Study of Red Sorghum (Sorghum Bicolor) Fermentation By Saccharomyces Cerevisiae Strain MTCC 170 and Its Effect on Total Polyphenolic Compound

Amesh V. Pawar*, Vishal R. Parate, Mohammad I. Talib
Department of Food Technology, University Institute of Chemical Technology
Jalgaon 425001, Country- India

***Abstract*-- The main objective of this work was to study the yeast fermentation and its effect on polyphenolic compound present in red sorghum (Sorghum bicolor (L.) monech) at different temperature ranges and also at different concentration of mash of red sorghum. During the study it was observed that the total polyphenolic compounds in fermentation broth were slightly increased up to 24 hrs. and rapidly increased till the end of 72 hrs. The changes occur in polyphenolic compound was different at different temperature. The maximum changes occur at 30^0 C and 25^0 C but there was minute increase at 35^0 C.It was also observed that the polyphenolic compound increase in concentration of mash. The maximum biomass and ethanol percentage were observed at 15% mash concentration at 30^0 C and at pH 6 followed by 25^0C and maximum sugar was consumed at the same.**

Keywords- Polyphenolic Compound, Temperature, Fermentation, Red Sorghum.

I. INTRODUCTION

The strain MTCC170 From IMTECH, Chandigarh of *Saccharomyces Cerevisiae* having capacity to produce Beta fructofuranosidases (invertase) enzyme that cleave "Beta-1,4- glucosidic linkage between D- glucose and D- fructose molecules of sucrose by hydrolysis producing glucose and fructose. Beta- fructofuranosidases are intracellular enzyme. It is used fermentation of cane molasses into ethanol, in calf feed preparation and also in manufacture of inverted sugar as food for honeybees [1].

India is semi-arid country and his main cereal crop is Sorghum (*sorghum bicolor* (L.) Monech). India is ranked

second in world to production of sorghum. Other cereals like wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) require more water and fertilizer than sorghum [2]. Red sorghum mainly contains tannins, which are located primarily in testa layer of the grain. Comparatively sorghum contain higher amount polyphenolic compound. The amount of polyphenolic compound in sorghum depends on genetic factor and environmental factor. Mainly sorghum ranges white color to red color and red color contain high amount of polyphenolic compound. There is direct relationship between polyphenolic compound and antioxidant activity and antimicrobial activity [3].

Polyphenolic compounds are very important in human diet as they have many medicinal properties such antioxidant, prevent heart diseases, anticarcinogenic. There two sources from where the polyphenolic compounds can be derived, they are phenylalanine and tyrosine [4][5][6] sorghum may contain benzoic acid phenylpropanoids, flavonoids,tannins,lignins Chemically, phenolics can be defined as substances possessing an aromatic ring bearing one or more hydroxyl groups, including their functional derivatives [7][8].Sorghum is very important grain for human consumption in Asian and African region and in U.S. it is used as source of animal feed [9] .Phenolics compound contributes to the flavor of the beer and also with implicated in nonbiological haze formation [10][11][12]. Phenolics substances in beer are present in various forms; theirolatility depends on their molecular weight Beer contains 283 mg/L of nontannin and nonflavonoid phenolics compounds [13]. Phenolics compounds of beer are classified as beneficial, harmful, or neutral in so far as their influence on beer stability and sensory properties is concerned [14].
Some beer phenolics may also act as antioxidants or may contribute to the formation of carbonyls in beer. It has been found that ferulic acid markedly increases the formation of carbonyls in beer aging under high and low air conditions. Moreover, ferulic acid and quercetin have been found to be active promoters of diacetyl formation in aging beer [15].

Some 67 different phenolics compounds have been identified in beer. Simple phenolics, aromatic carboxylic- and phenol carboxylic acids,hydroxycoumarins,catechins, leucoanthocyanidins,anthocyanidins,flavonols,flavonones,flavones,prenylated flavonoids and Phenolicglycosides are included in this list [16][17][18][19].

The objective of the present investigation was to determine the effect of yeast fermentation on total polyphenolic compounds of red sorghum.

II. MATERIALS AND METHOD

Strain

Saccharomyces Cerevisiae MTCC 170, IMTECH, Chandigarh.

Raw material

Red Sorghum was procured from local market of Jalgaon.

