1. Introduction

1.1 Background information

Finger millet (*Eleusine coracana*) is the fourth most important food crops of Nepal. It is a vital crop for mid-hills where it is commonly grown as a relay crop in maize/millet system and as a mono crop in the hilly areas of mid-western region. Finger millet occupied 265,496 ha of land and produced 291,098 MT with the productivity of 1,096 kg/ha (NARC, 2010). Use of microorganisms in preparing foods from locally available plant and animal materials is a traditional practice since pre-historic times. Development of spontaneous food fermentation was primarily governed by climatic conditions, the availability of typical raw materials, socio-cultural ethos, and ethnical preferences.

Fermented foods are defined as foods that have been subjected to the action of selected microorganisms or enzymes by which a biochemically and organoleptically modified substrate is produced, resulting in an acceptable product for human consumption. Growth and activity of microorganisms play an essential role in biochemical changes in the substrates during fermentation. Traditional fermented foods are generally nutritious and form the basic components of the diet as staple, adjunct, condiment and beverage, providing calories, proteins, vitamins, and minerals to the people (Tamang, 1998).

The food is considered fermented when one or more of its constituents have been acted upon by microorganisms to produce a considerably altered product acceptable for human use. Steinkraus (1997) defined fermented foods as the food substances that are overgrown by edible microorganisms whose enzymes hydrolyze the polysaccharides, proteins, lipids to non-toxic products with attractive flavor, aroma, and texture. Preparation of fermented foods and beverages involves technology from the most primitive to the most advanced, and achieving an astounding range of sensory and textural qualities in the final products. While most of these fermentations remained at household arts, others have achieved massive commercial application and play a significant part in most national economies. All such fermentations have been classified as indigenous, native to a country or culture.

Alcoholic beverages have played an important role in human spiritual and cultural life both in eastern and western society. Most Asian beer utilizes rice with molded starters. The industrial development of indigenous fermented foods and beverages can be

divided into four areas: raw material development, starter culture development, process development and finished product development. In many developed countries and even in some developing countries, their important indigenous fermented foods have been well investigated and statistical data on production, consumption, impact on socio-economy, biochemistry, nutritional profile, production method etc. are available. However, in Nepal many indigenous fermented foods are yet to be investigated.

Several traditional fermented foods and beverages are produced at household level. There are many regional variations to the production of each fermented products. Fermented foods make up an important contribution to the human diet in many countries because fermentation is an inexpensive technology that preserves food and improves its nutritional value and sensory properties (Murty and Kumar, 1995). The South African Council for Scientific and Industrial Research (CSIR) was tasked with developing the brewing of sorghum beer to an industrial level in 1954. This lead to the development of the industrially produced sorghum beer (opaque) that now well described and industrialized in South Africa, Botswana and Zimbabwe (Gadaga *et al*., 1999).

Fermentation of cereals leads a decrease in the level of carbohydrates as well as some non-digestible polysaccharides. Certain amino acids may be synthesized and the availability of B- complex vitamins may be improved. Fermentation also provides optimum pH conditions for enzymatic degradation of phytate, which is present in cereals in the form of complexes with polyvalent cations such as iron, zinc, calcium, magnesium, and proteins. Such a reduction in phytate may increase the amount of soluble iron, zinc, and calcium several folds. Fermentation significantly improves the protein quality as well as the level of lysine in maize, millet, sorghum, and other cereals (Hamad and Fields, 1979). During cereal fermentations several volatile contribute to a complex blend of flavors in the product (Chavan and Kadam, 1989).

In Nepal, the history of alcoholic beverages dates back to ancient times. These technologies were developed by ethnic groups while celebrating various festivals, feasts and settlement of marriage (Regmi, 2007). The knowledge of home brewing has been handed down from generation to generation without knowing science and technology. In fact, the exact nature of fermentation is still not fully known to them. Among the various fermented foods, *jand*, *toongba*, *nigar*, *hyaun thon,* and *raksi* are the major alcoholic beverages traditionally prepared and consumed in various parts of

Nepal. *Murcha* (a traditional alcoholic fermentation starter) is necessary for the preparation of these beverages in Nepal, some parts of India and China. Traditional alcoholic beverages have strong ritual importance and are deep-rooted in the cultural heritage of the various ethnic groups of people. Social activities require provision and consumption of appreciable quantities of alcoholic beverages by the *matwali* (alcohol drinkers of the non-Bramnin Nepali community mostly Limbu, Rai, Gurung, Tamang, Sunwar, Newar, Sherpa). *Jand* and *raksi* are essential to solemnize marriage ceremony of non-Bramnin Hindu Nepalese and the Bhuddhist tribes. Fermented beverages are offered to perform the *pitri puja* or *kul puja* (a religious practice to pray family God and Goddesses). Among the *Lepcha* tribe, *mong chee* (finger millet *jand*) is essential to perform various cultural functions. *Toongba* and rice-made *raksi* are among the important materials to perform the ritual practice of the Limbu called *tonsin mundhum*.

In East African countries, among other uses, millet is malted and used to brew various traditional beers (Ekundayo, 1996). Malting is simply a biochemical modification of the grain to produce malt that has enhanced nutritional quality compared to normal grain. During malting, the grain develops amylolytic enzymes, which hydrolyze starch to fermentable sugars. Some advantages brought about by malting include the improvement of grain nutritional quality by reducing the antinutritinal factors responsible for poor digestibility and increasing palatability of the grains. Use of millet to replace extensive imported barley malt in brewing has been tried (Skinner, 1976; Nout and Davies, 1982). Sorghum has substituted barley malt in the production of lager beer in Nigeria. The malting of finger millet is superior to other millet and is ranked next to barley (Malleshi et al., 1986). Taylor *et al*. (2006) also reported that finger millet malt has a higher level of β-amylase activity than that of sorghum and much less than that of barley. Millet in the form of malt is probably a much better option than using unmalted grain as adjunct in brewing (Taylor, 2009).

In many countries, millet has been used successfully as a substitute for barley, for instance, finger millet has been used in the East Africa (Kenya) and in India as a major ingredient in the traditional manufacture of malt. Local maltsters and brewers in Kenya claim that millet malt is preferred locally because of its higher enzymatic activity and flavor (Nout, 1981). Millet is also a good source of α- and β-amylases and malted millet is extremely used in weaning, infant, and supplementary food formulations (Malleshi, 2005). Pearl millet is widely home malted in sub-Saharan Africa for small scale

brewing of traditional African beer. A small amount is industrially malted in Zimbabwe for producing commercial opaque beer (Pelembe *et al*., 2004). Finger millet is important millet and its malting has been practiced both at home and industrial levels in India and in some of the African countries (Ravindran, 1991). Malted ragi flour or extract derived from it is extensively used in the preparation of weaning and infant foods, beverages or other pharmaceutical preparations (Narayanaswamy *et al*., 1971).

1.2 Problem statement

Notwithstanding the aforementioned scope, traditional food fermentations are not without shortcomings. Indigenous fermentations are uncontrolled and often unhygienic, labor intensive, primitive, and normally not integrated into the economic mainstream. Moreover, they have limited export potential and, in some cases, the impact of fermentation on nutritive value and safety is questionable. The microbiology of many indigenous fermentated foods is undoubtedly quite complex and in many cases specific information on microflora involved appears lacking. Further research should be directed towards identifying the benefits and risks associated with specific indigenous fermented foods and elucidating the contributions of microorganisms, enzymes and other food constituents in the fermentation processes.

Tribal brewing process lacks scientific backgrounds. Some forms of improvement are imperative to transfer household brewing art in the modern industry. The following requirements for industrializing traditional brewing technology can be suggested:

- Scientific knowledge of the basic raw materials
- Scientific production process and quality management system
- High production rate machinery of hygienic design
- Hygienic packaging and distribution system with special attention being paid to the shelf life.

In tribal cereal-based alcoholic fermentation, traditional fermentation starters are used as the starter culture. The starters are mixed cultures of a wide variety of microorganisms. Eventually, the use of such starters frequently leads to inconsistent product quality resulting in the loss of money and goodwill. Failure to adopt hygienic practices and controlled cultural and fermentation conditions (pH, temperature, etc.) makes the problem further worse. Although these technologies are primitive, they have played a major role in the socio-economic conditions of Nepalese people, especially in

hills. Such technologies are also receiving renewed interest from the scientific communities because indigenous foods are intimately associated with our identity and survival. Hence, efforts expended towards the development of indigenous foods are imperative.

In traditional method of cereal fermentation, all the procedures are followed as a code of practice under ambient conditions. Sanitary conditions are not strictly maintained. The use of *murcha* means the use of mixed micro-flora where the product quality never comes out to be uniform. Moreover, there will be an equal chance of being success or failure in obtaining a sound product. The use of mixed microflora may produce different metabolites that ultimately affect the quality of the end product. *Murcha* may vary in types and concentration of microflora that may result in inconsistent and poor quality products.

Although the technology of cereal fermentation has been known since antiquity in Nepal, its production is still limited to home brewing scale. No consolidated efforts have been made to commercialize traditional alcoholic beverages. One of the major factors in hindering its development is the low priority given to the research and development in our country. Japanese sake, which was a traditional product once, has now been commercialized and marketed worldwide. Due to rapid urbanization, many of the indigenous practices in developing countries are on the verge of extinction. Awareness on indigenous knowledge is likely to help preserve the identity of local communities.

A significant number of researches and reviews on *murcha* and similar amylolytic fermentation starters are now available (Bhatra and Millner, 1974; Karki, 1986; Gautam, 1987; Verma, 1991; Shrestha, *et al*., 2002; Shrestha and Rati, 2003; Dung *et al*; 2007; Tsuyoshi *et al*., 2005). These works, however, appear to be limited to either simple description or isolation and identification of *murcha*, or assessment of brewing value of *murcha.* Study on the preparation of fermentation starter using isolated cultures and physico-chemical and microbiological characteristics of the prepared product using the defined starter are relatively scarce.

Improvement on the traditional cereal fermentation will require certain scientific inputs. Thus, at a time when improvement of traditional technologies is being recognized as a powerful alternative to socioeconomic empowerment in the underdeveloped countries, research on indigenous starters such as *murcha* is essential. Fermentation starters

similar to *murcha* are found in many Asian countries. As a result of series of researches, many of them, for instance Chinese *chu*, Korean *nuruk*, Malaysian *ragi* and Japanese *koji*, have now evolved as commercial commodities. These starters contain yeasts, molds, and lactic acid bacteria and are used for the preparation of a wide variety of cereal based alcoholic beverages. A critical view of Nepalese traditional alcoholic cereal fermentation reveals that the production processes are laden with problems. The problems can be considered in the categories of production environment, microbiology, process control, and nutritional and toxicological status.

1.3 Significance of the study

Grains such as finger millet, broken rice, low quality maize, etc., are normally considered inferior for food use, but can be used for alcoholic fermentation to produce product of preference. The spent grains obtained after the preparation of alcoholic beverages can be utilized for cattle feed. Thus, cereal fermentation can become a viable option for the rural mass if adequate attention were given on its promotion, development, and standardization. The preparation of homemade beer (*jand*) and whisky (*raksi*) is mainly the business of women and it has played a significant role in gender empowerment. It is a traditional work developed into a new economic opportunity with market demands in the peri-urban and urban areas. The indigenous skill of brewing can be regarded as a human capital, substantiated as a key to successful livelihood diversification of the poor and marginal community. Such income generating capabilities of women also help improve the care and nutritional status of children.

Several traditional fermentation technologies from Asian and Africa have been upgraded to high technology production system because of the continual efforts on research and development. Their experience can be used to upgrade Nepalese indigenous fermented foods and beverages. The development of appropriate technologies aimed at upgrading the quality of Nepalese foods will be indispensable for the growth and survival of cottage level food fermentation businesses. The use of defined fermentation starter to carryout fermentation not only ensures safety of the product, but one can also incorporate desirable probiotic flora into the product. The fermentation not only enriches the diet through development of flavor, savory, and taste, but also improves the substrate biologically with protein, essential amino acids, essential fatty acids, and vitamins status (Steinkraus, 1995).

Development of stable fermentation starters will be an attractive proposition for use in small-scale food fermentation. A good defined starter may reduce fermentation time, minimizes dry matter losses, avoid the growth of undesirable microorganisms that causes off-flavor in the product. In the exercise of upgrading traditional food fermentation technique, it would, therefore, be worthwhile to investigate the effect of inoculum enrichment on product characteristics.

In many countries, millet malt has been successfully used as a substitute for barley malt, for instance, finger millet in East Africa and India (Nout, 1981). However, works related to the malting of finger millets are scarce in Nepalese context. In Nepal, there are five breweries in operation at present and produce about 500,000 hL of beer annually utilizing about 7,000 MT of barley malt that are imported from abroad. This proves that about 3,000 m Nepalese rupees (40 m US$) is being spent to purchase barley malt annually. Finger millet malt, if substituted to barley malt, partly if not totally, could help to reduce the overflow of a huge Nepali currency, thereby making benefit to both industrialists and farmers. Moreover, in the developing countries, today there is a growing demand for gluten-free food and beverages from people with celiac disease and other intolerances who cannot eat products from wheat, barley or rye (Fasano and Catassi, 2001).

2. Objectives of the study

In Nepal, finger millet is extensively used to prepare alcoholic beverages using traditional fermentation starter and studies related to its commercialization through product development are scarce. Therefore, the overall objectives of this study were to:

1. improve the safety and wholesomeness of fermented finger millet through the use of defined alcoholic fermentation starter, and
2. study the malting characteristics of Nepalese finger millet varieties with the view to determining their suitability in brewing lager beer.

The specific objectives of this study were to:

1. prepare defined cereal-based alcoholic fermentation starter using pure culture of yeast and mold,
2. study the effects of fermentation containers and raw materials on the chemical and sensory quality of fermented cereals,
3. investigate the effect of semi-solid state fermentation on the chemical and sensory quality of fermented millet,
4. clarify finger millet *jand* using different fining agents and to investigate the effect of clarification on the chemical and organoleptic quality of the millet *jand*,
5. study the storage stability of fermented millet at room temperatre,
6. compare the chemical and sensory quality of finger millet fermented by using defined starter and traditional starter (*murcha*),
7. study the effect of finger millet varieties and germination time on the enzymatic activities and chemical properties of their malt extracts,
8. investigate the effect of malt kilning temperature on enzyme activity of malt,
9. compare among different methods of finger millet malt mashing,
10. study the effects of gibberellic acid treatment on millet malt quality,
11. optimize the mashing process for finger millet malt using response surface methodology, and
12. analyze chemical, physical and sensory characteristics of the finger millet and barley beers.

3. Literature Review

3.1 Introduction to millet

The term “millet” is used for several small seeded annual grasses that are of minor importance in the West but a staple in the diets of African and Asiatic people (Casey and Lorenz, 1977). Five types of millets are common: common millet (*Panium miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), pearl millet (*Pennisetum typhoideum*) and barnyard millet (*Eichinochloa frumentacea*). In many part of the Asia, finger millet is often considered an antidiabetic food. It is believed to contain less carbohydrate and more fiber than other common cereals (Ravindran, 1991).

Finger millet was domesticated in Africa some 3000 years ago and in the same period it was introduced into India. Finger millet, also known as *ragi* in India, is another staple food in Eastern Africa and in Asia e.g. India, Nepal (Leder, 2004). Africa is the centre of origin and still today the major producing area for several cereal crops, notably sorghum, various millet spp., and African rice. These traditional crops have been called ‘orphan crops’ or even ‘lost crops’. Finger millet, named so because the plant head resembles the fingers of the hand, is widely cultivated in East Africa. It has some potentially very useful characteristics with respect to brewing. Research has shown that like sorghum, some finger millet species contain condensed tannins. Malts of the tannin-containing varieties have quite high diastatic power; especially the β-amylase activity of these types is much higher than in sorghum (Taylor *et al*., 2006).

Millet is a grain that has often been associated with food but it has a whole host of nutritional benefits for people as well. Nutritionist may recommend millet if one suffer from asthma, migraines, or thyroid problems. Millet is small, round in shape, and can be white, grey, yellow, or red. It is a tasty grain with a mildly sweet, nut like flavor. It is rich in fiber, B-complex vitamins (such as niacin, thiamine), lectin, and contains some vit. E. Millet is also high in minerals such as iron, magnesium, phosphorous and potassium (Ravindran, 1991).

In Nepal, finger millet, locally called as *kodo* is consumed generally by a small segment of the population in the form of dumpling, porridge, and *roti*. The production of paddy, maize, millet, wheat and barley in Nepal in the year 2008/09 B.S. were 45,23,693; 19, 30,669; 2,92,683; 13,43,862 and 23,224 metric ton respectively (www.moac.gov.np).

In recent years, finger millet has gained importance because of its nutritional strengths in terms of dietary fiber, functional fiber, and starch pattern as well as high calcium and iron contents.

3.2 Chemical composition of finger millet

3.2.1 Proximate composition

The average crude protein (% db), moisture (% wb), crude fat (%, db), crude fiber (%, db), ash (% db) and total carbohydrates (% db) contents were 9.8, 12.40, 1.6, 4.3, 2.8 and 81.5 respectively in three Sri Lankan finger millet varieties. Its crude fiber and mineral contents were markedly higher than those of wheat (1.2% fiber, 1.5% minerals) and rice (0.2% fiber, 0.6% minerals); its protein was relatively better balanced; it contained more lysine, threonine and valine than other millets (Ravindran, 1991). The range of protein content in finger millet has been reported to vary from 5.6 to 11.8% (Deosthale *et al.*, 1970; Pore and Magar, 1977). Protein content in Nigerian millet was reported to be 11.7% (Iwuagwu and Izuagbe (1986). According to McDonough *et al*. (2000), the protein content in finger millet ranged from 4.9 to 11.3%. USDA (2007) reported that protein (%), carbohydrates (%), fat (%), crude fiber (%), ash (%) and calcium (mg %) contents in finger millet (12% moisture content basis) were 7.3, 74, 1.3, 11.7, 2.6 and 358 respectively.

Protein, carbohydrate, fat, crude fiber, and mineral matter contents in finger millet were 7.3, 72, 1.3, 3.6 and 2.7% respectively. Similarly, calcium and phosphorus contents were 344 and 283 mg% respectively (http://www.hesco.in/ecofood.php., accessed on July 30, 2011) According to Leder (2004) protein, fat, carbohydrate, crude fiber and ash contents in finger millet were 6.0, 1.5, 75, 3.6 and 2.6 g/100 g edible portion at 12 % moisture content basis. According to Obilana and Manyasa (2002), the major nutrient contents in finger millet grain were 12% moisture, 74% carbohydrate, 7.3% protein, 1.3% fat, and 2.6% ash. However, finger millet also has a higher content of antinutrients, such as phytic acid (0.48%), that bind divalent cations and tannins (0.61%) which complexes proteins and carbohydrates, thereby decreasing nutrient bioavailability and present grounds for concern (Geeta *et al*., 1977; Ravindran, 1991).

Table 3.1 Proximate composition, vitamins and minerals composition of finger millet

Constituent	Content (db)
Protein (%)	12.0
Eather extractable lipids (%)	4.7
Fiber (%)	11.3
Carbohydrates (% by diff.)	67.0
Mineral matter (%)	5.0
Calcium (mg/100 g)	56
Phosphorus (mg/100 g)	32
Iron (mg/100 g)	7
Thiamin (mg/100 g)	0.45

Source: Matz, 1991)

Constituents	Content (db)
Vitamins (mg/100 g)	
Thiamin	0.19 – 0.62
Niacin	0.13 – 2.5
Riboflavin	0.06 – 0.16
Minerals (mg/100g)	
Calcium	220 - 855
Phosphorus	131 – 904
Iron	6.4 – 1.5

(Source: Hulse *et al*., 1980)

3.2.2 Phytic acid content

Phytic acid is present in grains and seeds of cereals, legumes, oilseeds, and nuts as the main phosphorus storage form. At pH values normally occurring in foods, as well as under physiological conditions, phytic acid is negatively charged and has the potential to bind cations or other positively charged functional groups of molecules. The average phytic acid content in three Sri Lankan finger millet varieties was 0.48% db. The average percentage of the phosphate present in the phytic acid form in finger millet was 60%. For this reason, though the phosphate content of millets is high, its availability at the absorption level would be low (Ravindran, 1991). Dietary phytic acid has been reported to inhibit intestinal absorption of certain divalent minerals such as Ca and Mg, limit protein and starch digestibility, hinder mineral bioavailability and inhibit proteolytic and amylolytic enzymes (Odeoemelan and Osu, 2009).

Mean phytate P content in 3 hill region Indian finger millet varieties was 224.6 mg%, while those of 7 base regions Indian finger millet var. was 194.9 mg% (Wadikar *et al.*, 2006). Udayasekhara and Deosthale (1988) have reported an average phytate content of 188 mg% in colored finger millet variety. Phytic acid content of few Kenyan finger millet var. ranged between 851.6 – 1419 mg% (Makokha *et al*., 2002). According to Leder (2004) phytic acid content in several finger millet ranged from 208 to 246 mg/100 g.

3.2.3 Minerals

Sripriya *et al.* (1997) reported that calcium, phosphorus, iron, copper, manganese, and zinc contents in finger millet (Co-13, brown var.) were 340.15, 276.41, 5.61, 0.54, 3.58, and 2.23 mg/100 g dm respectively. Finger millet is the richest source of calcium among cereals, of which only 162 mg/100 g is available in human body in native millet. They reported that germination was more effective in increasing the bioavailability of Ca, P, and Fe.

The average major minerals viz., K, Na, Mg, Ca, and P contents in three Sri Lankan finger millet finger millet varieties were 0.57, 0.07, 0.13 0.24 and 0.24 % m/m (db) respectively. Similarly, average trace minerals viz., Mn, Zn, Cu, and Fe contents were 5, 2, 4 and 5 mg/100 g dm respectively (Ravindran, 1991). Calcium and iron contents in ragi varieties have been reported to be 220 – 450 and 3 – 20 mg% respectively (Balakrishna Rao *et al.*, 1973). Makokha *et al.* (2002) reported that Mn, Fe, Ca, Na, K and Mg contents in four Kenyan finger millet var. ranged from 3.7 – 121.3; 1.9 – 4.6; 26.6 – 132; 274 – 380.6; 391.1 – 489 and 128 – 179.3 mg/100 g dm respectively. They also reported that natural fermentation of finger millet resulted in an increase in availability of iron, manganese, calcium, and magnesium.

US NRC (1996) reported that calcium, chlorine, copper, iodine, iron, magnesium, and manganese contents in finger millet grain were 358, 84, 0.5, 10, 9.9, 140 and 1.9 mg/100 g respectively. Samantray *et al*. (1989) has reported the following mineral contents in different finger millets and their malts (Table 3.2).

3.2.4 Tannin and oxalate

The range of tannin content in nine Nigerian millet varieties was from 0.48 to 0.53% (Odoemelan and Osu, 2009). Tannin content in brown finger millet variety ranged from 0.35 to 2.39% (Udayasekhara and Deosthale, 1988). The tannins reduce the nutritional quality of foods as they can bind both exogenous and endogenous proteins including enzymes in the digestive tract, affecting the utilization of proteins (Asquith and Buttler, 1986). The average tannin content in three Indian hill regions finger millet was 0.34% as tannic acid, while those of base region seven var. was 0.53% (Wadikar *et al*., 2006). Total and water-soluble oxalate contents in three Sri Lankan finger millet varieties were 29 and 15 g/100 g dm respectively (Ravindran, 1991).

Table 3.2 Mineral contents in different finger millets and their malts

Minerals	Contents (mg/kg)	
	Millet	Malt
1. Copper	9 – 10	7 – 9
2. Magnesium	1.9 – 2.9	2.5 – 2.8
3. Iron	0.2 – 2.0	0.2 – 0.45
4. Zinc	23 – 50	33 – 37
5. calcium	5.4 – 6.2	8.6 – 8.8
6. Potassium	4.8 – 6.0	3.3 – 3.9
7. Sodium	0.09 – 0.14	0.16 – 1.15
8. Sulphur	1.6 – 1.9	1.5 – 1.8
9. Phosphorous	0.15 – 0.406	0.25 – 0.775

(Source: Samantray *et al*., 1989)

3.2.5 Total phenolics

According to Odoemelan and Osu (2009) the mean total phenolics content in nine Nigerian millets was 2.4%. Polyphenols are known to inhibit the activity of digestive enzymes such as amylase, glucosidase, pepsin, trypsin and lipases and the subject has been studied extensively (Rohan *et al*., 2002). Synergy between phenolics may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of type II diabetes mellitus, which is characterized by high blood glucose level (Saito *et al*., 1998). Polyphenols may act as inhibitors of amylase and glycosidase leading to a decrease in post-prandial hyperglycemia (Bailey, 2001).

Phenolic compounds, present in all plants are of great importance for food and beverages derived from plants, since these compounds are responsible for their organoleptic properties. Consequently, they are closely related to the quality of such products, and thus their analysis is of considerable interest. Moreover, in recent years, numerous research studies have associated with the consumption of food rich in polyphenols with the prevention of cardiovascular diseases, certain types of cancer and other diseases related to aging due to their antioxidant properties (Borbolan *et al.*, 2003). Total phenolics in finger millet grain were 0.55 – 0.59 mg% db, as catechin equivalent (Siwela, 2009).

3.3 Traditional cereal-based alcoholic beverages

3.3.1 Classification of traditional beverages

Indigenous fermented foods may be classified according to a number of different criteria. They may be classified in accordance with the raw materials used, the major type of fermentation taking place, the usage of the product, and the district of production. In general, fermented products are classified according to the usage of the products and the major fermentation process taking place; e.g. alcoholic foods and beverages, vinegars, breads, fermented porridges and snacks, and lactic acid fermented fish products. The type of cereals used in the fermentation process and the region of the fermentation form the sub-classes of each category. Steinkraus (1983) has classified indigenous cereal-based alcoholic foods and beverages into three categories based on the way in which saccharification is carried out.

Those in which saliva is the amylolytic agent

It may come as a surprise to some readers to know that in ancient time's saliva was used as a source of diastage (amylase) for the conversion of starch to sugars in fermented alcoholic beverages such as Japanese *sake* (Kodama and Yoshizawa, 1977) and South American *chicha*. It may be more of a surprise to know that in the Andes regions of Bolivia and Peru, the Indians still make *chicha* by the salvation process. The process may have arisen when mother pre-chew grains for their infants and having pre-chewed material leftover found that it became sweet and, if left in liquid, it fermented spontaneously to give an alcoholic beverage.

Those in which starch hydrolysis is accomplished by malting

Malting is a means of converting starch to sugar, thus sweetening cereal grains – was most likely discovered soon after man started harvesting and storing grain. Grains stored in pots or in piles where first moistening and subsequent heating could occur and would undergo a natural malting step. Malted grain in free water would be fermented by omnipresent yeasts producing a very early, primitive beer. Examples of beverages of this categories are African *kaffir* (*kaffircorn*) (sorghum) beer also known as *bantu* beer, Ezyptian *bouza* (wheat-based), Kenyan *busaa* (maize and finger millet or a mixture of finger millet and sorghum),

Those in which starch hydrolysis and fermentation are accomplished by amylolytic molds and yeasts

This category ranges from very primitive Thai rice wines to highly sophisticated Japanese *sake*, which itself developed from a primitive beverage. In all cases, saccharification of the starchy substrate is due primarily to amylase produced by filamentous fungi. The essential amylase producers range from *Aspergillus oryzae* used in Japanese *sake* manufacture to molds of the genera *Mucor* and *Rhizopus*, to *Amylomyces* r*ouxii* and others that play varying roles in the fermentation. The essential alcohol producers vary from *A. rouxii* and *S. cerevisiae* to *Endomycopsis burtonii*, *E. fibuliger*a, *Candida lactosa*, and related yeasts. The starters are generally complex mixtures of essential and nonessential microorganisms. Depending upon the conditions of fermentation, the basic starchy substrates can be converted either to alcoholic beverages or to sweet/sour alcoholic pastes that are eaten with a spoon.

More than 200 alcoholic beverages are described in Korean literature written between the seventeenth centuries. Some of these beverages are identical but differ in nomeniclature; however, most of the products vary according to the methods of preparation, raw materials used, and the season of production (Lee and Kim, 1993). The most important fermentation products of cereals in the Asia-Pacific region are acids and alcohols, which are both flavor compounds and food preservatives. Alcohol fermentation is more important than acid fermentation in this region in terms of the relative amounts of cereals used for fermentation and the varieties of the products produced. European beer uses barley malt as the primary raw material, while Asian beer utilizes rice with molded starter as the raw material. Fermented products from maize are usually found in Africa and in Central and South America while those from sorghum and millet are found in Africa and South Asia. Food fermentations based on rice are practiced in India, China, South-east Asia, and the Far East, while those from wheat are particularly important in the Middle East, Turkey, and the Far East.

Finger millet being less preferred for human consumption as compared to other cereal grains is used for the production of alcoholic beverages (Osti, 2004). According to Pederson (1971), *toongba* is an alcoholic beverage prepared from millet in West Bengal. The millet seeds are boiled, cooled, and inoculated with yeast and fermented for 10 days in section of bamboo.

Table 3.3 Examples of cereal alcoholic beverages prepared in the Asia-Pacific region

Product name	Country	Major ingredients	Micro-organisms	Appearances and usage
Rice wine				
Shaosinghjiu	China	Rice	*S. cerevisiae*	Clear liquid
Chohgju	Korea	Rice	*S. cerevisiae*	Clear liquid
Sake	Japan	Rice	*S. sake*	Clear liquid
Rice beer				
Takju	Korea	Rice, wheat	Lactic acid bacteria, *S. cerevisiae*	Turbid drink
Tapuy	Philippines	Rice, glutinous rice	*Saccharomyces, Mucor, Rhizopus Aspergillus Leuconostoc L. plantarum*	Sour, sweet liquid, paste
Brebali	Indonesia	glutinous rice	*Mucor indicus Candida*	Dark brown liquid, sour alcoholic
Jaanr	India Himalaya	Finger millet	*Hansenula anomala*	Sweet-sour alcoholic paste; mixed with water
Alc. rice paste				
Khaomak	Thailand	Glutinous rice	*Rhizopus*, *Mucor*, *Saccharomyces*, *Hansenula*	Semisolid, sweet alcoholic
Tapai pulut	Malaysia	Glutinous rice	*Chlamydomucor*, *Endomycopsis*, *Hansenula*	Semisolid, sweet alcoholic
Tape-ketan	Indonesia	Glutinous rice	*A. rouxii*, *E. burtonii*, *E. fibulinger*	Sweet-sour alcoholic paste
Lao-chao	China	Rice	*Rhizopus*, *A. rouxii*	Paste
Bhattejaanr	India Sikkim	Glutinous rice	*Hansenula anomala*, *Mucor rouxianus*	Sweet-sour alcoholic paste

(Source: FAO, 1999)

The yeast is generally sold as small cakes in the market place which has been identified as *Endomycopsis fibuligera*. *Chhang* and *toongba* are the fermented products prepared in Sikkim. Similarly, *braga* is a fermented drink prepared from millet in Romania (Pederson, 1971). The South American Indians used corn in their preparation called

c*hica* or c*hichara*, a light beverage and s*ora*, a heavy beverage. *Pito* is the traditional beverage popularly consumed throughout Nigeria owing to its refreshing nature and low price. *Pito* is also widely consumed in Ghana (Mwesigye and Okurut (1995). Many alcoholic beverages prepared from rice have been reported. Clear products which are generally referred to as *Shaosingjiu* in China, *chongju* in Korea and *sake* in Japan contain at least 15% alcohol and are designated as rice wine. Turbid beverages such as *takju* in Korea, *jand* in Nepal and *tapuy* in the Philippines contain less than 8% alcohol along with suspended insoluble solids and live yeasts. Japanese *sake* is a clear, pale yellow, rice wine with a characteristic aroma, little acid and slight sweetness (Murakami, 1972).

3.3.2 Technology of traditional cereal-based alcoholic beverages making

Methods of preparing trational cereal-based alcoholic beverage in different parts of the world are presented in Fig. 3.1 to 3.3 (Saono *et al*., 1986; Tamang *et al*., 1996).

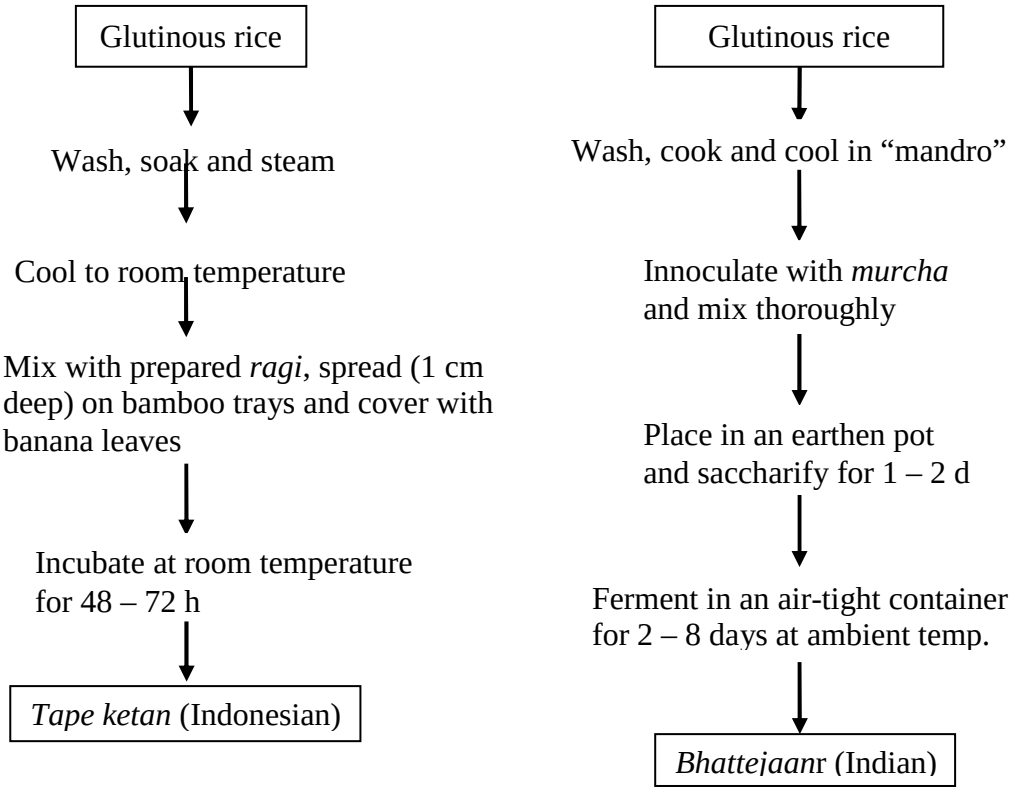


Fig. 3.1 Flow charts for the manufacture of Indonesian take *ketan* and Indian *Bhattejaanr*.

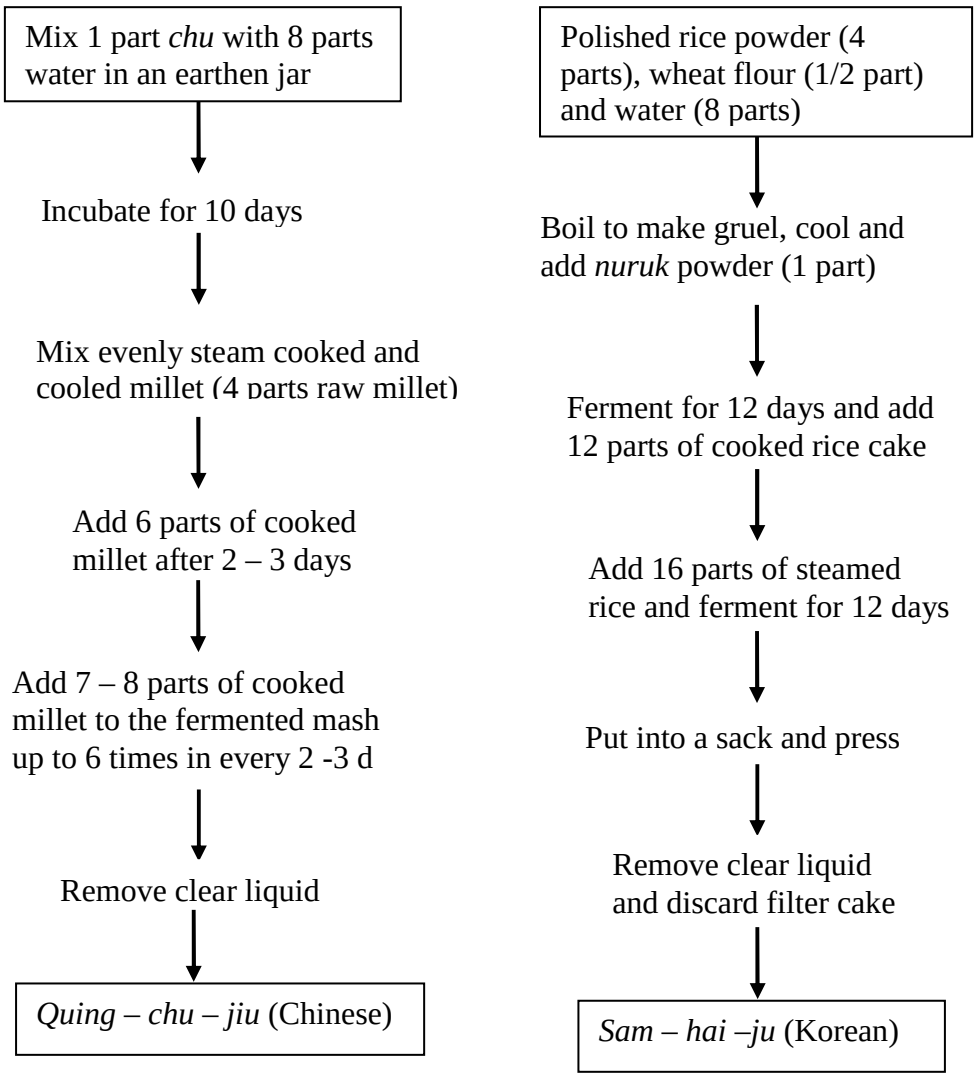


Fig. 3.2 Flow chart for the production of rice wines in China and Korea.

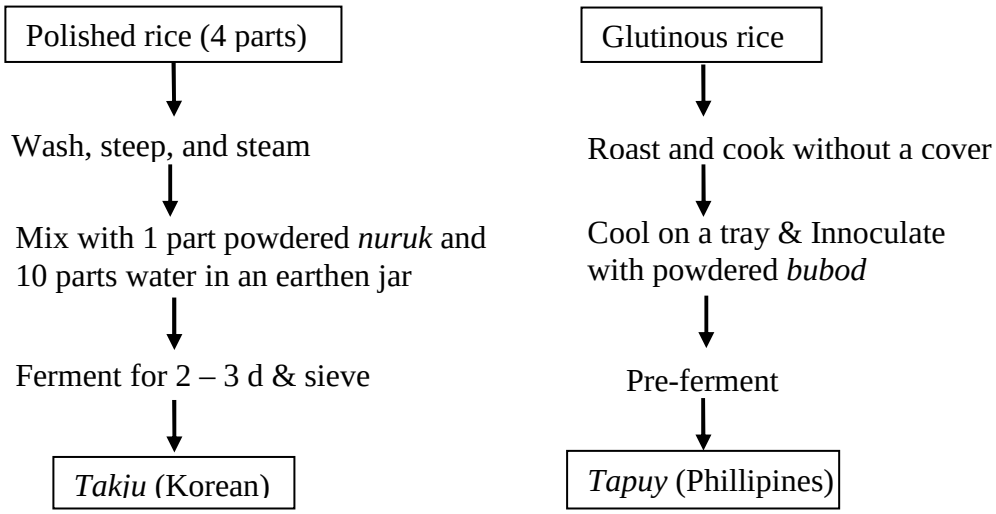


Fig. 3.3 Flow charts for the processing of *takju* and *tapuy*.

3.3.3 Traditional cereal-based alcoholic beverages of Nepal

In Nepal, the history of alcoholic beverages dates back to ancient times. These technologies were developed by ethnic groups while celebrating various festivals and settlement of marriage. The knowledge of home brewing has been passed on to generations but they are quite ignorant about the broad dimensions of microbial biochemistry or their complex mechanisms. In fact, the exact nature of fermentation is still not fully known to them. Among the various fermented alcoholic beverages, *jand*, t*oongba*, *hyaun thon* and *poko* are the major alcoholic fermented liquor traditionally consumed in various parts of Nepal. A wide range of raw materials can be used for fermentation. The materials may include: rice (*Sativa oryzae*), maize (*Zea mays*), wheat (*Triticum vulgar*), millet (*Eleusine coracana*) etc.

3.3.3.1 Traditional process of millet fermentation

A brief outline of the conventional method of finger millet fermentation in Nepal is shown in Fig 3.4. Millet is dehusked by pounding in a mortar and pestle locally known as *Okhli*. The husk, immature grains and other light particles are removed by winnowing using a woven bamboo tray called as *Nanglo*. The millet is washed with fresh water for several times to remove the adhered dirt. Sometimes, the millet is steeped in water for overnight to loosen the dirt and washed. Both red and black millet varieties are used for fermentation, however, black millet variety is said to produce better quality *jand* or *toongba* than that of red one.

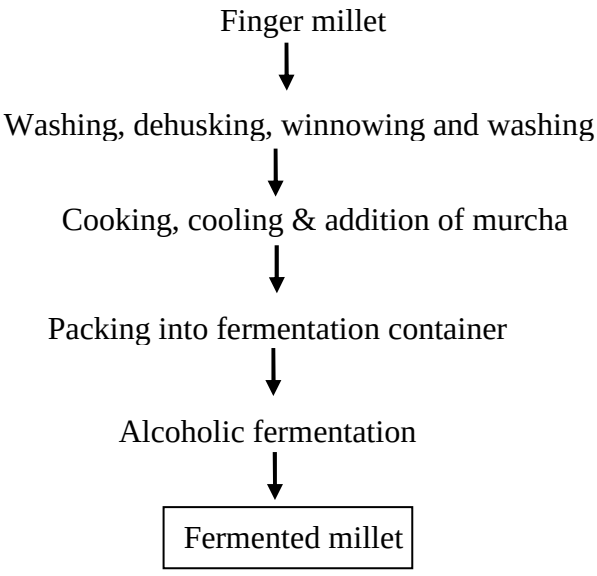
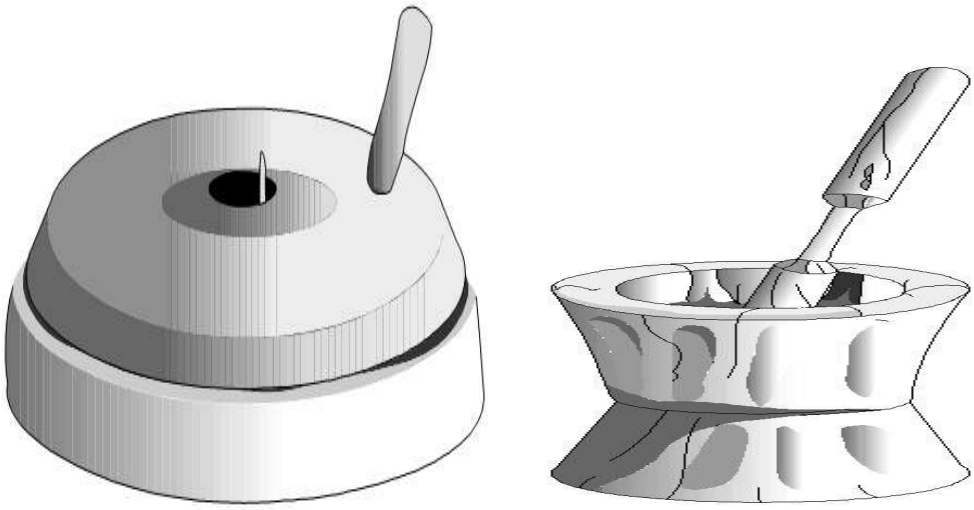


Fig. 3.4 Steps involved in the traditional finger millet fermentation process in Nepal.

The millet is cooked with the required amount of water until rupture. Some makers use a little amount of coriander seeds, (raw or roasted) during cooking in order to improve the taste and smell of the finished product. Another variation of millet cooking method is to heat the water about to boil, add the prepared millet, cook until the grains becomes soft, drain the excess water, cover and keep under mild heat until the grains are splited. Some part of the millet can be substituted with other cereals (viz., rice or wheat, singly or in combination, generally 10 to 20% by weight of total cereal) to improve the taste and smell of the product. It is said that addition of rice or wheat enhances the mouth feel and taste of the jand. Extent of cooking also depends on the type of beverages to be prepared from the fermented millet. For *toongba* making, the seeds are cooked just about to rupture, whereas for *jand* and *raksi* making, they are cooked until completely splitted. It is said that completely splitted millet grain can intensify the amount of solid content in the jand and hence could be used as a substitute for rice.



Janto *Okhal*

Fig 3.5 *Okhali and Janto*

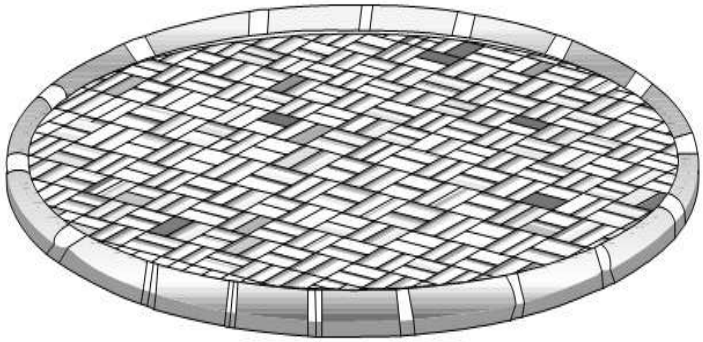


Fig. 3.6 *Nanglo*

The cooked millet is spread over a plastic sheet to cool the mass, powdered *murcha* sprinkled and mixed thoroughly. The inoculated mash is filled in a deep bamboo basket lined with banana leaves or heaped in a polyethylene sheet and left for 24 to 48 h for biomass development (aerobic fermentation). During winter season, the mass is either placed in the sun, near the fireplace or covered with warm clothes to increase the temperature. The presence of characteristic pleasing alcoholic smell and visible puffy colonies of mold indicate the end of biomass development. The amount of *murcha* used is governed by the prevailing fermentation temperature, quality of *murcha* used and the type of intended product. During winter, people use one-half of a *murcha* cake (i.e. 20 g) for *toongba* and a whole murcha cake (40 g) for *raksi* making to each 10 kg of millet used for fermentation, while during summer, the amount of murcha is reduced by half.

If the fermented millet is meant for *toongba*, a slow fermentation with little amount of *murcha* is preferred, while for *jand* making relatively a higher amount of *murcha* is used. Use of little *murcha* (10 to 15 g for every 10 kg millet) and longer fermentation time (>20 days) is said to give a better quality *toongba*. Millet wished-for *raksi* making generally requires the highest amount *of murcha* than those for *toongba* and *jan*d. For *rak*si making, some brewers add a little amount of *murcha* to the fermenting mash before 3 to 4 days of the completion of fermentation. Addition of extra starter during fermentation is thought to augment the yield and strength of the *raksi*.

The biomass-developed millet is now packed into a properly cleaned closed-necked earthen vessel locally called as *Ghyampo* or plastic bucket, the mouth is plugged and set aside for alcoholic fermentation. Fermentation is proceeded from 6 to 7 days (minimum) to as long as 6 to 12 months to obtain a highly matured *jand* (Rai, 1991; Yadav, 1993). Plastic containers of varying grades, strength, and capacity are abundantly available now a day. Traditionally, earthenwares have usually remained the

material of choice as fermentation vessel. The micro pores present therein play an important role in harboring some of the useful microorganisms from the previous fermenting lot and may help to inoculate them upon repetitive use of the same vessel. Some people ferment millet from Poush (December) to Falgun (February) and serve as *toongba* until Bhadra (August).

Fig 3.7 Biomass development of cooked millet.

Despite the method outlined above, variation may occur in practice from locality to locality depending up on the availability of raw material, geographical factors, types of *murcha* and the optional ingredients used to create special effects in the rural context. The final quality of the beverage mostly depends on the technical knowhow of the brewers and the choice of the raw materials.

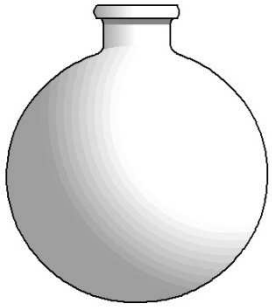


Fig 3.8 Ghyampo – a customary cereal fermentation vessel.

For *raksi* and *jand* (*chhyang*) making, Tamang households of Kathmandu district, Nepal, wet the cereals (e.g. wheat, millet, or maize) for a whole day. The grain is

steamed, cooled, ground and mixed with *manapu* (50 g *manapu* per 15.8 kg of steamed grain, i.e. 0.32% *manapu* by weight of cooked cereal for *jand* and 2.37% for *raksi* making). The mass is kept in a large earthen vessel (*Ghyampo*) and allowed to ferment for 3 to 6 days for *chhyang* and for 7 to 10 days for *raksi* preparation. For *chhyang* preparation, some amount of cold water is added to the fermented mash, stirred, and allowed to settle for overnight. On the next day, the clear white liquid called *chhyang* is racked and consumed (Rajbanshi, 2005).

3.3.3.2 Some important cereal-based alcoholic beverages of Nepal

Jand

Jand (also spelt *jandh*, *jnar*, *jaanr*, *jnard*) is a generic term that refers to sweet-sour cereal beer made by fermenting grains like finger millet (*Eleusine coracana*), rice (*Oryza sativa*), wheat (*Triticum spp*.), and maize (*Zea mays*) etc. using *murcha* (Rai, 2006). It finds a very prominent place in Limbu and Rai culture in particular and among other ethnic groups in general. The tradition of offering *jand* to guests is a unique way of showing hospitality. It is also used in several joyful events, ritual rites, settling disputes and appeasing deities. *Jand* is prepared by leaching out the readily extractable contents from the fermented mash with water (usually lukewarm). A strainer made of thin bamboo strips or perforated aluminum strainer is normally used for straining the liquor. This beverage is served in a vessel called *toongba* (a small brass bowl) or deep aluminum mug. The beverage is cloudy in appearance and has a very short shelf-life of about few hours (Rai, 1991).

Jand is a common drunk in the region, and is traditionally prepared by almost all Nepalese and Tibetans. This slightly acidic, alcoholic beverage is now prepared by small cottage industries. The word *jnard*, derived from the Mangaranti language (the Mangarantis being one of the ethnic groups of the Nepalese), is known by many synonyms *chhyang* by the Tibetans, *chii* by the Rong, and *toongba* by the Nepalese. Finger millet is sometimes supplemented with a small amount of wheat or corn (Tamang *et al*., 1988).

Some aspects of *jand* have been reviewed by Aidoo *et al.* (2005). They have described the role of *mucaraceous* fungi in producing amylases needed to saccharify and liquefy the starch. The amylase activity has been reported to reach its peak on the second day of fermentation. The authors have also mentioned the presence of mixture of yeasts (*Pitchia anomala*, *Saccharomyces cerevisiae*, *Candida galbrata*) and lactic acid

bacteria (*lactobacillus bifermentans*) in numbers exceeding 10^5 cfu/g in matured *jand*. According to Pederson (1971), *chhyan* is a millet beer brewed in Sikkim but Venkataramu and Basapu (1993) had defined the *chhyang* as a traditional fermented beverage made from ragi (finger millet) or barley and consumed by the population in the sub-himalayan region and other Tibetan settlements.The amount of *murcha* to be added to the cooked cereal is regulated by the prevailing temperature and the type of the beverage to be made. Generally, 10 to 20 g of powdered *murcha* is added to 1 kg of cooked cereal (Yadav, 1993). The sensory quality of *jand* naturally depends on its physico-chemcial properties, which in turn are dependent on several factors including the quality of m*urcha*. A simplified relation of this interdependency is shown in Fig. 3.9 and the components of the block are listed in Table 3.4 (Rai, 2006).

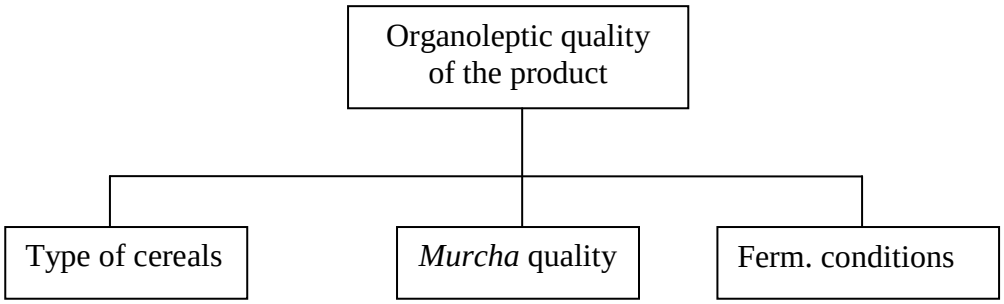


Fig. 3.9 Interdependency of various factors affecting *jand* quality.

According to Thapa and Tamang (2004) in the traditional process of finegr millet fermentation, the dry seeds are cleaned, washed and cooked with enough water. The excess water is drained off and the cooked seeds are spread on a bamboo mat for cooling to 20 to 25 $^{\circ}$C. Powdered *murcha* is mixed at the rate of 2% by weight of the cooked millet, filled into bamboo basket and left for 2 − 4 days for saccharification. The mass is then transferred into a closed earthen pot and fermented for 3 to 7 days at ambient condition (25 to 30 $^{\circ}$C).

Tamang *et al*. (1988) have described the following method of fermenting finger millet for *jand* and *toongba* making in Darjeeling and Sikkim of India. Finger millet seeds are boiled in an open cooker for about 30 min and spread on leaves preferably of banana plants. *Murcha* is powdered and sprinkled over the boiled and cooled seeds. After thorough mixing, the seeds are piled in a heap, kept for 24 h, placed in an earthen pot and covered with leaves followed by cow dung. In urban areas, the seeds are allowed to ferment in a polythene bag.

If air is allowed access and the pot is not kept airtight during fermentation, the product turns sour. The fermented cereal is placed in a bamboo vessel (*toongba*), water is added and after 10 min, the beverage is ready to drink (normally through a bamboo straw). This liquor is believed to be a good tonic, especially for post-natal women.

Table 3.4 Factors affecting overall sensory quality of *jand*

Factors	Components
Raw materials	Cereal substrates such as finger millet, wheat, rice, maize etc. used in the fermentation.
Fermentation conditions	Temperature, pH, aerobicity, duration of fermentation, solid-or semi-solid state of fermentation.
Murcha quality	Species and strains of the essential microorganisms (yeasts, molds, lactic acid bacteria), presence of extracllular enzymes, amylase in particular.
Physico-chemical properties of *jand*	Alcohol, acidity, pH, reducing and non reducing sugars, total soluble solids, esters, and other congeners.
Organoleptic properties of *jand*	Taste, smell mouth-feel and color

Accoridng to Rai (1991) finger millet is the material of choice for the preparation of *jand* and *toongba* because:

- It is a cheap raw material
- It is less preferred for food
- It can remain discrete (i.e. without much liquefaction) for a long time and thus promotes easy handling in the case of jand fermentation, and
- It imparts clean flavour and aroma.

Nigar

Nigar is the clear liquid that spontaneously accumulates during prolonged fermentation of cereal. The product likens *sake* and is highly prized by the consumers. *Nigar* can therefore, be classified as a cereal wine rather than a beer (Rai, 2006).

Toongba

Toongba is another variation of serving fermented finger millet. About 500 g of the fermented millet is transferred to a cylindrical bamboo or wooden barrel (Fig. 3. 10) containing about one-fourth of hot water. The juice gradually and spontaneously is extracted and after about 15 min, the extract is sucked in through a bamboo or metal

pipe called *peepa*. The mash can be repeatedly steeped and sucked in to exhaust the extract. People say that a good quality *toongba* tastes slight bitter and has peppery sensation.

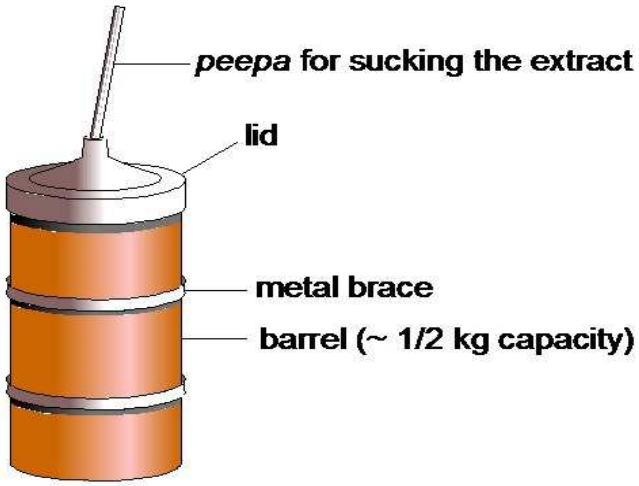


Fig 3.10 *Toongba* – a vessel used for serving fermented millet.

Rakshi

Raski is an unaged congeneric spirit obtained by pot distillation of the slurry of fermented cereal. The product likens whiskey and has varying alcohol contents, generally between 15 to 40% (v/v). Several basic researches have been done on *raksi* production from different cereals using *murcha* as well as pure cultures isolated from traditional fermentation starters (*murchas*) (Rai, 1984; Subba, 1985; Shrestha, 1985; Yadav, 1993, Bhandari, 1997).

After the completion of fermentation, the mash is mixed with some portion of water, poured into the *phoshi* (a flat bottom copper, brass or aluminium pot) to about 1/3rd of its volume and *paini* (earthen pot having holes at the bottom) is placed over it. *Nani* (earthen pot for collecting distillate) is kept inside the *paini* and *bata* (condenser) is placed on top of the *paini*. The *bata* is filled with cold water and firing is made. During distillation, water and other volatiles are evaporated, passed through the small holes of *paini* and condensed on the cold surface of *bata*, which in turn is collected in the *nani*. The water in the *bata* is chaged from time to time when its temperature exceeds 45 $^{\circ}$C. As the number of water changes increases, the distillate becomes weaker in alcohol content. A conventional distillation assembly is shown in Fig. 3.11.

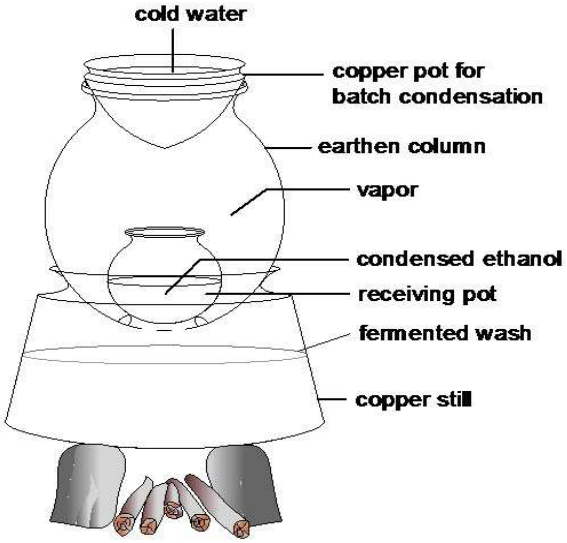


Fig 3.11 A conventioal assembly used for *raksi* distillation.

Poko

Poko is a traditional rice based beverage characterized by creamy color, soft texture, juicy and sour taste with mildly alcoholic and aromatic flavor. This product is widely used by the rural people of central Nepal, especially in Kathmandu valley during occasions like weddings, festivals, cultural celebrations, as well as special offerings to the goddesses. Nepalese people believe that *poko* promotes good health, nourishes the body, and gives vigor and stamina.

A protocol for poko preparation is given in Fig. 3.12 (Shrestha and Rati, 2003).

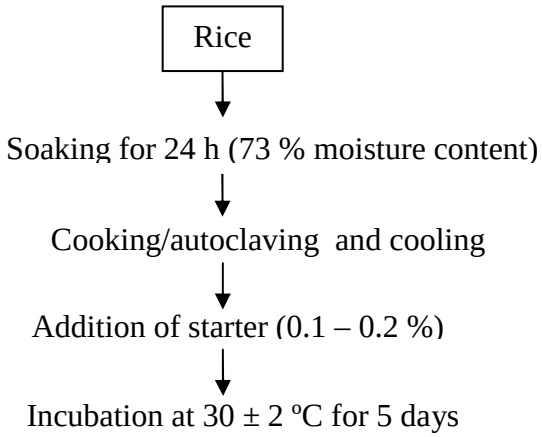


Fig. 3.12 A protocol for *poko* preparation.

Although it is a common dietary item, its production is confined to home scale so far. *Murcha*, the rice-based starters known as *manapu* or wheat- based starters known as mana bring about the traditional poko fermentation. Mixed types of microorganisms

belonging to *mucorales* group and yeasts followed by lactic acid bacteria are involved in poko fermentation (Gajurel and Baidya, 1979b; Hesseltine and Ray, 1988).

Hyaun thon

It is an undistilled alcoholic beverage indigenous to Nepal, particularly in Kathmandu valley among Newar community. It is prepared by the combination of solid and submerged state fermentation of red rice (*hakuwa*) using *mana*. The clear red colored liquid obtained after fermentation is called as *hyaun thon*. Among the Newar community, it is one of the socially and culturally accepted alcoholic beverages and is prepared traditionally using *hakuwa*, *mana* and water generally in the ratios of 1:1:3. According to Narayan Rajthala (personal communication 2008, Lubu, Lalitpur), the term *hyaun thon* is a Newari word meaning red rice wine).

A general method of preparing *hyaun thon* is outlined in Fig. 3.13 (Regmi, 2007).

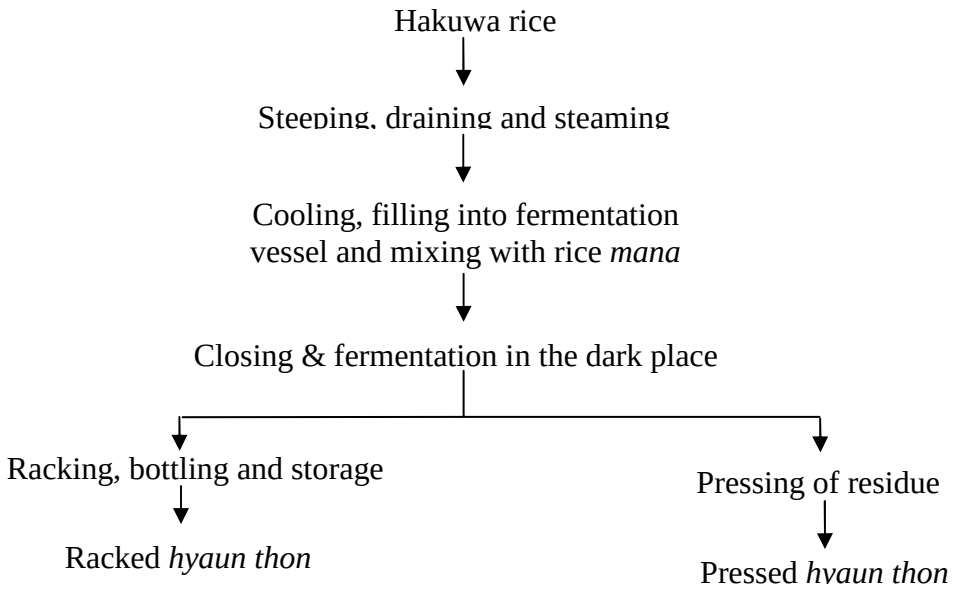


Fig. 3.13 An outline of traditional method of *hyaun thon* preparation.

Hakuwa does not refer to the wild variety of red rice (*Oryza rufipogon*) which is considered as the most weed pest of paddy in the North and South America. In Nepal, the purpose of reddening rice is especially for the preparation of *hyaun thon*. For the preparation of *hakuwa*, the paddy is threshed, heaped, and covered with paddy straw for about 8 – 10 days. In Eucador and Latin America, *hakuwa* is prepared by heaping moist rice (17 – 22% moisture) on open hard floors. The pile is covered with tarpaulin and turned after 4 to 5 days. The resultant product is light golden to cinnamon-brown in

color and is called *Sierra rice* (Regmi, 2007). Somewhat similar fermented rice is prepared in the Yangtse Delta of China where polished rice is mixed with rice bran in the proportion of 10 to 1, piled, and covered with rice-straw mat. After about 6 months, the rice is dried and screened. Delayed drying may result stack burning. Green peas are often added to *hyaun thon* to neutralize acidity. It is believed that good quality *hyaun thon* is possible during winter season (Personel communication, 2007). Sometimes red chilies are mixed with *mana* and it is believed that use of chili protects from evil eyes. Hari Gopal Maharjan (personal communication, 2008 Lubu, Lalitpur) has described the following method of preparing *hyaun thon*. It involves mainly three steps, viz., preparation of red rice, rice *mana* and *hyaun thon* fermentation. Preparation of red rice (*hakuwa*) involves the following process:

> The paddy is harvested, heaped, left for 6 − 9 days (during which the color of the paddy turns brown to red) and threshed. Alternatively, the harvested paddy is threshed and kept in a jute sack for 7 − 9 days. The paddy is now sun dried and milled to obtained red rice. The color of *hakuwa* depends on the length of time for which the paddy is left in heap or sack (for 5 - 6 days, the color becomes brown and for 9 - 11 days, the color turns red).

Red rice (*hakuwa*) is soaked in water for 2 h at room temperature, drained, steamed in a clay pot locally called as *hansi* and cooled. Rice *mana* (made from *hakuwa*) and cooked rice are mixed in equal parts, filled into properly cleaned and steam treated earthen container locally called as G*hyampo* (sometimes water is added to the rice-*mana* mixture at the rate of 3 parts water to each part of cooked rice), stoppered and left for fermentation. Now a cylindrical strainer made up of bamboo (called as *bacha* in Newari language) is inserted into the *Ghyampo*, pressed against the fermented mass and left for sometime during which clear *hyaun thon* is collected into the *bacha*.

If *hakuwa* rice flour is used instead of rice, the resulting beverage is called as *karthu* or *taku* (in Newari language, the term *taku* means thick *jand*). *Karthu* is turbid while *hyaun thon* is more or less clear. For *karth*u preparation, red rice is ground using requisite quantity of water and fermented with rice *mana* similar to that of *hyaun thon*. *Karthu* fermentation requires less time (15 - 30 days) to that that of *hyaun thon*. It is said that *hyaun thon* tastes sweet over 3 − 4 months of fermentation; thereafter it develops a slight bitter taste and again turns sweet taste. About 30 lit of *hyaun thon* are expected to obtain from 40 kilogram each of rice *mana* and *hakuwa*. *Hyaun thon*

prepared in months other than Magh (Jan – Feb) is belived to be either spoilt or not of a good quality.

3.4 Cereal based alcoholic fermentation starters

3.4.1 Introduction to traditional fermentation starters

It is often mentioned that today's modern biotechnology originated from the alcoholic fermentation of primitive peoples. Since indigenous fermented foods were produced by natural fermentation, the origin of cereal fermentation technology is obscure. Unlike fruit and milk fermentations, cereal fermentation requires a saccharification process, which is accomplished with some difficulty. One primitive method of saccharification would be chewing cereals and splitting them in order to allow sacharifiction to occur through the action of salivary amylase, followed by alcoholic fermentation by natural yeasts. Another method of cereal saccharification is through the malting process. However, in Asia the malting process is rarely used in traditional fermentation processes. Instead, fermentation starters prepared from the growth of molds and yeasts on raw or cooked cereals are commonly used. The use of fermentation starters might very well have its origin in the process of Euchok, the daughter of the legendary king of Woo of 4000 BC, known a the Goddess of rice-wine in Chinese culture (Lee, 1984).

Hamei is a traditional fermentation starter used for the preparation of various indigenous alcoholic beverages in North East India. Traditionally *hamei* is prepared from crused raw rice mixed with powdered bark of *yangli* (*Albizia myriophylla*) and a pinch of previously prepared powdered *hamei*. The dough is pressed into flat cakes and kept over rice husk in bamboo basket for 2 − 3 days at room temperature. (20 − 30 ºC) and then sun dried for 2 − 3 days. *Hamei* is used to prepare a rice-based beverage locally called *Atingba* and a distilled clear liquor called *Yu* in Manipur (Tamang *et al.*, 2007).

Murcha is a starter widely used in Nepal, India and Bhutan for the preparation of *jand* and *raksi*, both being alcoholic beverages form starchy grains, the later being a distilled one. In many Asian countries, mixed-culture dough inocula in the form of dry powder or hard balls are extensively used as starter cultures to prepare various fermented alcoholic beverages from starchy substrates. These starter cultures are known under different names such as *murcha*/*marcha*/*bhakar*/*phab* in India, Nepal, Bhutan and Tibet of China, *ragi* in Indonesia, *nuruk* in Korea, *bubod* in the Philippines, *chiu-yueh* in China and *loogpang* in Thailand. The sweet and sour alcoholic beverages prepared by

these starters are *tape ketan* in Indonesia, *jand/chhang* in the Himalayan regions of Nepal, India, and Bhutan, *krachae* in Thailand, *lao-chao* in China and Taiwan. Koji, an amylolytic mycotoxin-free *Aspergillus oryzae* culture on steamed rice is used along with a culture of *Saccharomyces* to prepare the common alcoholic drink *sake* in Japan (Inoue *et al*., 1992).

According to Karki (1994) two types of *koji*, a Chinese starter type called *manapu* (*murcha*) and a granular type called *mana* are prepared in Nepal. *Chu* is commonly used in the Asia-Pacific region as an enzyme source for the degradation of complex plant tissue to produce cereal-wines, soy sauce, fish and meat sauce, sour bread and fermented porridge and snacks.

The appropriate season for the preparation of *chu* is July in which the ambient temperature varies between 20 to 30 ºC in Northern China and the Korean peninsular. The first documentation of *chu* was found in Shu-Ching written in the Chou dynasty, in which it is stated that *chu* is essential for making alcoholic beverages. It is speculated that man must have discovered *chu* much earlier than was documented in the literature **(**Yokotsuka, 1985**).** According to Chi-Min-Yao-Shu written by Jia-Si-Xie of Late Wei kingdom in the 6th century, dozens of preparation method for *chu*, the cereal fermentation starter, were described (Yoon, 1993). Methodology for *chu* preparation is very similar to that for *shi* or Korean *meju*, which is a moldy starter prepared from soybeans, for soy sauce fermentation. According to Chi-Min-Yao-Shu (AD 530-550), *chu* was prepared from barley, rice and wheat and can be classified as described in Fig. 3.14 (Yoon, 1993).

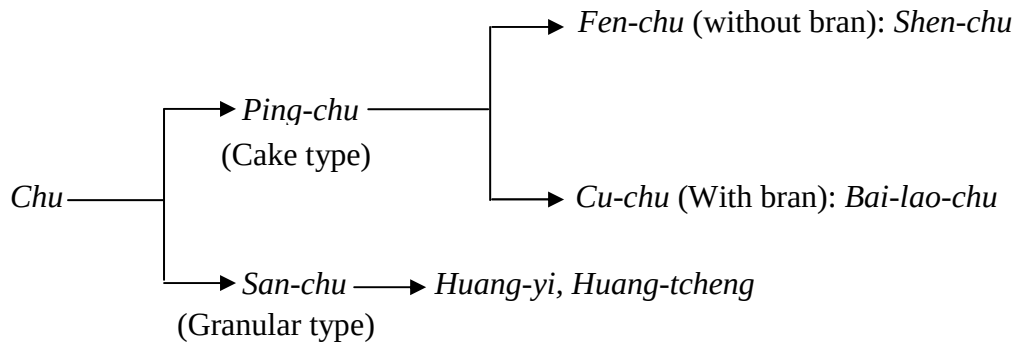


Fig. 3.14 Classification of fermentation starters as described in chi-Min-Yao-Su.

Table 3.5 summarizes the names of fermentation starters usd in different countries and their ingredients. In the preparation of fermentation starters, besides using microorganisms and binders, certain spices are also incorporated, namely garlic (*Allium sativum*), ginger (*Zingiber officinale, Rosc*), pepper. These spices probably inhibit contaminating organisms and stimulate the useful organisms in the starters.

Table 3.5 Fermentation starters used in different countries

Country	Name	Ingredients	Shape	Microorganisms
China	*chu*	Wheat, barley, millet, rice (whole, grits or flour)	Granular/cake	*Rhizopus, Amylomyces*
Korea	*nuruk*	Wheat, rice, barley (whole, grits or flour)	Large cakes	*Aspergillus Amylomyces* yeasts
	meiju	Soybean (whole)	Large balls	*Aspergillus Bacillus*
Japan	*koji*	Wheat, rice (whole , grits or flour)	Granular	*Aspergillus*
Indonesia	*ragi*	Rice flour	Small cakes	*Amylomyces, Endomycopsis*
Malasia	*ragi*	Rice flour	Small cakes	
Philippines	*bubod*	Rice flour	Small cakes	*Mucor, Rhizopus, Saccharomyces*
Thailand	*loopang*	Bran	Powder	*Amylomyces Aspergillus*
India	*murcha*	Rice	Flat cake	*Hansenula anomala, Mucor fragilis, Rhizopus arrhizus*

(Source: FAO, 1999)

3.4.2 Methods of preparing fermentation starters

According to Steinkraus (1983), methods of preparing traditional fermentation starters in different countries are given in Figs. 3.15 and 3.16.

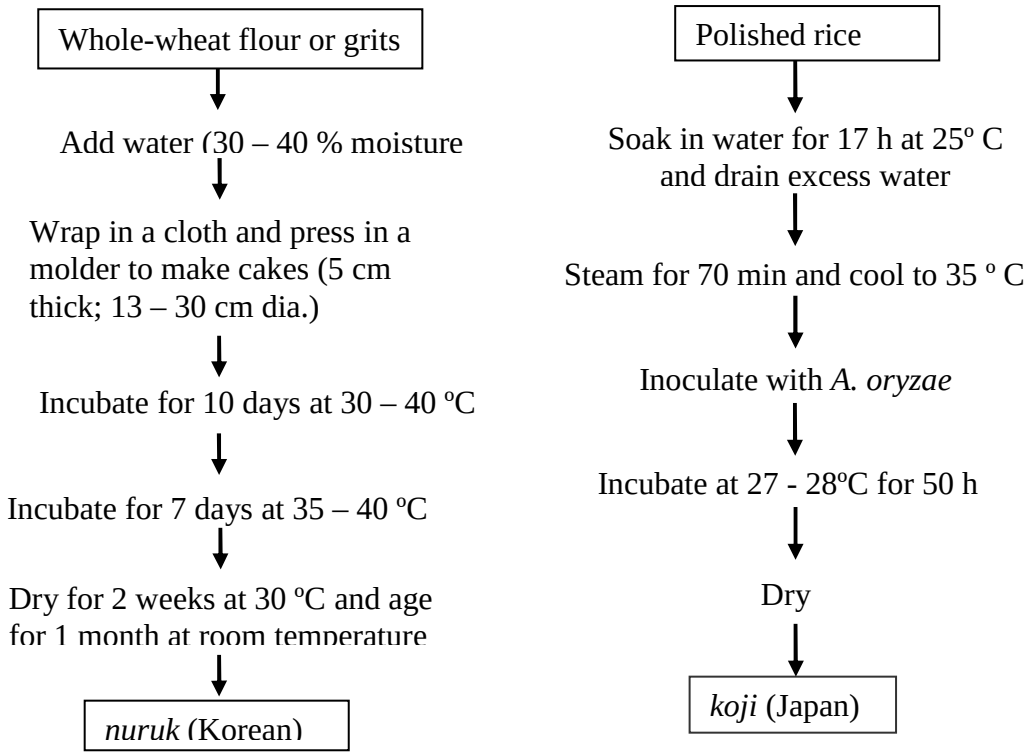


Fig. 3.15 Flow chart for the preparation of solid fermented starters in different countries.

