at 27^0C. MRS Agar (M641, Himedia, India) supplemented with $CaCO_3$ and bromocresol purple indicator was used for lactic acid bacteria (LAB) and these plates were incubated in an anaerobic gas pack system (LE012, Himedia, India) at 37^0C. SS Agar (Merck, Germany) for *Salmonella* and *Shigella*

species, Baird Parker Agar Base (M043, Himedia, India) for coagulase positive *Staphylococcus* species, McConkey Agar (M008S, Himedia, India) for lactose fermenting and lactose non-fermenting enteric bacteria, Modified MYP Agar (M1139, Himedia) for *Bacillus cereus* species and EMB Agar (M317, Himedia, India) for Enterobacteriaceae (plates incubated in an anaerobic gas pack system (LE012, Himedia, India) at 37^{0}C) was also used. The results obtained were expressed as colony forming units (CFU) per ml of sample. Colonies were isolated, examined under the microscope, purified and preserved in glycerol stocks at -80^{0}C for further investigation.

D. Preparation of the test samples for biochemical analysis

All the collected samples were made CO_2 free prior to analysis. This was done by transferring the test sample to a large flask and shaking, first gently and then vigorously, maintaining the temperature at 20-25^{0}C (AOAC Official Method 920.49, 2010).

E. Measurement of pH

The pH of the samples was determined according to AOAC Official Method 945.10 (2010). The undiluted test portions were tested in a digital pH meter (pH510, Eutech Instruments) using pH/reference electrode system. The pH meter was checked before and after use against standard potassium acid phthalate buffer.

F. Ashing of the samples

The ash content of the samples was estimated according to AOAC Official Method 920.54 (2010). The sample was weighed and heated in a platinum crucible at 100^{0}C. It was then heated slowly over a flame after adding a few drops of pure olive oil. The crucible was then placed in a muffle furnace (LEF – 105S-2, Labtech) at 525^{0}C until white ash was obtained. It was cooled in a desiccator and the weight was taken. The weight difference gave the content of ash. All the weight measurements were done at 20^{0}C.

G. Biochemical analysis

a. Total acidity

The indicator titration method under AOAC Official Method 950.07 was used to obtain the total acidity of the samples. The samples were mixed with boiling water and boiled for another 60 seconds. This was then cooled rapidly and 0.5%

phenolphthalein was added. This was titrated with 0.1M NaOH until the appearance of faint pink colour as the end point. The results were reported as % of lactic acid (1 ml of 0.1M alkali = 0.0090 g lactic acid).

b. Alcohol content

The volumetric content of ethanol was measured spectrophotometrically by the method described by Magri et al, (1997). The diluted sample is first mixed with 3.3mM aqueous $K_2Cr_2O_7$ and 65% perchloric acid, homogenized and allowed to stand for 20 minutes at 25°C. The absorbance (A_1) was measured at 267 nm against a 3 M perchloric acid solution. A second set or absorbance reading (A_0) was taken for samples which were made alcohol free by evaporating in a vacuum oven. The content of alcohol was calculated according to the formula given below.

Alcohol content (%) = $3(A_0-A_1)$ 11.51 D/0.78934V

Where, D = dilution factor of the sample and V = volume of the sample withdrawn for analysis.

c. Protein content

The protein content in the samples was determined by reaction with Folin-Ciocalteu reagent (FCR) according to the method of Lowry *et al.*, (1951). The proteins were extracted in a phosphate buffer, centrifuged and the aliquot was mixed with alkaline copper solution before reacting with FCR. The intensity of blue colour formed after 30 minutes of incubation was measured calorimetrically at 660 nm in a UV-Vis spectrophotometer (Spectrascan UV- 2600, Thermo Scientific). The amount of protein in the samples was calculated by comparison with a standard curve of bovine serum albumin (BSA).

d. Total fats content

The estimation of total fats content was done by slight modification of the method of Cohen (1971). The sample was first made moisture free by drying in a hot air oven at 60°C. The dried matter was taken on a cellulose extraction thimble and subjected to solvent extraction in automated solvent extraction system (SCS 6, Pelican

Instruments) using petroleum ether (b.p 60-80^{0}C) for 3 hours. The remaining ether was evaporated in a rotaevaporator (Roteva, Equitron) at 80^{0}C. The pre-weighed container was then dried in an oven at 100^{0}C for 1 hour and then weighed. The difference in weight gave the content of fats present in the sample.

e. ***Total Sugars, Reducing Sugars, Starch and Amylose content***

The total soluble sugars content was estimated by the anthrone method of Hedge and Hofreiter, (1962). The samples were first hydrolysed with 2.5N HCl in a boiling water bath and then neutralized with Na_2CO_3. They were then centrifuged and aliquot was mixed with anthrone reagent and kept on a boiling water bath for 8 minutes. The intensity of dark green colour was measured at 630 nm in a UV-Vis spectrophotometer (Spectrascan UV- 2600, Thermo Scientific) and results were obtained by comparing with a standard curve of glucose.

Reducing sugars were estimated according to the method of Nelson, (1944) and Somogyi, (1952). The sugars were first extracted with hot 80% alcohol several times and then re-dissolved in distilled water after evaporating the alcohol. The aliquot was mixed with alkaline copper tartarate reagent and placed in a boiling water bath for 10 minutes. Arsenomolybdic reagent was added after cooling the tubes and the intensity of blue colour formed was read after 10 minutes at 620 nm in a UV-Vis spectrophotometer (Spectrascan UV- 2600, Thermo Scientific). A standard curve of glucose was compared for obtaining the concentrations.

The amount of starch present was estimated according to the method of Hedge and Hofreiter (1962). The residue left after extraction of reducing sugars was mixed with water and 52% perchloric acid. Extraction was done at 0^{0}C for 20 minutes and then centrifuged. The supernatant was mixed with anthrone reagent and placed in a boiling water bath for 8 minutes. The intensity of dark green in a UV-Vis spectrophotometer (Spectrascan UV- 2600, Thermo Scientific) at 630 nm and results were quantified by comparision with a glucose standard curve.

The amylose content was determined by following the procedure of McCready *et al,* (1950). The sample was extracted in ethanol and 1N NaOH for overnight and

then centrifuged. The supernatant was neutralised with 0.1 N HCl by using phenolphthalein as an indicator. Then it was added with iodine reagent and the absorbance was read at 590 nm. An amylase standard curve was used for calculating the results.

f. Ascorbic acid content

Ascorbic acid was quantified according to the volumetric method of Harris and Ray, (1935). The sample was first extracted in 4% oxalic acid and then centrifuged. The supernatant was titrated against 2,6-dichlorophenol indophenols dye till a pinkish end point. Comparison was done with the titre value of a standard solution of ascorbic acid and the content of ascorbic acid was calculated based on the ratio between the two titre values.

g. Total phenols content

The concentration of total phenolic compounds was determined by the method of Bray and Thorpe, (1954). The sample was extracted in 80% ethanol and centrifuged. The supernatant was evaporated to dryness and the residue was dissolved in distilled water. The aliquot was first mixed with FCR and then 20% Na_2CO_3 solution was added followed by placing in a boiling water bath for 1 minute. The absorbance was read at 650 nm in a UV-Vis spectrophotometer (Spectrascan UV-2600, Thermo Scientific) and results were calculated by comparison with a standard curve of catechol.

h. Free radical scavenging activity

The free radical scavenging activity was measured as per the method of Brand-Williams et al., 1995. This assay is based on the ability of antioxidant to scavenge the DPPH cation radical. The sample was filtered and dissolved in ethanol to a desired concentration. This was mixed with 0.004% DPPH solution and left for 30 minutes in the dark. The absorbance was then measured at 517 nm in a UV-Vis spectrophotometer (Spectrascan UV- 2600, Thermo Scientific). Scavenging activity (SA) was calculated as percent inhibition relative to control using following equation and expressed as

RSA % (30 min) = control absorbance at 517nm − extract absorbance at 517nm / control abs at 517 nm ×100.

i. Organic acid analysis by HPLC

Extraction procedure: Extraction was carried out according to the method given by Nollet (2000). The samples were first mixed with a mixture of acetonitrile and type I water in a ratio of 70:30. This was mixed properly in a lab grinder and stirred continuously for two hrs. The mixture was then centrifuged for 10 mins at 10,000 rpm. The supernatant was then filtered through Whatman No.4 filter paper and the filtrate was again subjected to solid phase extraction using C18 SekPak cartridge. This extract was used for analysis of organic acid.

Analysis: The analysis of organic acids was carried out in a HPLC system (Ultimate 3000 Dionex Germany). The mobile phase used was 0.2 M Sodium sulphate with pH adjusted to 2.68 with methane sulphonic acid. The detector used was UV detector at 210 nm. A total of 9 standards were used, all of which were procured from Sigma U.S.A.

j. Colour measurement

The color measurement of the *Kachkal* (*Musa* ABB) samples at different growth stages were done by analyzing the colour of the samples in a Hunter Lab Color Quest (Model Ultrascan Vis- Model, USA). The results were expressed in L, a and b systems. L indicates the degree of lightness or darkness (L=0 indicates perfect black and L=100 indicates most perfect white); whereas "b" indicates degree of yellowness (+) and blueness (-); "a" indicates degree of redness (+) and greenness (-).

6. SURVEY WORK AND ANALYSIS OF SAMPLES

A. Methodology of rice beer preparation

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 For preparing the beer, rice (either glutinous or non-glutinous) are half cooked and allowed to cool. It is then mixed with powdered starter cakes and again spread for some time. The mixture is kept on an earthen pot and the mouth is sealed. This is kept in a closed room for a period of 3 to 5 days. After this some amount of water is added to the fermented mass and left for about 10 minutes. The mass is then strained and the liquid obtained is the rice beer. Some of the starter cultures used by different tribes and their methodology of preparation are mentioned here. All these cultures are in the form of cakes are reported to be used for up to a year after preparation.

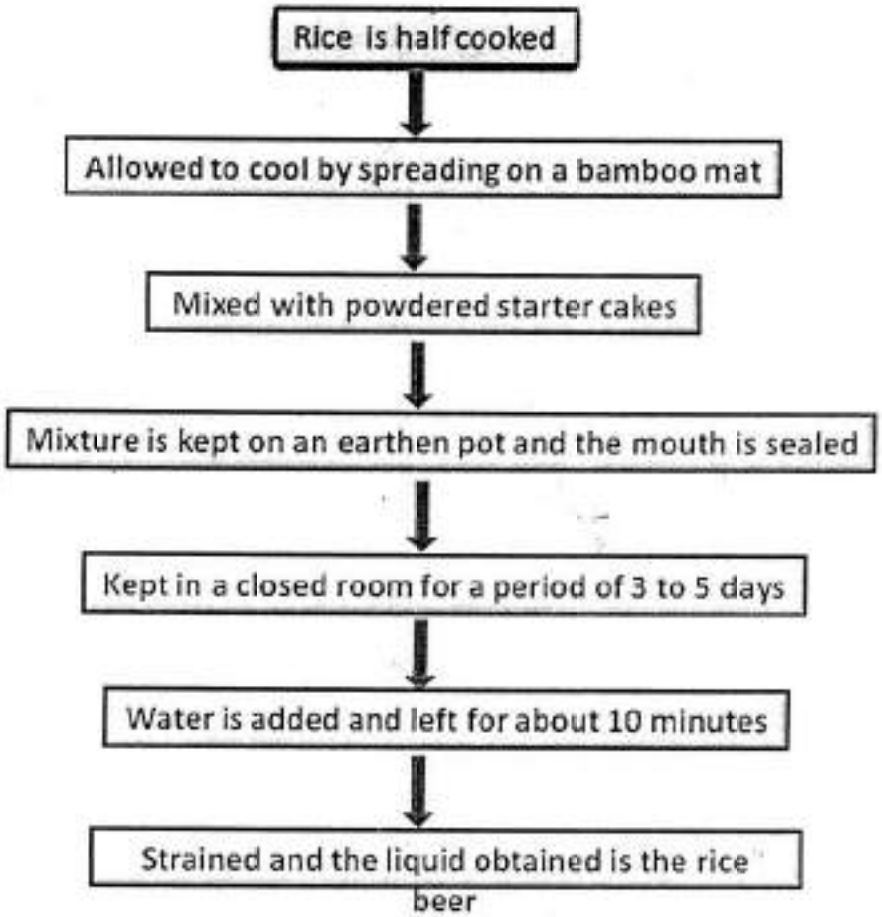


Fig 12: Flow chart of rice beer preparation

B. Analysis of the collected samples

1. Tribe : *Ao / Angami*

Place: Kohima, Nagaland

Nagaland is chiefly a mountainous state and is inhabited by many different Naga tribes. Each of these tribes has some common culture and traditions and they are all regarded as to having warrior background. This study was done in Kohima district of Nagaland, India.

Starter Culture: *Piazu*

This starter material used in the preparation of rice beer is known as *piazu*, which is basically sprouted rice. For preparing *piazu*, un-hulled rice is first soaked in water for a period of about 3-4 days. After this, some of the water is drained out and the grains are allowed to germinate. This may sometimes take about a week depending on the prevailing temperature. After being dried in the air, the sprouted grains are pounded on a wooden mortar with a pestle. The powder obtained is known as *piazu*.

Sometimes they also prepare starter cakes which are flattened and dried forms of the powder. The powdered rice is mixed with a little bit of water to make dough and then cakes are made out of the dough. They are then dried by covering with rice husk in a dark room.

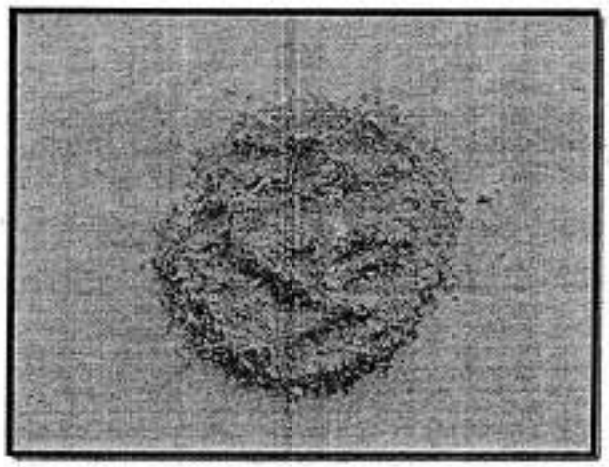


Fig 13: *Piazu*

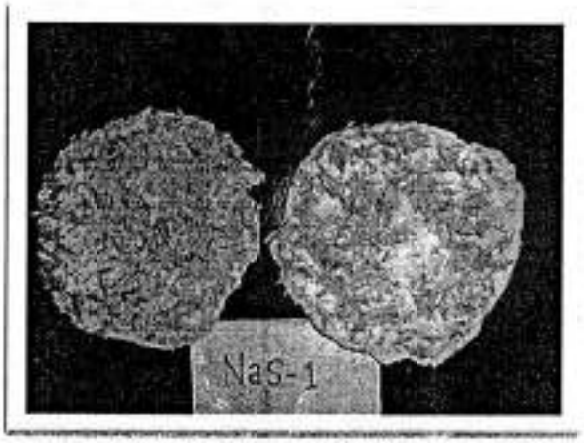


Fig 14: Cakes made of *piazu*

Rice beer: *Zutho / Litchumsu*

For preparing *zutho*, rice is first boiled and then allowed to cool by spreading on a bamboo mat. To this rice, *piazu* (about 10 g for 1 kg of rice) is added and mixed well. The amount of *piazu* added is needed more (almost double) during the months of winter. The mixture is then left to ferment in a closed earthen or wooden vessel for about 4 days in summer and about a week in winter. After completion of fermentation, some amount of water is added to the rice and is filtered by using a bamboo or plastic mesh and usually served in bamboo cups.