Malting

Malting involves germination, steeping and limiting growth of seed. During malting the enzymes are generated these are α -Amylase, β- Amylase, α-Glucosidase and Peroxidases [20] .α–Amylase catalyses random hydrolysis of starch at α (1, 4) linkage Amylase activity in sorghum malt is 25 to 183 U/g depending on sorghum variety [21].Beta amylase catalyses the hydrolysis of penultimate α (l, 4) glycosidic bond at non- β reducing end of polysaccharides causing the release of maltose. Amylase activity in sorghum malt is 11 to 41 SDU/g [Sorghum Diastatic Unit/g][22]. α Glucosidase in germinating grains catalyses hydrolysis of terminal, non-reducing a-(l, 4) glucosidic linkages in both oligosaccharides and α glucans yielding glucose [23]. Peroxidase catalyses the reductive destruction of hydrogen peroxide and invariably contributes to the defense system of living organism against peroxidation of unsaturated lipids involving oxygen radicals40. Lipid peroxidation is undesirable in malting and brewing and result in the production of hydroperoxides and their decomposition products, aldehydes[24].Lipase (triacylglycerol acyhydrolase) catalyses the hydrolysis of triacylglycerides to free fatty acids and Glycerol [25].Sorghum grains contain detectable lipase activity which vary slightly during 24 h steeping period at 30°C and increases during germination to about 4-fold after 96 h. However, lipase activity varies among different sorghum cultivars [26].

Mashing

Mashing in conventional brewing is basically by two methods, viz., decoction and infusion processes. During mashing water soluble substances dissolve, enzymes hydrolyse gelatinised starch and solubilised proteins and to a lesser extent other higher molecular weight substances essential for the type and character of beer, and finally dissolved substances are separated. Hydrolyses of substances involve enzymes such as amylases, proteases, peptidases, transglucosidases and phosphorylases which are regulated by factors like temperature, pH, time and concentration of the wort. Mashing extracts about 80% of the dry matter from the malt while cold water extracts about 15% [27]. Mashing of sorghum malt at 65°C and 70°C for 30 min each, at second and third stages respectively, of three stage decoction process, provides wort with complete hydrolysis [28].

Fermentation

Alcohol Fermentation is a process where different types of species of yeast are used to produce ethanol. The most economical species is *Saccharomyces Cerevisiae* which gives desirable amount of ethanol. The fermentation process is affected by various factors such as temperature, pH, sugar concentration, incubation period and media composition [1].

Determination of Alcohol

The alcohol content was calculated by potassium dichromate method. General principle includes that the potassium dichromate is yellowish color solution reacts with alcohol in presence of sulphuric acid and forms green color complex due to reduction of potassium dichromate and it is directly proportional to the content of alcohol. The absorbance is measured at 575 nm [29].

Determination of sugar

Sugar can be determined by phenol sulphuric acid method. Principal of this method is the carbohydrate means disaccharides polysaccharides and simple sugar reacts with strong acid and heat to generate furan derivatives that condensed to phenol which is yellow golden color complex which directly proportional to concentration of total sugar. The absorbance is measured at 550 nm [30].

Determination of biomass

Biomass produced during fermentation can be calculated by simple filtration method [31]. Simply filter the sample only for five minute at room temperature otherwise it will affect on the concentration of volatile component. Place the filter paper to hot air oven for drying for overnight. Next day simply weight the paper [32].

Determination of total polyphenolic compound Folin-Denis Assay

The Folin-Denis assay is the most widely used procedure for quantification of total phenolics in plant materials and beverages. Reduction of phosphomolybdic-phosphotungstic acid (Folin-Denis) reagent to a blue-colored complex in an alkaline solution occurs in the presence of phenolics compounds [33]. Folin-Ciocalteu reagent is not specific and detects all phenolics groups found in extracts including those found in the extractable proteins. A disadvantage of this assay is the interference of reducing substances such as ascorbic acid with the determinations. The total phenolics are assayed colorimetrically as modified [34] [35]. 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent, 2 mL of a 7.5% solution of sodium carbonate, and 0.5 mL of phenolics solution are mixed well. The absorbance is measured at 765 nm after a 15-min heating at 45°C; a mixture of water and reagents is used as a blank. The content of phenolics is expressed as Gallic acid or catechin equivalent.

Yield

Yield (Y_{PS}) was calculated by using following formula

$$Y_{PS} = \frac{\text{Product produced (ethanol)}}{\text{substrate consumed (sugar)}}$$

Substrate Conversion Ratio

Substrate Conversion Ratio (SCR) was calculated by using below formula

$$SCR = \frac{\text{Initial Sugar} - \text{Residual sugar}}{\text{Initial sugar}}$$

III. RESULT AND DISCUSSION

Effect of yeast fermentation at 25^0C and 24 hrs. on sugar, ethanol, polyphenolic compound and biomass

Table 1. Effect of Yeast Fermentation at 25^0C and 24 Hrs. On Sugar, Ethanol, Polyphenolic Compound and Biomass Values are Mean of Triplicate.