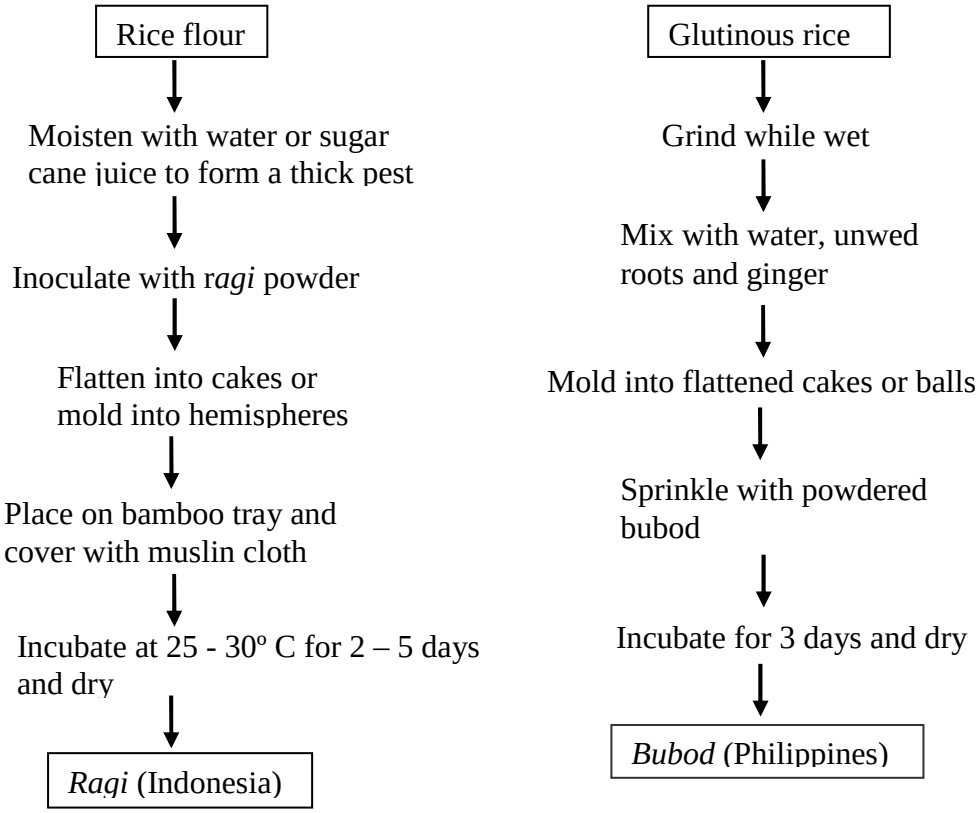


Fig. 3.16 Flow chart for the preparation of solid fermented starters in Indonesia and Philippine

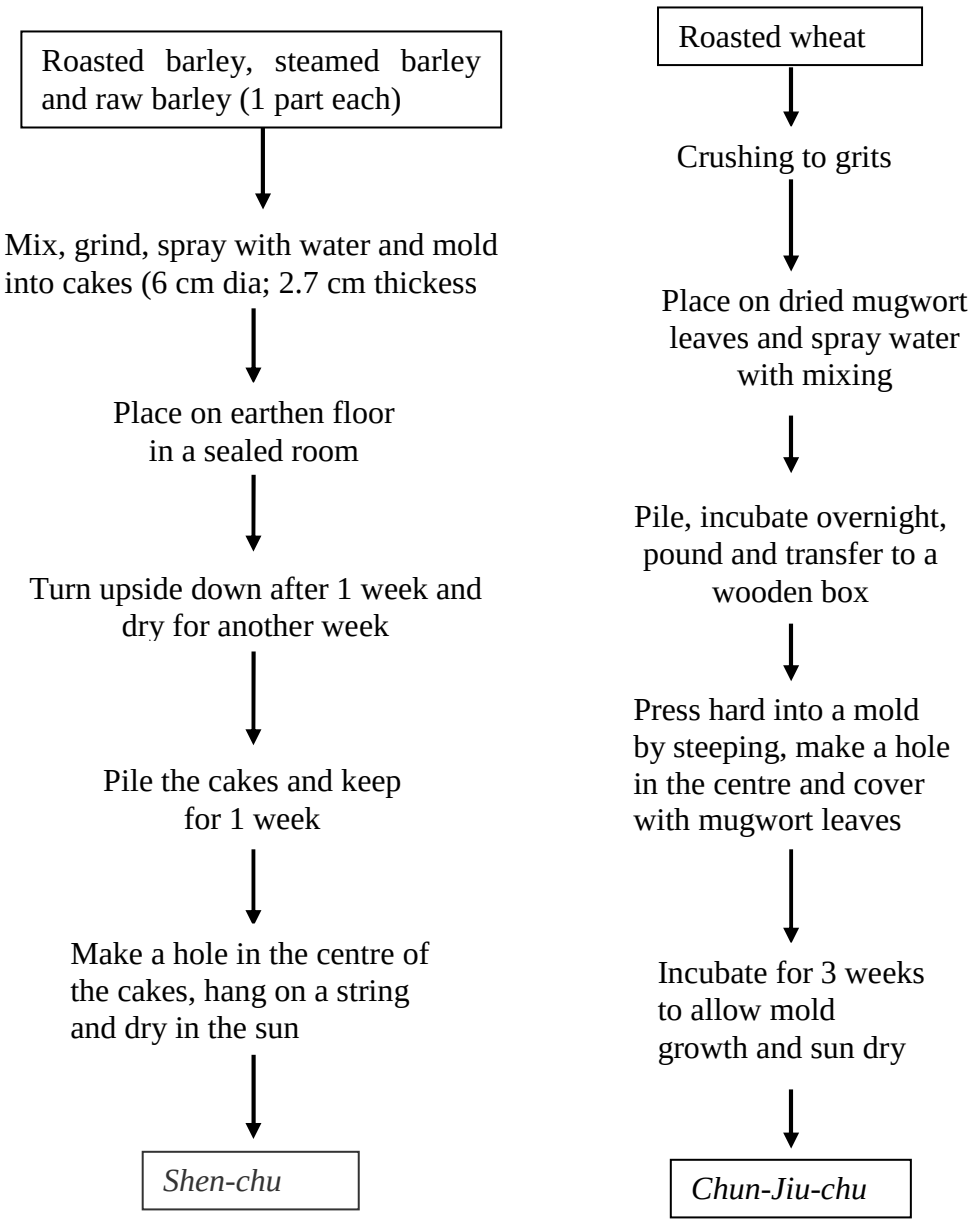


Fig. 3.17 Flow charts for the solid fermentation of *chu* preparation (Source: Yokotsuka, 1985)

3.4.3 Plants used for starter preparation in Nepal

The plants presently used vary widely both with respect to type and parts of the herbs employed for starter making. *Polygala areceata* (locally known as *turne*) has been identified among them. The roots of this herb in the past were used as fresh in the fermentation of starchy products. As lifestyle changed with time and emerged busy, they found it tedious to dig out the roots each time they needed. Furthermore, they could not carry the plant wherever they migrated. These eventually led to the development for preserving the natural micro flora of the herb root by subtracting them

in suitable starchy cereal flour as the media. According to Rai *et al*., (1999) a number of wild plants serve as the source of valuable microflora in the preparation *murcha*. Although the root and bark of *Polygala aricaeta* is probably most prized for *murcha* making, the whole plant of *Vernonia spp*. contains fermenting yeasts, namely *Saccharomyces cerevisiae* and *Pichia anamola*. The author further reported that the potential brewing yeasts presents in *Hisbiscus rosasinsis L., Scoparia dulcts L., Veronica cienrl L. Less, Veronica spp*. and *Polygalla arillata* were *Zygoascus hellenicus*; *Zygosaccharomyces bailii*; *Saccharomyces cerevisiae* and *Pichia* (syn. *Hansenula) anomola* and strains of *Filobasidium capsuligenum* respectively. KC *et al*. (2001) has documented 38 *murcha* plants from the Eastern region of Nepal which are given in Table 3.6. Of the various plants studied, the authors considered two species, viz., *Polygala arillata* and *Inula spp*. as important in *murcha* preparation. Out of some 16 ethnic groups interviewed, 12 of them were found to prepare and sell *murcha*. The list, in the descending order of involvement in the *murcha* trade is: Limbu, Tamang, Gurung, Majhi, Rai, Kumahal, Magar, Urau, Sherpa, Dhimal and Meche.

3.4.4 Traditional starter for cereal-based alcoholic fermentation in Nepal

Murcha, an amylolytic fermentation starter indigenous to Nepal, is used in the preparation of alcoholic beverages such as *jand* in the Darjeeling hills and Sikkim in India, Nepal, and Bhutan (Tamang *et al*., 1996). It is a dry, round to flattened, creamy white to dusty white, solid ball-like starter ranging from 1.9 to 11.8 cm in diameter and from 21.1 g in weight. *Murcha* is a Nepali word. Different ethnic communities of the region call it by their own dialect such as *khesung* by Limboo, *bharama* by Tamang, *bopkha* or *khabed* by rai, *phab* by Bhutia and Tibetans, and *buth/thanbum* by the Lepcha. *Marcha* is produced at home exclusively by women. This art is practiced as a hereditary trade that passes from mother to daughter (Tsuyoshi *et al*., 2005).

In Nepal, *murcha* starter cakes are of two types, *manapu* and *mana* (Karki, 1986; Shrestha *et al*., 2002). It has become a tradition to prepare *murcha* between September 10 and 20. There is a cultural belief that the quality of starter would be better if it is made during “Ganesh Chaturthi” (a famous Hindu festival), which generally falls in the month of September. The *manapu* is prepared from rice flour and millet grain; whereas the *mana* is prepared from wheat flakes or red rice locally known as *hakuwa* (Regmi, 2007). The method of *mana* and *manapu* preparation is kept so secrete by some people

of the Lubhu area of Kathmandu valley, Nepal, which it is not even taught to daughter but only to daughter-in-law.

Table 3.6 Plants used for *Murcha* making in the Eastern Nepal

Name	Vernacular name	Plant parts used
Ananas comosus (L) Mer	Anaras	Leaf
Anaphalis triplinevis	Buki Phul	Whole
Artocarpus heterophylus Lamk	Rukh Katahar	Fruit-stalk, leaf & bark
Asparagus racemosus Wild	Kurilo	Root
Buddleja asiatica Lour	Bhimsenpati	Leaf and tender shoot
Capsicum annum L.	Khursani	Fruit
Carica papaya L.	Mewa	Root
Centella asiatica L.	Ghodtapre	Whole
Clematis grewiaeflora	Mahagagro	Whole
Clerodendrum indicum (L.) Kize	Bhatu	Tender shoot
Christella appendiculata (Bl.) Holtt	Pire Unyu/ Uneu	Tender leaf
Dolichos lab-lab L.	Hiude simi	Root
Drymaria cordata Wild.	Abhijalo	Whole
Elephantopus scaber L.	Mulapate	Root
Elephantopus sp.	Anglah lena	Whole
Geniosporum coloratum D. Don	Sengreng	Tender shoot and flower
Ichnocarpus fructescens R. Br	Dudhe	Whole
Ichnocarpus sp.	Tite	Root-bark
Inula sp.	Chhatre	Whole
Juglan regia L.	Okhar	Bark
Ophiopogon parviflorus (Hook.f) Hara	Lasunpate	Root-tuber
Piper chaba Hunter	Chabo	Whole
Piper longum L.	Pipla	Whole
Piper nigrum L.	Marich	Whole
Plumbago zeylanica L.	Chitu	Whole
Polygala abyssynica Buch. Hum.	Gahate jhar	Whole
Polygala arillata Buch.Hum.	Khedei	Root-bark and flower
Polygala triphyla Buch. Hum.	Pulukna	Whole
Polygala sp.	Angtellek	Whole
Pteridium revolutum (Bl.) Nakai	Unyu	Tender leaf
Scoparia dulcis L.	Chini jhar	Whole
Sida acuta Burm F	Khareto	Leaf
Spergula arvensis L.	Lwangta	Whole
Spilathus acmella (L.) Less	Pire jhar	Whole
Vernonia cinerea (L.) Less	Phulange	Whole
Vernonia sp.	Nighare	Whole
Woodfordia fructicosa Kurz	Dhaenro	Flower
Zingiber officinale Roxb.	Adhuwa	Rhizome

(Source: KC *et al*., 2001)

Mana is generally used in *hyaun thon* making. According to Karki (1986) during *murcha* preparation rice flour is mixed with powdered *murcha* plant in the ratio of 90:3.5 (m/m). According to him, about 10% of the *murcha* production is industrialized and its shelf-life has been reported to be one year.

Thapa (2002) has described the following method of murcha preparation. Glutinous rice is soaked in water for 6 − 8 h and crushed in a foot-driven heavy wooden mortar and pestle. For 1 kg of rice, ingredients added include roots of *Plumbago zeylanica* L., 2.5 g; leaves of *Buddleja asiatica Lour*, 1.2 g; flowers of *Vernonia cinerea (L) Less*, 1.2 g; ginger, 5.0 g; red dry chilli, 1.2 g; and previously prepared *marcha* as a mother culture, 10.0 g. The mixture is kneaded, shaped in to flat cakes of varying sizes, placed individually on a platform suspended below the ceiling made up of bamboo strips above the kitchen and bedded with fresh fronds of ferns [*Glaphylopteriolopsis erubescens* (Wall ex Hook) Ching]. The bed of the cakes is covered with dry ferns and jute bag. These cakes left to ferment for 1 − 3 days, sun-dried for 2 - 3 days and stored in a dry place for more than a year.

According to Rai (2006), some *murcha* producers pound soaked cereals along with sun dried or fresh *murcha* plant and a small amount of *murcha* seed (about 1%). The molded mass (in the form of ball of about 1.5 cm dia. or flat cakes of 0.5 - 1 cm thick and 3 - 10 cm dia.) is placed in a single layer on a mat lined with fern leaves and covered with fern leaves. Data on *murcha* production in Nepal is scanty. However, it can assume that the annual production would not be less than 100 MT. This trade is one of the important economic activities in Matuwali communities (collective term for those with the tradition of drinking alcohol).

Rai (1984) has given the following method of preparing *murcha*.

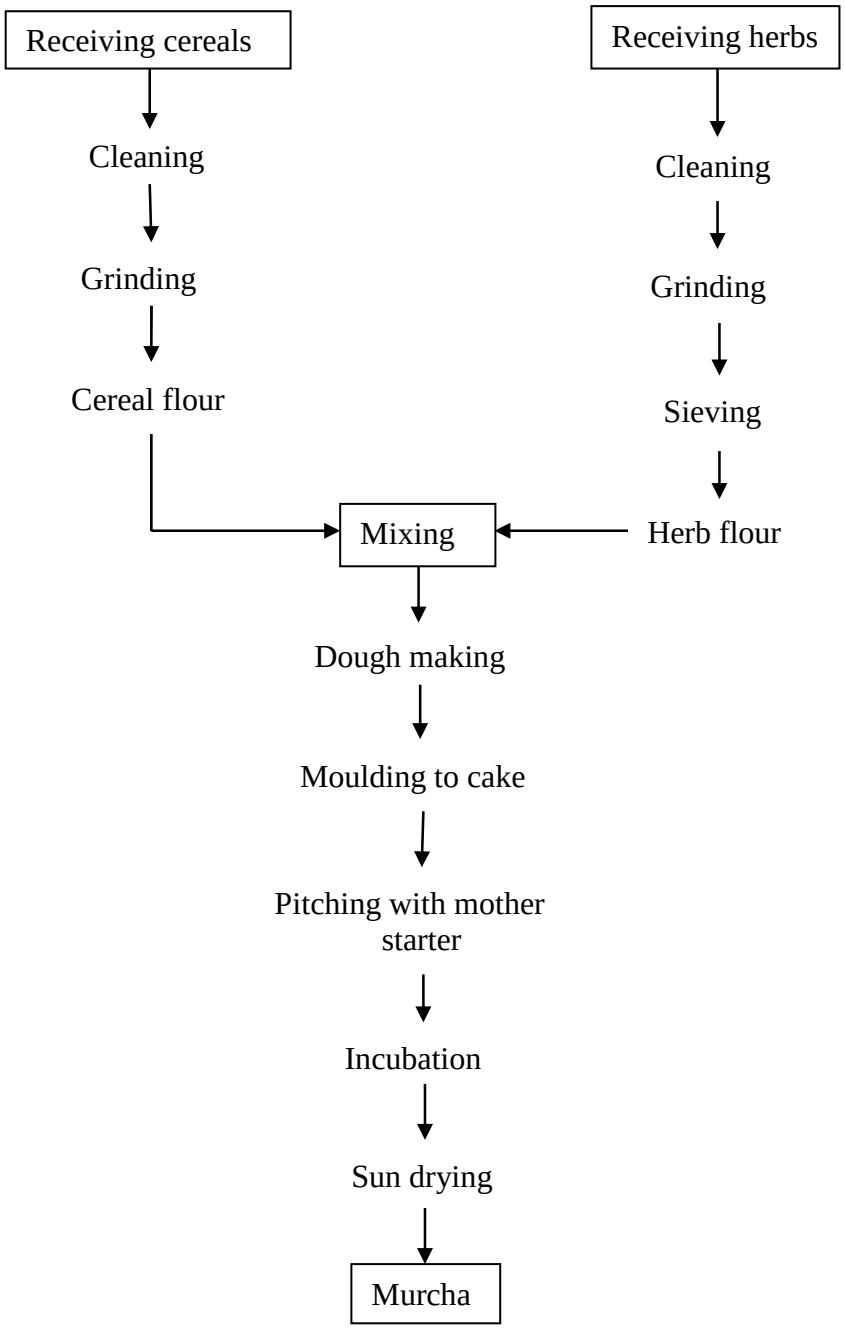


Fig. 3.18 Traditional method of *murcha* making in Nepal (Source: Rai, 1984).

Regmi (2007) has outlined the following method of preparing *mana* and *manapu* in Nepal (Fig. 19).

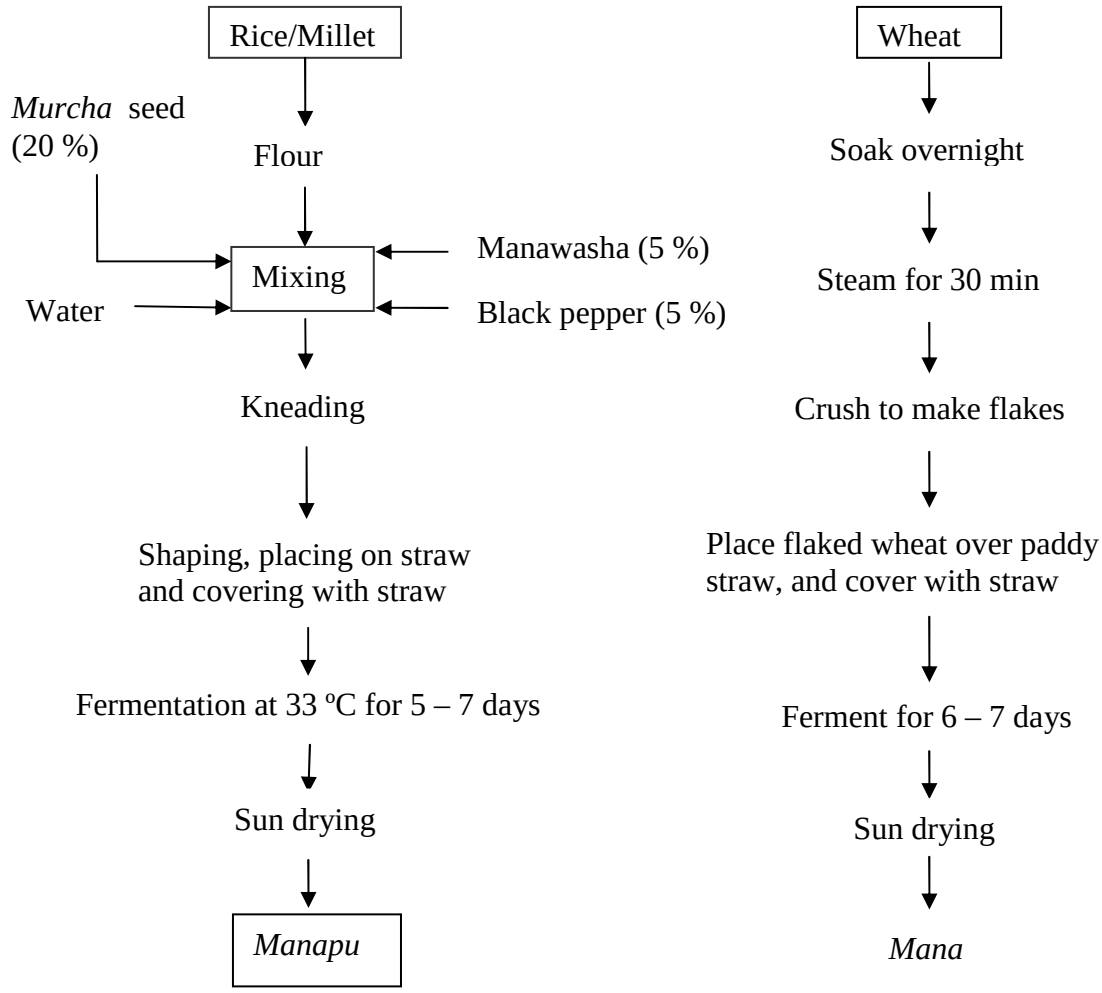


Fig. 3.19 Traditional method of preparing *manapu* and *mana.*

Newari murcha, white in color (*manapu*) is generally made from rice flour while *Tamang murcha* is made from millet flour and is dark in color. *Newari murcha* cakes are smaller, weigh about 6.7 g per cake while the Tamang *murcha* cakes are large, and weigh 25 g per piece (Rajbanshi, 2005). *Murcha* found in the Easter zones of Nepal is prepared by including a wide variety of plants in a starchy carrier medium. These plants serve as an inexhaustible source of fermenting microorganisms. Shresth and Rati (2003) prepared defined fermentation starters using *S. cerevisiae*, *Rhizopus chinensis* and *Pediococcus pentosaceus* in rice flour. In their preparation, 50 g of rice flour was sterilized and were added pure cultures of yeast, mold and lactic acid bacteria. The mass was then kneaded with the addition of required amount of water to prepare stiff dough. Round cakes (3 – 6 cm dia) were prepared and dried at 50 ± 2°C for 24 h.

Inoculation rates of 7 – 9, 9.6 – 11 and 6.0 $\log_{10}$ cfu/ml for mold, yeast and lactic acid bacteria respectively gave the best result for *poko* fermentation. It was reporte that the mixed cake inoculum at 0.1% gave the best quality *poko* product. *Poko* prepared by using defined fermentation starter was not only comparable to that of using traditional starter but was even better. Organoleptic score of *poko* remained at excellent point even on the fifth day of fermentation using defined starter.

Tamang and Sarkar (1995) and Thapa (2002) reported that generally two types of yeasts are involved in *jand* fermentation: amylolytic yeasts from *murcha* (mostly *Saccharomycopsis*) degrade starch and produce glucose, and then alcohol producing yeasts grow rapidly on the resultant glucose to produce ethanol. Tamang *et al.* (1988) reported that the microflora of *murcha* constitute mainly yeasts of the genera of *Pediococcus*, *Saccharomyces*, *Pitchia* and *Saccharomyces* and the moulds of the genera of *Rhizopus* and *Mucor*.

A typical recipe for the preparation of *murcha* as reported by Tamang *et al*. (1988) given in Table 3.7.

Table 3.7 A typical recipe for the preparation of *murcha*

Ingredients	Quantity (g)
Glutinous rice	1,000
Roots of *Plumbago zeylan*ica L.	2.5
Leaves of *Buddleja asiatica* Lour	1.2
Flowers of *Vernonia cinerea* (L.) Less	1.2
Ginger (*Zingiber officinale* Roxb)	5.0
Red dry chilli (*Capcicum spp*.)	1.2
Mother culture (previous batch *Murcha*)	10.0

(Source: Tamang *et al*.,1988)

***Manapu* making:** Miss Ganga Rajthaha (personal communication, 2008) has described the following process of preparing *manapu* (in Newari language, *murcha* is known as as *manapu*).

> About 40 kg of rice flour, 100 g of dried *murcha* plants and 3 pieces of old *murcha* cakes are mixed and kneaded by adding required amount of water in order to make stiff dough. The dough is shaped to small balls, spread over rice

straw bed, covered with rice straw and left for 6 days. The balls are sun dried and outer covering of black mycelia are removed by shoe brush.

***Mana* making** : Mr. Narayan Rajthala (personal communication, 2008, Lubu, Lalitpur) has described the following method of preparing *mana*.

Clean wheat is soaked in water for overnight, drained, and cooked. It is then flaked, spread over open floor, covered with straw mat/jute or plastic sacks and left for 6 days during which green mycelium appears on the surface of the wheat grain (first white then yellow and lastly green). The molded mass is then sun dried and sold as *mana*. Wheat based *mana* is mainly used for cereal fermentation for *raksi* making. According to him, *mana* is prepared during summer (from Jestha to Kartik: May - October). One kg of wheat costs Rs 21 whereas one kg of *mana* costs Rs. 33. Approximately 2,000 kg of *mana* is prepared by him yearly. Rice can also be used for *mana* making but it is mainly used for rice *jand* preparation.

A typical recipe and process for preparing *murcha* is as follows:

Table 3.8 A typical recipe for preparing *murcha*

Ingredients	Quantity
Rice	2,000 g
Dried Chitu jhar (*Plumbago zeylanica* L)	One handful
Fresh chini jhar (*Scoparia dulcis* L.)	One handful
Fresh fern leaves	Half handful
Fresh ginger (*Zingiber officinale* Roxb.)	125 g

(Source: Miss Ganga Rajthaha, personal communication, 2008, Lubu, Kathmandu, Nepal).

For the prepaqration of rice-based starter, the rice is soaked, drained, and pounded/milled with other ingredients. The flour is sieved and coarse particles are again pounded. Dough is prepared by adding the required amount of water, shaped into small cakes and the cakes are put on a bamboo tray/mat lined with fern/rice straw and again covered with the same bedding material followed by thick cloth. The cakes are left for 3 days during winter or for 1 day during summer. During this period the cakes raise in size (i.e. puffed), white cottony growth appears on the cakes and a distinct alcoholic smell emits. Then the bed coverings are removed and allowed to dry. It is said

that the higher the amount of *chitu* plant (*Plumbago zeylanica* L.) the stronger will be the *murcha*.

Dal Bahadur Tamang (Vijayapur, Dharan, personal communication) has given the following recipe and process of preparing fermentation starter.

Table 3.9 A typical recipe for preparing *murcha*

Ingradients	Amount (g)	Ingradients	Amount (g)
Broken rice	25,000	Chabo leaves (*Piper chaba Hunter*)	1000 g
Pipla leaves (*Piper nigrum L.*)	200	Chitu (*Plumbago zeylanica L.*)	200 g
Furke jhar	400	Guava leaves (*Carica papaya L.*)	One half handful
Chini jhar (*Scoparia dulcis L.*)	200	Bhimsenpate (*Buddleja asiatica Lour*)	One half handful

All plants are cut in to small pieces and pounded in *Okhali* or *Dhikki* (a traditional wooden device used to pound the cereals). Of the total 25 kg of broken rice used, 5 kg is soaked in water for sometime; excess water drained and pounded. Rest 20 kg of broken rice is milled in a mill (It is better to soak and pound the whole rice but due to much labor requirement; they soak and pound only about 20% of the total rice used). While pounding, 6 to 7 pieces of chilies are used in order to prevent from evil eye. Now the plant powder and rice flour (prepared by wet method) are mixed thoroughly and again pounded in *Okhali*. Once the pounding is completed, it is transferred into a large aluminum container containing water and mixed to form slurry. According to him, use of higher amount of chili causes headache after serving the fermented millet.

The dry milled rice flour is poured on a plastic sheet, a dich/pit is made at the centre of the mass, and the rice flour-plant slurry is poured into the ditch and mixed slowly. The mass is further kneaded using required amount of water and stiff dough is made. Now the mass is rolled, shaped into cakes, and put over jute sacks. The cakes are dusted on both sides with *murcha* powder (called as mother starter), placed on rice straw bed, and covered again with rice straw followed by thick cloth. The cakes are left for 2 − 3 days in winter and for 1 day in summer to raise the cake. Now the coverings are removed, the cakes turned upside down and

allowed to dry for 2 − 3 days in the sun. After drying, the *murcha* is ready for marketing. Now a day, people are making *murcha* using rice flour and old *murcha* only.

3.4.5 Physico-chemical and microbiological quality of fermentation starters

The microbial profiles of Nepalese traditional fermentation starters as reported by Shrestha *et al.* (2002) are given in Table 3.10.

Table 3.10 Microbial profile of traditional *murcha* starters

Microbial profiles	Microbial counts (cfu/g) in different traditional starters		
	Rice manapu	Millet manapu	Wheat mana
Mesophilic aerobes	1.8×10^{8} - 4×10^{10}	9.8×10^{8} – 1.7×10^{12}	1.4×10^{8} – 4.0×10^{8}
Lactics	3.6×10^{6} – 1.0×10^{9}	5.0×10^{7} – 3.0×10^{8}	Nil – 4.5×10^{8}
Yeast	5.0×10^{5} – 1.0×10^{8}	7.0×10^{7} – 1.0×10^{9}	8.0×10^{5} – 7.0×10^{6}
Mold	7.0×10^{5} – 1.0×10^{7}	2.0×10^{5} – 1.0×10^{7}	> 1.0×10^{7}
Coliforms	ND – 1.0×10^{2}	ND – 1.4×10^{5}	3.2×10^{3} – 1.6×10^{5}
E. coli	ND	ND – 1.0×10^{2}	ND – 1.0×10^{2}
S. aureus	ND	ND – 1.0×10^{2}	ND

ND = not detected (Source: Shrestha *et al.*, 2002)

Regmi (2007) prepared defined fermentation starter using pure culture of yeast (*S. cerevisiae*) and mold (*Rhizopus spp*.) isolated from traditional *mana* and the characteristics of the starters are presented in Table 3.11

Table 3.11 Characteristics of defined and traditional fermentation starters

Parameters	Fermentation starter types (*mana*)	
	Defined	Traditional
Moisture (% m/m)	5.03 - 5.13	5.33 - 5.90
Amylase activity (Units/g)*	22.49 - 36.13	17.90 - 33.76
Bulk density (g/L)	567.4 - 580.9	568.7 - 592.0
Yeast and mold counts (cfu/g)	3.6×10^{8} - 5.6×10^{8}	2.7×10^{8} - 3.2×10^{8}
Coliform count (cfu/g)	Nil	3 - 5
Total plate count (cfu/g)	30 - 41	> 300

*1 unit of amylase activity is equal to the 1 mg of maltose formed by 1 g of *mana* in 1% starch solution up on incubation for 15 min at 40°C.
(Source: Regmi, 2007)

Rai (2006) prepared defined fermentation starter using yeast and mold isolated from *murcha* using rice flour as carrier. The yeasts isolated from *murcha* samples were identified to be strains of *Sacharomyces cerevisiae*. The molds were identified to be the species of *Rhizopus*. The amylase activities of the starters were found to be in the range of 22.0 − 25.4 mg maltose in 15 min of incubation at 40 °C whereas those of traditional starter (*murcha*) ranged from 20.22 − 23.8 mg maltose under similar conditions. The physiochemical and microbiological characteristics of the *murcha* and defined starter are given in the Table 3.12.

Table 3.12 Physiochemical and microbiological properties of *murcha* and defined starter

Parameters	Type of fermentation starters	
	Traditional (*Murcha*)	Defined (pure culture)
Amylase activity[a]	20.2-23.8	22.0-25.4
Moisture (% m/m, wb)	16.4-16.54	16.26-16.31
pH[b]	5.0-5.5	5.2-5.5
Bulk density (g/L)	680-759	630-740
Total plate count (cfu/g)	1800-2010	104-367
Yeast and mold counts (cfu/g)	$1.7\times10^4 - 1.8\times10^4$	2.1×10^6-1.9×10^7
Coliform counts (cfu/g)	0-1	Nil

[a]Expressed as mg maltose formed by 1 g of *murcha* sample from 1% starch solution upon incubation at 40°C for 15 min. b: measured in 10% (m/v) aqueous solution.

(Source: Rai, 2006)

Study made by Karki (1984) has revealed that *murch* contains a diverse microflora namely, *Saccharomyces cerervisiae, Rhizopus spp., Endomycopsis fibuligera, Pediococcus pentosaceus*, and *Lactobacilliu plantarum*. Nikkuni *et al*. (1996) reported that a koji *mana* sample collected from Kathmandu valley, Nepal, contained 1.5×10^6 cfu/g of *Mucorales*, 3.5×10^7 cfu/g of *Aspergilli* and 1.1×10^5 cfu/g of lactic acid bacteria, while yeasts were present at less than 10^3 cfu/g. *A. oryzae* was found to be a dominant species in the *mana* sample prepared with steamed rice.

According to Yokotsuka (1985**)**, *chu* may either be yellow (*huang*) possibly due to *Aspergillus oryzae*, or white probably due to *Rhizopus* and *Mucor*. *Huang-chu* was widely used for alcoholic fermentation as well as for the preparation of soybean foods. Wheat *chu* originated in the Northern part of China and the Korean Peninsula., while

rice *chu* originated in the South. *Nuruk, ragi* and *bubod* are similar in that they are prepared by the natural fermentation of raw cereal powders, which are moulded into the shape of a cake or ball. *Koji*, on the other hand, is prepared by controlled fermentation of cooked cereals in a granular form, which are commonly inoculated with the mould, *Aspergillus oryzae*.

Numerous types of microorganisms (moulds, bacteria, and yeasts) are found in the naturally fermented products. *Aspergillus oryzae* (1×10^7 cfu/g), *Aspergillus niger* (1×10^7 cfu/g), *Rhizopus* (1×10^6 cfu/g), bacteria (1×10^7 cfu/g) and yeasts $1\times10^5 - 10^7$ cfu/g) were identified in *nuruk* **(**Kim, 1968). The number of moulds (1×10^3 - 10^7 cfu/g), yeasts (1×10^5 - 10^7 cfu/g) and lactic acid bacteria (1×10^5 - 10^7 cfu/g) were observed to vary with the source and district of collection of *bubod* (Tanimura *et al.*, 1978**)**. Important micro-organisms in *loog-pang* were *Amylomyces, Aspergillus, Rhizopus, Mucor* and *Absidia* (Pichyangkura and Kulprecha, 1977).

According to Tamang and Sarkar (1995), *murcha* cakes are mildly acidic (pH 5.2) and contain 13% moisture (m/m) and 0.7% ash (m/m, db). They have isolated a total of 194 bacteria, 110 yeasts, and 80 mold strains from 30 samples of *murcha*. The counts (cfu/g, wb) of microorganisms in the samples were $2.0\times10^7 - 4.2\times10^8$ for *Pediococcus pentosaceus*, 4.0×10^7-6.8×10^8 for *Saccharomyces fibuligera*, $2.0\times10^6 - 7.2\times10^7$ for *Pichia anomala*, $1.0\times10^6 - 4.1\times10^7$ for *Mucor circinelloides* and less than 10^6 for *Rhizopus chinensis*.

Fermentation starters were reported to contain mixed microflora of filamentous moulds such as species of *Amylomyces*, *Mucor*, *Rhizopus*, *Actinomucor*, yeasts such as, *Saccharomyces spp.*, *Pitchi spp*.; *Endomycopsis fibuligera*, *Homsemula spp*, *Saccharomycopsis fibuligera*, *Saccharomyces cerevisiae* and lactic acid bacteria, mostly spp. of *lactobacillus* and *Pedicoccus* (Tamang and Sarkar, 1995; Yokotsuka, 1985; Karki, 1986; Tamang *et al*., 1988).

3.5 Chemical composition of different indigenous cereal-based alcoholic beverages

Cereal beers are abundant in micronutrients such as the B vitamins, which are formed during fermentation. Table 3.13 shows the approximate chemical composition of *takju*, which contains 7% alcohol **(**Korea Rural Nutrition Institute, 1991). Indonesian *brem bali* contains 16 − 23% reducing sugars and 6 − 14% ethanol (Saono *et al.*, 1986).

Table 3.13 Approximate and mineral composition of *takju*

Nutrients	Content
Energy (kcal/100g)	55
Moisture (g/100 g)	90.7
Ethanol (%)	7.0
Protein (g/100g)	1.9
Fat (g/100g)	0.1
Carbohydrate (g/100g)	1.2
Ash g(100g)	0.1

Minerals	Content
Calcium (mg/100g)	14.0
Phosphorous (mg/100g)	28.0
Iron (mg/100g)	0.8
Thiamin (mg/100g)	0.01
Riboflavin (mg/100g)	0.03

(Source: Korea Rural Nutrition Institute, 1991)

Bhandari (1997) made a comparative study on the *raksi* production from different cereals using *murcha* and pure culture (*Saccharomyces cerevisie*, *S. sake* and *Aspergillus oryzae*) and reported the following composition.

Table 3.14 Chemical composition of the distillate

Cereals	Starter	Alcohol (% v/m)*	Methanol (g/100 L)	Higher alc. (% v/v)
Maize	*S. cerevisiae + A. oryzae*	5.42	12.1	> 0.1
Maize	*S.sake + A.oryzae*	8.71	9.6	,,
Maize	*Murcha*	12.05	2.4	,,
Finger millet	*S. cerevisiae + A. oryzae*	1.86	24.3	,,
Finger millet	*S. sake + A. oryzae*	7.48	19.4	,,
Finger millet	*Murcha*	7.22	9.6	,,
Rice	*S. cerevisiae + A. oryzae*	8.59	9.6	,,
Rice	*S. sake + A. oryzae*	11.17	7.2	,,
Rice	*Murcha*	10.01	Trace	,,

(Source: Bhandari, 1997).

*alcohol content was expressed as mL/100 g fermented millet.

Rai (1991) fermented unmalted and malted finger millet (*Eleusine coracana*) using defined fermentation starter prepared using *Aspergillus oryzae* and *Saccharomyces sake* and reported the following chemical characteristics (Table 3.15). The organoleptic quality of *jand* from fermented germinated millet was reported to be inferior to that of the ungerminated one probably due to the putrefaction of the sprouts present in the germinated millet. It seemed probably that the removal of sprouts before cooking could improve the quality of *jand* (Rai, 1991).

Table 3.15 Chemical characteristics of ungerminated and germinated finger millet during fermentation

Time[a]	Ungerminated millet			Germinated millet		
	Total acidity (% m/m)[b]	Red. sugar (% m/m)[b]	Alc. (% v/m)	Total acidity (% m/m)[b]	Red. sugar (% m/m)[b]	Alc. (% v/m)
2	0.98	0.20	0.50	0.84	0.56	0.44
4	0.99	0.79	0.65	0.85	1.01	1.31
8	1.14	1.20	1.82	0.99	1.95	2.48
12	1.26	1.91	2.76	1.02	3.08	3.30
14	1.27	2.11	2.86	1.11	4.80	3.92
16	1.30	3.46	3.3	1.14	4.33	4.00
18	1.35	4.8	4	1.15	3.80	4.70
20	1.52	3.8	4.33	1.23	2.88	5.17
22	1.59	3.6	5.3	1.25	2.59	5.3
24	1.6	2.94	5.57	1.34	2.24	5.88

[a]days after starter addition

[b]total acidity and reducing sugar are expressed as lactic acid and dextrose respectively.

(Source: Rai, 1991).

Venkataramu and Basapu (1993) made a comparative study of *chhang* fermentation of ragi using traditional and pure microbial inocula. Microorganisms viz., *Endomycopsis fibuligera*, *S. cerevisiae* and *Leuconostoc dextranicum*, isolated from the traditional fermentation starter, *phab*, were used in the fermentation and compared with that of '*phab*' as such using malted and unmalted ragi and their grits. Starch conversion efficiency was from 20 − 40% in the case of 20% grits medium than that in whole grain. Solid state fermentation temperature of 20 °C, pH of 6.7 (natural) and incubation period of 6 days were found to be optimum for fermentation. Malted grits further improved the starch conversion efficiency by 50 % with these inocula and also imparted better flavor and aroma to the product. Consistency of *chhang* in terms of ethanol (2.5 – 3.0%, v/v), total acidity (0.4 – 0.6%), volatile acidity (0.03 – 0.06%) and pH (3.8 – 4.1) were reported in the case of finger millet fermented using pure culture.

Manandhar (2002) prepared rice wine using saccharifying mold and fermentative yeast isolated from traditional starter (*murcha*) following sake fermentation procedure for 28 days. Alcohol (% v/v), total acidity as lactic acid (% m/v), pH, reducing sugar as dextrose (% m/v) and total sugar as dextrose (% m/v) contents in rice wine and strained

jand were 9.65 and 9.56; 0.72 and 0.70; 4.1 and 4.1; 2.05 and 1.53 and 3.03 and 4.47 respectively.

Mongar and Rai (2005) studied the preservation of millet *jand* by pasteurization. Two lots of *jands* were obtained from 1 kg of fermented millet by mixing with warm water and holding for 15 min followed by straining through muslin cloth. The *jand* was pasteurized (in-bottle at 75 °C for 15 min) and stored at – 4 °C (control), 10 °C and at room temperature. The characteristics of jand during storage are shown in Table 3.16. The author reported that all the characteristics were not significantly affect by storage temperature and methods.

Table 3.16 Chemical characteristics of finger millet *jand* during storage

Parameters	Condition	Values at different storage times (days)				
		0	12	24	36	48
pH	RT*	4.5	4.51	4.52	4.51	4.50
	10 °C	4.5	4.5	4.51	4.52	4.51
	- 4 °C	4.44	4.45	4.45	4.43	4.43
Total acidity as lactic acid (% m/m)	RT	0.6	0.6	0.6	0.61	0.61
	10 °C	0.6	0.61	0.61	0.6	0.62
	- 4 °C	0.6	0.61	0.61	0.62	0.61
Reducing sugar as dextrose (% m/m)	RT	1.52	1.51	1.50	1.49	1.50
	10 °C	1.5	1.52	1.51	1.50	1.51
	- 4 °C	1.48	1.47	1.49	1.48	1.43
Alcohol content (% v/m)	RT	4.03	4.0	4.0	4.0	4.03
	10 °C	4.0	4.06	4.04	4.06	4.0
	- 4 °C	4.1	4.09	4.13	4.08	4.1

*RT: room temperature (Source: Mongar and Rai, 2005)

Shrestha *et al.* (2002) reported that *poko* fermented for 3 days at 30 ºC using rice and millet *manapu* had total acidity in the range of 1 to 1.7% m/m as lactic and reducing sugar in the range of 9 to 15.6% m/m as dextrose. Shrestha and Rati (2003) prepared *poko* using traditional and defined starters and their chemical composition were analyzed. The ash, protein, crude fiber, and carbohydrate contents in sterilized rice were 0.26, 8.5, 0.17, and 86.4% (db) respectively. Proximate composition of *poko* fermented using traditional starter at 30 ºC for 5 days had ash, protein, crude fiber, fat

and total carbohydrates contents of 0.4, 11.8, 1.44, 0.35 and 77.2% (m/m, db) respectively. Similarly, *poko* prepared using defined fermentation starter had ash, protein, crude fiber, fat and total carbohydrates contents of 0.35, 10.0, 0.9, 0.4, and 77.7% (m/m, db) respectively. Fresh *poko* was found to have moisture content in the range of 70 to 73% (m/m).

Upadhyaya (2005a) fermented finger millet, rice, maize and wheat using traditional fermentation starter (*murcha*) and analyzed chemical and organoleptic quality of the *jands*. *Murcha* was added at the rate of 2% (m/m) by weight of cooked cereals and fermentation was carried out at 23 – 26 °C for 15 days. The *jand* was prepared by using fermented cereal and water in the ratio of 1:1 (m/v), stirred for 10 min and strained through muslin cloth and the chemical characteristics of jands are shown in Table 17.

Table 3.17 Chemical characteristics of different *jands*

Characteristics	Values for different jands samples			
	Wheat	Millet	Rice	Maize
pH	4.1	3.84	3.64	3.85
Total acidity as lactic acid (% m/v)	1.06	1.11	1.50	1.11
Fixed acidity as lactic acid (% m/v)	0.48	0.51	1.03	0.50
Volatile acidity as acetic acid (% m/v)	0.18	0.20	0.16	0.20
Alcohol (% v/v)	8.38	7.15	7.37	8.07
Methanol (g/L alc)	0.716	2.797	0.407	1.611
Total aldehydes as acetaldehyde (mg/L alc)	66.9	8.5	4.5	18.6
Esters as ethylacetate (mg/L alc)	189.00	98.0	310.4	283.5
TSS (°Bx)	8.00	6.00	7.00	5.00
Dry matter (% m/v)	8.80	4.91	6.56	4.73
Reducing sugar as dextrose (% m/v)	1.75	0.48	2.61	0.38
Total ash (% m/v)	0.29	0.21	0.22	0.05
Total nitrogen (% m/v)	1.37	0.38	0.59	0.71±0.01

(Source: Upadhyaya (2005a)

The composition of unfermented and fermented finger millet found in Darjeeling hills and Sikkim, India, as reported by Thapa and Tamang (2004) is given in Table 3.18.

Table 3.18 Composition of cooked and fermented finger millet

Parameters	Cooked millet	Fermented millet	Minerals (mg/100 g dm)	Cooked millet	Fermented millet
Moisture content, % m/m	67.3	69.7	Calcium	205.3	281.0
pH	6.4	4.1	Magnesium	76	118
Total acidity, as lactic, % m/m	0.01	0.3	Manganese	3.6	9
Alcohol content, % v/m	0.08	4.8	Copper	0.8	2.2
Ash, % db	5.0	5.1	Iron	8.7	24
Crude fat, % db	2.3	2.0	Zinc	1	1.2
Crude protein, % dm	10.2	9.3	Sodium	27.7	39
Crude fiber, % db	6.5	9.3	Potassium	391	398
Carbohydrates, % db	82.5	83.7	Phosphorous	228	326
Energy, kcal/100g dm	391.8	393.6			

(Source: Thapa and Tamang, 2004)

Shrestha and Rati (2003) reported that the overall vitamin content increased during fermentation process. Riboflavin is utilized during fermentation and was therefore not detected in the final fermented product. Cronk *et al*. (1977) reported an increase in the protein content to 16% in *tapeketan* fermentation. In cereals, the limiting amino acid is lysine. However, during fermentation of *tapeketan*, Cronk *et al*. (1977) found that lysine was selectively synthesized and increased by 11%. Similarly, thiamine showed an increase of 30% (from 0.04 to 0.13 mg/100 g) in *tapeketan*. According to Kodama and Yoshizawa (1977) total sugar as glucose (% m/v), fermentable sugar as glucose (% m/v), total nitrogen (% m/v), formol nitrogen (% m/v) and alcohol (% v/v) contents in *sake* were 4.2, 3.46, 0.0726, 0.0288, and 15 respectively. They further reported that *sake* contains various higher alcohols and esters: n-propanol (120 mg/L); isobutanol (64 mg/L); isoamyl alcohol (170 mg/L and 2 phenethanol (75 mg/L).

The chemical composition of *bhatte jaanr* prepared from glutinous rice using traditional starter reported by Tamang and Thapa (2006) is given in Table 3.19.

Table 3.19 Chemical composition of cooked and fermented rice

Parameters	Cooked rice	Bhatti jaanr	Minerals, (mg % db)	Cooked rice	Bhatti jaanr
Moisture content, % m/m	67.2	83.4	Calcium	2.5	12.8
pH	6.0	3.5	Magnesium	22	50
Total acidity, as lactic, % m/m	0.01	0.24	Manganese	0.4	1.4
Alcohol content, % v/m	0.00	5.90	Cupper	0.5	1.4
Ash, % db	0.6	1.7	Iron	2.2	7.7
Crude fat, % db	2.4	2.0	Zinc	0.6	2.7
Crude protein, % dm	9.5	9.5	Sodium	5.3	24.7
Crude fiber, % db	0.6	1.5	Potassium	57	146
Total carbohydrates, % db	87.5	86.9	Phosphorous	156	595

(Source: Tamang and Thapa, 2006)

Philippine *tapuy*, a highly acidic but sweet, aromatic, alcoholic rice wine was reported to contain reducing sugar, TSS, pH, total acidity and ethanol in the range of 4.1 to 5.2 % as glucose, 9.3 to 15 %, 3.3 to 4.9, 6.55 to 22.49 mL of 0.1N NaOH per 100 mL and 13.5 to 19.10 % (v/v) respectively (Tanimura *et al*., 1978).

Chemical characteristics of some traditionally prepared alcoholic beverages are presented in the following Tables.

Table 3.20 Chemical composition of 3 days fermented commercial Egyptian Bouza

Components	Content (% m/v)
Moisturre	87.5
Total solids	12.5
Protein (N x 5.7)	1.6
Fat	0.25
Fiber	0.40
Ash	0.26
Carbohydrates (by difference)	10.0

(Source: Morcos *et al*., 1973)

Table 3.21 Composition of Kaffir beer

Parameters	Range	Average
pH	3.2 – 3.9	3.5
Lactic acid (mg%)	164 – 250	213.10
Volatile acidity as acetic acid (%, m/v)	0.012 – 0.019	0.016
Total solids (%)	2.6 – 7.2	4.9
Insoluble solids (%)	1.6 – 4.3	2.3
Alcohol (% m/m)	2.4 – 4.0	3.2
Nitrogen (%)	0.065 – 0.115	0.084

(Source: Novellie, 1968)

Table 3.22 Chemical composition of Zambian opaque maize beer (7 day)

Component	Mean	Range
Moisture (% m/v)	93.2	90.7 – 95.9
Solids (% m/v)	5.68	3.73 – 10.26
Ash (% m/v)	0.232	0.612 – 0.300
Fat (% m/v)	0.43	0.11 – 1.04
Fiber (% m/v)	0.38	0.22 – 1.02
Protein (% m/v)	0.889	0.657 – 1.384
Total carbohydrates (% m/v)	4.14	2.71 – 8.56
Calcium (mg/100 ml)	1.9	1.0 – 2.9
Iron (mg/100 ml	9.8	3.3 – 26.0
Alcohol (% m/v)	1.86	0.96 – 2.86

(Source: Lovelace, 1977)

3.6 Flavoring compounds produced in alcoholic beverages

3.6.1 Esters

Esters are numerically the largest group of organoleptic compounds in alcoholic beverages. Lower esters have pleasant odors that are usually described as fruity (Bahl

and Bahl, 1997). Although some ester formation may occur during the distillation of spirits, the most common esters are produced by the yeast during fermentation stage. Esters, in general, have fruity and floral impact characteristics that are important in the sensory properties of wine. Wine esters may be categorized into two groups; (i) those arising from acetate and ethanol as well as fusel oils and (ii) those resulting from ethanol and straight-chain fatty acids precursors. Esters of the first group include ethyl-, isobutyl-, isoamyl-, 2-phenethyl-, and hexyl- acetate. Of those identified, ethyl acetate is generally present at the highest concentration. Examples of the second group include the ethyl esters of hexanoic, octanoic, and decanoid acids (Zoecklein *et al*., 1997).

3.6.2 Aldehydes

Alehydes are synthesized by yeast as intermediate in the fermentation of alcohols through the decarboxylation of keto acids. The majorities are further reduced by alcohol dehydrogenase, but a small amount may be oxidized to acids. During the active phase of fermentation, excess quantities can be excreted into the fermentation broth. The corresponding aldehydes to most of the alcohols formed by yeast have been detected in alcoholic fermentation (Engan, 1981). Generally, aldehydes have flavor threshold two to three orders of magnitude below the alcohols. The aroma of the lower aldehyde is generally perceived as fruity. Aldehyde has a characteristic pungent odor, but its solution in water has an agreeable fruity odor (Bahl and Bahl, 1997). Parameters which increases the initial fermentation rate, such as aeration, readily utilizable sugars and other nutrients, higher temperature, fast fermenting yeast strains and higher pitching rates results in increased accumulation of aldehydes (Engan, 1981).

3.6.3 Organic acids

According to Kirk and Sawyer (1991), free acidity as acetic acid was reported to be 0.05 – 0.10, 0.15 – 0.23, 0.09 – 0.15, 0.2 – 0.35, 0.05 – 0.15 and 0.03 – 0.20 % in Port, Sherry, Claret, Burgundy, Hock, and Champange sample of wines respectively. Similarly, fixed acidity was reported to be 0.35 – 0.55, 0.25 – 0.50, 0.30 – 0.5, 0.30 – 0.60, 0.25 – 0.45, and 0.30 – 0.45% as tartaric acid in Port, Sherry, Claret, Burgundy, Hock and Champange wine samples respectively. Volatile acidity refers to the volatility with steam of the fatty acids. Besides acetic and lactic, which are normal by-products of alcoholic fermentation, formic, butyric, propionic and traces of other fatty acids are present. The volatile acidity includes the fatty acids in the series starting with acetic but excludes lactic, succinic, carbonic and sulfurous acids. The amounts of acetic acid

produced during alcoholic fermentation are small usually less than 0.030 g per 100 mL. Acetic acid is a normal by-product of yeast growth and has its origin primarily in the early stages of fermentation (Zoecklein *et al*., 1997).

Table 3.23 Composition of different brandy samples

Parameters	Contents in different samples	
	Minimum	Maximum
Total acidity as acetic acid (g/100L alc)	86	160
Total esters as ethyl acetate (g/100L alc)	112	580
Total aldehyde as acetaldehyde (g/100L alc)	24	76
Higher alcohols (g/100L alc)	146	782

(Source: Kirk and Sawyer, 1991),

3.6.4. Higher alcohols

Quantitatively and qualitatively, fusel oils represent an important group of alcohols that may affect flavor. Quantitatively, isoamyl alcohol generally accounts for more than 50% of all fusel oil fractions in wine (Muller and Fugelsang, 1993). In table wines, the total fusel oil concentration is reported to range from 140 to 420 mg/L (Amerine and Ough, 1980).

Table 3.24 Origin of fusel alcohols from amino acid precursors

Amino acids	Fusel oil
Leucine	3-methyl-1-butanol
Isoleucine	2-methyl-1-butanol
Valine	2-methyl-1-propanol
Threonine	Propanol
2-phenylalanine	2-phenylethanol
Tyrosine	Tyrosol

(Source: Zoecklein *et al*., 1997)

Table 3.25 Acidity and volatile flavor compounds in different rum samples

Parameters	Rum samples		
	1	2	3
Total acidity as acetic acid (g/100L alc	22.4	193	174
Esters (g/100L alc)	241.3	275	190.3
Aldehydes (g/100L alc)	27.8	35.2	24.5
Higher alcohols (g/100L alc)	174.5	190.3	160.1

(Source: Kirk and Sawyer, 1991)

Table 3.26 Acidity and volatile flavor compounds in different rum, Scotch whisky and brandy (g/100L alcohol)

Pameters	Jamaica rum	Demerara rum	Scotch whisky	Cognac
Total acidity as acetic acid	80 - 180	90 - 240	30 - 70	5 - 100
Esters	120 - 200	40 - 70	10 - 20	-
Aldehydes	Up to 40	0 - 20	100 - 270	2 - 35
Fusel oil	140 - 200	100 - 160	-	-

(Source: Kirk and Sawyer, 1991)

Table 3.27 Volatile flavor compounds in different whisky samples (g/100 L alc)

Parameters	Sample number				
	1	2	3	4	5
Total esters as ethyl acetate	28	35.5	34.8	17.6	16.9
Total aldehyde as acetaldehyde	11.4	10.	9.7	4.9	3.1
Higher alcohols	129	130	186	41	44

(Source: Kirk and Sawyer, 1991)

3.7 Biochemical changes during cereal-based traditional alcoholic fermentation

3.7.1 Microbial changes

Tamang and Thapa (2006) prepared *bhatti jand* following traditional method using glutinous rice and local *murcha*. Fermentation was carried out for 8 days at 28 ºC after 2 days of biomass development. They reported that population of moulds decreased

significantly ($p<0.05$) during fermentation and disappeared after the fifth day of fermentation. Population of yeasts increased significantly from 10^5 cfu/g to 10^8 cfu/g on day 2 and decreased to a level of 10^5 cfu/g on day 10. It was assumed that two types of yeasts were involved in cereal fermentation: amylolytic (mostly *Saccharomycopsis*) degrade starch and alcohol producing yeasts then grow rapidly on the resultant glucose to produce ethanol.

Thapa and Tamang (2006) prepared *kodo ko jand* following traditional method using about 2% powdered *mucrha* and fermenting for 8 days at 28 ºC after 2 days of biomass development. They reported that mold count decreased from 4.2 log cfu/g on day 0 to 1.8 log cfu/g on day 3, but no mold were visible afterwards. Conversely, yeast count (5.2 log cfu/g on day 0) increased during fermentation reaching a maximum of 7.8 log cfu/g on day 2, remained constant over 4th day of fermentation and then declined to 7.3 log cfu/g on day 10.

Shrestha *et al*. (2002) studied the succession of different groups of microbes during *poko f*ermentation at 30 ºC using rice *manapu* and found that lactic acid bacteria increased during fermentation and were in the range of $3.5x10^6$ (day 1) to $5x10^7$ cfu/g (day 5). A similar trend was also reported for yeast counts with $1.8x10^6$ and $1.3x10^8$ on the 1st and 5th day of fermentation respectively. Mold counts increased from $6.3x10^5$ (day 1) to $1.3x10^6$ cfu/g (day 2), remained constant on day 3 and decreased to $1.3x10^3$ on day 5. In traditional sake fermentation, sake yeast (*Saccharomyse sake*) is added at the rate of 10^5 to 10^6 cfu/g and eventually propagates to levels of $3\ x10^8 – 4x10^8$ cfu/g (Murakami, 1972). During *takju* preparation (a Korean traditional wheat-based alcoholic beverage) molds disappeared after 2 or 3 days of fermentation (Park *et al*., 1977).

3.7.2 Physico-chemical changes

Tamang and Thapa (2006) analyzed pH, total acidity, alcohol and reducing sugar contents during traditional fermentation of *bhatti jand* and found that the pH decreased from 6.1 (day 0) to 3.96 (day 10) during fermentation at 28 °C. A large drop in pH was recorded in the first day of fermentation (from 6.1 to 3.36); there was no drastic change in pH was reported during succeeding fermentation. Similarly, total acidity increased substantially during the first day of fermentation (from 0.01 to 0.11% m/m as lactic acid) and reached up to 0.17% on the 10th day of fermentation. Alcohol content increased with fermentation time. There was a negligible amount of alcohol production

over the 1st day of aerobic fermentation (from 0.00% to 0.2%, v/m) and reached to 10.1 % v/m at the end of fermentation. Reducing sugar content increased with time attaining a max of 12.6%, m/m as dextrose and decreased sharply to 0.2% on day 10.

Thapa and Tamang (2006) analyzed reducing sugar, pH, total acidity and alcohol contents during traditional fermentation of finger millet (*Eleusine coracana*) and reported that the total reducing sugar increased from 0.4 (day 0) to 7.0% m/m as dextrose (day 7) and decreased afterwards reaching 1.0% on day 10. A significantly high reducing sugar production was reported to occur on the 1st day of aerobic fermentation. The pH of the mash decreased from 6.4 on day 0 to 4.1 on day 2 and remained constant throughout fermentation up to day 10. Total acidity (%, m/m as lactic) increased with time and reached up to 0.23% on day 10. Alcohol content was reported to increase with time significantly up to day 6 (6.9%, v/m), remained constant up to day 8 and decreased significantly up to 6.5% on day 10. The authors further fermented finger millet using pure cultures of *Rhizopus chinensis* MJ: R3 and *Saccharomyces cerevisiae* MJ: YS2 isolated from *murcha* and the change in pH, reducing sugar and alcohol after 2 and 6 days of fermentation were determined. pH, reducing sugar (%, m/m as dextrose) and alcohol (%, v/m) on 2 and 6 days fermented samples were 4.3 and 4.4; 4.21 and 3.57; and 2.5 and, 4.4 respectively.

Poko prepared using rice *manapu* (@ 0.2% by wt. of cooked rice) for 5 days at 30 ºC had pH contents of 4.3 on day 1, decreased with time giving final pH of 3.1 on day 5. Similarly, total acidity increased from 0.2 on day 1 to 1.7% (m/m) as lactic acid on day 5. Reducing sugar content increased from 0.4 on day 1 to a maximum of 15.6% (m/m) as dextrose on day 3 and decreased afterwards to zero on day 5. Alcohol content increased with fermentation time and reached up to 4.0% (v/m) on day 5 (Shrestha *et al*., 2002).

Khetarpaul and Chauhan (1990) fermented sterilized pearl millet flour (100 g + 900 mL water) using yeasts and lactic acid bacteria separately and in combination for 72 h at 30 ºC. pH decreased significantly from 6.42 to 4.41 while total acidity increased from 0.55 to 1.50% (m/v). Mixed fermentation further decreased pH and increased total acidity. Chavan and Kadam (1989) reported that total protein contents of cereals and millets were generally not affected by fermentation while a significant increase (from 10.8 to 12.4%) was reported by Agte and Sandhana (1997) in pearl millet. The increase in protein content can be attributed to microbial synthesis of proteins from intermediates during growth cycles (Zamora and Fields, 1979).

Das (2004) isolated four fermentative yeasts of *Saccharomyces* genera and one mold of *Rhizopus* genus and utilized for finger millet fermentation. The mold was inoculated to cooked millet, saccharified for 3 days at room temperature, then adjusted to 17 °Bx using molasses and pH from 4.7 – 4.8 using lactic acid. The millet was fermented for 8 days using different yeasts. Analytical results of the fermented millet are shown in the Table 3.28.

Table 3.28 Chemical characteristics of fermented millet

Parameters	Values at different fermentation times (days)			
	2	4	6	8
TSS (°Bx)	16 – 15	13.5 – 12	10 – 7	10 – 5
pH	4.5 – 4	4.3 – 3.5	3.9 – 3.2	3.7 – 2.9
Total acidity as lactic acid (% m/m)	1.73 – 0.88	2.57 – 1.5	2.9 – 1.8	3.8 – 1.9
Reducing sugar as dextrose (% m/m)	10.2 – 9.8	8.2 – 7.1	5.3 – 3.3	5 – 3
Alcohol, % v/m				8 – 1.9

(Source: Das, 2004).

Cai and Nip (1990) studied biochemical changes in the development of alcoholic fermented products from Taro (*Colocasia esculenta* L. Schott). Fresh taro was ground and cooked by steaming at 100 °C for 1 h and autoclaving at 118 °C for 20 min. The cooked taro was fermented using solid state and semi solid state (addition of 40% water by m/m) fermentations using defined fermentation starter (at the rate of 2% by weight of total taro solid) prepared using *Rhizopus tonkinensis*, *R. oryzae*, *R. chinensis* and *Saccharomyces cerevisiae* in rice bran and rice flour. The pH values of taro products fermented under different conditions decreased sharply during the first 3 days (from 5.9 to 4.5) but the decline was at a slower rate on the following fermentation time.

Total acidity of the products from solid-state fermentation was considerably higher than those of semi-solid fermented products, probably due to the uneven distribution of the desirable microorganisms in the sticky mass or substrate. Fermented taro product cooked by atmospheric steaming had 6.8% (w/w) of ethanol, while that cooked by autoclaving contained 6.6% (w/w) of ethanol after 3 dyas of semi-solid fermentation at room temperature. Taro grits cooked by autoclaving resulted 12.5 % (w/w) and 10.3%

(w/w) of ethanol in 12 days of fermentation using semi-solid and solid state fermentation respectively. Ethanol in all the samples increased sharply during the first 6 days of fermentation and decreased during the succeeding fermentation period. Sugar content in 6 days semi-solid fermented taro was less than 0.1% (as glucose), while it was more than 2.5% in solid state fermented one. Glucose content in the solid state fermented taro was reported to be 3.9% by the end of fermentation (15 days). Total oxalate decreased rapidly from approximately 40 mg/100 g of steamed taro (1h at 100 ºC) to 8 mg/100 g during the first day of fermentation. Presence of oxalic acid in foods is considered as an antinutrient. The decrease in total oxalate was attributed mainly to the metabolism of molds during taro fermentation.

Verma (1991) studied the chemical changes during rice fermentation using defined starter prepared from *A. oryzae* and *S. sake* in rice and following results were reported.

Table 3.29 Chemical changes during fermentation (n=3)

Time (days)	Average values			
	Total acidity (% m/m)	Starch (mg/g)	Total sugar as sucrose (% m/m)	Reducing sugar as dextrose (% m/m)
0	-	767.6	1.34	0.71
2	0.2	701.5	1.31	0.33
4	0.40	698.2	1.53	0.63
6	0.51	678.6	2.35	0.86
8	0.7	629.6	2.35	0.72
10	0.63	599.6	1.34	0.56
12	0.77	575.4	1.99	0.49
14	0.90	551.3	0.92	0.34

(Source: Verma, 1991).

In *tape ketan* fermentation the crude protein increased from 8.0 to 12% (db) in 96 h and further increased to 16.6% (db) in 192 h of fermentation using *Amylomyces rouxii* and *Endomycopsis burtonii*. (Cronk *et al*., 1977). According to Cronk *et al*. (1977) the alcohol and fusel oil contents during *tape ketan* fermentation at 30 ºC using *Amylomyces rou*xii mold in conjunction with various yeasts were as follows:

Table 3.30 Changes on alcohol and fusel oil contents during *tape ketan* fermentation

Microorganisms	Parameters	Contents at different fermentation times (h)		
		48	96	192
Amylomyces rouxii + Endomecopsis fibuliger	Fusel oil (mg/L distillate)	187	461	559
	Alcohol (% v/v)	3.7	7	7.7
A. rouxii + Candida lactosa	Fusel oil (mg/L distillate)	255	434	599
	Alcohol (% v/v)	3.3	7.4	7.3
A. rouxii + Hansenula anomalla	Fusel oil (mg/L distillate)	108	162	144
	Alcohol (% v/v)	2.6	3.2	3.7

(Souirce: Cronk *et al*., 1977)

Sripriya *et al.* (1997) soaked finger millet (Co-13, brown variety) for 12 h at 30 ºC, germinated for 24 h at 30 ºC and allowed to ferment by the natural grain flora at 30 ºC for different times. The pH dropped markedly from 5.8 to 3.87 during 48 h of fermentation. A similar trend was also reported for titratable acidity with the maximum increment being recorded during 6 to 12 h of fermentation (0.38 – 1.01%).

Fivefold increase in total and reducing sugars was reported during early stage of natural fermentation (0 – 6 h) (Sripriya *et al*., 1997). Starch content of sterilized autoclaved unfermented pearl millet flour was 63.3 % (m/m db) which decreased to 50.4, 54.2, 59.3 and 60.2 % (m/m db) over 72 h of fermentation using *Saccharomyces diastaticus*, *S. cerevisiae*, *Lactobacillus brevis* and *L. fermentum* respectively. Reducing sugar content increased from 0.36 to 2.01% (m/m db) in *S. cerevisiae* fermented sample (Khetarpaul and Chauhan, 1990). Lopez *et al.* (1983) observed that natural lactic acid fermentation decreased the phytic acid in corn meal due to phytase production by the microbes. Similarly, natural fermentation of the germinated finger millet resulted a mean decrease in phytic acid to 54.3% at 72 h and 72% at 96 h of fermentation (Makokha *et al*. (2002).

A significant decrease in phytic acid (786.2 to 393.1 mg/100 g) during 36 h of natural fermentation of pearl millet was reported by Agte *et al.* (1997). Elyas *et al.* (2002)

reported a significance decrease in phytate as a result of fermentation. A 50% reduction was observed during 36 h of natural fermentation of pearl millets. Khetarpaul and Chauhan (1990) reported an almost complete elimination of phytic acid in pearl millet flour during natural fermentation at 30 ºC for 72 h. Mahajan and Chauhan (1987) reported that endogenous phytase contributed significantly to the reduction of phytate content of natural fermented pearl millet flour. According to Daniels and Fisher (1981) and Lopez *et al.* (1983) microbial phytase was present in several microorganisms, which hydrolysed phytic acid during fermentation of autoclaved floura and accounted for the reduction in phytic acid content in the autoclaved fermented product. Fermentation led to increase in total phenolics contents in buckwheat, barley, and in rye (Zamora and Field, 1979). A significant decrease in total phenolics (TP) from 318.6 to 237 mg/100 g during natural fermentation of pearl millet was also reported by Agte *et al*., 1997). Sripriya *et al*. (1997) reported that natural fermentation caused increase in total phenolics (TP) from 1.43 g/100 g DM to 1.86g/100 g DM in finger millet.

According to Elyas *et al*. (2002), natural fermentation of pearl millet was found to cause a significant decrease in TP from 319 to 196 mg GAE/100 g after 24 h fermentation. Dhankher and Chauhan (1987) also reported a decrease in polyphenols in pearl millet with increasing fermentation time. The decrease in TP could be due to the activation of polyphenol oxidase. The higher increase at the end of fermentation could be attributed to higher loss in dry matter. They reported that 10 – 20% decrease in polyphenol content was found in the fermented pearl millet product, *rabadi*.

No significant change in tannin content during fermentation of pearl millet was reported by Agte *et al.* (1997). Similarly Elyas *et al.* (2002) also reported that natural fermentation of pearl millet for 36 h at 32 ± 2 ºC was found to cause no change in tannin content. Fermentation increased antioxidant activity (measured by DPPH method) in buckwheat (from 10 to 12.6%) and in barley (from 6.5 to 12.5%) (Agte *et al*., 1997).

3.8 Changes during cereal germination

3.8.1 Changes in protein and amino acids

Soluble proteins showed only marginal increase on germination of finger millet at 30 ºC for 24 h but a ten-fold increment occurred at 18 h of natural fermentation (1 part germinated millet flour + 2 parts water).This may be due to the increase in microbial enzyme during fermentation. The total free amino acids increased rapidly by about 4.5-

fold during germination and doubled at 18 h of fermentation, reaching a maximum at 36 h of fermentation (Sripriya *et al*., 1997). Dry matter loss, particularly carbohydrates, through respiration and leaching possibly caused the apparent increase in protein content on malting (Chavan and Kadam, 1989). Mbithi-Mwikya *et al.* (2000) reported a 29.5% overall increase in protein content during malting of finger millet. A significant decrease in protein and fat contents was reported by Choudhury *et al.* (2010) in foxtail millet (*Setaria italica*) than that of unmalted one. The changes in protein content during malting may be due to the loss of carbohydrates during germination and loss of low molecular weight nitrogenous compounds during soaking and rinsing of the grain. Similar findings were also reported by Kumari and Srivastava (2000). Roots and shoots are known to be rich in various nitrogenous compounds. During germination, the increase in the amount of free amino nitrogen in roots and shoots is the result of translocation of the products of storage protein breakdown from kernel (Pelembe *et al.*, 2002). This could be the probable reason for reduced protein content in malted sample.

3.8.2 Changes in carbohydrates

Starch content decreased from 81 to 71.3% (m/m) during germination of finger millet for 24 h at 30 ºC, which further decreased during natural fermentation. (Sripriya *et al.* 1997). The total and reducing sugars in 70% alcohol extracted sample of finger millet malt germinated at 25 ºC increased from 1.5 to 16% and from 1.44 to 8.36% respectively upon 96 h of germination. A 95- and 378-fold increase was observed in hexoses (glucose and fructose) and sucrose respectively (Nirmala *et. al.,* 2000). Starch content decreased from 81.1 to 71.3 g/100 g DM during germination of finger millet for 24 h at 30 ºC, which further decreased during natural fermentation. Dry matter loss, particularly carbohydrates through respiration and leaching during soaking and germination probably caused the apparent increase in fiber (Lorenz, 1983; Chavan *et al*., 1981).

Malleshi and Klopfenstein (1998) reported that on malting, the dietary fiber of sorghum, pearl millet and finger millet increased from 7.8 to 8.6%, 11 to 12% and 15.2 to 21.7% respectively. Starch content decreased from 65 to 40.43% after 4 days of malting of finger millet (Nirmala, *et al*., 2000). The reduction in starch content varied from 33 to 58.4% in 96 h germinated sorghum cultivars (*Sorghum bicolor*) as compared to ungerminated cereal (Subramanian *et al*. 1992). About 65% decrease in starch content was reported in germinating barley (Morall and Briggs, 1978).

3.8.3 Changes in mineral contents

In general, germination reduced mineral contents of pearl millet due to metabolic loss of nutrient. There was a significant reduction in total phosphorus and calcium contents during soaking and sprouting compared to the control. The reduction in mineral contents during soaking and sprouting treatments might be due to the leaching out of minerals in the soaking water (Chavan and Kadam, 1989; Malleshi, 1984; Samantray *et al*., 1989). Siwela (2009) reported that the mineral content of the malt was lower than that of the unmalted grain. Similarly Bhise *et al.* (1988) reported a decrease in ash content from 1.7 to 1% when sorghum was malted for 72 h. Badau *et al.* (2005) reported that calcium, iron, zinc, phosphorus and iodine contents decreased during germination of pearl millet.

There was a significant decrease in minerals content of malted samples of both yellow and purple varieties of foxtail millet (8.9 and 22.7% respectively) (Choudhury *et al.*, 2010). There was removal of seed coat during malting, which contributed towards the reduction in the total mineral content of malted samples (MacMasters *et al.*, 1971). Nithya *et al.* (2006) have given the following changes in minerals during germination and roasting of pearl millet

Table 3.31 Changes in mineral contents during germination and roasting of pearl millet* (mg/100g)

Parameters	Raw	Germinated	Roasted
Phosphorous	371	320	347
Iron	8.6	8.2	8.3
Calcium	32	19	21
Potassium	29	23	22
Magnesium	120	95	115
Zinc	5.20	4.47	4.80
Manganese	5.12	4.37	4.67

*Germinated pearl millet dried at 60ºC and roasted at 110ºC for 1h in hot air oven (Source: Nithya *et al.*, 2006).

3.8.4 Changes in phytate

Phytate complexes with essential elements such as Zn, Fe, and Ca and reduces their bioavailability which can be enhanced by degradation of phytate (Moeljiopawiro *et al*., 1987). Progressively decreased in phytin phosphorus content of finger millet varieties during germination were also reported by the Malleshi *et al*. (1986) and Udayasekhara and Deosthale (1988). Average decrease in phytic acid on malting of finger millet was 23.9% at 72 h and 45.3% at 96 h of germination (Makokha *et al.* (2002). According to Sripriya *et al.* (1997) phytic acid content in finger millet decreased from 0.61 to 0.55% (db) during 24 h of germination. Natural fermentation of the germinated millet flour further decreased the phytic acid to 0.24% (db). Germination significantly reduced the phytic acid content of pearl millet. An average decrease in phytic acid in the range of 83.71 to 86.01 % in 10 different pearl millet cultivars was reported by Badau *et al*. (2005).

Generally, cereals have been regarded as the major sources of dietary phytate (Harland, 1989; Reddy *et. al*., 1989). The majority of ingested phytate is undegraded during transition through gastrointestinal tract (Graf *et al.*, 1984). One phytate molecult can bind up to six divalent cations and the metal could possibly bridge at least two phytate molecules (Graf and Easton, 1990). Other researchers have reported decrease in the level of phytic acid during soaking (Khokhar and Chauhan, 1986; Ologhobo and Fetugal, 1984) and germination (Borade *et al*., 1984; Mandal *et al*., 1972) due to phytase activity in the germinating grain (Rao and Deosthale, 1982).

A lot of reports on the detrimental effects of phytic acid have been published (Clydesdale, 1988; Reinhold, 1988; Spencer and Karmer, 1988), but recent findings have shown that it has some beneficial effect. Recent information showed that phytates possess potential ability to lower blood glucose, reduce cholesterol and triglycerides, and reduce the risk of cancer and heart disease (Burgess and Gao, 2002; Cornforth, 2002, Jenab and Thompson, 2002).

3.8.5 Changes in total polyphenols (TP)

Nithya *et al.* (2006) reported that total phenolic contents in ungerminated, germinated and roasted pearl millet were 3.00, 0.68 and 2.27% m/m respectively. Sripriya *et al.* (1997) reported decrease in TP from 1.43 to 1.28 g/100 g DM in 24 h of germination at 30 ºC in finger millet (var. Co-13). According to Chethan *et al.* (2008) native millet contained about 2.5% polyphenols and nearly 44% was lost during the first 24 h of

germination at 25 ºC. The total phenolic content in 10 barley varieties grown in Kromeriz Czech Republic and their malts ranged between 211.4 – 572.8 and 358.3 – 668.5 mg gallic acid equivalent (GAE)/kg DM respectively (Dvorakova *et al*., 2008). This could be partially be explained by the enzymatic release of bound phenolic compounds during malting or under the effect of temperature during kilning (Maillard and Berset, 1995).