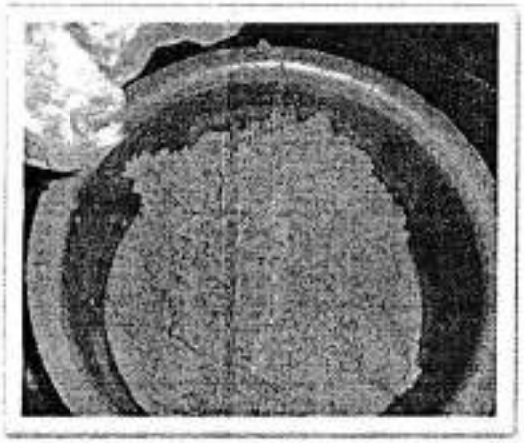


Fig 15: *Zutho* taken out to be added with water

Fig 16: Nags folks filtering *zutho*

Fig 17: *Zutho* being served in bamboo cups

Samples collected

a. Starter culture used by *Ao* Tribe (Code name: NaS1)

b. Rice beer prepared by *Ao* tribe (Code Name: NaB2)

c. Fermented rice of the *Angami* tribe (Code Name: NaR2)

d. Fermented rice of the *Ao* tribe (Code Name: NaR3)

e. Fermented rice of the *Rengma* tribe (Code Name: NaR4)

Microbial analysis of the samples

Table 1: Count of different group of microbes

Sample	CFU / ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobac-teriaceae	Yeasts	Moulds
NaS1	$4x10^8$	$5x10^7$	0	0	0	0	$6.2x10^8$	$9x10^5$
NaB2	$1.8x10^7$	$3.5x10^7$	$2x10^4$	$2x10^2$	0	0	$2.4x10^8$	0
NaR2	$1x10^6$	$3.1x10^7$	$1x10^3$	0	0	0	$2.7x10^7$	0
NaR3	$8x10^6$	$2.8x10^6$	0	0	0	0	$2.3x10^7$	0
NaR4	$1x10^6$	$1.1x10^5$	$2x10^3$	0	0	0	$3.5x10^7$	0

Biochemical analysis of the samples

Table 2: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
NaB2	4.63±0.006	0.5±0.006	4.24±0.056	0.22±0.003	0.51±0.006	0.76±0.015

Table 3: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
NaB2	1.29±0.105	0.21±0.007	1.38±0.084	0.52±0.006

Table 4: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
NaB2	1.98±0.344	1.83±0.150	81.11±1.508

Table 5: The content of different organic acids in the rice beer sample

Sample: NaB2	
Organic acid	Concentration in ppm
Lactic Acid	619.89
Propionic acid	20.19
Oxalic acid	0.13
Citric acid	ND
Tartaric acid	2607.7
Succinic acid	996.02
Pyruvic acid	6.94
Formic acid	63.57
Acetic acid	ND

*ND – Not Detected

Table 6: Result of colour measurement

Sample	L	a	b
NaB2	2.1	0.72	2.65

2. Tribe: *Dimasa*

Place: Dimapur, Nagaland

The *Dimasa Kacharis* are one of the earliest indigenous ethnic groups of North-Eastern India. They are mostly found in the North Cachar Hills of Assam and Dimapur in Nagaland. This study was done among the *Dimasas* residing in Dimapur, Nagaland, India.

Starter Culture: *Umhu* or *Humao*

The starter cake for preparing rice beer is called as *umhu* or *humao* and is a mixture of rice and bark of *thempra* plant. The barks are cut into small pieces and dried in the sun. Rice is soaked in water until it is softened. It is then grinded in a wooden or metallic mortal pestle called *rimin* along with the barks of *thempra* plant. A little water is added in order to make a paste. They are then made into cakes of appropriate sizes and allowed to dry for a period of one week. They can be stored for many months.

Fig 18: *Umhu*

Fig 19: A twig of *thempra* plant

Rice Beer: *Judima*

For preparing *judima*, rice is boiled and allowed to cool. It is mixed with powdered *humao* (one large sized *humao* is sufficient for 5 Kg of rice) and kept in a large container which is covered with jute gunny bags. After about a week, slightly yellowish juices come out of the mass which indicates the completion of fermentation. This can further be diluted with water and filtered for consumption.

Fig 20: A *Dimasa* woman serving *judima*

Identification of plant species

Table 7: Scientific name of the plant and its portion used

Local Name	Botanical Name	Family	Portions
Thempra	*Acacia pennata*	Fabaceae	Barks

Samples collected

a. Starter culture used by *Kachari* tribe (Code name: NaS2)

b. Rice beer prepared by *Kachari* tribe (Code Name: NaB1)

c. Fermented rice of the *Kachari* tribe (Code Name: NaR1)

Microbial analysis of the samples

Table 8: Count of different group of microbes

Sample	CFU / ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
NaS2	$1.6x10^8$	$5.67 x10^7$	0	0	0	0	$1x10^7$	$6 x10^5$
NaB1	$3.1x10^9$	$2.72 x10^5$	0	0	0	0	$3.6x10^6$	0
NaR1	$1x10^6$	$1.8 x10^3$	$2 x10^3$	0	0	0	$4x10^6$	0

Biochemical analysis of the rice beer sample

Table 9: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
NaB1	4.06±0.006	0.47±0.01	6±0.008	0.9±0.02	0.90±0.006	0.06±0.006

Table 10: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
NaB1	8.47±1.745	3.47±0.194	0.85±0.039	0.48±0.006

Table 11: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
NaB1	2.58±0.344	10.06±0.176	45.28±0.606

Table 12: The content of different organic acids in the rice beer sample

Sample: NaB1	
Organic acid	**Concentration in ppm**
Lactic Acid	4417.18
Propionic acid	ND
Oxalic acid	738.0
Citric acid	293.63
Tartaric acid	84.14
Succinic acid	ND
Pyruvic acid	201.52
Formic acid	ND
Acetic acid	ND

*ND – Not Detected

Table 13: Result of colour measurement

Sample	L	a	b
NaB1	78.75	0.11	23.5

Biochemical analysis of the plant samples

Table 14: Total polyphenol content and free radical scavenging activity (RSA) of the plant extracts

Plant species	Total polyphenols (mg/100g)	%RSA
Thempra	44.28±0.002	16.13±0.827

3. Tribe: *Bodo*

Place: Kokrajhar, Assam

The *Bodos* are one of the largest linguistic groups in North-East India and among the earliest settlers of Assam. They inhabit most of the regions in Assam but resides mostly in the Bodoland regions. This study was done among the *Bodos* residing in Kokrajhar district of Assam, India.

Starter culture: *Amou*

The starter cake used for preparing the local rice beer by the *Bodos* is known as *amou*. For preparing *amou*, different plant materials are said to be used based on their availability in different regions. However, the most common species are leaves of *agarsita* and *dongphang rakhep* and either roots or leaves of *lokhunath*. These plants are first washed properly and allowed to dry in the air. Rice grains are soaked for about 5 hours in normal temperature water and allowed to soften. This is then mixed with the plants and grinded together in a wooden mortar with a pestle and this set of apparatus is called *wayal*. Dough is made by adding a little water to the mixture. They are then made into round cakes of about 5.5 cm diameter and 0.5 to 1 cm thickness and covered with powder of the mixture to which water is not added. This is followed by covering with *gigab* (paddy straw) and allowed to dry for a period of 3–4 days. These can be stored in moisture free places for more than a year.

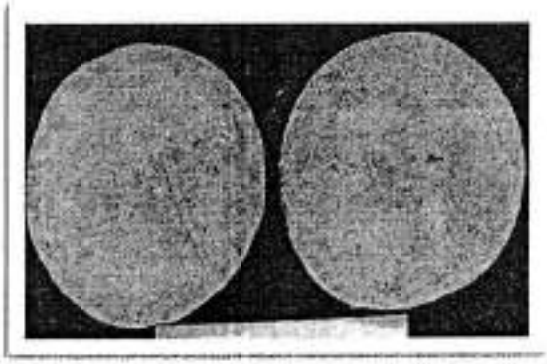


Fig 21: *Amou*

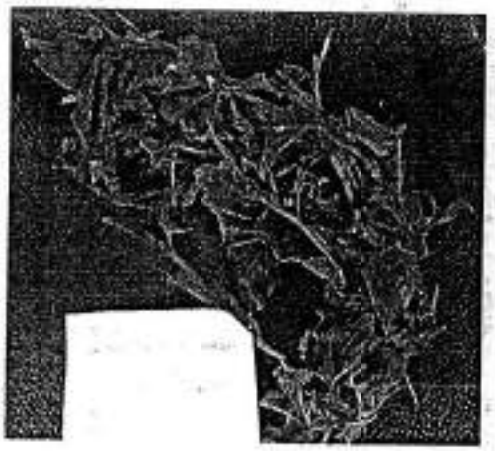


Fig 22: *Agarsita*

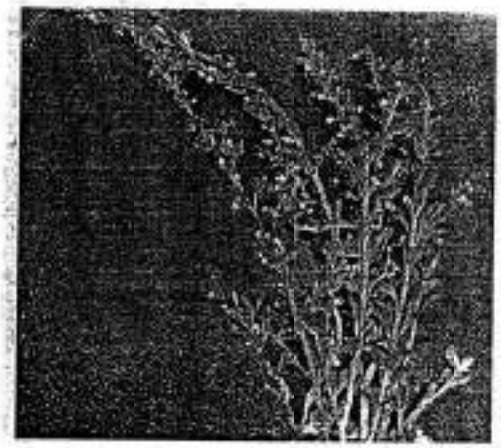


Fig 23: *Dongphang- rakhep*

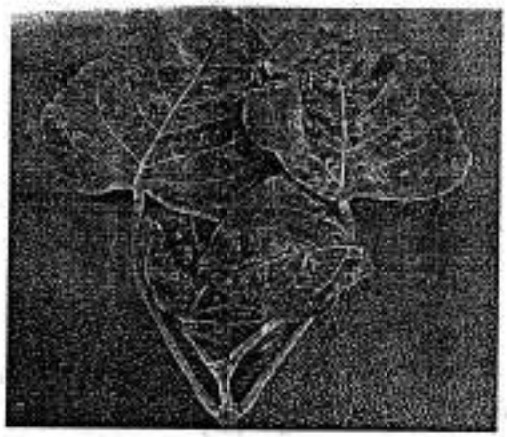


Fig 24: *Lokhunath*

Rice Beer: *Jou bishi*

For preparing the beer, either glutinous or non-glutinous rice can be used. When glutinous rice is used the product is known as *maibra jou bishi* and when non-glutinous rice is used it is known as *matha jou bishi*. The rice is first boiled with care not to allow it to overcook. It is then cooled and allowed to dry. To this powdered *amou* is added (about one *amou* for 1 kg of rice) and mixed well. This mixture is put inside a plastic bag and kept closed for one night. After this a little water is added to it and left in a *baiphu* (earthen pot) covered with banana leaves for a period of at least 3 days. The fermented mass if further mixed with water and strained in order to get the liquid *jou bishi*.

Fig 25: A *baiphu* filled with *jou bishi*

Identification of plant species

Table 15: Scientific names of the plants and their portions used

Local Name	Botanical Name	Family	Portions
Agarsita	*Xanthium strumarium*	Asteraceae	Whole plant
Dongphang-rakhep	*Scoparia dulcis*	Scrophulariaceae	Leaves
Lokhunath	*Clerodendrum viscosum*	Verbenaceae	Leaves/roots

Samples collected

a. Starter culture used by *Bodo* tribe (Code name: AsS2)

b. Rice beer prepared by *Bodo* tribe (Code Name: AsB1)

c. Fermented rice of the *Bodo* tribe (Code Name: AsR1)

Microbial analysis of the samples

Table 16: Count of different group of microbes

Sample	CFU/ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
AsS2	1.6×10^{6}	1.54×10^{7}	0	0	0	0	2.1×10^{8}	4×10^{7}
AsB1	1.9×10^{10}	6.3×10^{6}	2×10^{2}	1×10^{2}	0	0	5.3×10^{7}	0
AsR1	1.3×10^{8}	3.1×10^{6}	0	0	0	0	4.1×10^{7}	0

Biochemical analysis of the rice beer sample

Table 17: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
AsB1	4.28±0.0	0.5±0.006	4.3±0.024	0.17±0.004	0.45±0.01	0.36±0.017

Table 18: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
AsB1	0.63±0.18	0.05±0.02	0.7±0.01	0.52±0.006

Table 19: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
AsB1	2.38±0.001	2.19±0.204	90.95±0.387

Table 20: The content of different organic acids in the rice beer sample

Sample: AsB1	
Organic acid	**Concentration in ppm**
Lactic Acid	6441.44
Propionic acid	ND
Oxalic acid	4.31
Citric acid	490.71
Tartaric acid	ND
Succinic acid	ND
Pyruvic acid	1.09
Formic acid	103.25
Acetic acid	1182.8

*ND – Not Detected

Table 21: Result of colour measurement

Sample	L	a	b
AsB1	1.13	0.58	1.39

Biochemical analysis of the plant samples

Table 22: Total polyphenol content and free radical scavenging activity of the plant extracts

Plant	Total polyphenols (mg/100g)	% RSA
Agarsita	75.76±0.0035	76.22±0.29
Dongphang-rakhep	70.51±0.0029	41.56±2.609
Lokhunath	27.75±0.0008	10.5±0.58

4. Tribe: *Adivasi*

Place: Gossaigaion, Assam

The *Tea-tribes* are found mainly in almost all the districts of Assam. The so-called *Tea-tribes* were brought in by the colonial planters (British) as indentured labourers from the Chhota Nagpur Plateau region. This study was done among the *Tea-tribes* residing in Bongaigaon district of Assam, India.

