# STUDIES ON BEER PRODUCTION FROM DIFFERENT MINOR MILLETS

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# INTRODUCTION

Beer is an alcoholic beverage made from cereals like barley, corn, rice, oat, sorghum, *etc.* and tuber crops like cassava. Beer is made by fermentation and has an alcohol content of 2 to 6 per cent. Today, over 70 styles of beer are available in the market. Each brand has its unique characteristic from its ingredients and subtle differences in its brewing process. Different blends of barley malt, roasted barley, oat malt, caramel sugar and other cereals are used to prepare distinctive types of beer or ale.

Beer is the worlds most widely consumed alcoholic beverage. It is the third-most popular drink overall, after water and tea. It is thought to be the oldest fermented beverage. Some of the earliest known writings mentioned the production and distribution of beer. The Code of Hammurabi included laws regulating beer and beer parlors and "The Hymn to Ninkasi", a prayer to the Mesopotamian goddess of beer, served as both a prayer and as a method of remembering the recipe of beer in a culture with few literate people. Today, the brewing industry is a global business, consisting of several dominant multinational companies and many thousands of smaller producers ranging from brewpubs to regional breweries.

Many innovations paved the way for the proliferation of today's large brand beers. By early 1990's the largest American brewers, such as Anheuser-Busch and the Miller brewing company produced nearly 125 million barrels per year. In 2009, in the United States, 2100 brewers produced almost 196 million barrels of beer and on an average; annually each American consumed nearly 76 liters of beer. India is a home to nearly one sixth of the global population and is one of the most attractive consumer market in the world today.

In general, beer is found to have some medicinal values. It is found to increase the plasma HDL (High Density Lipoprotein) which is a scavenger of cholesterol and so can reduce risk of cardiovascular disease (Buemann *et al.*, 2002) and also as a diuretic agent for treating urolithosis (kidney stone). Besides, it has also been used as a traditional medicine against cold, cough, loss of appetite, skin blemishes *etc.* Traditional brewing ingredients of beer are barley and rice. The beer industry depends to a great extent on agricultural products in which barley malt with or without adjuncts predominates as the chief raw material. Along with the development of the beer industry and increase in population, these are insufficient resources. Minor millets are a new potential substitute for barley or rice, which can resolve not only the ingredient problem, but also raise economic benefits.

Worldwide the brewing industry is becoming more competitive and constantly looking for ways to improve beer quality and reduce manufacturing costs. In tropical countries, barley has to be imported from other temperate countries which involve drain of scarce foreign exchange. Minor millet is a new potential substitute for barley which can be used as an alternate substrate and also raise economic benefits.

Millets are unique agronomic group of small round seeded grasses that are able to grow well and set seed in the most difficult of terrains and harsh environments of rain fall and high temperature. Millets are probably the world's earliest food plants used by humans and certainly the first cereal grain that was used for domestic purposes. The world production is 28.38 million tons, out of which 11.36 million tons (40%) are produced in Africa. Millets provides 75 per cent of total calorific intake for the poor people living in the semi-arid tropics and sub-humid drought-prone areas. Millets alone provide 13.40 kg per year per capita food consumption. They generally contain high protein, calcium, phosphorus and potassium, iron and zinc.

In the region where millets are grown, it is used to prepare several types of food products some of which are fermented. Indeed, millets are used in brewing European-type lager beer. Other studies have suggested that millet has other important qualities and uses. Finger millet makes the best quality malt used in both brewing industry and making of digestible nutritious foods. Pearl millet has been shown to have similar diastatic power in pearl millet and similar to sorghum and makes just as good beer brewing with good quality malts. Millet malt produce wort that filter faster than sorghum malt wort and produce beers that had better foam properties than beers brewed from sorghum malt (Chavan *et al.*, 2010). Millet has some physical properties that are similar to those of sorghum, especially with regard to the gelatinization temperatures of the starches of sorghum and millet. Fermentation of sprouted (germinated) millets results in significant increase in protein and starch digestibility (Pradeep *et al.,* 2009).

In India, minor millets are becoming major crops in arid regions. It is essential to provide additional value to the grains as a part of preservation and commercialization to support the economy of farmers.

In this context attempts are made to screen, evaluate and explore the alternative crop varieties like Bajra, Finger millet, Foxtail millet and Little millet with following set of objectives.

1. To evaluate different minor millets for their suitability for beer production.
2. To screen different yeast strains and standardization of parameters for beer production in the selected minor millet.
3. Beer production from the selected minor millet under lab scale conditions.

# REVIEW OF LITERATURE

Millet is a collective term referring to a number of small-seeded annual grasses that are cultivated as grain crops, primarily on marginal hungry and thirsty soils lands in dry areas in temperate, subtropical and tropical regions. The most important species are great millet, pearl millet, finger millet, little millet, proso millet and foxtail millet. Finger millet [*Eleucine coracana* (L.) Gaertn.] is widely produced in the cooler, higher altitude region of Africa and Asia (International crop Research Institute for the semi-arid tropics (FAO, 1996).

Among the millets of the world, finger millet ranks fourth in importance after sorghum, pearl millet and foxtail millet (Upadhyaya *et al*., 2007). It is a grass crop grown in Africa, India, Nepal and many countries of Asia. The plant and grain is resistant to drought, pests and pathogens. In African countries, millet is malted and used to brew various traditional beers. It has some very potential useful characteristics with respect to brewing (Taylor and Belton, 2002).

One major use of the grain is the making of fermented beverages after malting. Food made from malted ragi is traditionally used for weaning and has been the source of low viscosity weaning foods that can deliver more energy per feed than those based on gelatinized starch. There is some evidence that food products from finger millet have a low gylcaemic index and are good for diabetic patients (Taylor and Belton, 2002).

## 2.1 Overview of finger millet production in the world

Finger millet is a staple food for millions of people in Africa, India and Nepal. Precise global area under finger millet is not known because this crop had often been clubbed with other millets. Global area under millet is 36.29 m ha (FAO, 2010). The estimated global annual production of finger millet is about 4.5 million tons of grain of which approximately two million tons is produced in Africa while the Asian continent (mainly India and Nepal) produces the remainder. It is the second important crop in Africa. Among the highest producers of finger millet in Africa: 405,000 ha in Uganda, 320,000 ha in Tanzania and 90,000 ha in Kenya (FAO, 2010). In Nepal, finger millet, locally known as kodo, is the fourth staple food crop after rice, maize and wheat. Here the crop is grown on about 26,000 ha of land with an average productivity of 1,100 kg per ha (Dida and Devos, 2006).

## 2.2 Nutritional aspects of finger millet

Finger millet malt is superior to other millet malts (Malleshi and Desikachar, 1986). Finger millet seeds are particularly rich for tryptophan, cystine, methionine and total aromatic amino acids (phenylalanine and tyrosine) compared to other cereals (McDonough *et al*., 2002). The two sulphur containing amino acids methionine and cystine especially are lacking in the diet of millions of the poor who live on starch foods such as cassava. Finger millet is therefore an important preventive against malnutrition, especially Kwashiorkor (Mgonja *et al*., 2007).

The seeds are exceptionally rich in calcium contains about 0.34 per cent, 5-30 times more than in most cereals. The ash content has been found to be nearly 1.7 to 4.13 per cent. The phosphorus and iron contents are also high. Iron containing 46 mg per kg (Upadhyaya *et al*., 2006), which is much higher compared to wheat and rice. Thus, finger millet is a good source of diet for growing children, expecting women's, old age people and patients (Desai *et al*., 2010).

Millet has been used as a major cereal in the traditional manufacture of malt in Kenya and in India. Millet malt is extensively used in weaning and infant food preparations and in various supplementary food formulations. The primary objective of malting is to promote the development of hydrolytic enzymes, which are present in much lower amounts and activities in non-*Triticeae* species. The development of the amylase enzymes during malting is of critical importance as these enzymes are required to hydrolyze the malt and adjuncts starch to fermentable sugars for brewing (Traore *et al*., 2004)

## 2.3 Processing methods for beer production

### 2.3.1 Malting

Malting is a traditional method and commercial activity of world-wide importance as malts are used in the manufacture of beers, whiskies, foodstuffs and non-alcoholic beverages. It is the controlled germination followed by controlled drying of the kernels. The main objective of malting is to

promote the development of hydrolytic enzymes, which are not present in non-germinated grain (Muhammad *et al.*, 2011).

Malting is the first step in the brewing process. The process of malting comprises three unit operations: steeping, germination and drying. Steeping of grains in water for certain time until it begins to germinate or sprout. During germination, enzymes within the grains convert the hard, starchy interior of the grain to a type of sugar called maltose. At this point, the grain is called malt. The sugars available in the malt can be fermented to produce alcohol. Amylase activity was reported to increase with increase in moisture content during malting. Since malting has traditionally been carried out for the purpose of brewing, previous studies have concentrated on producing a malt that has a balance of enzymes and polysaccharides, with due consideration to other parameters, such as malting losses, hot water extract, cold water extract, total nitrogen, total soluble nitrogen and fermentability that are of interest to the brewer (Dziedzoave *et al.*, 2010).

### 2.3.2 Steeping of grains

During steeping, kernels are immersed in water until imbibed with sufficient water to start the metabolic processes of germination and at the same time dirt, chaff and broken kernels are removed by washing and flotation.

Steeping of grains (cereals and legumes) in water followed by germination or sprouting is a common household practice in developing countries. Germination reduces the high viscosity and water-binding characteristic of starch based porridges. As soon as the cereal and legume seeds are hydrated, chemical changes occur, which result in partial breakdown of storage components, such as starch and proteins (Bernard *et al.,* 2010). Thus germination is mainly used to lower dietary bulk in cereals because it converts significant amounts of starch, which is principally responsible for viscosity in cereal gruels, to sugars and short chain oligosaccharides (Onyeka and Dibia, 2002 and Traore *et al.*, 2004).

Malting of red and white varieties of sorghum with an 18 hour steeping period and five days germination resulted in an □-amylase activity which peaked on third day in the red malts and on fourth day in the white malts (Uvere *et al.*, 2000).

Nso *et al.* (2003) compared the amylase activity of three sorghum cultivars Safrari, Madjeru and S-35 used for traditional beer production and found it to be 94.56, 56.59 and 51.70 (units $g^{-1}$) at the laboratory conditions. Roberta and Palmer (2005) used four barley varieties for determining the malting qualities of barley. Half cut grains were used for mashing and it was found that chariot variety showed highest starch breakdown of 78 per cent between two and three days of germination followed by decanter variety in which 53 per cent of starch breakdown was observed at the third day of germination.

During the Western beer brewing process, soaking of the barley in water for 2 days at 10-16 $^0$C in order to increase the moisture content to around 45 per cent periodically, the water is temporarily drained off and aeration is provided, thus preventing anaerobic conditions that can cause grain embryo damage (Waites *et al.*, 2001).

Hotz and Gibson (2007) concluded that soaking improves the absorption of iron, zinc and calcium in cereal-based foods prepared with a reduced phytate content. The content of other antinutrients such as saponins, trypsin inhibitors and some polyphenols and oxalates that inhibit iron and calcium absorption are reduced during soaking.

Eltayeb *et al.* (2007) reported that soaking of pearl millet reduce the phytic acid content in 39.47 and 24.17 per cent in two different varieties, while unrefined maize flour reduced phytate content by approximately 50 per cent (Hotz and Gibson, 2007). Soaking also significantly decrease tannin content in 22.72 per cent and 11.76 per cent in two varieties of pearl millet. Ali *et al.* (2009) also stated that, tannin content significantly decreased when the grains were soaked in either distilled water or NaOH for 8h and the reduction was more pronounced when the grains were cooked after soaking in NaOH.

Muhammad *et al.* (2011) conducted a study on the barley grains. The locally purchased barley grains were soaked for 48 h at low temperature (10-18$^0$C) to 40-45 per cent moisture and germinated for 2 days. The germinated grains were dried in a cabinet drier at 65$^0$C for 16 h. Dried grains were manually derooted and were ground by pin grinder. The raw, soaked and malted barley were analyzed for moisture, ash, crude protein, crude fat, crude fiber, starch, reducing sugar, phytic acid and for total iron. Analysis of the malted barley showed increase in reducing sugar and crude

fiber while decrease in ash, crude protein, starch and crude fat, while the total iron was also reduced during malting.

### 2.3.3 Germination

Germination involves the outgrowth of the plumule and radicle of the seedling until suitable enzymes (*e.g.* starch degrading enzymes and proteases) have been produced for the malt. Conditions that are necessary during the germination phase are moisture content, temperature, length of germination time and oxygen availability. Germination takes about 4-6 days and occurs rapidly between $20^0$C and $30^0$C with an optimum temperature of $25^0$C to $28^0$C (Onyeka and Dibia, 2002).

During germination, the hormone gibberellic acid (GA), at low concentration (0.1-0.2 ppm), induces the aleurone layer to produce endosperm-degrading enzymes such as □-amylase, protease, pentosanases and endo-□-glucanase, but this hormone plays no such role in enzyme development (Lyumugabe *et al*., 2010).

During germination amylolytic activity with the production of maltose, maltotriose and dextrin from starch hydrolysis and phytase activity can be identified. Several reports showed that germination alters the availability of minerals. Many simple processing techniques such as dehulling, soaking, malting, fermentation and autoclaving are used to minimize the interactions between phytate and divalent cations especially Fe++, Zn++ and Cu++ (Lyumugabe *et al*., 2010).

Mbithi-Mwikya *et al.* (2000) stated that sprouting finger millet resulted in lowered levels of the antinutreints namely tannins, phytate and trypsin inhibitors activity. These decreases were accompanied by an increase *in vitro* protein digestibility (IVPD) and HCl extractability of minerals and trace elements. In raw ungerminated finger millet tannin content was 14.4 mg per 100 g and on germination for 0, 24, 48 and 72 h it decreased by 20, 45, 62 and 72 per cent respectively.

Pelembe *et al.* (2004), observed the effect of germination moisture and time on pearl millet malt quality. Two pearl millet varieties SDMV 89004 and 91018 were germinated at $25^0$C under three different watering regimes for 5 days. As with sorghum malting, diastatic power, □-amylase activity, free □-amino nitrogen (FAN), hot water extract and malting loss all increased with level of watering. However, pearl millet malt has shown a much higher level of □-amylase and higher FAN than sorghum malt. Thus, it appears that pearl millet malt has perhaps even better potential than sorghum malt in beer brewing.

Brou Kouakou *et al.* (2008) steeped the millet grain before germination and fermented for 0 to 8 days. He has observed that, by increasing germination and fermentation time increased production of amylase and corresponded to increased content of soluble sugars *viz.,* reducing and total sugars. Since, germination provokes increasing the protein content.

Bernard *et al.* (2010) determined the cyanide contents of locally purchased brown finger millet (*Eleusine corocana* var. L. Gaertner) and brown speckled kidney bean seeds (*Phaseolus vulgaries* var. Rose Coco) using the raw, germinated and autoclaved samples. The aim was to establish the extent of cyanide content increase resulting from the germination process and the effectiveness of the autoclaving process on the reduction of cyanide levels in the samples. Autoclaving was carried out at $121^0$C for 20 minutes. It was found that germination increased the cyanide content by 2.11 to 2.14 fold in finger millet for laboratory processed samples. In the case of kidney beans the increment was 1.76 to 1.77 fold for laboratory samples. Autoclaving reduced the cyanide content to between 61.8 and 65.9 per cent of the original raw contents for finger millet and between 56.6 to 57.8 per cent in the case of kidney beans. The corresponding reductions for field samples were also found to be within the same ranges as the laboratory processed samples. It was concluded that autoclaving significantly reduced the cyanide levels in germinated finger millet and kidney beans.