3.8.6 Changes in crude fat

The increase in fat content during malting of finger millet grains may be attributed to the synthesis of lipids related to metabolism of starch (Chavan and Kadam, 1989). An increase in fat content because of malting was similarly reported in barley, wheat and oats but contradictory results were reported by other workers who found that fat content decreased when sorghum and millets were malted. Malleshi and Klopfenstein (1998) reported that there was a slight increase in fat content (1.5 to 1.7%) when finger millet was malted, whilst it decreased in sorghum (2.3 to 2.0%) and pearl millet (5.3 to 4.2%) on malting. An increase in fat content on malting finger millet may be undesirable as it may affect negatively the storage quality of the malt due to rancidity. Significantly, decrease in fat content (53.3%) was noted in malted millet of yellow variety than its raw counterpart on dry basis (Choudhury *et al.*, 2010). Hydrolysis of lipid and oxidation of fatty acids take place during germination of seeds. The hydrolyzed products do not accumulate in the seed, but the glycerol becomes a part of carbohydrate pool and the fatty acids are oxidized through α- and β- oxidation, resulting in decrease in fat on malting (Mayer and Mayber, 1963).

3.8.7 Changes in tannin

Tannin content in ungerminated, germinated, and roasted pearl millet was 1.52, 1.00, and, 1.30% m/m respectively. Decrease in TP and tannins in germinated pearl millet might be attributed to the leaching of polyphenols in soaking water (Jood *et al.*, 1987). In addition to leaching, increased enzymatic hydrolysis might have facilitated the reduction of total phenolics and tannins in germinated millets (Bishnoi *et al*., 1994; Fordham *et al*., 1975). Reduction in total phenolics and tannins during roasting might be due to the loss of compounds while treating at high temperatures (Shinde *et al.*, 1991). Udayasekhar and Deosthale (1988) reported a 54% decrease in tannin content during malting of brown finger millet variety.

3.8.8 Changes in enzyme activities

Alpha and beta amylase activities in ungerminated, germinated, and roasted pearl millet were 5.33 and 8.0, 14.67 and 14.33, and 3.00 and 6.33 µMol maltose/mL.min respectively (Nithya *et al.,* 2006). Seed germination triggers several metabolic changes and in turn increases the activity of various enzymes including α- amylase, β-amylase, acid phosphatase which are either present in the matured seed or induced during germination (Parvathy, 1995). According to Bewley and Black (1983), β-amylases were present in an inactive latent form and were activated during seed germination. There was a significant positive correlation between protease activity and FAN during the germination of minor millet (Parvathy, 1995).

According to Taylor and Robbins (1993), beta-amylase activity in sorghum malt was increased by germination time and steepout moisture content over the germination temperature range investigated (24 – 32 ºC) and 24 ºC germinated sorghum gave the highest β-amylase activity. Tiwari (2010) reported that maximum amylase activity in finger millet malts (var. *Kabre-1* and ACC#523-1) was observed for 36 h of germination at 27 ± 2 ºC. Alpha-amylase activity of Nepalese finger millet malts (var. *Kabre-1* and *ACC#523-1*) was in the range of 49.6 − 55.5 units/g DM (one unit means unit change in OD at 620 nm compared to control), while β-amylase activity was in the range of 194.5 − 215.5 mg maltose produced per gram dry matter. Similarly, the β-amylase activity of commercial barley malt was reported to be 354.8 mg maltose/g DM.

Acharya (2011) reported a maximum α-amylase activity of 59.93 units (change in OD at 620 nm per g of dry matter compared to control) in naked barley malt prepared by germinating at 25 ºC for 72 h. Again maximum β-amylase activity of 482.9 units (mg reducing sugar as dextrose produced per g of dry matter) was found in 72 h germination at 25 ºC. Amylase activity was maximum at 72 h of germination at 25 ºC and thereafter a slight decline was observed by Nirmala *et al.* (2000). The maximum development of amylase activity was noted after 6 − 7 days of germination in wheat, 4 days in corn and after 3 days in millet (Chavan and Kadam, 1989). The diastatic power (DP) of four different sorghum variety malts ranged from 13 to 18 ºL (Owuama and Adeyemo, 2009). Alpha- and beta- amylase activity in unmalted buckwheat, buckwheat malt, and barley malts kilned at 40 ºC were 0.1 and 5.3, 19.9 and 24.7, and 105.9 and 514 units/g respectively (Nic Phiarais, *et al*., 2005).

3.8.9 Changes in antioxidant activity

DPPH antiradical power was higher in barley malt compared to the corresponding native barley (Dvorakova *et al*., 2008). This increase could be due to the development of non-enzymatic browning products during malting, such as maillard products, particularly melanoidins, which can also act as antioxidants, (Goupy *et al.,* 1999). Samaras *et al.* (2005) reported that ferulic acid reacts with maillard reaction intermediates formed from glucose and proline at kilning temperatures leading to higher antioxidant activity. Increase or decrease of antioxidant activity depends mainly on the content of proanthocyanidins and on the release of phenolics from the bound form during malting (Friedrich *et al.*, 2000; Goupy *et al.,* 1999).

3.9 Malt composition

A primary objective of malting is to promote the development of hydrolytic enzymes that are not present in the ungerminated grain. The development of the amylase enzymes during malting is of critical importance. These enzymes are required to hydrolyze the malt and adjuncts starch to fermentable sugars (Briggs *et al.*, 1981). Analysis of malting and brewing characteristics of sorghum has shown that diastatic power, alpha-amylase, amyloglucosidase and protease activities increased with malting time (Nzelibe and Nwasike, 1995).

Cereal beta-amylases are important in brewing. As a major contributor to diastatic power of malt, their activity is essential for the generation of maltose and other easily fermentable sugars from cereal grain starch in the mashing process to fuel the production of alcohol by yeast. Two distinct categories of cereal beta-amylases have recently been recognized to exist. One is the classical endosperm beta-amylase of the *Triticeae* species, barley, wheat, and rye; which is present in high amounts and at high activities in the ungerminated seeds. Another, “tissue ubiquitous” form of the enzymes present in much lower amounts and activities in all cereals, appear to represent the entire enzyme complement of the seed of the non-*Triticeae* species (Daussant *et al*., 1994). Cereal beta-amylases are more acid stable and less heat stable than alpa-amylase and unlike these are susceptible to inactivation by sulphydryl group oxidizing reagents and Ca^{2+} chelating agents (Thomas *et al*., 1971). Beta-amylase activity extractable from the seeds in water/salt solutions increased considerably during germination (Evans *et al*., 1997). This is due to the release and activation of the ‘bound’ form of the enzyme. Beta-amylase released from the bound form is the primary factor in the increase of

activity upon germination. Aplha-amylase, debranching enzyme, alpha-glucosidase, celluloses, proteases, and nucleases are all synthesized in the aleurone layers and are secreted into the starchy endosperm. To enable maltose production to occur, alpha-amylase is the key enzyme involved in initiating the breakdown of starch in the cereal grain and since it is not present in the ungerminated grains (Zeigler, 1995) and its synthesis is the primary task of malting. Beta-amylase which can be extracted from even ungerminated cereals is thus a measure of the suitability of the grain for brewing purpose (Gibson *et al*., 1995). Gimbi and Kitabatake (2002) reported that highest alpha-amylase was exhibited in African finger millet germinated at 15 ºC for 9 days and at 20 ºC for 6 days, while the highest beta-amylase activity was displayed in the malt flour germinated for 5 days at 30 ºC.

There is no universally accepted specification for sorghum and millet malts. However, a minimum specification of DP of 28 sorghum diastatic unit (SDU)/g for malt for industrial sorghum or millet beer brewing appears to be widely used (Dewar *et al*., 1995). There is a significant difference in DP in millet malts among millet varieties. This is because each variety had its own ability to produce gibberlins, the hormones which during malting are produced in the germination of the grain and diffuse into the endosperm and aleurone layer. In the endosperm, they induce the synthesis of alpha-amylase (Dendy, 1995).

Okolo and Ezeogu (1995) studied malting of sorghum using 1, 2, 3 and 4 h of air-rest period. Cold water soluble protein content in ICSV400 and KSV8 sorghum cultivars were 1040 and 850; 1140 and 850; 1700 and 1000; and 1250, and 1040 mg% (db) in 1, 2, 3 and 4 h air-rest germinated sorghum malts respectively. Delvaux *et al.* (2004) prepared wheat malt by soaking for 29 h at 15 ºC, germinating at 15 º C for 6 days, and kilning for 17 – 19 h at 80 – 90 ºC. Moisture (%), extract (%) and total protein (%, db) contents in wheat malt were 5.3, 89 and 11.2 respectively. Morrall *et al.* (1986) reported that germination temperatures of 24 and 28 ºC were equally good for the development of DP, FAN and extract in sorghum malt and that higher temperatures were progressively worse. Demuyakor and Ohta (1992) noted that the optimum germination temperature for sorghum and millet malts were in the range of 20 – 30 ºC, however, higher germination temperatures resulted in higher malting losses.

Shukla *et al.* (1986) had given the following composition of eight improved millet malts collected from Jabalpur, India.

Table 3.32 Composition of millet malt

Parameters	Values
Germination	77.7 - 93
Amylase activity*	156 – 300.1
Protein (%, N x 6.25)	4.6 – 5.7
Ether extractive (%)	1.1 – 2.2
Total carbohydrate (%)	73.7 – 83.1
Soluble sugars (mg %)	2.9 – 9.9
Non-protein nitrogen (%)	0.148 – 0.346
True protein (%)	2.6 – 4.8

*amylase activity was expressed as mg maltose released by 1g of malt flour when acted on 1 ml of 1% starch at 37 ºC for 30 min. (Source: Shukla *et. al*., 1986).

Dewar *et al.* (1997) have given the following results of sorghum malt diastatic power (SDP).

Table 3.33 Effect of air-rest period on sorghum malt quality

Soaking time (h)	Sorghum Diastatic Power(SDU/g)					
	20ºC		25ºC		30ºC	
	AR	NA	AR	NA	AR	NA
16	29	26	27	33	35	33
24	32	28	31	36	38	38
40	35	31	37	43	43	39

Source: Dewar *et al.* (1997). AR = air-rest; NA = no air rest

Carbohydrate content in Nigerian millet (*Pennisetum typhoideum*) malt was 48.47% (Iwuagwu and Izuagbe, (1986). Ogu *et al.* (2006) studied the malting quality of different Nigerian sorghum and reported that moisture, malting loss, cold water extractives and diastatic power were 5.2 - 5.7%, 21.5 - 25.5%, 31 - 36% and 27 ºL respectively. Tiwari (2010) reported that the ash, crude fat, crude fiber, crude protein and total carbohydrate contents of unmalted and malted Nepalese finger millet (var. *Kavre-1*) were 1.98 and 1.98; 2.52 and 2.54; 3.12 and 3.38; 9.96 and 9.97 and 82.43

and 82.13% db respectively. Similarly ash, crude fat, crude fiber, crude protein and carbohydrate contents in unmalted and malted *ACC# 523-1* finger millet were 1.99 and 1.99; 2.45 and 2.46; 2.94 and 3.20; 9.94 and 9.96 and 82.71 and 82.39% db respectively.

Acharya (2007) had given the following characteristics of Nepalese naked barley and commercial barley malts.

Table 3.34 Chemical composition of naked barley and barley malts

Parameters	Naked barley malt	Barley malt
Aroma	Aromatic	Aromatic
Saccharification time (min)	10 − 15	0 − 5
Filtration time	Slow	Normal
Clarity of extract	Slightly hazy	Clear
pH of extract	6.1	6.62
Moisture content (%)	6.75	6.26
Starch (%)	65.43	55.05
Reducing sugar as maltose (%)	1.56	2.90
Ash content (%)	1.5	1.8
Protein (%)	11.92	11.16
Sp. gr of the extract	1.021	1.031
Extract (%, db)	56.33	60.76
Diastatic power (mg maltose/g dm)	47.33	55.20

(Source: Acharya, 2007)

Millet malt is a rich source of alpha-amylase besides being a good source of beta-amylases. The amylase activity increased with the period of germination up to a certain point and then decreased. The amylase activity of the millet increased rapidly during germination up to 96 h and subsequently decreased (Chethan *et al*., 2008). Ungerminated pearl millet did not have any diastatic power (DP) and the DP increased with germination time. The DP of pearl millet malt germinated at 25 ºC for 5 days with medium water regime was reported to be in the range of 27.5 to 40 PMDU/g dm (pearl millet diastatic unit). Ungerminated pearl mille did not exhibit any beta-amylase

(Pelembe *et al*., 2004). Ungerminated sorghum also did not exhibit beta-amylase activity (Taylor and Robbins, 1993). This is fundamentally different from barley where the ungerminated grain exhibits beta-amylase activity (Briggs, 1998; MacGregor, 1996). Total beta-amylase activity in pearl millet increased with increasing germination (Pelembe *et al*., 2004). Owuama and Adeyemo (2009) reported malting loss of 5.4 to 10.6% in four different sorghum varieties malts.

Acharya (2011) had given the following characteristics of naked and commercial barley malt worts. Mashing was done using malt: water ration of 1:5 (m/v).

Table 3.35 Chemical characteristics of Nepalese naked barley and commercial barley maltsworts

Parameters	Naked barley malt	Barley malt
Sp. gr.	1.0595	1.0631
TSS, ºBx	14.66	16.03
pH	6.03	5.79
Viscosity (cP)	3.73	1.73
Fermentable nitrogen (mg/L)	189.8	246.8
α-amylase activity (change in OD at 620 nm/ g dm)*	83.85	97.72
β-amylase activity (mg reducing sugar as dextrose/ g dm)*	534.96	564.95

*: enzyme activities determined in the malt

3.10 Wort composition

The composition of wort varies according to the ingredients used in its preparation as modified by processing and equipment the ingredients have encountered. According to Burger and La Berger, (1985) the extract, pH, iodine reaction and turbidity of various worts intended for the production of North American lager beers were 10.8 − 12.5 ºPlato, 5.2 − 5.7, negative and 12 − 48 FTU respectively. They also noted that total protein, free amino nitrogen, formol nitrogen and viscosity of the worts were 0.35 − 0.60%, 140 − 260 mg/L, 190 − 250 and 1.3 − 1.6 respectively. According to Hough (1985) fructose, glucose, maltose, total free amino acid, and total phenolic contents in a typical UK wort were 2.1, 9.1, 52.4, 1.65, and 0.25 g/L respectively. Ogu *et al*. (2006) reported that viscosity (cP), FAN (mg/L), glucose and maltose (g/L) contents in worts

obtained from four different sorghum malts mashed at 65 ºC (malt: water ration = 1:4) were 1.18 − 1.27, 42 − 80, 6.7 − 7.5 and 10.9 − 12.3 respectively. All worts showed +ve test with iodine. They further reported that sorghum malt mashed at 65 ºC with added commercial enzymes (20µL each of Bioglucanase ME 250 and Hi-tempase and Bioprotease N100L) had wort viscosity of 1.03 − 1.05 cP, FAN content of 84 − 152 mg/L, glucose content of 40.3 − 54.7 g/L and maltose content of 64.3 − 88.9 g/L. Again all worts showed positive reaction to iodine. Specific gravity of Nigerian millet (*Pennisetum typhoideum*) malt wort was 1.5927 (Iwuagwu and Izuagbe, 1986). Venkatanarayana *et al.* (1979) have reported the following composition of worts obtained using different proportion of finger millet and commercial barley malts with and without enzymes.

Table 3.36 Composition of worts derived from finger millet and barley malt combinations*

Barley : Finger millet malt	Enzymes	Sp.gr.	pH	α-amino nitrogen (mg/L)	Total carbohydrates (% m/v)
70: 30	With enzymes	1.047	6.0	107	11.1
	No enzymes	1.047	6.0	88	11.1
60:40	With enzymes	1.047	6.2	92	11.1
	No enzymes	1.048	6.2	77	11.1
50:50	With enzymes	1.047	6.2	77	11.1
	No enzymes	1.048	6.2	62	11.1

(Source: Venkatanarayana *et al*., 1979).

*200 mg% amylase and 100 mg% protease enzymes were mixed with the cooked and cooled ragi.

Okrah, (2008) noted that pH is very important during mashing since mashing is entirely an enzymic process and therefore it plays an important role. The pH of different sorghum malt worts was in the range of 5.39 – 6.09. The pH of mash should be between 5.3 – 5.4. It is the pH of the mash that matters, not the pH of the water before it enters the mash (http:// www.draymans.com/articles/art/05. html, accessed on July 30, 2011). According to Agu and Palmer (1998) sorghum malt wort had pH of 4.75.

Table 3.37 Composition of ragi wort derived from cooked ragi and barley malt with and without protease*

Wort	Sp.gr.	pH	α-amino nitrogen (mg/L)	Carbohydrates (% m/m)		
				Total	Fermentable	Non-fermentable
With enzymes	1.0566	6.6	175	13.9	12.0	1.9
No enzymes	1.0573	6.6	120	13.9	12	1.9
All malt (control)	1.0570	5.8	226	12.3	10.0	2.3

* 100 mg% protease by weight of cooked and cooled cereal used.
(Source: Venkatanarayana *et al*., 1979).

Protein and amino acids

Total nitrogen in malt and wort is an indicative of the extent of modification and the total proteolytic activity of malt (Okokon and EtokAkpan, 2004).The ratio of total soluble nitrogen in the wort and total nitrogen in malt of different sorghum varieties ranged from 25.33 to 48.9% (Okrah, 2008). Edney *et al.* (2005) reported an average FAN content of 262 mg/L in 108 Canadian barley malt worts. FAN contents in 40 ºC kilned buckwheat malt and barley malt worts were 107 and 106.7 mg/L respectively (Nic Phiarais *et al*., 2005). Addition of microbial protease increased α-amino nitrogen content of wort from 120 to 175 mg/L (Venkatanarayana *et al*., 1979).

Carbohydrates

The total reducing sugar contents in four different sorghum malt worts were reported to range from 20 to 21 g/L. There was a considerable increase in reducing sugar content of worts mashed with exogenous enzymes compared to untreated malt (Owuama and Adeyemo (2009).

3.11 Composition of beer

The ordinary beer of commerce made from pure barley malt and hops, had approximately 4.0% alcohol, 0.5% protein, 0.5% ash and 89% water (http://chestofbooks.com/food/beverages/Adulteration-Origin/Composition-Of-Beer.htlm, Accessed on 067/05/05). Matz (1991) has given the following composition American lager beer.

Table 3.38 Quality control test for American lager beer

Test and unit	Range	Typical value
Original extract (º Plato)	10.7 – 12.1	11.5
Apparent extract (º Plato)	1.9 – 3.0	2.08
Real extract (º Plato)	4.0 – 4.8	3.9
Alcohol (% by volume)	–	5.00
Alcohol (% by weight)	3.4 – 4.0	3.92
Apparent degree of ferm. (%)	–	81.9
Real degree of ferm. (%)	62 – 68	66.1
pH	3.9 – 4.4	4.26
Iodine reaction	Negative	
Reducing sugars (%	–	0.97
Protein (%)	–	0.37
Acid as lactic (%)	–	0.21
Diacetyl (ppm)	–	0.11
Bitterness units, EBC	–	11.9
Calcium (mg/L)	40 - 80	–
Sodium (mg/L)	40 - 220	–
Copper (mg/L)	0.15 max	–
Iron (mg/L)	0.10 max	–

Venkatanarayana *et al*. (1979) reported the following composition of ragi and barley beers (Table 3.39).

Table 3.39 Composition of ragi beer made with and without added barley malt

Components	Ragi beer*	Barley beer
Sp. gr.	1.0100	1.0059
pH	6.3	5.7
Alcohol (% m/m)	5.5	5.0
Real extract (%)	5.38	
Apparent extract (%)	2.56	1.52

*beer made using equal amounts of ragi and barley malts with 100 mg% protease enzymes. (Source: Venkatanarayana *et al*., 1979).

Nepal standard has given the following requirements for beers.

Table 3.40 Beer characteristics according to Nepal standard

Characteristics	Requirements
Ethyl alcohol (% v/v at 15ºC)	2.0 − 6.5
pH value	3.0 − 4.8
CO_2 (% w/v)	<0.3
Arsenic (ppm)	0.3 (max)
Lead (ppm)	0.5 (mx)
Copper (ppm)	2.0 (max)
Iron (ppm)	0.5 (max)

(Source: NS, 1983)

Acharya (2007) reported the following characteristics of beers made from Nepalese naked barley and commercial barley malts using malt: water ratio of 1:3 and *Saccharomyces cerevisiae* yeast.

Table 3.41 Chemical composition of naked barley and commercial barley beers

Parameters	Naked barley beer	Barley beer
pH	4.16	4.31
TSS, ºBx	5.8	6.7
Total acidity as lactic (% m/v)	0.27	0.30
Fixed acidity as lactic (% m/v)	0.21	0.24
Volatile acidity as acetic (% m/v)	0.04	0.04
Ester as ethyl acetate (mg/100ml)	19.7	14.9
Alcohol content (% v/v)	4.40	5.64
Reducing sugar as maltose (% m/v)	1.00	2.36
Starch content (% m/v)	3.36	4.67
Ash content (% m/v)	0.11	0.10
Protein content (% m/v)	0.35	0.56

(Source: Acharya, 2007)

Khanal (2008) prepared naked barley beer using malt to water ration of 1:3 and *S. cerevisiae yeast* and found that pH, TSS (ºBx), total acidity as lactic (%), volatile acidity as lactic (%) and fixed acidity as acetic (%) contents were 4.21, 5.8, 0.31, 0.28

and 0.027 respectively. Reducing sugar (% as maltose), alcohol (%, v/v), total ester as ethyl acetate (mg/100 mL), starch (%) and ash content (%) were reported to be 2.87, 6.45, 11.8, 3.32 and 0.11 respectively. Similarly, protein (%), total aldehyde as acetaldehyde (mg/100 mL), methanol (g/L alc) and fusel oil (g/L alc) were 0.38, 5.75, 3.67 and 8.71 respectively.

A typical American beer was reported to contain 91% water, 4.6% carbohydrates, 0.5% protein substances, 0.2% ash and 3.59% alcohol by wt. (http://www.tuntavern.com/pages/ hisbeer.htm, accessed on 2011/08/22). Novellie and De Schaepdrijver (1986) reported that the protein, ash, carbohydrates, iron, and zinc contents in 8 commercial sorghum beers were 5.4, 1.13, 47.6, 1.4 and 1.4 g/L respectively.

Tiwari (2010) prepared finger millet beer (var. *Kabre-1*) using *S. cerevisiae* yeast and malt to water ratio of 1:3. The pH, TSS (ºBx), total acidity as lactic (%), fixed acidity as lactic (%) and volatile acidity as acetic (%) were 4.28, 6.13, 0.33, 0.29, and 0.046 respectively. Similarly, alcohol content (% v/v), total ester as ethyl acetate (mg/100 mL alcohol), total aldehyde as acetaldehyde (mg/100 mL alcohol), ash (%), protein (%), reducing sugar as maltose (%), starch (g dextrose/100 mL) and sp. gr. were 6.54, 4.25, 9.31, 0.09, 0.40, 1.01, 3.45 and 1.0077 respectively.

Acharya (2011) reported the following characteristics of wort and beer prepared using 85% corn + 10% naked barley malt + 5% mold bran and 100% commercial barley malt.

Table 3.42 Chemical composition of wort and beer

Parameters	Wort		Beer	
	CNM*	Barley	CNM	Barley
TSS, ºBx	13.6	16.03	5.86	6.7
Sp. gr	1.0557	1.0631	0.9917	0.9908
pH	6.13	5.97	4.22	4.31
Fermentable nitrogen (mg/L)	170.12	246.75	–	–
Viscosity (cP)	1.44	1.73	–	–
Alcohol (% v/v)	–	–	5.82	6.39

*wort mashed using 85% corn, 10% naked barley malt, and 5% mold bran.
(Source: Acharya, 2011)

Mahato (2010) prepared beers from wheat malt (var. *NL-297*) and commercial barley malt using malt to water ratio of 1: 3 and *Saccharomyces cerevisiae* (Q8/T1501) and reported the following characteristics of the beers. The TSS and pH of the worts were maintained at 12 ºBx and 4.5 respectively.

Table 3.43 Chemical composition of wheat and barley beers

Parameters	Wheat beer	Barley beer
pH	4.04	4.2
TSS (ºBx)	6.4	5.2
Total acidity as lactic (%)	0.25	0.28
Fixed acidity as lactic (%)	0.21	0.21
Volatile acidity as acetic (%)	0.035	0.033
Esters as ethyl acetate (mg%)	16.5	15.2
Alcohol (% v/v)	5.55	5.60
Reducing sugar as maltose (%)	0.91	1.29
Ash (%)	0.11	0.10
Protein (%)	0.27	0.56

(Source: Mahato, 2010)

Kabelova *et al.* (2008) analyzed 35 commercial bottled beers available in the Czech market and reported that the alcohol and extract of original wort were in the range of 4.01 – 7.55% (v/v) and 11.02 – 16.11 ºPlato respectively. The pH of beers was in the range of 3.75 – 4.92. Goode and Arendt (2003) reported that the pH of sorghum and barley malt beers were 4.51 and 4.46 respectively. Beer prepared from Astoruca and Scarlett var. barley malts had pH in the range of 4.2 – 4.3 (Delvaux *et al*., 2004). Lager beer prepared using 70% barley malt and 30% corn grits had pH of 4.1 (Porter, 1975). The pH of gluten free beer made from rice malt and buckwheat malt was reported to be 4.1 (http://www.freepatentsonline.com/ EP0949328.html, accessed on 2011/09/22).

The alcohol contents in sorghum and barley beers were 4.3 and 4.89% (v/v) respectively (Goode and Arendt, 2003). Alcohol content in lager beer (70% barley malt + 30% corn grits) was 3.35% by wt as reported by Porter (1975). Alcohol content in gluten free beer made from rice malt and buckwheat using *S. carlsbergensis* yeast with added enzymes had alcohol content of 5% (v/v) (http://www.freepatentsonline.com/EP0949328.html, accessed on 2011/07/20). According to Matz (1991) the pH after bottom fermentation was normally in the range of 4.2 − 4.4, occasionally 4.0 or even

less. Starch test was positive in sorghum beer while it was negative in barley beer (Goode and Arendt, 2003). Glucose content was nil while maltose content was 0.166 and 0.089 mg/100 mL in sorghum and barley beers respectively. According to Goode and Arendt (2003), original extracts (º Plato) of sorghum and barley beers were 10.98 and 11.22 respectively. Original extracts of *Astorica* and *Scarlett* barley malt beers were 11.7 and 11.5 º Plato respectively (Delvaux *et al.*, 2004). Total carbohydrate content in Heineken's beer was 3.0 g/100 mL (Http://www.heinekeninter-national.com/faq page, accessed 2011/09/22). Lager beer brewed from 70% barley malt and 30% corn grits had reducing sugar and dextrin contents of 1.27 and 1.95% by wt. respectively (Porter, 1975).

Total soluble nitrogen in sorghum and barley beers were 478 and 716 mg/L respectively (Goode and Arendt, 2003). Porter (1975) reported 43.34 µMol/mL of total nitrogen and 3.47µMol/mL of formol nitrogen in lager beer brewed using 70% barley malt and 30% corn grits. Protein content in Heineken's beer was 0.5 g/100 mL (Http://www.heineken international.com/faq, accessed on 2011/09/22). FAN content of barley beer and beer prepared using 50% unmalted sorghum + 50% barley malt with bacterial amylase, protease and fungal amylase ranged from 69 – 76 mg/L (Goode and Arendt, 2003). Total nitrogen in gluten free beer made from rice malt and buckwheat was 200 mg/L. The nitrogen content of the wort decreased by about one third due mostly to the assimilation of amino acids and peptides by the yeast but partly to the precipitation of complex protein caused by low pH. After the completion of fermentation, the sp.gr. of the beer was in the range of 1.009-1.014 (Matz, 1991). Viscosity (mPa.s) in sorghum and barley beers were 1.67 and 1.76 respectively (Goode and Arendt, 2003). Sodium content in Heineken's beer was 3 mg/100 mL (Http://www.heinekeninternational.com/faq, accessed 2011/09/22.)

It has been claimed that beer is one of the richest sources of silicon in the diet. Commercial beers contain silicon ranging from 6.4 – 56.5 mg/L. Products derived from grist of barley tended to contain more silicon than did those from wheat-based grist, likely because of the high levels of silicon in the retained husk layer of barley (Casey and Charles, 2010). Flavour changes occur inevitably in beer during ageing and their nature depends on the type of beer and the storage conditions. An important type of change during beer staling is caused by aroma active carbonyl compounds, which can be formed by radical reactions (Hashimoto and Kuroiwa, 1975). Iron and copper ions are known to have a negative influence on beer flavor stability. Even concentrations of

below 50 ppb are reported to cause damage in the final product (Foster *et al*., 2001; Jacobsent and Lie, 1979; Uchida and Ono, 2000).

Beer has a complex mixture of phenolic compounds ranging from 150 to 330 mg/L in concentration. The majority (about 2/3rd) are malt derived especially from the husk material of the malt. The remainder (1/3rd) comes from the hops extracted during wort boiling (http:// www.draymans.com/articles/art/05. html, accessed on 2011/09/25). Due to their specific properties, brewers find polyphenols interesting technologically (foam maintenance, physical, and chemical stability and shelf life). Health researchers find them interesting because phenolic compounds can act as antioxidant in the human body, for example as protective agents against the oxidation of ascorbic acid and unsaturated fatty acids. They have the ability to reach with proteins during wort boiling to form the hot break; during cooling to form the cold break; during post fermentation they are involved in the formation of chill haze and permanent hazes, which can then be removed by filtration. Polyphenols on their own contribute too little to haze formation. Haze is composed fundamentally of complexes between condensed polyphenols and protein (http:// www.draymans.com/articles/art/05. html, accessed on 2011/09/25).

Beer is a source of readily absorbed antioxidants. Scientific studies indicate that the moderate consumption of beer help to promote cardiovascular health and to reduce the incidence of certain types of cancer (http://www.wikihw.com/Brew-Antioxidant-Rich-Beer, accessed on 2011/09/25). Total polyphenol content in beer as measured by the Folin-method varies between 12 to 52 mg/100 mL depending upon the beer type. Ale beer and dark beer are richer in polyphenols (52 and 42 mg/100 mL respectively). Regular beers contain about 28 mg/100 mL total polyphenols. Alcohol free beer contains about 12 mg/100 mL polyphenols (http:// www.henolexplorer.eu/reports/38, accessed on 2011/11/13).

The average concentration of total phenolics (TP) in lager and dark beers were 376 and 473 mg/L respectively. Antioxidant properties were dependent on the TP content of beers.(http://cat.inist.fr/?a Model=afficheN& cpsidt=15461475, accessed on 2011/08/22). TP and phenolic acid contents greatly vary among different beer types (e.g. TP from 366 µg/mL GAE for dealcoholized beers to 875 µg/ml GAE for bock beers (Piazzon *et al*., 2010). Flavour stability, one of the important characteristics in beer, is challenging brewers, and is one of the most important factors in determining the shelf life of the packaged beer. It has been widely accepted that the main factor responsible for beer flavor instability is oxidation during brewing although there is no

agreement on the main precursor of staling substances (Narziss *et al*, 1993). Consequently, a number of efforts have been made to avoid oxygen pick-up during brewing and packaging. The level of total oxygen might be as low as 0.1 ppm with modern filling equipment, but oxidative staling of beer remains difficult to control (Bamforth *et al*., 2000). Researchers are seeking the alternative ways to solve this problem by increasing the endogenous antioxidant activity of beer itself. Although both natural and synthetic antioxidants such as flavonoids, sulfites and ascorbate could be used in the brewing industry to improve beer flavour stability, there has been a trend towards minimizing the use of additives in brewing because of consumer demand stiffening regulations.

There are many endogenous antioxisdants such as phenolic compounds, maillard reaction products and sulfite present in beer (Vanderhaegen *et al*., 2006). Among the antioxidants, phenolic compounds are of particular interest to brewers because they play a key role in the brewing process by delaying, retarding, or preventing oxidation process (Guido *et al*., 2005). Zao *et al.* (2010) reported that total phenolic content varied from 152.01 mg/L as GAE for Reeb beer to 339.12 mg/L for Carlsberg beer. Grolsch, Heineken and Bitburger beers also had relatively higher TP contents (> 290 mg/L as GAE). Lugasi and Hovari (2003) reported that the TP contents of different beer samples were in the range of 270 − 600 mg/L. Similarly, Shahidi and Naczk (1995) reported that the TP contents of beers were 60 − 100 mg/L. The variations in TP contents may be due to the differences in the samples and TP content evaluation methods used. Gallic and ferulic acids are the phenolic constituents identified in beer and representing > 50% of the TP contents (Zao *et al*., 2010).

4. Materials and methods

4.1. Finger millet fermentation using defined starter

4.1.1. Materials

Finger millet (*Eleusine coracana*, var. *Kabre)* was obtained from National Agricultural Research Council (NARC), Kathmandu. Polyvinyl chloride (PVC) containers were obtained from Asian Thai Foods, Khanar, Sunsari and plastic, and wodden container were purchaged from local market of Dharan. Traditional cereal-based alcoholic fermentation starters (*murcha*) were collected from local market of Dharan, Bhedatar, Hile, Basantapur, Ilam and Bhojpur. Pure culture of *R. oryzae* ITCC No. 4408 was obtained from Indian Agricultural Research Institute, New Delhi, India and the yeast, *S. cerevisiae*, was isolated from traditional fermentation starters. Different fining agents (bentonite, gelatin, and tannin) were obtained from Central Department of Food Technology, Dharan.

4.1.2 Experimental methods

4.1.2.1 Selection of *murcha* for yeast isolation

Finger millet was fermented using different *murcha*. Millet was cleaned, thoroughly washed and stepped for 2 h in water. It was again washed, cooked using required amount of water and cooled to room temperature by spreading in an aluminum tray. To the cooled millet, were added different powdered *murcha* separately (@ 1 g/100 g cooked millet), mixed thoroughly and left for 2 days at 29 ± 1 ºC for biomass development. The tray was covered with moistened muslin cloth and the cloth was moistened twice a day. The biomass developed millet samples were filled in to plastic containers, closed tightly and allowed to under go alcoholic fermentation for 15 days at 26 ± 1 ºC. The fermented millets were analyzed for alcohol content and subjected to sensory evaluation. One part (by wt) of fermented millet was mixed with 1.5 parts (by vol) of potable water, gently mixed, held for 30 min and strained through plastic net with gentle pressing. *Murcha* giving higher alcohol content and better sensory quality was used for the isolation of fermentative yeasts.

4.1.2.2 Isolation and purification of yeasts

Ten gram of fermented millet (1 day after alcoholic fermentation) was mixed with 90 mL of sterile distilled water and shaken in a flask shaker for 10 min. One ml of the suspension was dispensed in to sterilized petri plates and overlaid with molten PDA supplemented with 0.5 mL of 5% aqueous solution of rose bengal and 100 mg chloramphenicol per liter of the medium. The plates were incubated for several days, yeasts were picked up separately and pure colonies obtained by using streak plate method as per Dubey and Maheshwari (2002).

4.1.2.3 Selection of the best yeast

Sugar fermentative yeasts were screened using basal growth medium as per Dubey and Maheshwari (2002). Purified yeasts were grown on molasses broth (6% TSS and pH 4.5) for 4 days at 27 ºC, kept under refrigeration, and the broth separated by decantation. The yeast residues were washed with sterile distilled water, re-suspended in sterile distilled water (yeast suspension), counted microscopically, and used for molasses fermentation. Sterile molasses broths containing 14% TSS and 4.5 pH were inoculated with these yeast isolates (10^6 cells/mL) in a flask and fermented at 27 ºC. After the completion of fermentation (7 to 10 days) the drop in TSS and alcohol content in the broth were determined and the highest alcohol yielding yeast was selected and characterized by classical techniques described by Barnet *et al*. (1990) and Harrinag and McCance (1976).

4.1.2.4 Preparation of mold *koji*

Sterile wheat bran was inoculated with *R. oryzae ITCC No. 4408* , incubated at 30 ºC till full sporulation (4 – 6 days), dried at 40 ºC for 12 h (mold *koji*) and packed in glass bottle.

4.1.2.5 Preparation of defined fermentation starter

Alcoholic fermentation starter was prepared using *R. oryzae ITCC No. 4408* mold and yeast (*S. cerevisiae*) in wheat bran-rice flour mixture (25:75 m/m). One hundred gram of wheat bran-rice flour mix was moistened with water, autoclaved at 121 ºC for 20 min and cooled. The medium was inoculated with yeast and mold (from mold koji), mixed and incubated at 30 ºC for 3 days. The starter was dried at 40 ºC for 12 h, packed in glass bottle and kept in refrigerator.

4.1.2.6 Effect of fermentation containers and raw materials on chemical and sensory quality of fermented cereals

Finger millet (var. *Kabre*) was fermented using defined fermentation starter in plastic, wooden and earthen containers as described in 4.1.2.1 and their chemical and sensory qualities were analyzed. Similarly, different cereals viz., millet, rice, wheat and maize were fermented in plastic containers and their chemical and sensory qualities were analyzed. Effect of rice and wheat addition on the quality of fermented millet was also studied. For this, rice and wheat were mixed with finger millet in different proportions [100% millet, 80 % millet + 20% rice, 80% millet + 20% wheat and 80% millet + 10% rice + 10% wheat], cooked and fermented in plastic containers as described earlier and their chemical and sensory characteristics were analyzed.

4.1.2.7 Solid vs semi-solid fermentations of finger millet

Finger millet was prepared, cooked and bio-mass developed as described in 4.1.2.1 using defined starter (@ 0.5 g/100 g cooked millet). For solid state fermentation, the bio-mass developed millet was tightly packed in plastic containers, while for semi-solid state fermentations, bio-mass developed millet was filled into plastic containers and sterile cool distilled water was added at the rate of 50% v/m (semi-solid$_1$) and 100% v/m (semi-solid$_2$). Fermentation was carried out at 26 − 28 ºC for 15 days and analyzed for chemical composition and sensory quality.

4.1.2.8 Storage stability of fermented millet packaged in polyvinyl chloride (PVC) containers

Finger millet was fermented in plastic containers for 15 days at 26 − 28 ºC using defined starter. The fermented millet was filled into PVC containers (the containers were washed, rinsed with 1000 ppm KMS solution and dried at 60 ºC), sealed with aluminum foil lined cover and capped. The containers were randomly divided into two lots: one lot was kept in deep freezer at – 30 ºC (control) and the other lot was kept at 25 ± 2 ºC and 75 – 85 % RH for 90 days. Both samples were analyzed for chemical and sensory characteristics after 90 days of storage.

4.1.2.9 Clarification of millet *jand* using fining agents

4.1.2.9.1 Preparation of millet *jand*

Finger millet fermented at 29 ± 1 ºC for 10 days using defined starter was brewed using 1.5 parts of potable water (by vol) to each part of the fermented millet (by wt). The mass was gently agitated, held for 30 min at room temperature (27 ± 1 º C), and strained through a plastic net with gentle pressing.

4.1.2.9.2 Selection of fining agents

Different fining agents, viz., bentonite, gelatin, tannin and tannin-gelatin combination were tried for clarification. Bentonite was added at concentrations ranging from 0 to 4 g/L, whereas for gelatin, tannin and tannin - gelatin clarifications, they were used at concentrations ranging from 0 to 0.4 g/L. Bentonite was used as 5% solution (m/v) in distilled water and tannin was used as 1 % solution (m/v) in 50% ethanol (v/v). In the case of gelatin, 1 g of gelatin was mixed with 75 mL of distilled water, warmed till dissolved and volume made up to 100 mL after the addition of 0.5 g of citric acid and 0.1 g of sodium benzoate.

A measured volume (100 mL) of millet *jand* was taken in several 250 ml conical flasks and fining agents were added to each flask at different concentrations and mixed thoroughly. The flasks were heated rapidly to 70 ºC, the mouth of the flask was closed and placed in a water bath maintained at 70 ºC for 10 min followed by immediate cooling to room temperature in a ice-water bath. The flasks were kept for 24 h at 27 ± 1 ºC and the turbidity of the supernatant was measured. The one showing minimum turbidity (i.e. greater brilliancy) at the lowest concentration of fining agents was chosen. For tannin-gelatin combination treatment, tannin was added first followed by gelatin in equal amounts.

4.1.2.9.3 Effect of bentonite concentration, *jand* acidity, holding time and temperature on millet *jand* clarification

In order to study the effect of bentonite concentration and *jand* acidity, four levels of bentonite concentrastions (0, 1, 2 and 3 g/L) and three levels of acidity (0.35, 0.45 & 0.55 % as lactic acid) were used. The required acidity levels were adjusted using 10% (m/v) lactic acid solution or 1N NaOH solution as required. Clarification was carried out as described in 4.1.2.9.2.

In order to examine the effect of holding time on clarification, the bentonite treated (3 g/L) and pasterurized millet *jand* (70 ºC for 10 min) samples were kept at 29 ±1 ºC and the turbidity of the supernatants were measured daily till 4 days. Similarly, two levels of bentonite (0 and 3 g/L) and two levels of temperature (29 ± 1 ºC and 5 ± 1 ºC) were used to study the effect of bentonite concentration and holding temperature. The treated samples were held at both temperatures for 24 h and turbidities of the supernatants were measured.

4.1.2.9.4 Effect of the sequence of bentonite addition

The acidity of the *jand* was adjusted to 0.4 % using lactic acid solution and it was divided into three lots. The first lot was pasteurized without bentonite treatment (control), the second lot was pasteurized at 70 ºC for 10 min after adding bentonite (3 g/L) and the third lot was pasteurized, kept for overnight for settling and bentonite treated (3 g/L) to the supernatant. All the treated samples were kept for 24 h at 27 ± 1 ºC and the turbidities of the supernatants were recorded. The chemical and physical characteristics of unclarified and clarified finger millet *jands* (3 g/L bentonite treated and held for 24 h at 27 ± 1 ºC) were also determined.

4.1.2.10 Bio-chemical changes during finger millet fermentation

Finger millet was fermented using defined fermentation starter in plastic container (as outlined earlier) at 27 ± 1 ºC for 12 days. Microbial changes were studied at 2 days of interval till 10 days, while chemical changes were studied at 3 days of interval till 12 days. Volatile constituents were determined at 3 and 12 days of fermentation.

4.1.2.11 Quality comparison between finger millet fermented using defined starter and market samples

Fermented finger millet using defined starter and those collected from different places, viz., Dharan, Bhedatar Hile and Basantapur, were analyzed for chemical and sensory characteristics.

4.1.3 Analytical methods

4.1.3.1 Analysis of proximate composition

Moisture content was determined by oven drying method at 105 ± 5 ºC (Ranganna, 1986). Total nitrogen and crude protein content was determined by micro-kjeldaha method according to Ranganna (1986) using 1 g of powdered sample and protein

content was found out by multiplying the percent nitrogen by 6.25. Crude fat content was determined by petroleum ether extraction method as per AOAC Method 945.16 (2005). Ash content was determined by direct method as per AOAC Method 923.03 (2005) using 5 g of powdered sample and a furnace temperature of 550 ± 5º C. Total carbohydrate content was determined by difference.

4.1.3.2 Determination of mineral

Phosphorous was determined colorimetrically (Genesis UV-10, Thermospectrionic, USA) as per Kirk and Sawyer (1991). Iron, manganese, sodium, potassium, and zinc contents were determined using AAS (Thermo Elemental Atomic Absorption Spectrometer, SOLLAR, Model No. 969, 2004, UK). Briefly, powdered sample (2.5 g) was mixed with 3 ml conc HNO_3 and 2 mL conc HCl, digested on hot plate and volume made up to 100 mL with distilled water. The solution was filtered using Whatman filter paper Grade 41 and used for the determination.

4.1.3.3 Determination of alcohol and other volatiles

Alcohol distillation was carried out as per Kirk and Sawyer (1991). Briefly, 40 g of the fermented samples was mixed with 100 mL distilled water, neutralized with 1M NaOH and transferred into a distillation flask using 50 mL of water. The sample was distilled; collecting at least 95 mL of the distillate in 100 mL volumetric flask and volume made up to 100 mL. The alcohol content in the distillates was determined by visible spectrometric – dichromate oxidation method (Zoecklein *et al*., 1997). Total ester was determined as per Kirk and Sawyer (1991). Total, free, and combined aldehydes were determined as per AOAC Official Method 972.08 (2005). Methanol was determined by chromotropic acid (The British Drug House, England) colorimetric method as per AOAC Official Method 958.04 (2005). Fusel oil was determined by spectrophotometric method as per AOAC Official method 963.10 (2005).

4.1.3.4 Determination of total solids, insoluble solids, total soluble solids, sugars and turbidity

Total solid was determined by drying 25 mL of the *jand* in a vacuum oven at 70 ºC as per Ranganna (1986). Insoluble solid was determined by drying the residue obtained after filtration of 25 mL sample through Whatman No. 41 filter paper in a vacuum oven at 70 ºC. TSS was determined by hand refractometer (Hanna Instrument, Portugal).Total reducing sugar, sucrose, and total sugars were determined by Lane and

Eynon method as per Ranganna (1986). Glucose (Glucose oxidase method) (Span Diagnostics Ltd, India) and fructose contents were determined as per Sadasivam and Manickam (1996). Turbidity was determined by using turbidity meter, (LP 2000, Hanna Instrument, Portugal) and the results were expressed as Formazine Turbidity Units (FTU).

4.1.3.5 Determination of pH, acidities and conductivity

Ten gram of the sample was homogenized with 20 mL of carbon dioxide free distilled water for 1 min and the pH of the slurry was determined by a digital pH meter (Hanna Instrument, Portugal) at 26 ± 1 ºC. For acidity determination, 25 g of well-mixed sample was ground in a mortar and pestle with CO_2-free distilled water, volume made up to 250 ml with distilled water and filtered through Whatman No. 40 filter paper. Total-, fixed- and volatile acidities were determined using 20 mL of the filtrate as per Kirk and Sawyer (1991). Conductivity of millet *jand* was determined by conductivity meter (Hanna Instrument, Portugal)

4.1.3.6 Determination of water soluble protein and other nitrogenous components

Water soluble protein was determined by Lowry's method as per Sadasivam and Manickam (1996) and the result was expressed as bovine serum albumin (Central Drug House Pvt. Ltd, New Delhi, India). Nitrate and nitrite contents were determined as per Ranganna (1986). Non protein nitrogen was determined according to Sadasivam and Manickam (1996). Total free amino acid (TFAAs) was determined as per AOAC Official Method 945.30 (2005) and the result was expressed as gallic acid (s.d. fine chem. Ltd., India) equivalent.

4.1.3.7 Determination of starch, total phenolics and antioxidant activity

Starch content was determined by direct acid hydrolysis method as per AOAC Official Method 920.83 (2005). Five hundred milligram of the sample was extracted with 10 mL of 80 % ethanol for overnight at room temperature, centrifuged and the supernatant separated. The residue was re-extracted with 5 mL of 80% ethanol for 12 h, centrifuged, the supernatants pooled together, and volume made up to 25 mL with 80% alcohol. Total phenolic content was determined as per Sadasivam and Manickam (1996) with slight modifications. Briefly, 0.5 mL of the extract was diluted to 3 mL with distilled water in a test tube and added with 0.5 mL of 0.5 N phenol reagent followed by 2 mL of 20% Na_2CO_3. The mixture was incubated for 2 h at room

temperature, the intensity of blue color recorded in a spectrophotometer at 760 nm and the result expressed as gallic acid equivalent.

Antioxidant activity was determined by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Wako Japan, Lot EpL2139) method as per Sing *et al*. (2008) with slight modification. Briefly, 0.5 ml of the alcoholic extract and 3 ml of 0.003% methanolic solution of DPPH were mixed in a test tube, incubated at room temperature in the dark for 30 min and absorbance taken in a spectrophotometer at 517 nm. The instrument was adjusted to 100% T with methanol and a control was prepared using distilled water instead of the sample. Tannin content was determined as per Ranganna (1986) and the result was expressed as tannin acid (Merck Pvt. Ltd, Mumbai, India).

4.1.3.8 Determination of phytic acid and total oxalate

Phytic acid was determined as per Sadasivam and Manickam (1996). Total oxalate was determined by permanganate titration method as per AOAC Official Method 974.24 (2005)

4.1.3.9 Enumeration of yeast and mold counts

Yeast and mold were enumerated by pour plate technique using PDA media supplemented with 100 mg chloramphenicol and 0.5 ml of 5% rose bengal solution per liter of the medium as per Dubey and Maheshwari (2002).

4.1.3.10 Sensory evaluation

Sensory evaluation of the *jands* was carried out by using hedonic rating test as per Ranganna (1986). Millet *jand* was prepared by mixing 1 part of fermented cereal to each 1.5 parts of water (m/v). The mixture was gently mixed and allowed to stand for 30 min and then strained through a plastic net. Ten semi-trained panelists consisting of administrative staffs, faculties and students of the college were ask to rate the acceptability of the product on a 9 point scale ranging from 1 (‘dislike extremely’) to 9 (‘like extremely’) in terms of color, taste and smell of the product.

4.1.3.11 Statistical analysis

The experiment was conducted in a RCBD with three replications. Experimental data were analyzed by analysis of variance (ANOVA) and means were compared by LSD and t-test as per Buysse *et al*. (2007).

4.2 Assessment of brewing potential of Nepalese finger millet varieties

4.2.1 Materials

Six finger millet varieties (*GPU 0025*, *GE 5016*, *Dalle*, *Okhle* and *Kabre* varieties from Hill Crops Research Program, Dolakha, and *Juwain* millet from Khotang district of Nepal) were collected. Barely malt and hops pallets were obtained from Gorkha Brewery, Nawalparasi, Nepal. Brewing yeast (bottom fermenting) was obtained from Sungold Brewery, pvt. Ltd, Nawalparasi, Nepal.

4.2.2 Experimental methods

4.2.2.1 Preparation of finger millet malts

The finger millets were cleaned, washed thoroughly, and steeped in surplus water at 26 to 28 ºC for overnight. After soaking, the grain was drained, spread on aluminum tray (7 ± 1 mm bed thickness), covered with moistened muslin cloth and germinated for different times (48, 72 and 84 h) in a BOD chamber maintained at 28 ± 1 ºC and 93 ± 2 % RH. During germination, the millet was turned, sprayed with water and covered with wetted muslin clothe twice a day.

Fig 4.1 Germination of finger millet

Fig 4.2 Drying of germinated finger millets

The germinated millet (green malt) was kilned in a mechanical dryer (REICO Drying Chamber, India) at 50 ± 2 ºC for 24 h and rootlets were removed by rubbing and winnowing. The malts were analyzed for alpha-amylase, beta-amylase and carboxypeptidase activities; diastatic power (DP); total free amino acids (TFAAs); free amino nitrogen (FAN) and malt extracts. Deferent chemical characteristics of native (ungerminated) and malted (germinated) millets were also analyzed.

4.2.2.2 Extraction of enzymes

One gram of powdered sample was ground in a pestle and mortar with distilled water, volume made up to 50 mL, filtered through Whatman No. 1 filter paper and the filtrate was used as enzyme source for α- and β-amylase activity determination. For carboxypeptidase activity , 2 g of powdered malt was extracted with 20 mL of 0.1M citrate-phosphate buffer (pH 7.0), filtered through Whatman No 1 filter paper and volume made up to 25 mL with 0.1 M citrate-phosphate buffer.

4.2.2.3 Kilning of millet malts at different temperatures

Finger millet (var.*Kabre*) was soaked for 12 h, germinated for 48 h at 28 ± 1 ºC and kilned by three different methods as follows:

Method$_1$: The green malt was dried at a constant temperature of 50 ± 2 ºC up to a final moisture content of 7 ±1 %

Method$_2$: The green malt was first dried at 50 ± 2ºC up to 22% moisture and then at 62 ± 2ºC up to 7 ±1 % moisture content

Method$_3$: The green malt was first dried at 50 ± 2ºC up to 22% moisture and final drying at 80 ±2 ºC up to 7 ±1 % misture content.

The malts were analyzed for α- and β- amylase activities, FAN and malt extracts.

4.2.2.4 Gibberellic acid (GA_3) treatment during millet germination

Finger millets of different varieties were germinated for 34, 48, and 56 h at 28 ± 1 ºC. During germination, 5 ppm of gibberellic acid solution (Loba Chemie Pvt. Ltd., Mumbai, India) in water was sprayed on to the bed of finger millet twice a day. The green malts (germinated millets) were dried at 50 ± 2 ºC for 24 h and analyzed for amylase activities FAN contents and malt extract characteristics.

4.2.2.5 Mashing of finger millet malt by different methods

Finger millet (var. *Kabre*) was germinated for 48 h at 28 ± 1 ºC and kilned at 50 ± 2 ºC for 24 h. The malts were ground in a coffee grinder and mashed by three different methods as follows:

I. Infusion mashing at 70 ºC

One hundred gram of malt flour was mixed with 500 mL distilled water at 45 ºC and rested at this temperature for 45 min. The temperature was increased slowly at the rate of 1 ºC per min to 70 ºC and rested for 1 h.

II. Decantation mashing at 80 ºC

One hundred gram of malt flour was mixed with 500 mL distilled water at 45 ºC, the temperature increased to 45 ºC and rested for 45 min. Thereafter, the clear enzymic supernatant was removed from the mash and the remaining mash was heated to 80 ºC, held at this temperature for 30 min, and cooled below 50 ºC. The clear enzyme extract was added to the mash, stirred and the temperature raised to 70 ºC as above. After 1 h, the mash was heated to 75 ºC and rested for 10 min. Finally the mash was cooled, filtered and analyzed.

III. US mashing process

The malt was ground in a coffee grinder and mashed using malt flour to water ration of 1:5 (m/v) as per Matz (1991) with minor modifications as follows:

Step 1: Albumin (protein) rest: The mash temperature was slowly increased to 45 ºC and held at this temperature for 45 min.

Step 2: Sugar rest: The mash temperature was slowly raised to 59 ºC with constant stirring and held for 18 min.

Step 3: Dextrinizing period: The mash temperature was slowly raised to 65 ºC and held for 30 min.

Step 4: Conversion Period: The mash temperature was slowly raised to 73 ºC and held for 15 min, and

Step 5: Mashing off: The mash was heated to 80 ºC and held for 8 min.

After mashing, the mashes were cooled, filtered through Whatman No. 4 filter paper and the worts analyzed.

4.2.2.6 Mashing of finger millet (var. *Kabre*) malt in combination with commercial barley malt and mold bran

Finger millet (var. *Kabre*) malt prepared as described in 4.2.2.5 was mashed in combination with commercial barley malt (0, 20, 30, and 40 % by weight of malt) using U.S. mashing process as outlined in 4.2.2.5. Effect of mold bran addition (0, 2.5, and 5% by weight of malt) on the chemical properties of millet malt wort was studied. Mold bran was prepared by inoculating *R. oryzae*, ITCC No. 4408 in to sterile wheat bran and incubating at 30º C. During incubation, α- and β- amylase activities in the wheat bran were determined. After attaining maximum amylase activities, the molded wheat bran (mold bran) was dried at 40 ºC for 24 h, and used for mashing. The mashes were filtered through Whatman No. 4 and analyzed for their chemical properties.

4.2.2.7 Optimization of mashing process

4.2.2.7.1 Optimization of holding time at constant mashing temperature and pH

One part of millet malt flour was mixed with five parts of potable water and the pH was maintained between 5.5 − 5.7 using 10% lactic acid. The mashing was carried out using US mashing process in which the mash was held for 0 to 90 min at 45 ± 1 ºC (protein rest), for 10 to 60 min at 58 ± 1 ºC (sugar rest), for 15 to 60 min at 65 ± 1 ºC (dextrinizing period) and finally for 10 – 40 min at 73 ± 1 ºC (Conversion period). After mashing, the mash was filtered through Whatman No. 4 filter paper and analyzed for different chemical properties.

4.2.2.7.2 Optimization of mashing pH and temperature

Response surface methodology (RSM) was adopted in the design of experimental combinations (Montgomery, 2001). A two variables (three levels of each variable), face centered experimental design was employed. The independent variables considered were mashing temperature (x_1), and pH (x_2) for protein rest, dextrinizing rest, and conversion rest periods respectively. The three levels of the process variables were coded as -1, 0, and +1. To examine the combined effects of the two independent variables on mashing, a face centered cube design with 13 experiments were performed. For protein rest period, independent variables included were, mashing temperature, $x_{1,}$ and pH, x_2. For protein rest period, temperature (x_1) was varied in three levels, viz., 40, 50 and 60 °C. For dextrinizing rest period temperature was varied in three levels, viz., 62, 65 and 68 °C and for conversion period temperaturer was varied in three levels, viz., 70, 73 and 76°C. For all above cases, pH ($x_{2,}$) was varied at 3 levels, viz., 4.5, 5.25, and 6. Different levels of independent variables and their coded and uncoded values are shown in appendix A.

Statistical Analysis of Responses

The responses such as free amino nitrogen (FAN), total soluble solids (TSS), total reducing sugar (RS), glucose and fructose for different experimental combinations were related to the coded variables (x_i, i = 1 and 2,) by a second degree polynomial equation as given below:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 . x_2 + \varepsilon. \text{ ----- (4.1)}$$

The coefficients of the polynomial were represented by β_0 (constant), β_1, β_2, (linear effects); β_{12}, (interaction effects); β_{11}, β_{22}, (quadratic effects); and ε (random error). The experiments were designed using the software, Design Expert Version 6.0.10 trial version (State-Ease, Minneapolis, MN). The same software was used for statistical analysis of experimental data.

Analysis of Data

A complete second order quadratic model was employed to correlate the independent process variables. The second order polynomial coefficient for each term of the equation was determined through multiple regression analysis using design expert. Experimental data were fitted to the selected models and regression coefficients were obtained. Statistical significance of the terms in the regression equation was examined

by analysis of variance (ANOVA) for each response. The adequacy of regression model was checked by R^2, adjusted R^2, adequate precision, and Fisher's F-test (Montgomery, 2001). The significances of all terms in the polynomial were judged statistically by computing the F-value at 5% level of significance. In this mashing, the sugar rest period was not included, as it had no significant effect on TSS production. The response variables used were, TSS and FAN in protein rest period; TSS, total reducing sugar, glucose, fructose and FAN in dextrinizing period; and TSS, reducing sugar, glucose, fructose and FAN in conversion period. After mashing, the mashes were filtered through Whatman No. 4 filter paper and analyzed for the respective response variables.

4.2.2.8 Preparation of millet and barley beers and their quality analyses

4.2.2.8.1 Preparation of millet malt

Finger millet (var. *Kabre*) was germinated at 28 ± 1 ºC for 48 h and kilned by two methods.

Method 1: The green malt was dried at 50 ± 2 ºC for 24 h ($Malt_1$).

Method 2: The green malt was kilned at successively increasing temperature from 50 to 80 ºC as per Matz (1991) in three stages as follows:

First stage: The moisture content of green malt was reduced from 42 to 22% at 50 ± 2 ºC.

Second stage: The moisture content was further reduced to 12% at 70 ºC, and

Third stage: The moisture content was finally reduced to 6 ± 1% at 80 ºC

4.2.2.8.2 Preparation of beer

Mashing: Barley and millet malts were ground in a coffee grinder and mashed by US mashing process as outlined in 4.2.2.5 using malt flour to water ratio of 1:5 (m/v). Mash pH was adjusted between 5.5 and 5.7 using 10% lactic acid solution.

Wort filtration: The mashes were strained through a double-folded muslin cloth, boiled for 5 min and filled into glass jar. The jar was closed tightly, held for 10 min, cooled and kept for overnight. On the next day, the supernatant was siphoned off using a PE pipe and used for wort boiling.

Wort boiling: TSS of the wort was adjusted to 14 ºBx. using table sugar and the pH was adjusted to 5.5 ± 1 using 10% lactic acid solution or sodium bicarbonate solution as required. Hops was added at the rate of 250 ppm and boiled for 1 h. The wort was filled into glass jar, held for 10 min and cooled.

Fermentation: Bottom fermenting yeast was inoculated into a PDA (supplemented with 0.5 ml 5% aqueous rose bengal and 100 mg chloramphenicol per litre of medium) slant and incubated at 20 ºC for 3 days. A slant full of yeast was aseptically transferred into 500 mL sterile wort (10 % TSS and 5.5 − 5.7 pH) in a 1L conical flask, cotton plugged, mixed and incubated for 2 days at 25 ºC. The wort was pitched at the rate of 1.6×10^6 yeast cells/mL, cotton plugged, thoroughly mixed and kept for fermentation for 12 days at 25 ºC. The fermentation jar was fitted with air-lock system containing 1000-ppm KMS solution.

Racking: After the completion of fermentation, the clear beer (green beer) was racked using PE pipe into glass jar (the glass jar was sterilized with boiling water) up to the brim, closed tightly, and kept under refrigeration for 15 days. The beer was again racked, pasteurized in bulk at 60 ºC for 20 min, cooled, filled into sterile bottles and stored under refrigeration. The chemical, physical and sensory properties of beers were analyzed.

4.2.2.9 Changes during millet and barley beer fermentation

Millet and barley beer fermentation was carried out as described in 4.2.2.8 and changes during fermentation were studied at 3 days of interval till 9 days.

4.2.3 Analytical methods

4.2.3.1 Determination of enzyme activities

4.2.3.1.1 Determination of α-amylase activity

Alpha-amylase activity was determined as per Mallik and Singh (1980). Briefly, a reaction mixture containing 2 mL of starch solution (150 mg starch, 600 mg KH_2PO_4, 20 mg anhydrous $CaCl_2$ dissolved in 100 mL distilled water, boiled for 1 min, cooled and filtered) and 1 mL of diluted enzyme was mixed in a test tube and incubated at 40 ºC for 30 min. The reaction mixture and enzyme were brought to 40 ºC before mixing. At zero and 30 min of incubation, 0.2 mL of the aliquot of reaction mixture was mixed with 3 mL of IKI solution (254 mg iodine and 4 g KI dissolved in 1 L of water) and

absorbance was measured at 620 nm using a digital spectrophotometer (M. S. Electronics, Pvt., Ltd, India, model 305). The instrument was adjusted to 100 % transparency with distilled water and enzyme activity was expressed in terms of decrease in optical density at 620 nm per min. per gram of dry malt.

4.2.3.1.2 Determination of β-amylase activity

Beta-amylase activity was determined as per Mallik and Singh (1980). Briefly, 1 mL of starch solution (1% in 0.067 M phosphate buffer, pH 6), 1 mL of undiluted enzyme extract and 1 mL of 0.1M EDTA were mixed in a test-tube and incubated at 37 ºC for 30 min (the substrate and enzyme were brought to 37 ºC before mixing) and the reducing sugar contents (as maltose) in the control (0 min incubation) and sample (30 min incubation) were determined by Nelson-Somogyi method as per Sadasivam and Manickam (1996) using a standard curve prepared from maltose sugar (Sisco Research Lab Pvt., Ltd., Mumbai, India)). Enzyme activity was expressed in terms of mg maltose produced per g of dry malt over 30 min of incubation.

4.2.3.1.3 Determination of total diastatic activity

Malts were ground in a coffee grinder and used for diastatic activity determination by ferricyanide modification method as per AOAC Official Method 935.31 (2005) using extra pure soluble starch (Merck Ltd., Mumbai, India).

4.2.3.1.4 Determination of carboxypeptidase activity

Carboxypeptidase activity was determined as per Okolo and Ezeogu (1995) with slight modifications. Two mL of the enzyme extract and 2 mL of 2.5 mg/mL bovine serum albumin (BSA) (Central drug house, Pvt. Ltd., India) in 0.1 M citrate-phosphate buffer, pH 7, were pipetted in a test tube, mixed and incubated for 1 h at 40 ºC after which the reaction was terminated by adding 2 mL of 15 % TCA. The mixture was centrifuged at 3000 rpm (Remi lab centrifuge, India), the supernatant separated and the residue washed with 2 mL of TCA followed by centrifugation. The supernatants were pooled together and the final volume made up to 10 mL with distilled water. Free amino nitrogen (FAN) content in the filtrate was determined and carboxypeptidase activity was calculated as the difference between the FAN (glycine equivalent) contents (µg FAN/h. g dry malt) of the filtrate from 1 and 0 h incubated reaction mixtures.

4.2.3.2 Determination of chemical characteristics of native and malted millets

The sample was extracted with 80 % alcohol as outlined in 4.1.3.7. Total free amino acids and FAN contents were determined from alcoholic extract as per AOAC Official Method 945.30 (2005). A portion of the alcoholic extract (10 mL) was evaporated on a water bath, the residue dissolved with 25 mL of distilled water and used for the determination of total reducing sugar, glucose, total phenolics, antioxidant activity, and flavonoids contents. Total reducing sugar was determined by Nelson-Somogyi method and glucose by glucose oxidase method using glucose oxidase peroxidase reagent (Span Diagnostics Ltd., India) as per Sadasivam and Manickam (1996). Total phenolics, antioxidant activity, starch and tannin contents were determined as described in 4.1.3.7. Total flavonoids was determined as per Shen *et al*. (2009) and the results were expressed as rutin (Loba Chemie Pvt., Ltd, Mumbai, India) equivalent. Amylose and amylopectin contents were determined as per Sadasivam and Manickam (1996).

4.2.3.3 Analysis of proximate composition

Moisture content was determined by air oven method as per AOAC Method 925.10 (2005) by drying 2 g of powdered sample for 1 h at 130 ±1 ºC. Crude protein, crude fat, total ash and total carbohydrates were determined as described in 4.1.3.1.

4.2.3.4 Determination of minerals contents

Calcium and magnesium contents were determined by titrimetric methods as per Ranganna (1986). Phosphorous, iron, manganese, sodium, potassium and zinc contents were determined as outlined in 4.1.3.2.

4.2.3.5. Analysis of malt extract, wort and beer

Specific gravity was determined by pycnometer method (50 mL capacity) as per AOAC Official Method 920.50 (2005) at 20 ºC. Viscosity was determined by viscometer method using Ostwald viscometer at 20 ºC as per AOAC Official Method 974.07(2005). Extract (º Plato) and extract yield of malt (% dry basis) was determined by reference to specific gravity value. Color measurement was done according to EBC Method 4.7.1 (1998) and the result was expressed as EBC units. TSS was determined using hand refractometer (Hanna Instrument, Portugal). Turbidity was determined using turbidity meter (Hanna Instrument, Portugal) at 26 °C. pH was determined by digital pH meter (Hanna Instrument, Portugal). Starch iodine test was performed by

mixing one drop of 0.01M I_2 solution with 2 drops of extract. Filtration rate was tested by filtering the mash through Whatman No. 4 filter paper.

Fructose and glucose were determined as per Sadasivam and Manickam (1996). Total reducing sugar, sucrose and total sugar were determined as per Ranganna (1986). Dextrin content was determined as per AOAC Official Method 920.52 (2005). Formol nitrogen was determined as per Zoecklein *et al.* (1995). Real and apparent extracts were determined as per AOAC Official Method 945.09 (2005). Original extract was determined as per AOAC Official Method 935.20 (2005). Real degree of fermentation was determined as per AOAC Official Method 950.06 (2005). Water-soluble protein was determined as described in Sadasivam and Manickam (1996). Formaldehyde was determined as per Jendral *et al.* (2011). Alcohol, total esters, total aldehydes, fusel oil and methanol contents were determined as outlined in 4.1.3.3. Vicinal diketones (VDK) was determined as per EBC Method 9.24.1(2005). Total-, fixed- and volatile acidities were determined as per Kirk and Sawyer (1991).

4.2.3.6. Sensory evaluation

Sensory evaluation of the beer was carried out by using hedonic rating test as per Ranganna (1986). Ten semi-trained panelists consisting of administrative staffs, faculties and students of the college were asked to rate the acceptability of the product on a 5 − point scale. (1 = poor, 2 = fair, 3 = satisfactory, 4 = good and 5 = Excellent) in terms of taste, smell, color, flavor, body and overall acceptability of the beer.

5. Results and discussion

5.1 Fermentation of finger millet using defined fermentation starter

5.1.1 Effect of fermentation containers and cereals on chemical and sensory quality of fermented finger millet

5.1.1.1 Effect of fermentation containers

The chemical characteristics of finger millet fermented using defined starter for 15 days at 26 ± 2 °C in plastic, wooden, and earthen containers were analysed and the results are shown in Table 5.1.

Table 5.1 Effect of fermentation container on chemical characteristics of fermented millet

Parameters	Values for different containers*		
	Plastic	Wooden	Earthen
Moisture content (% m/m)	75.43 [a] (0.74)	76.42 [a] (0.75)	75.23 [a] (0.27)
Total soluble solids (° Bx)	11.96 [a] (0.92)	9.70 [b] (0.61)	8.39 [b] (0.62)
Total acidity as lactic (% m/m)	1.05 [a] (0.09)	1.10 [a] (0.13)	1.58 [b] (0.32)
Fixed acidity as lactic (% m/m)	0.94 [a] (0.08)	1.00 [a] (0.14)	1.18 [a] (0.28)
Volatile acidity as acetic (% m/m)	0.075 [a] (0.006)	0.069 [a] (0.001)	0.296 [b] (0.014)
Alcohol (% v/m)	15.81 [a] (0.66)	14.21 [a] (0.54)	11.21 [b] (1.23)
Total esters (g ethyl acetate /L alc)	0.787 [a] (0.140)	0.912 [a] (0.075)	1.831 [b] (0.427)
Total aldehydes (g acetaldehyde/ L alc)	0.200 [a] (0.006)	0.577 [b] (0.01)	0.850 [c] (0.071)

*values are the means of three replications. Figures in the parentheses are the standard deviations. Means followed by similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

The moisture content of fermented millet was in the range of 75.23 to 76.42% m/m. Statistical analysis revealed that fermentation container had no significant effect ($p>0.05$) on moisture content of fermented millet. Millet fermented in plastic container had significantly higher ($p<0.05$) total soluble solids (TSS) than those of wooden and earthen whereas the values between the latter two did not differ. Both the total and volatile acidities were higher in millet fermented using earthen container compared to plastic and wooden containers but the values between the latter two did not differ significantly ($p>0.05$). No significant difference in the fixed acidity was found among the three samples.

Higher amounts of total and volatile acidities in the earthen container could be due to the incorporation of air through the pores of the container during fermentation. Alcohol contents between plastic (15.81% v/m) and wooden (14.21% v/m) containers were not different but it was significantly lower (11.21% v/m) in earthen container. Lower alcohol content in earthen container could partly justify for higher total and volatile acidities owing to the oxidation of alcohol. Millet fermented in earthen container had the highest total ester content (1.831 g ethyl acetate /L alc) of all the containers used but the values between plastic and wooden containers were statistically similar. Total aldehyde contents were found to be 0.20, 0.577 and 0.850 g acetaldehyde/L alc) in millet fermented using plastic, wooden and earthen containers respectively and the values were significantly different ($p<0.05$) from each other. Effect of fermentation containers on the sensory quality of millet *jand* is depicted in Fig. 5.1.

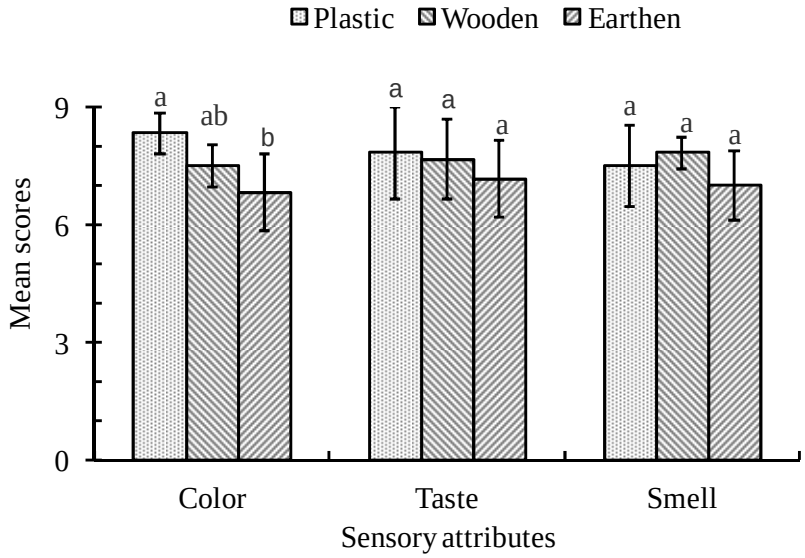


Fig. 5.1 Effect of fermentation containers on the sensory quality of finger millet *jands*.Values are the means (n=10) ± SD. Bars having different letters for any quality attributes are significantly different ($p<0.05$) by LSD.

The mean sensory scores for colour, taste and smell, out of a total possible score of 9, were found to be 8.33, 7.50 and 6.83; 7.83, 7.67 and 7.17; and 7.50, 7.83 and 7.00 for millet fermented in plastic, wooden and earthen containers respectively. Statistical analysis showed that fermentation container had a significant effect on colour, whereas taste and smell were not affected. LSD indicated that the mean colour scores between plastic and wooden and between wooden and earthen containers did not differ, but they were different between plastic and earthen containers.

Plastic containers are easy to clean and handle compared to wooden and earthen ones. Results of chemical and organoleptic analyses of fermented millets revealed that no remarkable improvement on the chemical and sensory quality was found using wooden and earthen containers over the plastic container. Hence, plastic containers could be regarded as an appropriate container for millet fermentation.

5.1.1.2 Effects of raw materials on the chemical and sensory quality of *jands*

Jands were prepared from fermented millet, rice, maize, and wheat by mixing 1 part of fermented cereal to each 1.5 parts of water and subjected for chemical and sensory analyses. Chemical characteristics of different j*ands* are shown in Table 5.2. *Jand* yield was found in the order of rice>maize>wheat>millet. Wheat *jand* had the highest TSS (3.97 ºBx) followed by millet (3.27 ºBx), while maize had the lowest TSS of 2.03 ºBx which was statistically similar to that of rice (2.10 ºBx). Raw materials showed a significant effect ($p<0.05$) on the pH of *jand*. Wheat *jand* had the maximum pH (4.67) whereas a minimum pH of 3.90 was recorded in rice *jand*. The pH values between millet and maize and between rice and maize *jands* were statistically not different. Total acidity was found to be 0.33, 0.24, 0.26, and 0.41% m/v (as lactic acid) in millet, rice, maize, and wheat *jands* respectively. Statistically, wheat resulted the highest total acidity of all the cereals. Millet *jand* had higher total acidity compared to rice and maize but no significant difference was found between the latter two samples.

A similar pattern to that of total acidity was also observed for fixed acidity but no significant difference in volatile acidity was found among the four *jand* samples. Moisture contents of rice (98.44% m/v) and maize (98.30% m/v) *jands* were significantly higher ($p<0.05$)) compared to millet (96.33% m/v) and wheat (95.93% m/v) but the values between the former two and between latter two samples were not different. Total ester contents (as ethyl acetate) were found to be 10.6, 15.9, 26.5 and 31.1 mg% (m/v) in millet, rice, maize, and wheat *jands* respectively and the values were significantly different from each other. The ester content of wheat *jand* was about 3-folds that of millet and 2- folds that of rice *jands*. Alcohol content was in the range of 5.53 to 6.20% (v/v) but the values were statistically not different among four *jand* samples. Analogous results of pH (3.8 – 4.1) and volatile acidity (0.03 – 0.06%, m/v as acetic acid) but a higher total acidity (0.4 – 0.6%, m/v as lactic acid) and lower alcohol content (2.5 – 3 % v/v) compared to those obtained in this study were reported by Venkataramu and Bassapa (1993) in finger millet *chhang*.

Table 5.2 Chemical characteristics of *jands* prepared from different cereals

Parameters	Values for different fermented cereals*			
	Millet	Rice	Maize	Wheat
Jand yield (%, v/m)	182.33^{a} (3.06)	233.73^{b} (7.34)	221.23^{c} (1.25)	202.70^{d}(6.95)
TSS (^{o}Bx)	3.27^{a} (0.06)	2.10^{b} (0.17)	2.03^{b} (0.06)	3.97^{c} (0.06)
pH	4.17^{a} (0.12)	3.90^{b} (0.17)	4.10^{ab} (0.1)	4.67^{c} (0.12)
Total acidity as lactic acid (%, m/v)	0.33^{a} (0.01)	0.24^{b} (0.01)	0.26^{b} (0.01)	0.41^{c} (0.02)
Fixed acidity as lactic acid (%, m/v)	0.26^{a} (0.02)	0.21^{b} (0.01)	0.22^{b} (0.01)	0.37^{c} (0.03)
Volatile acidity as acetic acid (%, m/v)	0.048^{a} (0.025)	0.020^{a} (0.001)	0.027^{a} (0.007)	0.025^{a} (0.004)
Moisture content (%, m/v)	96.33^{a} (1.07)	98.44^{b} (0.31)	98.30^{b} (0.31)	95.93^{a} (0.33)
Ester content (mg %, m/v)	10.6^{a} (0.85)	15.9^{b} (0.8)	26.5^{c} (0.53)	31.1^{d} (0.77)
Alcohol content (%, v/v)	6.20^{a} (0.75)	5.86^{a} (0.59)	5.53^{a} (0.41)	6.14^{a} (0.9)

*values are the means of three replications. Figures in the parentheses are the standard deviations. Means followed by different superscripts in a row are significantly different ($p<0.05$) by LSD.