Starter Culture:

Same preparation methodology as that of *Bodos*

Rice Beer: ***Lao pani***

Same preparation methodology as that of *Bodos*

Samples collected

a. Rice beer prepared by *tea tribe* (Code Name: AsB2)

b. Fermented rice of the *tea tribe* (Code Name: AsR2)

Fig 26: *Lao pani* in plastic container

Microbial analysis of the samples

Table 23: Count of different group of microbes

Sample	CFU / ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
AsB2	2.05×10^{9}	1.37×10^{7}	9×10^{4}	0	1.5×10^{5}	2.5×10^{5}	2.1×10^{8}	0
AsR2	4.6×10^{5}	5×10^{6}	1×10^{4}	0	0	0	1.19×10^{4}	0

Biochemical analysis of the rice beer sample

Table 24: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
AsB2	4.72±0.015	0.4±0.012	4.26±0.046	0.16±0.002	0.47±0.006	0.11±0.015

Table 25: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
AsB2	0.83±0.055	0.226±0.019	1.07±0.118	0.547±0.048

Table 26: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
AsB2	2.18±0.343	2.0±0.0154	90.29±0.535

Table 27: The content of different organic acids in the rice beer sample

Sample: AsB2	
Organic acid	**Concentration in ppm**
Lactic Acid	4540.60
Propionic acid	ND
Oxalic acid	ND
Citric acid	ND
Tartaric acid	80.52
Succinic acid	690.98
Pyruvic acid	ND
Formic acid	52.74
Acetic acid	ND

*ND – Not Detected

Table 28: Result of colour measurement

Sample	L	a	b
AsB2	5.3	0.41	3.24

5. Tribe: *Karbi*

Place: Karbi Anglong, Assam

The *Karbis* are one of the major tribes of Assam and are settled mostly in the districts of Karbi Anglong and North Cachar Hills. They prepare a traditional alcoholic beverage called *hor-alank*. This beverage is used as a refreshing drink and also bears significance in many social ceremonies and events. This study was conducted in Diphu sub-division of Karbi Anglong district in Assam, India.

Starter Culture: *Thap*

For preparation of rice beer, the yeast starter culture called *thap* first needs to be prepared. For preparing *thap*, rice is soaked in water for 1 day. The soaked rice is then mixed with leaves of *marthu, janphong, jockan, hisou-kẹhou* and barks of *themra* plant. The mixture is grinded together in a wooden mortal called "*long*" with a pestle called "*lingpum*" in order to make a paste. This paste is then made into small flat shaped cakes of about 6 cm in diameter and 0.5 cm in thickness. These are overlaid with powder of previous *thaps* and kept in a bamboo sieve called "*ingkrung*" and dried for about three days under the sun or above the fire place. These can be stored for about 1 year for further use.

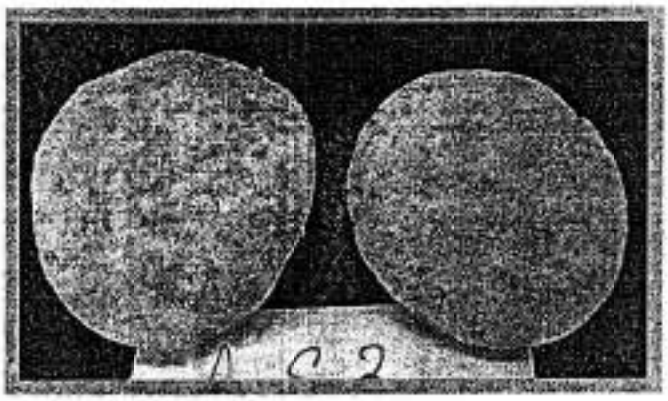


Fig 27: *Thap*

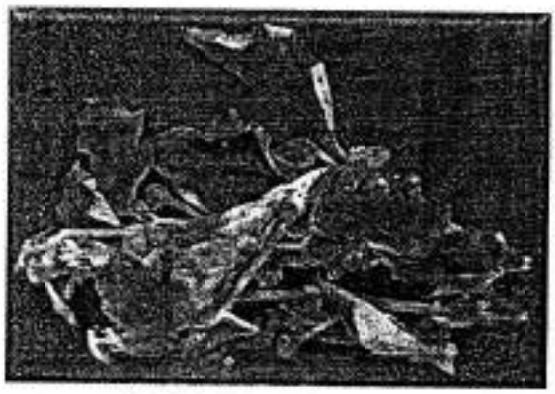


Fig 28: *Hisou-kehou*

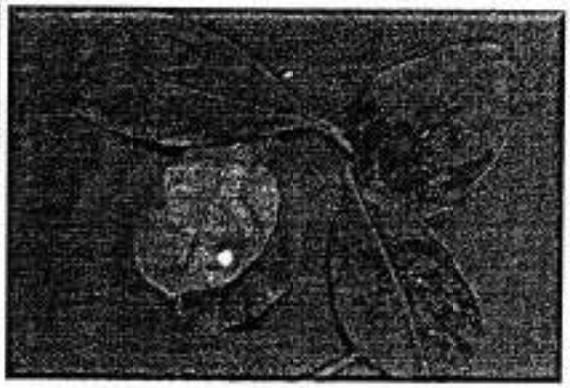


Fig 29: *Janphong*

Fig 30: *Jockan*

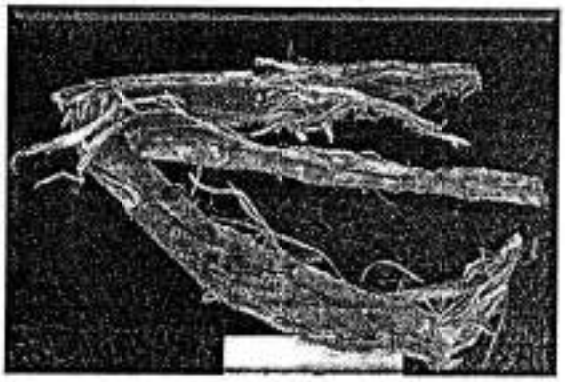


Fig 31: *Themra*

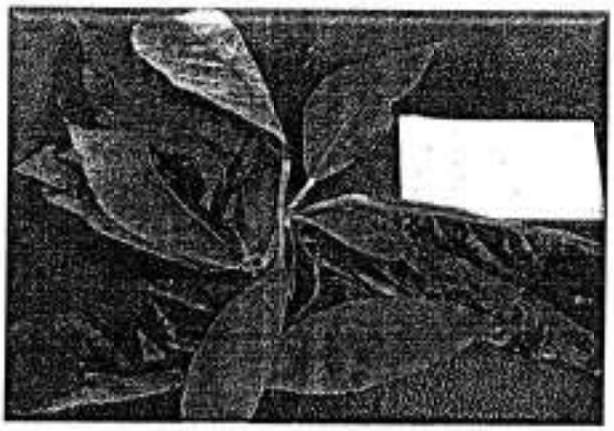


Fig 32: *Marthu*

Rice Beer: *Hor-alank*

For preparing *hor-alank*, rice is first boiled, then spread and allowed to cool. It is followed by with powdered *thaps* (5 Kg rice + 7 *thaps*). The whole mixture is kept in a large container and covered, first with plastic bags and then with sack. It is left to ferment for a period of 2 days at room temperature. After that it is mixed with water and further fermented for 2 (summer) to 4 (winter) days.

Identification of plant species

Table 23: Scientific names of the plants and their portions used

Local Name	Botanical Name	Family	Portions
Marthu	*Croton joufra*	Euphorbiaceae	Leaves
Janphong	*Artocarpus heterophyllus*	Moraceae	Leaves
Jockan	*Phlogocanthus thysiflorus*	Acanthaceae	Leaves
Hisou-kehou	*Solanum viarum*	Solanaceae	Leaves
Themra	*Acacia pennata*	Fabaceae	Barks

Samples collected

a. Starter culture used by *Karbi* tribe (Code name: AsS3)

b. Rice beer prepared by *Karbi* tribe (Code Name: AsB3)

c. Fermented rice of the *Karbi* tribe (Code Name: AsR3)

d. Fermented rice of the *Karbi* tribe (Code Name: AsR4)

Microbial analysis of the samples

Table 29: Count of different group of microbes

Sample	CFU / ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
AsS3	$1.41x10^7$	$6.3x10^6$	0	0	0	0	$8x10^7$	$4.1x10^7$
AsB3	$3x10^2$	$2x10^4$	0	$2x10^2$	0	0	$1.8x10^7$	0
AsR3	$1x10^5$	$1x10^5$	$1x10^2$	0	0	0	$5.6x10^7$	0
AsR4	$5x10^3$	$3x10^3$	$2x10^5$	0	0	0	$5.2x10^7$	0

Biochemical analysis of the rice beer sample

Table 30: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
AsB3	4.23±0.006	0.75±0.012	4.39±0.362	0.02±0.002	0.77±0.021	0.17±0.021

Table 31: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
AsB3	0.8±0.04	0.32±0.127	0.88±0.03	0.82±0.058

Table 32: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
AsB3	2.58±0.344	5.05±0.002	69.93±0.68

Table 33: The content of different organic acids in the rice beer sample

Sample: AsB3	
Organic acid	**Concentration in ppm**
Lactic Acid	4089.67
Propionic acid	ND
Oxalic acid	ND
Citric acid	366.89
Tartaric acid	ND
Succinic acid	532.91
Pyruvic acid	ND
Formic acid	204.37
Acetic acid	ND

*ND – Not Detected

Table 34: Result of colour measurement

Sample	L	a	b
AsB3	1.99	0.55	2.24

Biochemical analysis of the plant samples

Table 35: Total polyphenol content and free radical scavenging activity of the plant extracts

Plant species	Total polyphenols (mg/100g)	% RSA
Themra	44.29±0.0022	16.13±0.827
Hisou -kehou	33.89±0.0127	22.64±1.292
Janphong	55.02±0.0008	70.84±1.938
Jockan	24.84±0.0025	17.39±1.545
Marthu	38.78±0.0019	13.47±0.684

6. Tribe: *Ahom*

Place: Sibasagar, Assam

The *Ahoms* or *Tai-Ahoms* are an ethnic group settled in Assam and are of Tai origin. They are a part of the Assamese society and are found all over Assam. This study was carried out in Sibasagar district of Assam.

Starter Culture: Vekur Pitha

The *Ahoms* prepare rice beer in their own traditional way and the starter cake is known as *vekur pitha* and consists of various parts of several plant species. The mainly used are leaves *of banjaluk, kopou lota, horuminimuni, bormanmunii, tubuki lota* and seeds of *jaluk.* All these are washed and dried well and then grinded in an *ural* (wooden mortar) with a pestle and mixed with grinded rice and a little water in a vessel and made into a paste. From this, oval shaped balls of about 4.5 cm x 3 cm are made and placed on *kol pat* [banana (*Musa* sp.) leaves] and dried either in the sun or over the fire place by taking care not to bring them not to close to the fire. After a period of about 5 days they become hard and are ready to be used. This *vekur pitha* can be stored for up to a year and used when needed.

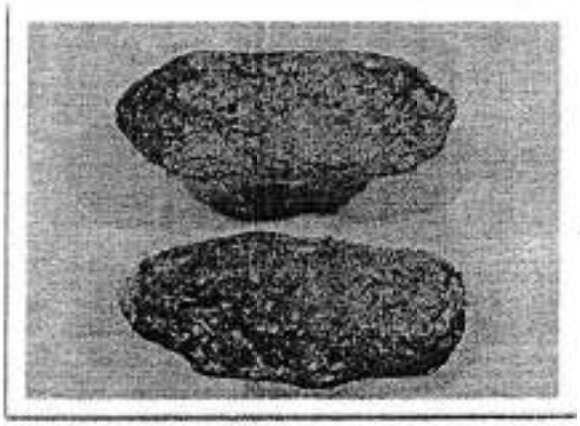


Fig 33: *Vekur- pitha*

Fig 34: *Banjaluk*

Fig 35: *Horumanimuni*

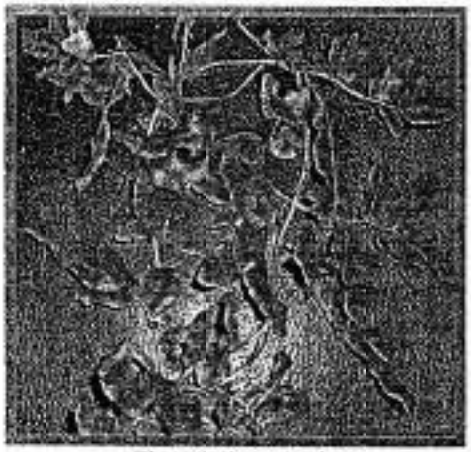


Fig 36: *Kopow-lota*

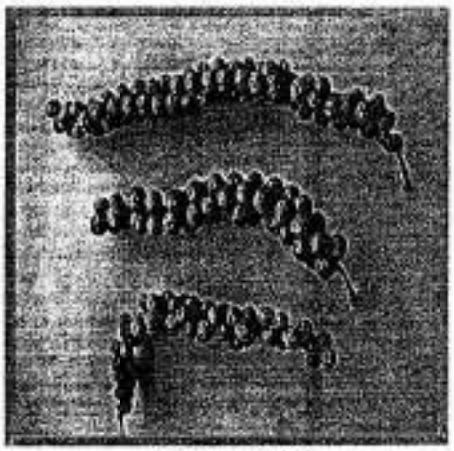


Fig 37: *Jaluk*

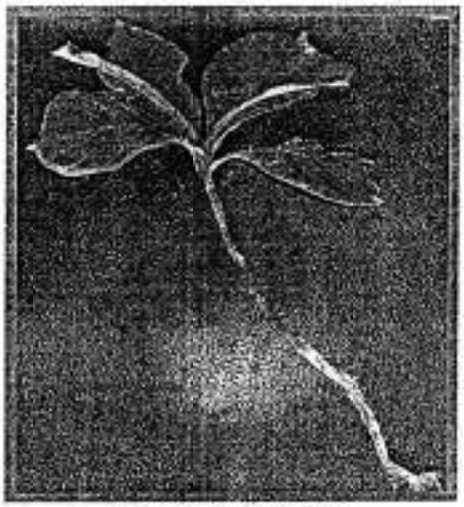


Fig 38: *Tubuki-lota*

Rice Beer: *Xaj pani*

The *Ahoms* prepare rice beer in their own traditional way and name it as *xaj pani* or *koloh pani*. For preparing *xaj pani*, rice (either glutinous or non-glutinous) are half cooked and spread on banana leaves to cool it down. It is then mixed with powdered *vekur pitha* (1 per Kg of rice) and again spread for some time. The mixture is kept on a *koloh* (earthen pot) and the mouth is sealed. This is kept in a closed room for a period of 3 to 5 days. After this some amount of water is added to the fermented mass and left for about 10 minutes. Filtration is done by straining the mass by using a cloth.