Muhammad *et al.* (2011) concluded that, germination is an important intermediate step in the preparation of malted barley. Malt has produced by the controlled germination of barley grains (*Hordeum vulgare* L.), which is initiated by steeping barley grains in water, followed by germination and kilning periods. The major objectives of the malting process are to hydrolyze barleys endosperm cell walls (predominantly (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)-□-glucan), hydrolyze a portion of endosperm protein; produce a quantity of enzymes within the grain that are further utilized during brewing *e.g.* □-amylase and to develop desirable malt colour and flavor.

Coulibaly and Chen (2011) has conducted the study to assess the energetic compounds *viz.,* protein, fat and carbohydrates some vitamins, minerals, antioxidant capacity, phytase and amylase activity during the germination of foxtail millet. Germination has found to increase nutritive qualities of

foxtail millet. One day soaking and germination up to 8 days increased soluble sugars (reducing and total sugars), amylase activity and phytase activity. However fat content and total phenolic content was decreased through germination.

Dhan and Ganga (2012) studied the chemical changes during germination and malting characteristics of six Nepalese finger millet varieties (*GPU 0025*, *GE 5016*, *Dalle*, *Okhle*, *Kabre* and *Juwain*). Millets were steeped in water at room temperature overnight, germinated for 48 h at 28±1$^0$C, kilned at 50±2$^0$C for 24 h and then, chemical characteristics of unmalted and malted millets were analyzed. Germination has found to increase total reducing sugar and glucose contents in all malted millet varieties. Total phenolics, total flavonoids and tannin were found lowest in malted millets.

### 2.3.4 Effect of germination on amylase activity

□-Amylase catalyzes the hydrolysis of □ -1,4-glucan bonds in amylosaccharide chains from the non-reducing ends of starch, glycogen, amylose, amylopectin and other malto-oligosaccharides and successively liberates □-anomeric maltose, leaving a □-limit dextrin. □-amylase is present in higher plants and microorganisms. It is used for the production of high maltose syrup from starch in combination with pullulanase, which can hydrolyze the □-1, 6-linkages.

Alkaline-stable □-amylase (EC 3.2.1.2) was purified to apparent homogeneity from malted African finger millet (*Eleusine coracana*) seed by ammonium sulfate fractionation and anion exchange and affinity chromatographies. Gel filtration chromatography together with SDS-PAGE revealed that the enzyme is monomeric, with a molecular weight of 59.1 kDa. The enzyme was stable at a pH range of 4.0–10.0 and temperature range of 30–70$^0$C (Ayodele *et al.,* 2011).

## 2.4 Mashing

Goode and Arendt (2003) carried out mashing of 50 per cent unmalted sorghum and barley grits using bacterial □-amylase, neutral protease and fungal □-amylase at 50$^0$C, 95$^0$C and 60$^0$C for each and proved that the mashing program was suitable for starch gelatinization in unmalted sorghum.

Pozo-Insfran *et al.* (2004) reported that addition of amyloglucosidase in the wort increased the initial content of fermentable carbohydrates by approximately 20 per cent and also significantly increased residual glucose and alcohol as compared with untreated worts. Extract remained fairly constant during the brewing period due to the usage of two external enzymes (Termamyl and Fungamyl) at a concentration of 0.1% w/w of malt for starch hydrolysis during mashing (Etokakpan, 2004).

Millet (*Pennisetum maiwa*) was malted for 5 days and mashed using the infusion, double-decoction and decantation mashing methods. Highest extract recovery was obtained in the decantation mashing system because in this mashing procedure, the enzymes of millet malt were protected and the starch adequately gelatinized. The decantation mashing method produced wort with lower values of soluble nitrogen and free amino nitrogen (FAN) products than the infusion mashing method because the proteins were partly denatured during the cooking process of the decantation mashing methods. The decantation mashing produced wort that filtered more slowly. The wort also had a darker colour because of a greater degree of Maillard reaction. Wet milling marginally produced extracts with higher values of the parameters tested than dry milling, but both the wet and dry milling procedures maintained a constant mass balance of the soluble nitrogen and FAN products (Eneje *et al*., 2001).

Dziedzoave *et al.* (2010) analyzed the activity levels of amylolytic enzymes and □-glucanase in malts prepared from four tropical cereal grains and assessed to establish the relative usefulness of these malts for production of glucose syrups. Rice malt showed the highest activity for the amylolytic enzymes, whilst millet and sorghum malts were richest in □-glucanase activity. Optimum amylolytic enzyme development in rice malts occurred between 9-13 days; and 11 days for optimum □-glucanase development in millet malt. □-Amylase was the predominant enzyme in all the cereal malts except maize, for which the predominant enzyme was a-amylase.

## 2.5 Effect of commercial enzymes

On quality of wort developed from replacement of malted barley (100%) with sorghum as adjunct 50, 60, 70 and 80 per cent was investigated for pH, colour, filtration rate, extract asin, dry extract, viscosity, total soluble nitrogen and free amino nitrogen. The wort pH (5.6 - 6.0) is relatively

stable, the colour is lighter with increment in proportion of the sorghum as it appears as colour diluents. The total soluble nitrogen and free amino nitrogen value increased with the use of commercial enzymes while the viscosity decreases with enzymes. The use of high percentage sorghum as adjunct with commercial enzymes is found to be useful in production of high quality wort with low cost and profitability.

Aguirre *et al.* (1987) used a-amylase treatment in processing of pre-cooked rice and maize flour in a double drum drier. The enzyme pre-treatment comprised of addition of □-amylase at 0.05, 0.5 and 1% of flour weight with a holding at 60-80$^0$C resulting in 50 per cent reduction in starch at 1% concentration.

Effect of temperature on the enzymatic hydrolysis of steam pretreated willow was investigated in the range of 40$^0$C to 60$^0$C. The temperature affected both the initial hydrolysis rate and the final glucose yield and highest glucose yield was obtained at 60$^0$C (Eklund *et al.*, 1990).

Two sorghum varieties were studied with a view to produce wort and evaporated wort. When mashed using commercial brewing enzymes, the sorghum samples produced sufficient sugars and amino acids required for yeast growth and alcohol production during fermentation. Fermentation studies showed that, the normal brewing wort of sorghum produced marginally higher levels of alcohol than the evaporated wort (extract) of sorghum. Also, the beer brewed from the normal wort of sorghum was lighter in colour than that brewed from the re-dissolved extract (evaporated wort) of sorghum. The lower values of alcohol or higher colour of the beer brewed from sorghum extract was linked to the Maillard reaction, which occurred during the process of evaporating the wort to produce the extract. Organoleptic assessment confirmed that the beer brewed using the extract was generally acceptable (Odibo *et al.*, 2002).

## 2.6 Fermentation

Fermentation is one of the oldest forms of food preservation technologies in the world. It can be described as desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes. It is the important step, by which yeast converts the sugars in the wort into ethyl alcohol. The natural flora of the malt carries out the fermentation at ambient temperature and the drink is consumed after standing for about 24 h. Indigenous fermented foods such as bread, cheese and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities (Yoshikawa and Okada, 2003). Fermentation is carried out to enhance taste, aroma, shelf life, texture and nutritional value. In Western breweries, the fermentation process is started by selected yeast strains (*S. cerevisae* or *S. carlsbergensis*) and the fermentation time ranges between 8-15 days at 10-16$^0$C (Waites *et al.*, 2001).

### 2.6.1 Fermentation parameters

Fermentation in the case of African traditional sorghum beers, sorghum wort is inoculated with a traditional leaven and fermentation time varies between 10 and 24 h in ambient temperature. African traditional leaven is a result of the spontaneous fermentation of sorghum malt wort (Kayodé, 2005; Lyumugabe *et al.*, 2010). The manufacturing methods of this leaven are diverse in Africa and depend on built-in ingredients.

African sorghum beers are typical examples of lactic fermentation followed by alcoholic fermentation in which initially, lactic acid bacteria (LAB) and later yeasts, play the dominant role (Kayodé *et al.*, 2005; Maoura and Pourquie, 2009). Due to their higher growth rate, bacteria typically dominate the early stages of fermentation.

## 2.7 Evaluation of different yeast strains on quality of beer

The most well-known and commercially significant yeasts are the species and strains related to *Saccharomyces cerevisiae.* These organisms have long been utilized to ferment the sugars of rice, wheat, barley and corn to produce alcoholic beverages. *Saccharomyces bayanus*, which is one of the yeast belongs to the genus of *Saccharomyces* and is used in wine and cider fermentation. It is closely related to *Saccharomyces cerevisiae* but because it has different genetic and metabolic characteristics, it is believed to have a hybrid origin. *Brettanomyces bruxellensis* (former *Dekkera bruxellensis*) plays a key role in the spontaneous fermentation of typical Belgian beer styles such as Lambic, Flanders red ales, Gueuze and Kriek. It competes with brewer's yeast, as well as other microorganisms, during the wort fermenting and gives the beer a distinctive taste.

Farid *et al.* (2002) obtained the alcohol production from starch by mixed cultures of *Aspergillus awamori* and immobilized *Saccharomyces cerevisiae* at different agitation speeds. Satyanarayana *et al.* (2004) reported that glucoamylase was optimally active at pH 7.0 and alpha amylase was optimum at pH 7.0 and saccarified starch efficiently. Sharma *et al.* (2002) used *Saccharomyces cerevisiae* strain SJ-31 which was potential for ethanol production from starch substrate because of its ability to produce amylase and glycoamylases. The ethanol produced was 3.4 per cent with fermentation efficiency of 91 per cent. Kayode *et al*., 2005 used *S. cerevisiae*, *Torulaspora delbruccki, S. pastorianus, L. divergens, L. fermentum, Lactobacillus* sp. for beer production.

Very high gravity (VHG) media with finger millet (*ragi*) flour were prepared and fermented with ethanol tolerant yeast, *Saccharomyces bayanus*. Maximum ethanol concentration of 15.6 per cent (v/v) was obtained in the nutrients supplemented with peptone and yeast extract. Supplementation with peptone and yeast extract showed 86.6 per cent fermentation efficiency, increase in the ethanol productivity and yeast viable cell concentration. Separate hydrolysis of the finger millet mash followed by fermentation was found to be better for ethanol production than simultaneous saccharification and fermentation (Reddy and Reddy, 2006).

Several investigators have observed that yeast extract, ammonium, calcium and magnesium have protective effect on growth, fermentation and on viability, which stimulate the fermentation rate and ethanol production. High ethanol concentrations are obtained by using yeast in 20 days fermentation. Increased ethanol production with supplementation of horse gram flour in VHG fermentation has been reported from our laboratory (Reddy and Reddy, 2006).

The effect of different inoculum sizes of 2, 4, 6, 8 and 10 per cent was studied on the ethanol production obtained from juice of sweet sorghum and a high alcohol concentration of 12.45 and 12.23 per cent at 6 and 2 per cent respectively and reported that 2 percent inoculum was sufficient for alcohol production (Nimbkar *et al*., 1989).

In order to investigate the influence of temperature on pH, alcohol production, carbohydrate profile and aroma compounds, a variety of top-fermenting yeast strains were used to ferment wort obtained from proso millet malt. *Saccharomyces bayanus*, *Saccharomyces cerevisiae* and *Brettanomyces bruxellensis* were used in two isothermal 12 and 18 $^{0}$C fermentation trials. During the fermentation, changes in pH, extract conversion and the monosaccharide profile were monitored. After 2 weeks of storage, the levels of higher alcohols and esters were measured. At higher temperatures *Brettanomyces* showed more rapid rates of sugar conversion. The values for aliphatic alcohols obtained during experimentation, ranged from 23 to 267 g per L (Zarnkow *et al*., 2010).

### 2.7.1 Tannin content

Mikyska *et al.* (2002) reported that malt polyphenols particularly polyphenols improved reducing activity values and carbonyl content in fresh and stored beers and that it had a positive impact on flavour stability.

### 2.7.2 Alcohol content

Haifeng Zhaoa *et al.* (2010) analyzed the 34 commercial beers obtained from malt, rice, hop, starch and corn of different brands of lager type for original gravity and it was ranged from 8-11 ºP and alcohol content was 3.1-8.5 per cent.

Very high gravity (VHG) media with finger millet (*ragi*) flour were prepared and fermented with ethanol tolerant yeast, *Saccharomyces bayanus*. Maximum ethanol concentration of 15.6 per cent (v/v) and 86.6 per cent fermentation efficiency was obtained in the nutrients supplemented medium. The supplementation of peptone and yeast extract led to increase in the ethanol productivity and yeast viable cell concentration. Separate hydrolysis of the finger millet mash followed by fermentation was found to be better for ethanol production than simultaneous saccharification and fermentation (Puligundla *et al.,* 2010).

Beers prepared from millet and barley malts and their chemical, physical and sensory characteristics were compared. Total reducing sugar (as maltose), dextrin and total acidity (as lactic acid) were 0.51, 1.69 and 0.24; and 0.82, 0.89 and 0.23 per cent (m/v) in millet and barley beers respectively. Millet and barley beers had similar alcohol contents (5.38 ± 0.26%, v/v). Taste, smell and flavor of millet and barley beers were similar.

# MATERIAL AND METHODS

The present study was conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad during 2011-13. The aim of the study was to hydrolyze the starch from minor millet grains to obtain maximum sugars and further conversion to beer which is a healthy fermentative food product.

The details of the materials used and methods employed during the course of investigation are presented herein.

## 3.1 Source of yeast cultures

| Sl. No. | Name of the culture | Source |
|---|---|---|
| 1 | *Saccharomyces cerevisiae* NCIM 3455 | NCIM, Pune |
| 2 | *Saccharomyces cerevisiae* NCIM 3551 | NCIM, Pune |
| 3 | *Saccharomyces cerevisiae* NCIM 3570 | NCIM, Pune |
| 4 | *Saccharomyces cerevisiae* NCIM 3580 | NCIM, Pune |
| 5 | *Saccharomyces cerevisiae* NCIM 3391 | NCIM, Pune |

### 3.1.1 Maintenance of cultures

Yeast cultures were maintained on yeast extract peptone dextrose agar (Y-E-P-D-A) (Appendix I). Overnight grown fresh yeast cultures grown in Y-E-P-D broth were used as inoculum.

## 3.2 Varieties of millet selected for the study

| Sl. No. | Name of the minor millet | Name of the variety | Source |
|---|---|---|---|
| 1 | Bajara (*Pennisetum glaucum*) | ACTP 8203 | National Seed Project (Crops) UAS, Dharwad |
| 2 | Finger millet (*Eleucine coracana)* | GPU-28 | National Seed Project (Crops) UAS, Dharwad |
| 3 | Foxtail millets (*Setaria italica*) | HMT-100-1 | National Seed Project (Crops) UAS, Dharwad |
| 4 | Little millet (*Panicum miliare)* | SUKSHEMA | National Seed Project (Crops) UAS, Dharwad |

### 3.2.1 Composition of millets grains as compared to Barley

| Name of grains | Moisture (%) | Carbo hydrate (g) | Protein (g) | Germi – nation (%) | Physical purity |
|---|---|---|---|---|---|
| Bajra | 12 | 69 | 7.3 | 75 | 98 |
| Finger millet | 13 | 75 | 11.7 | 75 | 97 |
| Foxtail millet | 12 | 71 | 9.7 | 75 | 98 |
| Little millet | 10 | 73 | 10.8 | 75 | 98 |
| Barley | 14 | 78 | 9.9 | 75 | 98 |

Source: wikipedia.org, millet Network of India, http://www.milletindia.org.