Similar results of pH and alcohol contents in millet, rice, maize and wheat *jands* prepared using fermented cereal and water in the ratio of 1:1 (m/v) were also reported by Upadhyaya (2005a), while total-, fixed- and volatile acidities and TSS were reported to be higher than those found in this study. Total ester contents found in this study were higher than those reported by Upadhyaya (2005a) and Rai (2006). Berry and Watson (1987) reported that total ester contents below 200 mg/L may be desirable but above this level appears to give a spoiled character to the wine. Amerine and Cruess (1960) reported that ethyl acetate levels of 150 – 200 mg/L of wine impart spoilage character to the wine. Based on the available literature, millet and rice *jands* seemed to contain total ester within the acceptable level, while those of maize and wheat *jands* had a bit higher ester contents.

The results of sensory evaluation of different *jand* samples are shown in Fig. 5.2. The mean color scores for millet, rice, maize and wheat *jands*, out of a total possible score

of 9, were 7.25, 7.50, 6.00 and 5.75 respectively. Statistically, the colour scores between millet and rice and between maize and wheat *jands* were similar but the colour scores of millet and rice j*ands* were significantly higher than those of maize and wheat samples. The mean sensory scores for taste were 7.5, 8.63, 4.75, and 6.63 for millet, rice, maize and wheat *jands* respectively and the values were significantly different from each other. Maize *jand* had the least taste preference (rated as dislike slightly) while rice had the highest taste preference (rated as like very much). The mean smell scores were 7.37, 8.13, 4.88, and 6.75 for *jands* made from millet, rice, maize, and wheat respectively. Statistically, the effect of cereals on the smell of *jand* was significant and the mean scores were significantly different from each other. Again, maize *jand* had the least smell preference whereas rice *jand* had the highest smell preference.

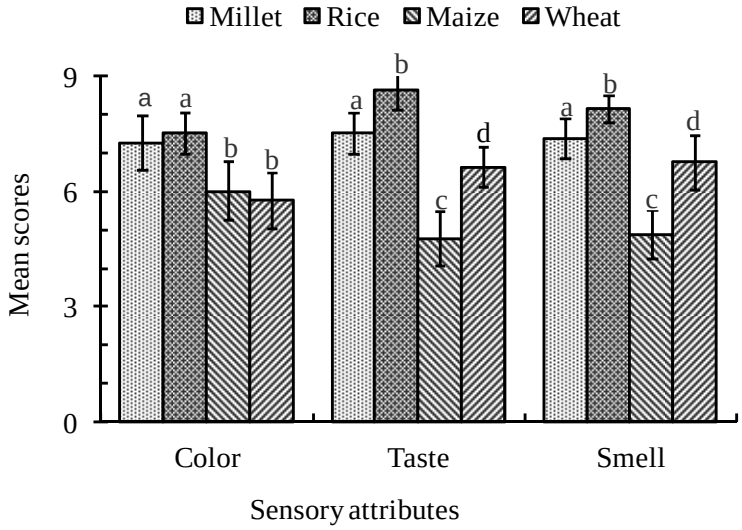


Fig. 5.2 Effect of cereals on the sensory quality of *jand*. Values are the means (n=10) ± SD. Bars having different letters for any quality attributes are significantly different ($p<0.05$) by LSD.

5.1.1.3 Effect of cereal combinations on the chemical and sensory characteristics of fermented cereals

Four lots of fermented millet samples were prepared by using millet alone [M], millet (80%) + rice (20%) [MR], millet (80%) + wheat (20%) [MW] and millet (80%) + rice (10%) + wheat (10%) [MRW] and analyzed for chemical and sensory quality of the fermented millets. Effects of cereal combinations on chemical characteristics of fermented millet are shown in Table 5.3.

Table 5.3 Effects of cereal combinations on the chemical characteristics of fermented cereals

Parameters	Values for different cereal combinations*			
	M	MR	MW	MRW
Moisture (% m/m)	70.78^{a} (0.41)	72.66^{b} (0.71)	81.16^{c} (0.66)	71.91^{ab} (1.56)
TSS (ºBx)	12.07^{a} (0.90)	14.60^{b} (0.46)	9.67^{c} (0.12)	14.10^{b} (0.3)
Total acidity as lactic acid (% m/m)	1.01^{a} (0.02)	0.89^{a} (0.02)	1.08^{a} (0.07)	1.03^{a} (0.01)
Fixed acidity as lactic acid (% m/m)	0.97^{a} (0.02)	0.81^{a} (0.04)	0.95^{a} (0.12)	0.87^{a} (0.11)
Volatile acidity as acetic acid (% m/m)	0.037^{a} (0.005)	0.044^{a} (0.019)	0.043^{a} (0.011)	0.079^{b} (0.009)
Alcohol (% v/m)	14.11^{a} (0.10)	14.19^{a} (0.93)	14.12^{a} (0.10)	14.41^{a} (0.72)
Ester as ethyl acetate (g/L alc)	0.863^{a} (0.168)	0.814^{ac} (0.103)	0.431^{b} (0.068)	0.651^{c} (0.061)
Aldehydes as acetaldehyde (g/L alc)	0.698^{a} (0.053)	1.545^{b} (0.224)	0.901^{a} (0.078)	1.275^{b} (0.169)
Fusel oil (g/L alc)	6.277^{a} (0.395)	9.370^{b} (0.582)	5.297^{c} (0.325)	7.335^{d} (0.673)
Methanol (g/L alc)	2.346^{a} (0.162)	2.873^{a} (0.444)	3.858^{a} (0.361)	3.839^{a} (0.261)
Reducing sugar as dextrose (% m/m)	5.98^{a} (0.18)	10.01^{b} (0.55)	2.04^{c}(0.02)	7.48^{d} (0.17)
Sucrose (% m/m)	1.62^{a} (0.25)	0.78^{b} (0.44)	0.82^{b} (0.01)	1.34^{a} (0.05)
Total sugar (% m/m)	7.62^{a} (0.09)	10.8^{b} (0.31)	2.86^{c} (0.03)	8.82^{d} (0.22)

*values are the means of three determinations. Figures in the parentheses are the standard deviations. Means followed by different superscripts in a row are significantly different ($p<0.05$) by LSD.

Sample notation

M = 100% millet; MR = 80% millet + 20% rice;

MW = 80% millet + 2% wheat, MRW = 80% millet + 10% rice + 10% wheat.

Fermented mash containing 80% millet + 20% wheat (MW) had significantly higher moisture content of all the cereal combinations but the values between fermented mashes containing 80% millet + 20% rice (MR) and 80% millet + 10% rice + 10% wheat (MRW) were statistically not different ($p>0.05$). Sample MR had significantly higher moisture content than that of sample M (100% millet). From Table 5.3 it can be

seen that finger millet substituted with 20% of either rice or wheat significantly increased the moisture content of fermented cereals compared to control (100% millet). Cereal combinations showed a significant effect ($p<0.05$) on the TSS of fermented mashes. Millet substituted with 20% rice resulted the highest TSS (14.60 ºBx) while fermented millet containing 20% wheat had the lowest TSS (9.67 ºBx) of all the samples. It was found that addition of rice at both levels (10 and 20%) significantly increased the TSS compared to control, whereas the TSS of samples MR and MRW were statistically not different. Total and fixed acidities were in the range of 0.89 – 1.08 and 0.81 – 0.97%, m/m (as lactic acid) respectively, but the values were statistically not different ($p>0.05$) indicating that cereal combination did not have a significant effect on total and fixed acid contents. Millet substituted with 10% each of wheat and rice (MRW) had the highest volatile acidity (0.079%, m/m as acetic acid) of all the samples but the values among the rest three samples were statistically not different.

Alcohol contents of fermented cereals were 14.11, 14.19, 14.12, and 14.41% (v/m) for samples M, MR, MW and MRW respectively, however, the values were statistically not different. Cereal combinations showed a significant effect on total ester content with a maximum value of 0.863 g/L alc (as ethyl acetate) in 100% miilet , while a minimum value of 0.431 g/L alc was found in fermented millet substituted with 20% of wheat. From Table 5.3 it is apparent that 20% wheat addition reduced the ester content by half, whereas addition of rice alone did not affect ester content compared to control (100% millet). Increasing wheat content from 10 to 20% decreased the total ester content by about 34% in the fermented millet. Total aldehyde content was significantly affected by cereal combinations. Fermented millet without cereal substitution (M) had the lowest total aldehyde content (0.698 g acetaldehyde /L alc), while a maximum value of 1.545 g/L alc was found in fermented millet containing 20% rice. LSD indicated that the values of total aldehyde contents between samples M and MW and between MR and MRW were statistically not different. Incorporation of rice significantly increased the aldehyde content suggesting that aldehyde formation was favored by the presence of rice (Table 5.3).

Fusel oil content in the fermented mash was significantly influenced by cereal combination and increased with increase in the proportion of rice, while a reverse was found in the case of wheat where fermented millet containing 20% wheat had the lowest fusel oil content (5.297 g/L alc) of all the treatment combinations. Methanol

content in the fermented mashes was in the range of 2.346 – 3.858 g/L alc with an average value of 3.229 g/L alc; however, the values were statistically not different. There was a wide variation in the total reducing sugar contents among four samples with a minimum value of 2.04% (m/m) as dextrose in 20% wheat incorporated sample and a maximum value of 10.01% in 20% rice substituted sample. LSD showed that addition of rice at both levels (10 and 20%) significantly increased ($p<0.05$) the reducing sugar contents over the control (100% millet). Furthermore, 20% addition of rice nearly doubled the reducing sugar content, while 20% addition of wheat reduced the reducing sugar content by half to that of control. Reducing sugar content in the fermented mash was found to be largely dependent on the extent of rice incorporation.

Total sugar content in the fermented cereal also followed similar trend to that of reducing sugar. Addition of both rice and wheat at 20% significantly decreased the sucrose content in the fermented mash over the control but the values between the former two samples were statistically not different. Similarly, sucrose contents between samples M (1.62%, m/m) and MRW (1.34%, m/m) were not different.The obtained values of methanol contents were analogous to that reported in wines (2 g/L alc) but were very low compared to fruit brandy (10 g/L alc) as reported by Kirk and Sawyer (1991). Kirk and Sawyer (1991) reported that the minimum and maximum fusel oil contents in different brandy samples were 1.46 and 7.82 g/ L alc respectively, which agreed to our findings. According to Cronk *et al.* (1979) fusel oil content in rice fermented at 30 ºC for 96 h using *Amylomyces rouxii* in combination with various yeasts was in the range of 4.714 – 6.740 g/L alc which is in agreement with our results.

Except in fermented millet (M), all other fermented cereals had higher total aldehyde than those reported in brandy samples (0.24 – 0.76 g/L alc as acetaldehyde). The obtained values of total esters in our study were lower than those reported in brandy (1.12 – 5.80 g/L alc as ethyl acetate) (Kirk & Sawyer, 1991). Cronk et al. (1979) also reported that total ester contents in rice fermented using *Amylomyces rouxii* in combination with *Hansenula anomala* and *H. subpelliculosa* at 30 ºC for 96 h were 26.843 and 21.548 g/L alc as ethyl acetate respectively which were quite higher than those obtained in this study. Effects of cereal combinations on the sensory quality of *jands* is depicted in Fig. 5.3. Mean sensory scores for colour, out of a possible total score of 9, were 7.86, 8.0, 7.29 and 7.14 for *jands* prepared from samples M, MR, MW and MRW respectively, however, the values were statistically not different indicating

that cereal combination had no significant effect ($p>0.05$) on the colour preference of *jand*. Mean sensory scores for taste were found to be 8.71, 8.14, 6.71, and 6.86 respectively for samples M, MR, MW, and MRW respectively.

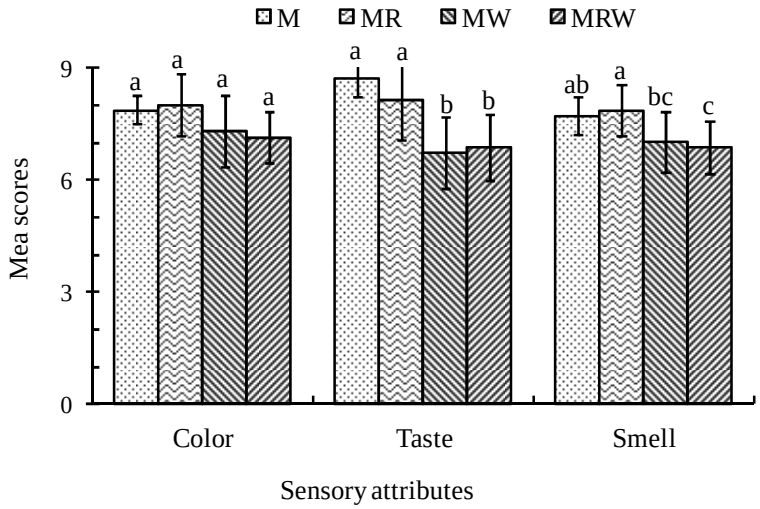


Fig. 5.3. Effect of cereal combinations on the sensory quality of millet *jand*. Values are the means (n=10) ± SD. Bars having different letters for any quality attributes are significantly different ($p<0.05$) by LSD.

Statistical analysis showed that cereal combinations had a significant effect ($p<0.05$) on the taste preference of *jand*. LSD indicated that the mean taste scores between samples M and MR (liked very much) and between MW and MRW (liked slightly) were not different, while the former two samples had higher taste scores compared to the latter two. Incorporation of wheat at either levels (10 and 20%) significantly decreased the taste preference of *jand*. Similarly, mean smell scorers were 7.71, 7.86, 7.0, and 6.86 for samples M, MR. MW, and MRW respectively. Statistically, the scores between samples M and MR and between MW and MRW did not differ. Similarly, *jands* prepared from samples M and MW were equally preferred based on smell. From Fig. 5.3 it revealed that addition of wheat significantly reduced the smell preference of millet *jand*.

5.1.2 Solid versus semi-solid fermentations of finger millet (*Eleusine coracana*)

5.1.2.1 Effect of solid and semi-solid fermentations on chemical characteristics of millet *jand*

The effects of solid and semi-solid state fermentations using defined fermentation starter on the physico-chemical characteristics of millet *jand* were analyzed and the results are shown in Table 5.4.

Table 5.4 Effect of solid and semi-solid fermentations on the chemical characteristics of finger millet *jand*

Parameters	Values*		
	Solid state	Semi-solid$_1$	Semi-solid$_2$
Moisture (% m/v)	96.33 ^{a} (1.07)	96.21 ^{a} (0.18)	96.28 ^{a} (0.23)
Total soluble solids (^{o}Bx)	3.27 ^{a} (0.06)	3.87 ^{b} (0.23)	4.03 ^{b} (0.06)
pH	4.17 ^{a} (0.12)	3.83 ^{b} (0.23)	3.37 ^{c} (0.12)
Total acidity as lactic acid (% m/v)	0.33 ^{a} (0.01)	0.72 ^{b} (0.01)	1.24 ^{c} (0.01)
Fixed acidity as lactic acid, (% m/v)	0.26 ^{a} (0.02)	0.50 ^{b} (0.001)	1.00 ^{c} (0.01)
Volatile acidity as acetic acid (% m/v)	0.048 ^{a} (0.025)	0.143 ^{b} (0.001)	0.156 ^{b} (0.002)
Alcohol (% v/v)	6.20 ^{a} (0.72)	7.13 ^{a} (0.3)	6.91 ^{a} (0.17)
Reducing sugar as dextrose (mg % m/v)	1582 ^{a} (128.1)	71 ^{b} (6.5)	69 ^{b} (5.1)
Esters, as ethyl acetate (mg/100 mL)	10.60 ^{a} (0.9)	13.30 ^{b} (0.7)	18.50 ^{c} (0.4)

*values are the means of three determinations. Figures in the parentheses are the standard deviations. Means followed by different superscripts in a row are significantly different ($p<0.05$) by LSD. Semi-solid$_1$ and Semi-solid$_2$ are the semi-solid state fermentations using 50 and 100% water addition respectively to the biomass developed millet during alcoholic fermentation.

Semi-solid state fermentations showed significant effects ($p<0.05$) on total soluble solids (TSS), pH, acidities, ester and reducing sugar contents of the finger millet *jands* (millet brews), whereas alcohol and moisture contents were not affected (Table 5.4). The average moisture contents of millet *jands* prepared by solid- and semi-solid state fermentations lied in the range of 96.21 to 96.33% (m/v) and the values were not significantly different ($p>0.05$) from each other. The TSS values were 3.27, 3.87, and 4.03 ^{o}Bx for solid state, semi-solid$_1$ and semi-solid$_2$ fermented *jands* respectively. Semi-solid fermentations incurred significantly higher TSS compared to control (solid

state); however, the values between semi-solid fermentations were not different. The pH decreased with increasing water addition and the values were significantly different from each other. Semi-solid fermentations significantly increased ($p<0.05$) the total acidity of millet brews compared to control. Millet fermented with 50% water addition had total acidity more than 2 times that of solid-state fermented one. Similarly, increasing water level increased the fixed acidity of millet *jands*. Furthermore, every 50% increase in water addition nearly doubled the fixed acidity of the millet *jand*. The volatile acidity of millet *jands* prepared by semi-solid state fermentations were significantly higher compared to control, whereas the values between semi-solid fermentations did not differ significantly. From Table 5.4 it can be envisaged that semi-solid fermentation had a profound effect on volatile acidity than those of total and fixed acidities.

The alcohol contents in the *jands* prepared from solid, semi-solid$_1$ and semi- solid$_2$ fermentations were 6.20, 7.13 and 6.91% (v/v) respectively, but the values were not statistically different. Reducing sugar contents decreased ($p<0.05$) with increase in water addition during alcoholic fermentation. Solid-state fermented *jand* contained higher reducing sugar than those of semi solid$_1$ and semi solid$_2$ fermented ones, however, the values between the later two were not significantly different. The lower reducing sugar contents in semi-solid fermentations could not be explained due to the lack of relevant information. The ester contents were 10.6, 13.3, and 18.5 mg ethyl acetate/100 mL in solid, semi-solid$_1$ and semi-solid$_2$ fermentations respectively and the values were significantly different from each other.

Due to the lack of relevant information, the results of this study could not be compared. However, similar result of alcohol content (6.8%, v/v) but higher values pH and total acidity were reported by Subba (1985) in traditionally prepared millet *jand*. According to Mongar and Rai (2005) the pH, total acidity (%, m/v as lactic acid), reducing sugar (%, m/v as dextrose) and alcohol (%, v/v) contents in millet *jands* fermented by using *murcha* were 4.5, 0.6, 1.52 and 4.03 respectively. The reported values of pH and reducing sugar contents seemed comparable, the acidity were quite higher, while alcohol contents were lower than those obtained for solid-state fermented millet.

Osti (2004) reported a similar alcohol content (6.68%, v/v) in millet *jand* fermented using starter made from *Rhizopus* and *Saccharomyces* spp. Cai and Nip (1990) reported analogous effect of semi-solid state fermentation on glucose in taro fermentation using

starter containing R. *tankinensis, R. oryzae, R. chinensis* and *S. cerevisiae,* but contrary to our finding, they reported a significantly higher yield of alcohol in semi-solid fermentation than that of solid state fermentation. Similar results of pH, and total acidity, but a lower value of alcohol (4.8%, v/v) in traditionally prepared *jands* were also reported by Thapa and Tamang (2004).

5.1.2.2 Effect of solid and semi-solid state fermentations on sensory quality of millet *jands*

Millet *jands* prepared by solid and semi-solid state [50% water addition, (semi-solid$_1$) and 100% water addition (semi-solid$_2$)] fermentations were subjected to sensory evaluation in terms of color, taste and smell using nine points hedonic method and the results are depicted in Fig. 5.4.

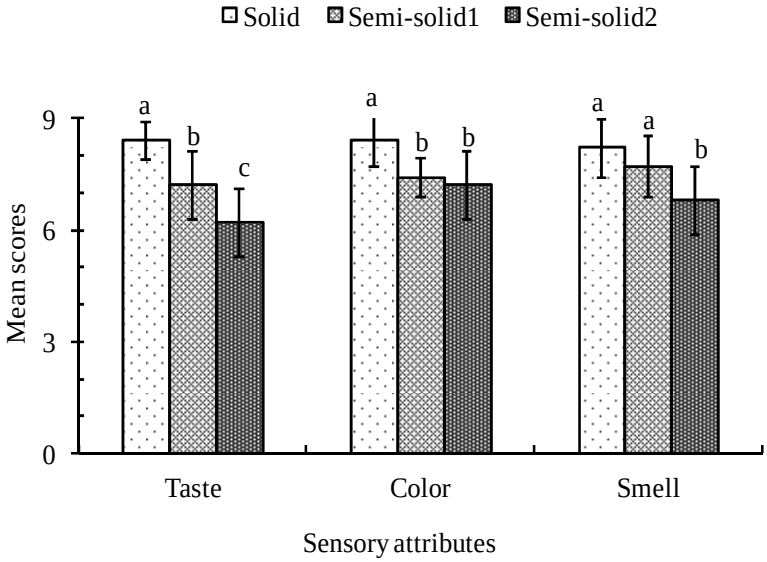


Fig. 5.4 Effects of solid and semi-solid state fermentations on the sensory quality of millet *jand*. Values are the means (n = 10) ± SD. Bars having different letters for any quality attribute are significantly different ($p<0.05$) by LSD. Semi-solid1 and Semi-solid2 are the semi-solid state fermentations using 50 and 100% water addition respectively to biomass developed millet during alcoholic fermentation.

Statistical analysis revealed that semi-solid fermentations had a profound effect on the organoletpic properties of the millet *jand*. Mean sensory scores for taste were found to be 8.4, 7.2, and 6.2 for solid, semi-solid$_1$ and semi- solid$_2$ fermented samples respectively. Increasing water level decreased ($p<0.05$) the taste preference and the scores statistically differed from each other. Higher acidity seemed to have a negative effect on the taste preference (Table 5.4).

The taste preference of the *jands* prepared by solid, semi-solid$_1$ and semi-solid$_2$ fermentations were rated as “liked very much”, “liked moderately” and “liked slightly” by the panelists. The average color preference scores were 8.4, 7.4, and 7.2 for *jands* prepared by solid, semi-solid$_1$ and semi-solid$_2$ fermentations respectively. Solid-state fermentation produced significantly better-colored *jand* compared to semi-solid ones, while the mean color scores between semi-solid fermented samples were not significantly different. Millet *jand* prepared by solid-state fermentation was “liked very much”, whilst those prepared by semi-solid fermentation were “liked moderately” by the panelists based on color preference. The mean scores for smell were 8.2, 7.7, and 6.8 for *jands* prepared by solid, semi-solid$_1$ and semi-solid$_2$ fermentations respectively.

The smell scores between solid and semi-solid$_1$ did not differ but the values were significantly higher than that of semi-solid$_2$ fermented millet. Although esters are responsible for the smell of the alcoholic beverages, higher contents seemed to be detrimental to the smell. Hence, low smell score for semi-solid$_2$ fermented product could be due to the presence of higher ester content (Table 5.4). Semi-solid state fermentations substantially increased the acidity (Table 5.4) resulting in reduced taste, color and smell preferences (Fig. 5.4) of the millet beer over the control (solid state fermentation). Alcohol content, one of the most important parameters of alcoholic beverages, was not significantly improved by semi-solid fermentations of finger millet. Moreover, semi-solid fermentation incurred a substantial increase in the ester content in the final product, which was beyond the acceptable limit as judged by the panelists. Hence, solid state fermentation could be regarded as the best method of finger millet fermentation than that of semi-solid fermentation.

5.1.3 Storage stability of fermented finger millet (*Eleusine coracana*) packaged in polyvinylchloride (PVC) container under ambient conditions

5.1.3.1 Chemical characteristics of fermented millet packaged in PVC container

Fermented millet packed in PVC container was kept at ambient (25 ± 2 ^{o}C) and at – 30 ^{o}C (control) for 90 days. The samples were analyzed for chemical and organoleptic characteristics and the results are shown in Table 5.5. Total soluble solids (TSS) increased significantly ($p<0.05$), whereas moisture content and total-, fixed- and volatile acidities did not change over the storage period. Reducing sugar content was

higher in room temperature stored sample, while total sugar remained unchanged indicating that slow saccharification of starch being taken place during storage.

Table 5.5 Chemical characteristics of PVC container packaged fermented millet after 90 days of storage

Parameters	Values*	
	Control#	Room temperature
Total soluble solids (ºBx)	15.67[a] (0.58)	17.9[b] (0.17)
Moisture content (% m/m)	75.66[a] (0.49)	75.6[a] (0.53)
Total acidity (% m/m as lactic acid)	1.01[a] (0.05)	1.03[a] (0.05)
Fixed acidity (% m/m as lactic acid)	0.73[a] (0.08)	0.79[a] (0.05)
Volatile acidity (% m/m as acetic acid)	0.163[a] (0)	0.163[a] (0)
Reducing sugar (% m/m as dextrose)	7.29[a] (0.12)	8.24[b] (0.32)
Total sugar (% m/m as dextrose)	10.43[a] (0.68)	9.66[a] (0.21)
Alcohol (% v/m)	14.44[a] (0.45)	13.17[b] (0.45)
Total aldehydes (g/100L alcohol as acetaldehyde	28.15[a] (1.37)	35.01[b] (1.00)
Esters (g/100L alcohol as ethylacetate	113.5[a] (10.53)	179.70[b] (11.97)

*values are the means of three determinations. Figures in the parentheses are the standard deviations. Means followed by the similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

#Control: −30 ºC and room temperature: 25 ± 2 ºC.

Both the total aldehydes and ester contents increased but alcohol content decreased significantly in room temperature stored sample compared to control. The decrease in alcohol content might have been attributed to the increase in ester and aldehyde contents at the expense of alcohol (Table 5.5).

5.1.3.2 Sensory quality of fermented milled packed in PVC container after 90 days of storage at 25 ± 2 ºC and at – 30 ºC

Millet j*ands* were prepared from both the fermented millet kept at – 30 ºC (control) and room temperature (25 ± 2 ºC) and the results of sensory evaluation are shown in Fig. 5.5. The mean sensory scores for color, taste, and smell for control and room temperature stored samples were found to be 7.0 and 8.4, 6.9 and 8.2, and 6.8 and 8.4 respectively. Room temperature stored sample had superior ($p<0.05$) color, taste and smell than that of control (− 30 ºC).

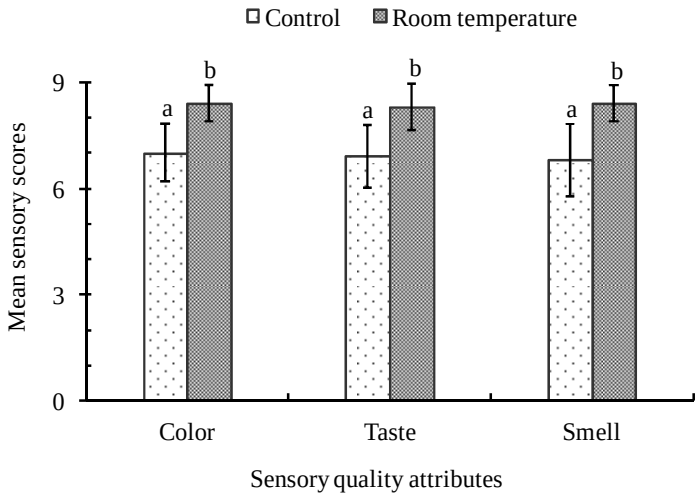


Fig.5.5 Effect of storage time on the sensory quality of fermented finger millet.

Values are the means (n = 10) ± SD. Bars with different letters for any quality attribute are significantly different ($p<0.05$).

Control: fermented millet kept at – 30 °C.

Room temperature: fermented millet kept at 25 ± 2 ºC.

Jand prepared from room temperature stored fermented millet had pinkish color whereas that of control sample had chalky white color. Higher taste and smell scores for room temperature stored sample could be due to the increase in ester and aldehyde contents (Table 5.5) because of ageing.

5.1.4 Clarification of finger millet *jand* using fining agents

5.1.4.1 Selection of fining agents

Effects of different fining agents on the clarification of millet *jand* are shown in Figs. 5.6 and 5.7. Turbidity of the clarified *jand* decreased with increasing concentration of both bentonite and gelatin. Bentonite greatly reduced turbidity compared to gelatin. But tannin and tannin-gelatin combination treatments increased turbidity with increasing concentration. Statistically, bentonite treatment showed a significant effect ($p<0.05$) on the clarification of millet *jand*. The mean turbidity values were 143, 131, 82, 62, and 48.33 FTUs for 0, 1, 2, 3, and 4 g/L bentonite respectively and the values were significantly different from each other by LSD. Due to the scarcity of the relevant literature, the obtained results were discussed with those available for wine clarification. Ough and Amerine (1960) reported that 12.5 lbs of bentonite per 1000 gallons (about 1.5 g bentonite/L) of must increased clarity and filter speed and

lightened the color of new wines. According to Weger (1965), bentonite can be used as needed in Italy, but German law limits it to 1.5 g/L and Austria to 2 g/L.

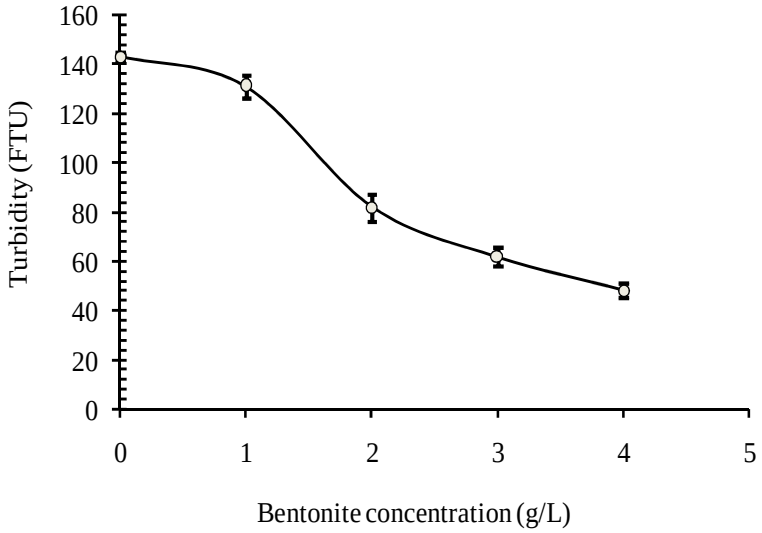


Fig. 5.6 Effect of bentonite concentration on the clarification of millet *jand*. Values are the means (n = 3) ± SD.

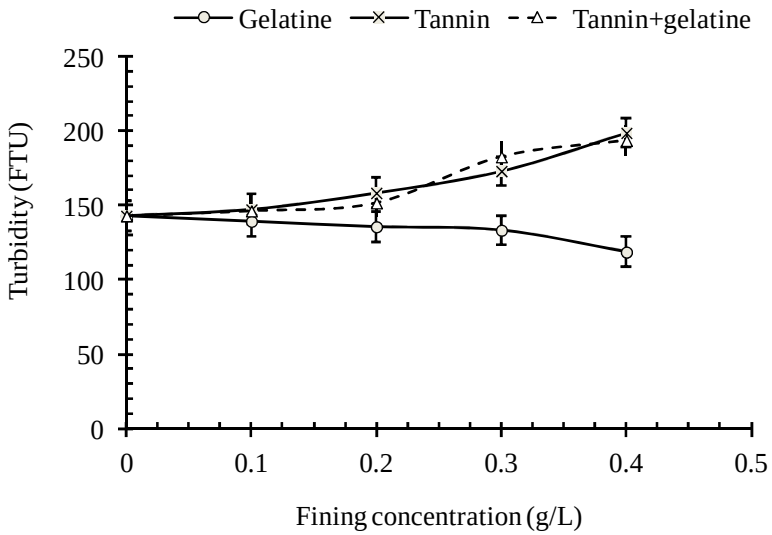


Fig. 5.7 Effect of tannin, gelatine and tannin-gelatine treatments on the clarification of millet *jand*. Values are the means (n = 3) ± SD.

For new cloudy dissert wines, the bentonite dose is usually 0.36 – 0.72 g/L. For well settled, fairly clear dessert wines about 0.36 – 0.60 g/L is often sufficient. Some wine makers warm dessert wines to about 100 ºF before adding the bentonite (Amerine *et al*., 1967). According to Zeocklein *et al.* (1997) addition of bentonite to red wines at levels

of 0.06 – 0.12 g/L improves membrane filterability due to reduction in colloidal suspended particles.

5.1.4.2 Effect of bentonite concentration and acidity on the clarification of millet *jand*

Bentonite concentrations of 0, 1, 2, and 3 g/L and acidity levels of 0.35, 0.45, and 0.55% as lactic acid were used to study the effect of bentonite concentration and acidity on clarification and the results are shown in Fig. 5.8. Bentonite concentrations, acidity and bentonite-acidity interaction all showed significant effect ($p<0.05$) on millet *jand* clarification. Increasing bentonite concentrations for any levels of acidity remarkably decreased the turbidity. Increasing acidity from 0.35 to 0.45% significantly reduced turbidity at all levels of bentonite, while further increase in acidity substantially increased turbidity irrespective of bentonite concentrations. Maximum reduction in turbidity was observed at 0.45% acidity and 3 g/L of bentonite of all the treatments used.

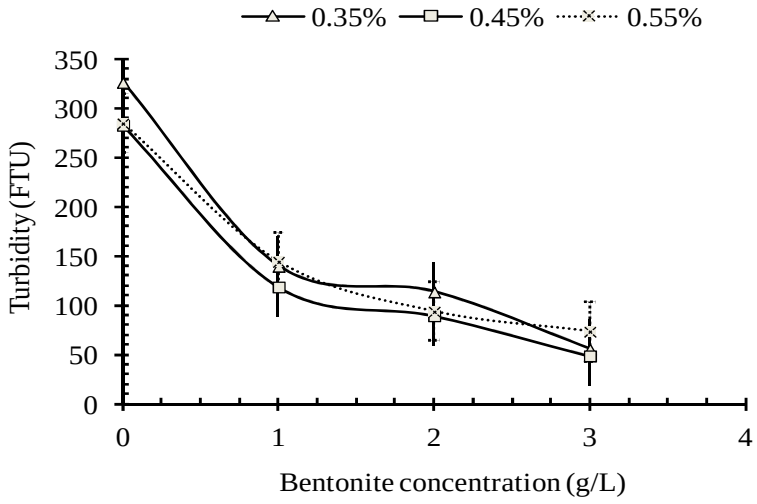


Fig. 5.8 Effect of bentonite concentration and acidity on the clarification of millet *jand*. Values are the means (n = 3) ± SD.

5.1.4.3 Effect of holding temperature, time and sequence of bentonite addition on the clarification of millet *jand*

The effect of holding temperature on the turbidity of millet *jand* treated with 0 (control) and 3 g/L of bentonite and held for 24 h at 29 ± 1 ºC (room temperature)and at 5 ± 1 ºC (refrigerated temperature) is shown in Fig. 5.9. The effect of bentonite treatment was significant ($p<0.05$), while those of temperature and bentonite-temperature interaction were insignificant ($p>0.05$). The turbidity of control samples

held at room and refrigerated temperature were 314 and 317.6 FTUs respectively, however the values were not significantly different. Similarly, the values for 3 g/L bentonite treated samples held at room and refrigerated temperature were 61 and 59.1 FTUs respectively but the values were not statistically different. From Fig. 5.9 it can be envisaged that bentonite treatment significantly reduced turbidity at both holding temperatures, while reducing holding temperature from room to refrigeration did not improve clarification.

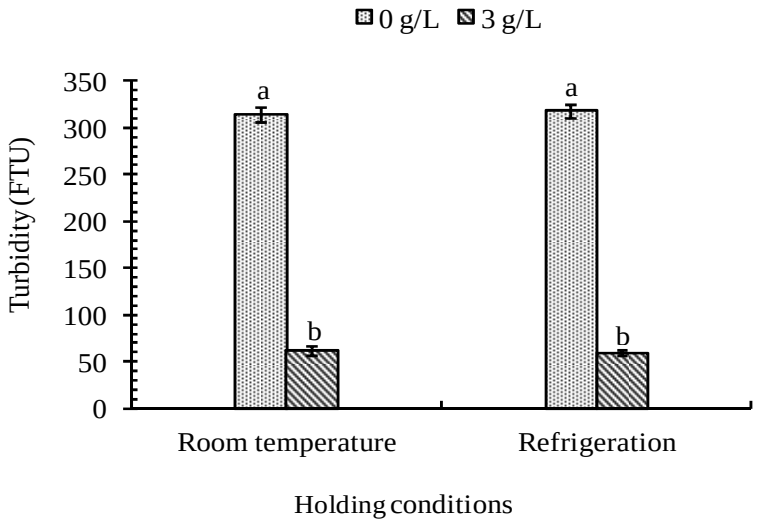


Fig. 5.9 Effect of bentonite concentration and holding conditions on the clarification of millet *jand*. Values are the means (n = 3) ± SD. Bars with different letters are significantly different ($p<0.05$).

Room temperature: 29 ± 1 ºC and refrigeration: 5 ± 1 ºC.

Effect of holding time on the clarification of finger millet *jand* is shown in Fig. 5.10. The mean turbidity values for first, second, third and fourth day held millet *jands* were 60.3, 55.87, 51.45, and 49.9 FTUs respectively. Statistical analysis revealed that holding time had a significant effect ($p<0.05$) on turbidity and it decreased with time up to the third day and then remained statistically unchanged. It was found that bentonite treatment of 3 g/L during pasteurization and a holding time of 3 days at room temperature were sufficient to clarify the millet j*and*.

The turbidity values of the supernatants obtained after pasteurization (control), bentonite treatment during pasteurization and bentonite treatment in pasterurized and gravity settled clear *jands* were measured and the results are shown in Fig. 5.11. Bentonite treatment had a significant effect on the clarification of millet *jand*. The mean turbidity values for control, bentonite treated (3 g/L) followed by pasteurization

and bentonite treatment in pasteurized and gravity settled samples were 319.33, 61 and 109.7 FTUs respectively. Statistically, the values were significantly different from each other. It was found that addition of bentonite during pasteurization considerably improved the clarity, while bentonite treatment to pasteurized and gravity settled *jand* failed to improve clarity.

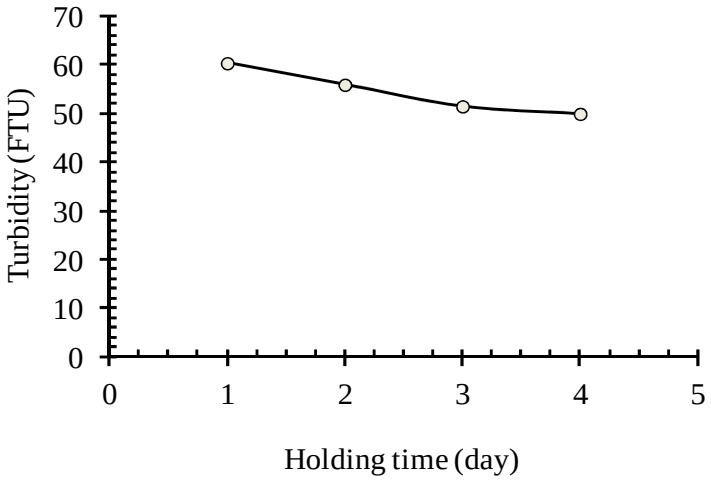


Fig. 5.10 Effect of holding time on the clarification of millet *jand*. Values are the means of three determinations ± SD.

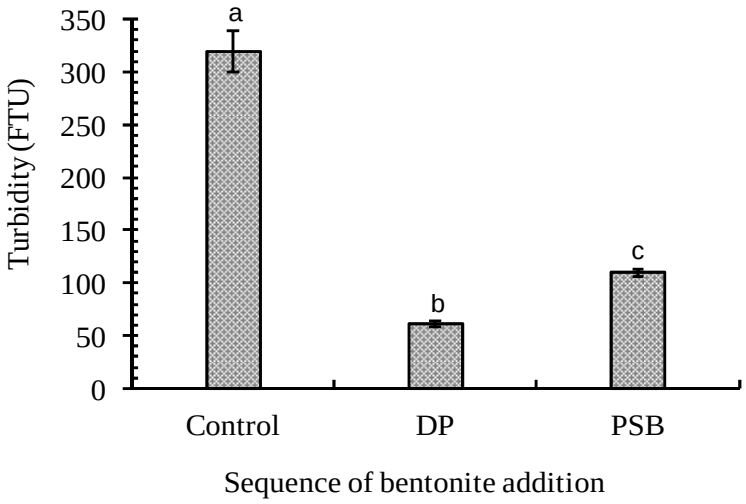


Fig. 5.11 Effect of sequence of bentonite addition on the clarification of millet *jand*. Values are the means (n = 3) ± SD. Bars having different letters are significantly different ($p<0.05$) by LSD. Control: Pasteurization only; DP: Bentonite treatment followed by pasteurization and PSB: Bentonite treatment in pasteurized and gravity settled *jand*.

5.1.4.4 Effect of bentonite treatment on chemical and sensory characteristics of finger millet *jand*

5.1.4.4.1 Effect of on chemical characterstics of millet *jand*

Effect of bentonite treatment on chemical characteristics of millet *jand* is shown in Table 5.6. Total soluble solids, pH and volatile acidity were not affected while total and fixed acidities were significantly decreased by clarification. Total reducing sugar, sucrose, total sugar, and glucose contents were lower in clarified *jand* compared to unclarified one. Similarly, bentonite treatment significantly reduced ($p<0.05$) water soluble protein, total nitrogen, non-protein nitrogen, total solids and insoluble solid contents in clarified *jand* than that of control (untreated) one. Starch content was 1.31% m/v in unclarified *jand*, while it was nil in the clarified *jand*. Total phenolics, tannin, antioxidant activity, and electrical conductivity were not affected by clarification. Alcohol, methanol and total ester contents were lower in clarified *jand* while fusel oil content remained statistically unchanged. A significant reduction in total, free and combined aldehydes contents occurred by bentonite treatments.

5.1.4.4.2 Effects on organoleptic quality of millet *jand*

Effect of bentonite treatment on organoleptic quality of clarified millet *jand* is shown in Fig. 5.12. The mean sensory scores for color, clarity, taste and smell for control and bentonite treated (3 g/L) samples were 7.43 and 7.86, 6.86 and 8.29, 7.86 and 7.71, and 8.29 and 8.29 respectively. Clarity significantly improved while taste, smell, and color remained unaffected by bentonite treatment (Fig. 5.12). According to Amerine *et al.* (1967), when properly used, bentonite has little or no adverse effect on the flavor and bouquet of table or dessert wines, which also supports our findings.

Table 5.6 Effect of bentonite treatment on the chemical characteristics of finger millet *jand*

Parameters	Values*	
	Unclarified	Clarified
TSS (º Bx)	4.97 [a] (0.06)	4.97 [a] (0.06)
pH	4.38 [a] (0.03)	4.38 [a] (0.03)
Acidities		
Total as lactic (% m/v)	0.34 [a] (0.006)	0.31 [b] (0.003)
Fixed as lactic (% m/v)	0.29 [a] (0.01)	0.27 [b] (0.01)
Volatile as acetic (% m/v)	0.0290 [a] (0.0035)	0.0297 [a] (0.0006)
Reducing sugar as dextrose (% m/v)	1.37 [a] (0.01)	1.32 [b] (0.01)
Sucrose (% m/v)	0.31 [a] (0.01)	0.28 [b] (0.01)
Total sugar (% m/v)	1.68 [a] (0.01)	1.60 [b] (0.06)
Glucose (mg/100 ml)	44.40 [a] (1.65)	38.9 [b] (1.22)
Water soluble protein (mg/100 ml)	301.3 [a] (31.5)	288.1 [a] (22.0)
Total nitrogen (% m/v)	0.06 [a] (0.001)	0.04 [b] (0.002)
Non-protein nitrogen (mg/100 ml)	4.93 [a] (0.11)	4.11 [b] (0.09)
Total solids (% m/v)	3.17 [a] (0.11)	2.40 [b] (0.05)
Insoluble solids (% m/v)	1.27 [a] (0.03)	0.22 [b] (0.02)
Starch (% m/v)	1.31 [a] (0.32)	0.00 [b] (0.00)
Total phenolics as GAE (mg%)	22.37 [a] (0.12)	22.80 [a] (0.52)
Tannin as tannic acid (mg%)	20.33 [a] (0.49	19.93 [a] (0.06)
Antioxidant activity (%)	68.59 [a] (4.24)	75.41 [a] (3.88)
Electrical conductivity (µS/cm)	14.6 [a] (0.01)	1.46 [a] (0.01)
Alcohol (% v/v)	7.90 [a] (0.01)	6.93 [b] (0.06)
Fusel oil (g/L alcohol)	5.061 [a] (0.002)	5.120 [a] (0.117)
Methanol (g/L alcohol)	2.511 [a] (0.230)	1.139 [b] (0.089)
Total esters as ethyl acetate (g/L alcohol)	2.477 [a] (0.057)	2.19 [b] (0.114)
Aldehydes as acetaldehyde (g/L alc)		
Total	0.601 [a] (0.001)	0.410 [b] (0.010)
Free	0.523 [a] (0.020)	0.361 [b] (0.019)
Combined	0.078 [a] (0.019)	0.048 [b] (0.010)

*values are the means of three determinations. Figures in the parentheses are standard deviations. Means followed by similar superscripts are not significantly different ($p>0.05$).

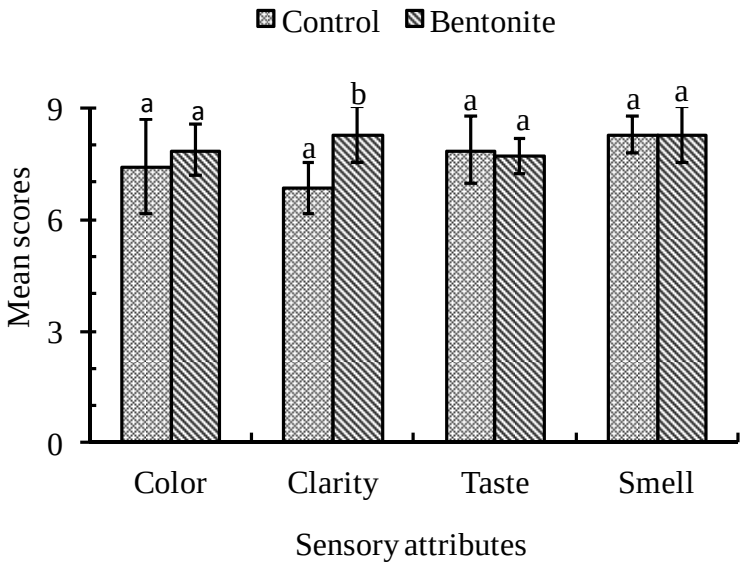


Fig. 5.12 Effect of bentonite treatment on the sensory quality of clarified *jand*.Values are the means (n = 10) ± SD. Bars having different letters for any attribute are significantly different ($p<0.05$).

5.1.5 Bio-chemical changes during finger millet fermentation

5.1.5.1 Changes in chemical characteristics during millet fermentation

Chemical changes during finger millet fermentation are shown in Table 5.7. Moisture content increased significantly ($p<0.05$) from 56.06% on day 0 (cooked millet) to 74.69% on day 12 during fermentation. Large increase in moisture content occurred up to 6 days of fermentation suggesting that liquefaction was at a faster rate during this stage. TSS increased ($p<0.05$) during fermentation with an average rate of 0.67 ºBx per day reaching a maximum value of 12 ºBx on day 12. Total acidity increased significantly from 0.12% on day 0 up to 0.82% on day 6 and remained statistically unchanged afterwards during fermentation. Fixed acidity content of cooked millet was equal to that of total acidity (0.12% m/m) on day 0 and it reached at maximum (0.90%, m/m) on day 9 and remained almost constant till day 12 during fermentation.

Volatile acidity was nil on day 0 and increased significantly during fermentation reaching a maximum value of 0.027% m/m on day 9 and remained fairly constant till day 12. From Table 5.7 it can be envisaged that total and fixed acidities produced at higher rates during the first 3 days of fermentation. Similar increase in total acidity with time was also reported in rice fermentation (Tamang and Thapa, 2006) and in finger

millet fermentation (Thapa and Tamang, 2006) but the values were quite lower (0.17% and 0.23% m/m as lactic acid in rice and millet respectively) than those obtained in this study. Analogous trends in total acidity development during fermentation were also reported by Das (2004), Verma (1991), and Koirala (2011) but the values reported by Koirala (2011) were higher than those obtained in this study.

Total reducing sugar content increased from 0.41% in cooked millet (day 0) to 3.68% on day 3, declined to 3.13% on day 6 and increased afterwards giving a maximum value of 4.49% on day 12. Increasing trend of total reducing content in *poko* fermentation was also observed by Shrestha *et al.* (2002), where it reached at a maximum value of 15.6% m/m on day 3 and subsequently decreased to zero on day 5. Similar results were also reported by Thapa and Tamang (2006) in finger millet and by Tamang and Thapa (2006) in rice. Gradual increase in reducing sugar content was also reported by Koirala (2011) with reducing content of 2.68% as maltose by the end of finger millet fermentation (day 12). Rai (1991) also reported analogous pattern in native and germinated fermented finger millet. Similarly, sucrose content increased from 0.37% (day 0) to 0.63% (day 3), remained constant until day 9 and decreased to 0.27% on day 12. Total sugar content followed the similar pattern to that of total reducing sugar giving a maximum value of 4.75% (m/m) on day 12. Glucose content of day 0 sample was 41.9 mg% (wb), which increased to 642.1 mg% on day 3, remained unchanged up to day 9 followed by increase on day 12 (692.8 mg%). The rate of glucose formation and its utilization by yeast seemed almost equal after day 3 until day 9 resulting constant glucose content over this period of fermentation (Table 5.7). Fructose content increased from 30.4 mg% (day 0) to 51.1 mg% on day 3, remained unchanged on day 6 and increased thereafter up to 74.2 mg% on day 12.

Starch content steadily decreased throughout fermentation. Maximum decline was found during the first 3 days of fermentation, while it decreased at a slower rate from 6 day onwards. Water-soluble protein increased from 0.50% (m/m) on day 0 to 0.99% m/m on day 3, remained constant on day 6 and again increased giving a maximum value of 1.15% (m/m) on day 9. Statistically, the water-soluble protein contents between 9 and 12 days fermented millets were not different. Similar increase in water-soluble protein during finger millet fermentation was also reported by Sripriya *et al.* (1997). Fermentation time showed a significant effect ($p<0.05$) on antioxidant activity and it increased from 59.88% on day 0 to a maximum value of 81.21% on day 12.

Table 5.7 Chemical changes during finger millet fermentation

Parameters	Values at different fermentation times (days)*				
	0	3	6	9	12
Moisture content (% m/m)	56.06 [a]	66.30 [b]	71.33 [c]	72.44 [d]	74.69 [e]
	(0.64)	(0.57)	(0.39)	(0.51)	(0.66)
TSS (º Bx)	3.93 [a]	6.30 [b]	8.33 [c]	10.43 [d]	12.00 [e]
	(0.81)	(0.30)	(0.12)	(0.12)	(1.32)
Total acidity as lactic acid (% m/m)	0.12 [a]	0.70 [b]	0.82 [bc]	0.96 [c]	0.93 [c]
	(0.01)	(0.11)	(0.07)	(0.09)	(0.09)
Fixed acidity as lactic (% m/m)	0.12 [a]	0.67 [b]	0.75 [bd]	0.90 [c]	0.87 [cd]
	(0.01)	(0.11)	(0.08)	(0.07)	(0.06)
Volatile acidity as acetic acid (% m/m)	0 [a]	0.014 [b]	0.023 [c]	0.027 [d]	0.026 [d]
	(0.00)	(0.001)	(0.003)	(0.001)	(0.001)
Reducing sugar as dextrose (% m/m)	0.41 [a]	3.68 [b]	3.13 [c]	3.74 [b]	4.49 [d]
	(0.02)	(0.26)	(0.06)	(0.23)	(0.12)
Sucrose (% m/m)	0.37 [a]	0.63 [b]	0.61 [b]	0.69 [b]	0.27 [c]
	(0.07)	(0.10)	(0.08)	(0.21)	(0.12)
Total sugar (% m/m)	0.78 [a]	4.31 [b]	3.73 [c]	4.44 [b]	4.75 [d]
	(0.05)	(0.33)	(0.03)	(0.03)	(0.03)
Glucose (mg/100g)	41.9 [a]	642.1 [b]	635.3 [b]	663.8 [bc]	692.8 [c]
	(4.2)	(39.9)	(10.3)	(3.4)	(7.6)
Fructose (mg/100g)	30.4 [a]	51.1 [b]	52.6 [b]	67.5 [c]	74.2 [d]
	(6.0)	(0.96)	(0.41)	(3.5)	(2.1)
Starch (% m/m)	31.23 [a]	22.94 [b]	17.40 [c]	15.00 [d]	14.00 [e]
	(0.51)	(0.11)	(0.32)	(0.43)	(0.70)
Water soluble protein (% m/m)	0.50 [a]	0.99 [b]	0.99 [b]	1.15 [c]	1.07 [bc]
	(0.05)	(0.03)	(0.09)	(0.04)	(0.05)
Antioxidant activity (%)	59.88 [a]	67.32 [b]	71.48 [c]	74.73 [c]	81.21 [d]
	(0.26)	(3.72)	(1.546)	(0.52)	(1.51)
Alcohol content (% v/m)	nd**	6.49 [b]	10.05 [c]	11.78 [d]	14.58 [e]
		(0.71)	(0.93)	(0.69)	(0.39)

*values are the means of three determinations. Figures in the parentheses are the standard deviations. Means followed by different superscripts in a row are significantly different ($p<0.05$) by LSD.

**nd means not determined.

Increase in antioxidant activity during fermentation in buckwheat and barley was also reported by Agte *et al*. (1997). Alcohol content increased significantly with fermentation time and the values were 6.49, 10.05, 11.78, and 14.58% (v/m) on 3, 6, 9 and 12 days fermentated millets respectively. Similar change in alcohol content was also reported by Thapa and Tamang (2006) in fermenting finger millet, where alcohol content increased till day 6 (6.9%, v/m), remained unchanged up to day 8 and decreased to 6.5% on day 10 but the reported values were lower than those obtained in this study. Tamang and Thapa (2006) and Shrestha (2002) also found similar trend in rice fermentation but the alcohols contents were lower compared to those obtained in our study. Cai and Nip (1990) reported increase in alcohol content during taro fermentation reaching 10.3% (m/m) alcohol on day 12. Koirala (2011) also obtained similar trend of alcohol change during finger millet fermentation with final alcohol content of 15.13% (v/m) on day 12.

5.1.5.2 Changes in volatile constituents during millet fermentation

Changes in volatile constituents during finger millet fermentation are depicted in Fig. 5.13.

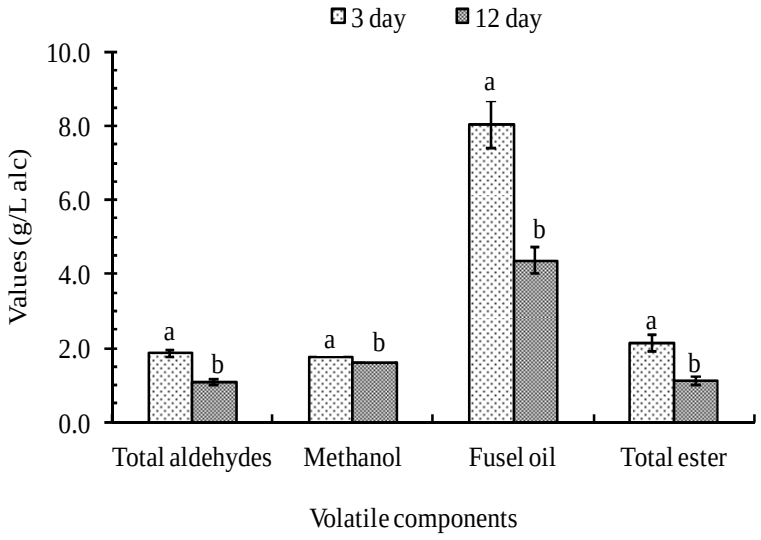


Fig 5.13 Changes in volatile constituents during millet fermentation. Values are the means of three determinations. Bars having dissimilar letters for any constituent are significantly different ($p<0.05$).

Total aldehydes (as acetaldehyde), total esters (as ethyl acetate), methanol and fusel oil contents in 3 and 12 days fermented finger millet were found to be 1.857 and 1.089, 2.121 and 1.124, 1.753 and 1.500, and 8.028 and 4.366 g/L alc respectively. It appeared

that concentrations of these volatile constituents were higher during initial stage of fermentation compared to later one. Fusel oil was approximately double in 3 days fermented millet compared to 12 days one. Koirala (2011) reported that total esters content in 3 and 12 days fermented finger millet were 1.404 and 1.164 g/L alc, which is analogous to our findings. Changes in phytic acid and total free amino acids (TFAAs) and total oxalates and non-protein nitrogen contents during fermentation are shown in Figs. 5.14 and 5.15 respectively.

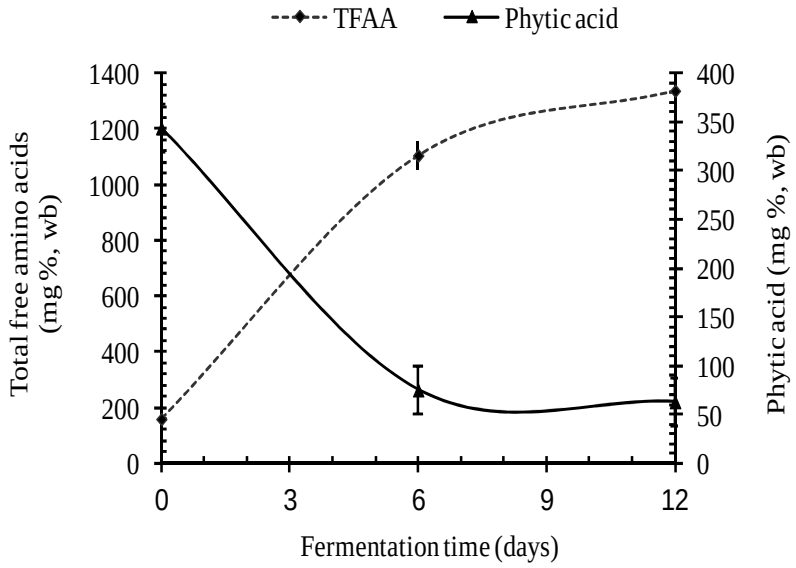


Fig. 5.14 Changes in phytic acid and total free amino acids (TFAAs) contents during millet fermentation. Values are the means (n=3) ± SD.

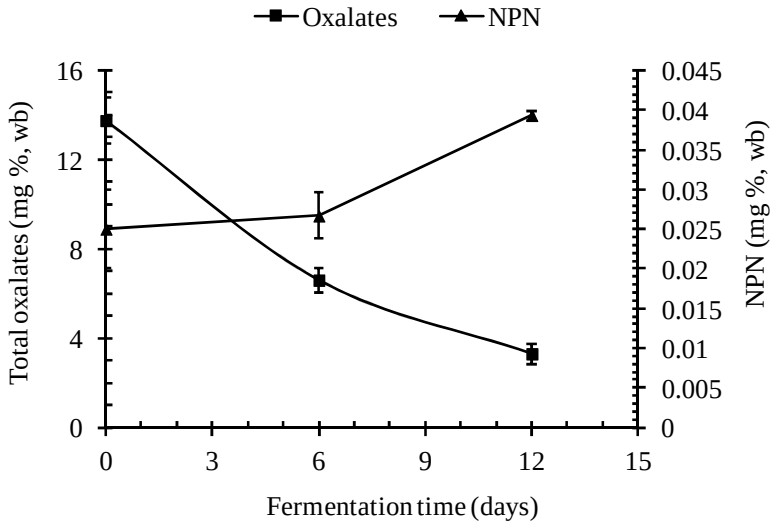


Fig. 5.15 Changes in total oxalates and non-protein nitrogen (NPN) during millet fermentation. Values are the means (n = 3) ± SD.

Phytic acid content of 343.6 mg% (wb) on day 0 decreased to 74.9 mg% on day 6 and further decreased to 62.7 mg% on day 12 during fermentation. It was found that a large amount of phytic acid reduction occurred over the first 6 day, while there was no significant ($p>0.05$) reduction afterwards. Lopez *et al.* (1983) also observed similar decrease in phytic acid during natural fermentation of corn meal due to phytase production by microorganisms. Makokha *et al*. (2002) reported a 72% decrease in phytic acid in natural fermentation of germinated finger millet, while it was 64.8% reduction in sorghum grain after 96 h of fermentation. *S. cerevisiae* can produce phytase and degrade phytate into its lower forms and inorganic phosphate, Pi, which is used by microorganisms for their growth. Significant reductions in phytic acid were also reported by Elyas *et al.* (2002) during natural fermentation of pearl millets. Phytate was reported to be present in several microorganisms (Daniels and Fisher, 1981; Lopez *et al.,* 1983).

Total free amino acids increased largely (about 6-folds) over 6 days of fermentation and further increased during fermentation but to a lesser extent (by about 21%). The increment could be attributed to the breakdown of protein by protease enzymes elaborated by molds as well as microbial synthesis of amino acids. Non-protein nitrogen contents between day 0 (0.025 mg%) and day 6 (0.027 mg%) fermented millets were not different ($p>0.05$) but the values were significantly lower to that of 12 days fermented millet (0.039 mg%). The increase in non-protein nitrogen could be due to the loss of dry matter and increase in moisture content during fermentation. Total oxalate contents in 0, 6, and 12 days fermented millets were 13.4, 6.6, and 3.3 mg/100 g respectively. Statistical analysis revealed that fermentation had a significant effect ($p<0.05$) on total oxlates reduction and decreased by about 50 and 75% over 6 and 12 days of fermentation respectively.

5.1.5.3 Changes in proximate composition and mineral contents

Proximate constituents and mineral contents in cooked, biomass developed and fermented finger millets are shown in Table 5.8. Except moisture content, protein, fat, ash, and total carbohydrate content did not change during aerobic fermentation (biomass development) but there was a significant increase in protein, fat and ash and decrease in total carbohydrate contents in fermented samples. Moisture content of cooked millet (55.44%, wb) increased by 3.18 and 17.49% (wb) during aerobic and

anaerobic fermentations respectively. Similar results of moisture increment were also reported in millet (Thapa and Tamang, 2004) and in rice (Tamang and Thapa, 2006).

Table 5.8 Changes in proximate composition and mineral contents during millet fermentation

Constituents	Values*		
	Cooked millet	Bio-mass developed	Fermented
Moisture content (% wb)	55.44[a] (0.47)	58.62[b] (0.54)	72.93[c] (0.81)
Crude protein (% db)	7.62[a] (0.52)	8.20[a] (0.50)	15.70[b] (0.75)
Crude fat ((% db)	1.45[a] (0.11)	1.26[a] (0.07)	2.14[b] (0.20)
Ash (% db)	2.76[a] (0.44)	3.34[a] (0.43)	4.62[b] (0.33)
Carbohydrate (% db)	87.84[a] (0.60)	87.20[a] (0.97)	77.54[b] (0.73)
Minerals (mg/100 g dm)			
Phosphate as PO_4-P	63.6[a] (4.9)	426.5[b] (13.7)	1846.6[c] (17.1)
Iron	15.8[a] (3.0)	8.8[b] (0.3)	16.1[a] (1.4)
Manganese	119.9[a] (4.5)	116.7[a] (2.5)	223.0[b] (7.3)
Sodium	19.7[a] (2.3)	25.6[b] (1.2)	25.8[b] (1.8)
Potassium	1017.6[a] (90.3)	1054.0[a] (62.0)	2132.6[b] (128.8)
Zinc	10.3[a] (0.5)	13.8[b] (0.3)	21.0[c] (0.5)

*values are the means of three determinations. Figures in the paranthesses are the standard deviations. Means followed by similar superscripts in a row are not significantly diiferent ($p>0.05$) by LSD.

There was about 2-fold increase in protein content in fermented millet compared to unfermented one. Fermentation resulted decrease in total carbohydrate content by 10.33% compared to cooked millet. About 1.8-folds increase in ash content in fermented rice was reported by Tamang and Thapa (2006), while no significant change in ash content in fermented finger millet was reported by Thapa and Tamang (2006). Contrary to our results of fat and protein contents, no remarkable increase due to fermentation was reported in millet (Thapa and Tamang, 2004) and in rice (Tamang and Thapa, 2006). Chavan and Kadam (1989) noted that protein content in millet was generally not affected by fermentation in pearl millet. The increase in protein content can be attributed to microbial synthesis of protein during growth cycles (Zamora and Fields, 1979). Elyas *et al.* (2002) reported that protein content increased from 10.8 to 12.4% during natural fermentation of Composite Population III variety of pearl millet,

while it increased from 14.92 to 17.75% in Maladi variety of pearl millet. Shrestha and Rati (2003) also reported similar results of increase in ash and protein and decrease in total carbohydrate contents during *poko* fermentation. Increase in protein content from 8.0 to 16.6% (db) in 192 h of *tape ketan* fermentation was reported by Cronk *et al*. (1977). Tamang and Thapa (2006) also reported a slight decrease in total carbohydrate content during rice fermentation, whereas Thapa and Tamang (2004) found a slight increment in fermented millet.

Phosphate content increased significantly ($p<0.05$) during biomass development which further increased during alcoholic fermentation. Iron content decreased in biomass developed samples compared to cooked and fermented finger millets, but the values between the later two did not differ significantly ($p>0.05$). Manganese content did not change remarkably during biomass development while it did significantly increase during fermentation. Sodium content increased from 19.7 mg% (db) in cooked millet to 25.6 mg% (db) in biomass developed millet but no further significant increment occurred on fermentation. Potassium did not change during biomass development but it increased by about 100% during fermentation. Zinc content was found to increase during both aerobic and anaerobic fermentations. Analogous results of increase in phosphate, manganese, sodium and potassium contents due to fermentation were also reported in rice (Tamang and Thapa, 2006) and in finger millet (Thapa and Tamang, 2004), but except for sodium content the reported values were quite lower than those found in this study. They further reported that iron contents were higher in fermented rice and millet over the unfermented substrate while no remarkable change was found in this study. Although similar results of increase in zinc due to fermentation were reported in rice (Tamang and Thapa, 2006) and in millet (Thapa and Tamang, 2004) but the reported values were lower than those obtained in our study.

5.1.5.4 Microbial changes during millet fermentation

Changes in yeast and mold counts during finger millet fermentation are shown in Fig. 5.16. Fermentation time showed a significant effect ($p<0.05$) on the yeast and mold counts. The mean yeast counts were 6.633, 7.959, 8.673, 8.447, 7.491, and 7.239 log cfu/g on day 0, 2, 4, 6, 8 and 10 respectively. Yeast population increased until day 4, remained constant on day 6 and then started decreasing. The initial mold count of 7.19 log cfu/g in biomass developed sample (day 0) decreased to 5.573 on day 2, while no growth was observed on day 4 and onwards. Thapa and Tamang (2006) reported that

mold counts were decreased from 4.2 log cfu/g on day 0 to 1.8 log cfu/g on day 3 and were not visible afterwards in fermenting finger millet. They also found that yeast count increased from initial count of 5.2 log cfu/g to a maximum value of 7.8 log cfu/g on day 2, remained constant until day 4 and then decreased reaching a final count of 7.3 log cfu/g on day 10.

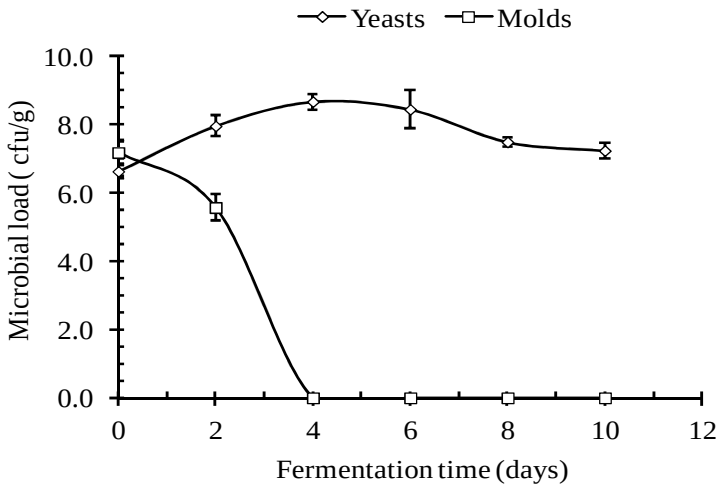


Fig. 5.16 Changes in yeast and mold counts during millet fermentation. Values are the mean (n = 3) ± SD. 0 day means biomass developed sample.

Tamang and Thapa (2006) also reported similar results of increase in yeast counts from 5 log cfu/g to 8 log cfu/g on day 2 and decreased to a level of 5 log cfu/g on day 10 in fermenting rice. Mold count decreased steadily during fermentation and was not visible after 4 days of fermentation. A similar trend was also reported by Shrestha *et al.* (2002) during *poko* fermentation using *manapu* starter in which yeast count increased till day 5 (end of fermentation) while mold count increased till day 2, remained fairly constant on day 3 and started decreasing afterwards. Koirala (2011) reported that both yeast and mold populations reached a maximum count on day 3 and then declined in which molds were completely disappeared on day 6 during finger millet fermentation.

5.1.6 Quality comparison of fermented finger millet using traditional and defined fermentation starters

5.1.6.1 Chemical characteristics of lab and market samples of fermented millet

Chemical characteristics of finger millet fermented using defined fermentation starter (Lab sample) and those collected from market (Market sample) were analyzed and the results are shown in Table 5.9. The mean values of moisture, TSS, acidities, total

reducing sugar and fusel oil contents between lab and markets fermented millets were not significantly different ($p>0.05$), but large variations existed among market samples than those of lab ones. Alcohol, total esters, total aldehydes, and methanol contents were significantly different between lab and market samples.

Table 5.9 Chemical characteristics of lab and market fermented finger millet*.

Parameters	Lab sample		Market sample	
	Mean ± SD	Range	Mean ± SD	Range
Moisture (% wb)	72.04[a] ± 1.78	70.78 – 74.69	68.11[a] ± 3.69	65.34 – 73.53
TSS (ºBx)	12.61[a] ± 1.13	12.00 – 14.30	14.72[a] ± 2.42	11.93 – 17.83
Total acidity as lactic acid (% wb)	0.95[a] ± 0.09	0.83 – 1.03	0.72[a] ± 0.19	0.57 – 0.99
Fixed acidity as lactic acid (% wb)	0.91[a] ± 0.07	0.82 – 0.97	0.67[a] ± 0.20	0.49 – 0.95
Volatile acidity as acetic acid (% wb)	0.03[a] ± 0.013	0.013 – 0.042	0.018[a] ± 0.01	0.013 – 0.026
Total reducing sugar (% wb)	6.06[a] ± 1.35	4.49 – 7.8	6.39[a] ± 3.45	3.42 – 11.21
Alcohol (% v/m)	14.43[a] ± 0.57	13.87 – 15.17	9.95[b] ± 0.89	9.25 – 11.25
Total esters as ethyl acetate (g/L alc)	0.946[a] ± 0.142	0.806 – 1.124	1.656[b] ± 0.498	1.203 – 2.305
Total aldehydes as acetaldehyde (g/L alc)	0.191[a] ± 0.013	0.174 – 0.201	0.343[b] ± 0.045	0.305 – 0.396
Methanol (g/L alc)	2.055[a] ± 0.521	1.633 – 2.641	5.119[b] ± 0.970	3.723 – 5.843
Fusel oil (g/L alc)	5.191[a] ± 0.959	4.366 – 5.717	5.355[a] ± 0.742	4.424 – 6.140

*values are the means (n = 4) ± SD. Means sharing similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

Millet fermented using defined starter resulted significantly higher alcohol content (14.43% v/m) than that of traditional starter (9.95%, v/m). Total ester content was higher in market sample (1.656 g/L alc) compared to lab sample (0.946 g/L alc). Traditionally fermented finger millet had about 80% higher total aldehyde than that of millet fermented using defined starter (0.191 g acetaldehyde/L alc). Similarly, methanol content was about 1.5 times higher in market sample (5.119 g/L alc) than that of lab fermented one (2.055 g/L alc).

5.1.6.2 Sensory quality of finger millet fermented using defined and traditional tarters

Sensory quality of *jands* prepared from lab and market fermented finger millet is depicted in Fig 5.17. The mean color, taste and smell scores, out of a total possible score of 9, for lab and market samples were 8.44 and 6.78, 8.56 and 6.67, and 8.11 and 6.44 respectively. Statistical analysis showed that lab fermented millet was significantly superior (rated as like very much) than that of market sample (rated as like moderately for color and taste, and like slightly for smell).

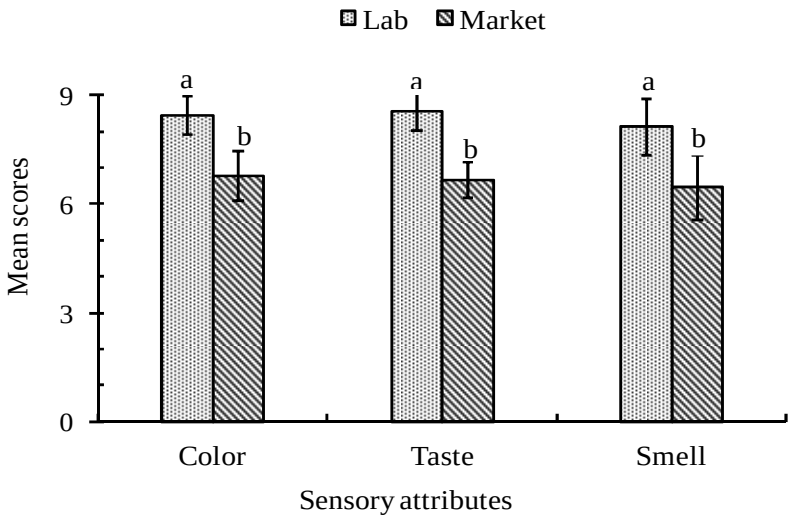


Fig. 5.17 Sensory quality of *jand* made from lab and traditionally fermented finger millet. Values are the means (n = 10) ± SD. Bars having dissimilar letters for any attribute are significantly different ($p<0.05$).

5.2 Assessment of brewing potential of Nepalese finger millet varieties

5.2.1 Effect of finger millet variety and germination time on enzymatic activities and chemical characteristics of millet malts

5.2.1.1 Effect on enzymatic activities

Different finger millet varieties were germinated for 0, 48, 72, and 84 h at 28 ± 1ºC, kilned at 50 ± 2 ºC and analyzed for α-amylase, β-amylase, total diastatic and carboxypeptidase activities, and total free amino acids (TFAAs) and free amino nitrogen (FAN) contents of millet malts. Alpha amylase activity of millet malts was significantly affected ($p<0.05$) by millet varieties and germination times, and the results are shown in Fig. 5.18.

