Chapter 5
5.0 PRODUCTION OF FERMENTABLE SUGARS FROM SORGHUM AND PADDY BY MALTING

In the present study, optimizing parameters like steeping temperature, time, alkali, germination time, mashing temperature and enzyme were studied.

5.1 Effect of temperature on steeping in sorghum malting:

The sorghum samples (10% w/v) were steeped at different temperatures ranging from 25-37°C and maximum yield of reducing sugars was obtained at 31°C. The steeping is done with distilled water, 0.1% KOH, 0.1% NaOH and maximum yield was obtained with 0.1% of NaOH. Steeping is the first stage of true malting process where the moisture content of grain is raised which encourage the germination to start (Fig.13). Traditionally, steeping was carried out about 13°C but, where possible, higher temperatures are used to accelerate water uptake. Earlier reports shows that increase of malt diastatic power with the steeping temperature up to 30°C and free amino nitrogen extract content peaked at steeping temperature of 25°C (Dewar et al.,1997) the optimum temperature is found to be between 29-32°C. With the raise in the temperature there is dramatic increase in the reducing sugars but after optimum temperature there is decrease in the reducing sugars concentration. The effect of various steeping conditions (time, temperature and aeration) on the quality of sorghum malt for brewing (in terms of diastatic power, free amino nitrogen and hot water extract) was examined. Steeping time and temperature had a highly significant effect on sorghum malt quality. In general, malt quality increased with steeping time (from 16-40 h). Malt diastatic power increased with steeping temperature (up to 30°C) and free amino nitrogen and extract content peaked at a steeping temperature of 25°C. Aeration during steeping appeared to enhance the extract and free amino nitrogen content of the finished malt. Sorghum malt quality was found to be directly related to the steep-out moisture of the grain. Sorghum, malted at 25°C showed a better rate of extract development than that of malted at 20°C up to the 4th day of germination after which malting temperature (20° or 25°C) had little influence on hot water extract development. The values showed a remarkable improvement over those reported by other workers who reported lower extract yields when sorghum malt was mashed at
the infusion temperature (65°C). This could be as a result of incomplete starch gelatinization.

Fig. 13: Effect of temperature on steeping in sorghum malting.

Fig. 14: Effect of steeping time on sorghum malting.
5.2 Effect of steeping time on sorghum malting:

Sorghum was steeped at different time intervals ranging from 12-24h and maximum yield of reducing sugars in malt was obtained at 18 h (Fig. 14). The steeping was done with distilled water, 0.1% KOH, and 0.1% NaOH and maximum yield was obtained with 0.1% of NaOH. Steeping is the first stage of true malting process where the moisture content of grain is raised which encourage the germination to start. Increased malt quality was observed with prolonged steeping time initially from 16 hrs up to 30 h (Dewar et al., 1997). The length of steeping had a significant influence on enzyme development and extract recovery. Traditionally, steeping was carried out about 13°C but, where possible, higher temperatures are used to accelerate water uptake. In general, malt quality increased with steeping time (from 16 to 40h).

5.3 Effect of steeping time (Distilled water) in paddy varieties at 32°C:

Four varieties of paddy were steeped at different time intervals ranging from 12-24 h and maximum yield of reducing sugars in malts was obtained at 20 h. The steeping was carried out with distilled water only (Fig.15). Steeping is the first stage of true malting process where the moisture content of grain is raised which encourage the germination to start. Increased malt quality was observed with prolonged steeping time initially from 16 hrs up to 30h (Dewar et al., 1997). The length of steeping had a significant influence on enzyme development and extract recovery. Traditionally, steeping was carried out about 13°C but, where possible, higher temperatures are used to accelerate water uptake.

5.4 Effect of steeping time (0.1% KOH) in paddy varieties at 32°C:

Four varieties of paddy were steeped at different time intervals ranging from 12-24 h and maximum yield of reducing sugars was obtained at 20 h (Fig.16). The steeping was done with 0.1% KOH only. Steeping is the first stage of true malting process where the moisture content of grain is raised which encourage the germination to start the yield of reducing sugars was little bit reduced because of toxicity of 0.1% KOH. Alkali steeping induces the alpha amylase but the effect is more with the NaOH rather KOH because the NaOH induced amylases are more toxic.
 thermo stable. The improvement in malt quality (high reducing sugars) about by steeping in dilute alkali is due to increased water uptake during steeping. This is presumed to be as a result of alkali disrupting the pericarp cell wall structure.