## 3.3 Preparation of the substrate

The grain varieties of minor millets were soaked separately in distilled water at different soaking periods and germinated subsequently for 2 and 3 days. Adjunct germinated grains were

kilned at $50^0$C and ground into grits. Mashing was performed using commercial □-amylase for hydrolysis of fermentable sugars.

### 3.3.1 Parameters for malting

#### 3.3.1.1 Optimization of steeping period

Grains were steeped in distilled water for 8 h, 12 h and 16 h separately. The optimum steeping period was determined in terms of amylase activity and reducing sugars produced.

#### 3.3.1.2 Optimization of germination period

The seeds steeped for different intervals were placed in the muslin cloth and tied with thread for optimization of germination period. The seeds were incubated for three days and maximum germination was determined in terms of amylase activity and reducing sugars at $2^{nd}$ and $3^{rd}$ day of germination.

## 3.4 Mashing parameters

Mashing is the process of combining a mix of milled grain and water, known as "liquor" and heating this mixture. Mashing allows the enzymes in the malt to break down the starch in the grain into sugars, typically maltose to create a malty liquid called wort. The mashing parameters such as enzyme concentration, temperature and incubation period were optimized to obtain maximum reducing sugars as per the protocol mentioned below.

### 3.4.1 Optimization of enzyme concentration

Commercial □-amylase Palkozyme (courtesy: M/s. Maps-India Ltd., Ahmedabad) was added at concentrations of 0.1, 0.5 and 1.0 per cent to the mashed 5 g of substrate for saccharification for 24 h. The optimum concentration was estimated in terms of release of maximum reducing sugars.

### 3.4.2 Optimization of temperature

The mashed substrate was kept for incubation at different temperature periods $30^0$C, $50^0$C and $70^0$C for 24 h release of reducing sugars. The optimum temperature was determined based on the release of maximum reducing sugars.

### 3.4.3 Optimization of incubation period for hydrolysis

Different incubation periods of 8, 16 and 24 hours were used during the mashing process. Optimal incubation period was identified based on the maximum reducing sugars released. The enzyme concentration of 1% and temperature of $70^0$C for 24 h of incubation was found most effective for saccharification.

### 3.4.4 Fermentation

After mashing, the wort that has obtained was boiled for 1 hour. After 30 minutes of boiling, hops (acid extracted, courtesy: M/s. U.B. Breweries, Mangalore) was added @ 40 ppm. Sugar was added as an adjunct to make up the brix to 20°. The wort was cooled, filtered and pH was adjusted with 4.5 – 5.0. The wort was then pitched with different yeast strains. The log phase of yeast inoculum was used and the wort was kept for aerobic fermentation for 24 h. Anaerobic fermentation was created after 24 h by sealing with rubber cork, making provision for trapping carbon dioxide. The flasks were incubated for 5 days till the carbon dioxide ceased to evolve.

## 3.5 Screening and selection of different yeast cultures for beer production

The above standardized parameters for soaking time, germination period and enzyme level yielding maximum fermentable sugars were adopted for further screening of yeast cultures. The hydrolysate/wort obtained was inoculated with five different yeast strains *viz., S. cerevisiae* NCIM 3455, *S. cerevisiae* NCIM 3551, *S. cerevisiae* NCIM 3570, *S. cerevisiae* NCIM 3580, *S. cerevisiae* NCIM 3391.

### 3.5.1 Standardization of inoculum size for beer production

The effect of different levels of inoculum on beer production was studied. Wort was pitched with inoculum size of 1.0, 1.5 and 2.0 per cent of overnight grown cultures separately and kept for

fermentation for 5 days at 32$^0$C. At the end of fermentation, beer samples were analyzed for pH, tannin content, alcohol content and residual reducing sugars.

## 3.6 Scale up production of beer adopting all the standardized parameters

The optimum parameters obtained from the previous experiments were adopted for the bench scale production wherein 3L fermenter bottles holding 1.5L unfermented wort was used. The pH, tannin content, alcohol content and residual reducing sugars were estimated and compared with commercial beer analytically. The beer was filtered and bentonite powder was added @ 200 ppm and allowed for settling for 5 days at 4$^0$C. The clear supernatant was decanted, stored in bottles and pasteurized at 65$^0$C for 30 minutes. The bottles were carbonated with carbon dioxide in a commercial juice bottling center and stored at 4$^0$C. This sample was further evaluated for organoleptic quality by a panel of judges.

## 3.7 Organoleptic evaluation

Sixteen point scale (Amerine and Ough, 1980) was selected which was based mainly on the physical characters and taste. The prepared sample and a commercial beer sample (standard) were used for organoleptic evaluation. The beer samples were evaluated by 8 test panel judges.

### 3.7.1 Sample presentation for judges evaluation

Both the samples were coded to keep the identity of the beer secret to the tasters and wafers were placed along to clean the mouth after the judgement of the first sample tasted. The judges were instructed to evaluate the samples by blind tasting as per scorecard.

### 3.7.2 Sensory evaluation

The beer samples were evaluated by a panel of 8 judges, who were acquainted with beer either occasionally or frequently. The numerical scoring method and the scorecard followed is given below.

Scorecard

Name of the judge date:

| | |
|---|---|
| Name of code of the sample | : |
| Appearance | : 2 |
| Colour | : 2 |
| Aroma | : 2 |
| Total acidity | : 2 |
| Body | : 2 |
| Flavour | : 2 |
| Astringency | : 2 |
| General quality | : 2 |
| Total score | : 16 |

## 3.8 Analytical procedures

The details of the reagent preparation and media composition are provided in Appendix I.

Amylase activity (□-amylase)

Amylase production was estimated by method described by Bernfield (1955).

Reagents

1. Sodium acetate buffer (0.1M) of pH 4.6
2. Starch solution (1%)
3. Di-nitro salicylic acid reagent

4. Rochelle salt solution 40%
5. Stock solution of maltose

### 3.8.1 Procedure

One g of sample material was extracted with 5-10 volumes of ice-cold 10 mM $CaCl_2$ solution overnight at $4^0$C and centrifuged further at 20,000 rpm for 20 min. Supernatant was used as enzyme source for estimation of amylase activity. One ml of starch solution and 1 ml of diluted extracted enzyme were mixed in test tubes and incubated at $27^0$C for 15 min. The reaction was stopped at the end of 15 minutes, by addition of 2 ml of dinitrosalicylic acid. The solution was then heated in boiling water bath for ten minutes. While the tubes were warm, 1 ml of Rochelle's salt solution was added and cooled under running tap water. The total volume was then made upto 10 ml with distilled water. Absorbance was read at 560 nm. The standard curve of maltose was plotted on graph.

### 3.8.2 Estimation of reducing sugars

The reducing sugars were estimated by following 3, 5, Dinitrosalicylic acid method (Miller, 1959).

Reagents

1. Di-nitro salicylic acid reagent
2. Rochelle salt solution 40 per cent
3. Stock solution of glucose.

3.8.2.1 Procedure

The sample of 100 mg was weighed and the sugars were extracted with hot 80 per cent ethanol twice (5 ml each time). Supernatant was collected and evaporated by keeping on a water bath at $80^0$C. Ten ml of distilled water was added and the sugars were dissolved. The extract was then pipetted out in separate test tube from 0.5 to 3 ml and the volume was equalized to 3 ml with distilled water in all the tubes. 3 ml of DNSA reagent was then added and the tubes were heated in a boiling water bath for 5 min. When the contents of the tubes were still warm, 1 ml of 40 per cent Rochelle salt solution was added. Tubes were then cooled and optical density was read at 510 nm using systronics UV visible spectrophotometer –117. The standard curve of glucose was plotted on graph.

### 3.8.3 pH

The pH of beer samples were recorded by using the pH meter of Analog model (Corion Research, USA). Standard solutions of pH 4.0 and 9.0 were used as reference.

### 3.8.4 Total soluble solids (°Brix)

Total soluble solids (TSS) of beer samples was determined with the help of ERMA hand refractometer having range of 0-32°Brix at $28^0$C.

### 3.8.5 Estimation of tannins

Tannins were estimated by Folin-Denis method (Schanderl, 1970).

3.8.5.1 Reagents

1. Folin-Denis reagent
2. Sodium carbonate solution
3. Standard tannic acid solution (mg $ml^{-1}$)
4. Working standard solution.

3.8.5.2 Procedure

A sample of 0.5 ml was transferred to a conical flask containing 75 ml of distilled water and boiled for 30 min. It was then centrifuged at 2,000 rpm for 20 min and the supernatant was collected in a 100 ml volumetric flask and the volume was made up. One ml of this sample extract was transferred to a 100 ml volumetric flask containing 75 ml of distilled water. A blank containing one ml distilled water was also maintained. To this, five ml of Folin- Denis reagent and ten ml of sodium

carbonate solution was added and diluted to 100 ml with distilled water. The absorbance was read at 700 nm after 30 min. The standard curve of tannin was plotted on graph.

### 3.8.6 Estimation of ethanol

The ethanol was estimated by colorimetric method as described by Caputi *et al.* (1968).

#### 3.8.6.1 Reagents

1. Potassium dichromate solution 0.23N
2. Preparation of stock solution of standard ethanol.

#### 3.8.6.2 Procedure

One ml of representative samples from each treatment was transferred to 250 ml round bottom distillation flask connected to the condenser and was diluted with 30 ml distilled water. The sample was distilled at 74-75$^0$C. The distillate was collected in 25 ml of 0.23 N $K_2Cr_2O_7$ reagent, which was kept at receiving end. The distillate containing alcohol was collected till total volume of 45 ml was obtained. Similarly, standard ethanol (20-100 mg ethanol) were mixed with 2 ml of $K_2Cr_2O_7$ separately. The distillate of samples and standards were heated in water bath at 60$^0$C for 20 minutes and cooled. The volume was made upto 50 ml with distilled water and the optical density was measured at 600 nm in a Spectrophotometer (Systronics Uv-Vis–117). The standard curve was plotted considering the concentration against absorbance.

### 3.8.7 Colour

The colour and brightness of the beer samples were measured with the help of Spectrophotometter (Onkarayya, 1985).

Colour of the beer samples were measured at 420 nm and 520 nm after diluting the samples to 1:1 with water. Similarly, commercial beer was also tested for comparison.

Colour = Absorbance was read at 420 nm.

Brightness = Sum of absorbance at 420 nm and 520 nm.

## 3.9 Statistical Analysis

The statistical analyzes of the data will be carried out by employing completely randomized design (CRD). The critical differences will be calculated at P = 0.01 and interpretations of the results were evaluated based on the significance of F tests (Panse and Sukhatme, 1985).

# EXPERIMENTAL RESULTS

The experimental results obtained by screening the different minor millets grains such as finger millet, foxtail millet, little millet and bajra for their suitability for beer production, standardizing the malting and mashing conditions and screening of yeast strains for beer production are being presented in this chapter. The results of organoleptic evaluation of the beer produced using all optimized parameters are also presented in this chapter.

## 4.1 Screening of the minor millets grains for beer production

The different millet grains were screened for their efficiency based on reducing sugars and amylase activity of the malt and maximum hydrolysis during mashing with commercial enzyme.

### 4.1.1 Effect of steeping period (soaking) and germination period on the reducing sugar content

The grains were soaked in distilled water for a soaking period of 8 h, 12 h and 16 h and germinated for 2 and 3 days. The results are presented in Table 1.

Among the millet grains, the finger millet recorded significantly higher reducing sugar (18.19 mg $g^{-1}$) followed by little millet (16.45 mg $g^{-1}$) at 16 h of soaking on the second day of germination, while bajra recorded the lowest reducing sugars (13.15 mg $g^{-1}$ of sample). Similar trend was also noticed on the third day of germination.

### 4.1.2 Effect of steeping period (soaking) and germination period on the amylase activity

The grains were soaked in distilled water for a soaking period of 8 h, 12 h and 16 h and germinated for 2 and 3 days. The results are presented in Table 2.

Among the millets, significantly higher amylase activity (21.33 mg of protein 15 $min^{-1}$ $g^{-1}$) was recorded in finger millet followed by little millet (20.14 mg of protein 15 $min^{-1}$ $g^{-1}$) at 16 h of soaking on the second day of germination while, bajra recorded the lowest amylase activity (13.05 mg of protein 15 $min^{-1}$ $g^{-1}$ sample). Similar trend was noticed on third day of germination.

## 4.2 Optimization of parameters for efficient hydrolysis of millet grains

Effect of commercial □-amylase (Palkozyme), incubation period and temperature on hydrolysis of millet grains during mashing was studied. Various parameters pertaining to hydrolysis of starch such as enzyme concentration, the incubation period and temperature were optimized and the results obtained were presented in Table 3a, 3b and 3c.

### 4.2.1 Optimization of enzyme concentration (□-amylase)

The results of the study clearly indicated that, the substrate subjected to □-amylase at three different concentrations (0.1, 0.5 and 1.0 per cent) had a significant influence on the release of reducing sugars from lower to higher concentrations. All the pretreated substrates showed significantly higher reducing sugars. Among the four millet grains, the finger millet grains treated with 1% of the commercial □-amylase recorded the highest reducing sugars (66.67 mg $g^{-1}$) which was significantly higher than finger millet treated with 0.5% (58.18 mg $g^{-1}$) and 0.1% (56.25 mg $g^{-1}$) of commercial □-amylase (Table 3a). Since 1 per cent amylase enzyme yielded maximum sugars, it was used in further experiment.

### 4.2.2 Optimization of incubation temperature

Significant increase was seen in the reducing sugars of the substrate incubated with commercial □-amylase at different temperature of $30^0$C, $50^0$C and $70^0$C (Table 3b). By enzyme hydrolysis at 1 per cent commercial enzyme, the highest reducing sugars was achieved in finger millet grains at $70^0$C (64.85 mg $g^{-1}$ of sample) followed by little millet (59.87 mg $g^{-1}$ of sample), While bajra and foxtail millet showed the lowest reducing sugars (49.46 mg $g^{-1}$ of sample) and (56.20 mg $g^{-1}$ of sample) which did not show any significant difference with reducing sugars obtained at $50^0$C and were on par with each other. Enzyme treatment at incubation of $30^0$C, recorded significantly lower reducing sugars in all millet grains. Since 1 per cent commercial enzyme @ $70^0$C yielded maximum reducing sugars, it was used for optimizing incubation period.