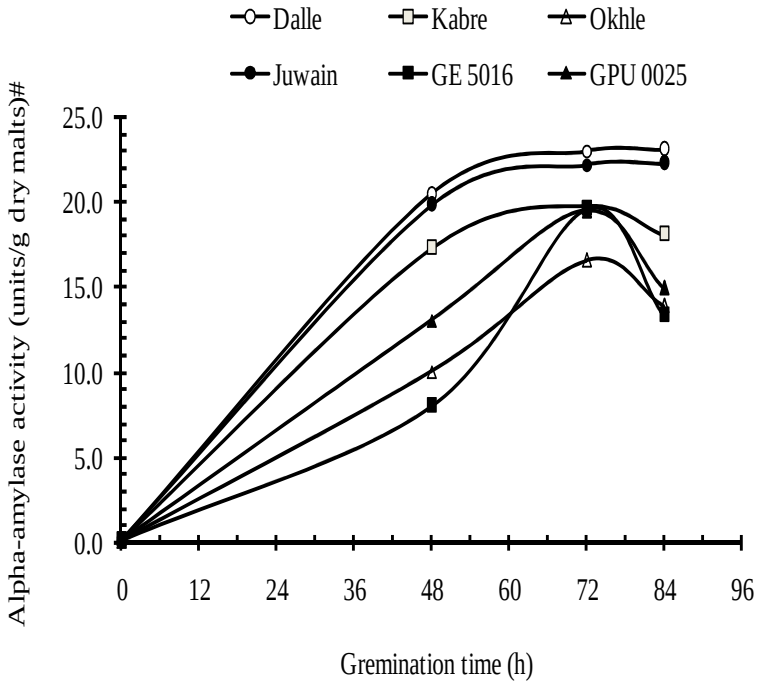


Fig. 5.18. Effect of millet variety and germination time on α-amylase activity of millet malts. Values are the means of three replications. #one unit of α-amylase activity was defined as one unit decrease in OD at 620 nm per min under the experimental conditions.

Alpha amylase activities of ungerminated millets were in the range of 0.05 – 0.11 unit/g dry malt but the values were not significantly different ($p>0.05$). Statistical analysis showed that both millet variety and germination time had a significant effect on α-amylase activity of malts. Increasing germination time up to 72 h significantly

increased the α-amylase activity in all millet varieties. Alpha amylase activities in 48 h germinated malts were 20.41, 17.23, 10.02, 19.80, 7.95, and 13.03 units/g dry malt in *Dalle*, *Kabre*, *Okhle*, *Juwain*, *GE 5016* and *GPU* 0025 millet varieties respectively. Similarly, the values in 72 h germinated malts were 22.96, 19.73, 16.55, 22.16, 19.54, and 19.47 units/g dry malt for the respective millet varieties. Germination beyond 72 h significantly reduced α-amylase activity in *Kabre*, *Okhle*, *GE 5016* and *GPU 0025* millet varieties while no remarkable changes were found in *Dalle* and *Juwain* millet malts. In *Dale*, *Kabre* and *Juwain* millets, large increment in α-amylase activity was observed during 48 h of germination and further germination up to 72 h increased α-amylase activity by about 2.5 units. In *Okhle*, *GE 5016* and *GPU 0025* varieties, a greater extent of α-amylase activity was found during 72 h of germination with about 6.5, 11.6, and 6.4 units/g dry malt higher α-amylase activity compared to the respective 48 h germinated millet malts. Alpha amylase activity of 72 h germinated *Kabre*, *GE 5016*, and *GPU 0025* malts were similar (19.6 ± 0.1 units). Likewise, the values for *Dalle* and *Juwain* malts were also similar (22.6 ± 0.4 units). It was found that 72 h germination of either *Dalle* or *Juwain* finger millet could give the maximum α-amylase activity in malts of all the treatment combinations studied.

Changes in β-amylase activity during germination of different finger millet varieties are depicted in Fig. 5.19. Like α-amylase activity, β-amylase activity was also significantly affected by both millet variety and germination time and their interaction effect was also significant ($p<0.05$). Except for *Juwain* millet, β-amylase activity was highest at 48 h of germination and further germination significantly reduced β-amylase activity in all millet varieties. Beta amylase activity in native millets was in the range of 0.1 – 2.4 units/g dry malt but the values were not significantly different ($p>0.05$). Beta amylase activities in 48 h germinated millet malts were 357.4, 385, 360.2, 244.9, 349.2 and 355.3 units/g dry malt in *Dalle*, *Kabre*, *Okhle*, *Juwain*, *GE 5016* and *GPU 0025* millet varieties respectively. Statistical analysis showed that β-amylase activity of *Kabre* malt was highest (385.0 units/g dry malt) of all the treatment combinations used, while the values among *Dalle*, *GPU 0025* and *Okhle* malts were not significantly different ($p>0.05$). From Fig. 5.19, it can be observed that *Kabre* and *Juwain* millet had an intermediate interaction, whereas the rest of the millet varieties showed a strong interaction with germination time on malt β-amylase activity.

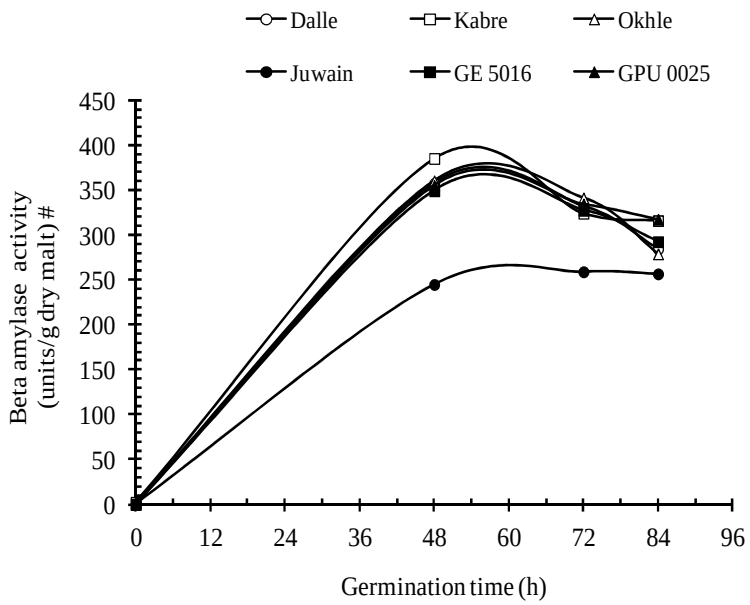


Fig. 5.19 Effect of millet variety and germination time on β-amylase activity of millet malts.Values are the means of three replications.

#One unit of β-amylase activity was defined as the production of 1 mg of maltose over 30 min of incubation under the experimental conditions.

Although, α-amylase activity was higher at 72 h of germination, β-amylase activity, TFAA and FAN contents (Figs. 5.19, 5.22 and 5.23 respectively) were significantly higher in 48 h germinated millet malts. Therefore, total diastatic power of 48 h germinated millet malts were determined and the results were found to be 12.3, 17.3, 8.3, 11.3, 8.3 and 15.0 ºDP (dry basis) for *Dalle*, *Kabre*, *Okhle*, *Juwain*, *GE 5016* and *GPU 0025* millet malts respectively (Fig. 5.20). Statistical analysis revealed that *Kabre* millet malt had the highest diastatic power followed by *GPU 0025* of all the malts but the values between *GE 5016* and *Okhle* and between *Juwain* and *Dalle* malts were not significantly different. Hence, based on amylase activity, *Kabre* millet germinated for 48 h could give the best millet malt of all the treatment combinations studied. Nic Phiarais *et al*. (2005) reported 0.1 and 5.3 units/g of α- and β-amylase activities in native and malted buckwheat respectively. Similarly, Nithya *et al*. (2006) also reported the presence of α-and β-amylase activities (5.33 and 8.0 µM maltose/mL·min respectively) in native pearl millet. Absence of β-amylase activity in native pearl millet (Pelembe *et al*., 2004) and sorghum (Taylor and Robbins, 1993) were also reported. Beta-amylase activity of commercial barley malt was reported to be 354.8 mg maltose/g dry matter (Tiwari, 2010) which was slightly higher than that of *Dalle* millet malt found in this study.

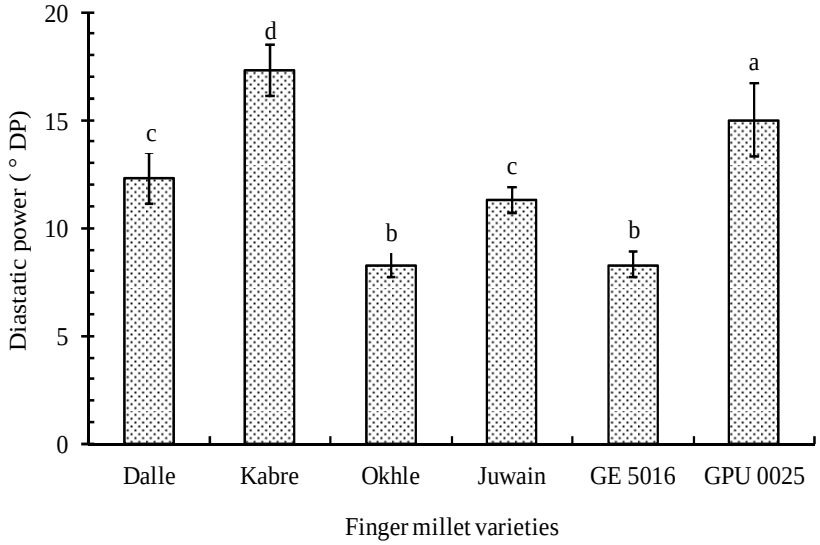


Fig. 5.20 Total diastatic power (DP) of millet malts germinated at 28 ± 1 ºC for 48 h. Values are the means (n = 3) ± SD. Bars sharing similar letters are not significantly different (p>0.05) by LSD.

Tiwari (2010) also reported α-and β-amylase activities in the range of 49.6 − 55.5 and 194.5 − 215.5 units/g dry matter in *Kabre-1* and *ACC#523-1* finger millet varieties respectively. Seed germination triggers several metabolic changes and in turn increases the activity of various enzymes including amylases. According to Bewley and Black (1983), β-amylases were present in an inactive latent form and were activated during seed germination. Daussant *et al.* (1994) reported that two distinct categories of cereal β-amylases have recently been recognized to exist. One is the classical endosperm β-amylase of the *Triticeae* species, barley, wheat, and rye; which is present in high amounts and at high activities in the ungerminated seeds. Another "tissue ubiquitous" form of enzymes present in much lower amounts and activities in all cereals appear to represent the entire enzyme complements of the seed of the non-*Triticeae* species. Zeigler (1995) who reported the lack of this enzyme in native grains also supported negligible α-amylase found in this study. B-amylase activity in Indian finger millet malts were in the range of 156 − 300 units (Shukla *et al*., 1986) which was in agreement to our findings. Pelembe *et al*. (2004) reported that both diastatic activity and β-amylase activity in pearl millet malt increased with germination time.

Diastatic activity was reported to increase with germination time in SE composite and SE 13 pearl millet varieties up to 6 days of germination at 28 – 30 ºC and decreased afterwards, while in the case of SE 2124 millet variety, diastatic power reached maximum in 2 days and decreased afterwards. Similar trend was also reported for α-

amylase activity in the three millet varieties. For SE composite pearl millet variety, β-amylase activity was reported to be maximum on day 2, while for SE 13 and SE 2124 millet varieties it reached at maximum on 4 and 8 days of germination respectively (Nzelibe and Nzelibe, 1995). According to Gimbi and Kitabatake (2002) the highest α-amylase activity in African finger millet was exhibited in finger millet malt germinated at 15 ºC for 9 days, and at 20 ºC for 6 days while the highest β-amylase activity being displayed in the malt germinated for 5 days at 30 ºC. However, in our experiment there was excessive root growth beyond 84 h of germination at 28 ± 1 ºC.

Wide variations in α- and β-amylase activities and total diastatic power (DP) among different millet malts could be due to their varying ability to produce gibberellins, the hormone, which is produced during germination of grain and diffused into the endosperm and aleurone layer. In the endosperm, they induce the synthesis of amylases (Dendy, 1995). Amylases are responsible for the generation of extract and fermentable extract during the conversion of starch to alcohol. There is no universally accepted specification for the sorghum and millet malts for their diastatic activity. However, a minimum specification of DP of 28 sorghum diastqtic unit (SDU)/g for malt for industrial sorghum or millet brewing appears to be widely used (Dewar *et al*., 1995). Dewar *et al*. (1997) reported that the diastatic activity of sorghum malt germinated at 30 ºC was highest (45 SDU/g) on day 4 and decreased to 39 SDU/g on day 6. The diastatic activity of millet malts obtained in this study cannot be directly compared with other reported values since the extent of DP is widely affected by millet variety, germination time, and temperature, steep-out moisture content, and method used in its determination. However, the total diastatic power of millet malts obtained in this study was lower to those reported for other cereal malts.

Effect of millet variety and germination time on carboxypeptidase activity is depicted in Fig. 5.21. Millet variety, germination time and their interaction all significantly affected carboxypeptidase activity of millet malt. Carboxypeptidase activity increased with germination time in *Kabre* millet variety with a maximum value of 134.2 units/g dry malt on 84 h of germination, while a maximum activity of 242.5 units/g dry malt was found on 48 h of germination in *Juwain* millet malt. In the case of *GPU 0025* millet variety, carboxypeptidase activity ranged from 66.6 to 73.4 units/g dry malt over the germination time of 48 – 84 h, and the values were statistically not different. Carboxypeptidase activity reached maximum at 72 h of germination and decreased

thereafter in *Okhle* and *GE 5016* millet with maximum activities of 60.1 and 42.4 units/g dry malt for the respective millet varieties.

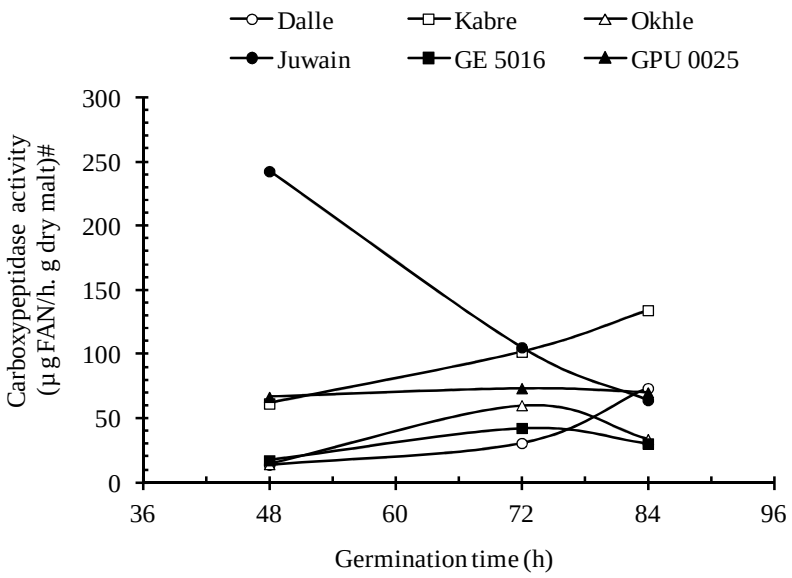


Fig. 5.21 Effect of millet variety and germination time on carboxypeptidase activity of millet malts. Values are the means of three replications.

#One unit of carboxypeptidase activity was defined as the production of 1 µg of free amino nitrogen (FAN) as glycine over 1 h of incubation at 40 ºC.

No remarkable difference in carboxypeptidase activity between 48 and 72 h germinated *Dalle* malt was observed with a maximum value of 73.4 units/g dry malt being found on 84 h of germination. Maximum carboxypeptidase activity of 242.5 units/g dry malt was found in 48 h germinated *Juwain* millet malt followed by 134.2 units/g dry malt in 84 h germinated *Kabre* millet malt. Carboxypeptidases are known to be responsible for the release of FAN from solubilized proteins during malting. Carboxypeptidase activity in ICSV 400 and KSV 8 sorghum malts prepared using 1 h of air-rest period during germination were reported to be 428 and 222.5 mg FAN/3h/g dry malt respectively (Okolo and Ezeogu, 1995) which were quite higher than those of *Dalle, Okhle, GPU 0025* and *GE 5016*, malts but were similar to those of *Kabre* and *Juwain* finger millet malts found in this study.

5.2.1.2 Effect on total free amino acids (TFAAs) and free amino nitrogen (FAN) contents of millet malts

Figs. 5.22 and 5.23 illustrate the effect of millet variety and germination time on total free amino acids (TFAA) and free amino nitrogen (FAN) contents in millet malts. Both the millet variety and germination time showed a significant effect on the TFAAs contents in millet malts. Except for *Juwain* millet, the TFAAs contents were not different ($p>0.05$) among other native millets and the values were in the range of 53.9 – 57.9 mg glycine equivalent/100 g dry malt while, *Juwain* millet variety had the highest TFAAs content (87.1 mg glycine/100 g dry malt) of all the millet varieties. TFAAs contents reached maximum at 48 h of germination and decreased significantly on further germination in all millet varieties. TFAAs contents in 48 h germinated millet malts were 188.7, 209.0, 178.8, 310.0, 195.3 and 186.2 mg glycine/ 100 g dry malt in *Dalle*, *Kabre*, *Okhle*, *Juwain*, *GE 5016* and *GPU 0025* millet varieties respectively. Statistical analysis indicated that *Juwain* millet malt had the highest TFAAs content (310.0 mg glycine/100 g dry malt) of all the malts, while the values among *Dalle*, *Okhle*, *GE 5016* and *GPU 0025* and between *Kabre* and *GE 5016* malts were not significantly different ($p>0.05$).

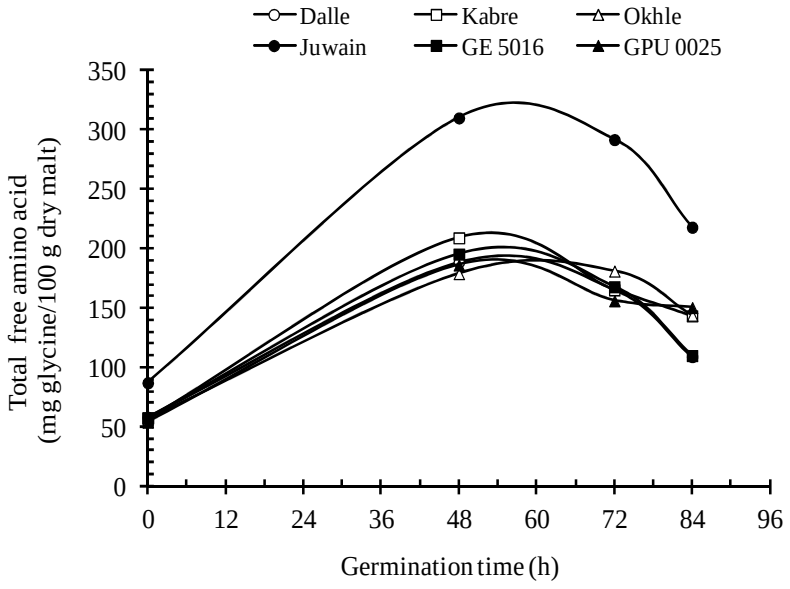


Fig. 5.22 Effect of millet variety and germination time on total free amino acids (TFAAs) contents of millet malts.

Juwain millet showed an intermediate interaction while rest of the millet varieties showed a strong interaction with germination time on TFAAs contents of malts (Fig 5.

22). A similar trend to that of TFAAs contents was also found for FAN contents in millet malts (Fig. 5.23). Except in *Juwain* millet, FAN contents in other native millet varieties were in the range of 10.1 – 10.9 mg glycine/100 g dry malt and the values were statistically not different, while a maximum FAN content of 16.4 mg glycine/100 g dry malt was found in *Juwain* millet malt. FAN content reached maximum at 48 h of germination and further germination resulted a significant reduction in all millet varieties. FAN contents in 48 h germinated malts were 35.2, 39.0, 33.4, 57.8, 36.4 and 34.7 mg glycine/100 g dry matter in *Dalle*, *Kabre*, *Okhle*, *Juwain*, *GE 5016* and *GPU 0025* millet varieties respectively. *Juwain* malt had the highest FAN contents of all the malts, while the values among *Dalle*, *Okhle*, *GE 5016*, and *GPU 0025*, and between *Kabre* and *GE 5016* malts were not different. *Juwain* millet showed an intermediate interaction, while all other millet varieties showed a strong interaction with germination time on FAN contents of malt. Hence, 48 h germinated *Juwain* millet resulted the maximum TFAAs and FAN contents in the malt.

Amino acid content is an important malt parameter for the growth and metabolism of yeast in malt wort. To increase fermentability and efficiency, malts with high levels of free amino nitrogen and amino acids are essential. Total soluble nitrogen and free amino acids increase with increasing germination time due to more extensive protein hydrolysis (Nie *et al*., 2010). Amino acid content plays a crucial role in yeast nutrition (Clapperton, 1971). FAN contents in millet malts were reported to vary from 87 to 155 mg/100 g by Morral *et al.* (1986) and the reported results were quite higher than those found in our experiment (maximum value of 57.8 mg glycine equivalent/100 g dry malt). The FAN content of the malt is the product of the catabolic processes, which degrade the storage proteins into new proteins in roots and shoots. Okolo and Ezeogu (1995) reported that the FAN content in ICSV 400 and KSV 8 sorghum malts were 181.1 and 140.8 mg% (db) using 1 h of air rest period during malting which were quite higher than those found for millet malts in this study. According to Pelembe *et al.* (2004) FAN increased with germination time in pearl millet. Similar trend was also reported in sorghum malt (Dewar *et al*., 1997; Morrall *et al*., 1986; Nout and Davis, 1982) and in finger millet malt (Nout and Davis, 1982). Adequate FAN content is necessary to support yeast growth during fermentation (Shayo *et al.*, 2001).

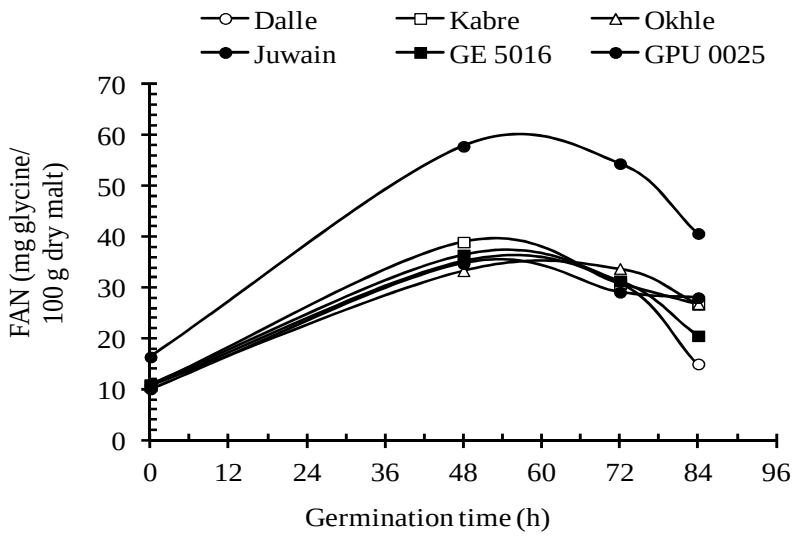


Fig. 5.23 Effect of millet variety and germination time on free amino nitrogen (FAN) contents of millet malts. Values are the means of three determinations.

5.2.1.3 Effect of finger millet varieties on chemical characteristics of their malts

5.2.1.3.1 Changes in carbohydrates during malting of finger millets

Chemical constituents of unmalted and malted finger millets were determined and the results are shown in Table 5.10.

Total reducing sugar

Total reducing sugar content in unmalted millets varied from 70.0 in *Dalle* to 130.0 mg dextrose/100 g dry matter in *GE 5016* millet, with a mean of 106.1 mg dextrose/100 g dry matter. Except in *Dalle*, there was no significant difference ($p>0.05$) on the content of total reducing sugar among the millet varieties. For malted millet, total reducing sugar varied from a minimum of 1410.0 mg dextrose/100 g dry matter in *GE 5016* millet malt to a maximum of 5953.3 mg dextrose/100 g dry matter in *Juwain* millet malt. The mean total reducing sugar content in malted millets was 2516.7 mg dextrose/100 g dry malt. Malting incurred a significant increase in total reducing content in all millet cultivars. The extent of increment differed with millet varieties, with *Juwain* millet malt having the highest increase of about 53 -fold, while *GE 5016* had the lowest increment of about 10-fold. Total reducing sugar contents between *GE 5016* and *Dalle* and between *GPU 0025* and *Okhle* millet malts did not differ ($p>0.05$) (Table 5.10). Our study revealed that the extent of total reducing sugar content in the malts was related with the degree of starch loss during germination. Increase in

reducing sugar content in malted finger millet was also reported by Nirmala *et al*. (2000).

Table 5.10 Chemical changes during malting of finger millets*

Parameters	Sample	Values for different finger millet varieties**					
		GPU 0025	*GE 5016*	*Juwain*	*Okhle*	*Kabre*	*Dalle*
Reducing sugar as glucose (mg %)	Millet	101.7^{a} (2.9)	130.0^{a} (30.0)	110.0^{a} (20.0)	105.0^{a} (5.0)	120.0^{a} (10.0)	70.0^{b} (10.0)
	Malt	2073.3^{c} (160.1)	1410.0^{d} (55.7)	5953.3^{e} (197.3)	1996.7^{c} (193.0)	2116.7^{b} (160.1)	1550.0^{d} (287.9)
Glucose (mg %)	Millet	50.8^{a} (5.9)	67.7^{a} (1.5)	46.8^{a} (3.2)	86.7^{a} (2.9)	47.0^{a} (4.2)	28.7^{a} (0.2)
	Malt	1340.0^{b} (334.2)	883.3^{c} (196.6)	4553.3^{d} (274.3)	1243.3^{b} (285.7)	1723.3^{e} (25.2)	990.0^{c} (165.2)
Starch (%)	Millet	72.93^{a} (3.54)	71.32^{a} (1.85)	79.86^{b} (0.18)	72.88^{a} (2.16)	71.88^{a} (3.07)	72.60^{a} (2.52)
	Malt	64.08^{c} (3.20)	67.12^{c} (1.81)	63.74^{c} (0.88)	66.80^{c} (2.05)	66.22^{c} (2.94)	65.22^{c} (1.95)
Amylose (%)	Millet	21.48^{a} (1.38)	22.22^{e} (0.76)	24.13^{d} (0.08)	21.36^{ae} (0.84)	20.42^{af} (1.17)	20.39^{af} (0.95)
	Malt	18.77^{c} (1.29)	17.87^{bc} (0.67)	19.27^{cf} (0.37)	16.62^{b} (0.73)	18.74^{cg} (1.14)	16.87^{b} (0.71)
Amylopectin (%)	Millet	51.45^{af} (2.16)	49.11^{bf} (1.09)	55.72^{d} (0.11)	51.52^{af} (1.32)	51.46^{af} (2.58)	52.21^{a} (1.57)
	Malt	45.30^{e} (1.91)	49.25^{bf} (1.14)	44.47^{e} (0.52)	50.18^{acf} (1.33)	47.47^{b} (1.80)	48.44^{bc} (1.23)
Total phenolics as gallic acid (mg%)	Millet	103.7^{a} (9.6)	229.2^{b} (15.9)	60.9^{c} (10.2)	165.1^{df} (10.6)	165.9^{d} (5.2)	87.3^{e} (5.5)
	Malt	148.6^{fh} (12.2)	247.8^{g} (8.8)	135.3^{hj} (7.5)	158.7^{df} (8.1)	185.9^{i} (6.8)	123.1^{j} (14.3)
Total flavonoids as rutin (mg%)	Millet	55.3^{a} (6.1)	141.7^{b} (20.3)	35.2^{a} (5.3)	95.8^{c} (3.1)	93.9^{c} (7.0)	52.2^{a} (10.8)
	Malt	98.0^{c} (15.4)	236.3^{d} (33.5)	50.1^{a} (7.0)	120.7^{bc} (8.7)	114.8^{bc} (1.7)	109.1^{c} (41.9)
Tannin as tannic acid (mg%)	Millet	227.1^{ae} (14.4)	566.0^{b} (11.9)	169.9^{c} (20.1)	373.3^{d} (12.1)	374.9^{d} (6.5)	245.7^{e} (16.5)
	Malt	212.2^{a} (12.5)	301.8^{f} (3.2)	173.7^{c} (1.1)	235.1^{e} (10.7)	245.2^{e} (7.5)	178.1^{c} (8.5)
Antioxidant activity (%)	Millet	55.39^{a} (2.82)	77.30^{b} (3.97)	18.23^{c} (3.02)	74.46^{b} (2.14)	71.62^{b} (5.07)	53.18^{ae} (6.59)
	Malt	30.25^{gd} (7.22)	73.05^{b} (15.42)	16.49^{c} (4.81)	42.83^{ef} (7.29)	40.69^{fd} (8.58)	28.86^{gc} (6.01)

*results are expressed as dry weight basis.

**values are the means of three replications. Figures in the parentheses are the standard deviations. Means followed by similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

Glucose

Glucose content in native millets ranged from 28.7 to 86.7 mg dextrose/100 g dry matter, with a mean of 54.6 mg dextrose/100 g dry matter; however, the values were not statistically different ($p>0.05$). Glucose content in malted millets was highest in *Juwain* (4553.3 mg dextrose/100 g dry matter), followed by *Kabre* (1723.3 mg dextrose/100 g dry matter), while it was lowest in *GE 5016* (883.3 mg dextrose/100 g dry matter), with a mean content of 1788.9 mg dextrose/100 g dry matter for the six millet malts. Malting led to a significant increase in glucose content in all millet varieties. A maximum glucose increment of 96-fold was observed in *Juwain* millet malt; while a minimum of 12-fold was found in *GE 5016* millet malt (Table 5.10). Glucose contents between *GPU 0025* and *Okhle* and between *GE 5016* and *Dalle* millet malts were not significantly different. From Table 5.10 it can be envisaged that the average ratios of total reducing sugar to glucose were 2:1 and 3:2 for native and malted millets respectively indicating that glucose was being utilized at a greater extent during seed germination.

Starch

Starch content in the native millets was in the range of 71.32 – 79.86% (db), with a mean content of 73.58% (db). *Juwain* millet had the highest starch content (79.86%, db) of all the millet varieties, while the values among other cultivars did not differ ($p>0.05$). Similarly, starch content in malted millets ranged from 63.74 to 67.12% (db), with a mean value of 65.53% (db); however, the values were not significantly different. Germination significantly reduced starch content in all millet varieties. A maximum starch loss of 20.19% was found in *Juwain* malt, while a minimum of 5.89% was found in *GE 5016* malt, with a mean loss of 10.77% among the six millet varieties. Sripriya *et al*. (1997) reported that the starch content of finger millet was 81% (db) which decreased to 71.3% (db) on germination at 30 °C for 24 h. Decrease in starch content by 33.85% after 4 days of malting was reported in finger millet (Nirmala *et al*., 2000). Reductions in starch content ranging from 33 to 58.4% in 96 h germinated sorghum cultivars were also reported by Subramanian *et al.* (1992). Morall and Briggs (1978) reported a 65% decrease in starch in germinated barley. The decrease in starch content in the grain was due to hydrolysis by the native enzymes (α-and β- amylases) during germination resulting increase in reducing sugars (Dewar *et al*., 1995).

The minimum amylose content in native millets was 20.39% (db) in *Dalle*, while the maximum was 24.13% db in *Juwain*, with a mean content of 21.67% (db). Germination significantly decreased amylose in all millet malts compared to their respective unmalted millets. Amylose content in malted millets varied from 16.62% (db) in *Okhle* to 19.27% (db) in *Juwain*, with a mean value of 18.02% (db). Amylose reduction ranged from 8.23 to 22.19%, with the minimum reduction being found in *Kabre* and the maximum in the *Okhle* millet malt. This study revealed that amylose content accounted for 28.19 to 31.25% of the total starch content in native millet, with a mean of 29.47%. Similarly, in malted millets, amylose accounted for 24.88 to 30.23% of the total starch, with a mean of 27.53%. Amylose contents in sorghum and millets were: proso millet 28%, sorghum 24%, pearl millet 21.1%, foxtail millet 17.5%, kodo millet 24 .0% and finger millet 16.0% (wb) (Zarnkow *et al*., 2007) and the obtained results appeared a bit higher than that reported for finger millet.

Amylopectin contents in native millets varied between 49.11% (db) in *GE 5016* millet and 55.72% (db) in *Juwain* millet, with a mean content of 51.91% (db). Similarly, malted millets had amylopectin contents ranging from 44.47% (db) in *Juwain* to 50.18% (db) in *Okhle*, with a mean content of 47.52% (db). Except for *GE 5016* and *Okhle* millets, malting significantly decreased ($p<0.05$) amylopectin in all other varieties. Maximum amylopectin reduction was observed in *Juwain* millet malt (20.19%), while a minimum reduction was found in *Dalle* malt (7.22 %). It was observed that, amylopectin content accounted for 68.75 to 71.81% of the total starch in native finger millet, with a mean of 70.53%; while it accounted for 69.68 to 75.12% in malted millets, with a mean of 72.45%. Moreover, amyloses to amylopectin ratios in native and malted millet starch were found to be 29:71 and 28:72 respectively. Zarnkow *et al*. (2007) noted that Proso millet starch contained 72% amylopectin, but the data for finger millets were scarce.

5.2.1.3.2 Changes in phenolic compounds and antioxidant activity

Total phenolics

Total phenolics (TPs) contents greatly varied among native millet cultivars ranging from 60.9 mg gallic acid equivalent (GAE)/100 g dry matter (DM) in *Juwain* to 229.2 mg GAE/100 g DM in *GE 5016*, with a mean content of 135.4 mg GAE/100 g DM. TPs contents between *Okhle* and *Kabre* millets were not different ($p>0.05$), while they were significantly different among other native millets.

Similarly, TPs contents in malted millets ranged from 123.1 mg GAE/100 g DM in *Dalle* to 247.8 mg GAE/100 g DM in *GE 5016*, with a mean of 166.6 mg GAE/100 g DM. Except in *Okhle*, malting significantly increased ($p<0.05$) TPs contents in all other millet varieties. The minimum TPs increment was 8.12% in *GE 5016* malt, while the maximum was 122.17% in *Juwain* malt. Analogous result of increase in phenolics during malting of barley was reported by Dvorakova *et al.* (2008). Nwanguma and Eze (1996) also observed TPs increments between 7- and 14- fold in sorghum cultivars. Contrary to our findings, Sripriya *et al.* (1997) reported a decrease in total phenolics in finger millet malt. Decline in phenolics on malting of millet were also reported by Chethan *et al.* (2008) and Chukwura and Muller (1982).

Increased in TPs during germination in this study may be expected as a result of loss of dry matter as well as hydrolysis of condensed tannins due to germination which can be justified for decrease in tannin on malting (Table 5.10). Furthermore, it can be anticipated that during germination different enzymes were produced and contributed to the modification of grain composition resulting in the release of bound phenolics and facilitate extraction of phenolics than that of native grain. According to Maillard and Berset (1995), increase in total phenolics on malting may be due to enzymatic release of bound phenolic compounds during seed germination. Maillard *et al.* (1996) reported that polyphenols in millet occur both in free and in bound forms. Increase in malt TPs may be due to the action of induced esterase activity on bound phenolics, which act on various phenolic acid esters linked to either arabinoxylans or other non-strach polysaccharides.

Total flavonoids

Total flavonids (TFs) contents in unmalted and malted millets ranged from 35.2 mg rutine equivalent (RE)/100 g DM in *Juwain* to 141.7 mg RE/100 g DM in *GE 5016* millet and from 50.1 mg RE/100 g DM in *Juwain* to 236.3 mg RE/100 g DM in *GE 5016* millet malt respectively (Table 5.10). The mean TFs contents in six native and malted finger millets were 79.0 and 121.5 mg RE/100 g DM respectively. The TFs content was not affected by malting in *Juwain*, *Okhle* and *Kabre* millets, while it was significantly increased ($p<0.05$) in other millet varieties. Results (Table 5.10) indicated that the TFs increment was highest in *Dalle* malt (109%), followed by in *GPU 0025* (77.22%), while it was lowest in *GE 5016* millet malt (66.76%). It was further found that both the TPs and TFs contents in malted millets were related with that of their

contents in the native millets (i.e. the higher the TPs and TFs content in the native millets, the higher their contents in the malts).

Tannin

The tannin contents in native millets ranged from 169.9 mg tannic acid equivalent (TAE)/100 g DM in *Juwain* to 566.0 mg TAE/100 g DM in *GE 5016* millet, with a mean of 326.2 mg TAE/100 g DM. Similarly, tannin content in malted millets was in the range of 173.7 (*Juwain*) − 301.8 mg TAE/100 g DM (*GE 5016*), with a mean content of 224.6 mg TAE/100 g DM. Except in *GPU 0025* and *Juwain*, malting significantly decreased tannin contents in other millet varieties. The maximum tannin reduction of 46.7% was observed in *GE 5016* malt, while a minimum of 27.5% was found in *Dalle* malt. The results of tannin contents in native millet varieties were analogous to those reported by Odoemelam and Osu (2009) in Nigerian millet variety (0.48 − 0.53%), Wadikar *et al.* (2006) in three Indian hill region finger millets (average value of 0.34%) and Udayasekhara and Deosthale (1988) in brown finger millet varieties (0.35 − 2.39%). Reichert *et al*. (1980) also noted a 25% tannin reduction in germinating barley and they noted that the loss could partly be due to polymerization of the tannins in water and/or their possibly carbohydrates and proteins. Larger tannin polymers or complexes of tannin with other biopolymers would be insoluble and thus not extractable.

Antioxidant activity

Large variations in antioxidant activity among unmalted (18.23 to 77.30%) and malted (16.49 to 73.05%) finger millets were observed.

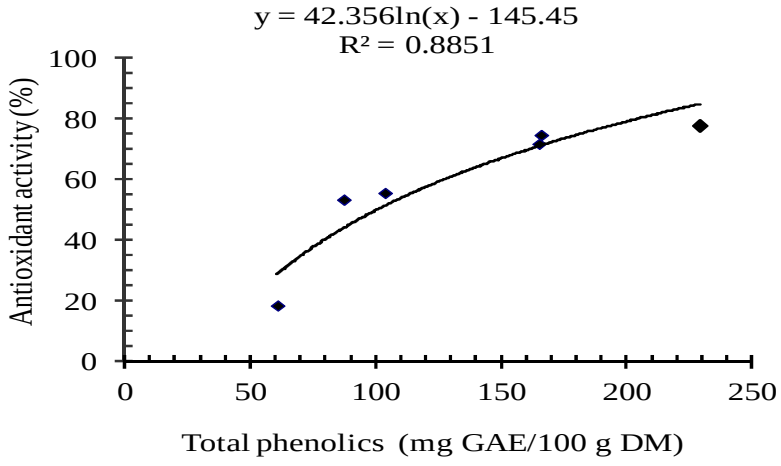


Fig. 5.24 Relationship between total phenolics and antioxidant activity in native millets.

Except for *GE 5016* and *Juwain* native millets, malting significantly decreased ($p<0.05$) antioxidant activity in other varieties. The antioxidant activity of native millet was exponentially related to its TPs content (Fig. 5.24), however, this relationship was not apparent in malted millet. In contrast to our findings, increased in DPPH antiradical power in barley malt was reported by Dvorakova *et al*. (2008). Of the various chemical characteristics of malts, FAN, tannin and total phenolic contents can be taken as prominent ones, since FAN is essential for the growth of yeast during brewing and excessive presence of tannin and total phenolics will pose problem of beer haze formation.

5.2.1.4 Effect of millet varieties on chemical characteristics of their malt extracts

Millet malts prepared by germinating for 48 h at 28 ± 1 ºC were used for extract of malt analysis and the results are shown in Table 5.11. All extracts were found to be aromatic. Starch-iodine test showed negative results (yellow to brick red coloration) indicating the absence of starch in malt extracts. Except for *Juwain malt*, filtration rate was normal in other malt extracts. Extracts TSS ranged from 7.90 to 8.9 ºBx, with a mean value of 8.22 ºBx. There was a significant difference ($p<0.05$) in color among malt extracts. Maximum color was recorded in *GE 5016* millt malt extract (5.78 EBC units) while a minimum value of 2.77 EBC units was found in *Dalle malt*. The extract color was within the range reported for different British floor malts (2.5 − 7.0 EBC units). Turbidity significantly varied ($p<0.05$), ranging from 9.97 FTU in *Dalle* malt extract to 24.10 FTU in *Juwain* malt extract, with a mean of 16 FTU. Extract pH was in the range of 5.76 − 6.31. The pH of *GPU 0025* and *Kabre*, and that of *GE 5016* and *Okhle* malt extracts were not different ($p>0.05$).

Total free amino acids (TFAAs) varied widely among the malt extracts. The lowest TFAAs was 13.9 mg glycine equivalent (GE)/100 mL in *GE 5016*, while the highest was 48.4 mg GE/100 mL in *Juwain* millet malt extract. TFAAs contents between *GPU 0025* and *Kabre*, and between *Okhle* and *Dalle* malt extracts were not different ($p>0.05$). It was found that TFAAs content in the extract was related with that of its content in the malt [i.e. malts containing higher TFAAs also resulted higher TFAAs in the extract. FAN content (glycine equivalent) lied between 2.6 and 9.0 mg/100 mL, with minimum content being found in *GE 5016* extract and the maximum in *Juwain extract*.

Table 5.11 Physico-chemical characteristics of finger millet malt extracts

Parameters	Mean values*					
	GPU 0025	*GE 5016*	*Juwain*	*Okhle*	*Kabre*	*Dalle*
TSS (ºBx)	8.12^{ac} (0.10)	7.90^{b} (0.10)	8.90^{d} (0.10)	8.03^{ab} (0.06)	8.10^{a} (0.10)	8.27^{c} (8.27)
Color (EBC unit)	3.92^{a} (0.03)	5.78^{b} (0.02)	4.38^{c} (0.03)	5.67^{d} (0.01)	3.17^{e} (0.02)	2.77^{f} (0.02)
Turbidity (FTU)	13.05^{a} (0.66)	21.49^{b} (0.30)	24.10^{c} (0.14)	15.64^{d} (0.09)	11.73^{e} (0.12)	9.97^{f} (0.16)
pH	6.16^{a} (0.03)	6.31^{d} (0.01)	5.76^{c} (0.01)	6.29^{d} (0.01)	6.14^{a} (0.01)	6.21^{b} (0.01)
TFAA as glycine (mg /100 mL)	36.7^{a} (3.5)	13.9^{b} (0.9)	48.4^{c} (2.4)	25.8^{d} (0.5)	33.6^{a} (1.8)	24.1^{d} (2)
FAN as glycine (mg/100 mL)	6.8^{a} (0.5)	2.6^{b} (0.1)	9^{c} (0.3)	4.8^{d} (0.1)	6.2^{a} (0.3)	4.5^{d} 90.3)
Glucose (g/100 mL)	1.62^{a} (0.03)	1.22^{b} (0.05)	2.51^{c} (0.01)	1.52^{d} (0.12)	1.78^{e} (0.03)	1.48^{f} (0.01)
Reducing sugar (g maltose/100 mL)	5.48^{a} (0.13)	4.50^{b} (0.13)	6.93^{c} (0.22)	5.27^{a} (0.25)	6.27^{d} (0.39)	5.13^{a} (0.02)
Extract yield of malt (%, db)	83.67^{a} (0.29)	87.80^{b} (0.26)	88.83^{c} (0.15)	82.17^{d} (0.67)	82.10^{d} (0.46)	80.20^{e} (0.26)
Viscosity (cP)	1.21^{ac} (0.02)	1.22^{a} (0.01)	1.24^{b} (0.01)	1.22^{a} (0.01)	1.20^{c} (0.01)	1.18^{d} (0.01)
Starch-iodine test	−	−	−	−	−	−
Filtration rate	++	++	+	++	++	++
Aroma	√	√	√	√	√	√

*values are the means of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

Note: − : negative test, ++ : normal rate, + : slow rate, √ : characteristics aroma.

Glucose content differed significantly ($p<0.05$) among the malt extracts, ranging from 1.22 g/100 mL in *GE 5016* extract to 2.51 g/100 mL in *Juwain* malt extract. Similarly, millet variety had a significant effect ($p<0.05$) on the total reducing sugar content in the extract. The reducing sugar content was in the range of 4.5 − 6.93 g maltose/100 mL, where the values among *GPU 0025*, *Okhle* and *Dalle* malt extracts were not different. Extract's viscosity was in the range of 1.18 − 1.24 cP, with a mean of 1.21 cP.

The highest viscosity observed in *Juwain* malt extract could be attributed to the presence of higher soluble solids in the extract (Table 5.11). Extract yield of malt ranged from 80.20% (db) in *Dalle* to 88.83% (db) in *Juwain* malt extract. Except between *Okhle* and *Kabre*, the extract yields were significantly different among other malts. Despite lower β-amylase and diastatic power of *Juwain* millet malt it exhibited the highest extract yield of all the malts studied. Regarding the results of malt extract analysis, all extracts had color well above the minimum required value (2.5 EBC units). Although, clarity of *Dalle* malt extract was significantly superior (i.e. the lowest turbidity value), it had lower FAN content and extract yield (Table 5.11). Hence, in order to select the best millet malt for further study, mashing was carried out using Dalle, *Kabre* and *Juwain* millet malts.

5.2.1.5 Effect of millet varieties on the chemical properties of their malt worts

Chemical characteristics of worts derived from *Dalle*, *Kabre* and *Juwain* millet malts are shown in Table 5.12. TSS, extract, total free amino acids and FAN contents of the worts derived from *Juwain* malt were significantly higher ($p<0.05$) than those of *Dalle* and *Kabre* malts, while the values between the latter two did not differ significantly. Wort pH was in the range of 5.44 − 5.66, with an average value of 5.53. Total reducing sugar was maximum in *Kabre* malt wort (10.45 g maltose/100 mL); while a minimum of 7.64 g maltose/100 mL was found in *Dalle* malt wort. Wort composition varies according to the ingredients used in its preparation. Berger and La Berger (1985) reported that the extract, pH and FAN contents of various worts intended for the production of North American lager beer were 10.8 − 12.5 °P, 5.2 − 5.7 and 140 − 260 mg/L respectively which are comparable to our results. The results of wort FAN contents are in close agreement with those reported by Eneje *et al.* (2001), who reported the FAN content in the range of 14.8 – 16.8 mg/100 mL in millet (*Pennisetum maiwa*) malt worts.

The pH and FAN contents in typical UK wort derived from finger millet and barley malts combination (70:30 to 50:50) were reported to be 6.0 − 6.2 and 88 − 62 mg/L respectively. pH and FAN of an all-malt wort were 5.8 and 226 mg/L respectively (Venkatanarayana *et al.*, 1979). Similarly, a FAN content of 10.7 mg/100 mL was also reported in buckwheat malt wort (Nic Phiarais *et al.*, 2005). Analogous results of pH in different sorghum malt worts (5.39 − 6.09) were also reported by Okrah (2008). Hough (1985) also reported total free amino acids content of 1.65 g/L in typical UK wort.

Therefore, the obtained results of pH, extract, FAN and reducing sugar contents of millet malt worts were found to be within the reported range for different cereal malt worts. Hence, based on the results of malt analyses; *Kabre* finger millet was found superior of all millet varieties for malting to produce lager beer.

Table 5.12 Chemical properties of finger millet malt worts

Parameters	Values for different millet malt worts*		
	Dalle	*Kabre*	Juwain
TSS (°Bx)	$12.43^{a} \pm 0.06$	$12.43^{a} \pm 0.06$	$13.07^{b} \pm 0.12$
pH	$5.44^{a} \pm 0.01$	$5.66^{b} \pm 0.01$	$5.49^{a} \pm 0.01$
Extract (g/100 g)	$12.00^{a} \pm 0.10$	$12.20^{a} \pm 0.10$	$12.70^{b} \pm 0.10$
TFAA as glycine (mg/100 mL)	$104.90^{a} \pm 0.90$	$106.00^{a} \pm 0.70$	$108.00^{b} \pm 0.30$
FAN as glycine (mg/100 mL)	$19.50^{a} \pm 0.20$	$19.80^{a} \pm 0.10$	$20.30^{b} \pm 0.10$
Reducing sugar as maltose (g/100 mL)	$7.64^{a} \pm 0.10$	$10.45^{b} \pm 0.04$	$9.66^{c} \pm 0.36$

*values are the means (n = 3) ± S.D. Means followed by similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

5.2.2 Proximate and mineral compositions of unmalted and malted finger millet (variety *Kabre*)

5.2.2.1 Proximate composition of unmalted and malted finger millet

Table 5.13 shows the changes in proximate composition and mineral contents during malting of finger millet (variety *Kabre*). Crude protein contents in native millet, millet $malt_1$ and millet $malt_2$ were 9.80, 9.85, and 8.26% (db) respectively. The protein contents between millet and millet $malt_1$ were statistically not different ($p>0.05$), while the values were significantly higher than that of millet $malt_2$. On the contrary, increase in protein content from 8.6 to 11.8% (db) was reported by Opoku *et al.* (1981) in millet (*Pennisetum typhoides*), while decrease in protein content from 7.0 to 5.8% (db) in finger millet (Malleshi and Klopfenstein, 1998) and from 11.1 to 9.7% (db) in foxtail millet (Choudhury *et. al*., 2010) were reported.

Table 5.13 Proximate composition of native and malted finger millets (% db)

Parameters	Values*		
	Millet	$Malt_1$	$Malt_2$
Crude protein	9.80 (0.49)[a]	9.85 (0.13) [a]	8.26 (0.27)[b]
Crude fat	2.73 (0.21) [a]	2.29 (0.06) [b]	2.28 (0.03) [b]
Total ash	3.05 (0.32) [a]	2.13 (0.06) [b]	2.03 (0.15) [b]
Crude fiber	3.00 (0.07) [a]	3.65 (0.04) [b]	3.71 (0.01) [b]
Total carbohydrate**	84.42 (0.20) [a]	85.73 (0.18) [b]	86.00 (0.41) [b]

*values are the means of triplicate determinations. Figurers in the parentheses are the standard deviations. Means sharing similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

**: by difference

$Malt_1$: Green malt kilned at 50 ± 2 ºC.

$Malt_2$: Green malt kilned using standard barley malt kilning procedure (Matz, 1991).

Crude fat contents in native millet, $Malt_1$ and $Malt_2$ were 2.73, 2.29, and 2.28% (db) respectively. Significantly lower ($p<0.05$) crude fat contents were found in malts than its raw counterpart, whereas the values between millet malts were not different. There are conflicting reports about the changes in crude fat on malting. Opoku *et al.* (1981) and Choudhury *et al.* (2010) reported a decrease in crude fat content of up to 66.7% on malting of millets, while Malleshi and Klopfenstein (1998) and Tiwari (2010) did not find noticeable difference in Nepalese finger millets. In cereals, fat content is reported to be more in outer seed coat, hence higher fat content in unprocessed cereal (MacMaster *et al*., 1971). Hydrolysis of lipid and oxidation of fatty acids take place during seed germination. The hydrolyzed products do not accumulate in the seed, but the glycerol becomes a part of carbohydrate pool and the fatty acids are oxidized through α- and β-oxidation, resulting in decrease in crude fat content (Mayer and Mayber, 1963). Total ash contents were found to be 3.05, 2.13, and 2.03% (db) in unmalted millet, $Malt_1$ and $Malt_2$ respectively. There was a significant reduction in total ash content in both malts compared to unmalted millet but the values between malts did not differ. Analogous results of decrease in ash content ranging from 9.1 to 21.6% were also reported by Malleshi and Klopfenstein (1998), Choudhury *et al*. (2010) and Opoku

et al. (1981). Crude fiber contents were found to be 3.00, 3.65 and 3.71% (db) in native millet, $malt_1$ and $Malt_2$ respectively and the values between malts were not statistically different but they were significantly lower compared to ungermnated millet. Similar results were also reported by Opoku *et al.* (1981) in millet (*Pennisetum typhoides*), Shyao *et al.* (2001) in five Tanzania finger millets varieties, Malleshi and Klopfenstein (1998) in Indian finger millet (var. Indaf, brick red color) and Tiwari (2010) in Nepalese finger millet varieties. Contrary to our results, Choudhury *et al*. (2010) reported up to 12.5% decrease in crude fiber on malting of yellow and purple foxtail millets. Increase in crude fiber content may be attributed to the loss of soluble solids during steeping and dry matter during germination. Total carbohydrate contents in native millet, $Malt_1$ and $Malt_2$ were 84.42, 85.73, and 86.00% (db) respectively. Malting significantly increased total carbohydrate content, whereas malt kilning method had insignificant effect ($p>0.05$) on total carbohydrate. Increase in carbohydrate content on malting was also reported by Choudhury *et al.* (2010) in Indian foxtail millets, while Okopu *et al.* (1981) reported about 10% reduction in carbohydrate on malting of millet (*Pennisetum typhoides*).

5.2.2.2 Mineral composition of unmalted and malted finger millet (variety *Kabre*)

Table 5.14 shows the mineral composition of native and malted finger millets. Phosphorus decreased by about 14 %, while calcium increased by 15 % on malting. Nithya *et al.* (2006) also reported 15% decrease in phosphorus in pearl millet malt. Iron content in unmalted millet was 11 mg/100 g dry matter (DM) and increased by about 84%, while no appreciable change in manganese occurred on malting. Increase in iron content up to 5% in pearl millet malt (Nithya *et al*., 2006) and up to 1.6-fold in finger millet malts (Samantray *et al*., 1989) have been reported. Malting increased sodium content by about 9%, while it decreased potassium content by about 60% compared to unmalted one. Samantray *et al.* (1989) also reported about 46% increase in sodium and 29% decrease in potassium contents in Indian finger millet malts. Both zinc and magnesium contents decreased ($p<0.05$) from 3.2 to 2.6 and from 260.3 to 244.1 mg/100 g DM respectively on malting. Nithya *et al.* (2006) also reported about 16 and 26% reduction in zinc and magnesium contents respectively in pearl millet malts.

Table 5.14 Mineral composition of native and malted finger millet (mg/100 g DM)

Minerals	Values*	
	Native millet	Malted millet**
Phosphorous	273.0 (8.0)[a]	235.0 (9.0)[b]
Calcium	247.0 (2.1)[a]	283.4 (4.8)[b]
Iron	11.0 (0.2)[a]	20.2 (0.3)[b]
Manganese	41.7 (0.2)[a]	42.6 (0.9)[a]
Sodium	271.9 (1.8)[a]	297.0 (5.3)[b]
Potassium	619.5 (4.0)[a]	248.7 (4.4)[b]
Zinc	3.2 (0.2)[a]	2.6 (0.1)[b]
Magnesium	260.3 (1.1)[a]	244.1 (4.1)[b]

*values are the means of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by similar superscripts in a row are not significantly different (p>0.05) by LSD.

**Green millet malt kilned at 50 ± 2°C for 24 h.

5.2.3 Effects of kilning methods on enzyme activities, FAN contents and extract quality of finger millet malts

Kabre millet was soaked for 12 h, germinated for 48 h at 28 ± 1 ° C, and kilned by three different methods as follows:

Method 1: Drying at a constant temperature of 50 ± 2 ºC up to 7% moisture content.

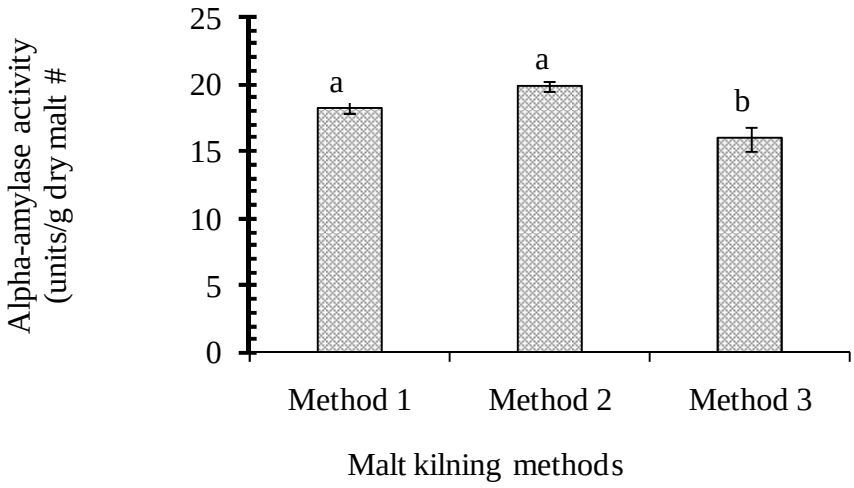
Method 2: First drying at 50 ± 2 ºC to 24% moisture content and final drying at a temperature of 62 ± 2 ºC.

Method 3: First drying at 50 ± 2 ºC to 24% moisture content and final drying at a temperature of 80 ± 2 ºC.

The malts were analyzed for amylase and carboxypeptidase activities, FAN contents and chemical properties of malt extracts.

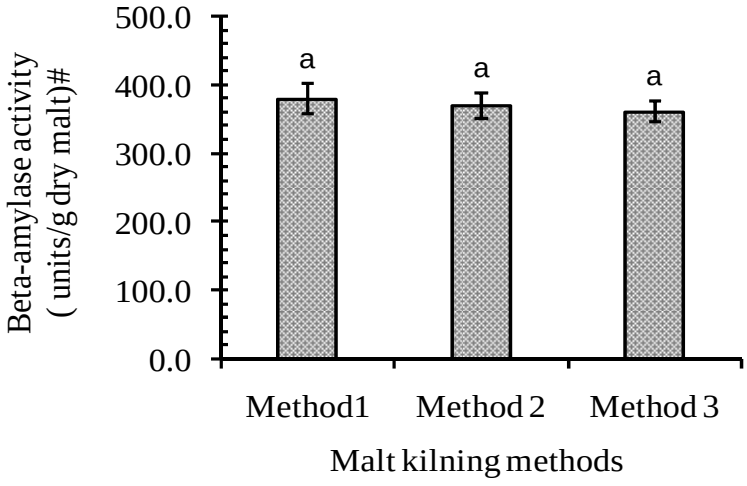
5.2.3.1 Effects of kilning methods on enzymes activity and FAN content of millet malts

Alpha-amylase, beta- amylase and carboxypeptidase activities and FAN contents of millet malts kilned by different methods were analyzed and the results are shown in Fig. 5.25.



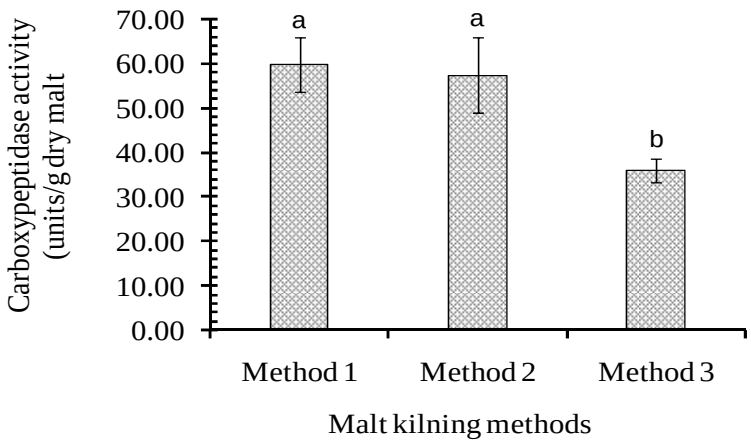
(A)

#One unit of α-amylase activity was defined as one unit decrease in OD at 620 nm per min under the assay conditions.



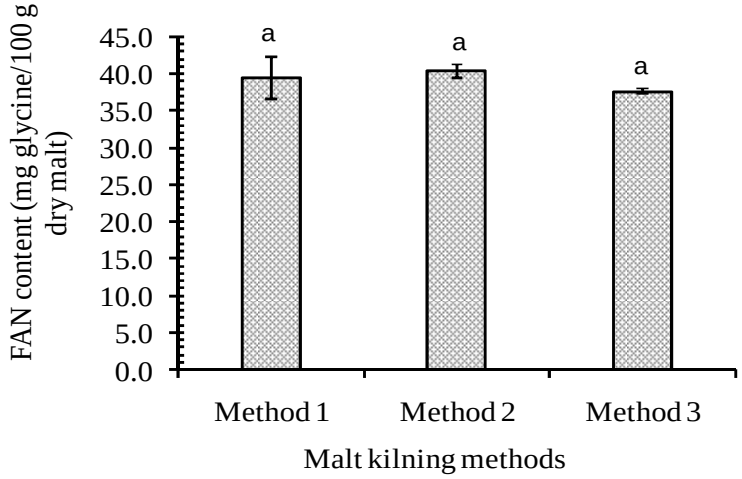
(B)

#One unit of β-amylase activity was defined as the production of 1 mg of maltose over 30 min of incubation under the experimental conditions.



(C)

[#]One unit of carboxypeptidase activity was defined as the production of 1 µg of FAN (as glycine) per hour under ther assay conditions.



(D)

Fig. 5.25 Effects of kilning methods on (A): α- amylase activity, (B): β-amylase activity (C): Carboxypeptidase activity and (D): FAN content of finger millet malts.

Kilning methods

Method 1: Drying at a constant temperature of 50 ± 2 ºC.

Method 2: First drying to 24% moisture content at 50 ± 2 ºC and final drying at 62 ± 2 ºC.

Method 3: First drying at 50 ± 2 ºC to 24% moisture content and final drying at 80 ± 2 ºC.

Alpha-amylase activity of malts kilned by Method 1, Method 2 and Method 3 were 18.26, 19.81 and 15.93 units/g dry malt respectively (Fig. 5.25A).

Statistical analysis showed that kilning method had a significant effect ($p<0.05$) on α-amylase activity of malt. LSD test indicated that α-amylase activity of malts kilned by Method 1 and Method 2 were not significantly different ($p>0.05$) but the malt kilned by Method 3 had significantly lower α-amylase activity of all the malts. Beta-amylase activity of malts was in the range of 360.8 – 379.6 units/g dry malt and the values were statistically not different (Fig. 5.25B). Carboxypeptidase activities were found to be 59.7, 57.4, and 35.7 units/g dry malt for malts kilned by Method 1, Method 2 and Method 3 respectively. Malts kilned by Method 1 and Method 2 had statistically similar carboxypeptidase activity but the one kilned by Method 3 had significantly lower carboxypeptidase activity than those of former two methods (Fig. 5.25 C) indicating that higher kilning temperature was detrimental to carboxypeptidase activity. FAN content of malts ranged from 37.6 to 40.4 mg glycine equivalent/100 g dry malt but the values were statistically not different (Fig. 5.25 D).

5.2.3.2 Effect of kilning methods on chemical characteristics of malt extracts

Finger millet malts kilned by different methods and commercial barley malts were subjected for extracts of malt analyses and the results are shown in Table 5.15. All millet malt extracts were aromatic. The extract obtained from Malt 3 was more aromatic, while that of barley malt had grassy odor. pH of millet malt extracts was in the range of 5.63 – 5.73 and the values were not different ($p>0.05$) but they were significantly higher than that of barley malt extract. TSS of malt extracts were in the range of 7.73 – 8.13 ºBx and the values were not significantly different. Malt kilning method had a significant effect on the color of the extracts and increasing temperature increased the extract color. Barley malt extract had the lowest color (4.60 EBC units) of all the malt extracts. FAN contents of extracts obtained from millet malts kilned by Method 1 and Method 2 were not different but the values were significantly higher than that obtained by Method 3.

Barley malt extract had the highest ($p<0.05$) FAN content (16.1 mg glycine/100 mL) of all the malt extracts. Total reducing sugar content of the malt extracts was in the range of 6.09 – 7.11 g maltose/100 mL but the values were not significantly different from each other. Similarly, kilning methods did not have significant effect on glucose content of millet malt extract (2.05 – 2.09%, m/v) but the values were significantly higher than that of barley malt extract (0.83%, m/v). Millet malts kilned by Method 2 and Method 3 resulted comparable extract gravity and extract yield to that of

commercial barley malt extract, but the extract obtained from millet malt kilned by Method 1 had the lowest gravity (6.83 °P) and extract yield (62.2% db) of all the malt extracts.

Table 5.15 Effects of kilning methods on the chemical characteristics of millet malts

Parameters	Mean values*			
	Method 1	Method 2	Method 3	Barley malt
Odor	Aromatic	Aromatic	Strong aromatic	Grassy
pH	5.63 (0.06)[a]	5.63 (0.06) [a]	5.73 (0.06) [a]	5.53 90.06) [b]
TSS, ºBx	7.73 (0.06) [a]	8.03 (0.06) [a]	8.03 (0.06) [a]	8.13 (0.23) [a]
Color, EBC unit	4.80 (0.04) [a]	5.16 (0.03) [b]	5.70 (0.04) [c]	4.60 (0.05) [d]
FAN, mg% as glycine	9.1 (0.4) [a]	8.9 (0.2) [a]	7.1 (0.1) [b]	16.1 (0.9) [c]
Total reducing sugar, as maltose, % m/v	6.87 (0.12) [a]	7.11 (0.15) [a]	6.59 (0.12) [a]	6.09 (0.33) [a]
Glucose, % m/v	2.06 (0.01) [a]	2.09 (0.07) [a]	2.05 (0.05) [a]	0.83 (0.06) [b]
Gravity, ºPlato	6.83 (0.07) [a]	8.28 (0.09) [b]	7.87 (0.41) [b]	7.73(0.48)[b]
Extract, % db	62.22 (4.04) [a]	78.07 (0.98) [b]	73.29 (4.09) [b]	75.41(5.03) [b]

*values are the means of three replications. Figures in the parentheses are the standard deviations. Means followed by similar superscripts in a row are not significantly different by LSD ($p>0.05$).

Kilning Methods

Method 1: Drying at a constant temperature of 50 ± 2ºC.

Method 2: First drying at 50 ± 2ºC to 24% moisture content and final drying at a temperature of 62 ± 2ºC.

Method 3: First drying at 50 ± 2ºC to 24% moisture content and final drying at a temperature of 80 ± 2ºC.

5.2.4 Effect of Gibberellic acid (GA_3) treatment on enzymatic activity and chemical properties of finger millet malts

5.2.4.1 Effect on amylase activity and FAN contents

Finger millets of different varieties were malted as described earlier. During germination, 5 ppm of gibberellic acid solution in water was sprayed on to the bed of finger millet twice a day and germinated for different times.

Alpha- and beta- amylase activities and FAN contents in the malts were determined. Changes in α-amylase activities with time in different finger millet varieties treated with 5 ppm of gibberellic acid are depicted in Fig. 5.26.

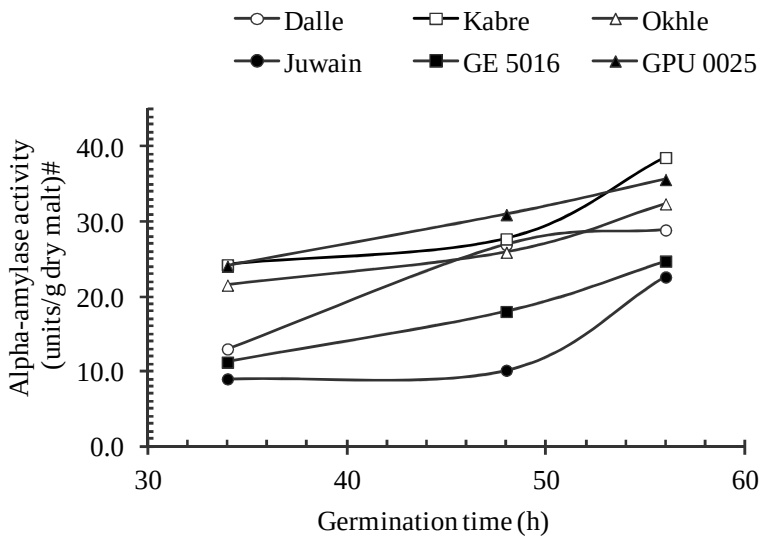


Fig. 5.26 Effect of varieties and germination time on alpha-amylase activity in gibberellic acid treated finger millet malts. Values are the means of three determinations.

#one unit of alpha-amylase activity was defined as one unit decrease in optical density at 620 nm per min under the assay conditions.

From Fig. 5.26 it is apparent that alpha-amylase activity increased significantly with germination time in all millet varieties. Moreover, there was a wide variation in varietal response to gibberellic acid (GA_3) in triggering alpha-amylase production. The alpha-amylase activities in 34, 48 and 56 h germinated millet malts were 13.01, 26.94 and 28.89 units/g dry malt in *Dalle* millet malt respectively, while those of *Kabre* malts had alpha amylase activity of 24.25, 27.69 and 38.57 units/g dry malt for the respective germination times. In *Dalle* millet malt, alpha-amylase activity increased abruptly at 48 h of germination and it did not change appreciably on further germination. In *Kabre* millet, alpha-amylase activity increased steadily throughout the germination period and showed a strong response to GA_3 treatment. The alpha amylase activities in *Okhe* and *Juwain* millet malts germinated for 34, 48, and 56 h were 21.52, 25.91 and 32.38; and 9, 10.15 and 22.61 units/g dry malt respectively. In *Juwain* millet, the alpha amylase activity did not change remarkably over the germination time from 34 to 48 h, whereas it did increase by about 10 units on 56 h of germination suggesting that it had longer incubation time. Similarly, alpha amylase activities in 34, 48 and 56 h germinated *GE*

5016 millet variety were 11.23, 18.01 and 24.72 units/g dry malt respectively, whereas that of *GPU 0025* millet had alpha amylase activates of 24.05, 30.94 and 35.63 units/g dry malt for the respective germination times. It was found that finger millet varieties having higher alpha-amylase activities during the initial stage of germination also showed higher activity at the end of germination. From Fig. 5.26 it is clear that *Juwain* and *GE 5016* millet varieties had mild interaction while other varieties exhibited a strong interaction with germination time towards the production of alpha-amylase when GA_3 was applied. All finger millet cultivars germinated for 56 h exhibited the maximum alpha amylase activity, with the highest activity being found in *Kabre* malt (38.57 units/g dry malt), followed by *GPU 0025* (35.63 units/g dry malt), while the lowest activity was found in *Juwain* malt (22.61 units/g dry malt). When GA_3 was not treated, the maximum alpha amylase activities were found at 72 h of germination in all millet varieties and the values were 22.96, 19.73, 16.55, 22.16, 19.54, and 19.47 units/g dry malt for *Dalle*, *Kabre*, *Okhle*, *Juwain*, *GE 5016* and *GPU 0025* finger millet varieties respectively (Fig. 5.18). On comparing the results of alpha-amylase activities between GA_3 treated and untreated millet malts, GA_3 treatment substantially enhanced the alpha-amylase activity in all millet varieties than those of their untreated counterparts. GA_3 treatment increased alpha-amylase activity by 100% in *Kabre* and Okhle millets compared to their untreated ones.The effects of millet varieties and germination time on beta-amylase activity of GA_3 treated millet malts are shown in Fig. 5.27.

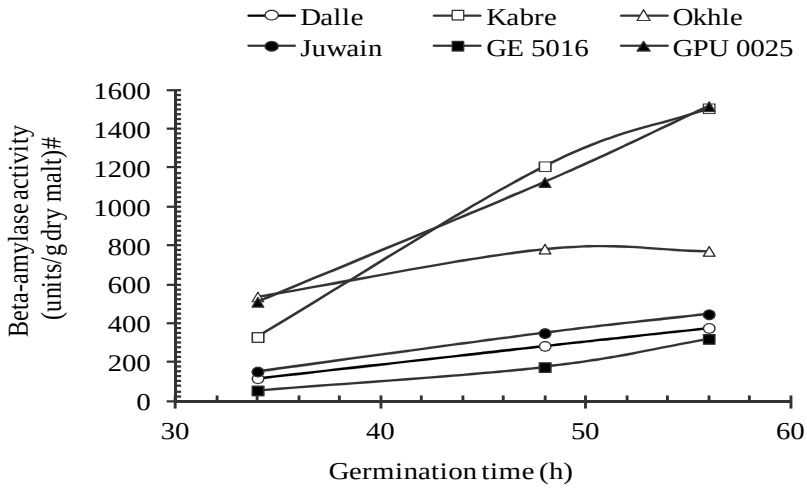


Fig. 5.27 Effect of varieties and germination time on beta-amylase activity in gibberellic acid treated finger millet malts. #one unit of beta-amylase activity was defined as the production of 1 mg of maltose over 30 min of incubation period under the assay conditions.

Like alpha-amylase activity, beta-amylase activity increased with germination time in all millet varieties. The beta-amylase activities in *Dalle* and *Kabre* millets germinated for 34, 48 and 56 h were 117, 284 and 376; and 329, 1208 and 1506 units/g dry malt respectively. The beta amylase activity in 34, 48, and 56 h germinated *Okhle* and *Juwain* millets were 534, 784 and 772; and 152, 349 and 446 units/g dry malt respectively. Similarly, beta-amylase activities in *GE 5016* and *GPU 0025* millet varieties germinated for 34, 48 and 56 h were found to be 54,174 and 319; and 511, 1127 and 1517 units/g dry malt respectively. From Fig 5.27 it revealed that *Kabre* and *GPU 0025* millets highly responded with GA_3 treatment, followed by *Okhle*, while the least response to GA_3 was exhibited by *Juwain*, *GE 5016* and *Dalle* millet varieties. Statistical analysis indicated that beta-amylase activities between 56 h germinated *Kabre* and *GPU 0025* millet malts were not different ($p>0.05$) but they were significantly higher among all millet mallts.

When GA_3 was not applied, maximum production of beta-amylase activity occurred during 48 h of germination in all millet varieties and the values were 357.4, 385.0, 360.2, 244.9, 349.2 and 355.2 units/g dry malt for *Dalle*, *Kabre*, *Okhle*, *Juwain*, *GE 5016* and *GPU 0025* millet varieties respectively (Fig. 5.19). While comparing the beta-amylase activities between GA_3 treated and untreated finger millet malts, it was found that GA_3 treatment decreased beta-amylase activity in *GE 5016* millet varieties, while it extensively enhanced in all other varieties, the extent being highly varietal dependent. Moreover, it was observed that the effect of GA_3 treatment was more pronounced on beta-amylase activity than on alpha-amylase activity. The maximum beta- and alpha-amylase activity enhancement by GA_3 treatment were about 4- and 2-fold respectively in *Kabre* millet malt compared to its untreated malt.

FAN contents were significantly influenced by both germination time and millet varieties when GA_3 was applied. The FAN contents for *Dalle* and *Kabre* millet varieties germinated for 34, 48 and 56 h were 35.3, 63.7 and 69.1; and 78.8, 93.5 and 113.0 mg glycine equivalent (GE)/100 dry matter (DM) respectively. Similarly, the FAN contents in 34, 48 and 56 h germinated *Okhle* and *Juwain* millet cultivars were 81.7, 89.7 and 98.4; and 20.2, 44.4 and 66.9 mg GE/100 g DM respectively. The FAN contents in *GE 5016* and *GPU 0025* millet malts germinated for 34, 48 and 56 h were 50.1, 78.2, and 74.8; and 82.7, 88.0 and 86.3 mg GE/100 g DM respectively. From Fig. 5.28 it can be seen that FAN contents in *GE 5016* and *GPU 0025* millet varieties

attained the maximum value at 48 h of germination and further germination did not increase significantly ($p>0.05$); while in other varieties, the FAN contents increased gradually all through the germination time. Statistical analysis revealed that 56 h germinated *Kabre* millet had the highest FAN content (113 mg GE/100 g DM) of all the treatment combinations studied.

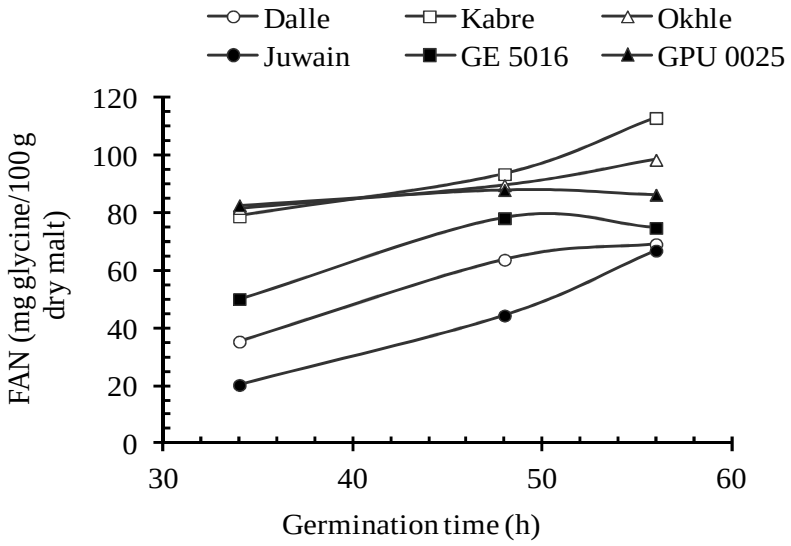


Fig. 5.28 Eeffect of varieties and germination time on FAN contents in gibberellic acid treated finger millet malts.