Fig 39: An *Ahom* woman filtering *xaj-pani*

Identification of plant species

Table 36: Scientific names of the plants and their portions used

Local Name	Botanical Name	Family	Portions
Banjaluk	*Oldenlandia corymbosa*	Rubiaceae	Leaves
Kopou-lota	*Lygodium sp*	Lycopodiaceae	Leaves
Horuminimuni	*Hydrocotyle sibthorpioides*	Apiaceae	Whole plant
Bormanmunii	*Centella asiatica*	Mackinlayaceae	Whole plant
Tubuki-lota	*Cissampelos pareira*	Menispermaceae	Leaves
Jaluk	*Piper nigrum*	Piperaceae	Seeds

Samples collected

a. Starter culture used by *Ahom* tribe (Code name: AsS4)

b. Rice beer prepared by *Ahom* tribe (Code Name: AsB4)

c. Fermented rice of the *Ahom* tribe (Code Name: AsR5)

Microbial analysis of the samples

Table 37: Count of different group of microbes

Sample	CFU / ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
AsS4	2×10^6	5×10^5	0	0	0	0	7×10^4	1×10^2
AsB4	2.6×10^8	4.5×10^4	0	3.4×10^3	0	0	7.0×10^6	0
AsR5	1.4×10^6	3×10^4	7×10^4	0	0	0	1×10^7	0

Biochemical analysis of the rice beer sample

Table 38: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
AsB4	4.27±0.0	0.55±0.01	2.5±0.01	0.53±0.00	1.02±0.015	0.86±0.015

Table 39: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
AsB4	4.0±0.087	1.67±0.071	0.94±0.045	0.69±0.074

Table 40: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% FRSA ± SD
AsB4	2.78±0.343	4.71±0.018	63.7±2.493

Table 41: The content of different organic acids in the rice beer sample

Sample: AsB4	
Organic acid	**Concentration in ppm**
Lactic Acid	9105.35
Propionic acid	ND
Oxalic acid	258.19
Citric acid	ND
Tartaric acid	ND
Succinic acid	ND
Pyruvic acid	2.63
Formic acid	92.33
Acetic acid	ND

*ND – Not Detected

Table 42: Result of colour measurement

Sample	L	a	b
AsB4	0.92	1.73	1.53

Biochemical analysis of the plant samples

Table 43: Total polyphenol content and free radical scavenging activity of the plant extracts

Plant species	Total polyphenols (µg/100g)	% RSA
Banjaluk	67.06±0.016	32.19±2.477
Jaluk	43.84±0.004	64.58±0.936
Bormanimuni	11.9±0.003	19.35±2.008
Tubuki- lota	23.25±0.001	17.52±1.872
Kopou- lota	147.94±0.0027	77.86±0.974

7. **Tribe:** ***Mising***

Place: Lakhimpur, Assam

Although inhabiting in many districts of Assam, the *Misings* are concentrated mostly in the districts of Dhemaji, Lakhimpur and Jorhat. They are said to have migrated to Assam from the state of Arunachal Pradesh. This study was undertaken among the *Mising* communities residing in the district of Lakhimpur in Assam.

Starter Culture: ***Apop-pitha***

For preparing rice beer, the *Misings* use starter cakes known as *aopo pitha*. The different leaves needed for preparing *apop pitha* are of the plants *bormanimuni, horumanimuni, banjaluk , kuhiar, dhapat tita , bhilongoni, bam kolmou, senikuthi , lai jabori, jalokia, anaras* and *kopou dhekia.* All these leaves are cleaned and dried by placing on a bamboo mat called *opoh*. They can be either used freshly or dried in the sun before addition. Soaked rice and the leaves are grinded separately in a *kipar* (wooden grinder) and they are mixed together in a vessel with little water. From the dough, oval shaped balls of about 6 cm x 3 cm are made and dried in the sun.

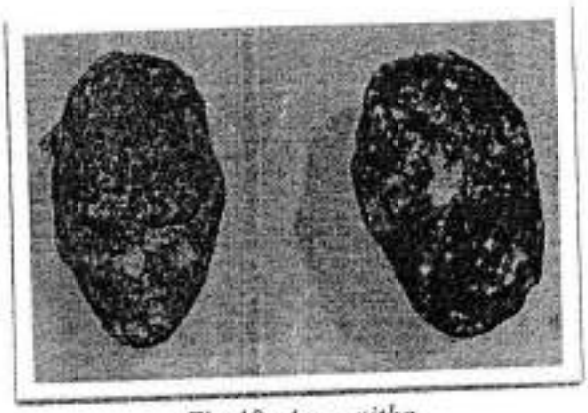


Fig 40: *Apop-pitha*

Fig 41: *Lai-jabori*

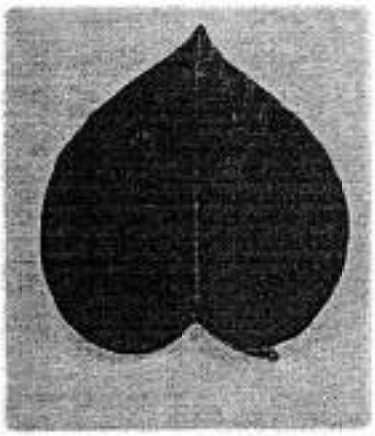


Fig 42: *Bam-kolmou*

Fig 43: *Tita-phul*

Fig 44: *Dhopat-tita*

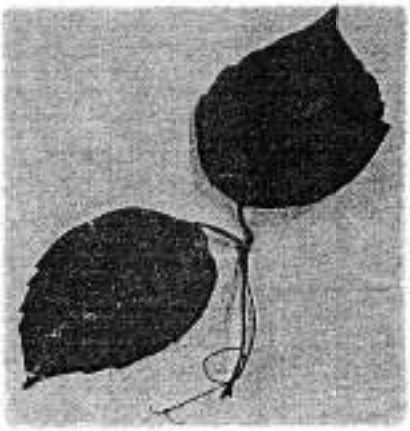


Fig 45: *Ranram-kuchere*

Fig 46: *Senikuthi*

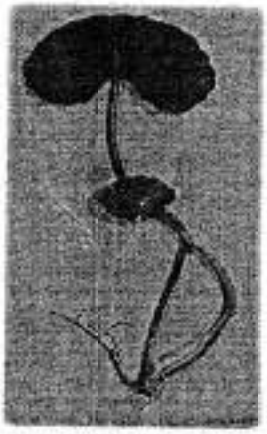


Fig 47: *Bormanimuni*

Rice Beer: *Apong*

The rice beer prepared by the *Misings* is known as *apong*. Before starting the fermentation process, the *kiling* (earthen pot) used for fermentation is first fumigated by placing it on a *torap* (a bamboo frame constructed over the fire place) until the pot turns blackish. After that boiled rice is spread on a *kol pat* (banana leaf) and allowed to cool. To this powdered *apop pitha* is added (1 *apop pitha* for 1 kg of rice) and the whole mixture is kept inside the *kiling* and the mouth of the pot is covered with banana leaves or leaves of *bhilongoni*. This is left for fermentation to take place for a period of about 5 days. A little water is added to the fermented product and is filtered to get the *apong*.

Fig 48: A *Mising* woman filtering *apong*

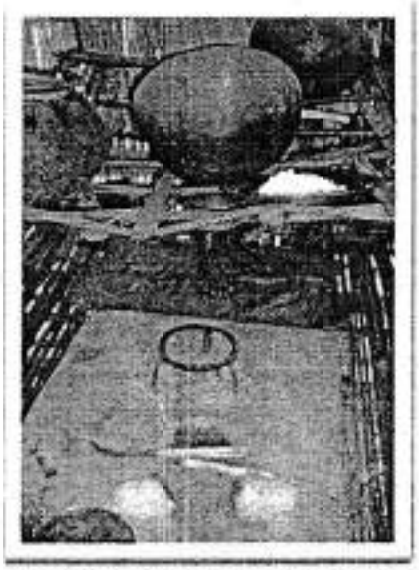


Fig 49: A *kiling* being fumigated

Identification of plant species

Table 44: Scientific names of the plants and their portions used

Local Name	Botanical Name	Family	Portions
Bormanimuni	*Centella asiatica*	Mackinlayaceae	Whole plant
Horumanimuni	*Hydrocotyle sibthorpioides*	Apiaceae	Whole plant
Banjaluk	*Oldenlandia corymbosa*	Rubiaceae	Leaves
Kuhiar	*Saccharum officinarum*	Poaceae	Leaves
Dhapat-tita	*Clerodendrum viscosum*	Verbenaceae	Leaves
Bhilongoni	*Cyclosorus exlensa*	Thelypteridaceae	Leaves
Bam-kolmou	*Ipoemea sp*	Convulvulaceae	Leaves
Senikuthi	*Scoparia dulcis*	Scrophulariaceae	Leaves
Lai-jabori	*Drymeria cordata*	Caryophyllaceae	Leaves
Jalokia	*Capsicum annuum*	Solanaceae	Leaves
Anaras	*Ananas comosus*	Bromeliaceae	Young leaves
Kopou-dhekia	*Lygodium flexuosum*	Lycopodiaceae	Leaves

Samples collected

a. Starter culture used by *Mising* tribe (Code name: AsS5)

b. Rice beer prepared by *Mising* tribe (Code Name: AsB5)

c. Fermented rice of the *Mising* tribe (Code Name: AsR6)

Microbial analysis of the samples

Table 45: Count of different group of microbes

Sample	CFU / ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
AsS5	$1x10^5$	5.3×10^3	0	0	0	0	0	$1x10^4$
AsB5	2.0×10^7	8.5×10^6	1.6×10^3	$3x10^2$	0	0	4.4×10^7	0
AsR6	2.3×10^7	1.2×10^7	$1x10^5$	0	$1.2x10^5$	$4.5x10^5$	$1.25x10^8$	0

Biochemical analysis of the rice beer sample

Table 46: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
AsB5	3.6±0.006	0.58±0.01	4.37±0.015	0.13±0.001	0.35±0.006	0.43±0.021

Table 47: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
AsB5	0.98±0.078	0.2±0.006	1.36±0.049	0.68±0.068

Table 48: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
AsB5	2.18±0.344	0.93±0.08	47.21±2.929

Table 49: The content of different organic acids in the rice beer sample

Sample: AsB5	
Organic acid	Concentration in ppm
Lactic Acid	6026.90
Propionic acid	ND
Oxalic acid	134.76
Citric acid	ND
Tartaric acid	424.18
Succinic acid	ND
Pyruvic acid	34.64
Formic acid	502.14
Acetic acid	533.37

*ND – Not Detected

Table 50: Result of colour measurement

Sample	L	a	b
AsB5	0.83	0.66	1.13

Biochemical analysis of the plant samples

Table 51: Total polyphenol content and free radical scavenging activity of the plant extracts

Plant species	Total polyphenols (mg/100g)	%RSA
Senikuthi	33.62±0.0004	44.97±5.02
Dhopat-tita	41.2±0.0008	17.39±0.11
Bam-kolmou	33.92±0.0012	13.22±0.61
Banjaluk	67.06±0.0016	32.19±2.477
Horumanimuni	17.38±0.001	11.57±0.329
Bormanumuni	11.9±0.003	19.35±2.008
Lai-jabori	29.61±0.0063	9.17±0.856
Ramram-kuchere	27.75±0.0004	12.14±4.74
Bilongoni	67.09±0.004	80.71±0.718
Tita-phul	58.34±0.0018	34.41±1.079

8. Tribe: *Deori*

Place: Lakhimpur, Assam

Being one of the oldest settlers of Assam, the *Deoris* are mostly inhabitant of Lakhimpur, Sibasagar, Dibrugarh, and Tinsukia districts of Assam, India. Information was collected from the *Deori* communities residing in Lakhimpur district, Assam, India.

Starter Culture: *Perok-kushi*

The indigenous rice beer of the *Deoris* is prepared using the starter material known as *perok kushi.* The plant materials used for preparing *perok kushi* are leaves *of bhatar duamali, thok thok, tesmuri, zing zing, zuuro, bhilongoni, sotiona* and roots of *dubusiring* and the stem and rhizome of the plant *jomlakhoti.* All these are washed and cut into small pieces. They are then grinded in a specialized wooden grinder called as *dheki.* The mixture is then soaked in water in a vessel until the water becomes coloured. The whole mixture is added to grinded rice in a vessel in order to make dough. Round balls of about 4 cm diameter is made out of this and dried either in the sunlight or over the fire hearth by placing in a bamboo mat called as *aaphey.* After getting dried they are placed in a bamboo container called as *kula* the inside of which is laid with *kher* (paddy straw). Its mouth is again covered with *kher* and is kept over the hearth for storage. They can be kept in this manner for many months and can be used as and when required.