**Plate 1. Germinated Minor millets used for beer production**

**Table 1: Effect of soaking period and germination on yield of reducing sugars**

| Millets | Reducing sugar (mg $g^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | At germination period 2 days | | | At germination period 3 days | | |
| | Soaking period (h) | | | Soaking period (h) | | |
| | 8 | 12 | 16 | 8 | 12 | 16 |
| Bajra | 9.21 | 10.60 | 13.15 | 11.47 | 14.11 | 15.18 |
| Finger millet | 14.30 | 17.10 | 18.19 | 16.30 | 19.87 | 25.30 |
| Foxtail millet | 11.35 | 13.95 | 14.33 | 13.09 | 15.85 | 20.19 |
| Little millet | 13.70 | 15.60 | 16.45 | 14.90 | 16.07 | 23.16 |
| SEm± | 0.145 | 0.177 | 0.190 | 0.142 | 0.11 | 0.137 |
| CD (0.01) | 0.425 | 0.519 | 0.571 | 0.410 | 0.35 | 0.401 |

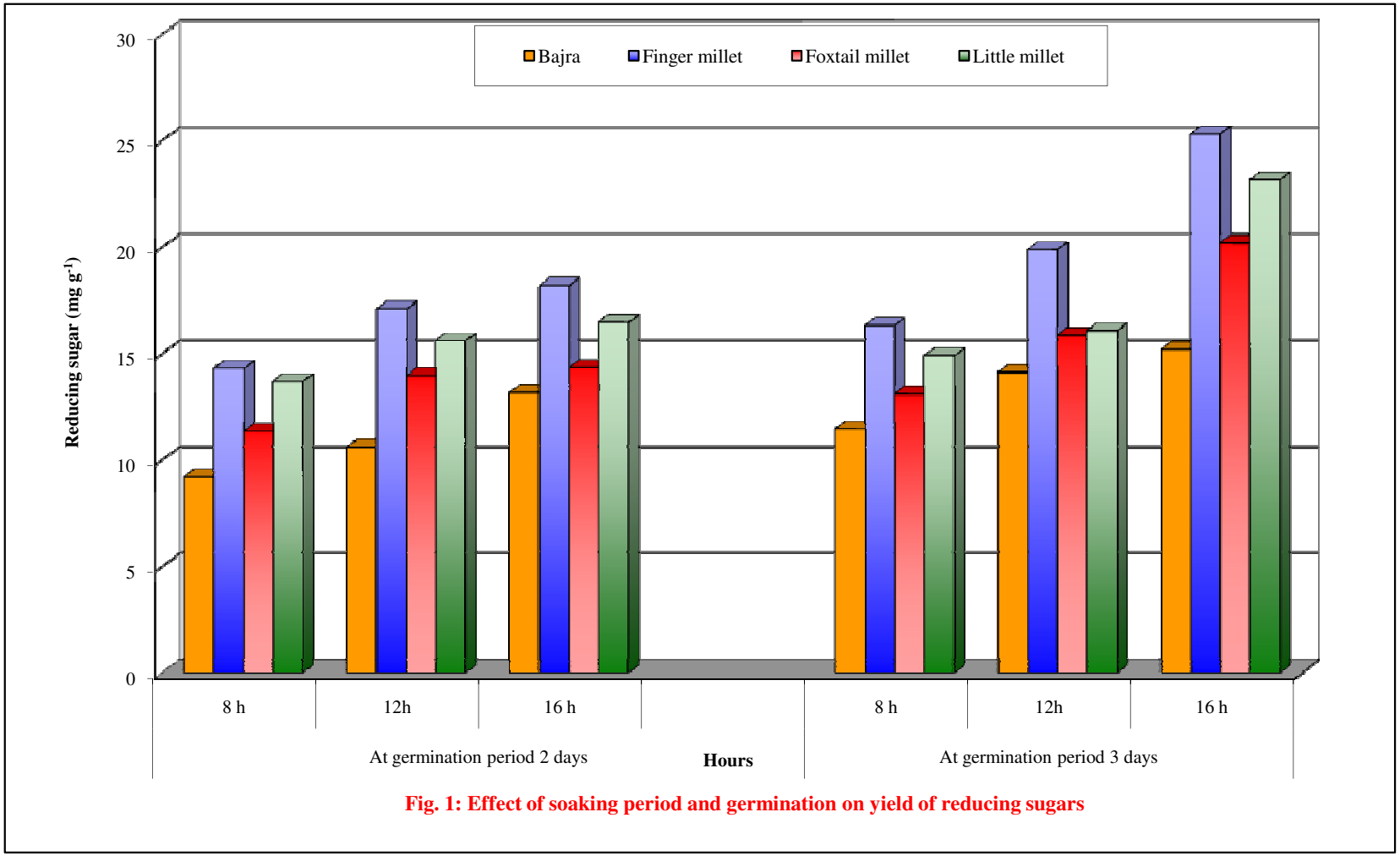


**Fig. 1: Effect of soaking period and germination on yield of reducing sugars**

**Table 2: Amylase activity at different periods of soaking and germination**

| Millets | Amylasae activity (mg of protein $g^{-1}$ of sample) | | | | | |
|---|---|---|---|---|---|---|
| | At germination period 2 days | | | At germination period 3 days | | |
| | Soaking period (h) | | | Soaking period (h) | | |
| | 8 | 12 | 16 | 8 | 12 | 16 |
| Bajra | 12.40 | 12.77 | 13.05 | 12.90 | 13.56 | 13.62 |
| Finger millet | 15.27 | 20.91 | 21.33 | 16.47 | 23.20 | 23.50 |
| Foxtail millet | 13.71 | 19.27 | 19.39 | 14.06 | 20.14 | 20.03 |
| Little millet | 14.20 | 19.78 | 20.14 | 14.96 | 19.83 | 20.10 |
| SEm$\pm$ | 0.0568 | 0.037 | 0.048 | 0.056 | 0.060 | 0.048 |
| CD (0.01) | 0.172 | 0.131 | 0.194 | 0.175 | 0.190 | 0.152 |

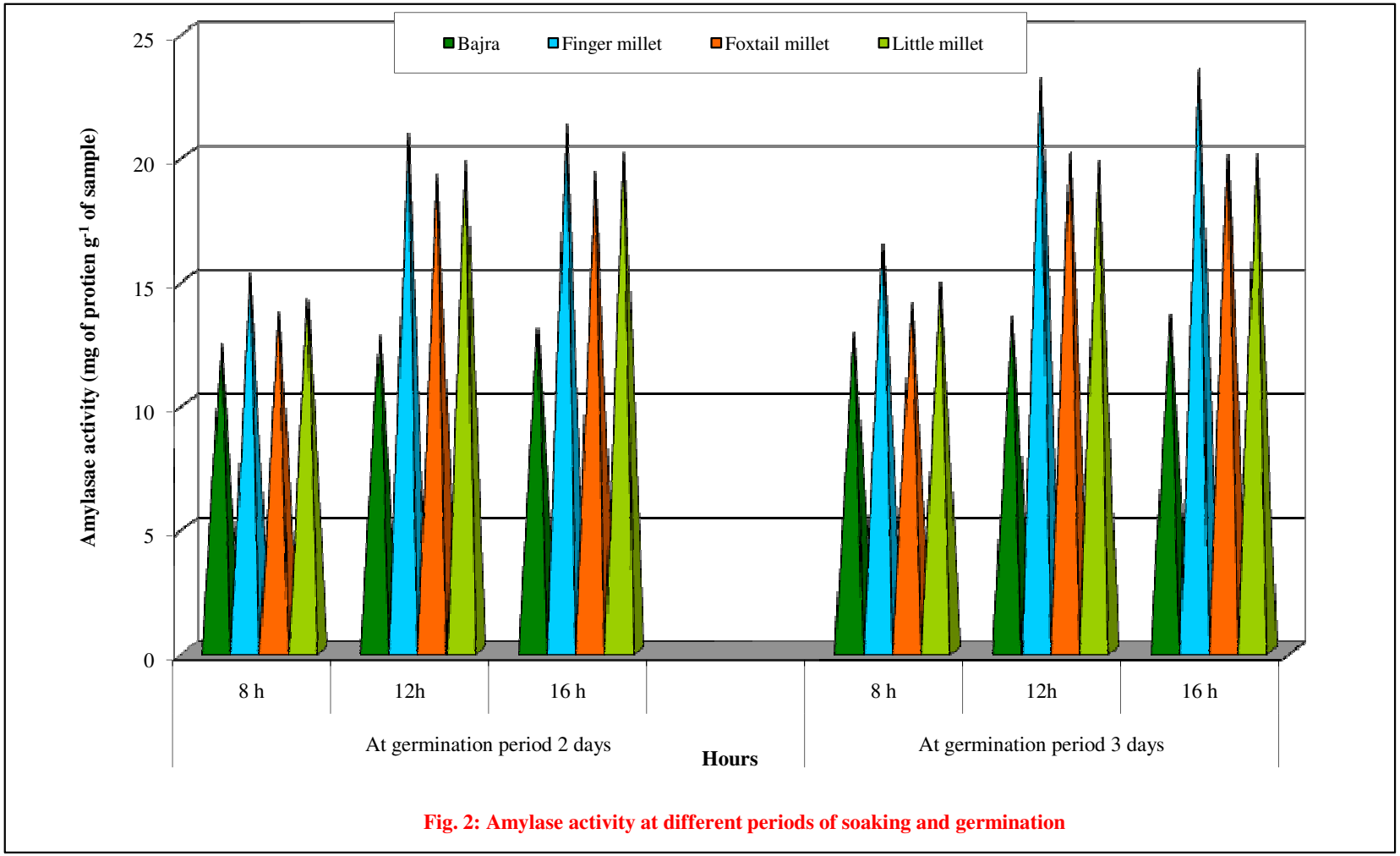


**Fig. 2: Amylase activity at different periods of soaking and germination**

**Table 3a: Optimization of enzyme concentration for saccharification by commercial α-amylase**

| Sl. No. | Millets | Reducing sugar (mg $g^{-1}$) | | |
|---|---|---|---|---|
| | | Concentration of α- amylase (%) | | |
| | | **0.1** | **0.5** | **1.0** |
| 1. | Bajra | 42.32 | 54.16 | 57.45 |
| 2. | Finger millet | 56.25 | 58.18 | 66.67 |
| 3. | Foxtail millet | 46.20 | 57.12 | 62.85 |
| 4. | Little millet | 49.10 | 60.34 | 61.80 |
| | SEm± | 0.16 | 0.15 | 0.23 |
| | CD (0.01) | 0.47 | 0.44 | 0.69 |

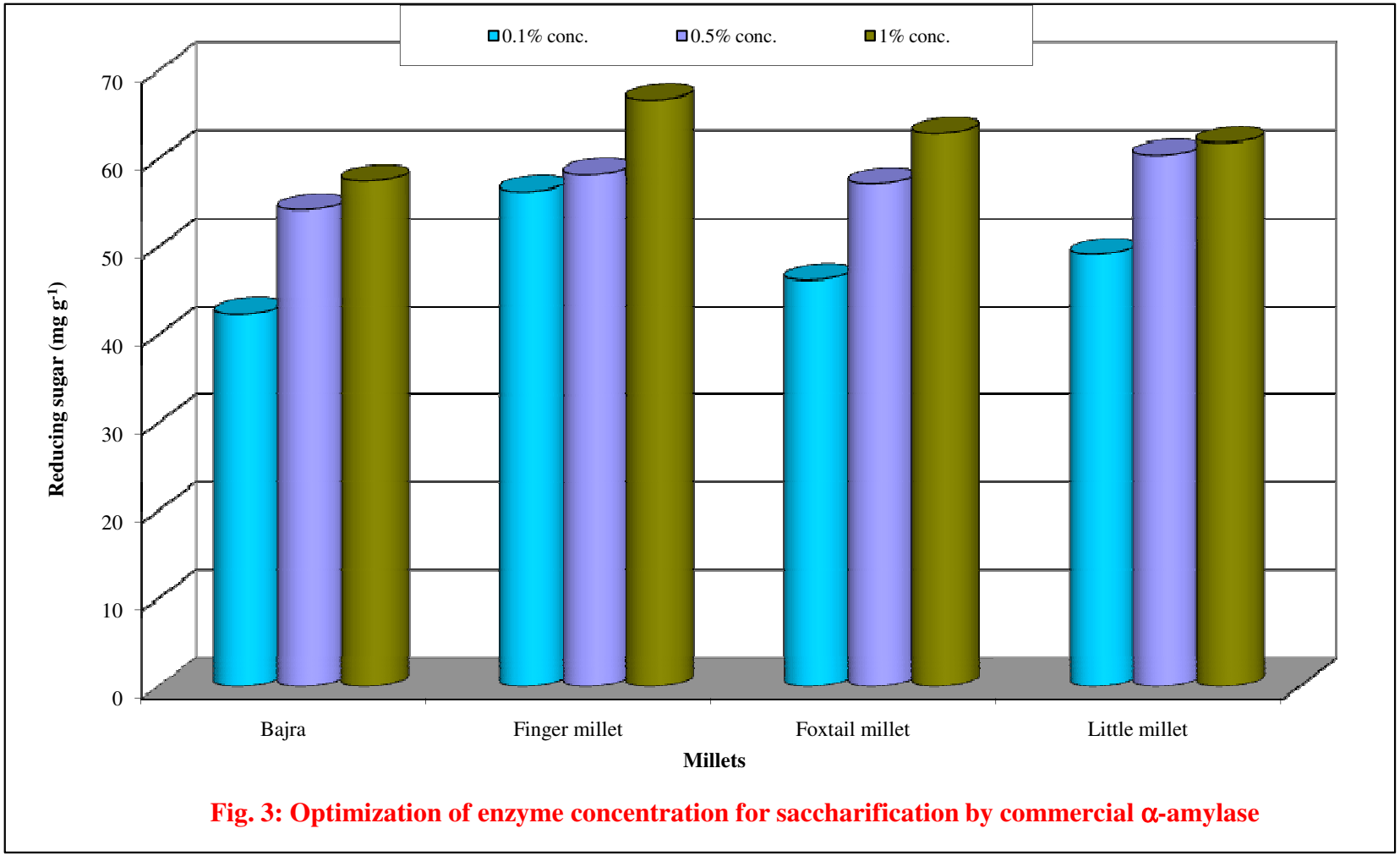


**Fig. 3: Optimization of enzyme concentration for saccharification by commercial α-amylase**

**Table 3b: Performance of commercial α-amylase (1%) on yield of reducing sugars in millets of different incubation periods**

| Sl. No. | Millets | Reducing sugar (mg $g^{-1}$) | | |
|---|---|---|---|---|
| | | Incubation temperature ($^{O}C$) | | |
| | | 30 | 50 | 70 |
| 1. | Bajra | 46.76 | 49.06 | 49.46 |
| 2. | Finger millet | 55.10 | 64.78 | 64.85 |
| 3. | Foxtail millet | 49.12 | 56.14 | 56.20 |
| 4. | Little millet | 53.63 | 59.66 | 59.87 |
| | SEm$\pm$ | 0.15 | 0.13 | 0.14 |
| | CD (0.01) | 0.46 | 0.39 | 0.42 |