Kabre, *Okhle* and *GPU* millet varieties exhibited the strong interaction, while *Juwain*, *GE 5016* and *Dalle* millet varieties had the medium interaction with germination time in FAN development during germination (Fig. 5.28).When the GA_3 was not applied during germination, maximum FAN contents of 35.2, 39.0, 33.4, 57.8, 36.4 and 34.7 mg glycine/ 100 g DM were found in 48 h germinated *Dalle, Kabre, Okhle, Juwain, GE 5016* and *GPU 0025* finger millet malts respectively (Fig. 5.23). On comparing the maximum FAN contents between GA_3 treated and untreated millet malts, it was found that GA_3 treatment caused only a marginal increase in FAN content in *Juwain* millet, while there were large increments in all other millet varieties (96 to 195%). Except for alpha-amylase in *Dalle* millet, for beta-amylase in *Okhle* and for FAN contents in *GE 5016* and *GPU 0025* millets, the enzyme and FAN production did not appear to be completed deven after 56 h of germination when the experiment was terminated owing to the excessive development of root and shoot. Our study revealed that both the amylase and FAN production in finger millets were extensively increased by GA_3 treatment.

Agu *et al.* (1993) reported that the highest diastage activity was found in 0.2 ppm GA_3 treated millet (*Pennisetum maiwa*) and sorghum (*Sorghum bicolor*). Agu and Ezeanolue (1993) also noted improvement on malting performance of millet (*Pennisetum maima*) by using 0.2 ppm GA_3. Rapid secretion of alpha-amylase enzyme from the aleurone tissue occurred in 1 µM GA_3 treated wheat seeds compared to untreated ones (Mirbahar and Laidman, 1982). Similar findings of enhanced alpha-amylase activity in GA_3 treated germinated cereals were also reported by Bloch and Morgan (1967) and Moro *et al*. (1963). Sahai Srivastava and Meredith (1962) reported increased alpha- and beta-amylase activities by GA_3 treatment in barley seeds. Most of the earlier workers have reported increased amylase activity by GA_3 treatment but the literature concerning beta-amylase activity is limited.

Interestingly our study revealed that GA_3 treatment had a profound effect on beta amylase than that on alpha-amylase activity in finger millet varieties. Banasik (1969) reported increase in protease activities when GA3 was treated in the steeping cereals for malt production. Kringstand *et al.* (1960) reported a several fold increase in protease activity in malted barley. The results of amylase activities (Figs. 5.26 and 5.27) and FAN production (Fig. 5.28) during germination of different finger millet cultivars treated with 5 ppm of GA suggested that 56 h germinated *Kabre* millet had significantly higher alpha-amylase activity and FAN content of all the millet malts, while the beta-amylase activity between *Kabre* and *GPU 0025* millets germinated for 56 h did not differ significantly. Hence, in order to select the best finger millet variety, extract of malt analysis was performed using *Kabre* and *GPU 0025* millet malts.

5.2.4.2 Effect of GA_3 treatment on the extract quality of *Kabre* and GPU 0025 millet malts

Table 5.16 shows the chemical properties of malt extracts derived from 56 h germinated *Kabre* and *GPU 0025* millet malts. The TSS and total reducing sugar contents in both *Kabre* and *GPU 0025* millet malt extracts did not differ ($p>0.05$), while the FAN content of *Kabre* extract (18.90 mg glycine/100 mL) was higher to that of *GPU 0025* (17.60 mg glycine/100 mL). The TSS and total reducing sugar contents of malt extracts derived from GA_3 untreated *Kabre* and *GPU 0025* millet malts were 8.10 ºBx and 6.27 mg maltose/100 mL) ande 8.12 ºBx and 5.48 mg maltose/100 mL) respectively (Table 5.16). The results clearly indicated that GA treatment significantly improved the TSS and total reducing contents in the extract than those of control.

Table 5.16 Chemical properties of malt extracts obtained from *Kabre* and *GPU 0025* millet malts germinated for 56 h at 28 ± 1 ºC

Parameters	Mean values*	
	Kabre extract	*GPU 0025* extract
TSS (º Bx)	9.27 (0.25)[a]	9.48 (0.15) [a]
Total reducing sugar (g maltose/100 mL)	9.94 (0.12) [a]	9.79 (0.20) [a]
FAN (mg glycine/100 mL)	18.90 (0.24) [a]	17.6 (0.52) [b]
Color (EBC, unit)	4.93 (0.09) [a]	3.61 (0.09) [b]
Extract yield of wort (º Plato)	8.95 (0.10) [a]	9.30 (0.11 [a]
Extract yield of malt (% db)	86.63 (1.13) [a]	87.82 (1.74) [a]

*values are the means of three determinations. Figures in the parentheses are the standard deviations. Means followed by similar superscripts in a row are not significantly ($p>0.05$) different.

Both the FAN and color of *Kabre* malt extract were significantly higher ($p<0.05$) than those of extract derived from *GPU 0025* millet malt. It is interesting to note that the FAN content of GA_3 treated *Kabre* malt extract (18.90 mg glycine/100 mL) was about 2-fold higher to that of GA_3 untreated (6.2 mg glycine/100 mL). Again, the color values of GA_3 treated malt extracts were remarkably higher than those of control (GA_3 untreated millet malt extracts). The extract yield of malts between GA_3 treated *Kabre* and *GPU 0025* malt extracts were not different, but they were significantly higher compared to to their respective GA_3 untreated counterparts. Hence, the results of malt and extract analyses suggested that GA_3 treatment substantially enhanced the amylase activities and FAN contents in the finger millet malts. On the whole, *Kabre* millet exhibited better response to GA_3 towards the production of amylase and FAN during germination as well as had better malt extract properties.

5.2.5 Effect of mashing methods on the chemical properties of finger millet (variety *Kabre*) malt wort

Finger millet malt was mashed by infusion at 70 °C, decantation at 80 °C and US mashing process, and the chemical properties of the worts were determined. The chemical properties of finger millet worts prepared by three different methods are given in Table 5.17.

Table 5.17 Effect of mashing methods on chemical properties of finger millet malt wort

Parameters	Mean values for different mashing methods*		
	Infusion	Decantation	US mashing
Total reducing sugar (%, m/v)	8.47[a] (0.40)	8.80 [b] (0.10)	9.27 [c] (0.18)
Glucose (%, m/v)	3.82 [a] (0.14)	3.99 [a] (0.07)	4.73 [b] (0.14)
Fructose (mg%, m/v)	11.0 [a] (0.1)	12.6 [b] (0.1)	13.6 [b] (0.4)
Sucrose (%, m/v)	0.72 [a] (0.16)	0.68[a] (0.06)	0.55 [a] (0.05)
Total sugar as glucose (%, m/v)	9.18 [a] (0.2)	9.43 [a] (0.05)	9.80 [b] (0.14)
Dextrin (%, m/v)	2.07 [a] (0.3)	2.59 [b] (0.1)	3.49 [c] (0.1)
Color (EBC unit)	4.94 [a] (0.10)	6.03 [b] (0.09)	5.69 [c] (0.10)
FAN as glycine (mg%, m/v)	15.1 [a] (0.9)	14.1 [a] (1.2)	17.4 [b] (0.9)
Turbidity (FTU)	9.99 [a] (0.36)	23.77 [b] (0.44)	22.4 [c] (0.39)
pH	5.33 [a] (0.06)	5.36 [a] (0.06)	5.56 [a] (0.06)
Tannin as tannic acid (mg%, m/v)	35.1 [a] (0.5)	36 [a] (1.8)	38.3 [b] (0.6)
Extract (^{o} Plato)	10.71 [a] (0.21)	11.69 [b] (0.2)	12.13 [c] (0.11)

*values are the means of three determinations. Figures in the parantheses are the standard deviations. Means followed by the similar superscripts in a row do not differ ($p>0.05$) by LSD.

Mashing methods showed a significant effect on chemical characteristics of millet malt worts. Infusion mashing resulted wort with considerably lower total reducing sugar (8.47%, m/v), while a maximum was found in US mashing method (9.27%, m/v). The highest glucose content was found in wort derived from US mashing method (4.73%, m/v), whereas the values for infusion and decantation mashing did not differ ($p>0.05$). The fructose contents between decantation (12.6 mg%, m/v) and US mashing (13.6 mg%, m/v) methods were not different, while it was lowest ($p<0.05$) in infusion mashing (11.0 mg% m/v). The sucrose contents in worts prepared by infusion (0.72%, m/v), decantation (0.68%, m/v) and US mashing (0.55%, m/v) methods did not differ. US mashing method produced higher total sugar (9.80%, m/v as glucose) than those of infusion and decantation methods.

Dextrin contents were found to be 2.07, 2.59, and 3.49 % (m/v) in worts prepared by infusion, decantation, and US mashing methods respectively. Statistical analysis revealed that the dextrin contents were significantly different from each other. The lowest color was obtained in infusion mashing (4.94 EBC units), while the highest was in decantation mashing (6.03 EBC units). Both the infusion and decantation methods produced wort of similar FAN contents, while the wort obtained by US method had the highest FAN content (17.4 mg glycine/100 mL). Wort turbidity was maximum in decantation mashing (23.77 FTU), while it was lowest in infusion mashing (9.99 FTU). US mashing method resulted wort with higher pH (5.56) and tannin (38.3 mg tannic acid/100 mL) contents than those of infusion and decantation methods. Maximum extract was obtained with US mashing process (12.13 º Plato), while the minimum was found with infusion mashing (10.71 º Plato).

The maximum color found in decantation and US methods over infusion method could partly be due to higher mashing temperature (80 ºC) to that of infusion method (70 ºC) resulting in higher degree of maillard reaction . The higher extract, total reducing sugar and FAN contents in US mashing method may be due to the successive exposure of the mash at temperatures that are optimal for proteinases and starch hydrolyzing enzymes. However, the level of FAN obtained for millet malt worts were within the adequate level (130 – 150 mg/L) required for optimal yeast growth and fermentation efficiency. (O'Connor-Cox, 1989). Eneje *et al.* (2001) also reported similar result of FAN content in millet malt wort mashed by decantation method (147 mg/L). Igyor *et al.* (2001) and Palmer (1989) also reported higher level of extract with a reasonable proportion of fermentable sugar in decantation mashing over infusion mashing of sorghum malt. It is obvious from the results of Table 5.17 that the US mashing method significantly enhanced the extract, FAN and total reducing sugar contents of the wort.

5.2.6 Effect of barley malt addition on millet malt wort quality

Finger millet malt was substituted with commercial barley malt at different proportions, mashed and the chemical properties of the resulting worts were analyzed. Table 5.18 shows the chemical properties of millet malt worts. The TSS of the control wort (without barley malt addition) was 11.87 ºBx and it increased by 0.46, 0.56, and 0.26 º Bx in 20, 30 and 40% barley malt added worts respectively. From Table 5.18 it is clear that addition of barley malt up to 40% did not improve the wort TSS remarkably.

Table 5.18. Chemical properties of millet malt worts mashed in combination with commercial barley malt*

Parameters	Barley malt substitution (%)			
	0	20	30	40
TSS (º Bx)	11.87 (0.12)[a]	12.33 (0.1)[a]	12.43 (0.23)[a]	12.13 (0.22)[a]
Total reducing sugar as maltose (%, m/v)	8.31 (0.10)[a]	8.74 (0.10)[b]	9.02 (0.15)[bc]	9.27 (0.23)[c]
Glucose (%, m/v)	4.73 (0.2)[a]	4.07 (0.23)[b]	3.79 (0.2)[c]	3.67 (0.26)[d]
Color (EBC units)	5.69 (0.22)[a]	6.89 (0.32)[b]	6.39 (0.15)[b]	6.51 (0.34)[b]
Extract (%, m/m)	11.95 (0.36)[a]	12.13 (0.22)[a]	12.70 (0.36)[b]	12.73 (0.39)[b]
FAN as glycine (mg /100 mL)	17.35 (0.98)[a]	22.24 (1.1)[b]	23.37 (1.5)[b]	27.42 (2.01)[c]

*values are means of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by the similar letters are not significantly different ($p>0.05$) by LSD.

Addition of barley malt at the rate of 20% increased the total reducing sugar content by 0.43% over the control, while 40% barley malt addition increased the reducing sugar content by about 1% in the wort. Addition of barley malt showed adverse effect on wort glucose content (Table 5.18). The color increased significantly ($p<0.05$) from 5.69 EBC units in control to 6.89 EBC units in 20% barley malt added wort, while the color values among the barley malt added worts were not different. The extract contents of control (11.95%, m/m) and 20% barley malt added worts (12.13%, m/m) were not different, while 30% barley malt addition significantly increased the wort extract. The FAN contents in worts were significantly affected by the levels of barley malt addition. FAN content increased by about 28% on adding 20% barley malt with respect to control, while it increased by about 58% in 40% barley malt added wort. Although statistical analysis showed significant differences among the treatment means, from Table 5.18 it is clear that no remarkable improvement on chemical properties of worts was observed on adding barley malt up to 40% except FAN, which increased appreciably.

5.2.7 Effect of mold bran addition on chemical properties of millet malt wort

5.2.7.1 Preparation of wheat mold bran using *R. oryzae*, ITCC No. 4408 mold

Sterile wheat bran was inoculated with *R. oryzae*, ITCC No. 4408, incubated at 30º C and alpha and beta amylase activity were measured daily. Fig. 5.29 shows the development of alpha and beta amylase activity in wheat bran inoculated with R. oryzae, ITCC No. 4408. From Fig. 5.29 it is evident that both the alpha- and beat-amylase activities increased steadily, reached at maximum on day 4, and then decreased. Then wheat mold bran was prepared in bulk amount by incubating up to the pre-determined incubation time (4 days at 30 °C) and used for mashing.

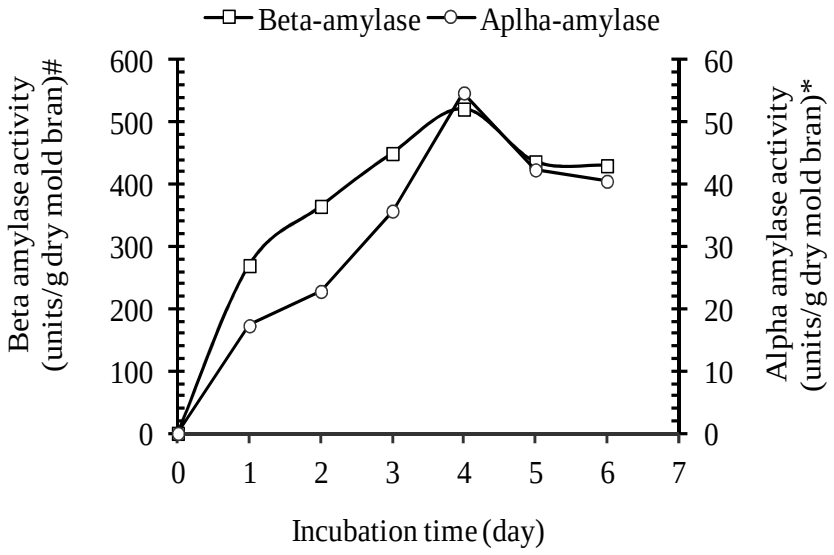


Fig. 5.29 Development of α- and β-amylase activities in wheat bran during incubation at 30º C.

[#]One unit of beta amylase activity was defined as the production of 1 mg reducing sugar as maltose over 30 min of incubation under the assay conditions.

[*]One unit of alpha amylase activity was defined as 1 unit decrease in OD at 620 nm per min under the assay conditions.

5.2.7.2 Mashing of finger millet (Variety *Kabre*) malt with mold bran

Millet malt was mashed in combination with mold bran using US mashing procedure (Matz, 1991) and the chemical properties of the worts were analyzed. From Table 5.19 it can be envisaged that addition of mold bran up to 5% did not improve the TSS, total

reducing sugar, glucose and pH contents, while it remarkably improved the FAN, formol nitrogen and viscosity of the worts compared to control. FAN contents increased by about 80 and 128% by the addition of 2.5 and 5% mold bran respectively compared to control. Similarly, formol nitrogen increased by 44 and 102% by incorporating 2.5 and 5% mold bran respectively compared to control.

Table 5.19 Chemical properties of millet malt wort mashed with mold bran*

Parameters	Mold bran incorporation (%, m/m)		
	0	2.5	5
Total soluble solids (º Bx)	12.00 (0.90)[a]	12.55 (0.62)[a]	12.7 (0.45)[a]
Total reducing sugar as maltose (%, m/v)	7.37 (0.37)[a]	7.87 (0.20)[a]	7.87 (0.30)[a]
Glucose (%, m/v)	3.73 (0.10)[a]	3.66 (0.07)[a]	3.65 (0.09)[a]
pH	5.85 (0.25)[a]	5.93 (0.30)[a]	5.96 (0.32)[a]
FAN as glycine (mg/100 mL)	15.9 (0.7)[a]	28.7 (1.1)[b]	36.3 (0.8)[c]
Viscosity at 20 ºC (cP)	1.41 (0.01)[a]	1.30 (0.04)[b]	1.30 (0.02)[b]
Formol N (mg/L)	109.1 (2.2)[a]	157.1 (1.9)[b]	220.3 (3.5)[c]

*values are the means of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by the similar letters in a row are not significantly different ($p>0.05$) by LSD.

Wort viscosity decreased significantly by mold addition compared to control (100% millet malt wort), while no significant ($p>0.05$) decrease in wort viscosity was observed between 2.5 and 5% mold bran added worts. Insignificant changes in TSS, reducing sugar and glucose contents by adding mold bran could be due to the unfavorable conditions for fungal amylases present in the mold bran. As reduced viscosity assists in wort filtration and increased FAN and formol nitrogen promote yeast growth, addition of mold bran up to 2.5% seems appropriate. Addition of mold bran beyond 2.5 % adversely affected the color of the wort.

5.2.8 Optimization of mashing stages

5.2.8.1 Changes in free amino nitrogen (FAN) and total soluble solids (TSS) during millet and barely malt mashing

The main objective of mashing is primarily to convert the starch and protein into simpler form so that they are easily assimilated by the yeast for their growth and product formation. The two most vital elements are the amino acids and sugars present in the wort. Hence, the FAN and TSS contents were monitored at each stage of mashing in order to see their evolution during mashing. Mashing was carried out according to the US mashing procedure as described by Matz (1991) using the average time and temperature given for each of the stages. The FAN and TSS were monitored at different times of each stage and the results are depicted in Figs 5.30 and 5.31 respectively.

Fig 5.30 shows the changes in FAN during finger millet and barley malt mashing.

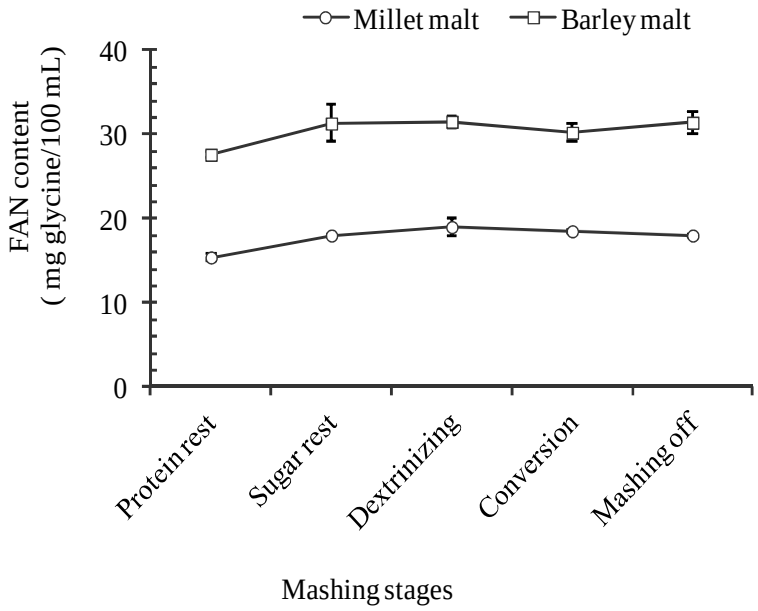


Fig. 5.30 Evolution of FAN during finger millet and barley malts mashing.

The FAN contents at protein rest, sugar rest, dextrinizing, conversion and mashing off periods for millet malt worts were 15.4, 18, 19, 18.5 and 18 mg glycine/100 mL wort respectively, while the values for barley malt were 27.6, 31.3, 31.5, 30.2 and 31.4 mg glycine /100 mL wort respectively for the respective mashing stages. FAN contents increased sharply during the protein rest period in both malts (Fig. 5.30). There was a slight increase in FAN contents during sugar rest and dextrinizing periods in millet malt mash but not in barley malt mash. Changes in TSS during mashing are dipected in Fig.

5.31. The TSS of millet malt mash were 1.1, 1.7, 4.1, 11.3, and 12.1 ºBx at protein rest, sugar rest, dextrinizing, conversion, and mashing off periods respectively, whereas the values for barley malt mash were 5.5, 11.1, 12.3, 12.7 and 13.2 º Bx for the respective mashing stages.

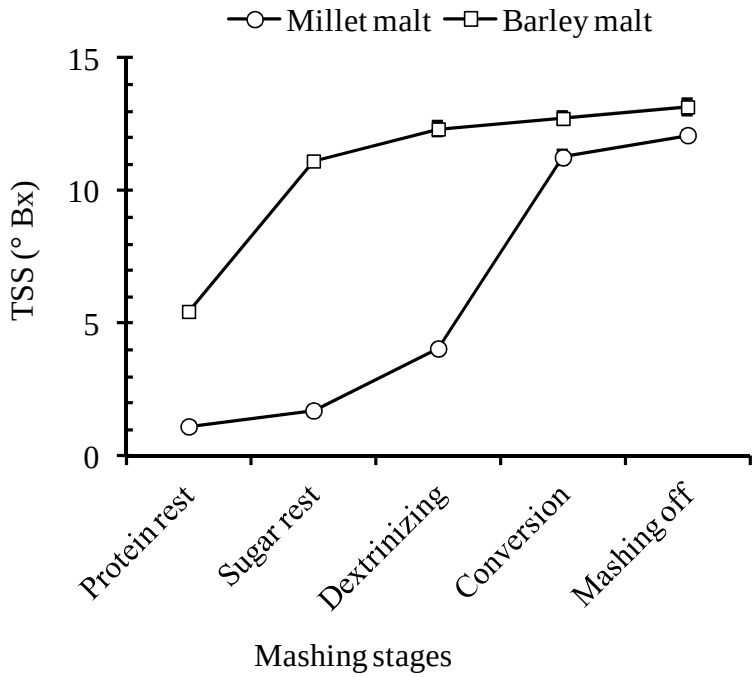


Fig. 5.31 Changes in TSS during millet and barley malts mashing.

For finger millet malt, there was no significant increase in TSS during protein and sugar rest periods, whereas a slight increase in TSS took place during dextrinizing period. A sudden upsurge in TSS took place during conversion period and the TSS approached closer to that of barley malt mash (Fig. 5.31). In the case of barley malt, maximum TSS increment occurred during the sugar rest stage and there was a slight increase in the subsequent stages (Fig. 5.31). This study demonstrated that finger millet starch has gelatinization temperature around 70 ºC. Moreover, starch-hydrolyzing enzymes present in the millet malt must have their optimum temperature around 70 ºC. The gelatinization temperature of barley starch is 51 – 60 ºC. For starch to be rapidly hydrolyzed by amylases enzymes the starch granules first be fully hydrated to disrupt the internal molecular structure (gelatinize), then brokendown and the starch molecule become solubilized (Taylor, 2009). The preferred conditions for β-amylase are 50 – 63 ºC (Matz, 1991) and these temperature ranges were provided during sugar rest and dextrinizing periods that is why the TSS of barley malt mash increased rapidly at these

stages. The millets, like sorghum, have high starch gelatinization temperature: pearl millet (*Pennisetum glaucum*) 61 – 68 ºC and finger millet 65 – 69 ºC (Serna-Saldivar and Rooney, 1995), therefore, millet mash TSS increased abruptly during conversion period.

5.2.8.2 Optimization of holding time for millet malt mashing at each stage of US mashing process

In order to arrive at a suitable holding time for each mashing temperature, finger millet malt mash was held for different times at each of the mashing stages and the evolution of FAN and total reducing sugars were monitored. Changes in FAN and total reducing sugar during mashing are depicted in Figs 5.32 – 5.35. FAN content (glycine equivalent) increased from 5.5 to 13.2 mg/100 mL during the first 30 min, while it increased with an average rate of 1% per 20 min of time interval in the subsequent periods of protein rest period (Fig. 5.32). Statistical analysis revealed that 50 min of holding time in protein rest period was optimum for FAN production in finger millet malt. Total reducing sugar (as maltose) increased very slowly from 0.51 to 2.27% (m/v) over 90 min of incubation in protein rest period.

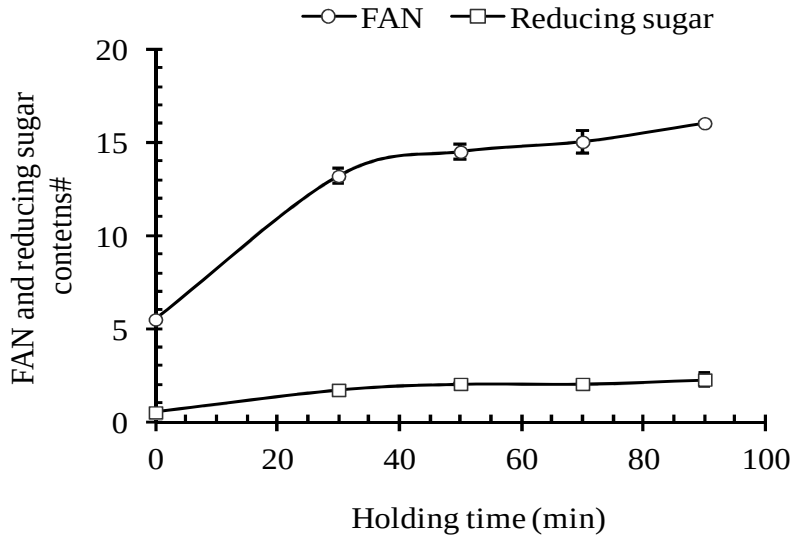


Fig. 5.32 Changes in FAN and total reducing sugar contents during protein rest period.
#FAN and total reducing sugar contents are expressed as mg glycine equivalent/100 mL and g maltose/100 mL respectively.

Changes in FAN and total reducing sugar contents during sugar rest period are shown in Fig. 5.33. The FAN content increased from 5.5 to 13.8 mg glycine/100 mL over the first 10 min of holding, while it increased very slowly (at an average rate of 0.7 mg% per 10 min of holding time) during the following time intervals. Similarly, total reducing sugar (as maltose) increased from 0.51 to 2.15% over the first 10 min and increased thereafter very slowly (at an average rate of 0.57 % per 10 min). It appeared that sugar rest period had no significant contribution in FAN and reducing sugar production in finger millet malt.

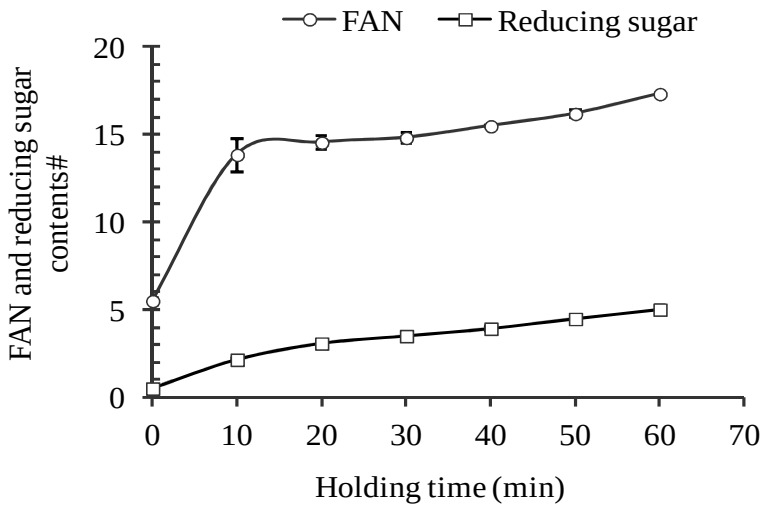


Fig. 5.33 Changes in FAN and total reducing sugar contents during sugar rest period. #FAN and total reducing sugar contents are expressed as mg glycine equivalent/100 mL and g maltose/100 mL respectively.

Fig. 5.34 shows the evolution of FAN and total reducing sugar during dextrinizing period. Both the FAN and reducing sugar contents followed the similar trend to those of previous mashing stages. The FAN and sugar contents increased from 5.5 to 15.7 mg glycine/100 mL and 0.51 to 5.99 g maltose/100 mL respectively over 60 min of holding time. Changes in FAN and reducing sugar during conversion period are depicted in Fig. 5.35. It was observed that FAN content did not changed appreciably, while total sugar content changed abruptly during conversion period (From Fig. 5.35). The total reducing sugar content (as maltose) increased from 0.51 to 8.89 mg% (m/v) over the first 10 min, while it increased by about 0.43% per 10 min afterwards during conversion period (Fig. 5.35

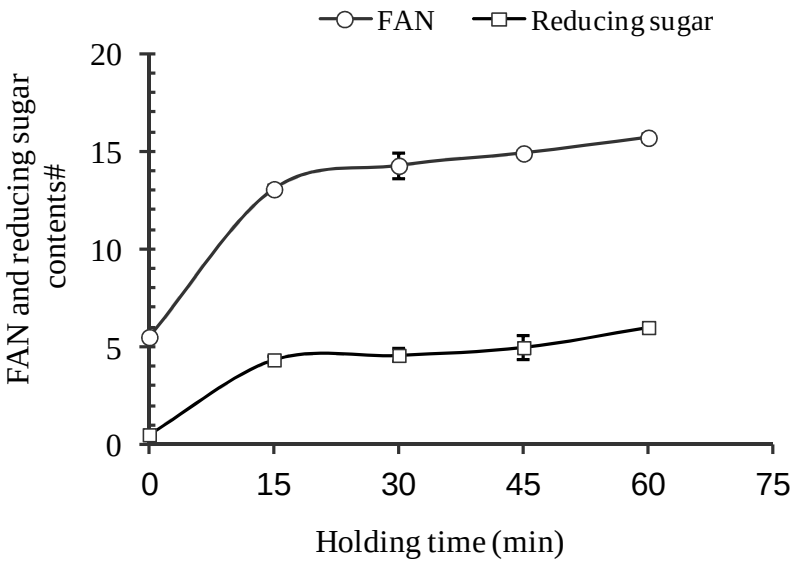


Fig. 5.34 Changes in FAN and total reducing sugar contents during dextrinizing period.

#FAN and total reducing sugar contents are expressed as mg glycine equivalent/100 mL and g maltose/100 mL respectively.

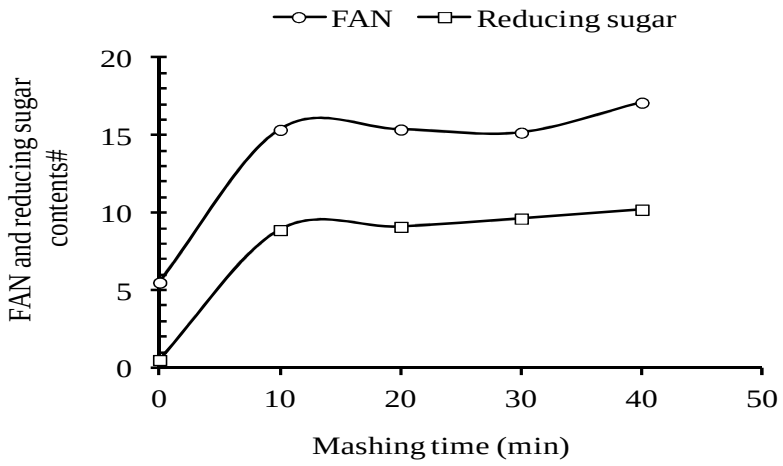


Fig. 5.35 Effect of holding time on FAN and total reducing sugar production during conversion period.

#FAN and total reducing sugar are expressed as mg glycine equivalent/100 mL and g maltose/100 mL respectively.

5.2.9 Optimization of pH and temperature at pre-selected mashing time using response surface method (RSM)

Designed experiments were conducted to investigate the effects of temperature (x_1) and pH (x_2) on different mashing responses viz., FAN (Y_1), total soluble solids (Y_2), total reducing sugar (Y_3), glucose (Y_4) and fructose (Y_5). A second order polynomial model for the dependent variables was tested to fit the experimental data and validity of the model was checked using coefficient of determination (R^2), adjusted R^2, model F-value, adequate precision, and lack of fit F-value.

5.2.9.1 Effect of process variables on different responses during protein rest period

5.2.9.1.1 Effect of process variables on free amino nitrogen (FAN) content during protein rest period

The FAN contents of mash during protein rest period varied from 10.8 to 15.72 mg glycine/100 mL. The coefficients of the model and other statistical attributes of FAN are shown in Appendix B, Table 1. Regression model fitted to the experimental data of FAN indicated that the model was significant ($p<0.05$), whearas the lack-of-fit was not significant ($p>0.05$) (Appendix B, Table 2). The chance of large model F-value due to noise was only 0.01%. The fit of model was also expressed by the coefficient of determination (R^2) which was found to be 0.9904, indicating that 99.04% of the variability of the response could be explained by the model. The adjusted R^2 was 0.9835 and adequate precision was 32.883, which showed an adequate signal. A ratio greater than 4 is desirable and hence this model could be used to navigate the design space. Considering all the above criteria, the model (Eq 5.1) was selected for representing the variation of FAN during protein rest period of mashing.

The quadratic model obtained from regression analysis for FAN content in terms of coded levels of the variables was as follows:

$$\text{FAN (mg gly/100 mL)} = 15.38 + 0.17x_1 + 0.39x_2 - 2.67x_1^2 - 0.51x_2^2 - 0.99x_1x_2 \quad \text{(Eq 5. 1)}$$

Where x_1 and x_2, are the coded values of temperature (°C) and pH respectively.
In this case, x_2, x_1, ^{2} x_2, ^{2} and x_1x_2 were significant terms. Quadratic term of temperature had highly negative significan effect ($p<0.001$) followed by negative significant effect ($p<0.001$) of the interaction term (x_1x_2), positve linear effect ($p<0.05$)) of pH (x_2) and negative significant quadratic effect ($p<0.05$) of pH (x_2^2). Results showed that the linear

term of temperature (x_1) had no significant effect on FAN content ($p>0.05$). Negative coefficient of quadratic terms (x_1^2, x_2^2) and interaction term (x_1x_2) indicated a convex shaped variation in FAN content with process variables (Fig. 5.36). Normal plot of residuals (Fig 5.37) indicated that studentized residuals were normally distributed. The variation of FAN content with process variables (temperature and pH) are shown in Fig 5.38. From Fig. 5.38 it can be seen that maximum FAN formation during protein rest period was found to be at pH of about 5.63 and temperature of around 50 °C. The contour plots for FAN formation during protein rest period is shown in Appendix C, Fig. 1.

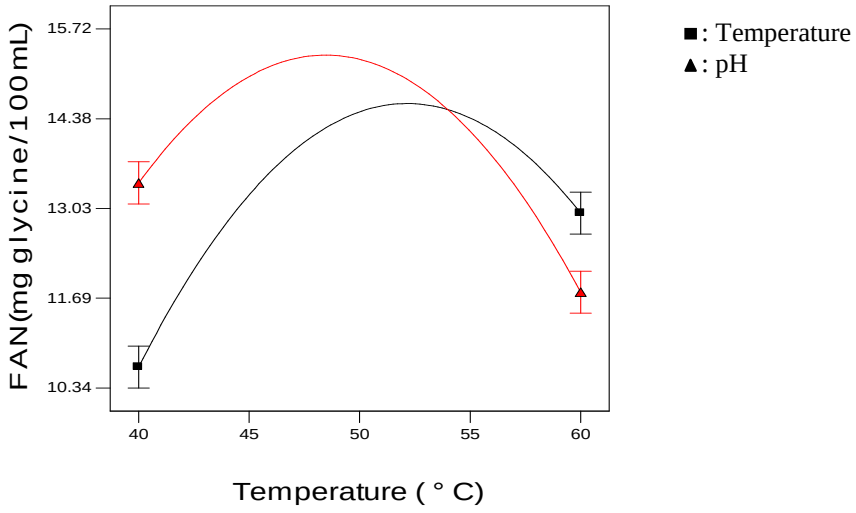


Fig. 5.36 Interaction plot of temperature and pH for FAN formation during protein rest period.

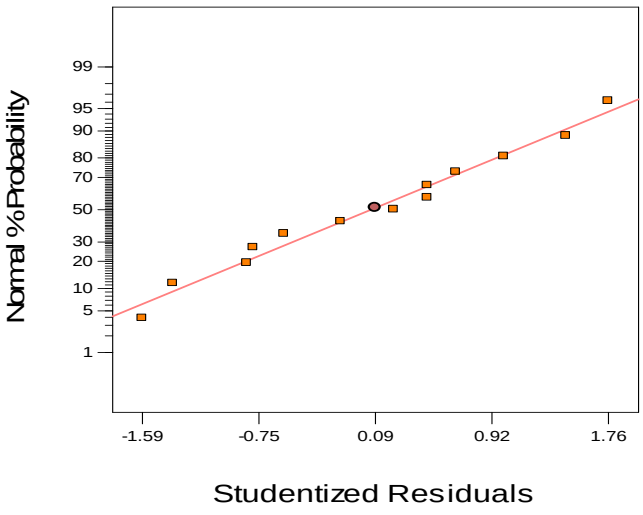


Fig. 5.37 Residual plot for FAN contents during proterin rest period.

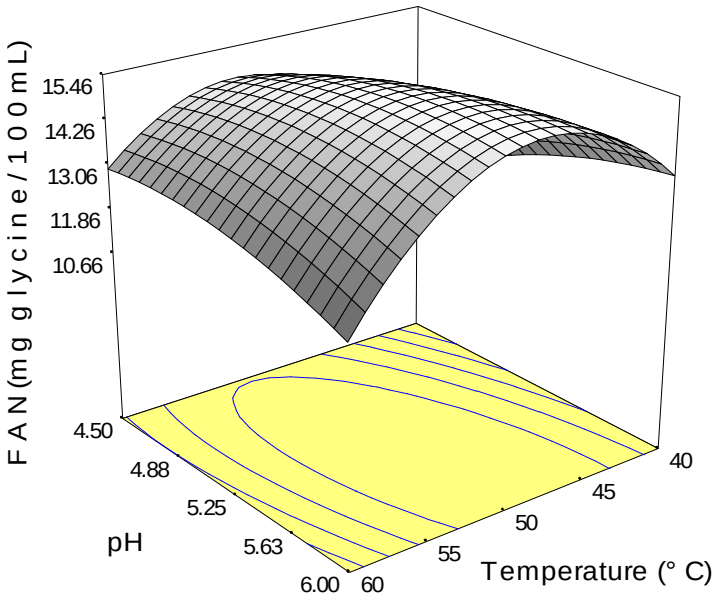


Fig. 5.38 Response surface plot for FAN content as a function of temperature and pH during protein rest period.

5.2.9.1.2 Effect of process variables on total soluble solids (TSS) during protein rest period

Total soluble solids of the mash during protein rest period varied from 1.4 − 5.8 °Bx. The coefficients of the model and other statistical attributes of TSS are shown in Appendix B, Table 1. Regression model fitted to the experimental data of TSS showed that model was significant ($p<0.05$), whereas lack-of-fit was not significant ($p>0.05$) (Appendix B, Table 2). The fit of model was also expressed by the coefficient of determination, R^2, which was found to be 0.9585, indicating that 95.85% of the variability of the response could be explained by the model. The adjusted R^2 (0.9288) and adequate precision (17.385) showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq 5. 2) was selected for representing the variation of TSS. The quadratic model obtained from regression analysis for TSS content in terms of coded levels of the variables was as follows:

$$\text{TSS (° Bx)} = 1.95 + 1.63x_1 + 0.22x_2 + 1.33x_1^2 - 0.12x_2^2 + 0.60x_1x_2 \qquad \text{(Eq 5. 2)}$$

Where x_1, x_2, are coded value of temperature (°C) and pH respectively.

In this case, x_1, x_1^2 and x_1x_2 were significant terms. Mashing temperature had highly significant linear positive effect followed by positive significant effect of quadratic term of temperature (x_1^2) and positive significant effect of the interaction term (x_1x_2). Results showed that pH (x_2) and its quadratic term (x_2^2) had no significant effect on TSS content ($p>0.05$). Positive coefficient of the interaction term (x_1x_2) indicated a concave shaped variation in TSS with process variables (Fig. 5.39).

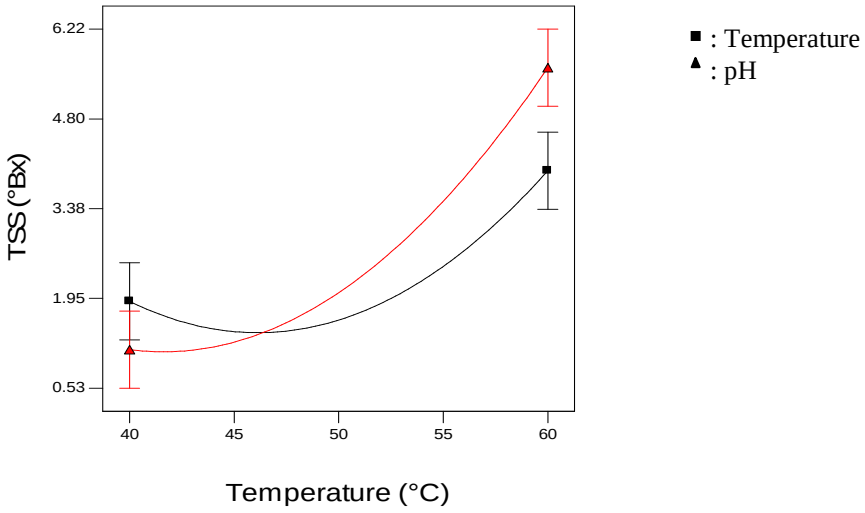


Fig. 5.39 Interaction plot of temperature and pH for TSS during protein rest period.

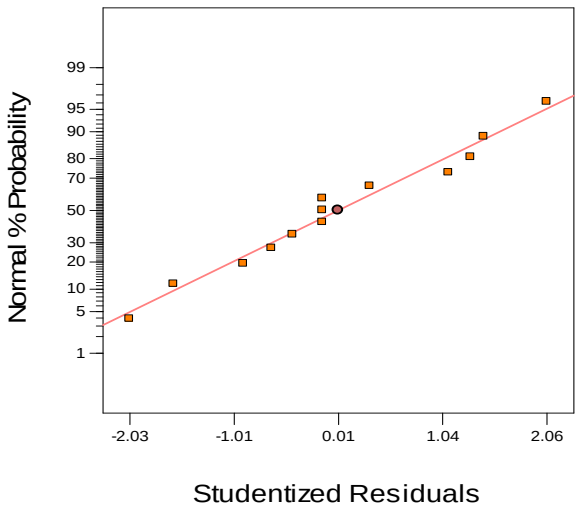


Fig. 5.40 Residual plot for TSS contents during proterin rest period.

Proximity of residuals around prediction line in normal plot of the residuals indicated that studentized residuals were normally distributed (Fig. 5.40). Fig 5.41 shows the variation of mash TSS with process variables during protein rest period of mashing.

TSS increased with increase in mashing temperature, while it decreased with increase in pH. Maximum TSS was found at higher temperature at all levels of pH. Appendix C, Fig. 2 shows the contour plot for TSS contents during protein rest period.

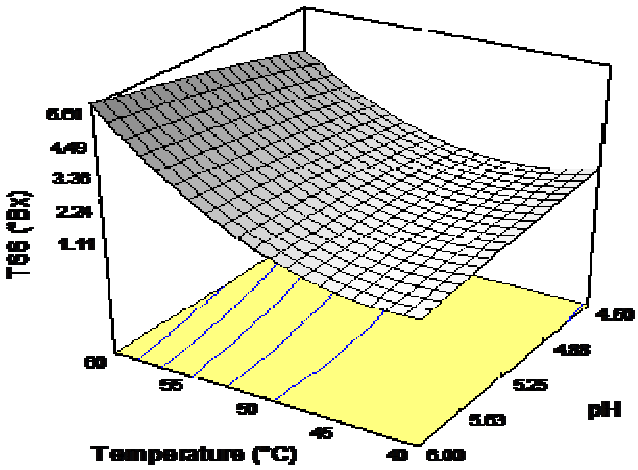


Fig. 5.41 Response surface plot for TSS content as a function of temperature and pH during protein rest period.

5.2.9.2 Effect of process variables on different response variables during dextrinizing period of mashing

5.2.9.2.1 Effect of process variables on FAN content during dextrinizing period

The FAN contents in worts during dextrinizing period varied from 9.3 to 14.0 mg glycine/100 mL. The coefficients of the model and other statistical attributes of FAN are given in Appendix B, Table 3. Regression model fitted to the experimental results of FAN showed that the model was significant ($p<0.05$), whearas the lack-of-fit was not significant (Appendix B, Table 4). The chance of large model F-value due to noise was only 0.01%. The fit of model was also expressed by the coefficient of determination (R^2) which was found to be 0.9748, indicating that 97.48% of the variability of the response could be explained by the model. The adjusted R^2 (0.9568) and adequate precision (18.943) showed an adequate signal. A ratio greater than 4 is desirable and hence this model could be used to navigate the design space. Considering all the above criteria, the model (Eq 5. 3) was selected for representing the variation of wort FAN during dextrinizing period of mashing. The quadratic model obtained from regression analysis for FAN content in terms of coded levels of the variables was as follows:

FAN (mg gly./100 mL) = 9.62 - 0.97x_1 - 0.52x_2 + 2.90x_1^2 + 0.006x_2^2 - 0.30x_1x_2 (Eq 5. 3)

Where x_1 and x_2, are the coded value of temperature and pH respectively.

In this equation x_1, x_2 and x_1^2 were significant terms. Analysis of varience of the equation 5.3 indicated that the quadratic term of temperature (x_1^2) had highly significant positive effect ($p<0.001$) followed by negative linear effects of temperature and of pH. Positive coefficient of quadratic term of temperature and negative coefficient of interaction term (x_1x_2) indicated that the overall variation of FAN content followed a concave shaped curve without having interaction (Fig. 5.42). Situation of studentized residual along the predicted line in normal plot was ideal condition for charecterising residuals as unbiased. Most of the residuals were along the predicted line (Fig. 5.43) indicating that the residuals were normally distributed. Fig. 5.44 shows the variation of FAN contents as functions of temperature and pH during dextrinizing period. Wort FAN content was lowest at intermediate temperature (65 ^{O}C) for all pH regimes. Increasing mash pH appeared to decrease FAN content at all temperatures. Maximum FAN production occurred at the lowest pH and temerature regimes. (Fig 5.44). Contour plot of FAN during dextrinizing period is shown in Appendix C, Fig 3.

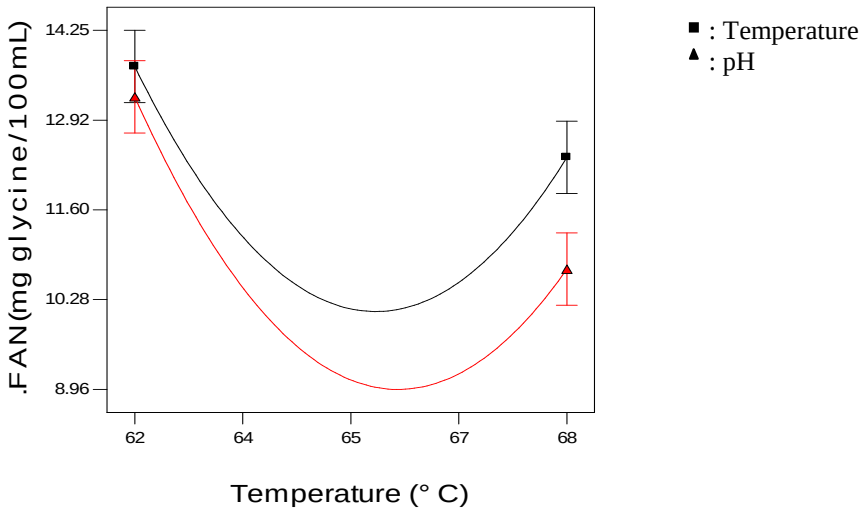


Fig. 5.42 Interaction plot of temperature and pH for FAN content during dextrinizing period.

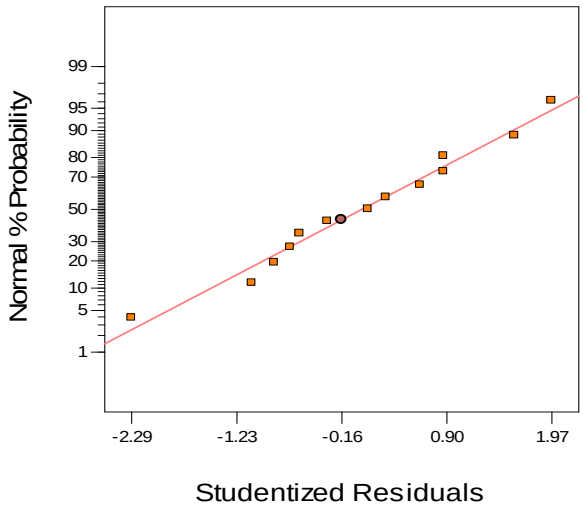


Fig. 5.43 Residual plot for FAN content during dextrinizing period of mashing.

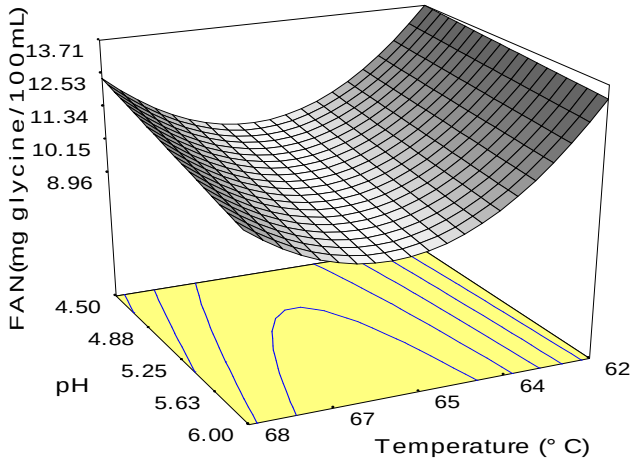


Fig. 5.44 Response surface plot for FAN content as a function of temperature and pH during dextrinizing period.

5.2.9.2.2 Effect of process variables on TSS during dextrinizing period

The total soluble solid (TSS) of mash during dextrinizing period ranged from 2.26 to 7.73 °Bx. The coefficients of the model and other statistical attributes of TSS are shown in Appendix B, Table 3. Regression model fitted to the experimental data of TSS showed that the model was significant ($p<0.05$), whereas lack-of-fit was not significant ($p>0.05$) (Appendix Table, 4). The fit of model was also expressed by the coefficient of determination which was found to be 0.9861, indicating that 98.61% of

the variability of the response could be explained by the model. The adjustedj R^2 was 0.9761 and adequate precision was 26.89, which showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq 5.4) was selected for representing the variation of TSS during dextrinizing period of mashing.

The quadratic model obtained from regression analysis for TSS content in terms of coded levels of the variables was as follows:

$$TSS\ (^\circ Bx) = 6.95 + 2.73x_1 - 0.33x_2 - 1.88x_1^2 + 0.26x_2^2 - 0.082x_1x_2 \qquad \text{(Eq 5.4)}$$

Where x_1 and x_2 are the coded values of temperature and pH respectively.

In this case, x_1, x_2 and x_1^2 were significant terms. Analysis of varience of equation 5.4 indicated that the temperature (x_1) had highly significant linear positive effect ($p<0.05$) followed by negative quadratic effect of temperature (x_1^2) and linear negative effect of pH (x_2). Negative coefficient of quadratic term of temperature and negative coefficient of interaction term (x_1x_2) showed that the overall variation in wort TSS was of a convex shaped. Mashing temperature and pH had no interaction effect on TSS (Fig. 5.45). Situation of studentized residual along the predicted line in normal plot was ideal condition for charecterising residuals as unbiased and most of the residuals were along the predicted line (Fig. 5.46) indicating that the residuals were normally distributed..

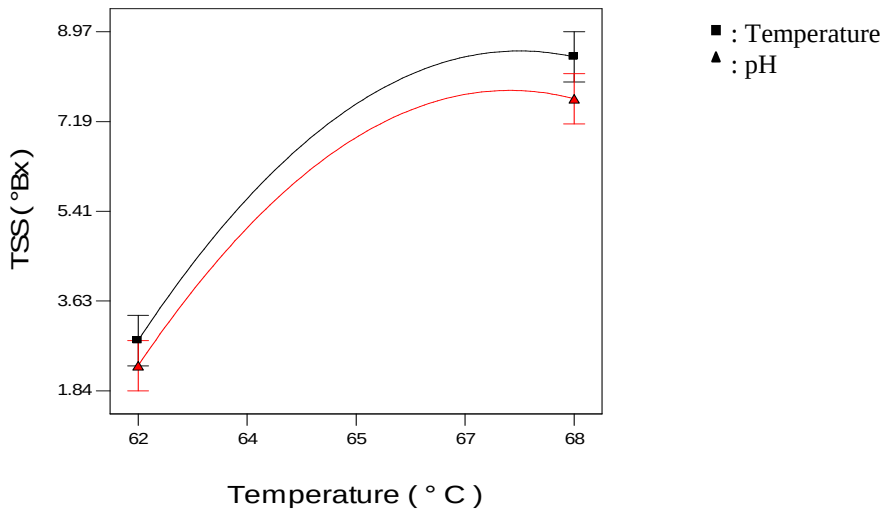


Fig. 5.45 Interaction plot of temperature and pH for TSS content during dextrinizing period.

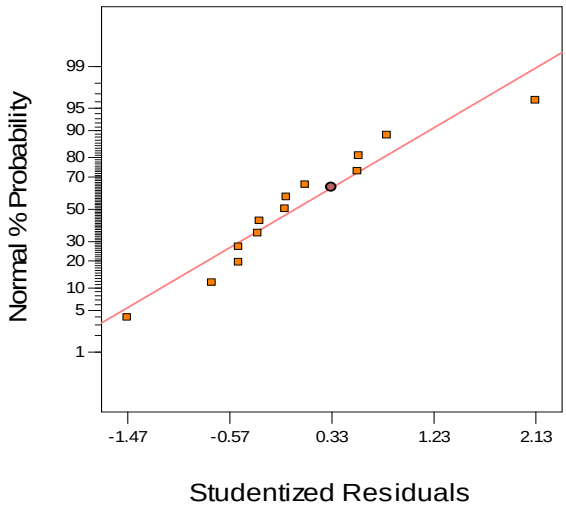


Fig. 5.46 Residual plot for TSS content during dextrinizing period of mashing.

Fig. 5.47 shows the variation of TSS during dextrinizing period of mashing, and it was found that wort TSS coud be maximized by using higher mash temperature for any pH regimes. Contour plot of TSS during dextrinizing period is shown in Appendix C, Fig 4

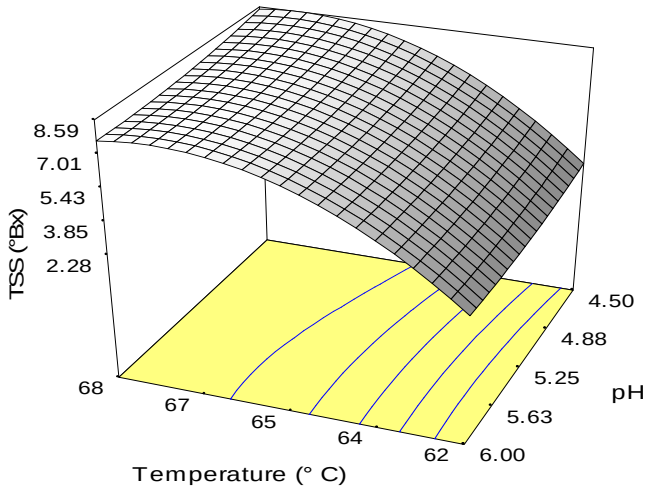


Fig. 5.47 Response surface plots for TSS content as a function of temperature and pH during dextrinizing period.

5.2.9.2.3 Effect of process variables on total reducing sugar (RS) during dextrinizing period

Total reducing sugar (RS) contents of mashes during dextrinizing period ranged from 2.25 to 7.06 g maltose/100 mL. The coefficients of the model and other statistical

attributes of total reducing sugar are shown in Appendix B, Table 3. Regression model fitted to the experimental results of RS showed that the model was significant, while the lack-of-fit was not significant ($p>0.05$) (Appendix B, Table 4). The chance of large model F-value due to noise was only 0.01%. The fit of model was also expressed by the coefficient of determination which was found to be 0.9882, indicating that 98.82% of the variability of the response could be explained by the model. The adjusted R^2 was 0.9798 and the adequate precision was 28.68, which showed an adequate signal. A ratio greater than 4 is desirable and hence this model could be used to navigate the design space. Considering all the above criteria, the model (Eq 5.5) was selected for representing the variation of total reducing sugar in worts during dextrinizing period of mashing. The quadratic model obtained from regression analysis for total reducing sugar contents in terms of coded levels of the variables was as follows:

$$\text{RS (g maltose/100 mL)} = 5.86 + 2.10x_1 + 0.088x_2 - 1.37x_1^2 + 0.0012x_2^2 + 0.2x_1x_2 \quad \text{(Eq 5.5)}$$

Where x_1, and x_2 are the coded values of temperature and pH respectively.

In this case, x_1 and x_1^2 were significant terms. Analysis of varience of equation 5.5 showed that temperature had a highly significant positive linear effect ($p<0.05$) followed by its significant negative effect of quadratic term (x_1^2). In this case, pH had no significant effect on RS content. Mash temperature and pH showed a strong interaction effect towards total reducing sugar development during dextrinizing period of mashing (Fig. 5.48). Situation of studentized residual along the predicted line in normal plot was ideal for charecterising the residuals as unbiased. Most of the residuals lay along the predicted line (Fig. 5.49) indicating that the residuals were normally distributed. Fig. 5.50 shows the variation of wort total reducing sugar during dextrinizing period as functions of temperature and pH. Increasing mashing temperature in the range of 62 to 68 °C profoundly increased total reducing sugar at all pH levels between 4.5 and 6.0. Contour plot of TSS during dextrinizing period is shown in Appendix C, Fig 5.

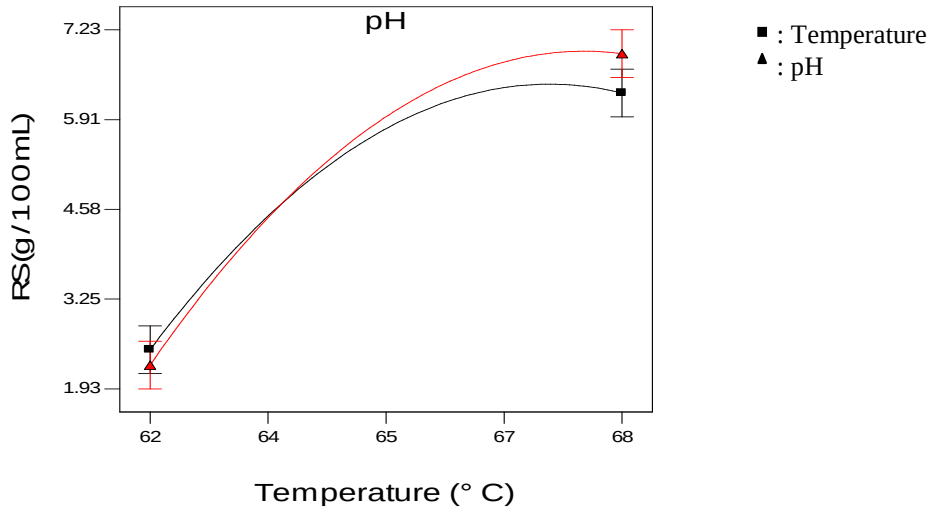


Fig. 5.48 Interaction plot of temperature and pH for total reducing sugar (RS as maltose) content during dextrinizing period.

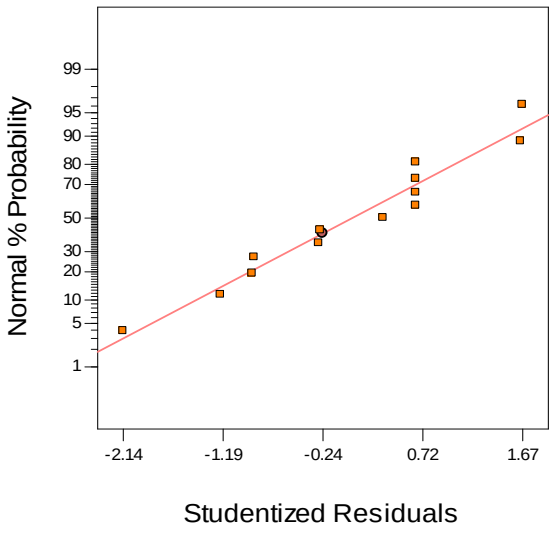


Fig. 5.49 Residual plot for total reducing sugar content during dextrinizing period of mashing.

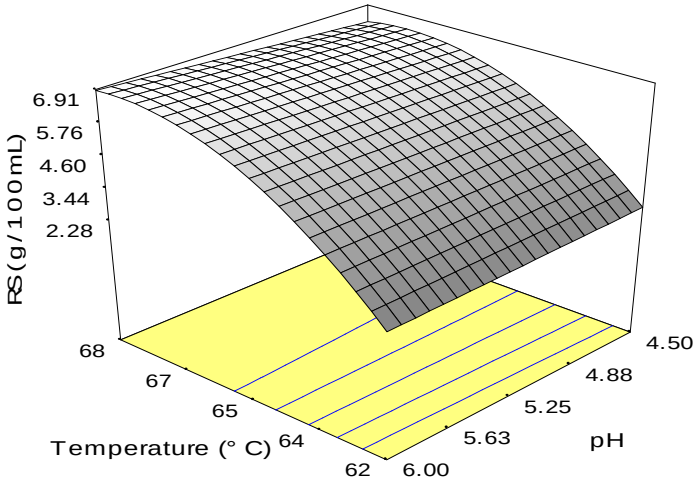


Fig. 5.50 Response surface plot for total reducing sugar (RS as maltose) content as a function of temperature and pH during dextrinizing period.

5.2.9.2.4 Effect of process variables on glucose content during dextrinizing period

The glucose contents during dextrinizing period ranged from 1.55 to 4.3 g /100 mL. The coefficients of the model and other statistical attributes of glucose are given in Appendix B, Table 3. Regression model fitted to the experimental results of glucose indicated that the model was significant ($p<0.05$), whereas lack-of-fit was not significant (Appendix B, Table 4). The fit of the model was also expressed by the coefficient of determination and was found to be 0.9808, indicating that 98.08% of the variability of the response could be explained by the model. The adjusted R^2 was 0.9671 and the adequate precision was 24.089, which showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq 5.6) was selected for representing the variation of glucose during dextrinizing period of mashing. The quadratic model obtained from regression analysis for glucose content in terms of coded levels of the variables was as follows:

$$\text{Glucose (g/100 mL)} = 3.49 + 1.13x_1 - 0.15x_2 - 0.78x_1^2 + 0.17x_2^2 - 0.10x_1x_2 \qquad \text{(Eq 5.6)}$$

Where x_1 and x_2 are the coded values of temperature and pH respectively.

In this case, x_1 and x_1^2 were significant terms. Analysis of varience of equation 5.6 showed that temperature had a highly significant positive linear effect ($p<0.05$) followed by its significant negative effect of the quadratic term (x_1^2), while mash pH had no significant effect on glucose content. Overall variation of RS followed a convex

shaped curve and the interaction effect of temperature and pH on wort glucose content was intermediate (Fig. 5.51). Situation of studentized residual along the predicted line in normal plot was ideal for charecterising residuals as unbiased. Most of the residuals were along the predicted line (Fig. 5.52) indicating that the residuals were normally distributed. The variation of glucose with mash temperature and pH during dextrinizing period is shown in Fig. 5.53. Glucose formation was found to increase with increasing mash temperature irrespective of pH indicating that evolution of glucose was significantly influenced by mash temperature during dextrinizing period of mashing. Contour plots for mash glucose content during dextrinizing period is shown in Appendix C, Fig 6.

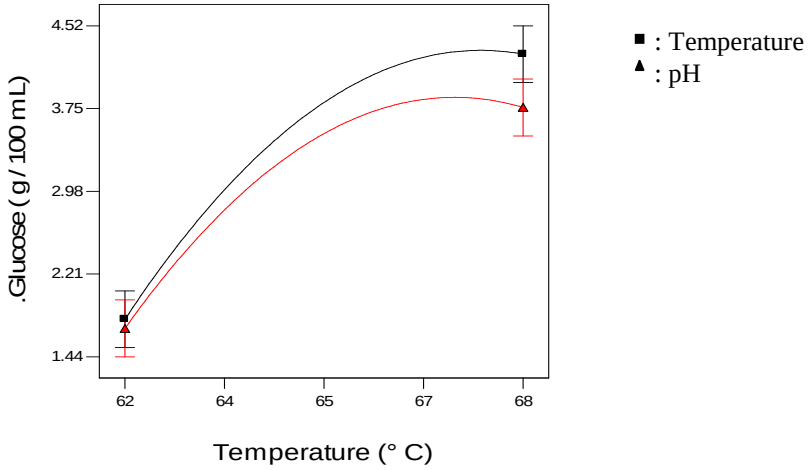


Fig. 5.51 Interaction plot of temperature and pH for glucose content during dextrinizing period.

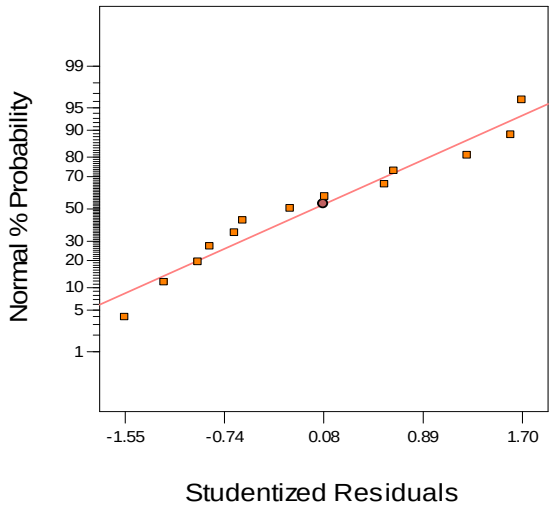


Fig. 5.52 Residual plot for glucose content during dextrinizing period of mashing.

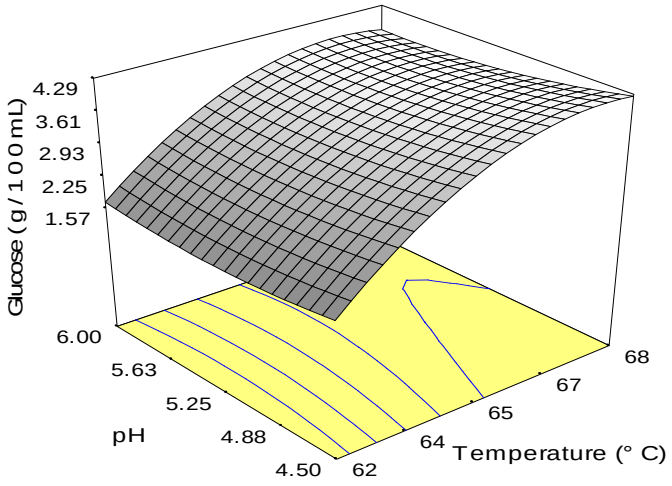


Fig. 5.53 Response surface plot for glucose content as a function of temperature and pH during dextrinizing period.

5.2.9.2.5 Effect of process variables on fructose content during dextrinizing period.

The fructose contents of mashes during dextrinizing period were in the range of 0.24 to 0.53 g/100 mL. Appendix B, Table 3 shows the coefficients of the model and other statistical attributes of fructose during dextrinizing period of mashing. Regression model fitted to experimental data of fructose contents showed that the model was significant, whereas the lack-of-fit was not significant ($p>0.05$) (Appendix B, Table 4). The chance of large model F-value due to noise was only 0.05%. The fit of model was also expressed by the coefficient of determination which was found to be 0.9366, indicating that 93.66% of the variability of the response could be explained by the model. The adjusted R^2 (0.8913) and the adequate precision (15.839) showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq 5.7) was selected for representing the variation of fructose contents during dextrinizing period.

The quadratic model obtained from regression analysis for fructose content in terms of coded levels of the variables was as follows:

$$\text{Fructose} = 0.44 + 0.053x_1 - 0.080x_2 - 0.032x_1^2 - 0.028x_2^2 - 0.0023x_1x_2 \quad \text{(Eq 5.7)}$$

(g/100 mL)

Where x_1 and x_2 are the coded values of temperature and pH respectively.

In this case, x_1 and x_2 were significant terms. Analysis of varience of equation 5.7 showed that pH had a highly significant negative linear effect ($p<0.05$) followed by significant positive linear effect of temperature, while the quadratic terms of temperature (x_1^2) and pH (x_2^2), and the iteraction term (x_1x_2) had no significant effect on fructose content ($p>0.05$). No interaction effect of temperature and pH on fructose content of mash during dextrinizing period was found (Fig. 5.54). Situation of studentized residual along the predicted line in normal plot was ideal for charecterising the residuals as unbiased and most of the residuals were along the predicted line (Fig. 5.55).

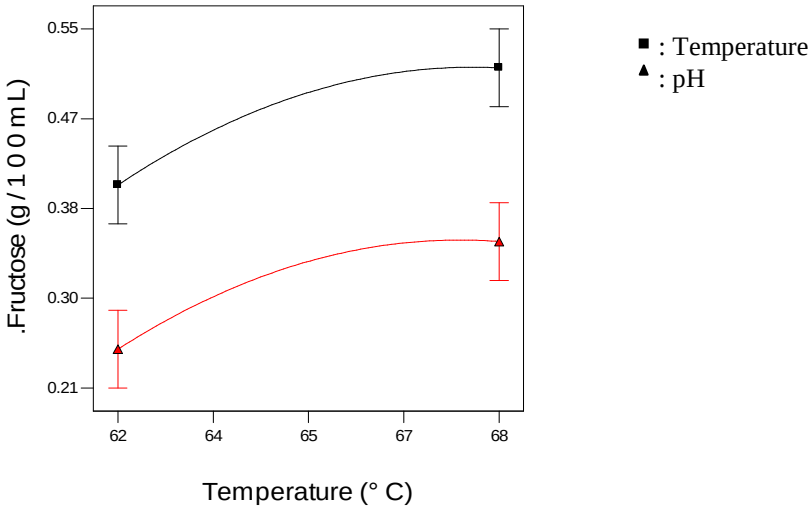


Fig. 5.54 Interaction plot of temperature and pH for fructose content during dextrinizing period.

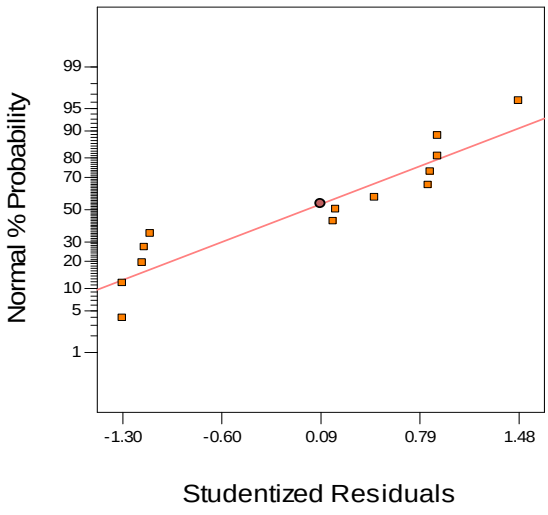


Fig. 5.55 Residual plot for fructose content during dextrinizing period of mashing

The variation of fructose with temperature and pH during dextrinizing period is depicted in Fig. 5.56. Millet malt mashed at higher temperature and lower pH regimes seemed to maximize fructose content in the wort. Contour plot of mash glucose content during dextrinizing period is shown in Appendix C, Fig 7.

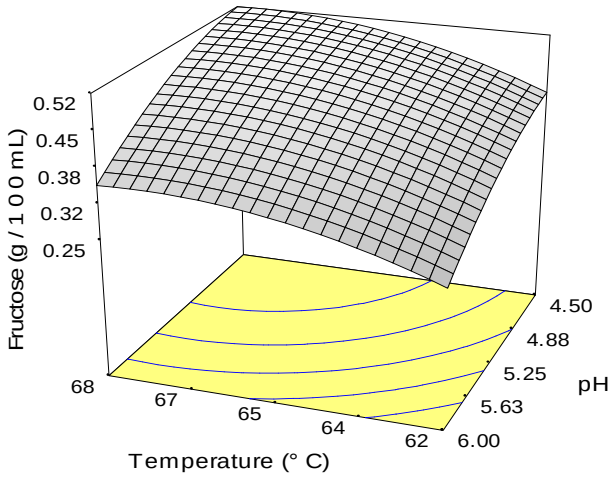


Fig. 5.56 Response surface plot for fructose content as a function of temperature and pH during dextrinizing period.

5.2.9.3 Effect of process variables on different responses during conversion period

5.2.9.3.1 Effect of process variables on FAN content during conversion period

The FAN contents of mash during conversion period varied from 10.3 to 13.5 mg glycine/100 mL. Appendix B, Table 5 shows the coefficients of the model and other statistical attributes of FAN. Regression model fitted to experimental results of FAN showed that the model was significant, while the lack-of-fit was not significant ($p>0.05$) (Appendix B, Table 6). The chance of large model F-value due to noise was only 0.01%. The fit of model was also expressed by the coefficient of determination which was found to be 0.9597, indicating that 95.97% of the variability of the response could be explained by the model. The adjusted R^2 was 0.9309 and adequate precision was 20.118, which showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq. 5.8) was selected for representing the variation of FAN during conversion period of mashing. The quadratic model obtained from regression analysis for FAN content in terms of coded levels of the variables was as follows:

$$FAN = 11.65 - 0.73x_1 + 0.65x_2 + 0.99x_1^2 - 0.86x_2^2 - 0.22x_1x_2 \quad \text{(Eq 5.8)}$$

(mg gly./100 mL)

Where x_1, and x_2, are the coded values of temperature (°C) and pH respectively.

In this case, x_1, x_2, x_1^2 and x_2^2 were significant terms. Analysis of varience of equation 5.8 showed that temperature had highly significant negative linear effect followed by its positive significant quadratic effect, positive linear effect of pH (x_2) and negative quadratic effect of pH (x_2^2). Results showed that the interaction term (x_1x_2) had no significant effect on FAN content. From Fig. 5.57 it can be envisaged that mash temperature and pH had no interaction effect on FAN formation during conversion period. Normal plot of residuals (Fig. 5.58) indicated that studentized residuals were normally distributed.

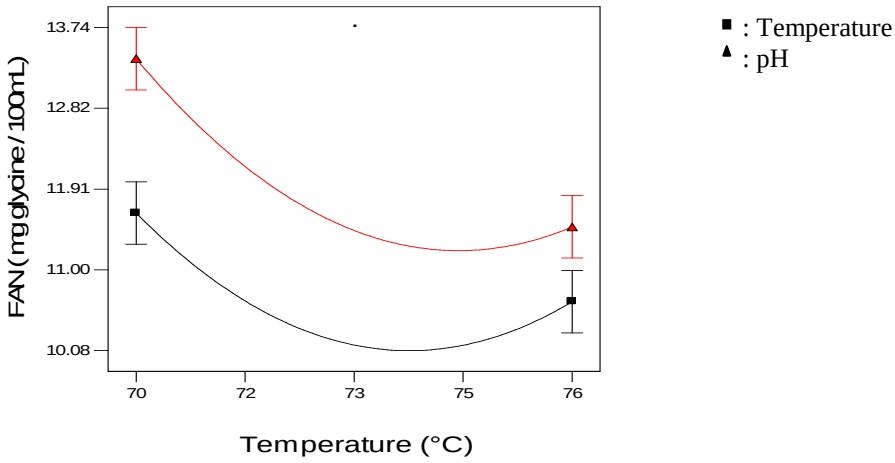


Fig. 5.57 Interaction plot of temperature and pH for FAN content during conversion period.