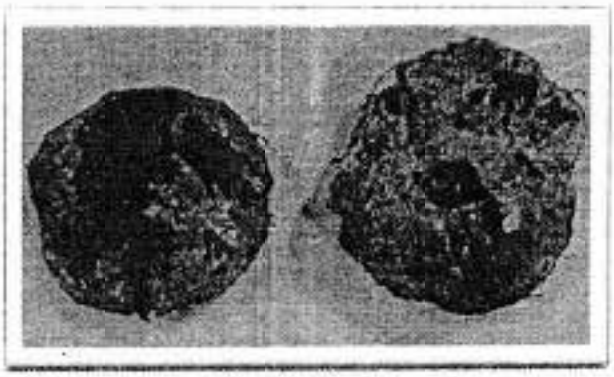


Fig 50: *Perok-kushi*

Fig 51: *Zing-zing*

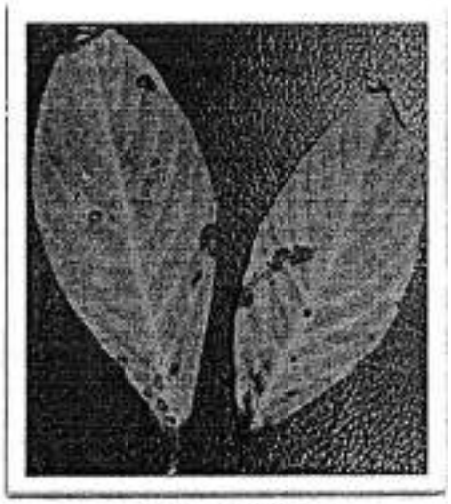


Fig 52: *Zuuoro*

Fig 53: *Bhilongoni*

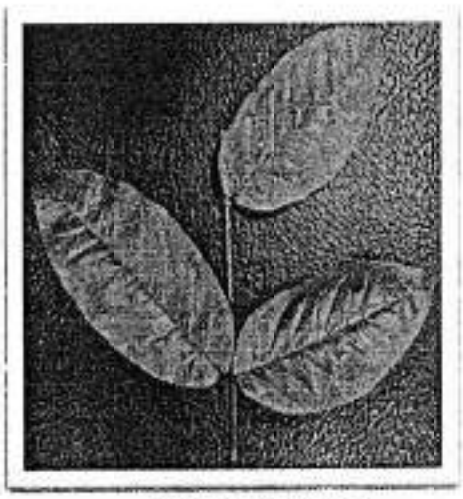


Fig 54: *Tesmuri*

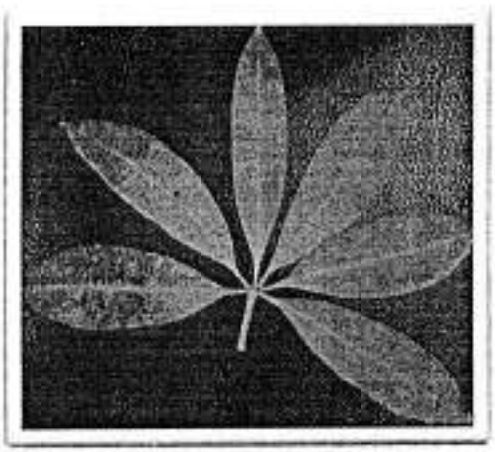


Fig 55: *Sotiona*

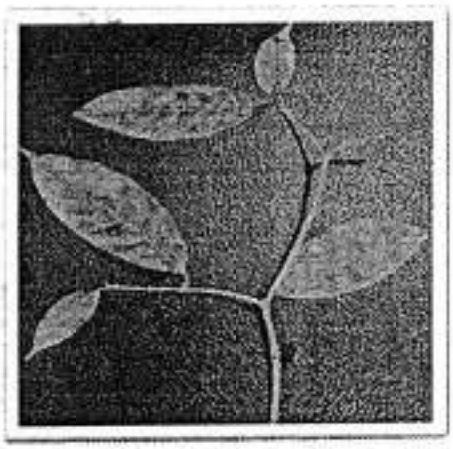


Fig 56: *Pheru-eba*

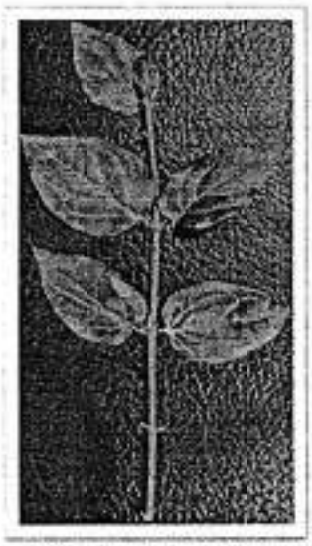


Fig 57: *Bhator-duamali*

Fig 58: *Zomlakhoti*

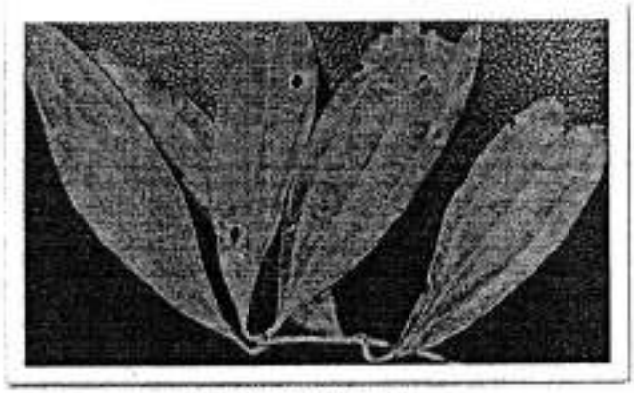


Fig 59: *Thok-thok*

Fig 60: *Dubusiring*

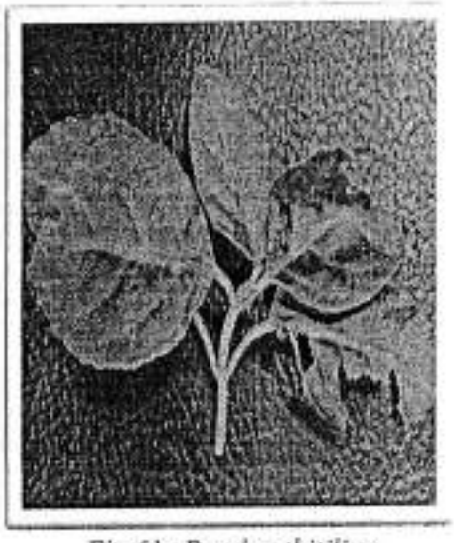


Fig 61: *Bandar-thitiling*

Rice beer: *Sujen*

For fermentation of *sujen*, an earthen pot (*disoh*) is first sterilized by washing it with ash and placing it over the hearth for drying and fumigation. Rice is first boiled and then allowed to cool by spreading on banana leaves placed above an *aaphey*. This is followed by addition of powdered *perok kushi* to the cooled rice (1 starter per 3 Kg of rice). The mixture is kept in a *disoh*, the mouth of which is sealed with *kol pat* (banana leaves) and left for fermentation to take place for about 4 to 5 days. It can then be diluted and filtered. It is said that the fermented mass in the *disoh* can be stored for up to 1 to 2 months at room temperature.

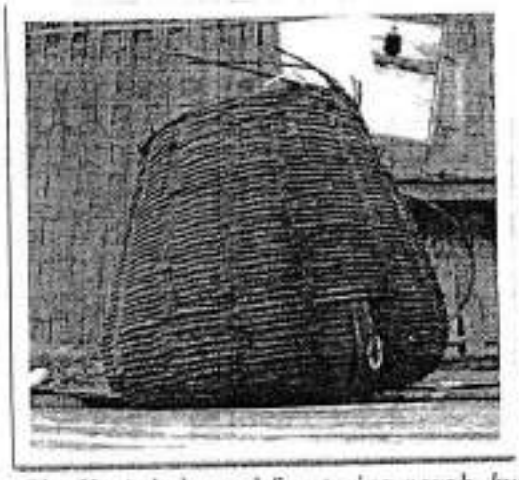


Fig 62: A *kula* used for storing *perok -kushi*

Fig 63: A *Deori* woman filtering *sujen*

Identification of plant species

Table 52: Scientific names of the plants and their portions used

Local Name	Botanical Name	Family	Portions
Bhatar-duamali	*Jasminum sambac*	Olaceae	Leaves
Thok-thok	*Cinnamomum byolghata*	Lauraceeae	Leaves
Tesmuri	*Zanthoxylum hamiltonianum*	Rutaceae	Leaves
Zing- zing	*Lygodium flexuosum*	Lycopodiaceae	Leaves
Zuuro	*Acanthus leucostychys*	Acanthaceae	Leaves
Bhilongoni	*Cyclosorus exlensa*	Thelypteridaceae	Leaves
Sotiona	*Alstonia scholaris*	Apocynaceae	Leaves
Dubusiring	*Alpinia malaccensis*	Zingiberaceae	Roots
Jomlakhoti	*Costus speciosus*	Costaceae	Stem, rhizome

Samples collected

a. Starter culture used by *Deori* tribe (Code name: AsS6)

b. Rice beer prepared by *Deori* tribe (Code Name: AsB6)

c. Fermented rice of the *Deori* tribe (Code Name: AsR7)

Microbial analysis of the samples

Table 53: Count of different group of microbes

Sample	CFU/ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
AsS6	7.5×10^6	3.2×10^6	0	0	0	0	0	2.7×10^6
AsB6	2×10^7	2.2×10^6	7×10^5	0	0	0	5×10^7	0

Biochemical analysis of the rice beer sample

Table 54: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titratable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
AsB6	4.82±0.014	0.52±0.012	5.06±0.058	0.1±0.005	0.88±0.028	0.53±0.019

Table 55: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
AsB6	2.58±0.053	0.60±0.008	0.48±0.011	0.21±0.003

Table 56: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
AsB6	2.44±0.006	5.11±0.186	71±1.198

Table 57: The content of different organic acids in the rice beer sample

Sample: AsB6	
Organic acid	**Concentration in ppm**
Lactic Acid	5481.2
Propionic acid	ND
Oxalic acid	ND
Citric acid	457.91
Tartaric acid	118.92
Succinic acid	ND
Pyruvic acid	ND
Formic acid	341.21
Acetic acid	ND

*ND – Not Detected

Table 58: Result of colour measurement

Sample	L	a	b
AsB6	1.21	0.86	2.65

Biochemical analysis of the plant samples

Table 59: Total polyphenol content and free radical scavenging activity of the plant extracts

Plant species	Total polyphenols mg/100g	% RSA
Zing-zing	147.93±0.0027	77.86±0.974
Jom-lakhoti	9.95±0.0003	18.85±9.095
Zuuro	44.29±0.0031	19.99±0.718
Bilomoni	67.09±0.004	80.71±0.718
Sotiona	57.68±0.0005	25.68±2.319
Tesmuri	57.38±0.0005	23.4±0.897
Bhator-duamali	154.62±0.0003	84.12±0.548
Pheru-eba	34.64±0.0002	68.69±2.052
Dudu-siring	73.24±0.006	82.29±0.11
Bandar-thitiling	18.55±0.0006	11.95±2.052
Thok-thok	38.03±0.0008	13.16±2.879

9. **Tribe:** ***Adi-Galo***

Place: Pasighat, Arunachal Pradesh

Located in the far North-East India, Arunachal Pradesh is inhabited by many different tribes and each of these bears their own cultural resemblance. This study was done in Pasighat sub-division of East Siang district and the contribution came from the *Adi-Galo* tribe residing in that area.

Starter Culture: ***Siiyeh/ Opop***

The local rice beer prepared by this tribe is prepared using the starter cake known as *siiyeh* or *opop*. For preparing *opop*, leaves and barks of the plants *Dhapat* and *Lohpohi* are washed, sun dried and then made into powder. This is then mixed with powdered rice and a little bit of previously prepared *opo* in order to make a paste. From this flat cakes of about 10 – 11 cm diameter are made and placed upon bamboo mats. The mats are then kept in the hearth for about 3 – 4 days, when the cakes become hardened. These can be stored for many months.

Fig 64: A *Siiyeh*

Rice Beer: ***opo***

For preparing *opo*, rice husk called *ampe* is half burnt till they become black in colour. After that, rice is boiled and then spread on a bamboo mat called as *peche*. After the rice gets

cooled, it is mixed with the burnt husk in 1:1 ratio. To this powdered *opop* is added (about 100 g of the starter for 10 kg of the mixture) and mixed well. This mixture is then put in a plastic container, the walls of which are covered with leaves of a locally available plant called as *oko* (Zingiberaceae family). The mouth is also sealed with *oko* leaves and is left undisturbed for about 5 days. After this the contents are mixed well and are again left in the same manner for a longer duration. The product becomes ready after about 20 days of fermentation. It is also kept for longer durations for production of more alcohol. For filtration, a special type of funnel called as *perpur* is used where *oko* leaves are used as the filter. The fermented mass is first placed on the *perpur* and then hot water is poured over it slowly in order to obtain the *opo* as the filtrate. The quantity of water poured depends on the desired concentration of the final product.

Fig 65: An *Adi* woman filtering *opo*

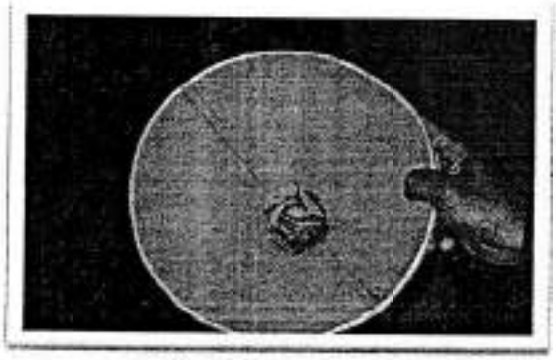


Fig 66: A *perpur*

Identification of plant species

Table 60: Scientific names of the plants and their portions used

Local Name	Botanical Name	Family	Portions
Dhapat	*Clerodendron viscosum*	Verbenaceae	Leaves/barks
Lohpohi	*Veronia* sp.	Asteraceae	Leaves

Samples collected

a. Starter culture used by *Adi-Galo* tribe (Code name: ArS1)

b. Starter culture used by *Adi-Galo* tribe (Code name: ArS2)

c. Starter culture used by *Adi-Galo* tribe (Code name: ArS3)

d. Rice beer prepared by *Adi-Galo* tribe (Code Name: ArB1)

e. Fermented rice of the *Adi-Galo* tribe (Code Name: ArR1)

Microbial analysis of the samples

Table 61: Count of different group of microbes

Sample	CFU / ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
ArS1	$1.5x10^7$	$5.4 x10^4$	0	0	0	0	$1.44x10^8$	$2x10^5$
ArS2	$7.3x10^6$	$3.36 x10^6$	0	0	0	0	$2.1x10^7$	$2 x10^3$
ArS3	$1.3x10^8$	$7 x 10^5$	0	0	0	0	$1x10^6$	$2 x10^3$
ArB1	$4.37x10^6$	$1.14x10^6$	0	0	0	0	$2.7x10^6$	0
ArR1	$2 x10^5$	$2 x10^3$	$4 x10^3$	0	0	0	$1x10^2$	0

Biochemical analysis of the rice beer sample

Table 62: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
ArB1	5.07±0.005	0.32±0.006	4.35±0.023	0.37±0.001	0.28±0.006	0.32±0.015

Table 63: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
ArB1	0.7±0.021	0.21±0.006	1.21±0.039	0.76±0.237

Table 64: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
ArB1	1.39±0.344	2.62±0.133	56.89±2.572

Table 65: The content of different organic acids in the rice beer sample

Sample: ArB1	
Organic acid	**Concentration in ppm**
Lactic Acid	1812.02
Propionic acid	ND
Oxalic acid	150.93
Citric acid	ND
Tartaric acid	ND
Succinic acid	921.59
Pyruvic acid	ND
Formic acid	92.65
Acetic acid	ND

*ND – Not Detected

Table 66: Result of colour measurement

Sample	L	a	b
ArB1	14.44	2.13	10.07

10. Tribe: *Khasi*

Place: Shillong, Meghalaya

The *Khasis* are an indigenous group of tribal people, the majority of whom live in the State of Meghalaya. They are also found in small populations in Assam, and in parts of Bangladesh. They call themselves *Ki Hynñiew trep*, which means "the seven huts" in the Khasi language. The main crops produced by the Khasi people are betel leaf, areca nut and oranges. This study was done among the Khasi people of Shillong in Meghalaya.

Rice Beer: *Kiad*

For brewing *kiad*, 4-5 kgs of *kho-so* (local variety of rice) is mixed with spring water and cooked in a metallic vessel with continuous stirring. The cooked rice is then spread on a *malieng* (round basket) for cooling and drying. Then to this 2-3 cakes if finely crushed *thiat* (yeast inoculum) is mixed. The mixture is then put in a cone shaped basket called *shang*. The whole basket is covered with a cloth and left for 2-3 days. The fermented mash known as *jyndem* is distilled in a set of apparatus called *shet-kiad* which is made by piling different sized vessels one above another. The distillate is known as *kiad*.