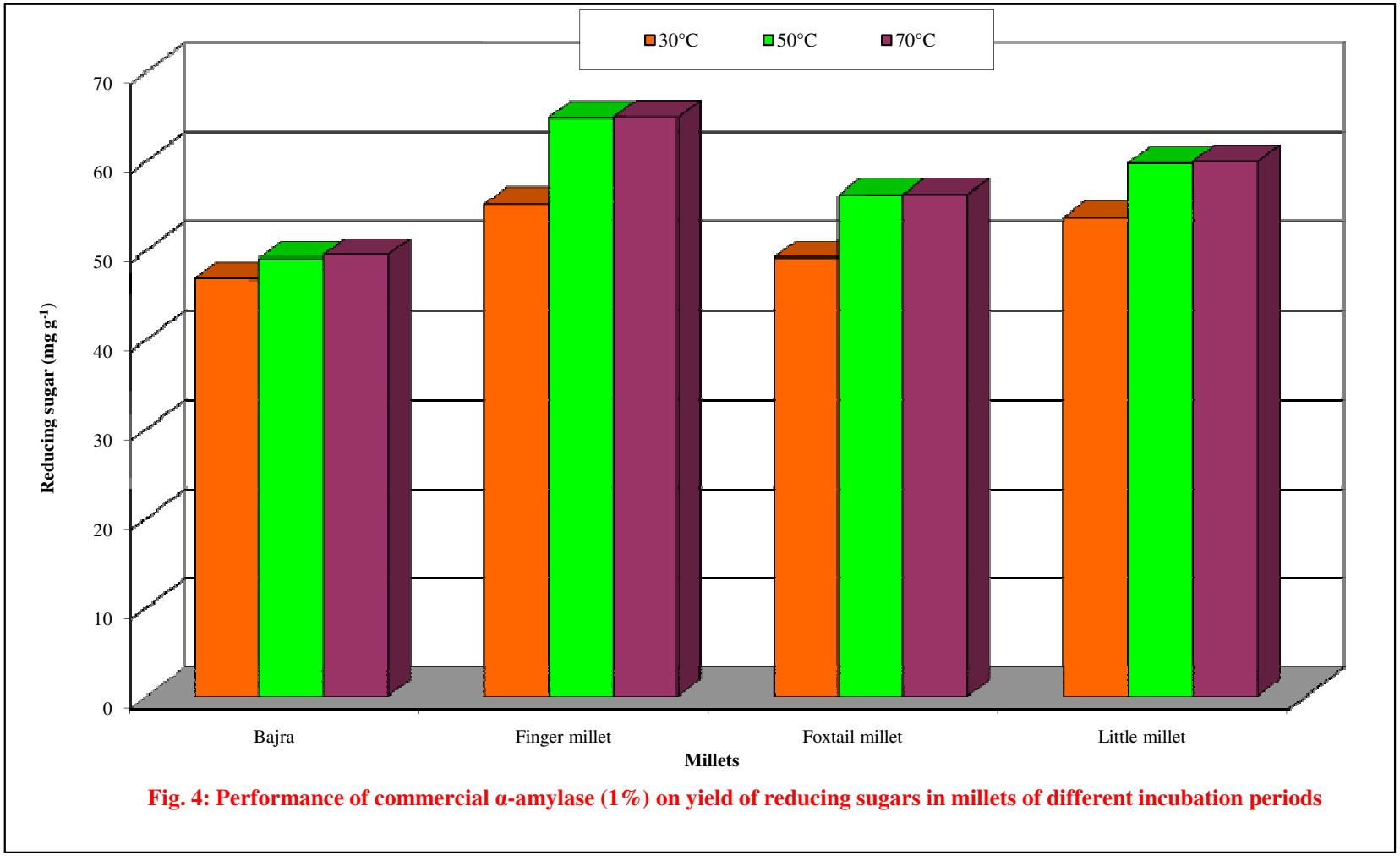


**Fig. 4: Performance of commercial α-amylase (1%) on yield of reducing sugars in millets of different incubation periods**

### 4.2.3 Optimization of incubation period

Different incubation periods were used to test the optimum incubation period for maximum hydrolysis of the substrate (Table 3c). From the results, it was observed that maximum saccharification took place at an incubation period of 24 hours in finger millet (74.19 mg $g^{-1}$ of sample) followed by little millet (69.23 mg $g^{-1}$ of sample) which was found to be significantly higher than all other treatments. The lowest reducing sugar was observed at 8 h of incubation in bajra (41.61 mg $g^{-1}$ of sample).

## 4.3 Effect of commercial □-amylase on hydrolysis of minor millets using standardized parameters

All the optimized parameters for the hydrolysis of millet grains using commercial □-amylase were used for saccharification of the millets grains to obtain maximum sugars (Table 4).

From the results, it is clear that commercial enzyme had an effect on the release of sugars between the millet grains. The highest reducing sugars were obtained in the Finger millet (63.21 mg $g^{-1}$) which was higher than the other millets. While the Little millet showed high reducing sugars (53.10 mg $g^{-1}$) next only to Foxtail millet, while the least was recorded in Bajra (49.02 mg $g^{-1}$). Since, finger millet showed maximum sugars, it was selected for pilot scale studies.

### 4.3.1 Chemical analysis of wort prepared from finger millet.

Finger millet was found to be the best among the millets tested as a brewing material for beer production. The chemical analysis of the wort prepared from this grain was done. The results are presented in Table 5.

### 4.3.2 pH, tannin content, TSS and reducing sugars

The pH of wort initially was 4.5 and was adjusted to 5.00. The tannin content of wort was 9.15 mg per 100 ml and TSS of 8.50°Brix (which was later adjusted with cane sugar to 20°Brix) was recorded. The reducing sugar was found to be 64.58 mg $g^{-1}$.

## 4.4 Screening the yeast strains for their suitability for beer production

### 4.4.1 Standardization of the inoculum level of different yeast strains for the production of beer

The results of the experiment are presented in Table 6a, 6b and 6c.

#### 4.4.1.1 Tannin content

Inoculum level had a significant effect on the tannin content of the beer for different yeast strains. From the results, it is clear that tannin content decreased significantly with inoculum size. The yeast strain *Saccharomyces cerevisiae* NCIM 3570 recorded the highest tannin content of 5.14 mg per 100 ml @ 1 per cent level of Inoculum while *Saccharomyces cerevisiae* NCIM 3551 recorded the lowest tannin content (3.06 mg 100 $ml^{-1}$) in 2 per cent Inoculum level. The effect inoculum level and the yeast strains were found significant (Table 6a).

#### 4.4.1.2 Alcohol content

With the increase in inoculum size, there was significant increase in the alcohol content of the beer. Among the yeast strains, *Saccharomyces cerevisiae* NCIM 3551 recorded the highest alcohol content (3.32%) in 2 per cent Inoculum which was significantly higher than the other yeast strains. While lowest alcohol content was observed in *Saccharomyces cerevisiae* NCIM 3570 (2.32 %) @ 1 percent inoculum. The effect of inoculum level and yeast strains was significant (Table 6b).

#### 4.4.1.3 Residual reducing sugars

Table 6c displays the residual reducing sugars of the beer obtained from the Finger millet at different inoculum levels of yeast strains.

In general, the residual reducing sugars decreased significantly with increase in the inoculum level. Inoculum level of 1 per cent recorded the highest residual reducing sugars (7.91 mg $g^{-1}$ of sample) in *Saccharomyces cerevisiae* NCIM 3570 while the lowest residual reducing sugars were obtained from the beer treated with 2 per cent inoculum level (5.06 mg $g^{-1}$ of sample) in

**Table 3c: Performance of α-amylase (1%) at $70^0$C at different period of incubation on yield of reducing sugars**

| Sl. No. | Millets | Reducing sugar (mg $g^{-1}$) | | |
|---|---|---|---|---|
| | | Incubation period (h) | | |
| | | 8 | 16 | 24 |
| 1. | Bajra | 41.61 | 45.11 | 59.86 |
| 2. | Finger millet | 53.19 | 58.15 | 74.19 |
| 3. | Foxtail millet | 45.12 | 49.21 | 63.34 |
| 4. | Little millet | 51.04 | 56.91 | 69.23 |
| | SEm± | 0.102 | 0.089 | 0.11 |
| | CD (0.01) | 0.313 | 0.270 | 0.32 |

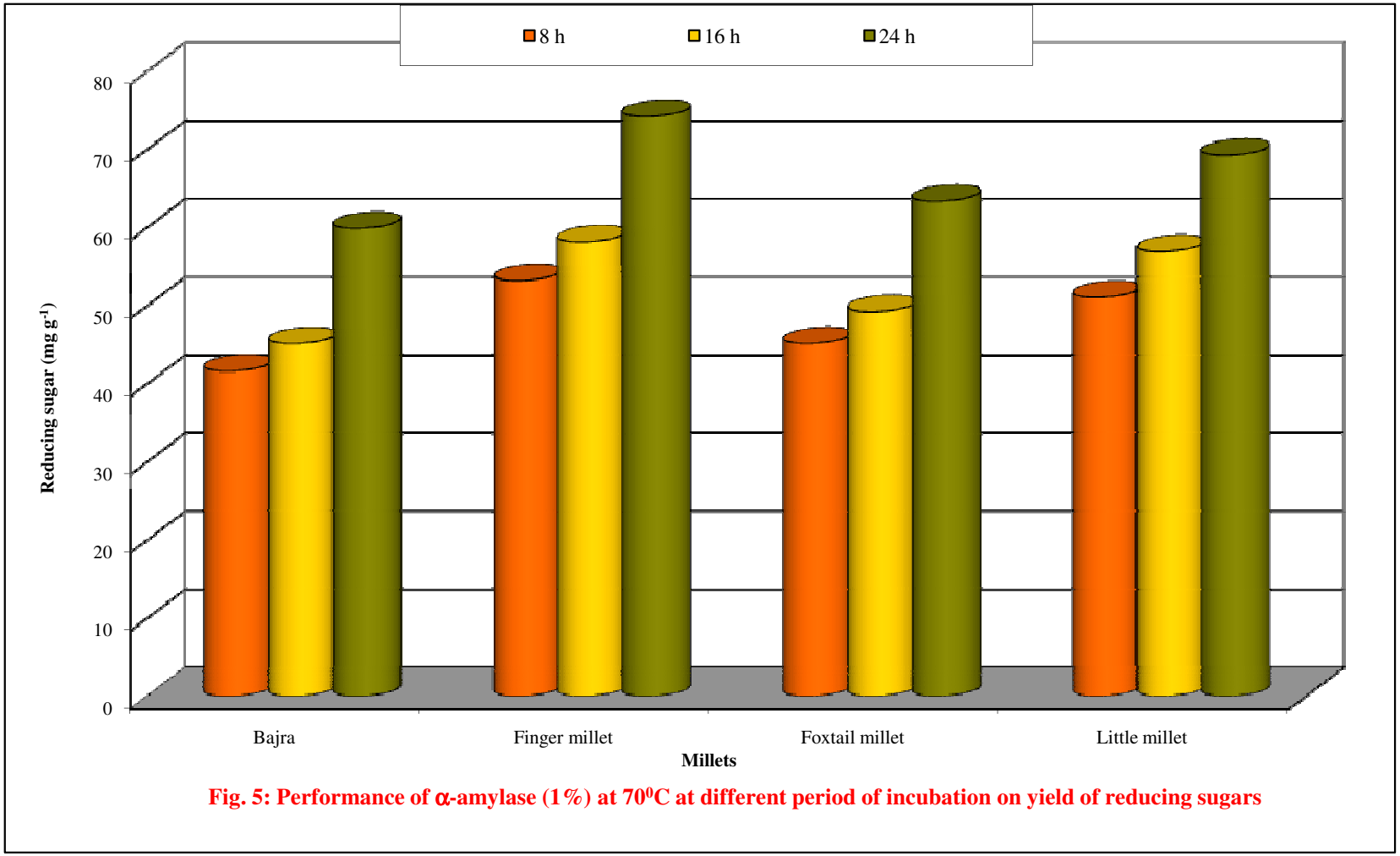


**Fig. 5: Performance of α-amylase (1%) at 70$^0$C at different period of incubation on yield of reducing sugars**

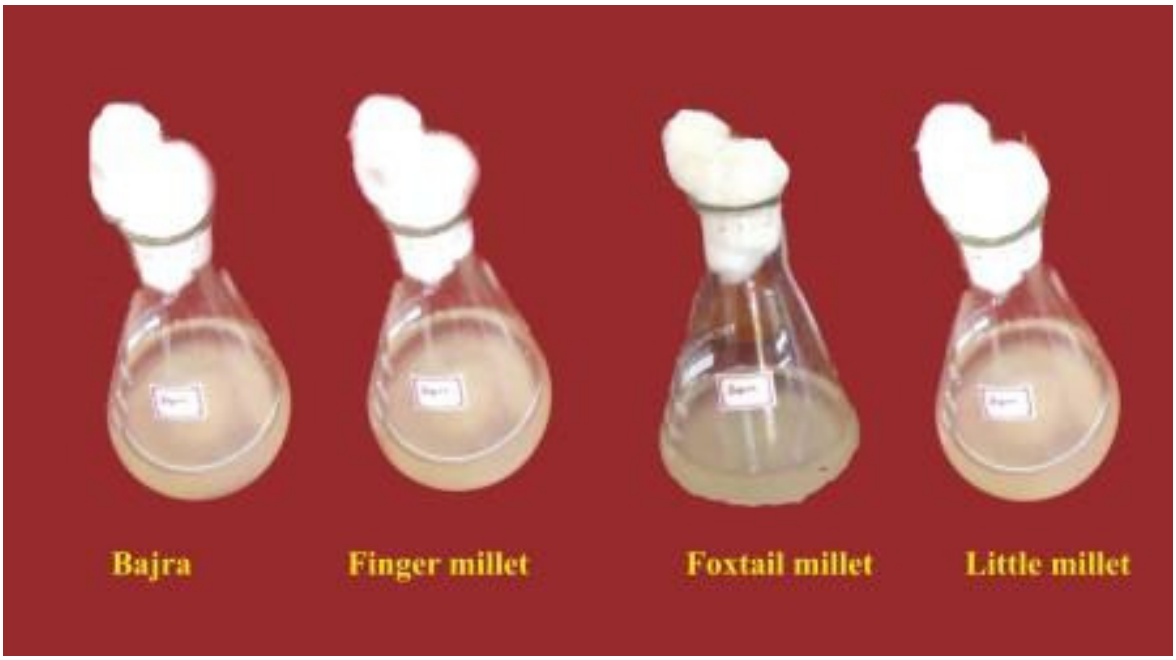


**Plate 2. Wort treated with commercial α-amylase**

**Table 4: Saccharification of different millets under all optimized condition**

| Sl. No. | Millets | Reducing sugars ( mg $g^{-1}$) |
|---|---|---|
| 1. | Bajra | 49.02 |
| 2. | Finger millet | 63.21 |
| 3. | Foxtail millet | 58.16 |
| 4. | Little millet | 53.10 |

Note: Note : α-amylase = 1%
Incubation temperature = $70^0$C
Incubation period = 24 hr