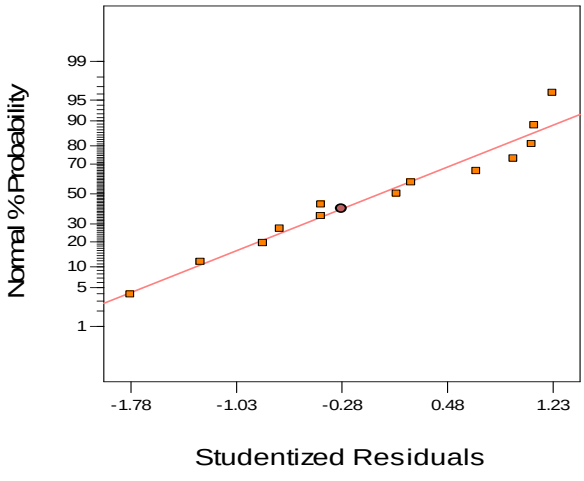


Fig. 5.58 Residual plot for FAN content during conversion period of mashing.

Fig. 5.59 shows the variation of FAN with process variables during conversion period of mashing where the maximum FAN content was obtained at lower temperature (around 70 °C) and intermediate pH value (about 5.25). Contour plot for wort FAN during conversion period of mashing is shown in Appendix C, Fig. 8.

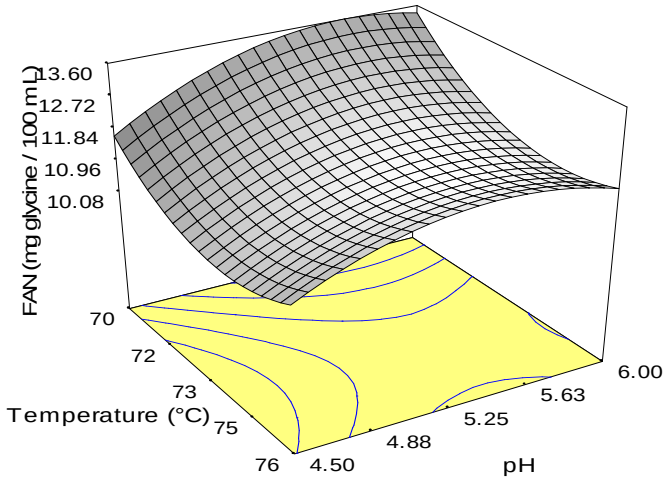


Fig. 5.59 Response surface plot for FAN content as a function of temperature and pH during conversion period.

5.2.9.3.2 Effect of process variables on TSS content during conversion period

The total soluble solid (TSS) of mash during conversion period ranged from 8.3 to 10.80 °Bx. The coefficients of the model and other statistical attributes of TSS are shown in Appendix B Table 5. Regression model fitted to the experimental results of TSS showed that the model was significant, whereas the lack-of-fit was not significant ($p>0.05$) (Appendix, Table 6). The fit of the model was also expressed by the coefficient of determination which was found to be 0.9870, indicating that 98.70% of the variability of the response could be explained by the model. The adjusted R^2 was 0.9778 and adequate precision was 35.2, which showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq. 5.9) was selected for representing the variation of TSS.

The quadratic model obtained from regression analysis for TSS content in terms of coded levels of the variables was as follows:

$$TSS\ (°\ Bx) = 10.22 + 0.80x_1 - 0.16x_2 - 0.30x_1^2 - 0.39x_2^2 + 0.23x_1x_2 \qquad \text{(Eq 5.9)}$$

Where x_1 and x_2, are the coded values of temperature and pH respectively.

In this case, x_1, x_2, x_1^2 x_2^2 and x_1x_2 were all significant terms. Analysis of varience of the equation 5.9 indicated that temperature (x_1) had highly significant linear positive effect followed by negative quadratic effect of pH (x_2), negative quadratic effect of temperature (x_1^2), positive effect of interaction term (x_1x_2) and linear negative effect of pH (x_2). It was found that mash temperature and pH showed an interactive effect on the evolution of TSS during conversion period of mashing (Fig. 5.60). Situation of studentized residual along the predicted line in normal plot was ideal condition for charecterising residuals as unbiased. Most of the residuals lay along the predicted line indicating that the residuals were normally distributed (Fig. 5.61). Fig. 5.62 shows the variation of TSS during conversion period as functions of temperature and pH. From Fig. 5.62 it revealed that maximum TSS could be obtained using higher temperature and intermedite pH regimes. Contour plot for total soluble solids during conversion period of mashing is shown in Appendix C, Fig 9.

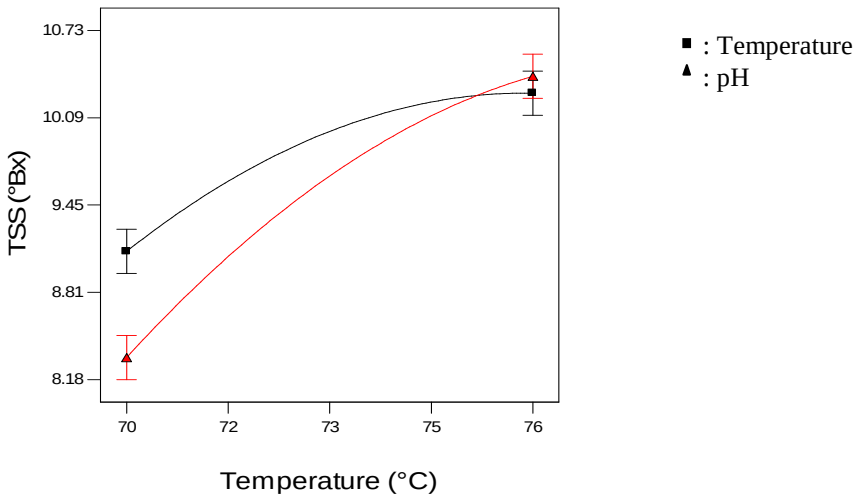


Fig. 5.60 Interaction plot of temperature and pH for total soluble solid (TSS) content during conversion period of mashing.

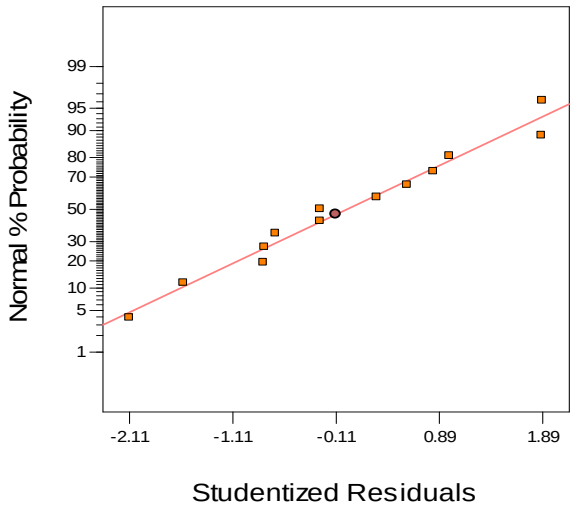


Fig. 5.61 Residual plot for total soluble solid (TSS) content during conversion period of mashing.

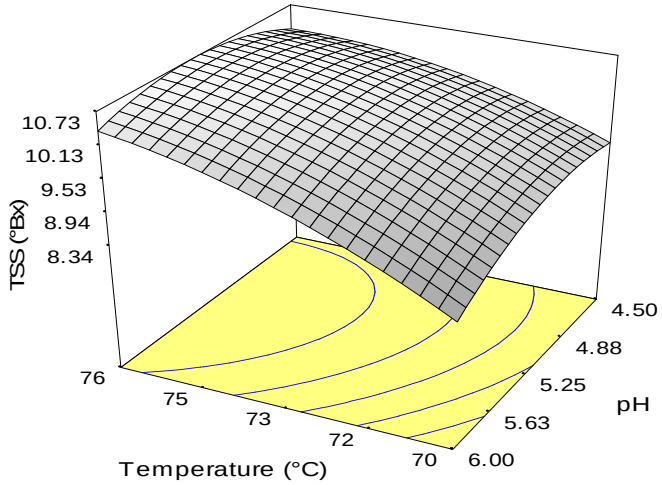


Fig 5.62 Response surface plot for TSS content as a function of temperature and pH during conversion period.

5.2.9.3.3 Effect of process variables on total reducing sugar (RS) contents during conversion period

The total reducing sugar (RS) content during conversion period ranged from 4.79 to 8.16 g maltose/100 mL. The coefficients of the model and other statistical attributes of total reducing sugar are shown in Appendix B, Table 5. Regression model fitted to the experimental data of total reducing sugar showed that the model was significant ($p<0.05$), whereas lack-of-fit was not significant (Appendix B, Table 6). The chance of large model F-value due to noise was only 0.01%. The fit of model was also expressed

by the coefficient of determination (R^2) which was found to be 0.9598, indicating that 95.98% of the variability of the response could be explained by the model. The adjusted R^2 (0.9310) and adequate precision (18.187) showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq 5.10) was selected for representing the variation of total reducing sugar during conversion period of mashing. The quadratic model obtained from regression analysis for RS content in terms of coded levels of the variables was as follows:

Reducing sugar (g maltose/100 mL) = $7.60 - 1.20x_1 - 0.019x_2 - 0.57x_1^2 - 0.47x_2^2 - 0.37x_1x_2$ (Eq 5.10)

Where x_1 and x_2 are the coded values of temperature and pH.

In this case, x_1, x_1^2 , x_2, ^{2} and x_1x_2 were significant terms. Analysis of varience of equation 5.10 showed that temperature had highly significant negative linear effect followed by significant negative effect of quadratic term of temperature (x_1^2), quadratic term of pH with negative coefficient and interaction term (x_1x_2) with negative coefficient. In this case, pH had no significant effect on RS content at 95% level of confidence. Significant negative linear and quadratic effects of temperature as well as negative coefficient of interaction term (x_1x_2) indicated that overall variation of RS with temperature and pH resulted a convex shaped curve. Mash temperature and pH showed a high interaction effect on total reducing sugar development during conversion period of mashing (Fig. 5.63). Normal plot of residuals indicated that studentized residuals were normally distributed (Fig. 5.64).

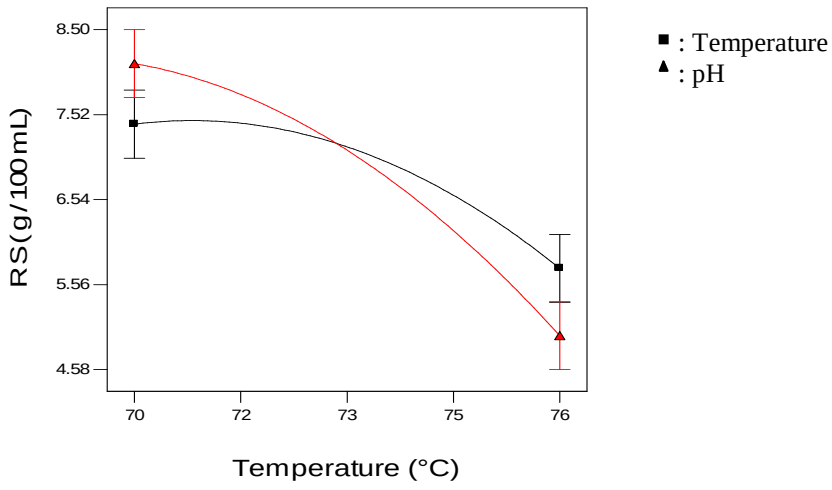


Fig. 5.63 Interaction plot of temperature and pH for total reducing sugar (RS as maltose) content during conversion period of mashing.

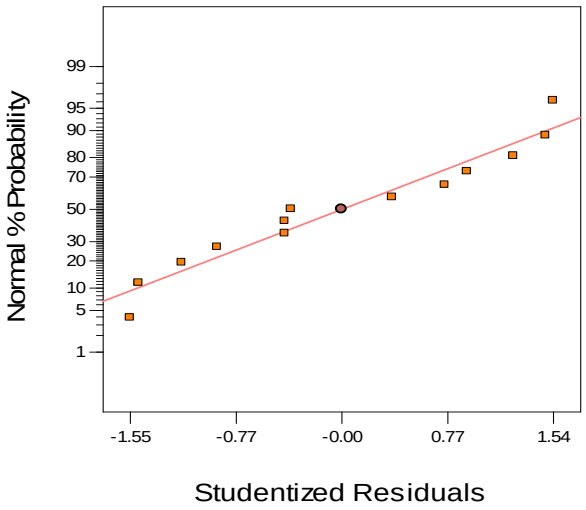


Fig. 5.64 Residual plot for total reducing sugar content (RS as maltose) during conversion period of mashing.

The variation of total reducing sugar with temperature and pH during conversion period is shown in Fig. 5.65. It was found that the evolution of reducing sugar was higher at lower range of temperature and middle range of pH (5.25 – 5.63) within the selected design space. Contour plot of total reducing sugar content during conversion period is shown in Appendix C, Fig 10.

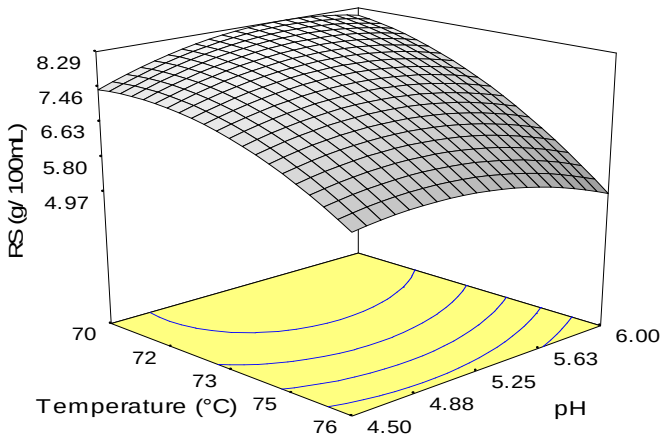


Fig. 5.65 Response surface plot for total reducing sugar content (RS as maltose) as a function of temperature and pH during conversion period of mashing.

5.2.9.3.4 Effect of process variables on glucose content during conversion period

Glucose contents in worts during conversion period ranged from 2.35 to 3.17 g/100 mL. The coefficients of the model and other statistical attributes of glucose are given in

Appendix B, Table 5. Regression model fitted to the experimental results of glucose showed that the model was significant ($p<0.05$), whereas lack-of-fit was not significant (Appendix B, Table 6). The fit of model was also expressed by the coefficient of determination, which was found to be 0.9650, indicating that 96.50% of the variability of the response could be explained by the model. The adjusted R^2 (0.9400) and adequate precision (22.584) showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to navigate the design space. Considering all the above criteria, the model (Eq 5.11) was selected for representing the variation of mash glucose during conversion period. The quadratic model obtained from regression analysis for glucose content in terms of coded levels of the variables was as follows:

$$\text{Glucose (g/100 mL)} = 2.94 - 0.20x_1 + 0.083x_2 - 0.028x_1^2 - 0.22x_2^2 + 0.053x_1x_2 \quad \text{(Eq 5.11)}$$

Where x_1 and x_2 are the coded values of temperature and pH respectively. In this equation x_1, x_2, and x_2^2 were significant terms. Analysis of varience of equation 5.11 showed that temperature had highly significant negative linear effect followed by significant negative effect of quadratic term of pH (x_2^2) and significant positive linear effect of pH.

In this case, quadratic effect of temperature and interaction term (x_1x_2) had no significant effect ($p>0.05$) on glucose content. Since linear term of temperature was very high in comparison to its quadratic term of pH (x_2^2) and linear term of pH (x_2), conversion period was mainly influenced by temperature. Mash temperature and pH had intermediate interaction effect towards glucose formation during conversion period of mashing (Fig. 5.66). Situation of studentized residual along the predicted line in normal plot was ideal condition for charecterising the residuals as unbiased and most of the residuals lay along the predicted line indicating that the residuals were normally distributed (Fig. 5.67). From Fig 5.68 it was apparent that increaseing temperature decreased the wort glucose content during conversion period and maximum glucose formation was at lower temperature and at middle pH regimes within the defined design space. Contour plot of mash glucose content during conversion period of mashing is shown in Appendix C, Fig 11.

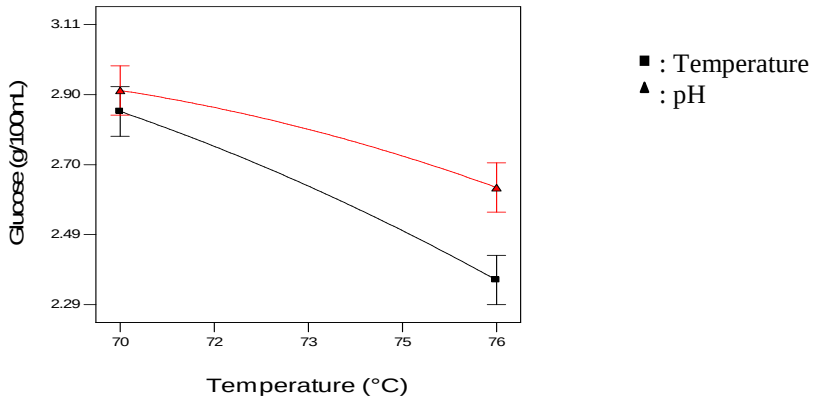


Fig. 5.66 Interaction plot of temperature and pH for glucose formation during conversion period of mashing.

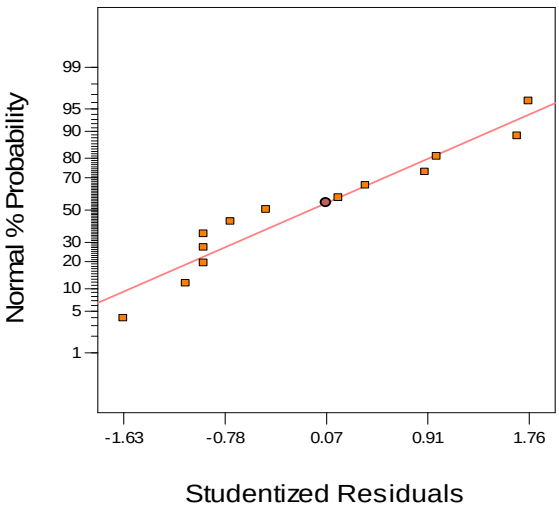


Fig. 5.67 Residual plot for glucose content during conversion period of mashing.

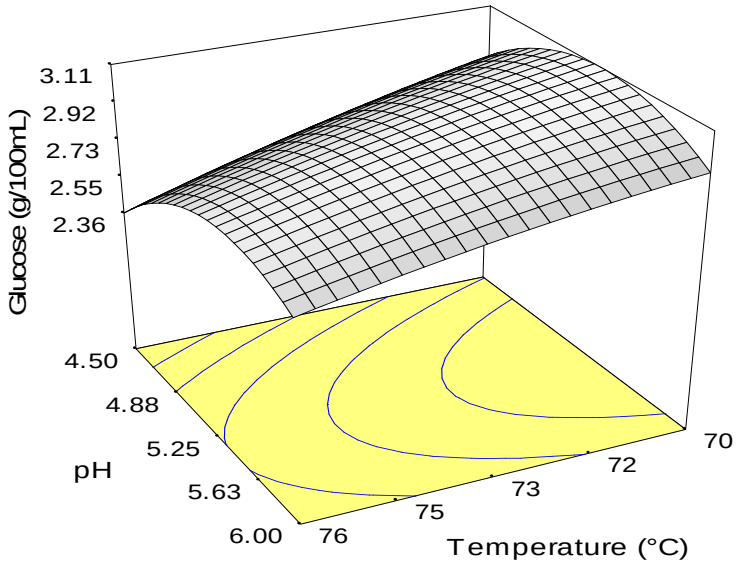


Fig. 5.68 Response surface plot for glucose content as a function of temperature and pH during conversion period of mashing.

5.2.9.3.5 Effect of process variables on fructose content during conversion period

The fructose contents in mashes during conversion period ranged from 0.3 to 0.53 g/100 mL. Appendix B, Table 5 shows the coefficients of the model and other statistical attributes of fructose. Regression model fitted to experimental results of fructose showed that the model was significant, whereas the lack-of-fit was not significant ($p>0.05$) (Appendix B, Table 6). The chance of large model F-value due to noise was only 0.01%. The fit of model was also expressed by the coefficient of determination which was found to be 0.9765, indicating that 97.65% of the variability of the response could be explained by the model. Adjusted R^2 and adequate precision were 0.9597 and 23.47 respectively, which showed an adequate signal. A ratio greater than 4 is desirable and hence this model could be used to navigate the design space. Considering all the above criteria, the model (Eq 5.12) was selected for representing the variation of fructose in the wort during conversion period. The quadratic model obtained from regression analysis for fructose content in terms of coded levels of the variables was as follows:

$$\text{Fructose (g/100 mL)} = 0.37 + 0.047x_1 + 0.017x_2 + 0.11x_1^2 - 0.037x_2^2 - 0.02x_1x_2 \quad \text{(Eq 5.12)}$$

Where x_1 and x_2 are the coded values of temperature and pH respectively.

In this case, x_1, x_2, x_1^2, x_2^2 and x_1x_2 were all significant terms. Analysis of varience of equation 5.12 showed that the quadratic term of temperature had highly significant positive linear effect followed by its significant positive linear effect, significant negative quadratic effect of pH, significant linear positive effect of pH (x_2) and significant negative effect of the interaction term(x_1x_2). Since F-value of linear effect of temperature was higher than other terms and its quadratic term was also significant, fructose formation followed concave shaped variation (Fig. 5.69). Mash temperature and pH showed interaction effect towards fructose development during conversion period.

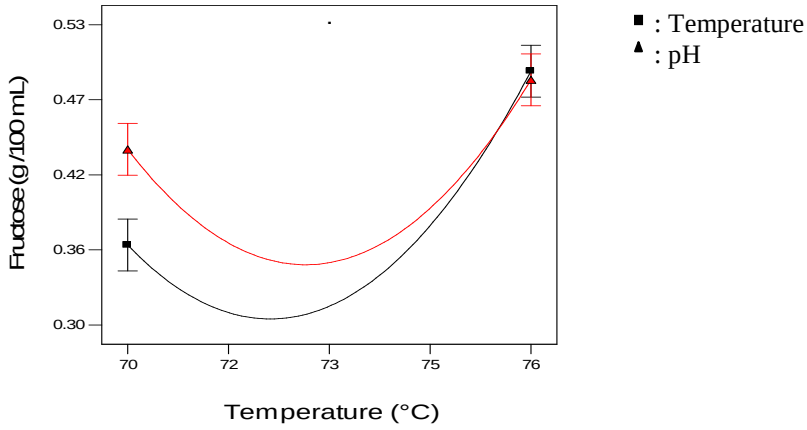


Fig. 5.69 Interaction plot of temperature and pH for fructose content during conversion period of mashing.

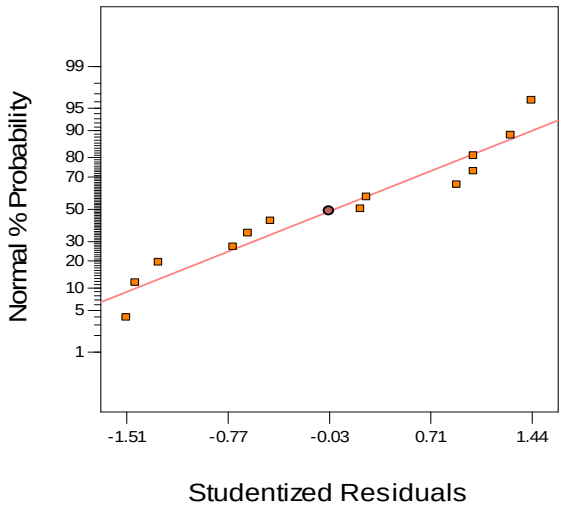


Fig. 5.70 Residual plot for fructose content during conversion period of mashing.

Situation of studentized residual along the predicted line in normal plot was ideal for charecterising the residuals as unbiased. Most of the residuals were along the predicted line (Fig. 5.70) indicating that the residuals are normally distributed. Fig. 5.71 showed that the development of fructose during conversion period was maximum at higher temperature and intermediate pH ranges, while it was minimum at middle temperature range for all pH values. Contour plot for fructose development during conversion period of mashing is shown in Appendix C, Fig. 12.

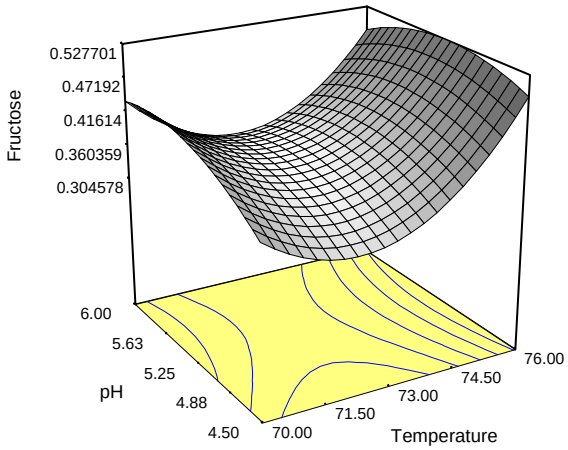


Fig. 5.71 Response surface plot for fructose content as a function of temperature and pH during conversion period.

5.2.9.4 Optimization of process variables

A numerical multi-response optimization technique was applied to determine the optimum combination of temperature and pH for FAN and TSS contents during protein rest period, for FAN, TSS, RS, glucose, and fructose contents during dextrinizing and conversion periods of mashing respectively.

5.2.9.4.1 Compromized optimum conditions for protein rest period

The assumptions were to develop a mashing condition with respect to mashing temperature and pH that would result maximum values of FAN and TSS in the mash. Under these criteria, the uncoded optimum operating conditions for mashing were 57.62 °C temperature and pH of 5.47. The responses predicted by the software for these optimum process conditions resulted FAN content of 13.8 mg glycine/100 mL and TSS of 4.1 °Bx with desirability of 0.62.

Table 5.20 Multiresponse optimization constraints of protein rest period

Variables	Goal	Lower limit	Upper limit	Weight	Importance
Temp.	is in range	40	60	1	3
pH	is in range	4.5	6	1	3
FAN	maximize	10.8	15.72	1	3
TSS	maximize	1.4	5.8	1	3

Verification of the results

The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables and was also used to validate experimental and predicted values of the responses. The experimental sample under the optimum process condition resulted FAN content, of 13.4 mg glycine/100 mL and TSS of 4 °Bx.

Table 5.21 Predicted and actual values of the responses at the optimized condition for protein rest period

Process variables	Coded	Uncoded	Responses	Predicted value	Actual value	Variation (%)
Temp.	+0.76	57.62°C	FAN	13.8	13.4	2.90
pH	+0.17	5.47	TSS	4.1	4	2.44

5.2.9.4.2 Compromized optimum conditions for dextrinizing period

The assumptions were to develop a mashing condition that would maximize FAN, TSS, RS, glucose, and fructose contents in the wort. Under these criteria, the uncoded optimum operating conditions for mashing were 68 °C temperature and pH of 4.5. The responses predicted by the software for these optimum process conditions were FAN content of 12.4 mg glycine/100 mL, TSS of 8.47 °Bx, RS of 6.29% as maltose, glucose of 4.25% and fructose of 0.51 % with the desirability of 0.88.

Table 5.22 Multiresponse optimization constraints of dextrinizing period

Variables	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Temp.	is in range	62	68	1	1	3
pH	is in range	4.5	6	1	1	3
TSS	maximize	2.26	8.46	1	1	3
RS	maximize	2.25	7.06	1	1	3
Glucose	maximize	1.55	4.3	1	1	3
Fructose	maximize	0.235	0.525	1	1	3
FAN	maximize	8.825	13.975	1	1	3

Verification of the results

The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables and was also used to validate experimental and predicted values of the responses. The predicted and actual values of the responses at the optimized condition for dextrinizing period are shown in Table 5.23.

Table 5.23 Predicted and actual values of the responses at the optimized conditions for dextrinizing period

Process variables	Coded	Uncoded	Responses	Predicted value	Actual value	Variation (%)
Temp.	+1	68 °C	FAN	12.4	12.2	1.61
pH	-1	4.5	TSS	8.47	8.2	3.19
			Reducing sugar	6.29	6.13	2.54
			Glucose	4.25	4.31	1.39
			Fructose	0.51	0.49	3.92

5.2.9.4.3 Compromized optimum conditions for conversion period

The assumptions were to develop a mashing condition, which would have maximum values of FAN, TSS, RS, glucose, and fructose contents in the wort. Therefore, among responses, these parameters were attempted to be maintained. Under these criteria, the uncoded optimum operating conditions for mashing were 70 °C temperature and pH of 5.28. The responses predicted by the software for these optimum process conditions resulted FAN content of 13.40 mg as glycine/100 mL, TSS of 9.10 °Bx, total reducing sugar content of 8.24 % as maltose, glucose content of 3.10 % and fructose content of 0.43 % with desirability of 0.703.

Table 5.24 Compromized optimum condition for conversion period

Variables	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Temp.	is in range	70	76	1	1	3
pH	is in range	4.5	6	1	1	3
TSS	maximize	8.3	10.8	1	1	3
RS	maximize	4.795	8.16	1	1	3
Glucose	maximize	2.35	3.17	1	1	3
Fructose	maximize	0.3	0.53	1	1	3
FAN	maximize	10.33	13.49	1	1	3

Verification of the results

The suitability of the model developed for predicting the optimum response values was tested using the recommended optimum conditions of the variables and was also used to validate experimental and predicted values of the responses. The predicted and actual values of the responses at the optimized condition for conversion period are shown in Table 5.25.

Table 5.25 Predicted and actual values of the responses at the optimized condition for conversion rest period

Process variables	Coded	Uncoded	Responses	Predicted value	Actual value	Variation (%)
Temp.	-1	70°C	FAN	13.40	13.41	0.07
pH	+0.04	5.28	TSS	9.10	9.15	0.55
			RS	8.24	8.3	0.72
			Glucose	3.1	3.15	1.59
			Fructose	0.43	0.42	2.33

5.2.10 Chemical, physical and sensory quality analyses of finger millet and barley beers

5.2.10.1 Chemical characteristics of millet and barley beers

The chemical characteristics of finger millet and barley beers are shown in Table 5.26. Total soluble solids (TSS) ranged between 5.60 and 5.93 ºBx among the three beers, however, the values were not statistically different ($p>0.05$). Analogous results of TSS were also reported in naked barley beer (5.80 ºBx), and barley beer (6.70 ºBx) (Acharya, 2007); wheat beer (6.40 ºBx) (Mahato, 2010) and in millet beer (4.28 ºBx) (Tiwari, 2010). Total reducing sugar content in beer brewed from millet malt kilned by standard barley method (millet $beer_2$) was similar to that of barley malt beer, while it was significantly lower in millet beer brewed using millet malt kilned at 50 ± 2 ºC (millet $beer_1$). Acharya (2007) also reported similar reducing sugar content in naked barley beer (1% m/v as maltose) and Mahato (2010) in wheat beer (0.91% m/v, as maltose. Matz (1991) also reported similar reducing sugar in American lager beer (0.97%). Glucose contents in millet beers were similar, while it was significantly lower ($p<0.05$) in barley beer than those of millet beers (Table 5.26). The obtained results of glucose content were quite lower than those reported for sorghum beers (0.16 – 0.31 g/100 mL) (Veith, 2009). Fructose content was highest in barley beer (135.3 mg/100 mL) followed by millet $beer_2$, while it was lowest in millet $beer_1$ (53.4 mg/100 mL).

Both the total sugar and sucrose contents were higher ($p<0.05$) in barley beer than those of millet beers. Again, millet $beer_2$ had higher sucrose and total sugar compared to millet $beer_1$ (Table 5.26). Dextrin content was lower ($p<0.05$) in barley beer (0.89 g /100 mL) than those of millet beers. Millet $beer_1$ contained higher dextrin (1.69 g/100 mL) than millet $beer_2$ (1.14 g/100 mL).

Table 5.26 Chemical characteristics of finger millet and barley beers

Parameters	Mean values*		
	Millet beer$_1$**	Millet beer$_2$***	Barley beer
TSS (^{o} Bx)	5.93 (1.59)^a	5.60 (0.26)^a	5.83 (0.93)^a
Reducing sugar as maltose (% m/v)	0.51 (0.01)^a	0.84 (0.05)^b	0.82 (0.01)^b
Glucose (mg%, m/v)	10.0 (0.1)^a	10.9 (0.8)^a	4.5 (0.1)^b
Fructose (mg%, m/v)	53.4 (0.7)^a	85.7 (0.9)^b	135.3(4.3)^c
Sucrose (mg%, m/v)	44.5 (5.6)^a	61.3 (9.4)^b	117.3 (11.1)^c
Total sugar (mg%, m/v)	561.3 (11.1)^a	872.7 (55.3)^b	934.0 (16.9)^c
Dextrin (%, m/v)	1.69 (0.1)^a	1.14 (0.05)^b	0.89 (0.02)^c
pH	4.02 (0.11)^a	3.97 (0.06)^a	4.06 (0.05)^a
Total acidity as lactic (%, m/v)	0.24 (0.01)^a	0.16 (0.04)^a	0.23 (0.06)^a
Fixed acidity as lactic (%, m/v)	0.21 (0.01)^a	0.15 (0.03)^a	0.20 (0.05)^a
Volatile acidity as acetic	0.021(0.001)^a	0.015 (0.003)^a	0.023 (0.005)^b
Water soluble protein#(mg/100 mL)	224.3 (3.7)^a	244.0 (3.1)^b	299.9 (10.1)^c
FAN as glycine (mg%, m/v)	3.1 (0.1)^a	2.2 (0.1)^b	9.4 (0.6)^c
Formol N (mg/L)	26.0 (1.0)^a	19.5 (2.5)^a	64.5 (12.0)^b
Total phenolics as gallic acid (mg%, m/v)	43.1(2.0)^a	54.1 (3.27)^b	59.2 (1.9)^c
Tannin as tannic acid (mg%, m/v)	42.5 (2.7)^a	52.0 (2.1)^b	67.5 (2.6)^c
Total flavonoids as rutin (mg%, m/v)	33.8 (1.4)^a	41.5 (1.0)^b	27.3 (2.2)^c
Antioxidant activity (%)	18.09 (1.67)^a	26.85 (1.62)^b	33.17 (2.22)^c
Extract (%, m/m)	1.80 (0.01)^a	1.80 (0.01)^a	1.84 (0.04)^a
Real extract (%, m/m)	3.76 (0.1)^a	3.89 (0.1)^a	3.12 (0.06)^a
Apparent extract (%, m/m)	3.23 (0.90)^a	3.61 (0.41)^a	3.01 (0.23)^a
Original extract (%,m/m)	11.56 (0.53)^a	13.08 (0.37)^b	12.31 (0.47)^{ab}
Real degree of ferm. (%)	71.73 (6.26)^a	71.90 (2.5)^a	76.26 (3.8)^a
Formaldehyde (mg/L)	0.24 (0.05)^a	0.23 (0.01)^a	0.33 (0.04)^b

*values are the means of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by the similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

**millet $beer_1$: beer made from millet malt kilned at 50 ± 2 °C.

***millet $beer_2$: beer made from millet malt kilned by standard barley malt kilning procedure.

#water soluble protein was calculated as bovine serum albumin (BSA).

pH values did not differ among the three beers. The pH of millet beers were within the requirement for Nepalese beer standard (NS, 1983) and similar to naked barley beer (Acharya (2007), wheat beer (Mahato, 2010) and American lager beer (Matz, 1991), but lower than millet (*Pennisetum maiwa*) beer (Agu and Ezeanolue, 1993), sorghum beer (Goode and Arendt, 2003). Similar results of pH contents were also reported by Delvaux *et al.* (2004) in barley beer. Total and fixed acidities ranged from 0.16 to 0.24, and 0.15 to 0.21% (m/v) as lactic acid, but the values were not significantly different ($p>0.05$) among the three beers. Total acid contents of millet beers were similar to those reported by Agu and Ezeanolue (1993) in millet beer and Matz (1991) for American lager beer (0.21% as lactic acid. Tiwari (2010) also reported similar results of total and fixed acidities in millet beer. Volatile acid contents were found to be 0.021, 0.015 and 0.023% (m/v) as acetic acid in millet $beer_1$, millet $meer_2$ and barley beer respectively and the value for barley beer was higher ($p<0.05$) compared to millet beers. Analogous result was also reported by Tiwari (2010) in millet beer.

Water-soluble protein was highest in barley beer (299.9 mg/ 100mL), followed by millet $beer_2$ (244.0 mg/100 mL), while it was lowest in millet $beer_1$ (224.3 mg/100 mL). The FAN content differed significantly among the three beers. The highest FAN content was in barley beer (9.4 mg glycine/100 mL), while the lowest was in millet $beer_2$ (2.2 mg glycine/100 mL). Formol nitrogen was higher in barley beer (64.5 mg/L) compared to millet beers, while the values between millet beers did not differ ($p>0.05$). Barley beer contained significantly higher ($p<0.05$) total phenolics (TPs) (59.2 mg GAE/100 mL) and tannin (67.5 mg tannic acid/100 mL) compared to millet beers. Again, millet $beer_2$ had higher total phenolics and tannin contents than that of millet $beer_1$. Zao *et al*. (2010) also reported TPs contents in beers ranging from 15.2 – 33.9 mg GAE/100 mL, which also supported our findings. Analogous results of TPs contents were also reported by Lugasi and Hovari, (2003) (39.2 – 47.3 mg GAE/100 mL) in dark and lager beers and by Piazzon *et al.* (2010) in Italian beers. The variations in TPs contents in beers may be due to differences in samples and TPs content evaluation methods. Moreover, the brewing process itself may influence the final TPs content and antioxidant activity of beers (Vanbeneden *et al.*, 2007). Total flavonoids contents (rutin equivalent) varied significantly among beers, with the highest value being found in millet $beer_2$ (41.5 mg /100 mL) and the lowest value in barley beer (27.3 mg/100 mL). Barley beer exhibited the highest ($p<0.05$) antioxidant activity (33.17%),

followed by millet beer$_2$ (26.85%), while it was lowest in millet beer$_1$ (18.09%). It appeared that antioxidant activity was in parallel with TPs and tannin contents in beers. Lugasi and Hovari (2003) also reported strong correlation between TPs content and antioxidant activity in beers and wines. Beers rich in phenolic antioxidants show higher quality, more stable sensory properties (such as flavor and aroma), foam stability, and longer shelf life with respect to beers with lower antioxidant activity (Goupy *et al.*, 1999). Extract contents were in the range of 1.80 – 1.84 g/100 g, but the values were not different ($p>0.05$). Real extract, apparent extract and real degree of fermentation ranged between 3.12 – 3.89, 3.01 – 3.61 and 71.73 – 76.26% m/m respectively among the three beers, and the values were statistically not different. The results agreed to those reported by Tiwari (2010) in millet beers.

The original extracts were 11.56, 13.08 and 12.31 % (m/m) respectively in millet beer$_1$, millet beer$_2$ and barley beer respectively. Millet beer$_2$ had higher original extract compared to millet beer$_1$, but the values between millet beer$_2$ and barley beer were not different. Original extracts of millet beers were similar to that reported for American lager beer (10.7 – 12.1 ^{o}Plato) (Matz, 1991), for barley beers (11.5 – 11.7 ^{o}Plato) (Delvaux *et al.*, 2004) and for sorghum beers (10.98 – 11.22 ^{o}Plato) (Goode and Arendt, 2003). Barley beer contained higher ($p<0.05$) formaldehyde (0.33 mg/L) then those of millet beers, but the values between millet beers were similar. Jendral *et al.* (2011) reported that in 132 samples of beer, wine, spirit, and unrecorded alcohol analyzed, formaldehyde content was in the range of 0 – 14.7 mg/L, with an average of 0.27 mg/L.

More recently, formaldehyde has been implicated by the International Agency for Research on Cancer (IARC) as a causative agent of leukemia as well as nasopharyngeal cancer in humans (Baan *et al.*, 2009). The WHO has established a tolerance concentration of 2.6 mg/L in ingested products based on animal experiments (Til *et al.*, 1988). Hence, the millet and barley beers brewed under the experimental conditions were safe as far as the formaldehyde content was concerned.

5.2.10.2 Mineral contents of millet and barley beers

Mineral contents in millet and barley beers are shown in Table 5.27. Total phosphorous content differed significantly among the three beers, with the highest content being found in barley beer (122.4 mg/100 mL) and the lowest content in millet beers. The

highest ($p<0.05$) iron content was found in millet beer$_1$ (5 mg/100 mL), followed by barley beer (1.2 mg/100 mL), while it was lowest in millet beer$_2$ (0.4 mg/100 mL). The obtained values of iron contents in beers were substantially higher than that reported by Matz (1991). Iron is known to have a negative influence on beer flavor stability and even concentrations of below 50 ppb are reported to cause damage in the final product (Foster *et al*., 2001). Millet beer$_1$ had higher manganese content (0.7 mg/100 mL), than that of barley beer (0.1 mg/100 mL) but the manganese contents of millet beers were not statistically different ($p>0.05$). Sodium content was maximum in millet beer$_2$, (21.8 mg/100 mL) followed by barley beer (5.2 mg/100 mL), while it was minimum in millet beer$_1$ (4.6 mg/100 mL). The sodium contents of beers found in this study were similar to those reported for American lager beers (40.220 mg/L), but the values were higher than that reported for Heineken's beer (3 mg/100 mL) (Http://www.heinekeninternational. com/faq Accessed on 2011/09/22). Barley beer contained significantly higher ($p<0.05$) potassium (53.3 mg/100 mL) compared to millet beers. Millet beer$_2$ had higher potassium than millet beer$_1$. The zinc contents between millet beers were not different, while it was significantly lower (0.03 mg/100 mL) in barley beer over the millet beers.

Table 5.27 Mineral composition of millet and barley beers (mg/100 mL)

Parameters	Mean values*		
	Millet beer$_1$**	Millet beer$_2$***	Barley beer
Phosphorous	65.6 (0.2)^a	70.7 (0.2)^b	122.4 (1.2)^c
Iron	5.0 (0.1)^a	0.4 (0.03)^b	1.2 (0.06)^c
Manganese	0.7 (0.06)^a	0.6 (0.06)^a	0.1 (0.01)^b
Sodium	4.6 (0.06)^a	21.8 (0.1)^b	5.2 (0.1)^c
Potassium	24.3 (0.1)^a	29.9 (0.1)^b	53.3 (0.1)^c
Zinc	0.07 (0.005)^a	0.08 (0.001)^a	0.03 (0.006)^b

*values are the mean of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by the similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

**Millet beer$_1$: beer made from millet malt kilned at 50 ± 2 °C

***Millet beer$_2$: beer made from millet malt kilned by standard barley malt kilning procedure.

5.2.10.3 Volatile constituents of millet and barley beers

The volatile constituents of millet and barley beers are shown in Table 5.28. Alcohol contents in millet beer$_1$, millet beer$_2$ and barley beers were 5.12, 5.60 and 5.64% (v/v) respectively but the values were not different ($p>0.05$). The minimum fusel oil content was 228.85 g/100 L alc in barley beer, while the maximum was 809.41 g/100 L alc in millet beer$_1$. The highest methanol content was found in barley beer (249.71 g/100 L alc), while the lowest was in millet beer$_1$ (213.75 g/100 L alc). Similar result of fusel oil (871.00 g/100 L alc) and methanol (367.00 g/100 L alc) contents were also reported by Khanal (2008) in naked barley beer. Total ester contents in barley beer and millet beer$_2$ were similar, while it was significantly lower in millet beer$_1$ (Table 5.28). Total ester contents (as ethyl acetate) found in this study in millet beers were considerably higher than those reported for sorghum beers (3.55 – 11.05 g /100 L alc), but were similar to barley beers (15.86 – 44.83 g/100 L alc) (Igyor *et al*., 2001) and millet beers (64.98 g/100L alc) (Tiwari, 2010). Considerably high total ester contents were reported by Acharya (2007) in naked barley beer, Mahato (2010) in wheat beer and Khanal (2008) in naked barley beer.

Table 5.28 Volatile constituents of finger millet and barley beers

Parameters	Mean values*		
	Millet beer$_1$**	Millet beer$_2$***	Barley beer
Alcohol content (%, v/v)	5.12 (0.19) [a]	5.60 (0.36) [a]	5.64 (0.26) [a]
Fusible oil (g/100L alc)	809.41 (46.65) [a]	582.47 (64.34) [b]	228.85 (5.58) [c]
Methanol (g/100L alc)	213.75 (22.03) [a]	229.81 (42.10) [b]	249.71 (44.95) [c]
Total esters as ethyl acetate (g/100L alc)	39.85 (8.45) [a]	52.19 (7.44) [b]	50.39 (7.18) [b]
Total aldehydes as acetaldehyde (g/100L alc)	8.80 (1.15) [a]	18.85 (1.56) [a]	32.23 (9.79) [b]
Vicinal diketones (mg/L)	0.33 (0.01) [a]	0.27 (0.01) [b]	0.44 (0.01) [c]

*values are the means of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by the similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

**Millet beer$_1$: beer made from millet malt kilned at 50 ± 2 °C.

****Millet beer$_2$: beer made from millet malt kilned by standard barley malt kilning procedure.

Total aldehydes content was significantly higher ($p<0.05$) in barley beer (32.23 g acetaldehyde/100 L alc) than those of millet beers, but the values between millet beers were not different. Surprisingly higher total aldehydes were reported by Khanal (2008) in naked barley beer (89.15 g/100 L alc as acetaldehyde) and Tiwari (2010) in finger millet beer (142.35 g/100 L alc as acetaldehyde). Vicinal diketones (VDK) significantly differed among the beers. Barley beer contained significantly higher VDK (0.44 mg/L) compared to millet $beer_1$ (0.33 mg/L) and millet $beer_2$ (0.27 mg/L) and the values were significantly different ($p<0.05$). *Lekkas et al. (2007)* reported about 0.34 mg/L of VDK in barley beer.

5.2.10.4 Physical properties of millet and barley beers

The physical properties of finger millet and barley beers are shown in Table 5.29.

Table 5.29 Physical properties of finger millet and barley beers

Parameters	Mean values*		
	Millet $beer_1$**	Millet $beer_2$***	Barley beer
Turbidity (FTU)	119.67 (2.8)[a]	52.00 (1.73) [b]	16.33 (1.15) [c]
Viscosity at 20 ºC (cP)	1.39 (0.01) [a]	1.37 (0.01) [a]	1.30 (0.02) [b]
Color (EBC units)	7.17 (0.30) [a]	10.03 (0.10) [b]	10.22 (0.1) [b]
Starch-iodine test#	+	+	-

*values are the mean of triplicate determinations. Figures in the parentheses are the standard deviations. Means followed by the similar superscripts in a row are not significantly different ($p>0.05$) by LSD.

#+ : positive test, - : negative test

** Millet $beer_1$: beer made from millet malt kilned at 50 ± 2 °C

***Millet $beer_2$: beer made from millet malt kilned by standard barley malt kilning procedure.

The turbidity significantly differed among the three beers. Millet $beer_1$ had the highest ($p<0.05$) turbidity (119.67 FTU), while the lowest turbidity was found in barley beer (16.33 FTU). Similarly, barley beer possessed the minimum viscosity (1.30 cP), than those of millet beers, while the values between millet beers did not differ. The highest viscosity and turbidity in millet beers may be due to the presence of higher amounts of dextrin and other unconverted starch (Table 5.26 and 5.29). Millet $beer_1$ had lower

color value than those of millet beer$_2$ and barley beer, but the values between the latter two were not different ($p>0.05$). The color of millet beer$_1$ was comparable, while that of millet beer$_2$ was higher than those reported by Agu and Ezeanolue (1993) for different millet beers (6.5 – 8 EBC units). Delvaux *et al.* (2004) reported lower color values in beers brewed using wheat and barley malts (4.5 – 6.0 EBC units) than those found in this work. Similarly, the obtained color values for millet beer were higher than those reported for sorghum and barley beers (5.49 – 5.65 EBC units) (Goode and Arendt, 2003). Barley beer showed negative starch – iodine test (no change in color), while both millet beers showed positive test (reddish tinge) indicating that barley beer did not have residual starch, while millet beers contained some unconverted starch. Similar results were also reported in millet beers (Agu and Ezeanolue. 1993) and in sorghum beer (Goode and Arendt, 2003).

5.2.10.5 Sensory quality of millet and barley beers

The mean sensory quality scores for millet and barley beers are shown in Table 5.30.

Table 5.30 Sensory evaluation scores of millet and barley beers

Attributes	Mean scores*		
	Millet beer$_1$**	Millet beer$_2$***	Barley beer
Taste	3.5 (0.4)^{a}	3.8 (0.6)^{a}	4.0 (0.4)^{a}
Smell	3.9 (0.7)^{a}	4.0 (0.7)^{a}	3.5 (0.7)^{a}
Color	2.9 (0.6)^{a}	4.1 (0.3)^{b}	4.3 (0.7)^{b}
Flavor	3.4 (0.5)^{a}	3.8 (0.6)^{a}	3.5 (0.5)^{a}
Body	3.8 (0.4)^{a}	4.0 (0.6)^{a}	3.2 (0.5)^{b}
Overall acceptance	3.4 (0.4)^{a}	4.0 (0.3)^{b}	4.0 (0.4)^{b}

*values are the means of 10 sensory panelists. Figures in the parentheses are standard deviations. Mean followed by similar superscripts in a row do not differ ($p>0.05$) by LSD.

**Millet beer$_1$: beer made from millet malt kilned at 50 ± 2 °C .

***Millet beer$_2$: beer made from millet malt kilned by standard barley malt kilning procedure.

The mean sensory scores for taste of the beers, out of a possible score of 5, were 3.5, 3.8 and 4.0 for millet beer$_1$, millet beer$_2$ and barley beer respectively, however, the values were not statistically different ($p>0.05$). Similarly, the mean scores for smell preference ranged from 3.5 to 4.0 among the beers, however, the values were statistically not different. The color of millet beer$_1$ was least preferred (color score 2.9), while those of the millet beer$_2$ (color score 4.1) and barley beer (color score 4.3) were equally preferred by the sensory panelists. Despite higher VDK content of barley beer (Table 5.28), the flavor preference of the three beers was identical with mean scores ranging from 3.4 to 3.8. Body of the millet beer$_1$ and millet beer$_2$ was alike but it was better than that of barley beer. The lowest body score for barley beer may be attributed to the presence of lower amount of dextrin. Millet beer$_1$ had the least preference, while millet beer$_2$ and barley beer were similar in overall acceptability. Hence, from the results of sensory evaluation, it revealed that millet malt kilned by standard barley kilning method would result a beer as good as barley beer with respect to their sensory quality attributes.

5.2.11 Changes during finger millet and barley beer fermentation

Changes in total soluble solids (TSS) and total reducing sugar (TRS) during millet and barley beer fermentation are shown in Fig 5.72.

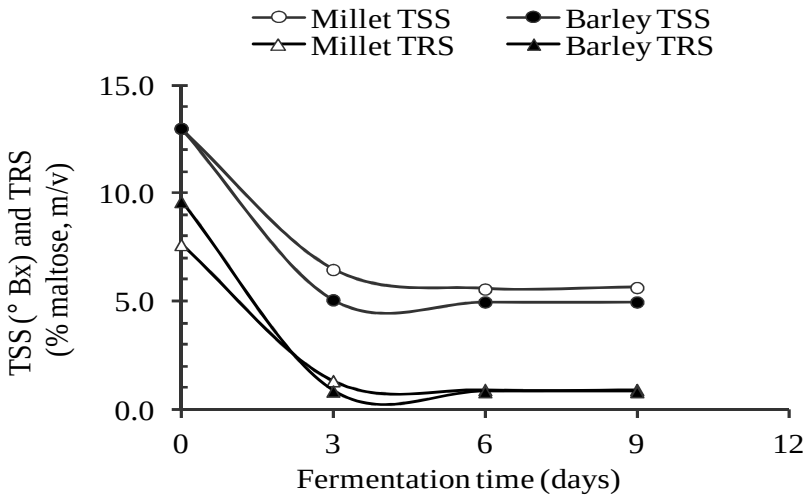


Fig. 5.72 Changes in total soluble solids (TSS ° Bx) and total reducing sugars as maltose, % m/v (TRS) during millet and barley beer fermentation.

TSS decreased from 13 ºBx on day 0 to 5.57 ºBx on day 6 and remained almost constant on further fermentation in millet wort, while in barley malt wort TSS decreased up to 5.07 ºBx on day 3 and did not change afterwards. It appeared that the rate of TSS decline was relatively faster in barley wort than in millet wort (Fig.5.72). Total reducing sugar was higher in barley malt wort than in millet wort and it decreased by about 83 and 91% in millet and barley wort respectively over the first 3 days of fermentation. Again, the reduction in reducing sugar followed the similar trend to that of TSS during beer fermentation.

Fig. 5.73 shows the glucose and fructose profiles during millet and barley beer fermentation. In millet wort, glucose content decreased gradually from 3.29 to 0.03 g/100 mL by the end of fermentation, while in barley malt wort it decreased from 1.22 to 0.02 g/100 mL on day 6 and remained virtually constant on further fermentation. From Fig 5.73 it is apparent that large amount glucose was exhausted over the first 3 days of fermentation in both fermentations. Fructose content decreased significantly in both millet (from 0.42 to 0.10 g/100 mL) and barley (from 0.75 to 0.14 g/100 mL) on day 6 and remained almost unchanged on further fermentation.

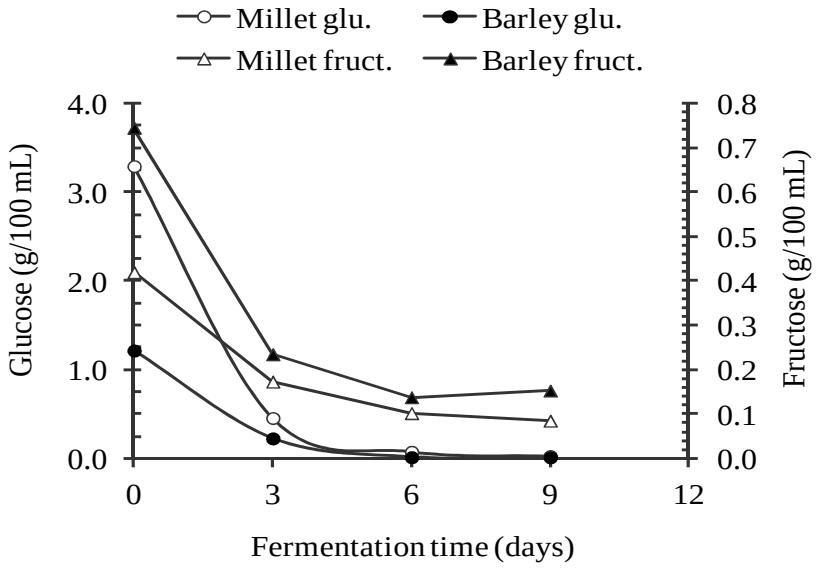


Fig. 5.73 Glucose and fructose profiles during millet and barley beer fermentation.

Changes in total acidity during millet and barley beer fermentation are depicted in Fig. 5.74 The total acidity (as lactic acid) increased gradually and reached at 0.29% (m/v) in millet and at 0.27% (m/v) in barley malt worts respectively by the end of fermentation. FAN contents decreased abruptly during the first 3 days of fermentation and then declined at a slower rate reaching final concentrations of 2.8 and 8.3 mg glycine/100 mL in millet and barley beers respectively (Fig. 5.75).

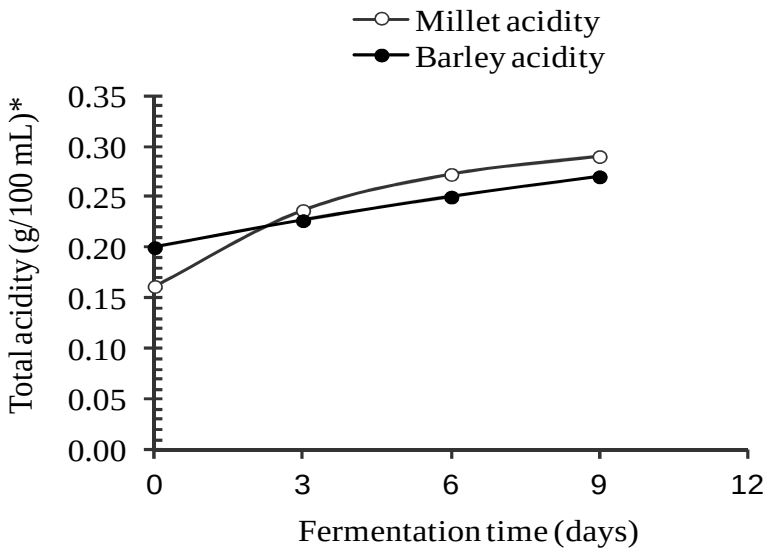


Fig. 5.74 Changes in total acidity (as lactic acid) during millet and barley beer fermentation.

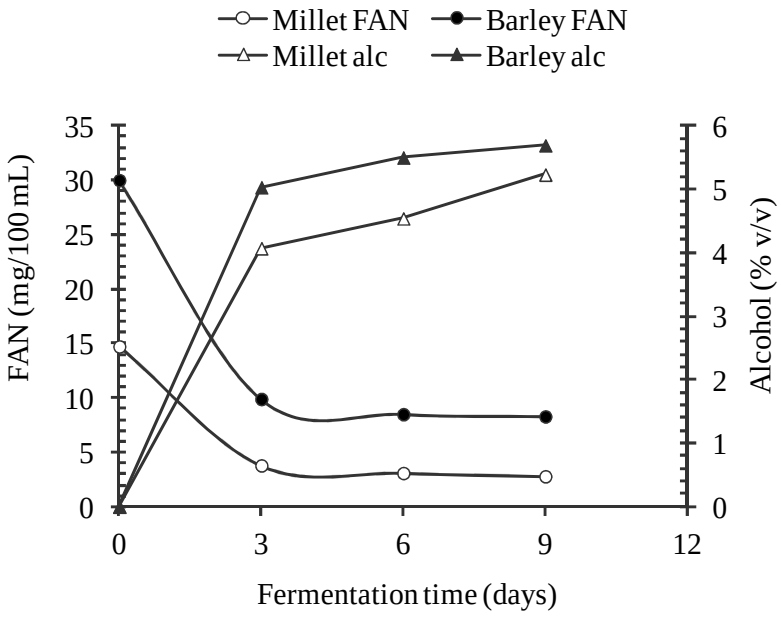


Fig. 5.75 Changes in free amino nitrogen (FAN) and alcohol during millet and barley beer fermentation.

Alcohol content increased significantly during fermentation and reached at 5.23% (v/v) on day 9 in millet wort and at 5.5% (v/v) on day 6 in barley wort. Of the total alcohol formed, about 78% was produced in millet wort, while about 91% was formed in barley wort over the first three days of fermentation (5.75). Fig. 5.76 shows the profiles of fusel oil and methanol contents during millet and barley beer fermentation. In millet malt wort, fusel oil increased sharply up to 912.28 g/100 L alc on day 3 and decreased reaching at 741.61 g/100 L alc by the end of fermentation. While in the case of barley malt wort, fusel oil increased up to 511.15 g/100 L alc on day 6 and then decreased to 446.31 g/100 l alc) on day 9.

Methanol production also followed similar trend to that of fusel oil and reached at 182.69 and 196.94 g/100 L alc in millet and barley malt worts respectively by the end of fermentation. Total ester contents increased significantly from 3 days onwards and reached at 85.27 and 76.74 g/100 L alc in millet and barley worts respectively on day 9 (Fig. 5.77). Total aldehydes formation was higher in barley beer than in millet beer. Aldehydes increased significantly throughout the fermentation and reached at 25.60 and 40.98 g/100 L alc in millet and barley worts respectively on day 9 (Fig. 5.77). Similar to other volatiles, vicinal diketones was also maximum at the initial stage of fermentation and it decreased gradually afterwards reaching at 0.25 and 0.32 mg/L on day 9 in millet and barley beers respectively (Fig. 5.78).

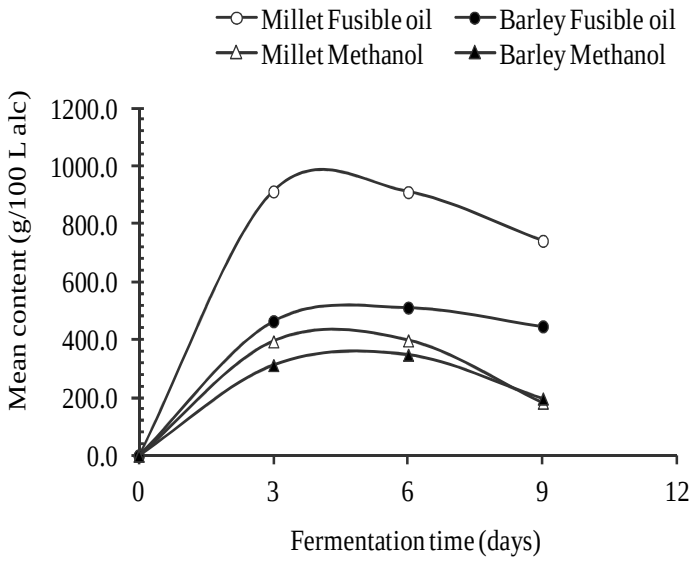


Fig. 5.76 Changes in fusel oil and methanol during millet and barley beer fermentation.

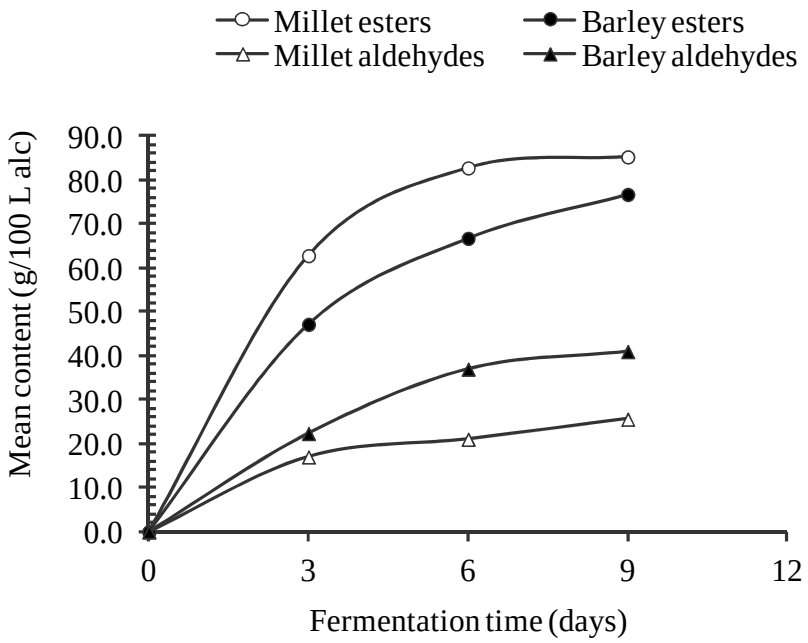


Fig. 5.77 Changes in total esters (as ethyl acetate) and total aldehydes (as acetaldehyde) during millet and barley beer fermentation.

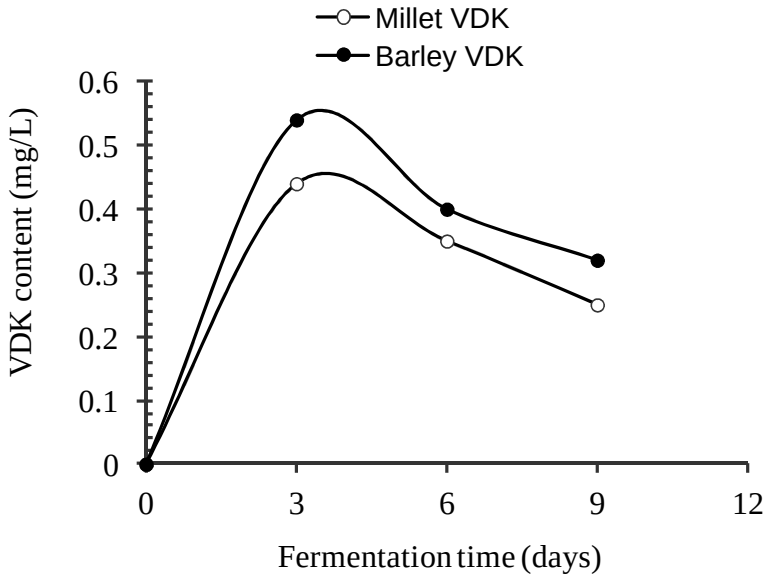


Fig. 5.78 Changes in vicinal diketones (VDK) during millet and barley beer fermentation.

6. Conclusions

The following conclusions were drawn from this study

1. Fermentation container had no significant effect on the sensory quality of fermented millet. The sensory quality of rice *jand* was highly preferred, while that of maize was least preferred among millet, rice, maize and wheat *jands*. Addition of rice and wheat to millet up to 20% (singly or in combination) did not improve the overall chemical and sensory quality of fermented millet.
2. No significant improvement on chemical and sensory quality in fermented millet was found by semi-solid fermentation over solid state. Polyvinylchloride (PVC) containers could be a promising packaging material for extending the shelf-life of fermented finger millet.
3. Addition of bentonite @ 3 g/L was adequate to clarify millet *jand* without scarifying chemical and sensory properties.
4. Defined fermentation starter resulted safer and more aesthetic product compared to traditional starter. Fermentation remarkably increased phosphorous, magnesium, sodium, potassium and zinc contents compared to raw counterpart.
5. *Kabre* finger millet variety germinated for 48 h at 28 ± 1 ºC produced the best malt of all the varieties investigated.
6. US mashing process used for American lager beer was more suitable for finger millet mashing compared to infusion and decatation mashing.
7. Except for FAN content, incorporation of barley malt to millet malt up to 40% did not improve the chemical properties of millet malt wort. Addition of mold bran to millet malt up to 2.5% appreciably increased fermentable nitrogen and decreased viscosity of the wort.
8. A 5-ppm gibberellic acid (GA_3) solution when applied to germinating millets significantly enhanced amylase activities and malt extract properties.
9. Chemical changes occurring during millet and barley beer fermentation exhibited similar trends; however, the changes were relatively faster in barley beer. Chemical and sensory characteristics of millet and barley beer were comparable.

7. Summary and recommendations

The study constitutes two parts. The first part deals with the quality improvement of fermented finger millet using defined alcoholic fermentation starter and the second part deals with the investigation on the brewing potential of some Nepalese finger millet varieties.

Finger millet (*Eleusine coracana*) is the fourth most important food crops of Nepal. The use of millets is limited only to some traditional alcoholic beverages, like *jand* (a traditional alcoholic fermented undistilled alcoholic beverages), *rakshi* (distilled alcoholic beverage) *roti* and *dhido* (traditional food preparations). Cereal based alcoholic beverages, like *jand*, *toongba*, *rakshi*, have a long tradition in Nepal. It is an important source of income in both hills and Terai of Nepal. Of the various cereals used for alcoholic fermentation, finger millet has remained the raw material of choice due to its low cost and clean aroma. Detailed study on the bio-chemical changes during millet fermentation, quality and safety aspects of these products are limited. Moreover, comparative study on cereal fermentation using traditional starter and defined starter is lacking. Similarly, investigation on the brewing quality of Nepalese finger millet varieties is scanty. Hence, this study is aimed at assuring quality and safety aspects of fermented millet by using defined fermentation starter and investigating the brewing potential of some Nepalese finger millet varieties.

Defined alcoholic fermentation starter for cereal fermentation was prepared by using *S. cerevisiae* yeast and *R. oryzae* mold (ITCC N0. 4408) in wheat bran – rice flour mixture (25:75) and used for finger millet fermentation. Effect of fermentation containers and and raw materials on the chemical and sensory quality of fermented cereals were studied. Finger millet (var. *Kabre*) was dehusked, cleaned, washed with water, soaked for 2 h, cooked, and cooled to room temperature. Fermentation starter was added at the rate of 1% by weight of millet, biomass developed for 2 days at 29 ± 1ºC, filled into plastic, wooden and earthen containers and fermented at 26 ± 1ºC for 15 days. Finger millet fermented in plastic container had higher alcohol (15.81% v/m) and TSS (11.96 ºBx) than that of earthen container, while moisture and fixed acidity remained unaffected. Total esters (1.813 g ethyal acetate/L alc), total aldehyde (0.850 g acetaldehyde/L alc), total acidity (1.58% m/m as lactic acid), and volatile acidity (0.296% m/m as acetic acid) were higher in millet fermented in earthen container.

Fermentation containers had no significant effect ($p>0.05$) on the taste and smell of millet *jand* but color was comparatively superior in plastic container. Chemical and sensory quality of *jands* prepared from fermented millet, rice, maize and wheat were compared. Results indicated that total ester (31.1 mg ethyl acetate/100 mL), TSS (3.97 ºBx), pH (4.67), total acid (0.41% m/v as lactic acid)) and fixed acid (0.37% m/v as acetic acid) were significantly higher in wheat *jand* compared to millet, rice, and maize *jands*, whereas no noticeable differences in volatile acidity (0.02 – 0.048% m/v as acetic acid) and alcohol (5.53 – 6.20% v/v) were found among four *jands*. Sensory evaluation revealed that taste and smell of rice *jand* were superior (liked very much) while that of maize was dislike slightly by the panelists.

Effects of rice and wheat incorporation to millet (10 and 20%, singly and in combination) on the chemical and sensory quality of fermented millet were studied. Addition of rice significantly increased the TSS of fermented millet while total acidity, fixed acidity, and alcohol contents were not affected by cereal combinations. Twenty percent wheat addition decreased the total ester content by 50% to that of control (100% millet) (0.863 g/L alc). Total aldehyde content was significantly increased by both rice and wheat addition with a maximum value of 1.545 g acetaldehyde/L alc being found in fermented millet containing 20% rice. Finger millet substituted with 20% of rice had the maximum fusel oil (9.370 g/L alc); while a minimum of 5.297 g/L alc was found in 20% wheat substituted fermented millet. Cereal combination did not have significant effect on methanol content and the values were in the range of 2.346 – 3.858 g/L alc. Addition of rice significantly increased the reducing and total sugar contents while 20% wheat addition decreased both the reducing and total sugar contents in fermented millet. Sensory evaluation revealed that cereal combination did not affect the color preference, whereas addition of wheat significantly impaired the taste and smell of the *jand*.

Finger millet was fermented under solid and semi-solid states (50 and 100%, v/m sterile water added to the biomass-developed millet) using defined fermentation starter for 10 days and effect of semi-solid fermentations on chemical and sensory quality of fermented millet were evaluated. Results indicated that a substantial increase in TSS, total –, fixed- and volatile-acidities and total esters contents occurred in semi-solid fermentations compared to solid state. Millet fermented with 50 and 100% water addition had about 1.2 and 2.8 times higher total acidity than that of solid-state

fermented millet. Sensory evaluation revealed that semi-solid fermentations did not improve the sensory quality of fermented millet over solid-state fermentation. Fermented finger millet was packed in polyvinylchloride (PVC) container and stored for 90 days at 25 ± 2 ºC, while the control sample was kept – 30 ºC. Chemical and sensory qualities of the samples were evaluated after 90 days of storage. The moisture, total-, free- and volatile acidities and total sugar did not changed. Alcohol decreased by about 9%, while total esters and total aldehydes increased ($p<0.05$) by about 58 and 24 % respectively over 90 days of storage. Sensory evaluation revealed that a remarkable improvement on the organonlepic quality of fermented millet occurred during room temperature storage (25 ± 2 °C).

Millet *jand* was prepared using 1 part of fermented millet and 1.5 parts potable water and clarified using different fining agents (bentonite, tannin, gelatin, and tannin – gelatine combinations). Addition of bentonite @ 3g/L resulted the best clarification of all the fining agents, while tannin-gelatine combination showed adverse effect on clarification. Holding of bentonite treated *jand* beyond 3 days at 29 ± 1 ºC did not improve clarity. Total phenolics, tannin, and antioxidant activity did not change, while total esters, aldehydes, methanol and alcohol decreased by bentonite clarification of millet *jand*. Starch was practically nil in clarified *jand*. Finger millet was fermented in plastic container at 26 – 28 ºC and bio-chemical changes during fermentation were studied at 3 days of intervals until 12 days. Starch content decreased throughout fermentation with maximum reduction being found during the first 3 days of fermentation.

Alcohol content increased significantly and reached at 14.58% (v/m) on day 12. Total aldehydes, esters, methanol, and fusel oil were higher at the beginning and decreased on subsequent fermention. Phytic acid decreased by about 3.6-fold, while total free amino acid increased by 6-fold over 6 days of fermentation. Total oxalate decreased by 51% on day 6. Crude protein, crude fat, and total ash increased, whereas total carbohydrate decreased in fermented millet compared to raw counter part. Yeast count increased until day 4 and then started declining, while mold count decreased by 23% on day 2 and vanished completely on day 4. Phoshporous, manganese, sodium, potassium and zinc contents increased, while iron remained unchanged on fermentation. Chemical and sensory analyses of millet fermented using defined fermentation starter (lab sample) and traditionally fermented millet (market samples) were made. The average

alcohol, total aldehydes (as acetaldehydes) and total esters (as ethyl acetate) contents in finger millet fermented using defined fermentation starter and market samples were 14.43 and 9.95% (v/m), 0.191 and 0.343 g/L alc, and 0.946 and 1.656 g/L respectively. Similarly, methanol and fusel oil contents were 2.055 and 5.119, and 5.191 and 5.355 g/L alc in lab and market fermented millet respectively. Defined fermentation starter produced millet *jand* of superior sensory quality than those of market samples. It was concluded that millet fermented using defined starter had significantly better chemical and sensory quality as well as safer than that fermented using traditional starter.

Six different Nepalese finger millet varieties (*Dalle*, *Okhle*, *Kabre*, *Juwain*, *GE 5016*, and *GP 0025*) were evaluated for their brewing potential. The millets were soaked for 12 h at room temperature (26 – 28 ºC), germinated at 28 ± 1º C for different times, kilned at 50 ± 2 º C for 24 h and their enzymatic activities and chemical properties were studied. All native millet had negligible alpha-amylase activity. *Dalle* finger millet germinated for 72 h had the highest alpha-amylase activity (22.96 units/g dry malt) of all the millet varieties. Beta-amylase activity in native millet were 0.1 – 2.4 units/g dry malt and it reached at maximum at 48 h of germination in all millet varieties, with the highest activity being found in *Kabre* millet (385 units/g dry malt). Diastatic activity ranged between 8.3 – 17.3 ºDP among the six millet varieties. Forty-eight hour germinated *Juwain* millet exhibited the maximum carboxypeptidase activity (242.5 units/g dry malt) and FAN contents (57.8 mg glycine/100 g dry malt) of all the millet vareties.

The chemical characteristics of native and 48 h germinated millet malts were analyzed. Results indicated that starch, amylose, and amylopectin contents in native millets were 71.32 – 79.86, 20.39 – 24.13 and 49.11 – 55. 72% (db) respectively, whereas those in malted millets were 63.74 – 67.12, 16.62 – 19.27 and 44.47 – 50.18% (db) respectively. Similarly, total phenolics (as gallic acid), total flavonoids (as rutin) and tannin (as tannic acid) contents in native millet ranged from 60.9 – 229.2, 35.2 – 141.7 and 169.9 – 566.0 mg% (db) respectively, while the values for malted millet ranged from 123.1 – 247.8, 50.1 – 236.3 and 173.7 – 301.8 mg% (db) respectively. Amylose to amylopectin ratios in native and malted millet starch were found to be 29:71 and 28:72 respectively. Millet malts were subjected for malt extract analysis and were found to be aromatic. The color (EBC unit), FAN (as glycine), and total reducing sugar (as maltose) contents in the extracts ranged between 2.77 – 5.78, 2.6 – 9.0 mg% (m/v) and 4.5 – 6.93 mg%

(m/v) respectively. The pH, extract, and FAN contents in worts obtained from *Dalle*, *Kabre* and *Juwain* millet malts were comparable to those of other cereal malt extracts. Based on the results of enzymatic activities, extract and wort analyses, *Kabre* millet had better malting quality of all the finger millet varieties.