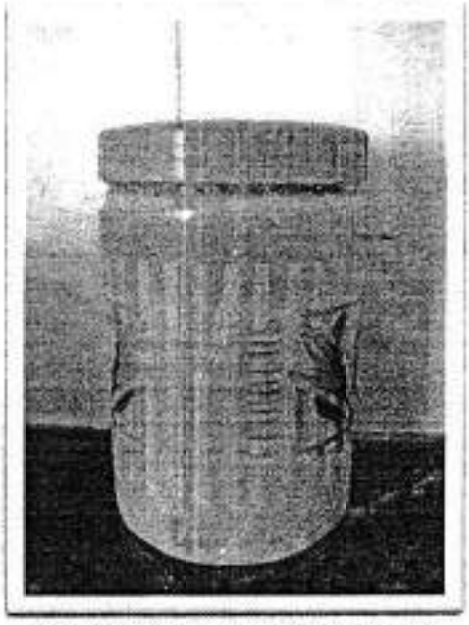


Fig 67: *Kiad* in a plastic container

Samples collected

a. Rice beer prepared by *Khasi* tribe (Code Name: MIB1)

b. Fermented rice of the *Khasi* tribe (Code Name: MIR1)

Microbial analysis of the samples

Table 67: Count of different group of microbes

Sample	CFU/ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
MIB1	6.2×10^7	3.6×10^6	0	0	0	0	1.13×10^8	0
MIR1	4×10^5	1.8×10^7	0	0	0	0	4×10^6	0

Biochemical analysis of the rice beer sample

Table 68: The pH, alcohol content, ash content, crude protein content and fats content in the rice beer sample

Sample	pH ± SD	Titrable acidity ± SD (% lactic acid)	Alcohol (%) ± SD	Ash (%) ± SD	Crude Protein (%) ± SD	Fats (%) ± SD
MIB1	3.35±0.01	0.76±0.015	4.35±0.026	0.04±0.002	0.25±0.006	0.37±0.012

Table 69: The content of total sugars, reducing sugars, starch and amylose in the rice beer sample

Sample	Total Sugars (%) ± SD	Reducing Sugars (%) ± SD	Starch (%) ± SD	Amylose (%) ± SD
MIB1	1.37±0.335	0.2±0.004	0.74±0.006	0.84±0.037

Table 70: The content of ascorbic acid and total polyphenols, and percentage of free radical scavenging activity of the rice beer sample

Sample	Ascorbic acid (mg/100g) ± SD	Total Polyphenols (mg/100g) ± SD	% RSA ± SD
MlB1	2.381±0.004	0.91±0.04	44.38±0.412

Table 71: The content of different organic acids in the rice beer sample

Sample: MlB1	
Organic acid	**Concentration in ppm**
Lactic Acid	7432.13
Propionic acid	ND
Oxalic acid	5.43
Citric acid	ND
Tartaric acid	36.15
Succinic acid	ND
Pyruvic acid	2.98
Formic acid	ND
Acetic acid	1319.19

*ND – Not Detected

Table 72: Result of colour measurement

Sample	L	a	b
MlB1	1.44	0.83	1.83

11. Tribe: *Nepali*

Place: Ranipool, Sikkim

Apart from being found in Nepal, the *Nepalese* people also inhabits the states of Sikkim, West Bengal and other states of Northeast India. The two major groups in Nepalese society are Tibeto-Burmans, or Mongoloids from the north, and Indo-Aryans from the south. This study was done in Ranipool area of Gangtok in Sikkim.

Starter Culture: *Murcha*

For preparing *murcha,* glutinous rice is soaked in water for 6 to 8 hours and pounded on a foot-driven heavy wooden mortar and pestle. To 1 kg of the grinded rice, is added roots of *Plumbago zeylanica,* leaves of *Buddleja asiatica,* flowers of *Vernonia cinerea* , rhizome of *Gingiber officinale*, red dry chilli and previously prepared *murcha.* The mixture is kneaded into flat cakes placed on a bamboo mat lined with fresh fronds of ferns (*Glaphylopteriolopsis erubescens*) and covered with dry ferns and jute bags. This is placed above the kitchen and allowed to ferment for 1 to 3 days. These are then sun-dried for 2 to 3 days. The product is called *murcha* and can be stored in a dry place for more than a year.

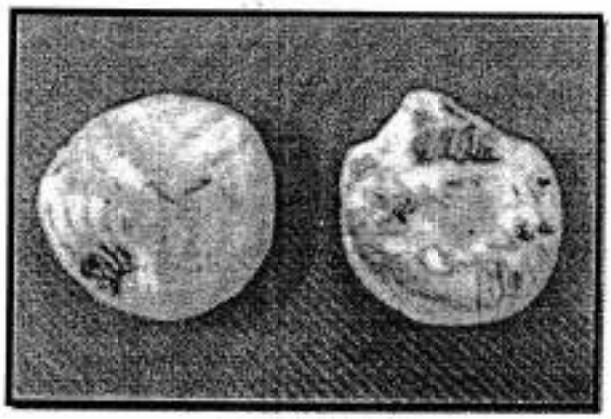


Fig 68: *Murcha*

Samples collected

a. Starter culture used by *Nepali* tribe (Code name: SkS1)

Identification of plant species

Table 73: Scientific names of the plants and their portions used

Local Name	Botanical Name	Family	Portions
Guliyo	*Plumbago zeylanica*	Plumbaginaceae	Roots
Bheemsen	*Buddleja asiatica*	Scrophulariaceae	Leaves
Sengreknna	*Vernonia cinerea*	Asteraceae	Flowers
Aduwa	*Zingiber officinale*	Zingiberaceae	Rhizome
Khorsani	*Capsicum* sp.	Solanaceae	Fruit

Microbial analysis of the samples

Table 74: Count of different group of microbes

Sample	CFU/ml							
	General aerobes	*Lactobacillus* sp.	*Staphylococcus* sp.	*Bacillus* sp.	*Salmonella* and *Shigella* sp.	Enterobacteriaceae	Yeasts	Moulds
SkS1	6.5×10^5	8×10^6	0	0	0	0	2.54×10^6	3.5×10^5

7. FERMENTATION OF CASSAVA

A. Cassava varieties used

Three different varieties of cassava (*Manihot esculenta*) were obtained from sub-station of Assam Agriculture University, *Gossaigaon*, Assam during the month of December 2010. The varieties were *Joya* , *Sri Bijoya* and *Gossaigaon Local*.

Fig 69: Dried cassava chips of the three varieties

B. Preparation of cassava chips

The cassava tubers after being brought to the laboratory were washed properly with tape water and then with distilled water. They were then dried at room temperature to remove the surface water. They were then peeled with a stainless steel knife and sliced into thickness of 2mm using a mechanical slicer. The slices were dried in a tray dryer at 45^0 C for 7 hrs.

C. Mixed microbial starter

A starter cake of mixed microbial inocula was prepared in the laboratory using rice and different plant materials. This was prepared in accordance to the method followed by the *Karbi* people of Assam. The amount of different ingredients added was standardized in the laboratory. This inocula was used for fermenting the cassava chips.

D. Methodology

The cassava chips were subjected to fermentation using starter culture used for rice beer production. 30g sample of each variety were taken in Erlenmeyer flask in replicates of three and the mouths of the flasks were sealed with cotton plugs and then autoclaved. To this was

added separately sterilized 160ml distilled water. Then the whole content was boiled for 10 minutes. This was allowed to cool and 3g of the starter culture was added to each flask left for fermentation to take place at 27°C and 60 rpm. The Microbial load and other biochemical parameters were estimated on 0th, 3rd, 6th, and 9th day of fermentation.

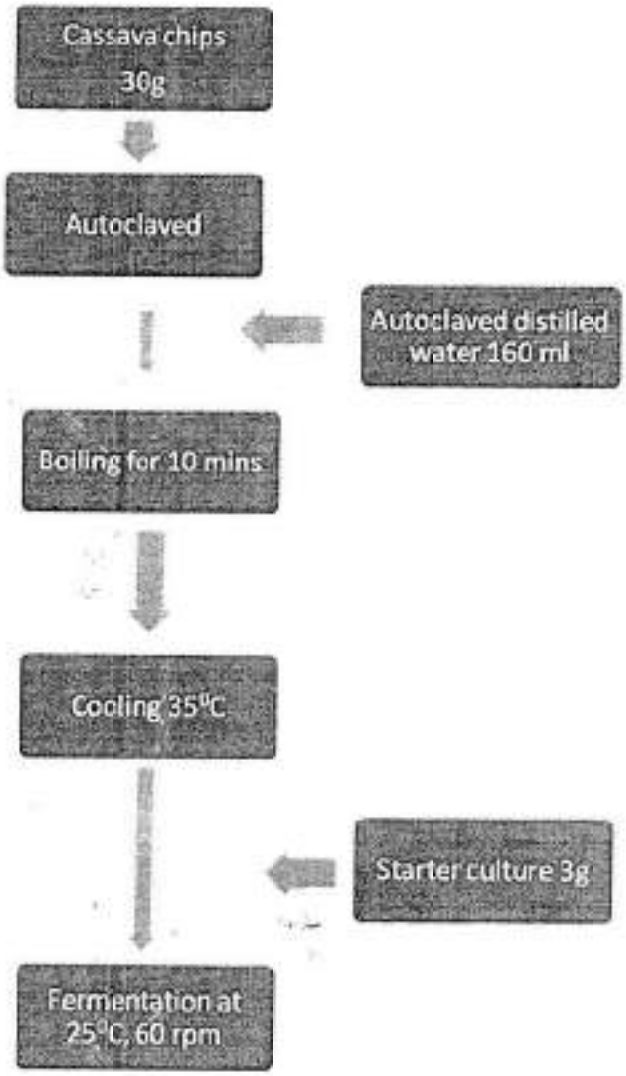


Fig 70: Flow diagram for the fermentation of cassava chips with rice beer starter culture

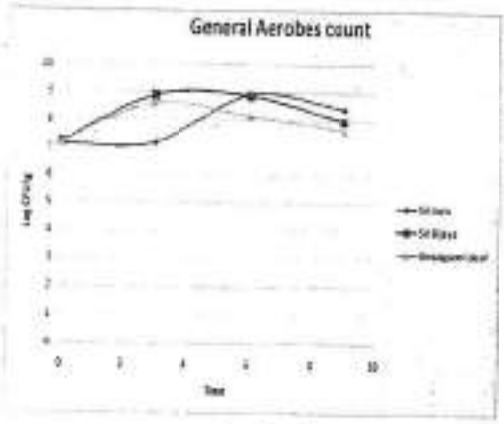
F. Count of different group of microbes at different time interval

The general Aerobes population showed a gradual increased up to 6th day from 7.15 log CFU/g to 8.34 log CFU/g and then decreased in the 9th day to 7.92 log CFU/g. Lactic acid bacteria were found to increase gradually from 6.83 log CFU/g to a maximum count of CFU/g log 8.48 in the 10th day sample. Yeast count shows a decrease in load in the 0th h of incubation than the starter culture then however it gradually increased to a maximum value of log7.9 log CFU/g. Moulds count was found to decrease gradually starting from7.61 log CFU/g in the starter culture and did not appear in the 10th day.

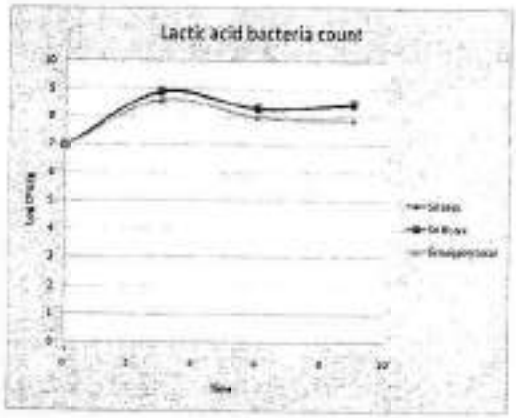
Table 75: Microbial count in cassava fermented with starter cake

Variety	Microbial Count expressed as Log CFU/g				
	General Aerobes				
	Starter	0 Day	3 Days	6 Days	9 days
Joya	7.15	7.20	8.94	8.43	7.92
Sri Bijoya	7.15	7.20	8.91	8.89	7.97
Gossaigaon Local	7.15	7.20	8.62	8.15	7.64
	Lactic Acid Bacteria				
Joya	6.83	6.95	8.94	8.32	8.41
Sri Bijoya	6.83	6.95	8.91	8.30	8.48
Gossaigaon Local	6.83	6.95	8.62	8.00	7.89
	Yeasts				
Joya	7.90	6.76	7.48	7.72	7.90
Sri Bijoya	7.90	6.76	7.76	7.71	7.91
Gossaigaon Local	7.90	6.76	7.62	7.72	7.70
	Moulds				
Joya	7.61	5.83	5.78	4.00	0.0
Sri Bijoya	7.61	5.83	5.00	0.0	0.0
Gossaigaon Local	7.61	5.83	5.95	4.30	0.0

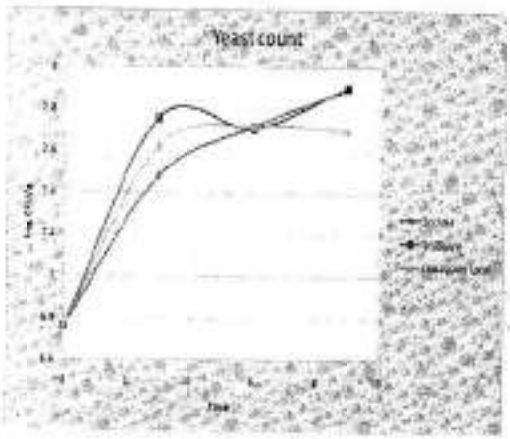
Results in replicate of three ± SD



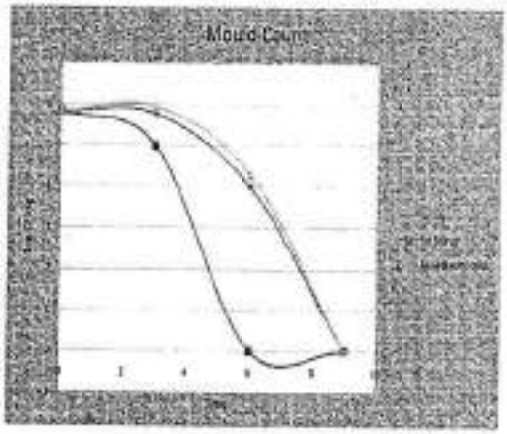
Graph 1



Graph 2



Graph 3



Graph 4

Fig 71: Graphs showing the sussession of the microbiota during fermentation

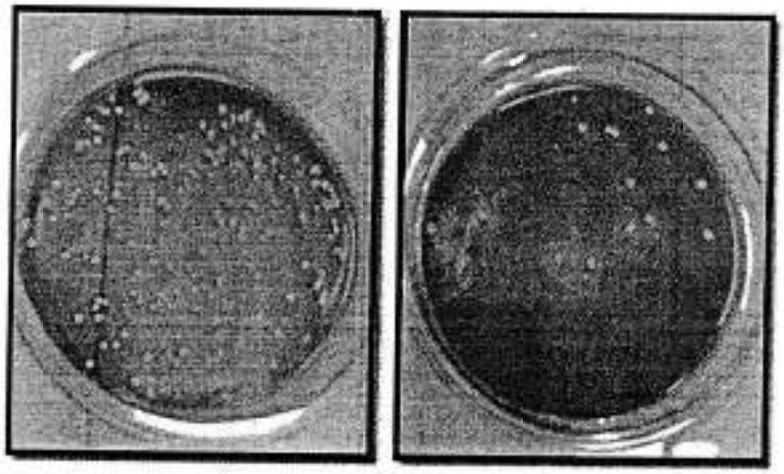


Fig 72: *Lactobacillus* colonies on MRS plates

G. Changes in protein content during fermentation

Protein content shows a similar trend of gradual increase as seen in case of enzyme treated cassava flour fermentation.