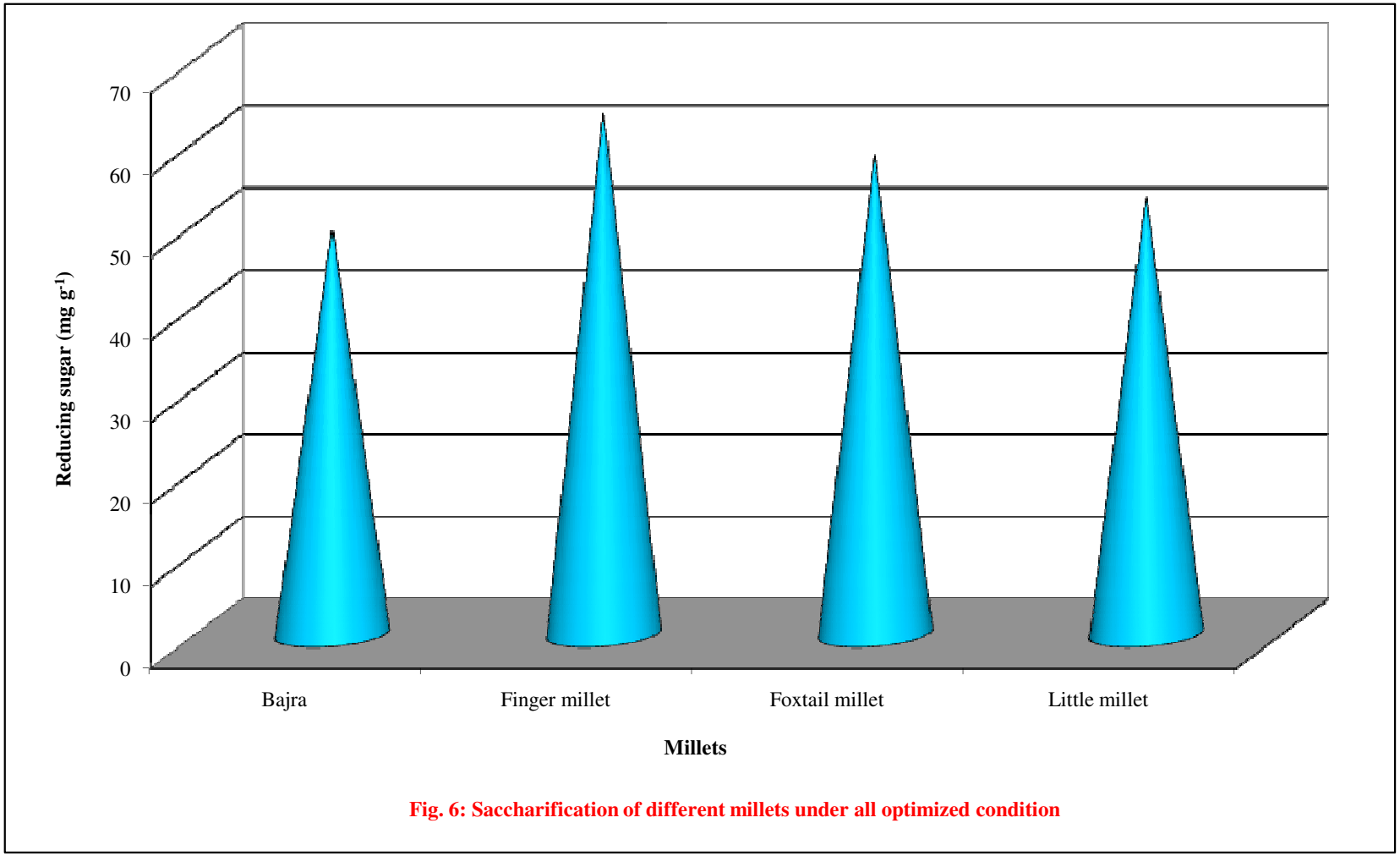


**Fig. 6: Saccharification of different millets under all optimized condition**

**Table 5: Chemical analysis of wort prepared from finger millet for beer production**

| Sl. No. | Parameters | Values |
|---|---|---|
| 1. | pH | 4.50 |
| 2. | Total Soluble Sugars (TSS) | 8.5$^{o}$Brix |
| 3. | Tannin content | 9.15 mg 100 ml$^{-1}$ |
| 4. | Reducing sugars | 64.58 mg g$^{-1}$ |

**Table 6a: Performance of yeast strains at different levels of inoculum on tannin content of finger millet beer**

| Inoculum level (%) | Tannin content (mg 100 ml$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | ***S.cerevisiae* NCIM 3570** | ***S.cerevisiae* NCIM 3551** | ***S.cerevisiae* NCIM 3455** | ***S.cerevisiae* NCIM 3580** | ***S.cerevisiae* NCIM 3391** |
| 1. | 5.14 | 3.57 | 4.10 | 4.40 | 3.62 |
| 1.5 | 4.16 | 3.48 | 3.53 | 3.83 | 3.29 |
| 2. | 3.37 | 3.06 | 3.10 | 3.43 | 3.50 |
| SEm$\pm$ | 0.036 | 0.088 | 0.043 | 0.04 | 0.039 |
| CD (0.01) | 0.11 | 0.27 | 0.131 | 0.13 | 0.121 |

Initial tannin content: 9.20 mg 100 ml$^{-1}$

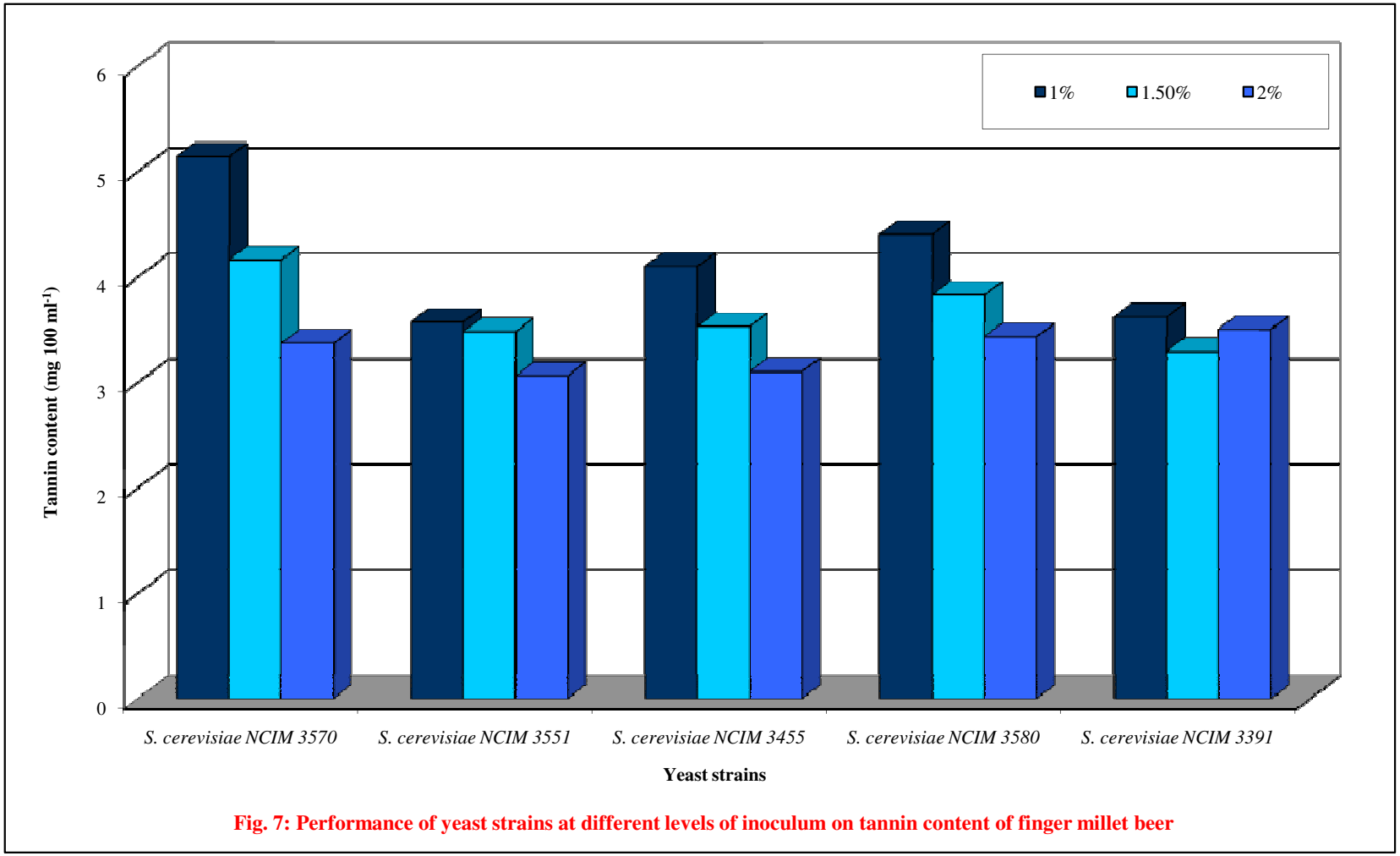


**Fig. 7: Performance of yeast strains at different levels of inoculum on tannin content of finger millet beer**

**Table 6b: Effect of differ rent yeast strains and inoculum size on alcohol content in finger millet beer**

| Inoculum level (%) | Alcohol content (g $g^{-1}$) | | | | |
|---|---|---|---|---|---|
| | ***S.cerevisiae* NCIM 3570** | ***S.cerevisiae* NCIM 3551** | ***S.cerevisiae* NCIM 3455** | ***S.cerevisiae* NCIM 3580** | ***S.cerevisiae* NCIM 3391** |
| 1. | 2.32 | 3.16 | 2.60 | 2.83 | 2.60 |
| 1.5 | 2.36 | 3.26 | 2.61 | 2.90 | 2.64 |
| 2. | 2.45 | 3.32 | 2.68 | 2.93 | 2.70 |
| SEm± | 0.068 | 0.019 | 0.108 | 0.124 | 0.078 |
| CD (0.01) | 0.213 | 0.057 | 0.325 | 0.372 | 0.241 |

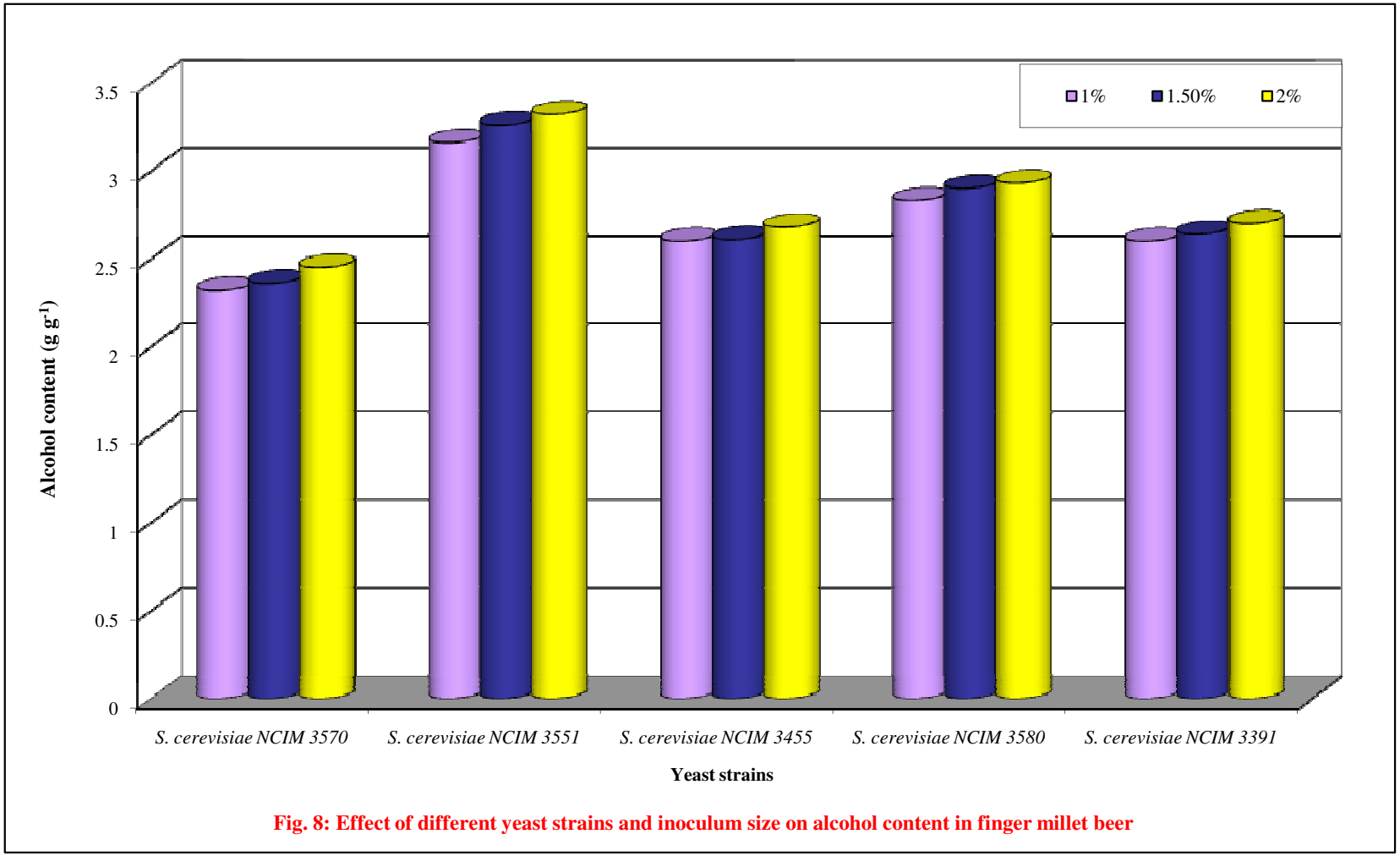


**Fig. 8: Effect of different yeast strains and inoculum size on alcohol content in finger millet beer**

**Table 6c: Effect of different yeast strains and inoculum size in residual reducing sugar in finger millet beer**

| Inoculum level (%) | Residual reducing sugar (mg $g^{-1}$) | | | | |
|---|---|---|---|---|---|
| | ***S.cerevisiae* NCIM 3570** | ***S.cerevisiae* NCIM 3551** | ***S.cerevisiae* NCIM 3455** | ***S.cerevisiae* NCIM 3580** | ***S.cerevisiae* NCIM 3391** |
| 1. | 7.91 | 6.21 | 6.44 | 7.01 | 7.46 |
| 1.5 | 6.98 | 5.83 | 6.05 | 6.86 | 6.98 |
| 2. | 6.33 | 5.06 | 5.31 | 6.51 | 5.19 |
| SEm± | 0.03 | 0.046 | 0.0154 | 0.017 | 0.0125 |
| CD (0.01) | 0.09 | 0.0142 | 0.047 | 0.05 | 0.038 |

Initial reducing sugars: 62.41 mg $g^{-1}$

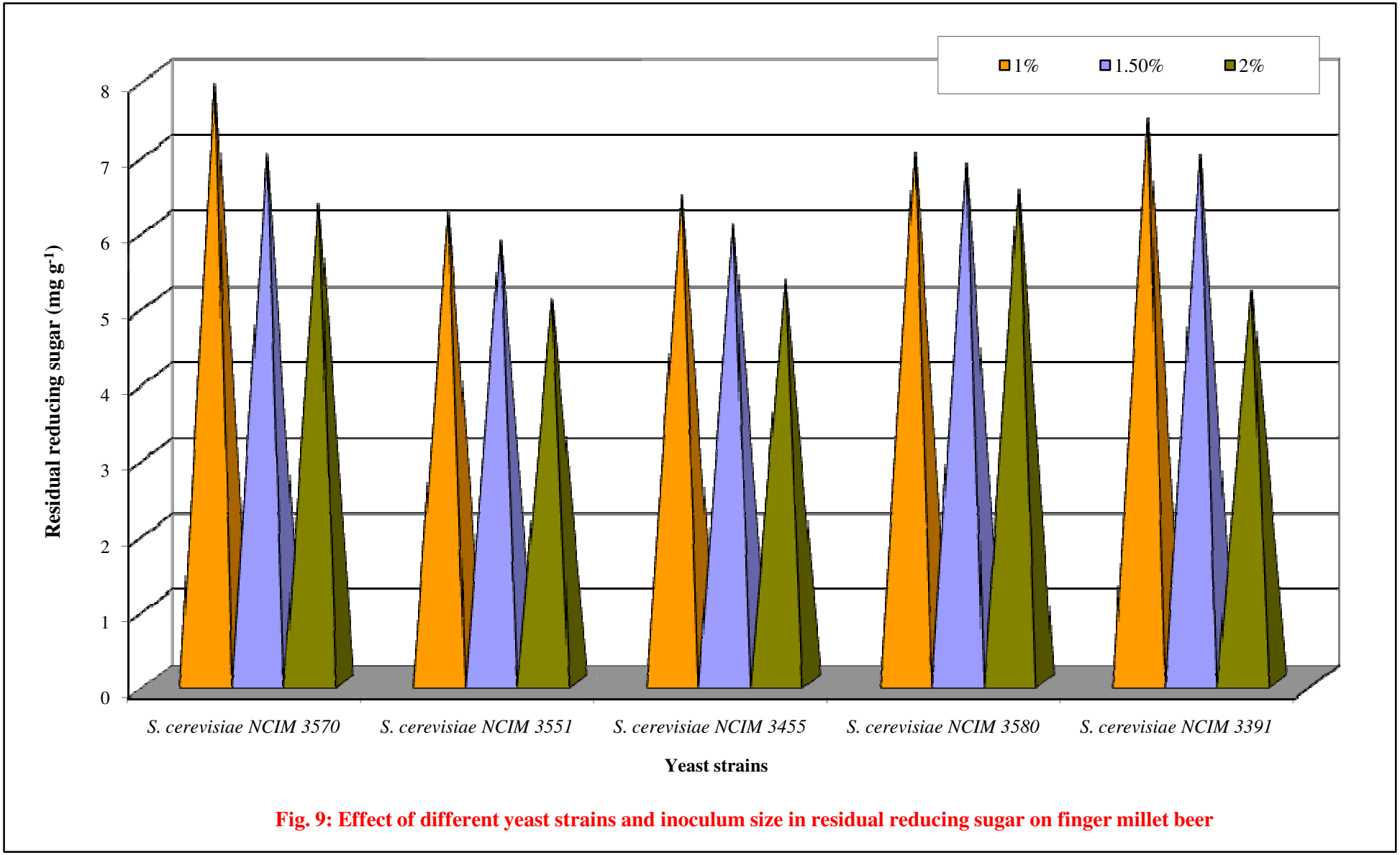


**Fig. 9: Effect of different yeast strains and inoculum size in residual reducing sugar on finger millet beer**

**Plate 3. Experimental set up for beer production from finger millet**

*Saccharomyces cerevisiae* NCIM 3551. Significant difference was found between the residual reducing sugars of different yeast strains.