Effect of kilning temperature on enzyme activity and extract properties were evaluated. The green malt (var. *Kabre*) was kilned by three different method viz., (a) at 50 ± 2 ºC; (b) first drying at 50 ± 2 ºC to 24 moisture and then at 62 ± 2 ºC, and (c) first drying at 50 ± 2 ºC to 24 moisture followed by at 80 ± 2 ºC. The moisture contents of the malts were between 6 – 8%. Malts kilned at higher temperature had significantly lower alpha-amylase and carboxypeptidase activities, while no remarkable differences were found in beta amylase activity and FAN contents among the three malts. The chemical properties of the millet and barley malt extracts were compared. It was found that higher kilning temperature produced stronger aroma and darker extract color, while decreased FAN contents in the malt extracts. Extract content was lowest in 50 ± 2 ºC kilned millet malt (6.83 °Plato). Total reducing sugar contents in millet malt extracts were similar to that of barley malt extract (6.09 – 7.11%, m/v as maltose).

Effects of mashing methods on the chemical properties of millet (var. *Kabre*) malt wort were studied. Mashing was carried out by three different methods (infusion at 70 ºC, decantation at 80 ºC and by US mashing method). The US mashing method resulted the highest total reducing sugar (9.27%, m/v as maltose), glucose (4.73%, m/v), FAN (17.4 mg%, m/v as glycine) and dextrin (3.49%, m/v) contents in the wort. Color was minimum in infusion mashing (4.94 EBC units). Hence, US mashing process was found to be suitable for finger millet mashing. Effects of barley malt addition on the properties of millet malt (var. *Kabre*) wort were evaluated. Mashing was carried out according to US mashing procedure by replacing millet malt with commercial barley malt at the rate of 0, 20, 30, and 40% by weight of millet malt. Forty percent barley malt addition increased the wort reducing sugar content by 1% compared to control (100% millet malt wort). Barley malt addition remarkably increased the FAN contents, while no appreciable improvements were found on other wort properties. Effect of mold bran addition on the chemical properties of millet malt wort mashed by US method was studied by incorporating mold bran (0 - 5% by weight of the millet malt). Mold bran was prepared by growing *R. oryzae* ITCC No. 4408 in sterile wheat bran for 4 days. Results indicated that addition of mold bran up to 5% did not improve the TSS,

total reducing sugar, and glucose while it remarkably improved the FAN content and viscosity of the wort.

Effect of gibberellic acid (GA_3) treatment on the enzyme activities and chemical properties of millet malts were studied. During germination, 5 ppm of gibberellic acid solution was sprayed twice a day. Millet were germinated for different times (34, 48 and 56 h) at 27 – 29 ºC, kilned at 50 ± 2 ºC for 24 h and analyzed for enzyme activities and chemical characteristics of the malts extracts. GA_3 treatment significantly increased ($p<0.05$) the alpha-and beta-amylase activities and FAN contents in all millet varieties compared to control (GA_3 untreated malts). The effect of GA_3 treatment was more pronounced on beat-amylase than on alpha-amylase. GA_3 treatment enhanced the beta-amylase activity by about 4-fold in *Kabre* millet malt compared to its control. *Kabre* millet germinated for 56 h showed the highest FAN content (113 mg GAE/100g dry matter) of all the treatment combinations used. GA_3 treatment increased the FAN contents in malts ranging from 96 to 195% compared to their control malts. Chemical analysis of extracts made from GA_3 treated *Kabre* and *GPU 0025* millet malts showed that FAN and color were higher in *Kabre* millet malt extract but no significant differences were found in total reducing and gravity of the extract. *Kabre*r millet was found to have better response to GA_3 treatment of all the millet varieties.

Optimization of pH and temperature for millet malt mashing was carried out for each stage of US mashing process using response surface methodology (RSM). The independent variables included were mashing temperature (x_1) and pH ($x_{2,}$). Temperature was varied in three levels (40°, 50° and 60 °C for protein rest period, 62, 65 and 68 °C for dextrinizing period and 70, 73 and 76 °C for conversion period. Similarly, pH was varied in three levels, viz., 4.5, 5.25 and 6.0 for all mashing stages. The measured FAN and TSS contents during protein rest period ranged from 10.8 − 15.72 mg glycine/100 mL and 1.4 − 5.8 °Bx respectively. FAN, TSS, and total reducing sugar contents in worts during dextrinizig period were 9.34 to 13.97 mg glycine/100 mL, 2.26 to 7.73 °Bx and 2.25 to 7.06 g maltose/100 mL respectively. Similarly, glucose and fructose contents ranged from 1.55 to 4.3 and 0.24 to 0.53% (m/v) respectively. The FAN, TSS, and total reducing sugar contents during conversion period were 10.33 to 13.49 mg glycine/100 mL, 8.3 to 10.80 °Bx and 4.79 to 8.16 g maltose/100 mL respectively. Similarly, glucose and fructose contents during conversion period were 2.35 to 3.17 and 0.3 to 0.53% (m/v) respectively. The

optimized temperature and pH for protein rest, dextrinizing and conversion periods in US mashing process for millet malt were 57.62 ºC and 5.47, 68 ºC and 4.5, and 70 ºC and 5.28 respectively.

Millet (var. *Kabre*) was geminated for 48 h at 27 − 29 ºC and kilned by two method (a) by drying at 50 ± 2 ºC to 7% moisture content and (b) by drying at successively increasing temperature from 50 to 80 ºC similar to that used for barley malt kilning. Beers were made from barley and millet malts and their chemical characteristics were analyzed. Glucose content was higher in millet beers than in barley beer, while fructose content was higher in barley beer. Dextrin content was higher in millet beers (average value of 1.42%, m/v) than in barley beer (0.89%, m/v). FAN content was higher in barley beer (9.4 mg glycine/100 mL) while it was lowest in millet beer brewed from millet malt kilned by standard barley method (2.1 mg glycine/100 mL). Barley beer contained higher total phenolics (59.2 mg GAE/100 mL) and tannin (67.5 mg tannic acid/100 mL), while it had lower total flavonoids (27.3 mg rutin/100 mL) than that of millet beer. Barley beer showed higher antioxidant activity (33.17%) than those of millet beers (average of 22.47%). Formaldehyde content was higher in barley beer (0.33 ppm) compared to millet beers (average of 0.24 ppm). Barley beer contained lower fusel oil (228.85 g/100 L alc), but higher methanol (249.71 g/100 L alc) and total aldehydes (32.23g/100 L alc) than those of millet beers. Sensory evaluation revealed that taste, smell, flavor, and color of millet beers were comparable to barley beer, but the body of millet beer was better than that of barley beer.

Chemical changes during millet and barley beer fermentation was studied at 3 days of interval until 9 days. FAN and sugars decreased largely over the first 3 days of fermentation while total acidity increased gradually reaching at 0.29 and 0.27% (m/v) as lactic acid in millet and barley beers respectively. Over the first 3 days of fermentation, about 78% of the total alcohol was produced in millet beer, while it was 91% in barley beer. Fusel oil and methanol increased up to day 6, while total esters increased gradually during fermentation in both beers. Vicinal diketones was highest during the initial stage and it decreased over the succeeding periods of fermentation.

Recommendations

1. Pasteurization and shelf-life study of clarified millet *jand* can be studied using different packaging materials.

2. Malting of finger millet at different temperatures with varying concentrations of gibberellic acid treatments at can be studied.

3. Development of millet malt mashing procedure in combination with mold bran and its impact on beer quality can be carried out.

4. Use of commercial amylase and protease enzymes in millet malt mashing can be studied.

References

Acharya D (2007). Study on the brewing quality of naked barley (*Hordeum vulgare*). B. Tech (Food) Dissertation, Central Campus of Technology, Tribhuvan University, Nepal.

Acharya D (2011). Preparation and quality evaluation of whiskey from naked barley *(Hordeum vulgare*). Masters' Thesis, Central Department of Food Technology, Tribhuvan University, Nepal.

Agte VV and Sandhana RJ (1997). Effect of traditional food processing on phytate degradation of wheat and millets. *Food Chem.*, 45: 1659-1661.

Agte VV, Gokhale MK and Chiplonkar SA (1997). *J. Nutr. ???* In: Elyas SHA, Tinay AHEI, Yousif NE and Elsheikh EAE (2002) Effect of natural fermentation on nutritive value and in vitro protein digestibility of pearl millet. Food Chem., 78: 75 – 79.

Agu RD and Ezeanolue JC (1993). Combined mashing of millet (*Pennisetum maiwa*) malts prepared with potassium bromate and gibberellic acid (GA_3) as additive. *Process Biochem.*, 28: 475-479.

Agu RC, Okeke BC, Nwufo SC, Ude CM and Onwumelu AH (1993). Influence of gibberellic acid (GA_3) on diastase and cellulase development of Nigerian millet (*pennisetum maiwa*) and Sorghum (*Soghum bicolor*). *Process Biochem.*, 28: 105 – 108.

Agu RC and Palmer GH (1998). A re-assessment of sorghum for lager brewing. *Bioresource Technol.*, 66: 253-261.

Aidoo KE, Nout MJR and Sarkar PK (2005). Occurance and function of yeast in Asian indigenous fermented foods. A mini-review. *FEMS Yeast Res*., 6: 3039.

Amerine, M.A. and Cruess, W.V. (1960). The Technology of Wine Making, 2nd edn. Westport, CT: AVI Publishing CO.

Amerine MA, Berg HM and Cruess WV (1967). The Technology of Wine Making, 2nd edn. AVI Publishing Co., Westport, Connecticut.

Amerine MA and Ough CS (1980). Mehtods for Analysis of Musts and Wines. New York, John Willy and Sons.

AOAC (2005). Official Methods of Analysis. AOAC International Suite 500 481 North Frederick Avenue. Gaithersburg, Maryland 20877-2417, USA.

Asquith TN and Buttler LC (1986). Interaction of condensed tannins with selected protein. *Phytochem.,* 25: 1591-1593.

Baan R, Grosse Y and Straif K (2009). A review of human carcinogens – part F: chemical agents and related occupations. *The Lancet Oncology*, 10(12): 1143-1144.

Badau MH, Nkama I and Jideani IA (2005). Phytic acid content and hydrochloric acid extractability of minerals in pearl millet as affected by germination time and cultivar. *Food Chem*., Vol. 3, Issue 3: 425-435.

Bahl BS and Bahl A (1997). Advanced Organic Chemistry, 12th edn. S. Chand and Company Ltd., New Delhi, India.

Bailey CJ (2001). New approaches to the pharmacotherapy of diabetes, In: Pickup JC and William G (eds). The Text Book of Diabetes, 3rd edn. Vol. 2. U.K., Blackwell Science Ltd., pp. 73.1-73.2.

Balkrishna Rao K, Mithyantha MS, Devi LS and Peru NG (1973). Nutrient content of some new ragi varieties. *J. Agric. Sci.,* 7: 562-565.

Bamforth CW, Muller RE and Walker MD (2000). Oxygen and oxygen radicals in malting and brewing. A Review. *J. Am. Soc. Brew. Chem.,* 53: 79-88.

Banasik OJ (1969). Recent advances in the biochemistry of malting. *Brewer's Digest*, 44 (1): 52

Barnett A, Payne RW and Yarrow D. (1990). Yeasts: Characteristics and Identification. Cambridge, pp 18-29.

Berger WC, La Berger DE (1985). Malting and Brewing Quality. In: Barley, Rasumusson D (ed). Am. Soc. Agronomy, Madison,

Berry DR and Watson, DC (1987). Production of organoleptic compounds. In: Berry DR, Russell I and Stewarts GG (eds). Yeast Biotechnology. Elsevier Applied Science Publishers, London, New York.

Bewely JD and Black M (1983). Physiology and biochemistry of seed development, germination and growth. Springer-Verlag Publishers, New York. In: Nithya KS, Ramachandramurthy B and Krishnamurthy V (2006). Assessment of

antrinutritional factors, minerals and enzyme activities of the traditional (Co7) and hybrid (Cohcu-8) pearl millet (*Pennisetum glaucum*) as influenced by different processing methods. *J. Appl. Sci. Res.*, 2(12): 1164-1168.

Bhandari S (1997). Comparative study on raksi production from different raw materials using *murcha* and pure culture. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Bhatra LR and Millner PD (1974) Some Asian femented foods and beverages, and associated fungi. *Mycologia*, 6; 942-950.

Bhise VJ, Chavan JK and Kadam SS (1988). Effect of malting on proximate composition and invitro protein and starch disestibity of grain sorghum. *J. Food Sci. Technol.*, 25: 327-329.

Bishnoi S, Khetarpaul N and Yadav RK (1994). Effect of domestic processing and cooking methods on phytic acid and polyphenol contents of pea cultivars (*Pisum sativum*). *Plant Foods for Hum. Nutr.*, 47: 381-388.

Bloch F and Morang AI (1967). Germination inhibition in wheat and barley during steeping and alpha-amylase development in the presence of gibberellic acid. *Cereal Chem*., 44: 61

Borade VP, Kadam SS and Salukhe DK (1984). Changes in phytate phosphorus and minerals during germination and cooking of horse gram and moth bean (Qual Plant). *Plant Foods for Hum. Nutr.*, 34: 151-157.

Borbolan AMA, Zorro L, Guillen DA and Barroso CG (2003). Study of the polyphenol content of red and white grape varieties by liquid chromatography-mass spectrography and its relationship of antioxidant power. *J. Chromatogr.*, 1012:13-38.

Briggs DE, Hough JS, Stevens R and Young TW (1981). Malting and Brewing Science, Vol. I. London, Chapman and Hall.

Briggs DE (1998). Malts and Malting. Blackie Academic and Professional, London.

Burgess JR and Gao F (2002). The antioxidant effects of inositol phosphate. In: Reddy NR and Sathe SK (eds). Food Phytates. CRC Press, pp. 205-214.

Buysse W, Stern R, Coe R and Matere C (2007). Genstat Discovery Edition 3rd for everyday use. ICRAF Nairobi, Kenya.

Cai T and Nip WK (1990). Biochemical changes in the development of alcoholic fermented products from taro [*Colocasia esculenta (L0 Schott*]. *Tro. Sci.,* 30: 379-390.

Casey TR and Charles WB (2010). Silicon in beer and brewing. *J. Sci. Food Agric*., 90(5): 784-788.

Casey P and Lorenz K (1977). Millet – Functional and nutritional properties. *Baker Digest*, 51: 45-45.

Chavan JK, Kadam SS and Daluke DE (1981). Changes in tannin, free amino acids, reducing sugar and starch during germination of low and high tannin cultivers of sorghum. *J. Food Sci.*, 46: 638-9.

Chavan JK and Kadam SS (1989). Nutritional improvement of cereals by fermentation. Critical Review in Food Science. In: Matz SA (ed). The Chemistry and Technology of Cereals as Food and Feed, 2nd edn ((1996). CBS Publishers and Disttributors, New Delhi, India, pp. 259-284.

Chethan S, Sreeram YN and Malleshi NG (2008). Mode of inhibition of finger millet malt amylases by the millet phenolics. *Food Chem.,* 111: 187-191.

Choudhury M, Das P and Baroova B (2010). Nutritional evaluation of popped and malted indigenous millet of Assam. *J. Food Sci. Technol.,* DOI 10.1007/S13197-010-0157-3.

Chukwura EN and Muller HG (1982). Effect of tannic acid on a low tannin African sorghum variety in relation to carbohydrate and amylase. *J. Food Sci.,* 47: 1380-1381.

Clapperton AO (1971). Simple peptides of wort and beer. *J. Inst. Brew.,* 77: 177-180.

Clydesdale FM (1988). Minerals: Their chemistry and fate in food. In: Smith KD (ed). Trace Minerals in Foods. Marcel Dekker Inc, New York and Basel, pp. 57-94.

Cornforth DP (2002). Potential use of phytate as an antioxidant in cooked meals. In: Reddy NR and Sathe SK (eds). Food Phytates. CRC Press, pp. 214-215.

Cronk TC, Steinkraus H, Hackler LR and Mattic lR (1977). Indonesian *tape ketan* fermentation. *Appl. Environ. Microbiol.,* 33: 1067-1073.

Cronk TC, Mattic LR, Steinkraus KH and Hackler LR (1979). Production of higher alcohols during the Indonesian *tape ketan* fermentation. *Appl. Environ. Microbiol*., 37: 892 – 896.

Daniels DGH and Fisher N (1981). Hydrolysis of the phytate of wheat flour during bread making. *British J. Nutr.,* 46: 1-6.

Das DK (2004). Improvement in traditional method of preparing *jand* from finger millet. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Daussant J, Sadowski J and Ziegler P (1994). Cereal β-amylases: Diversity of the β-amylase isozyme status within cereals. *J. Plant Physiol.*, 143: 585-590.

Delvaux F, Cambes FJ and Delvaux FR (2004). The effect of wheat malting on the colloidal haze of wheat beers. MBAA TG, 4(1): 27-32.

Demuyakor B and Ohta Y (1992). Malt characteristics of sorghum vulgare varieties from Ghana. *J. Sci. Food Agric.,* 457 – 462.

Dendy DA (1995). Sorghum and Millet: Chemistry and Technology, Am. Assoc. Cereal Chem., Inc, St. Paul Minnesota, U.S.A., p. 406.

Deosthale YG, Nagarajan V and Pant KC (1970). Nutrient composition of some varieties of ragi. *Indian J. Nutr. Diet.,* 7: 80.

Dewar J, Taylor JRN and Joustra SM (1995). Accepted Methods of Sorghum Malting and Brewing Analysis. CSIR Food Science and Technology, Pretoria, South Africa.

Dewar J, Taylor JRN and Berjak P (1997). Effect of germination conditions, with optimized steeping on sorghum malt quality with particular reference to free amino nitrogen. *J. Inst. Brew.*, 103: 171-175.

Dhankherer N and Chauhan BM (1987). Effect of temperature and fermentation time on phytic acid and polyphenol content of rabadi - a fermentated pearl millet food. *J. Food Sci.*, 52: 828-829.

Dubey RC and Mheshwari DK (2002). Practical Microbiology. S. Chand and Company Ltd. Ram Nagar, New Dlhi-110 055.

Dung NTP, Rombouts FM and Nout MJR (2007). Characteristics of some traditional Vietnamese starch-based rice wine fermentation starters (men). *LWT*., 40: 130-135

Dvorakova M, Guido LF, Dostalek P, Skulilova Z, Moreira MM and Barros AA (2008). Antioxidant properties of free, soluble ester and insoluble bound phenolic compounds in different barley varieties and corresponding malts. *J. Inst. Brew.*, 114 (1): 27-33.

EBC (1998). European Brewery Convention (EBC), Anylitica, 5th edn. Verlag Hans Carl, Nurnberg, Germany.

EBC (2005). Analytica EBC Method No 9.24.1.

Edney MJ, Legge WG and Rossnagel BG (2005). Amino acid levels in wort and their significance in developing malting barley varieties. Paper Presented at the 18th North American Barley Researchers Workshop, July 17-20.

Ekundayo JA (1996) The production of *pito*: Nigerian fermentd beverages. *J. Food Technol.,* 4: 217-225

Elyas SHA, EL Tinay EI, Yousif NE and Elesheikh EAE (2002). Effect of natural fermentation on nutritive value and in vitro protein digestibility of pearl millet. *Food Chem.,* 78: 75-79.

Eneje LO, Obeiekezie SO, Aloh CU and Agu RC (2001). Effect of milling and mashing procedures on millet (*Pennisetum maiwa*) malt wort properties. *Process Biochem.,* 36: 723-727.

Engan S (1981). Production of organoleptic compounds: In: Breey DR, Russell I and Stewart GG (eds). Yeast Biotechnology. Allen and Unwin, London.

Evans DE, Maclead LC, Eglinton JK, Gibson CE, Zang X, Wallace W, Skerritt JH and Lance RCM (1997). Measurement of β-amylase in malting barley (*Hordeum vulgare* L.). I. Development of a quantitative ELISA for β-amylase. *J. Cereal Sci.,* 26: 229-239

FAO (1999). Fermented Cereals: A Global Perspective. FAO Agricultural Services Bulletin No. 138. Food and Agriculture Organization of the United Nations, Rome, p. 67.

Fasano A and Catassi C (2001). Current approaches to diagnosis and treatment of celiac disease. An evolving spectrum. *Gastroenterology,* 120: 636-651.

Fordham JR, Wells CC and Chen LH (1975). Sprouting of seeds and nutrient composition of sees and sprouts. *J. Food Sci.,* 40: 550-552.

Foster RT, Samp EJ, Patino H and Barr DP (2001). Electron paramagnetic resonance (EPR) profiling of potential flavour stability improvement in beer. *Tech. Q. Master Brew. Assoc. Am.*, 38(4): 247-250.

Friedrich W, Eberhardt A and Galensa R (2000). Investigation of proanthocyanidins by HPLC with electrospray ionization mass spectrometry. *Eur. Food Res. Technol.*, 211: 56-64.

Gadaga TH, Mutukumira AN, Nar vhus JA and Feresu SB (1999). A review of traditional fermented foods and beverages of Zimbabwe. *Int. J. Food Microbiol.*, 53: 1-12

Gajurel C and Baidya K (1979b). Traditional Technology of Nepal (In Nepali). Yeast *Mana Manapu* Technology, 191-195.

Gautam G (1987) Efficiency of ethanol production by *murcha* yeast. B. Tech (Food) Dissertation, Central Campus of Technology, T.U., Nepal

Geeta R, Virupaksha TK and Sadaksharaswamy M (1977). Relationship between tannin levels and in vitro protein digestibility in finger millet (*Eleusine coracana* Gaertn). *J. Agric., Food Chem.,* 25: 1101-1104.

Gibson TS, Solah J, Glennie Holmes MR and Taylor HR (1995). Diastatic power in malted barley: Contributions of malt parameters to its development and the potential of barley grain β-amylase to predict malt diastatic power. *J. Inst. Brew.*, 101: 277-280.

Gimbi DM and Kitabatake N (2002). Changes in α and β- amylase activities during seed germination of African finger millet. *Int. J. Food. Sci. Nutr.,* 53(6): 481-488.

Goode DL and Arendt EK (2003). Pilot scale production of lager beer from a grist containing 50% unmalted sorghum. *J. Inst. Brew.,* 109(3): 208-217.

Goupy P, Hugus M, Boivin P and Amiot MJ (1999). Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *J. Sci. Food Agric.*, 70: 1625-1634.

Graf E, Mahoney JR, Bryant RG and Easton JW (1984). Iron catalyzed hydroxyl radical formation stringent requirement for free iron coordination site. *J. Biol. Chem.,* 259: 3629\0-3624.

Graf E and Easton JW (1990). Antioxidant function of phytic acid. *Free Radical Biology and Medicine,* 8: 61-69.

Guido LF, Boivin BN, Goncalves CR and Barros AA (2005). An early development of the nonenal potential in the malting process. Eur. Food Res. Technol., 220: 200-206.

Hamad AM and Fields MI (1979). Evaluation of protein quality and available lysine of germinated and ungerminated cereals. *J. Food Sci.,* 4: 456-459.

Harland DF (1989). Dietary fiber and mineral availability. *Nutr. Res. Rev.,* 2: 133-147.

Harrigan WF and McCance ME (1976). Laboratory Methods in Food and Dairy Microbiology. Academic Press, London, pp. 107, 108, 139, 227-303.

Hashimota N and Kuroiwa Y (1975). Pathways for the formation of volatile aldehydes during storage in bottled beer. *Rept. Res. Lab. Kirin Brew.,* 18: 1-11.

Hesseltine CW and Ray M (1988). Lactic acid bacteria in *murcha* and ragi. *J. Appl. Bacteriol.,* 64: 395-401.

Hough JS (1985). The Biotechnology of Malting and Brewing. Cambridge Univ. Press, Cambridge, England.

Hulse JH, Laing EM and Pearson OE (1980). Sorghum and the Millets: Their composition and nutritive value. Academic Press, NYC.

Igyor MA, Ogbonna AC and Palmer GH (2001). Effect of malting temperature and mashing methods on sorghum wort compostion and beer flavour. *Process Biochem.,* 36(11): 1039-1044.

Inoue T, Tanka J and Mitsui S (1992). Recent Advances in Japanese Brerwing Technology. Gordon and Breach Science Publishers, Tokyo.

Iwuagwu YOU and Izuagbe YS (1986). Evaluation of Nigerian millet (*Pennisetum typhoideum*) as a raw material for beer production. *Acta Biotechnologica (Jan),* 6(3): 299-303.

Jacobsen T and Lie S (1979). Metal binding in wort – an evaluation of practical stability constants. EBC Proceedings of the European Brewery Convention Congress. Fach Verlag Hans Carl: Nurnberg, Germany, pp. 117-129.

Jenab M and Thompson LU (2002). Role of phytic acid in cancer and other diseases. In: Reddy NR and Sathe SK (eds). Food Phytates. CRC Press, pp. 261-275.

Jendral JA, Monakhova YB and Lachenmeier DW (2011). Formaldehyde in alcoholic beverages: large chemical survey using purpald screening followed by chromotrophic acid spectrophotometry with multivariate curve resolution. *Int. J. Ana. Chem.,* 2011: 11 pages

Jood S, Chauhan MB and Kapoor CA (1987). Polyphenols of chickpea and blackgrams as affected by domestic processing and cooking methods. *J. Sci. Food Agric.,* 39: 145-149.

Kabelova I, Dvorakova M, Cizkova H, Dostalek P and Melzoch K (2008). Determination of free amino acids in beers: A comparision of Czech and foreign brands. *J. Food Comp. Anal.*, 21(8): 736-741.

KC JB, Subba DK and Rai BK (2001). Plants used *in murcha* preparation in eastern Nepal. *J. Hill Res.*, 14 (2): 107 – 109.

Karki TB (1984). Some fermented foods and beverages of Nepal. Paper presented at the First National Seminar on Food Industries and Food Technology Organized by Dharan Campus, Nepal, pp. 178-180.

Karki TB (1986). *Murcha*. In: Saono S, Hull RR, and Dhamcharee B (1986). A Concise Handbook of Indigenous Fermented Foods in the ASCA Countries. The Government of Australia, Canberra, Australia, p. 136.

Karki TB (1994). Food Processing Industries in Nepal. In: Komataga K, Yoshida T, Nakase T and Osada H (eds). Porceeding of the International Workshop on Application and Control of Microorganisms in Asia, Science and Technology Agency. The Institute of Physiacl and Chemical Research and Japan International Science and Technology Exchange Centre, pp. 71-87.

Khanal H (2008). Study on the brewing quality of naked barley (*Hordeum vulgare*, var. solu uwa), M. Tech. (Food) Dissertation, Central Department of Food Technology,T.U., Nepal.

Khetarpaul N and Chauhan BM (1990). Improvement on HCl-extractability of minerals from pearl millet by natural fermentation. *Food Chem.*, 37: 69-75.

Khokhar S and Chauhan BM (1986). Nutrient composition, protein fraction and antinuttritional factors of moth bean (*Vigna aconitifocia*). *Bull. Grain Technol.*, 24: 3-11.

Kim CJ (1968). Microbiological and enzymological studies on *takju* brewing. *J. Korean Agric. Chem. Soc*., 60: 69-99.

Kodama K and Yoshizawa K. (1977). Sake. In: Rose AH (ed). Economic Microbiology. Academic Press, New York. Cited In: Steinkraus KH. (1996). Handbook of Indigenous Fermented Foods, 2nd edn. Marcel Dekker, New York, pp. 446-447.

Koirala P (2011). Preparation of fermentation starter using yeast and mold isolated from different traditional starter (*murcha*). B. Tech (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Korea Rural Nutrition Institute (1991). Food Composition Table, 4th edn., Seoul, Korea.

Krik RS and Sawyer R (1991). Pearson's Composition and Analysis of Foods, 9th edn., Addison Wesley longman Ltd. Edinburg Gate, Harlow, England.

Kringstad H, Busengdal H and Rasch S (1960). Effect of gibberellic acid on barley during malting. *J. Inst. Brew.*, 66: 477-80.

Kumari S and Srivastava S (2000). Nutritive value of malted flours of finger millet genotypes and their use in the preparation of *Burfi*. *J. Food Sci. Technol.*, 37: 419-422.

Leder I (2004). Sorghum and millets in cultivated plants primarily as food sources. In: Gyorgy F (ed). Encyclopedia of Life Support System (EOLSS) Developed under the Auspicious of the UNESCO. EOLSS Publishing, Oxford, U.K. (http://www.eloss.net).

Lee SW (1984). Hankuk Sikpum Munhwasa (Korean Dietary Culture). Kyomunsa, Seoul, Korea

Lee CH and Kim GM (1993). Korean rice-wine, the types and processing methods in old Korean literature. *Bioindustry*, 6(4): 8-25.

Lekkas C, Stewart GG, Hill AE, Taidi B and Holdgson J (2007). Elucidation of the role of nitrogenous wort components in yeast fermentation. *J. Inst. Brew.*, 113(1): 3-8.

Lopez Y, Gordon DT and Fields M (1983). Release of phosphorous from phytate by natural lactic acid fermentation. *J. Nutr.,* 48: 953-954.

Lorenz K (1983). Tannins and phytate content in proso millets (*Panicum miliaceum*). *Cereal Chem.*, 60: 424-426.

Lovalace CEA (1977). Estimation of nutrient content of two fermented beverages from Zambia opaque maize beer and *munkoyo*. Symposium on Indigenous Fermented Foods, Bangkok, Thailand. In: Steinkraus KH (1996). Handbook of Indigenous Fermented foods, 2nd edn. Marcel Dekker, Inc, New York, p. 437.

Lugasi A and Hovari J (2003). Antioxidant properties of commercial alcoholic and non-alcoholic beverages. *Food/Nahrang*, Vol. 47, Issue 2: 79-86.

MacGregor AW (1996). Malting and Brewing Science: Challenges and opportunities. *J. Inst. Brew.*, 102: 97-102.

MacMasters MM, Hinton JJC and Bradbury D (1971). Microscopic structure and composition of the wheat kernel. In: Pomerang Y. (ed). Wheat: Chemistry and Technology, 2nd edn. American Association of Cereal Chemists, St. Paul, pp. 51-113.

McDonough CM, Rooney LW and Serna-Saldivar SO (2000). The Millets. In: Kulp K and Ponte Jr JG, (eds), Handbook of Cereal Science and Technology, 2nd edn., Marcel Dekker, New York, pp 177-195.

Mahajan S and Chauhan BM (1987). Phytic acid and extractable phosphorus of pearl millet as affected by natural lactic acid fermentation. *J. Food Sci. Agric.*, 41: 381-386.

Mahato BM (2010). Study on brewing quality of some Nepalese wheat varieties (*Triticum aestivum*). B. Tech. (Food) Dissertation, Central Campus of Technology, TU, Nepal.

Maillard MN and Berset C (1995). Evaluation of antioxidant activity during kilning: Role of insoluble bound phenolic acids of barley and malt. *J. Agric. Food Chem.*, 43: 1789-1793.

Maillard MN, Soum MH, Boivin P and Berset C (1996). Antioxidant activity of barley and malt relationship with phenolic content. *Levnesmitted-wissenschaft Und-Technologic*, 29: 238-244.

Makokha AO, Oniango RK, Njoroge SM and Kamar OK (2002). Effect of traditional fermentation and malting on phytic acid and mineral availability of sorghum (*sorghum bicolor*) and finger millet (*Eleusine coracana*) grain varieties grown in Kenya. *Food Nutr. Bull.*, 23: S241-245.

Malleshi NG, Desikachar HSR and Tharanathan RN (1986). Free sugars and non-sugar starch polysaccharides of finger millet (*Eleusine coracana*), pearl millet (*Pennisetum typhoideum*), foxtail millet (*Setaria italica*) and their malts. *Food Chem.*, 20: 253-261.

Malleshi NG and Klopfenstein CF (1998). Nutrient composition and amino acid and vitamin content of malted sorghum, pearl millet, finger millet and their rootlets. *Int. J. Food Sci. Nutr.*, 49: 415-422.

Malleshi NG (1984). Studies on malting of millet grains. Ph.D. Dissertation, The Univ. of Mysore, India.

Malleshi NG (2005) Finger millet (ragi). The Wonder Grain. IFIS, http://www.ieis.org/fsc.ixid , Accessed on Aug 20, 2010

Mallik CP and Singh MB (1980). Plant Enzymology and Histo-enzymology. Kalyani Publications, New Delhi, India, pp. 68-69.

Manandhar O (2002). Preparation and quality evaluation of rice wine using pure cultures of yeast and mold isolated from *murcha*. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Mandal NC, Burman S and Biswas BM (1972). Isolation, purification and characterization of phytase from germinating mungbean. *Phytochem.*, 11: 495-502.

Matz SA (1991). The Chemistry and Technology of Cereal as Food and Feed, 2nd edn. CBS Publishers and Distributors, New Delhi, India.

Mayer AM and Mayber PA (1963). The germination of seeds. MacMillan Co., New York. In: Choudhury M, Das P and Baroova B (2010). Nutritional evaluation of popped and malted indigenous millet of Assam. *J. Food Sci. Technol.*, DOI 10.1007/S13197-010-0157-3.

Mbithi-Mwikta S, Van Camp J, Yiru Y and Huyghebaert A (2000). Nutrient and antinutrient changes in finger millet (*Eleusine coracana*) during sprouting. *Lebensmittel-Wissenschaft + I.E Und Technologie* 33: 9-14.

Mirbahar RB and Laidman DL (1982). Gibberellic acid-stimulated alpha-amylase secretion and phospholipids metabolism in wheat aleurone tissue. *Biochem. J.*, 208: 93 – 100.

Mongar G and Rai BK (2005). Preservation of strained *jand* by pasteurization. *J. Food Sci. Technol.*(Nepal) 1(1): 58-61.

Montgomery DC (2001). Introduction to statistical quality control. In: Design and Analysis of Experiments, 5th edn., John Wiley and Sons: Chichester, UK, pp. 457 – 461.

Moeljiopawiro S, Gordon DT and Fields ML (1987). Bioavailability of iron in fermented soybeans. *J. Food Sci.*, 52: 102-105.

Morall P and Briggs DE (1978). Changes in cellwall polysaccharides of germinating barley grains. *Phytochem.*, 17: 1495-1502.

Morrall P, Boyd HK, Taylor JRN and Van der Walt WH (1986). Effect of germination time, temperature and moisture on malting of sorghum. *J. Inst. Brew*., 92: 439-445.

Morcos SR, Hegazi SM and El-Damhougy ST (1973). Fermented foods of common use in Egypt. II. The chemical composition of *bouza* and its ingredients. *J. Sci. Food. Agric.*, 24: 1157-1161.

Moro MS, Pomeranz Y and Shellenberger JA (1963). The effects of gibberellic acid on alpha-amylase in wheat endosperms. *Phyton (Buenos Aires)*, 20: 59.

Muller CJ and Fugelsang KC (1993). Gentisic acid: an aspirin-like constituent of wine. *Practical Winery and Vineyard*, Sep/Oct: 45-46.

Murakami H (1972). Fermented Foods: Traditional and Current Practices. In: Dasilva EJ, Dammergues YR, Nyn EJ and Ratledge CR (eds). Microbial Technology in Developing World. Oxford Science Publications, 179-180.

Murthy DS and Kumar KA (1995) Traditional uses of sorghum and millets. In: Dendy DA (ed). Sorghum and Millets: Chemistry and Technology. Am. Assoc. Cereal Chem., St. Paul, Minnesota, USA, pp 185-221

Mwesigye RK and Okurut TO (1995). A survey of the production and consumption of alcoholic beverages in Uganda. *Process Biochem.*, 30(6): 497-501.

Narayanaswamy D, Somakurien Daniel VA, Rajalakshimi D, Swaminathan M and Parpia HA (1971). Supplementary value of a low cost protein food based on blend of wheat and soybean flours to poor rice and ragi diets. *Nutr. Rep. Int.*, 4: 109-116

NARC (2010). Nepal Agricultural Reseach Council, Hill Crops Research Program, Kabre, Nepal. Annual Report No. 14 (July 16, 2008 - July 15, 2009

Narziss L, Meidaner H, Graf H, Eichhorn P and Lusting S (1993). Technological approach to improve flavor stability. *MBAA Tech. Quart.*, 30: 48-53.

Nic Phiarais BP, Wijngaard HH and Arendt EK (2005). The impact of kilning on enzyme activity of buckwheat malt. *J. Inst. Brew.*, 111(3): 290-298.

Nie C, Wang C, Zhou G, Dou F and Huang M (2010). Effects of malting conditions on the amino acids composition of final malt. *African J. Biotechnol.*, 9(53): 9018-9025.

Nikkuni S, Karki TB, Terao T and Suzuki C (1996). Microflora of *mana* - a Nepalese rice *koji*. *J. Ferm. Bioengg.*, 81(2): 168 – 170.

Nirmala M, Subba Rao MVSS and Muralikrishna G (2000). Carbohydrates and their degrading enzymes from native and malted finger millet (ragi, *Eleusine coracana*, Indaf-15). *Food Chem.*, 69(2): 175-180.

Nithya KS, Ramachandramurthy B and Krishnamurthy V (2006). Assessment of antrinutritional factors, minerals and enzyme activities of the traditional (Co7) and hybrid (Cohcu-8) pearl millet (*Pennisetum glaucum*) as influenced by different processing methods. *J. Appl. Sci. Res.*, 2(12): 1164-1168.

Novellie L (1968). Kaffir beer brewing, ancient art, and modern industry. *Wallerstein Lab. Comm.*, 31: 17-29. In: Steinkraus KH (1996). Handbook of Indigenous Fermented Foods, 2nd edn. Marcel Dekker, Inc, New York, p. 413.

Nout MJR (1981). Aspects of the manufacture and consumption of Kenyan traditional beverages. Wageningen, The Netherlands.

Nout MJR and Davis (1982) Malting characteristics of finger milet, sorghum and barley. *J. Inst. Brew.*, 88: 157-163

Novellie L and De Schaepdrijver P (1986). Modern development in traditional African beer. In: Adams MR (ed). Progress in Industrial Microbiology. The Elsevier Applied Science Publisher, p. 23.

NS (1983). Nepal Standard for beer. NS-23-1983.

Nwanguma BC and Eze MO (1996). Changes in the concentrations of the polyphenolic constituents of sorghum during malting and mashing. *J. Sci. Food Agric.*, 70; 162-169.

Nzelibe CN and Nwasike CC (1995). The brewing potential of *Acha* (*Digitaria exilis*) malt compared with pearl millet (*Pennisetum typhoides*) malt and sorghum (*sorghum bicolor*) malts. *J. Inst. Brew.*, 101: 345-350.

Obilana AB, and Manyasa E (2002). Millets. In: Belton PS and Taylor JRN (eds). Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential. Springer – Verlag, Berlin, pp. 176-217.

Odeoemelan SA and Osu CI (2009). Evaluation of the phytochemical content of some edible grains marketed in Nigeria. *E-journal Chem.*, 6(4): 1193-1199.

O'Connor-Cox ESC and Ingledew WM (1989). Wort nitrogenous sources – Their use by brewing yeasts: A review. *J. Am. Soc. Brew. Chem.*, 47: 102-108

Ogu EO, Odiba FJC, Agu RC and Palmer GH (2006). Quality assessment of different sorghum varieties for their brewing potential. *J. Inst. Brew.*, 112(2): 117-121.

Okokon U and Etokakpan OU (2004). Changes in sorghum malt during storage, *J. Inst. Brew.*, 110(3); 189-192.

Okolo BN and Ezeogu LI (1995). Effect of air-rest period on the mobilization of sorghum reserve proteins. *J. Inst. Brew.*, 101: 463-468.

Okrah SGA (2008). Screening of six local sorghum varieties for their malting and brewing qualities. M.Sc. Dissertation, Department of Biochemistry and Biotechnology, Kwame Nkrumah Univ. of Science and Technology, Ghana.

Ologhobo AD and Fetuga Bl (1984). Distribution of phosphorus and phytate in some Nigerian varieties of legums and some effects of processing. *J. Food Sci.*, 49: 199-201.

Opoku AR, Ohenhen SO and Eijofor N. (1981). Nutrient composition of millet (*Pennisetum typhoides*) grain and malt. *J. Agri. Food Chem.*, 29(6): 1247-1248.

Osti SC (2004). Saccharification of finger millet by using mold *koji*. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Ough CS and Amerine MA (1960). Experiments with controlled fermentation IV. *Am. J. Enol. Vitic.*, 11: 5 -14. In: Amerine MA, Berg HM and Cruess WV (1967). The Technology of Wine Making, 2nd. edn. AVI Publishing Co., Westport, Connecticut, pp. 313.

Owuama CI and Adeyemo O (2009). Effect of exogenous enzymes on the sugar content of wort of different sorghum varieties. *World Appl. Sci. J.*, 7(11): 1392-1394.

Palmer GH (1989). Cereals in malting and brewing. In: Palmer GH (ed). Cereal Science and Technology. Aberdeen University Press, Aberdeen, Scotland, pp. 61-242

Park KI, Mheen TI, Lee KH, Chang CH, Lee SR and Kown TW (1977). Korean *yakju* and *takju*. Symposium on Indigenous Fermented Foods, Bangkok, Thailand, pp. 447-449.

Parvathy P (1995). Homologies among seed storage proteins and comparison of biochemical constituents in developing and germinating millets. Ph.D. Thesis, Tamil Nadu Agric. Univ., Coimbatore, India.

Pederson CS (1971). Microbiology of Food Fermentation. AVI Publishing Company, Inc., Westport Connecticut.

Pelembe LAM, Dewar J and Taylor JRN (2002). Effect of malting conditions on pearl millet malt quality. *J. Inst. Brew.*, 108: 7-12.

Pelembe LAM, Dewar J and Taylor J RN (2004). Effect of germination moisture and time on pearl millet malt quality with respect to its opaque and lager beer brewing potential. *J. Inst. Brew.*, 110(4): 320-325.

Pichyangkura S and Kulprecha S (1977). Survey of mycelial molds in *loopang* from various sources in Thailand. Symposium on Indigenous fermented Foods, Bangkok, Thailand.

Piazzon A, Forte M and Nardini M (2010). Characterization of phenolics content and antioxidant activity of different beer types. *J. Agric. Food Chem.*, 58(19): 106773-83.

Pore MS and Magar NG (1977). Nutritive value of hybrid varieties of finger millet. *Indian J. Agric. Sci.*, 147: 226.

Porter SC (1975). Accelerated fermentation of brewer's wort by *Saccharomyces carlbergensis*. *Appl. Microbiol.*, 30(6): 970-974.

Rai BK (1991). Preparation and quality evaluation of *jand* from malted and non-malted millet (*kodo*) using *A. oryzae* and *S. sake*. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Rai BK, Subba DK and KC, JB (1999). Isolation of fermentative yeasts from some plants. *Tribhuvan Univ. J.*, (Nepal), 22: 37-40.

Rai BK (2006). Preparation of starter culture using yeasts and molds isolated from local *murcha*. M. Tech (Food) Dissertation, Central Department of Food Technology, T.U., Nepal.

Rai RK (1984). To study *raksi* distilled liquor-making process in Eastern Nepal. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Rajbanshi A (2005). Women's livelihood from homemade beer (*chhyang*) and whiskey (*raksi*) in a peri-urban village: A case study of Ghumrachowk in Bajrayogini village development committee, Kathmandu district, *Tribhuvan Univ. J.*, (Nepal), 15(1): 61-77.

Ranganna S (1986). Handbook of Analysis and Quality Control for Fruit and Vegetable Products, 2nd edn. Tata McGraw-Hill Publishing Co., Limited, India

Rao PU and Deosthale YG (1982). Tannin content of pulses, varietal differences, and effects of germination and cooking. *J. Sci. Food Agric.*, 33: 7013-7016.

Ravindran G (1991). Studies on millets: Proximate composition, mineral composition and phytate and oxalate contents. *Food Chem.*, 39: 99-107.

Reddy NR, Pierson MD, Sathe SK and Salukhe DK (1989). Occurance, distribution, content, and dietary intake of phytate. Phytates in Cereals and Legumes. CRC Press, Boca Ration, pp. 39-56.

Regmi P (2007). Preparation and quality evaluation of fermentation starter using yeast and mold isolated from *mana*. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Reichert RD, Fleming SE and Schwab DJ (1980). Tannin deactivation and nutritional improvement of sorghum by anaerobic storage H_2O – HCl, or NaOH treated grain. *J. Agric. Food Chem.*, 28: 824-829.

Reinhold JG (1988). Problems in mineral nutrition: A global perspective. In: Smith KD (ed). Trace Minerals in Foods. Marcel Dekker Inc, New York and Basel, pp. 57-94.

Rohan S, Rawel HM and Krol J (2002). Inhibitory effect of plant phenols on the activity of selected enzymes. *J. Agric. Food Chem.*, 50: 3566-3571.

Sadasivam S and Manickam A (1996). Biochemical Methods, 2nd edn. New Age International (P) Limited, Publishers, New Delhi, India.

Sahai Srivastava BI and Meredith WOS (1962). Mechanism of action of gibberellic acid: Inhibition of alpha-amylase development during germination of barley by chloramphenicol and its reversal by gibberellic acid. *Canadian J. Bot.*, 40(9): 1257-1265.

Saito N, Sakai H, Sekihara H and Yajima Y (1998). Effect of α-glucosidase inhibitor (voglibose) in combination with sulphonilurease on glycemic control in type 2 diabetes patients. *J. Int. Medical Res.*, 26: 219-232.

Samantray GT, Misra PK and Patnaik KK (1989). Mineral composition of ragi. *Indian J. Nutr. Diet.*, 26: 113-116.

Samaras TS, Gordon MH and Ames JM (2005). Antioxidant properties of malt model system. *J. Agric. Food Chem.*, 53: 4983-4945.

Saono S, Hull RR, and Dhamcharee B (1986). A Concise Handbook of Indigenous Fermented Foods in the ASCA Countries. The Government of Australia, Canberra, Australia, p. 136.

Serna-Saldivar S and Rooney LW (1995). Structure and chemistry of millets. In: Dendy DA (ed), Sorghum and Millets: Chemistry and Technology. Am. Assoc. Cereal Chem., st. Paul, MN, USA, pp. 69-124.

Shayo NB, Tiisekwa BPM, Laswai HS, Kimaro JR (2001). Malting characteristics of Tanzania finger millet varieties. *Food Nutrition Journal of Tanzania*, 10 (1): 1-3.

Shinde GB, Adsule RN and Kale AA (1991). Effect of dehulling and cooking treatments on phytate phosphorus, polyphenols and trypsin inhibitor activity of cowpea seeds. *Indian Food Packer*, 36: 63-65.

Shahidi F and Naczk M (1995). Phenolic compounds of beverages. In: Lancaster PA (ed). Food Phenolics, Sources, Chemistry, Effects and Applications. Technoming Publishing Co., pp. 128-136.

Shen Y, Jin L, Xiao P, Lu Y and Bao J (2009). Total phenolics, flavonoids, antioxidant capacity in rice grain, and their relations to grain color, size, and weight. *J. Cereal Sci*., 49: 106-111.

Shrestha B (1985). Study on raksi production from rice by traditional method. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Shrestha H, Nand K and Rati ER (2002). Microbiological profile of *murcha* starters and physico-chemical characteristics of *poko* - a rice-based traditional fermented food product of Nepal. *Food Biotechnol.*, 16(1): 1-15.

Shrestha H and Rati ER (2003). Defined microbial starter formulation for the production of *poko* – a traditional fermented food product of Nepal. *Food Biotechnol.*, 17(1): 15-23.

Shukla SS, Gupta OP, Sawarkar NJ, Tomar AK and Sharma YK (1986a). Malting quality of ragi varieties: Nutrient and mineral composition of their malts. *J. Food Sci. Technol.*, 23: 235-237.

Sing G, Kapoor IPS, Sing P, de Heluani CSL, de Lampasona MP (2008). Chemistry, antioxidant and antimicrobial investigation on essential oil and oleoresins of *zingiber officinale*. *Food and Chem. Toxicol.*, 47:3295-3302.

Siwela M (2009). Finger millet grain phenolics and their impact on malt and cookie quality. Ph.D. Thesis, Dept. of Food Science, Faculty of Natural and Agricultural Sciences, Univ. of Pretoria, Pretoria, South Africa.

Skinner R (1976) Tropical lager beer brewing with sorghum malt. *Brew. Dist. Int.*, 6: 26-27

Spencer H and Karmer L (1988). Calcium, phosphorus, and fluoride. In: Smith KD (ed). Trace Minerals in Foods. Marcel Dekker Inc, New York and Basel, pp. 40-46.

Sripriya G, Antony U and Chandra TS (1997). Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination. *Food Chem.*, 58(4): 345-350.

Steinkraus KH (1983). Handbook of Indigenous Fermented Foods. Marcel Dekker Inc., New York.

Steinkraus KH (1995). Handbook of Indigenous Fermented Foods., 2nd edn. Marcel Dekker, New York

Steinkraus KH (1997). Classification of fermented foods: Worldwide review of household fermentation techniques. *Food Control.*, 8(5/6): 311-317

Subba C (1985). *Raksi* production from finger millet (*kodo*) by traditional method. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Subramanian V, Murthy DS, Rao S and Jambunathan R (1992). Chemical changes and diastatic ativity in grains of sorghum (*Sorghum bicolor*) cultivars during germination. *J. Sci. Food Agric.*, 58: 35-40.

Tamang JP, Dewan S, Tamang B, Rai A, Chillinger U and Holzpafel WH (2007). Lactic acid bacteria in *hamei* and *murcha* of North East. *Indian J. Microbiol.*, 47: 119-125.

Tamang JP and Thapa S (2006). Fermentation dynamics during production of bhaati jaanr - a traditional fermented rice beverage of the Eastern Himalayas. *Food Biotechnol.*, 20: 251-261.

Tamang JP (1998). Role of microorganisms in traditional fermented foods. *Indian Food Industry*, 17(3): 162-167.

Tamang JP, Thapa S, Tamang N and Rai B (1996). Indigenous fermented food beverages of Darjeeling hills and Sikkim - process and product characterization. *J. Hill Res.*, 9(2): 401-411.

Tamang JP and Sarkar PK (1995). Microflora of *murcha* - an amylolytic fermentation starter. *Microbios.*, 81: 115-122.

Tamang JP, Sarka PK and Hesseltine C (1988). Traditional fermented foods and beverages of Darjeeling and Sikkim – a review. *J. Sci. Food Agric.*, 44(4): 375-385.

Tanimura W, Sanches PC and Kozaki M (1978). The fermentd foods in the Philippines (part I) *tapuy* (rice-wine). *J. Agric. Soc*. (Japan), 22: 118-133.

Taylor JRN, Schober TJ and Bean SR (2006). Novel food and non-food uses for sorghum and millets. *J. Cereal Sci.*, 44: 252-271.

Taylor JRN and Robbins DJ (1993). Factors influencing beta-amylase activity in sorghum malt. *J. Inst. Brew.*, 99: 413-416.

Taylor JRN (2009) Development in Africa's cereal crops – potential sustainable resources for brewing in tropical and sub-tropical countries. The Institute of Brewing and Distilling Africa Sect. – 12th Scientific and Technicl Convention, 2009

Thapa S (2002). Microbiological and biochemical studies of indigenous fermented cereal-based beverages of the Sikkim Himalayas. Ph. D. Thesis, Food Microbiology Laboratory, Sikkim Government College, North Bengal Univ. India, p. 190.

Thapa S and Tamang JP (2004). Product characterization of *kodo ko jaanr* : fermented finger millet beverage of the Himalays. *Food Microbiol.*, 21: 617-622.

Thapa S and Tamang JP (2006). Microbiological and physico-chemical changes during fermentation of kodo ko jaanr - a traditional alcoholic beverages of the Darjeeling hills and Sikkim. *Indian J. Microbiol*., 46 (4): 333 – 341.

Thomas JA, Spradlin JE and Dygert S (1971). Plant and animal amylases. In: Boyer PD (ed). The Enzymes, 3rd edn., Vol. 5. Academic Press, New York, pp. 115-189.

Til HP, Woutersen RA and Feron VJ (1988). Evaluation of the oral toxicology of acetaldehyde and formaldehyde in a 4-week drinking water study in rats. *Food and Chem. Toxicol.*, 26(5):447-452

Tiwari MP (2010). Study on the brewing quality of different Nepalese finger millet [*Eleusine coracana (L) Gaertn*, kodo]. M.Sc. Dissertation, Central Department of Food Technology, T.U., Nepal.

Tsuyoshi N, Fudou R, Yamanake S, Kozaki M, Tamang N, Thapa S and Tamang JP (2005). Identification of yeast strains isolated from *murcha* in Sikkim - a microbial starter for amylolytic fermentation. *Int. J. Food Microbiol.*, 99(2): 135-146.

Uchida M and Ono M (2000). Technological approach to improve flavor stability: Analysis of the effect of brewing process on beer flavor stability by the electron spin resonance method. *J. Am. Soc. Brew. Chem.*, 58: 8-13.

Udayasekhara P and Deosthale YG (1988). In vitro availability of iron and zinc in white and color ragi (*Eleusine coracana*): role of tannin and phytate. *Plant Food Hum. Nutr*., 38: 35-41.

Upadhyaya A (2005a). Effect of raw materials on the quality of *jand*. B. Tech (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

USDA (2007). www.nal.usda.gov/fnic/foodcomp/search. United States Department of Agriculture (USDA).

US NRC. (1996). Lost Crop of Africa, Vol. I: Grains. National Research Council Washington, DC: National Academy Press.

Vanderhaegen B, Neven H, Verachtert H and Derdelinckx G (2006). The chemistry of beer aging – A critical review. *Food Chem.*, 95: 357-381.

Vanbeneden N, Gils F, Delvaux F and Deldvux RF (2007). Variability in the release of free and bound hydroxycinnamic acids from diverse malted barley (*Hordeum vulagre L.*) cultivars during wort production. *J. Agric. Food Chem.*, 55; 11002-11010.

Venkatanarayana S, Sreenivasa Murthy V and Satyanarayana Rao BA (1979). The use of ragi (*Eleusine corana*) in brewing. *J. Food Sci. Technol.*, 16: 204-206.

Venkataramu K and Basapu SC (1993). A comparative study of *chhang* fermentation of ragi using natural (traditional) and pure microbial inocula. IFCON 1993 Poster Sessions Abstract, AFST (India), pp. 65-65.

Verma SK (1991). Study on the preparation and efficiency of starter cake from *A. oryzae* and *S. sake* using rice as a binder. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Veith KN (2009). Evaluation of four sorghum hybrids through the development of gluten-free beer. Master's Thesis, Food Science Institute, Grain Science and Industry. Kansas State Univ. http://krex.k-state.edu/dspace/handle 2097/2281, accessed on 9/7/2011.

Wadikar DD, Vasudish CR, Premavalli KS and Bawa AS (2006). Effect of variety and processing on antinutrients in finger millet. *J. Food Sci. Technol.*, 43(4): 370-373.

Weger, B (1965). Calcium und Natriumbentonite. Wein-Wissen, 20: 545 – 559. In: Amerine *et al*. (1967)…….

Yadav BK (1993). Study on some physico-chemical indices of locally produced raksi quality. B. Tech. (Food) Dissertation, Central Campus of Technology, T.U., Nepal.

Yokotsuka T (1985). Non-proteinaceous fermented foods and beverages produced with *koji* molds: In: Arora DK, Mukerji KG and Marth EH (eds). Handbook of Applied Mycology. Marcel Dekker, Inc., New York, pp. 293-328.

Yoon SS (1993). CheMin YoSul - A translation of Chi-Min-Yao-Shu in Korean. MinEumsa, Seoul, Korea.

Zamora AF and Fields ML (1979). Nutritional quality of fermented cowpeas and chickpea. *J. Food Sci.*, 4: 234-236.

Zao H. Chen W, Lu L and Zhao M (2010). Phenolic profiles and antioxidant activities of commercial beers. *Food Chem.*, Vol. 119, Issue 3: 1150-1158.

Zarnkow M, Kebler M and Burberg F (2007). The use of response surface methodology to optimize malting conditions of Proso millet (*Panicum miliaceum* L.) as a raw material for gluten free foods. *J. Inst. Brew*., 113(3): 280-292

Zeigler P (1995). Carbohydrate degradation during germination. In: Kigel J and Galili G (eds). Seed Development and Germination. Marcel Dekker Inc., New York, pp. 447-474.

Zoecklein BW, Fugelsang KC, Gump BH and Nury FC (1997). Wine Analysis and Production. CBS Publishers and Distributors, New Delhi, India.

Appendices

Appendix A: Experiment design for response surface analysis

Appendix A, Table 1 Experimental design in coded form for response surface analysis

Coded variables				
x_1	x_2	Combinations	Replications	No. of experiments
±1	±1	4	1	4
±1	0	2	1	2
0	±1	2	1	2
0	0	5	1	5

Code '0' is for centre point of the parameter range investigated, '±1' for factorial points.

x_1 = temperature (^{0}C) and x_2 = pH.

Appendix A, Table 2 Values of independent variables at three levels of the center composite face centered design (CCFCD)

Independent variables	Code	Levels in coded form		
		-1	0	+1
Protein rest				
Temperature (°C)	x_1	40	50	60
pH	x_2	4.5	5.25	6
Dextrinizing preiod				
Temperature (°C)	x_1	62	65	68
pH	x_2	4.5	5.25	6
Conversion period				
Temperature (°C)	x_1	70	73	76
pH	x_2	4.5	5.25	6

Appendix A, Table 3 Experimental design for mashing with coded and uncoded variable levels

	Coded levels		Uncoded levels					
			Protein rest		Dextrinizing period		Conversion period	
Run	x_1	x_2	x_1	x_2	x_1	x_2	x_1	x_2
1	-1	-1	40	4.5	62	4.5	70	4.5
2	1	-1	60	4.5	68	4.5	76	4.5
3	-1	1	40	6	62	6	70	6
4	1	1	60	6	68	6	76	6
5	-1	0	40	5.25	62	5.25	70	5.25
6	1	0	60	5.25		5.25	76	5.25
7	0	-1	50	4.5	65	4.5	73	4.5
8	0	1	50	6	65	6	73	6
9	0	0	50	5.25	65	5.25	68	5.25
10	0	0	50	5.25	65	5.25	73	5.25
11	0	0	50	5.25	65	5.25	73	5.25
12	0	0	50	5.25	65	5.25	73	5.25
13	0	0	50	5.25	65	5.25	73	5.25

Appendix B : Analysis of variences (ANOVA) Tables

Appendix B Table 1 Analysis of variance for FAN and TSS models during protein rest period

	FAN		TSS	
Factor	Coeff.	p-value	Coeff.	p-value
x_1	0.167	0.095	1.633	< 0.001
x_2	0.393	0.003	0.217	0.203
x_1^2	−2.669	< 0.001	1.331	0.001
x_2^2	−0.509	0.005	−0.119	0.617
x_1x_2	−0.985	< 0.001	0.600	0.016

Appendix B, Table 2 Regression model fitted to FAN and TSS data during protein rest preiod

	p-values	
Responses	Regression	Lack of fit
FAN	< 0.001	0.300
TSS	< 0.001	0.090

Appendix B Table 3 Analysis of variance for FAN, TSS, RS, glucose and fructose models during dextrinizing period

	FAN		TSS		RS		Glucose		Fructose	
Factor	Coeff	p -value	Coeff	p-value	Coeff	p-value	Coeff	p-value	Coeff	p-value
x_1	−0.97	0.003	2.73	<0.001	2.10	<0.001	1.13	<0.001	0.053	0.001
x_2	−0.52	0.009	−0.33	0.046	0.088	0.390	−0.15	0.067	−0.080	<0.001
x_1^2	2.90	<0.001	−1.88	<0.001	−1.37	<0.001	−0.78	<0.001	−0.032	0.068
x_2^2	0.006	0.980	0.26	0.247	0.0012	0.994	0.17	0.126	−0.028	0.106
x_1x_2	−0.30	0.138	−0.082	0.638	0.20	0.130	−0.10	0.251	−0.002	0.861

Appendix B, Table 4 Regression model fitted to FAN, TSS RS, glucose and fructose data during dextrinizing preiod

	p-values	
Responses	Regression	Lack of fit
FAN	<0.001	0.096
TSS	<0.001	0.948
RS	<0.001	0.178
Glucose	<0.001	0.404
Fructose	0.001	0.551

Appendix B Table 5 Analysis of variance for FAN, TSS, RS, glucose and fructose models during conversion period

	FAN		TSS		RS		Glucose		Fructose	
Factor	Coeff	p -value	Coeff	p-value	Coeff	p-value	Coeff	p-value	Coeff	p-value
x_1	−0.73	0.001	0.80	<0.001	−1.20	<0.001	−0.20	<0.001	0.047	<0.001
x_2	0.65	0.001	−0.16	0.005	−0.019	0.864	0.083	0.004	0.017	0.019
x_1^2	0.99	0.001	−0.30	0.002	−0.57	0.009	−0.028	0.365	0.11	<0.001
x_2^2	−0.86	0.001	−0.39	0.001	−0.47	0.021	−0.22	0.001	−0.037	0.003
x_1x_2	−0.22	0.100	0.23	0.003	−0.37	0.027	0.053	0.069	−0.020	0.020

Appendix B, Table 6 Regression model fitted to FAN, TSS RS, glucose and fructose data data during conversion preiod

	p-values	
Responses	Regression	Lack of fit
FAN	<0.001	0.696
TSS	<0.001	0.060
RS	<0.001	0.322
Glucose	<0.001	0.069
Fructose	<0.001	0.439

Appendix C: Contour plots for various response variables

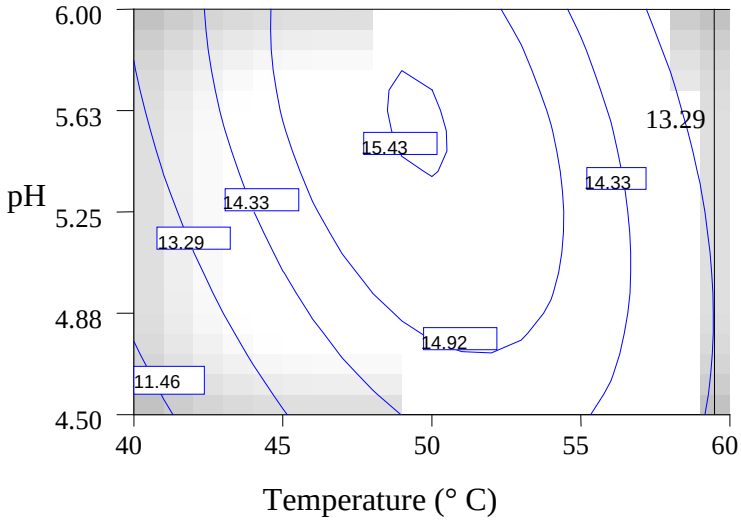


Figure 1 Contour plots for FAN content during protein rest period of mashing.

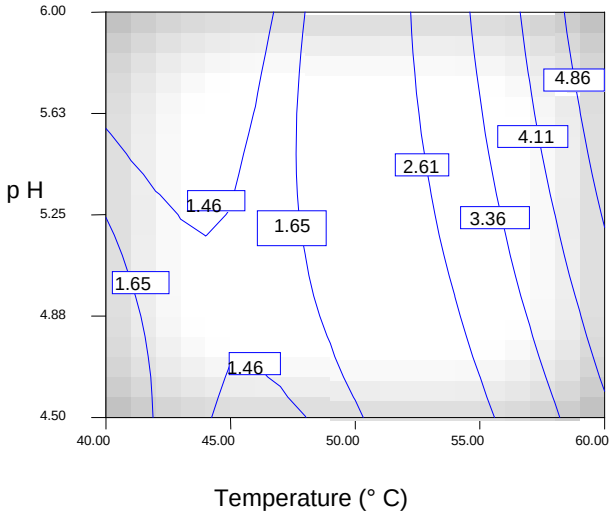


Figure 2 Contour plots for TSS content during protein rest period of mashing.

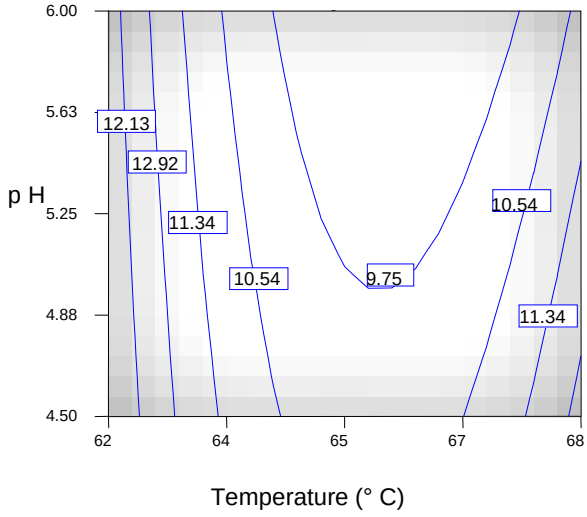


Figure 3 Contour plots for FAN content during dextrinizing period of mashing.

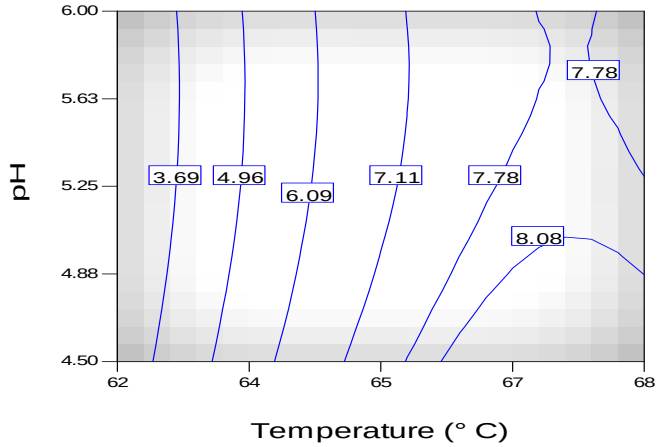


Figure 4 Contour plots for TSS content during dextrinizing period of mashing.

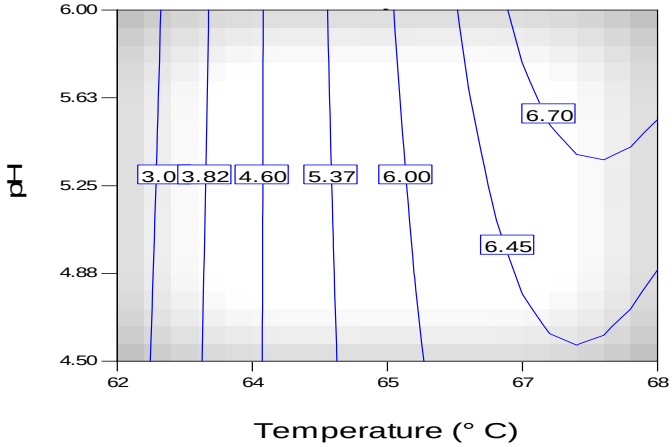


Figure 5 Contour plots for total reducing sugar (RS) content during dextrinizing period of mashing.

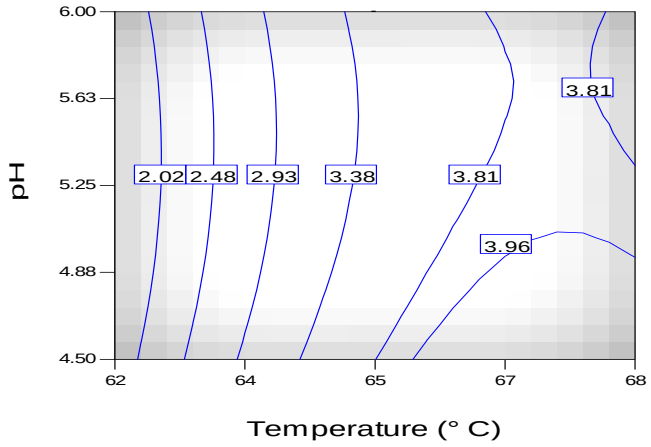


Figure 6 Contour plots for glucose content during dextrinizing period of mashing.

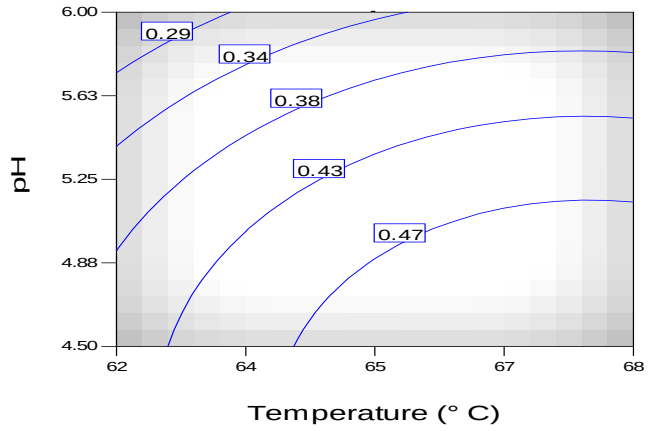


Figure 7 Contour plots for fructose content during dextrinizing period of mashing.

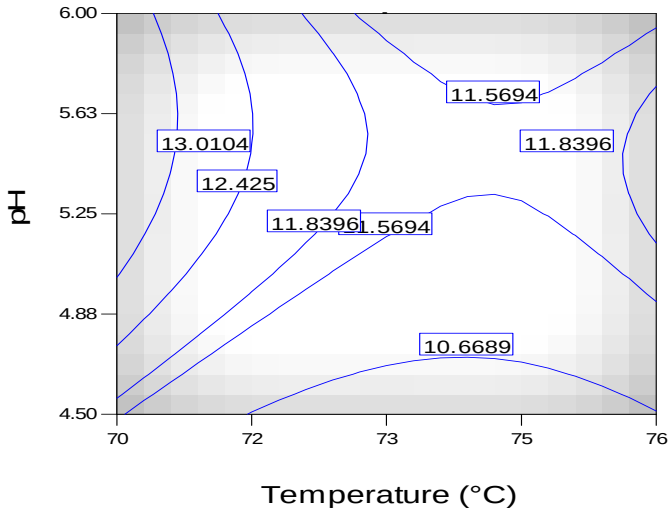


Figure 8 Contour plots for FAN content during conversion period of mashing.

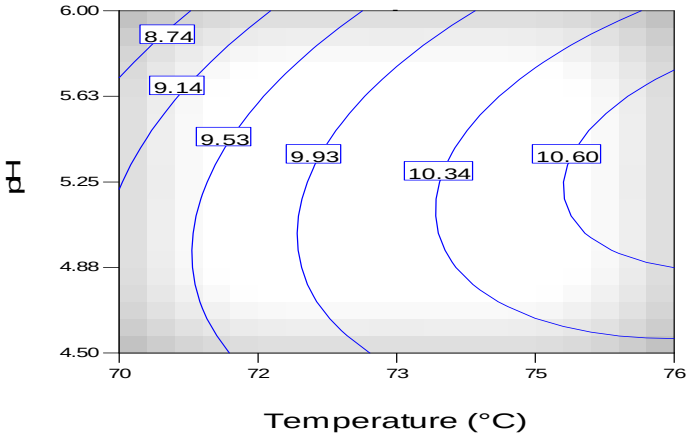


Figure 9 Contour plots for TSS content during conversion period of mashing.

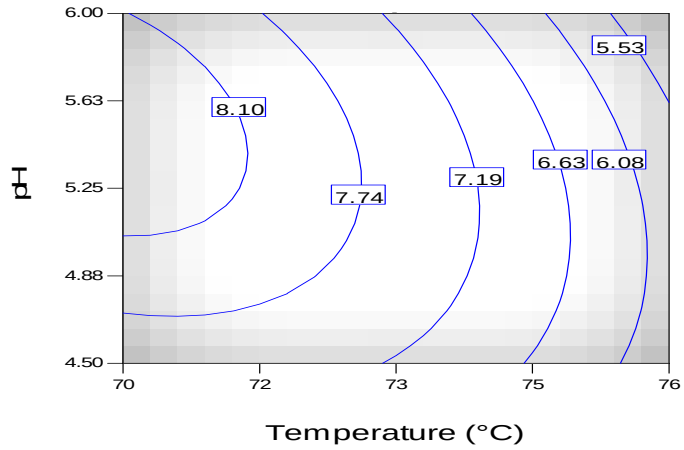


Figure 10 Contour plots for total reducing sugar (RS) content during conversion period of mashing.

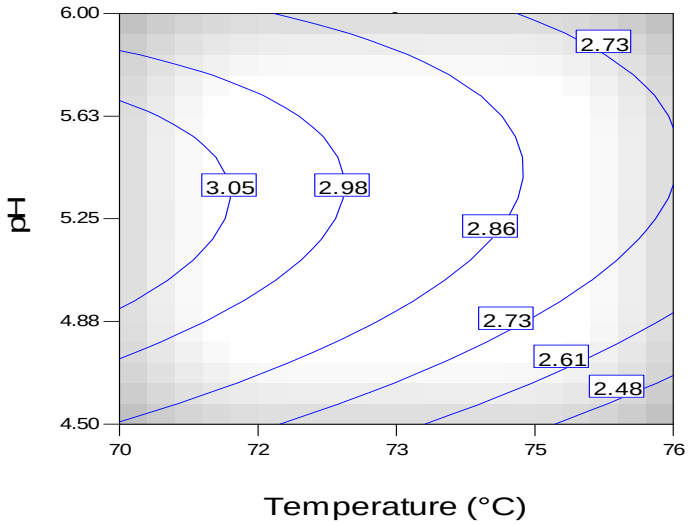


Figure 11 Contour plots for glucose content during conversion period of mashing.

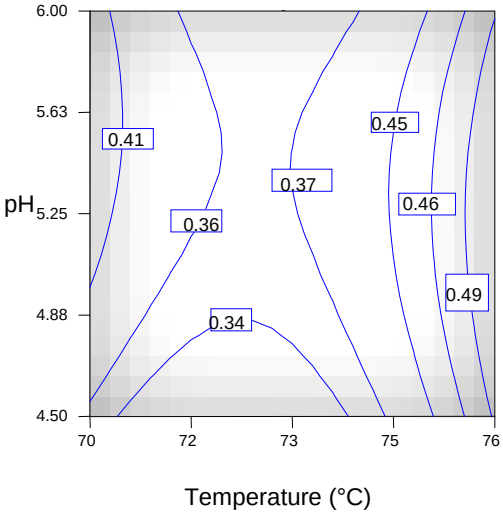


Figure 12 Contour plots for fructose content during conversion period of mashing.

Appendix D: Sensory evaluation score sheet

Hedonic Rating Test

Name of the panelist:
Product: Beer Date:
Taste these samples and give the appropriate score to show your attitude about the sample.

Attributes	Sample codes
1. Taste	
2. Smell/odor	
3. Flavor	
4. Color	
5. Clarity	
6. Body	
7. Overall acceptance	

Please use the following numeric values to express your feeling about the sample

Feeling	Score
Excellent	5
Good	4
Satisfactory	3
Fair	2
Poor	1

(Signature)

List of publications from this work

Papers published in international journals

1. Effect of finger millet varieties on chemical characteristics of their malts. *African Journal of Food Science* (ISSN: 1996-0794), 6(11): 308-316, 2012.

2. Physico-chemical properties of finger millet (*Eleusine coracana*) and barley beers. *International Journal of Biotechnology and Allied Fields* (ISSN: 2320-0774), 1(1): 19-35, 2013.

3. Malting characteristics of some Nepalese finer millet (*Eleusine coracana*) varieties. *International Journal of Current Research* (ISSN: 0975-833X), 5(5):1054-1059, 2013.

Papers published in national journals

1. Storage stability of fermented finger millet (*Eleusine coracana*) packaged in PVC container under ambient conditions. *Journal of Food Science and Technology, Nepal* (ISSN 1816-0727), 4: 70-73, 2008.

2. Solid versus semi-solid fermentation of finger millet (*Eleusine coracana*). *Journal of Food Science and Technology, Nepal* (ISSN 1816-0727), 6: 31-35, 2010.

3. Effect of fermentation containers and raw materials on the chemical composition and sensory quality of fermented cereals. *Nepal Journal of Science and Technology* (ISSN 1994-1412), 12: 330-339, 2011.

Paper presented at conferences

1. Clarification of finger millet (*Eleusine coracana*) jand using fining agents. The fifth national conference on Science and Technology, Nov. 10-12, 2008, Kathmandu, Nepal. Organized by Nepal Academy of Science and Technology (NAST), Nepal.

2. Bio-chemical changes during finger millet (*Eleusine coracana*) fermentation using defined fermentation starter. Proceeding of the National Conference on Food Science and Technology (Food Conference-2012, 10-11 August, Kathmandu, Nepal. Organized by Nepal Food Scientists and Technologists Association (NEFOSTA), Nepal; Deparment of Food Technology and Quality Control (DFTQC), Nepal and Central Campus of Technology (CCT), Dharan, Nepal.