Table 76: Protein content starter culture fermented samples

Variety	% of Protein			
	0 Day	3 Days	6 Days	9 days
Joya	1.09±0.012	1.15±0.056	1.16±0.052	1.41±0.085
Sri Bijoya	1.07±0.037	1.11±0.019	1.17±0.019	1.22±0.012
Gossaigaon Local	1.12±0.001	1.12±0.020	1.14±0.014	1.15±0.013

Results in replicate of three ± SD

H. Changes in starch content during fermentation

The starch content of the samples subjected to starter culture fermentation shows gradual decrease in amount, which is quite significant because it shows the conversion of starch to its simpler units (Ezekiel *et al.*, 2009) without the use of commercial enzyme, which was not observed during the fermentation by *L. plantarum* and *S. cerevisiae* without enzyme treatment.

Table 77: Starch content starter culture fermented samples

Variety	% of Starch			
	0 Day	3 Days	6 Days	9 days
Joya	10.21±0.182	9.39±0.228	8.96±0.065	8.45±0.093
Sri Bijoya	10.16±0.016	7.05±0.032	6.60±0.032	5.21±0.463
Gossaigaon Local	10.98±0.021	8.38±0.201	6.20±0.021	5.29±0.212

Results in replicate of three ± SD

I. Total carbohydrate content at different time interval

There is a gradual decrease in total carbohydrates of all the samples which signifies the process of fermentation. Carbohydrate has been utilised by the microbes to produce ethanol (Pandey *et al.*, 2000, Ueda *et al.*, 1981) or protein or fat (Lehninger, 1987).

Table 78: Total carbohydrate content starter culture fermented samples

Variety	% of Total Sugars			
	0 Day	3 Days	6 Days	9 days
Joya	13.95±0.310	11.03±0.303	8.14±0.669	7.39±0.816
Sri Bijoya	12.15±0.685	10.11±0.098	9.14±0.187	8.36±0.141
Gossaigaon Local	12.72±0.464	10.37±0.733	9.01±0.885	8.33±0.517

Results in replicate of three ± SD

J. Changes in organic acids content during fermentation

The main organic acids found to be present in the fermenting samples are the Lactic acid and Tarteric acid. The formation of lactic acid bacteria is may be due to the action of the LAB. Tartaric acid is a common organic acid often found during wine alcohol/ wine production. There is a gradual increase in the lactic acid concentration of the samples in all the cases. For tartaric acid in *Joya* and *Gossaigaon local* varieties decreased in first three days of fermentation and then increase in the concentration in subsequent days is observed.

Table 79: Organic acid content of the samples

Variety	Time	Concentration in PPM							
	(Days)	Lactic acid	Oxalic acid	Tartaric acid	Pyruvic acid	Succinic acid	Formic acid	Citric acid	Propionic acid
Joya	0	10912.38	427.23	7654.90	8.96	ND	ND	64.43	116.24
	3	11152.24	456.94	6930.70	173.35	ND	ND	ND	ND
	6	11493.28	407.05	6856.20	170.68	ND	ND	ND	ND
	9	11704.69	392.83	6790.30	160.77	ND	ND	ND	ND
Sri Bijoya	0	6311.19	253.97	4912.30	22.43	ND	ND	27.20	51.13
	3	7993.28	294.77	5198.10	128.75	24.89	ND	ND	ND
	6	8476.14	274.42	5906.50	127.51	111.72	ND	ND	ND
	9	8773.84	266.46	6615.30	125.06	191.44	ND	ND	ND
Gossaigaon Local	0	7361.55	244.23	4433.20	ND	0.82	100.34	42.59	67.35
	3	8876.69	272.83	4354.70	2.35	126.44	84.24	ND	ND
	6	9210.41	195.64	4551.20	2.201	208.44	76.28	ND	ND
	9	9961.44	182.49	4638.30	ND	490.51	54.63	ND	ND

*ND – Not Detected

8. PREPARATION OF STARTER CAKES

Various types of starter cakes for fermenting rice beer were prepared in the laboratory. The methodology followed for preparing these cakes was in accordance to the traditional preparation procedure followed by the indigenous people and the plant materials were also selected based on the survey work done. To following cakes were prepared.

a. Starter 1

This starter was prepared by mixing plants with rice flour in the ratio of 1:3.

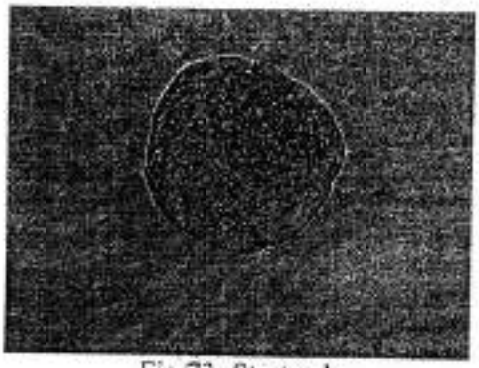


Fig 73: Starter 1

b. Starter 2

This starter was prepared by mixing plants with cassava flour in the ratio of 1:3.

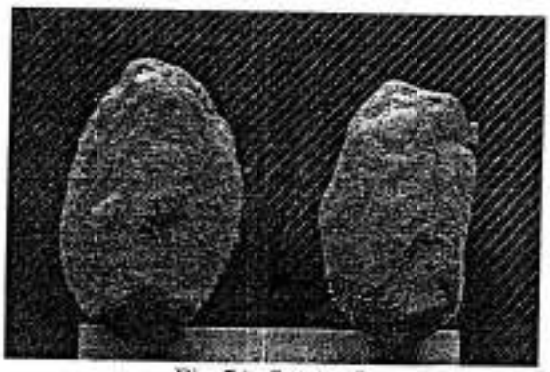


Fig 74: Starter 2

c. **Starter 3**

This starter was prepared by mixing plants with cassava flour and rice flour in the ratio of 1:2:2.

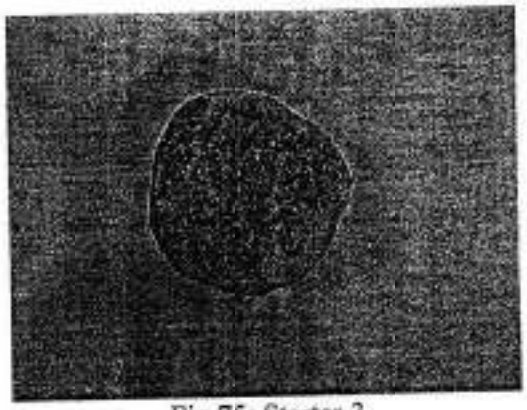


Fig 75: Starter 3

9. DEVELOPMENT OF MINI PILOT PLANT

A mini pilot plant (lab scale) has been developed in the department. This is for the purpose of bottling of the prepared rice beer and also for packaging of the starter cakes. Some photographs of the plant are given below.

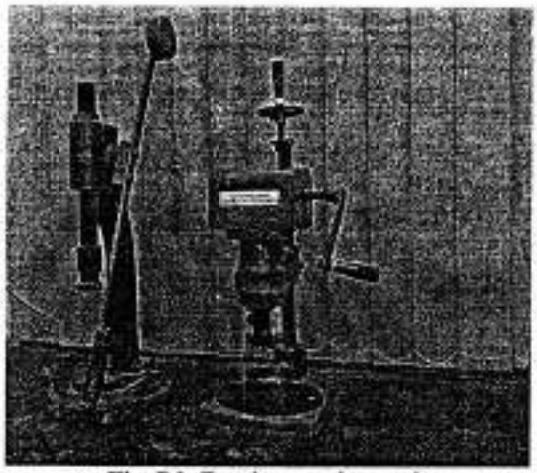


Fig 76: Bottle capping unit

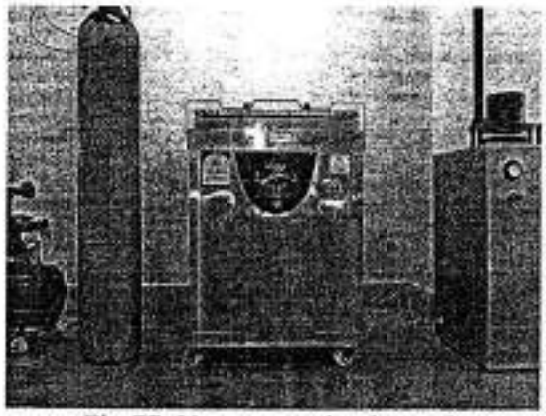


Fig 77: Vacuum packaging unit

Fig 78: Bottles containing rice beer

10. DISCUSSIONS

It was observed that the process of rice beer preparation followed by different ethnic tribes residing in different states of North-East India is more or less similar. The only difference is the ingredient in the form of different parts of various plants species. The tribes in different regions use different plant species based on their availability. This has been reported earlier by Tanti *et al.* 2010. Some of the plant species documented in this article have also been mentioned earlier by different authors like Saikia *et al.*, 2007 about the *Ahoms of* Assam, Deori *et al.*, 2007 about the *Deoris* of Assam, Teron, 2006 about the *Karbis* of Assam, Tiwari and Mahanta, 2007 about the *Arunachalis* of Arunachal Pradesh and Tanti *et al.* 2010 about the *Misings* of Assam. The knowledge of the indigenous people in the use of the starter cultures as a source of yeast is very interesting. The local brews such as rice beer bears very significant resemblance of the culture and traditions of the tribal people residing in this part of the country. Each of the beverages prepared is rooted with the socio-cultural practices of the individual tribes and also on various environmental factors. It has been found that the preparation of rice beer is considered as sacred by all the tribes and it occupies special recognitions in many of the occasions like rituals, festivals, marriages and communal gathering. The consumption of mild amount of alcohol in the form of rice beer gives some relaxation to the hard working population of these states and practically has no side effect on their health. Apart from imparting colour, flavour and sweetness to the beer, the various plants used in the starter culture are also said to have many medicinal properties. Also some of the plant extracts may also provide certain nutrients for the survival of the microflora present in the starter cakes. The quality of the starter culture is said to be dependent on the variety of plant parts used and also on the maintenance of proper sanitary conditions. The preference of the variety of rice used for fermentation also differs from communities to communities. However, it is seen that glutinous rice is preferred more than non-glutinous rice, owing to the taste and alcohol content of the product. Further studies on the plants used and the final product may reveal some other important properties and beneficial effects of this traditional beverage. Furthermore the preparation and local marketing of this product serve as a source of income and livelihood to many of the families living in the rural regions.

For microbiological analysis, all the analyses were done in replicates of three and the mean values were taken. The place count of aerobes showed a lowest value of $2x10^4$ CFU

ml^{-1} in the sample AsB3, while the sample AsB1 had the highest count with a value of $1.9x10^{10}$ CFU ml^{-1}. The count of mesophilic aerobes in fermented *poko*, a rice based fermented food of Nepal, had been reported to start from a count of $7.9x10^{7}$ CFU g^{-1} on the first day of fermentation and decrease to a count of $1x10^{7}$ CFU g^{-1} on the fifth day by Shreshta *et al.*2002. Thapa and Tamang, 2004 has also reported the total count of aerobes in *kodo ko jaanr*, a fermented finger millet beverage to be around 7.4 log CFU g^{-1}.Yeasts were the dominant microbes in all the samples and there was not much variation among the samples with values ranging from $2.7x10^{6}$ CFU ml^{-1} in the sample ArB1 to 2.4×10^{8} CFU ml^{-1} in the sample NaB2. Tamang and Thapa, 2006 studied the count of yeasts in *bhaati jaanr*, which is a type of rice beer made in the Eastern Himalayas, and found that their population increased from 10^{5} CFU g^{-1} on day 1 of fermentation to 10^{8} CFU g^{-1} on day 2, and then gradually decreased to level of 10^{5} CFU g^{-1} on day 10. Shreshta *et al.*, (2002) has found an increase in the population of yeasts from $1.8x10^{6}$ to $1.3x10^{8}$ CFU g^{-1} from the first to the fifth day of fermentation of *poko*. The kind of yeast strain present in the final product will differ based on the type of starter used in the process (Tsuyoshi et al, 2005). Moulds were found to be absent from all the samples. the mucorales have roles in the initial phase of fermentation, mostly in saccharification and their disappearance from the final product have been reported by authors like Tamang and Thapa, 2006, Thapa and Tamang 2004. The *Lactocacillus* sp.were present in all the samples in considerable high number ranging from $2x10^{4}$ to $3.5x10^{7}$. One of the samples AsB3 had a count of LAB even higher than the general aerobes. The population of LAB in the fermentation mixture was found to increase from an initial value of $3.5x10^{6}$ on day 1 to a value of $5x10^{7}$ on day 5 by Shreshta *et al.*, 2002, while Thapa and Tamang, (2004), reported the counts of LAB in *kodo ko jaanr* to range from 4.1 to 6.5 log CFU g^{-1}. Tamang and Thapa, (2006), reported the population of LAB (*Pediococcus pentosaceus* and *Lactobacillus bifermentans*) to be highest at a value of 8 log CFU g^{-1} during the second day of fermentation. *Lactobacillus* and *Pediococcus* are recognised as the most common contaninants of beer and are responsible for approximately 70% of spoilage due to microorganisms (Back, 1994). The common food contaminants of enterobacteriaceae and *Salmonella* and *Shigella* species were found in only one sample (AsB2) in low counts of $2.5x10^{5}$ CFU g^{-1} for and 1.5×10^{5} CFU g^{-1} respectively. *Staphylococcus* species were found to be present in the samples NaB2, AsB1, AsB2 and AsB5 in low count. The presence of some contaminants in a few samples may be attributed to the nutritive content of the product. However, water source or the degree of hygiene maintained during production also may

result in the presence of certain unwanted microbes. Thus it is necessary to educate the people involved in rice beer making about proper sanitary conditions.