### 4.4.2 Effect of efficient yeast strain and fermentation parameters on production of beer on scale up studies

From the above results, it was found that the yeast strain *Saccharomyces cerevisiae* NCIM 3551 was most efficient for beer production as compared to the other yeast strains and was used for scale up production of beer.

#### 4.4.2.1 Chemical analysis of Finger millet beer in comparison with commercial beer (barley)

Results of the experiment are presented in Table 7.

##### 4.4.2.1.1 pH

There was a fall in the pH from 5.00 to 4.42 at the end of fermentation in finger millet beer, while the commercial beer recorded a pH of 4.13.

##### 4.4.2.1.2 Tannin content

From results it was clear that tannin content from the wort decreased during fermentation and was found to be 4.06 mg 100 $ml^{-1}$ in the beer prepared from Finger millet and in commercial beer the tannin content was found to be 3.95 mg per 100 ml.

##### 4.4.2.1.3 Alcohol content

An alcohol content of 3.32 per cent was recorded in finger millet beer which was lower than the alcohol content of commercial beer (8 %).

##### 4.4.2.1.4 Residual reducing sugars

From the experiment, the residual reducing sugars was found to be 3.75 mg $g^{-1}$ in the beer prepared from Finger millet, whereas, the commercial beer recorded 2.90 mg $g^{-1}$ of residual reducing sugars.

##### 4.4.2.1.5 Colour and brightness

The beer prepared from the finger millet recorded a colour intensity of 0.056 (optical density at 420 nm) and brightness of 0.083 (optical density at 420 + 520 nm). While, the colour and brightness of commercial beer were 0.091 and 0.101 respectively.

Organoleptic evaluation of Finger millet beer in comparison to commercial beer. The results of the organoleptic evaluation are presented in Table 8.

##### 4.4.2.1.6 Appearance

The beer representing sample B scored highest points (1.85 out of 2.00) as compared to sample A (1.60 out of 2.00) with respect to appearance of beer.

##### 4.4.2.1.7 Colour

Maximum score for characteristic beer colour was recorded by sample B (2.00 out of 2.00), while sample A recorded an average score (1.75 out of 2.00).

##### 4.4.2.1.8 Aroma

Pleasing aroma was recorded in both the beer sample A and B. Both the samples scored (1.25 and 1.95 out of 2.00) with respect to aroma.

##### 4.4.2.1.9 Total acidity

The beer sample B scored slightly higher points (1.83 out 2.00) while sample A scored lower points (1.63 out of 2.00).

##### 4.4.2.1.10 Body

Among the two different beer samples, sample B scored higher points (1.80 out of 2.00) than sample A (1.70 out of 2.00) which recorded slightly lower body characteristics.

**Table 7: Chemical parameters of finger millet beer in comparison with commercial beer**

| Sl. No. | Parameters | Finger millet beer | Commercial beer |
|---|---|---|---|
| 1. | pH | 4.42 | 4.13 |
| 2. | Tannin content (mg 100 $ml^{-1}$) | 4.06 | 3.95 |
| 3. | Alcohol content (g $g^{-1}$) | 3.32 | 8.0 |
| 4. | Residual reducing sugars (mg $g^{-1}$) | 3.75 | 2.90 |
| 5. | Colour (optical density | 0.056 | 0.091 |
| 6. | Brightness (optical density) | 0.083 | 0.101 |

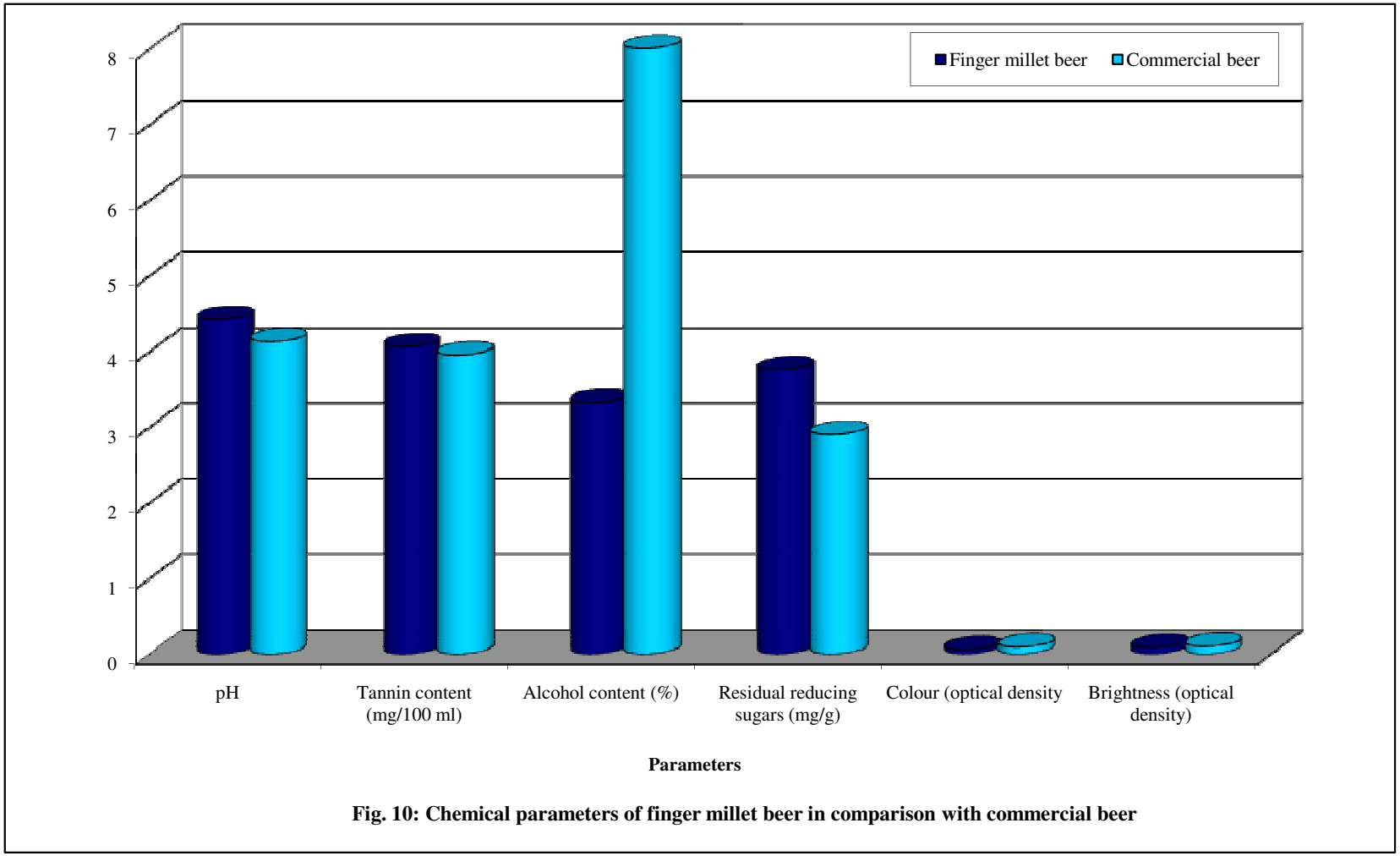


**Fig. 10: Chemical parameters of finger millet beer in comparison with commercial beer**

**Table 8: Organoleptic evaluation of finger millet beer in comparison with commercial beer**

| Sl. No. | Characteristics | Total score | Average score given by panel judges based on 16 point scale | |
|---|---|---|---|---|
| | | | Finger millet beer | Commercial beer |
| 1. | Appearance | 2.. | 1.60 | 1.85 |
| 2. | Colour | 2. | 1.75 | 2 |
| 3. | Aroma | 2. | 1.25 | 1.95 |
| 4. | Total acidity | 2. | 1.63 | 1.83 |
| 5. | Body | 2. | 1.70 | 1.80 |
| 6. | Flavour | 2. | 1.48 | 2 |
| 7. | Astringency | 2. | 1.59 | 1.90 |
| 8. | General quality | 2. | 1.55 | 1.70 |
| 9. | Overall acceptability | 16. | 12.55 | 15.03 |

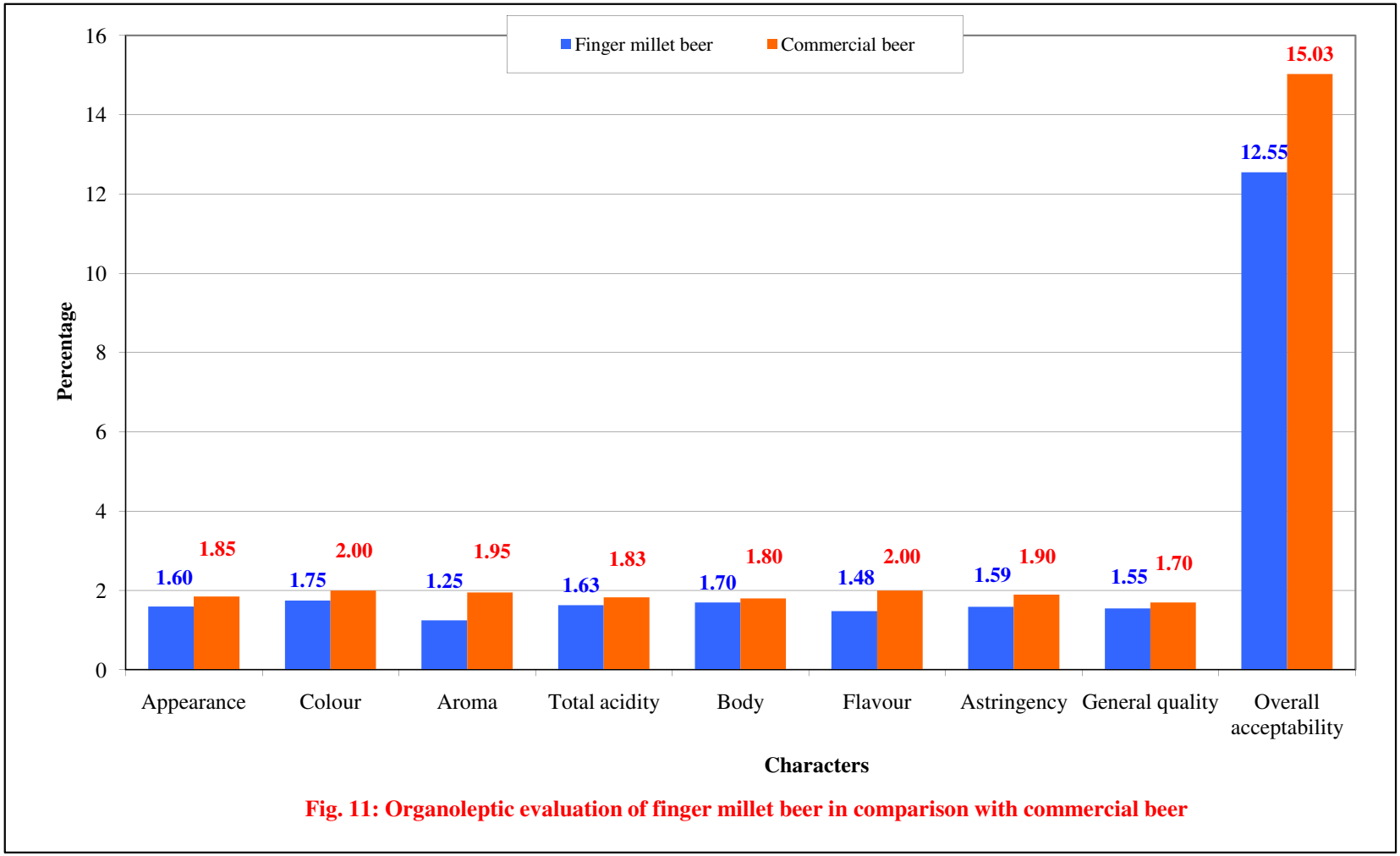


**Fig. 11: Organoleptic evaluation of finger millet beer in comparison with commercial beer**


**Plate 4. Comparison of beer prepared from finger millet and commercial beer**

4.4.2.1.11 Flavour

The beer sample B scored maximum points (2.00 out of 2.00) in terms of flavour than sample A (1.48 out of 2.00) which was less flavoured than sample B.

4.4.2.1.12 Astringency

With regard to astringency, both A and B beer samples evaluated recorded (1.59 and 1.90 out of 2).

4.4.2.1.13 General quality

In terms of general quality, sample B scored higher points (1.70 out of 2.0) than sample A, which recorded comparatively lower points (1.55 out of 2.00).

4.4.2.1.14 Overall acceptability

With respect to overall acceptability, there was not much difference between sample B (commercial barley beer) and sample A (Finger millet beer). Sample B scored maximum points (15.03 out of 16.00) than sample A (12.55 out of 16.00). The results confirm that beer from Finger millet cannot totally replace barley beer but the beer was comparable oragnoleptically with commercial beer and was acceptable by panel judges.

# DISCUSSION

It is general practice throughout the world that suitable type of alcoholic beverage native to its region is prepared and consumed. Beverages are prepared by fermentation of a variety of foods or blends of fruits, cereals, milk, sap, honey and other foods containing fermentable carbohydrates. Many foods undergo fermentation to produce alcoholic beverages like wine, beer *etc.* Beer is an alcoholic beverage produced from cereals. The brewing industry is one of the great advances accomplished in an industry. Malt is called the "soul of beer". Malting has been practiced for centuries while hops are believed to have been used since 3000 B.C. Barley and other cereals were used for beer preparation and during the middle ages, oats were commonly used by the poor people. Egyptians are known to be associated with beer and religion. They added spices and herbs to offset the sweet taste of malt. Beer was frequently used to administer drugs orally. The drugs from beer are a source of vitamin B and a number of valuable antibiotic substances (Haifeng Zhaoa *et al.*, 2010).