![Graph](image)

**Fig. 15:** Effect of steeping time (Distilled water) in paddy varieties at 32°C.

![Graph](image)

**Fig. 16:** Effect of steeping time (0.1% KOH) in paddy varieties at 32°C.
Fig. 17: Effect of steeping time (0.1% NaOH) in paddy varieties at 32°C.

Fig. 18: Effect of germination time at optimized steeping conditions in sorghum malt.
5.5 Effect of steeping time (0.1% NaOH) in paddy varieties at 32°C:

Four varieties of paddy were steeped at different time intervals ranging from 12-24 h and maximum yield of reducing sugars was obtained at 20 h (Fig. 17). The steeping was done with 0.1% NaOH only. Steeping is the first stage of true malting process where the moisture content of grain is raised which encourage the germination to start the yield of reducing sugars was little bit increased because of toxicity of 0.1% NaOH. Alkali steeping induces the alpha amylase but the effect is more with the NaOH rather KOH because the NaOH induced amylases are more thermostable. The improvement in malt quality (high reducing sugars) about by steeping in dilute alkali is due to increased water uptake during steeping. This is presumed to be as a result of alkali disrupting the pericarp cell wall structure.

5.6 Effect of germination time at optimized steeping conditions in sorghum malt:

The sorghum was germinated in relative humid chamber at different time an interval ranging from 24-120h. maximum yield of reducing sugars was obtained at 72 h i.e., 3days (Fig.18). Germination is a second stage of malting process where the conversion of starch to sugars occurs. During germination the iodine test is positive indicating that only small amount of starch is converted to sugars. In traditional British malting, germination temperature of 12-13°C over 7-10 days were preferred, but in modern practice temperatures of up to 18-20°C are common depending on whether additives abrasion and germination accelerating schedules have been used. Previous results showed that optimum germination time at temperature 15°C was four (or) five days. After this time, the grains were sufficiently modified but nutrients have not yet been exhausted germination at 25°C produced a higher rate of enzyme development during the first 4 days of germination. From the 5th day, germination at 20°C induced higher enzyme development. α-Amylase development continued to increase throughout the germination period while β-amylase reached its peak on the 6th day. Earlier reports showed that optimum temperature for sorghum germination and development of sufficient diastatic power range from 25 to 30°C although Aisien and Ghosh (1978) reported an optimum temperature of 22°C for the germination of a particular variety of sorghum. Glennie and Wright (1986) reported that sorghum malt contains α- and β- amylases in approximate ratio of 4:1 which is a reversal of the ratio
in barley. A ratio of approximating 2:1 was observed at the 8th day of germination for the particular variety under study.

5.7 Effect of germination time at optimized steeping conditions in paddy malts:

Four varieties of paddy were germinated at optimized steeping conditions in relative humid chamber at different time an interval ranging from 24-120h (Fig.19). Maximum yield of reducing sugars was obtained at 72 h i.e., 3 days for paddy Sona Masoori variety. Germination is a second stage of malting process where the conversion of starch to sugars was occurs. During germination the iodine test is positive indicating that only small amount of starch is converted to sugars. Germination temperature of 12-13°C over 7-10 days were preferred, but in modern practice temperatures of up to 18-20°C are common depending on whether additives abrasion and germination accelerating schedules have been used. Pervious results showed that optimum germination time at temperature 15°C was four (or) five days.

5.8 Effect of temperature on sorghum malt mashing:

The sorghum was malted at optimized conditions on subjected to different range of mashing temperature ranging from 55-65°C (Fig.20). Maximum yield of reducing sugars was obtained at 59°C. Mashing is a final stage of malting process where the conversion of starch to simple reducing sugars occurs. Pervious results show that mashing temperature of 60°C at pH 4.0 was used in free alpha amino nitrogen production in sorghum beer mashing (John et al., 1976). In sorghum mashing beta amylase is more thermo liable then alpha amylase. So higher mashing temperature destroys the enzyme activity and temperature more than 60°C are not preferred in sorghum mashing process.

5.9 Effect of temperature on various paddy malts with Bio-Glucanase enzyme:

Four varieties of paddy malted at optimized conditions on subjected to different range of mashing temperature ranging from 55-65°C with 2 μl of bio-glucanase enzyme maximum yield of reducing sugars was obtained at 61°C which is higher than the mashing with out the enzyme (Fig. 21). 18.5g/l without enzyme and 55.6 g/l with enzyme in paddy malt, 11.5 g/l without enzyme and 34.8 g/l with
enzyme in sorghum malts at 61° C. Mashing is a final stage of malting process where the conversion of starch to maltose occurs. The addition of commercial β-amylase enhances the yield of reducing sugars.