The pH of all the samples was found to be low, with the sample MlB1 having a value of 3.35. The sample ArB1 had the highest pH with a value of 5.07. The low pH of beer has been reported earlier by Teramoto et al., (2002), who found the pH of *jutho* prepared in Nagaland to be 3.6. Sanchez *et al.*, 1988 also found the pH of 10 different varieties of *tapuy* (Plilippine rice beer) to be in between 4.6 to 5.0. The total acidity of the of the samples expressed as % of lactic acid was however found to be lower than the samples described by Teramoto et al., 2002, and Shreshta *et al.*, (2002) but had similar values to *yakju* (Korean rice beer) brewed with different wild type yeast strains (Kim *et al.*, 2010). All the samples had similar alcohol content within the range of 3.93 - 4.39%. This content was less than that found in samples of *yakju* of Korea (Kim *et al.*, 2010), *Ou* of Thailand (Chuenchomrat et al., 2008) and *tapuy* of Philippines (Sanchez et al., 1988). The alcohol content was however found to be similar to that of *zutho* (Teramoto et al., 2002) and *poko* (Shreshta *et al.*, 2002).

The ash content in the samples ranged from 0.02% in sample AsB3 to 0.37% in sample ArB1. Crude protein in the samples was found to be present in the range of 0.25% to 1.02%. The highest content was found in the sample AsB4. Chuenchomrat et al. (2008), also studied the ash and protein content of *Ou* samples and found the ash content to range in between 0.1% to 0.3% and the protein content in between 0.45 to 0.99%. The samples NaB2 and AsB4 were found to have the highest content of fats with values of 0.76 and 0.86% respectively.

Total sugars and reducing sugars were found in varying amount in all the samples. The sample NaB1 contained the highest amount of total sugars (8.5%) and reducing sugars (3.4%). Whereas, the lowest amount of total sugars and reducing sugars was found in the samples AsB1 (0.63%) and AsB2, MlB2 (0.2%) respectively. The content of total and reducing sugars during *poko* fermentation was found to start from an initial concentration of 3.2% and 0.4% on day 1 and end with 1.8% and 0.0% on day 5 (Shreshta *et al.*, 2002). Tamang and Thapa (2006) also found the total and reducing sugar concentration to start with an initial value of 64.1% and 0.01% respectively on the first day and end with values of 13.4% and 0.2% respectively on the tenth day during fermentation of *bhaati jaanr*. The concentration of total sugars in samples of *tapuy* has been reported to be in between 0.9% to

0.25% and 0.07% to 0.21% respectively (Sanchez *et al.*, 1988). Teramoto et al. (2002), has also reported the content of total sugars (39.7 mg/ml) and reducing sugars (6.3 mg/ml). The starch and amylose content did not show much variation among the samples. Starch content ranged in between 0.74% in sample MlB1 and 1.38% in sample NaB2 and amylose content varied depending on the content of starch.

The ascorbic acid content in all the samples was within the range of 1.3mg/100g to 2.7 mg/100g. The sample NaB1 had the highest content of polyphenols (10.06 mg/100g) followed by AsB3 (5.05 mg/100g) and AsB4 (4.71 mg/100g). The presence of ascorbic acid and phenolic compounds in the samples may be attributed to the high free radical scavenging activity exhibited by them.

The content of different organic acids present in the samples as detected by HPLC analysis has shown that lactic acid was found in high concentration and other acids showed variations with samples.

It was also seen that the 5th day of fermentation was found to be more suitable in terms of nutritional aspects. The traditional starter culture used for rice beer preparation was found suitable for fermenting cassava which broadens the prospect of using cassava for beer making.

The biochemical parameters of the samples showed that rice beer produced in North-East India can be a good source of nutrition along with mild intoxication. The process of brewing is of major commercial importance in many countries and standardization of the method of rice beer making and its preservation can lead to its commercialization which again through taxation can be an important source of revenue to the government.

Biochemical analysis of the plants used in preparing starter cakes has shown that total polyphenols was present in all the samples in high amount. The highest concentrations were found in samples of *Bhator duamali* (155mg/g) and *Zing –zing* (148mg/g). Both these plants are used by the *Deori* tribe of Assam.

The DPPH free radical scavenging activity was also exhibited by all the plant species and the highest radical scavenging activity of 84.12 % was shown by the extracts of the plant *Bhator duamali*. *Dudu-siring* used by the *Deori* tribe and *Bilongoni* used both by the *Deori*

and *Mising* tribe have also shown good free radical scavenging activity of 82.29% and 80.71% respectively. This shows that the starter cakes also have some antioxidant properties.

11. PUBLICATIONS MADE FROM THE PROJECT

The following publications were made by work done under the project

- Das, A. J., Deka, S. C. and Miyaji, T. (2012). Methodology of rice beer preparation and various plant materials used in starter culture preparation by some tribal communities of North-East India: A survey. *International Food Research Journal* 19(1):101-107
- Das, A. J. and Deka, S. C. (2012) Mini Review: Fermented foods and beverages of the North-East India:. *International Food Research Journal* 19(2):377-392.
- Deka., S. C. (2011).A myriad of starter cultures used in rice beer preparation of North-East India. Invited paper, at 5th International Conference on "Fermented Foods, Health Status and Social Well Being: Challenges and Opportunities" held at CFTRI, Mysore, India December 15-16, 2011
- Das, A.J., Deka, S.C., Khawas, P. and Sit, N. (2012). Studies on some microbiological and biochemical properties of different varieties of rice beer from North-East India. Abstract presented at 18th International Conference (POST ISCBC) Perspective and Challenges in Chemical and Biological Sciences, Innovation Cross Roads, Held at IASST Guwahait from 28th - 30th January, 2012
- Deka, P., Das, A.J., Deka, S.C. and Khawas, P. (2012). Effect of fermentation on some local cassava (*Manihot esculenta*) varieties of Assam vis-à-vis quality attributes. Abstract presented at 18th International Conference (POST ISCBC) Perspective and Challenges in Chemical and Biological Sciences, Innovation Cross Roads, Held at IASST Guwahait from 28th - 30th January, 2012

12. EVALUATION OF 'SCHEME FOR RESEARCH & DEVELOPMENT' UNDER MINISTRY OF FOOD PROCESSING INDUSTRIES

As per direction of MoFPI, New Delhi; a visit was made by ICRA Management Consulting Service Limited on July 30, 2011 to assess the project. Mr. Siddharth Dhyani visited and assessed the project and the evaluation report was submitted to the Ministry by ICRA.

13. CONCLUSION

The North-Eastern states are mostly agrarian with almost two-thirds of the population engaged in agriculture and allied activities. Majority of the land in these states is covered with forests which are rich source natural resources. The fermentation technologies practices by the ethnic people reveal a strong correlation of these people with nature and the assessment of microbial benefits. The rich microbial diversity in various sources of fermented foods and beverages reflects that the indigenous people have been harnessing indigenous microbiota for spontaneous fermentation. Climatic conditions also play major role in the type of fermented foods produced in the temperate, sub-tropical and tropical climates of this region. Besides the food items mentioned here, other products like. Modern science and technological knowledge should be united to produce beneficial results. Development of value added products by selecting productive microbial strains, genetic improvement, process improvement, raw material improvement, improving process control, the use of immobilised systems and/or enzymes, study of probiotic activity and use of genetically modified organisms will lead to industrialization of these food products. Multi-institutional collaborative research will lead to standardization of the fermented food products and increase their shelf life. At present these products are produced only for local consumption. A commercial unit of the traditional fermented foods of the North-Eastern states should be developed which would in turn help in proper marketing of the products in packed form. This would contributes to subsistence of regional economy and prove as a boost to the livelihood of the rural people. Up gradation of the technologies involved can be brought about without damaging the existing form of product. Different kind of neutraceuticals and novel compounds may be produced from fermented foods if proper research is meted out. A database can be developed listing all the fermented foods available in the region, along with their place of origin and production, raw materials used, microorganisms involved, nutritional value and the cost involved. These traditional methods of fermentation and preservation can be commercialised and productivity can be maximised if contributions in terms of financial support and technological development is provided by various governing bodies and institutes.

14. REFERENCES CITED UNDER *LITERATURE SURVEY, METHODOLOGIES* AND *DISCUSSIONS*

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No. 3482 Dated 15th February, 2012

To
Mr. Vijay Kumar
Section Officer
Ministry of Food Processing Industries
Panchsheel Bhawan
August Kranti Marg, New Delhi – 110049

Subject: Submission of Final Report, Statement of Expenditure and Utilization Certificate for release of 3rd installment

Ref: R&D project "Quality Improvement of Traditional Method of Rice Beer Production by the Tribal People of North-East India" (File No. 12/MFPI/R&D/2009 Dated 26th February, 2010)

Sir,

I am sending herewith the Final Report, Statement of Expenditure and Utilization Certificate for release of 3rd instalment for the project entitled "Quality Improvement of Traditional Method of Rice Beer Production by the Tribal People of North-East India" (File No. 12/MFPI/R&D/2009 Dated 26th February, 2010).

I may request you to release the 3rd instalment to meet the deficit amount on time.

This is for favour of your kind information and necessary action.

Thanking you,

Encl: As stated

Yours faithfully,

15/2/2012

(S. C. Deka)
Professor & Head
HEAD
Deptt. of Food Processing Technology
Tezpur University
Napaam, Tezpur-784 028.

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PROPFORMA AS PER GFR 19 – A

(See Rule 212 (1)

Form of Utilization Certificate

Sl.	Letter No. & Date	Amount
1.	**12/MFPI/R&D/2009, dated 24th February, 2011**	**Rs. 30,34,432.00**

Certified that out of **Rs. 30,34,432.00** of grants-in-aid sanctioned during the years 2011-12 in favour of the Registrar, Dept. of Food Processing Technology, Tezpur University, Napaam-784028, Sonitpur, Assam under this Ministry's letter No.**12/MFPI/R&D/2009, dated 24th February, 2011** given in the margin and **Rs. 1,12,981.00** on account of unspent balance of the previous year , a sum of **Rs. 40,47,248.00** has been utilized for the purpose of purchase of equipments, chemicals, glasswares, stationery, raw materials and expenses on manpower and tour expenses for which it was sanctioned, that the balance of Rs. **NIL** remaining unutilized at the end of the year has been surrendered to the Government, will be adjusted towards the grants - in-aid payable during the next year 2012. A minus balance of **(-) Rs.8, 99,835.00** which is due to the Tezpur University.

2. Certified that I have satisfied myself that the conditions on which the grants- in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised that following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised.

1. Accounts audited by qualified Chartered Accountant appointed by this University as Internal Auditor
2. All the equipments, chemicals, consumables etc purchased from the grant are entered in the stock register of the Department.

15.2.2012

(S.C. Deka)
Professor & Head
HEAD
Deptt. of Food Processing Technol
Tezpur University
Napaam, Tezpur-784 028

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NO. 3743
18/7/2012

PROPFORMA AS PER GFR 19 – A
(See Rule 212 (1)
Form of Utilization Certificate

Sl.	Letter No. & Date	Amount
1.	**12/MFPI/R&D/2009, dated 24th February, 2011**	**Rs. 30,34,432.00**

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2. Certified that I have satisfied myself that the conditions on which the grants- in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised that following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised.

1. Accounts audited by qualified Chartered Accountant appointed by this University as Internal Auditor

2. All the equipments, chemicals, consumables etc purchased from the grant are entered in the stock register of the Department.

(S.C. Deka)
Professor & Head
HEAD
Deptt. of Food Processing Technology
Tezpur University
Napaam, Tezpur-784 028

Statement of expenditure till 31st December, 2011

Actual amount spent till December 31st, 2011 against the project, "Quality improvement of traditional method of rice beer preparation by the tribal people of North-East India"

Total grant-in-aid of the project: **Rs. 78, 68,534.00** (File no. 12/MFPI/R&D/2009, dated 26th February, 2010)

Year wise distribution of budget:

Expenditure Head	Amount (Rs. in Lakhs)		Cost (Rs. in Lakhs)
	First year (I)	Second year (II)	I & II Total
Equipments	69,30,534.00	--	69,30,534.00
Manpower (SRF)	1,44.000.00	1,44.000.00	2,88,000.00
Consumables			5,80,000.00
Raw Materials	40,000.00		
Stationery	20,000.00		
Chemicals	3,00,000.00	40,000.00	
Glassware	1,50,000.00	30,000.00	
Travelling allowances			70,000.00
Total			**78,68,534.00**

Amount of money released from MFPI:

Installment	**Amount Released**
1st installment:	**Rs. 39, 34,267.00** (File No. 12/MFPI/R&D/2009, dated 23rd March, 2010)
2nd installment:	**Rs. 30, 34,432.00** (File No. 12/MFPI/R7D/2009, dated 24th February, 2011)
Total	**Rs. 69,68,699.00**

Amount of Money Spent from February, 2011 till December, 2011 (Current Year):

A.	**Budget Head : Equipments/Consumables**			
Sl No	Name of the equipment	TU Purchase order No.	Supplier	Amount in INR
1	Accessories for HPLC system	TU/11-15/Pur/FPT/2011/5060-A, dated 07.10.2010	Dionex India Pvt. Ltd., Mumbai	1,12,500.00
2	Fermentor	TU/11-15/Pur/FPT/2011/831-A, dated 30.05.2011	M/s Eppendorf India Limited, Chennai	16,50,000.00
3	Vertical Autoclave	TU/11-15/Pur/FPT/2011/832-A, dated 30.05.2011	M/s North East Enterprise, Guwahati, Assam	56,224.00
4	Trinocular Microscope	TU/11-15/Pur/FPT/2011/833-A, dated 30.05.2011	M/s Leika Mikrosysteme, Germany	17,46,250.00
5	Custom duty for Trinocular Microscope remitted by TU	Vide challan no. 2002025021	-	85,694.00
6.	Chemicals etc	On process	North East Chemicals, Guwahati	2,64,580.00
B.	**Budget Head: Manpower (SRF)**			
	Arup Jyoti Das	Expenses from 01.02.2011 to 30.12.2011 (in INR)		1,32,000.00
Total (A+B):				**Rs.40,47,248.00**

Abstracted Summary of the Previous Year & Current Year:

Amount Actually Received		**Amount Actually Spent**		**Total Sanctioned Amount of the Project**
(i) During 1st Installment	Rs. 39,34,267.00	Actual Expenses during 1st Installment Period	Rs. 38,21,286.00 (UC submitted vide letter No. 2795 dated 8.02.2011)	Rs.78,68,534.00
(ii) During 2nd Installment	Rs. 30,34,432.00	Actual Expenses during 2nd Installment Period	Rs.40,47,248.00	
Total Amount Actually Released	**Rs. 69,68,699.00**	**Total Amount Actually Spent**	**Rs.78,68,534.00**	
		Minus Balance Rs (-)	**Rs. 8,99,835.00**	
Minus Balance of (-) Rs. 8, 99,835.00 may be released to Tezpur University. (Rupees eight lakhs ninety nine thousand eight hundred and thirty five only)				

15.2.2012

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