The fundamental basis of the liquor industry is the conversion of sugars to ethyl alcohol and carbon dioxide by the action of enzymes. Malt is mostly prepared from barley grains but other cereals and starch containing substances are also used now days. These include corn, rice, wheat, sorghum, finger millet and cassava. The brewing industry is constantly looking for ways to improve beer quality and reduce manufacturing costs. Minor millet crops, which grow well in tropical countries and containing good amount of reducing sugars, can become a substitute for barley in beer production. Hence, minor millet are good alternative for barley as a brewing source.

The present study aimed at producing beer from minor millet grains using different yeast strains. The various malting, mashing and fermentation parameters were standardized for good quality beer production.

Among the millet grains, the finger millet recorded significantly high reducing sugars (25.30 mg $g^{-1}$) and amylase activity (23.50 mg of protein 15 $min^{-1}$ $g^{-1}$) at 16 h of soaking on the third day of germination. The difference in amylase activity may be because of the soluble fraction of amylase enzyme that increases during germination. While the insoluble fraction of enzyme remains constant on further malting. Uvere *et al.* (2000) determined □- amylase activity in the red and white varieties of sorghum, steeped in water for 18 h and germinated up to 5 days and found that □-amylase activity was maximum on the third day and was higher in white sorghum variety. Nso *et al.* (2003) compared the amylase activity of three sorghum cultivars *i.e.*, Safrari, Madjeru and S-35, which were germinated for 5 days for traditional beer production and found it to be 94.56, 56.59 and 51.70 (units $g^{-1}$) in laboratory conditions.

The fermentation potential of wort is strongly influenced by its initial concentration of total fermentable carbohydrate and its composition. Brewers utilize amylase enzymes to improve the production and final characteristics of beer. Various parameters were studied for optimizing the concentration of commercial □-amylase for saccharification. The effective saccharification was obtained by using enzyme concentration of one per cent, at $70^0$C for 24 h of incubation. These optimized parameters of commercial □-amylase were used for saccharification of the millet grains to release maximum sugars. The highest reducing sugars were recorded in finger millet (63.21 mg $g^{-1}$) followed by little millet (53.10 mg $g^{-1}$). The variation in reducing sugars among the millet grains could be due to the fact that the reaction of enzymes may be based on the composition starch content in the grains. Pozo-Insfran *et al.* (2004) reported that addition of amyloglucosidase in the wort increased the initial content of fermentable carbohydrates by approximately 20 per cent and also significantly increased residual glucose and alcohol as compared with untreated worts.

Wort was prepared using Finger millet and optimized parameters for saccharification using commercial □-amylase. The characteristics of wort showed a pH of 4.50, TSS of 8.5°brix and a tannin content of 9.15 mg 100 $ml^{-1}$. The reducing sugars released were found to be 64.58 mg $g^{-1}$. This may be probably due to the composition of mash which contained sugars, polyphenolics. The reducing sugars in malt beer and adjunct beer prepared from sweet sorghum were 0.05 per cent and 0.02 per cent respectively (Anonymous, 2002). Also, Pozo-Insfran *et al.* (2004) recorded a pH of 5.20 and a glucose content of 20.4 g $L^{-1}$ wort in sorghum wort.

Small amount of tannins are important for good colour and flavour of beer. But high amounts make beer bitter and also inhibit amylase enzymes. From the results, the tannin content decreased significantly with inoculum size. The lowest tannin content was obtained at 2 per cent inoculum level in *Saccharomyces cerevisiae* NCIM 3551 recorded (3.06 mg 100 $ml^{-1}$). The variation in tannin content with inoculum size could be due to the utilization of considerable amount of tannins by the yeast cells

during fermentation. Uvere *et al.* (2000) reported that tannin content decreases considerably during malting and fermentation.

With increase in inoculum level, there was significant increase in the alcohol content of beer. The yeast strain *Sacharomyces cerevisiae* NCIM 3551 recorded highest alcohol (3.32 %) content @ 2 per cent among the other strains. The alcohol production by different yeast strains at different inoculum level may be due to variation in their rate of sugar utilization and alcohol tolerance limits by the yeast strains. A maximum alcohol recovery of 5.85 per cent at 5 per cent yeast concentration was obtained during fermentation of yam to ethanol (Ramanathan, 2000).

Reducing sugars are prime component of fermentation, after consumption of which, alcohol is produced. Some non-utilized sugars remain as residual reducing sugars in beer which in high amounts decrease market quality of beer. With increase in inoculum level, residual reducing sugars decreased significantly. In the present investigation. Among all yeast strain *Saccharomyces cerevisiae* NCIM 3551 recorded the lowest residual sugars (5.06 mg $g^{-1}$ of sample) at 2 per cent inoculum. This difference may be due to the initial sugar concentration of wort, efficiency of fermentation and the capacity of yeast strains to utilize sugars. The residual sugars in malt beer and adjunct beer prepared from sweet sorghum were 1.54 and 0.95 per cent respectively (Anonymous, 2002).

A scale up study was made incorporating all optimized parameters. A pH of 4.50 was obtained in wort used for pilot scale production of beer. This would be due to the presence of sugars and acids in wort. The TSS of the wort was observed to be 8.5°Brix. This could be due to the presence of sugars produced during mashing.

The tannin content of Finger millet wort was 9.15 mg 100 $ml^{-1}$. Among millets grains contain the highest amount of tannins, which reduces to certain extent during malting and fermentation. The results are in confirmation with the findings of Uvere *et al.* (2000) who reported that, tannin content decreases considerably during malting and fermentation.

Beer from Finger millet was obtained and compared analytically with commercial brand of beer (barley) in terms of pH, tannin content, alcohol content, residual reducing sugars and colour and brightness. The beer prepared from Finger millet was compared closely with commercial beer analytically. Since beer is for consumption, the effect of perceived flavour gathers a lot of importance and hence the beer was also subjected for organoleptic evaluation by 8 panel judges along with the commercial beer. From the results it was confirmed that beer made from Finger millet was not superior to commercial beer (barley beer). This could be due to the reasons like low diastatic power of Finger millet, comparatively less amylase activity, higher amount of tannin content than barley and less sophisticated methods for brewing as compared to industrial production (Shayo *et al*., 2001). However, Finger millet beer was comparable to barley beer analytically as well as organoleptically and acceptable by panel of Judges. Arendt (2003) on the basis of organoleptic evaluation concluded that sorghum beer was comparable with barley beer and acceptable to the public.

It can be concluded that, the millets were found to be best suited for beer production. The results of the present investigation revealed that, 16h soaked with 3 day germinated millet seeds and with set of optimized mashing parameters such as 1 per cent commercial alpha amylase, $70^0$C temperature and 24 h incubation with inoculation of yeast strain *S. cerevisiae* NCI 3551 @ 2 per cent are well suited for quality beer production. Beer prepared from finger millet was found to have higher alcohol content with low residual sugars and tannin content as compared to other millets. The production/preparation of value added millet based products such as beer may pave the way for commercial cultivation of millets and high returns to the farmer.

# SUMMARY AND CONCLUSIONS

The brewing of beer is considered as an art in many parts of the world from the ancient days. For centuries brewing has been conducted on the basis of experience. Several types of beer are consumed today like porter, stout, mild ale, pale ale, brown ale, pilsner, bock beer, ice beer *etc.* The most common beer in the European and African countries is sorghum beer. Sorghum beer in Africa is an acid type of beer, which is quite different from traditional European beer. Chinese sorghum beer is similar to European beer in colour, flavour and quality.

In the present investigation, the rich carbohydrates present in minor millet grains are converted to reducing sugars by the action of endogenous enzymes activated during malting and commercial enzymes and fermented with efficient strains to produce beer.

The reducing sugars of malted millet grains were tested at different soaking periods and germination periods. Among the millet grains, the highest reducing sugars was observed in finger millet (25.30 mg $g^{-1}$ of sample) followed by little millet (23.16 mg $g^{-1}$ of sample) at 16 h of soaking on third day germination. And lowest reducing sugars was observed in bajra (15.18 mg $g^{-1}$ of sample) on three days of germination.

The amylase activity of malted grains were tested at different soaking period and germination day's. Among the treatments, 16 h of soaking and 3 days germination was found to have significantly high □-amylase activity. Among all these millets, the finger millet (25.50 mg of protein $g^{-1}$ of sample) followed by little millet (20.10 mg of protein $g^{-1}$ of sample) was recorded highest amylase activity at 16 h on third day of germination. The lowest amylase activity was found in bajra (13.62 mg of protein $g^{-1}$ of sample) at 3 days of germination.

Millets has low amount of saccharifying enzymes and hence, commercial □-amylase was used to release maximum sugars, Standardization for optimizing the conditions for utilizing commercial enzyme was done.

The saccharification carried out with 1 per cent commercial □-amylase showed the highest reducing sugars. Among the millet grains, Finger millet recorded significantly higher reducing sugars (66.67 mg $g^{-1}$ of sample) than other millet grains. The optimum incubation temperature for maximum release of sugars in finger millet (64.85 mg $g^{-1}$ of sample) was $70^0$C. An incubation period of 24 h was found to be optimum for saccharification recording significantly higher reducing sugars (74.19 mg $g^{-1}$ of sample) than the other incubation periods.

These optimized parameters were used for all the four millet grains. The highest reducing sugars were obtained in the finger millet (63.21 mg $g^{-1}$ of sample). Hence, this finger millet was used for further studies.

The wort prepared from Finger millet showed pH of 4.50, total soluble sugars of 8.50°Brix (which was later adjusted with cane sugar up to 20°Brix) and tannin content of 9.15 mg 100 $ml^{-1}$. The reducing sugars of wort were found to be 64.58 mg $g^{-1}$.

Tannin content decreased significantly with increase in inoculum level. The least amount of tannins was recorded at 2 per cent inoculum level. While, the yeast strain *Saccharomyces cerevisiae* NCIM 3551 recorded lowest tannin content (3.06 mg 100 $ml^{-1}$) compared to other yeast strains. Yeast strain *Saccharomyces cerevisiae* NCIM 3551 recorded significantly higher alcohol content (3.32%) than other yeast strains. There was an increase in alcohol content with increase in inoculum level. Accordingly, residual reducing sugars decreased significantly with increase in inoculum level. The amount of residual reducing sugars in the beer influences the organoleptic properties of beer and indicates the completion of fermentation. Accordingly, residual reducing sugars decreased *significantly* with increase in inoculum level. At an inoculum level of 2 per cent, least residual sugars were recorded. Among the yeast strains, *Saccharomyces cerevisiae* NCIM 3551 recorded the lowest reducing sugars (5.06 mg $g^{-1}$ of sample).

With the above-standardized parameters, beer was prepared on a scale up production. This product was further pasteurized (water bath at $65^0$C for 30 min) and used for further analysis. The beer was found to have a pH of 4.42, tannin content of 4.06 mg 100 $ml^{-1}$, an alcohol content of 3.32 per cent and residual sugars of 3.75 mg $g^{-1}$. The colour and brightness of the beer were 0.056 and 0.083, respectively. Similarly, the commercial brand of beer (barley beer) was also tested for the above mentioned parameters which shown little difference with that of finger millet beer. From the organoleptic evaluation, it was found that finger millet beer was comparable with that of commercial beer except differing slightly in aroma and flavour which can be further improved. Thus, grains of finger millet can be used for commercial beer production as an alternate to barley.

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## Appendix I: Media composition

### 1. Yeast Extract-Peptone-Dextrose (Y-E-P-D) Agar

| Composition | Quantity |
|---|---|
| Yeast extract | 10 g |
| Peptone | 20 g |
| Dextrose | 20 g |
| Agar | 18 g |
| pH | 5.4 |
| Distilled water | 1000 ml |

### 2. Sodium acetate buffer (0.1M) of pH 4.6

Stock solutions of 0.2 M solution of acetic acid (11.55 ml in 1000 ml dist. water) and 0.2 M solution of sodium acetate (16.4 g of $C_2H_2Na$ in 1000 ml dist. water) were prepared. 25.5 ml of 0.2 M Acetic acid and 24.5 ml of 0.2M sodium acetate were mixed and a total volume of 100 ml was made up to obtain 0.1M buffer.

### 3. 1 per cent starch solution

A fresh solution containing 1 g soluble starch was dissolved in 100 ml of acetate buffer.

### 4. Di-nitro salicylic acid reagent

One gram of 3, 5 dinitrosalicylic acid (DNSA) 200 mg of crystalline phenol and 50 mg of sodium sulphite in 100 ml of 1 per cent Sodium hydroxide were dissolved and stored at $4^0$C. For long storage, sodium sulphite was added at the time of use.

### 5. Rochelle salt solution 40%

Solution was prepared by dissolving 40 g of potassium sodium tartarate in 100 ml of distilled water.

### 6. Stock solution of maltose

Maltose of 50 mg was dissolved in 50 ml distilled water in a standard flask.

**7. Stock solution of glucose**

Standard stock solution was prepared @ 1 mg $ml^{-1}$ by dissolving 100 mg of D-glucose in distilled water and final volume was made up to 100 ml with distilled water.

**8. Folin-Denis reagent**

Sodium tungstate of 100 g and 20 g of phospho molybdic acid was dissolved in 750 ml of distilled water in a suitable flask and 50 ml of phosphoric acid was added to it. The mixture was refluxed for 2 h and the volume was made up to one litre with distilled water.

**9. Sodium carbonate solution**

Sodium carbonate of 35 g was dissolved in one litre of distilled water at 70-80$^0$C and filtered through glasswool after allowing it to stand overnight.

**10. Standard tannic acid solution (mg $ml^{-1}$)**

Tannic acid of 100 mg was dissolved in 100 ml of distilled water.

**11. Working standard solution of tannic acid**

The stock solution of 10 ml was diluted with 100 ml of distilled water. Each ml of the solution contained 100 □g tannic acid.

**12. Potassium dichromate solution**

Thirty four grams of $K_2Cr_2O_7$ was dissolved in 500 ml distilled water and 325 ml of sulphuric acid was added and volume was made upto 1000 ml with distilled water to give 0.23N $K_2Cr_2O_7$.

**13. Preparation of stock solution of ethanol**

Standard stock solution of 100 per cent pure analytical grade (containing 789 mg $ml^{-1}$) ethanol was prepared by dissolving 12.6 ml of ethanol in 100 ml distilled water, which resulted in 100 mg $ml^{-1}$ of standard ethanol.

# STUDIES ON BEER PRODUCTION FROM DIFFERENT MINOR MILLETS

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## ABSTRACT

The minor millet grains were screened for their suitability for beer production based on the amylase activity and reducing sugars of malted grains. Standardization of the parameters for malting and mashing were also carried out along with optimization of fermentation parameters for good quality beer production. The millet grains "Finger millet" showed the highest amylase activity (23.50 mg protein/15 min/g sample) and reducing sugars (25.30 mg/g) for a soaking period of 16h and germination period of two days which was followed by 'Little millet', 'Foxtail millet' and Bajra'. The 'Finger millet' was further used to standardize other parameters. The commercial $\alpha$-amylase (MAPS India Ltd., Ahmedabad) was used during mashing process, at a concentration of 1 per cent at 70 ℃ for an incubation period of 24 h which released maximum reducing sugars (74.19 mg/g) than other treatments. Five strains of Yeast was screened for fermentation of the above hydrolysis and the optimization of the inoculum level and fermentation period were carried out. Among the inoculum levels used (1, 1.5 and 2%), the tannin content and residual reducing sugars of beer decreased significantly up to 2 per cent inoculum. The alcohol content increased as inoculum level increased. Among the yeast strains, *Saccharomyces cerevisiae* NCIM 3551 performed best when inoculated at 2 per cent inoculums level. The final beer produced was compared with the commercial beer and was favourable when evaluated organoleptically.