Fig. 19: Effect of germination time at optimized steeping conditions in paddy malts.

Fig. 20: Effect of temperature on sorghum malt mashing.
Fig. 21: Effect of mashing temperature on various paddy malts.

Fig. 22: Effect of mashing temperature on sorghum malt with Bio-Glucanase.
Bio-Glucanase will further act on the unhydrolysed starch and increase the reducing sugar concentration makes the substrate ready for the fermentation with optimised level of reducing sugars which can be converted in value-added product citric acid. Mashing involves basically two methods; the decoction method and the infusion method (Hough et al., 1971). Mashing consists of (a) dissolving those substances directly soluble in water (b) enzyme breakdown followed by solution of a series of substances important for the type and character of the beer and (c) separation of the dissolved substances. Enzymes involved in the hydrolysis of substances include amylases, proteases, peptidases, transglucosidases and phosphorylases. These enzymes are regulated by temperature, pH, time and concentration of the wort (Hoyrup 1964; Hough et al., 1971; Mandl and Wagner 1978). During mashing, rapid degradation of solubilized starch and proteins and less-extensive hydrolyses of other high-molecular substances occur. Mashing extracts about 80% of the dry matter from the malt while 15% of it can be extracted by cold water (Wainwright, 1971). Okafor and Aniche (1980) produced beer from sorghum using the already-established three-stage decoction method; they did not however show that other mashing methods are inferior to the three-stage decoction method.
5.10 Effect of temperature on various sorghum with Bio-Glucanase enzyme

The sorghum malted at optimized conditions on subjected to different range of mashing temperature ranging from 55-65°C with 2 µl of bio-glucanase enzyme maximum yield of reducing sugars was obtained at 59°C which is 2 times higher than the mashing without the enzyme (Fig. 22). Mashing is a final stage of malting process where the conversion of starch to maltose occurs. The addition of commercial β-amylase enzyme Bio Glucanase will further act on the unhydrolysed starch and increase the reducing sugar concentration makes the substrate ready for the fermentation with optimized level of reducing sugars which can converted in value-added product citric acid.

5.11 Effect of mashing temperature on various paddy malts:

The 4 Varieties was malted at optimized conditions on subjected to different range of mashing temperature ranging from 55-65°C maximum yield of reducing sugars was obtained at 61°C (Fig. 23). With the increase in temperature there is decrease in the reducing sugars concentration because of the inactivation of enzyme at higher temperature. Mashing is a final stage of malting process where the conversion of starch to simple reducing sugars occurs. The varieties differ also play a major in the yield of reducing sugars the yield is more with the variety Sona Masoori followed by varieties Budalu, Gilakara Masoori and Sananulu. Mashing involves basically two methods; the decoction method and the infusion method (Hough et al., 1971). Mashing consists of (a) dissolving those substances directly soluble in water (b) enzyme breakdown followed by solution of a series of substances important for the type and character of the beer and (c) separation of the dissolved substances. Enzymes involved in the hydrolysis of substances include amylases, proteases, peptidases, transglucosidases and phosphorylases. These enzymes are regulated by temperature, pH, time and concentration of the wort (Hoyrup 1964; Hough et al., 1971; Mandl and Wagner 1978). During mashing, rapid degradation of solubilized starch and proteins and less-extensive hydrolyses of other high-molecular substances occur. Mashing extracts about 80% of the dry matter from the malt while 15% of it can be extracted by cold water (Wainwright, 1971). Okafor and Aniche (1980) produced beer from sorghum using the already-established three-stage decoction method; they did not
however show that other mashing methods are inferior to the three-stage decoction method.

5.12: Paper chromatography: The sugars like maltose and glucose found to be present in the malted media based on the chromatogram result. But still traces of starch to be unhydrolyzed form the sorghum and paddy which can be broken down into simple fermentable sugar in mashing. (Fig.24)

![Paper chromatogram](image)

Fig. 24: Paper chromatogram of saccharides present in malts of Paddy (P), Ragi (R) and Sorghum (S) (Mixture of standards (M) used are Sucrose, Maltose and Glucose).