Mash conc. (%)	Initial sugar (gm)	Residual sugar (gm)	Alcohol (%)	Yps	SCR	Total polyphenolic compound (µgm/ml)	Biomass (gm)
5	3.5159	2.1718	0.5728	0.4261	0.3822	1.0733	0.3645
10	7.0318	5.3618	0.7451	0.4461	0.2374	1.2992	0.2501
15	10.5477	8.066	1.087	0.4369	0.2358	1.4247	0.4482
20	14.0636	12.4705	0.7078	0.4442	0.1132	1.8686	0.2540
25	17.5795	16.9037	0.2851	0.4218	0.0384	1.9546	0.0411

Table no. 1 showing that the maximum sugar was consumed; maximum alcohol production and high biomass were produced at 15% concentration. Maximum yield was at 10% mash concentration and maximum substrate conversion was at 5% mash concentration. The maximum polyphenolic compounds are observed at 25% mash concentration.

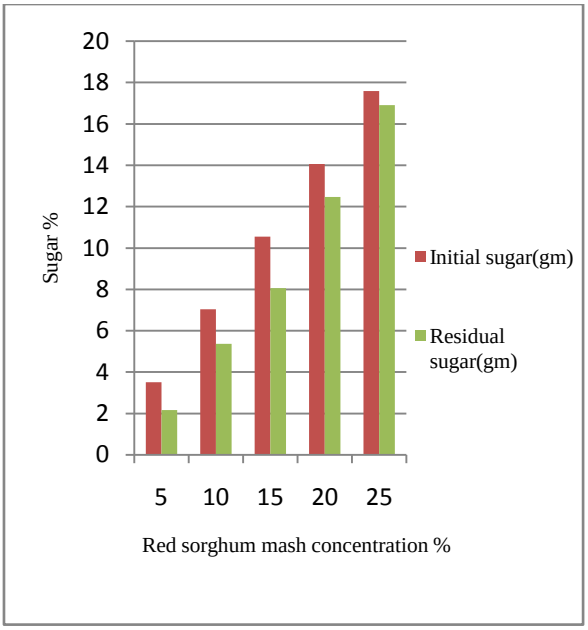

Figure 1. Effect of fermentation at 25^{0}C and time 24 hrs. on sugar, determined by phenol sulphuric method. Values are mean of triplicate.

Fig. no. 1 showing that the maximum sugar was consumed at 15% mash concentration and minimum at 25% mash concentration.

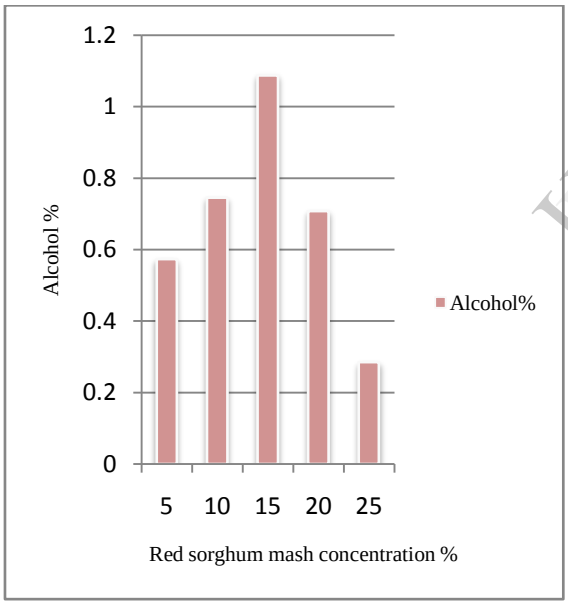

Figure 2. Effect of fermentation at 25^{0}C and time 24 hrs. on alcohol, determined by potassium dichromate method. Values are mean of triplicate

Fig. no. 2 showing that the maximum alcohol was produced at 15% mash concentration and minimum at 25% mash concentration.

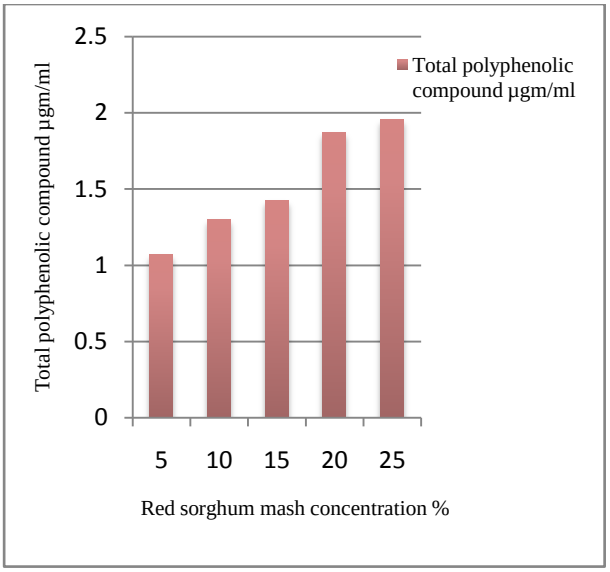

Figure 3. Effect of fermentation at 25^{0}C and time 24 hrs. on total polyphenolic compound, determined by Folin-Ciocalteu method. Values are mean of triplicate

Fig. no. 3 showing that the maximum total polyphenolic compound found at 25% mash concentration and minimum at 5% mash concentration.

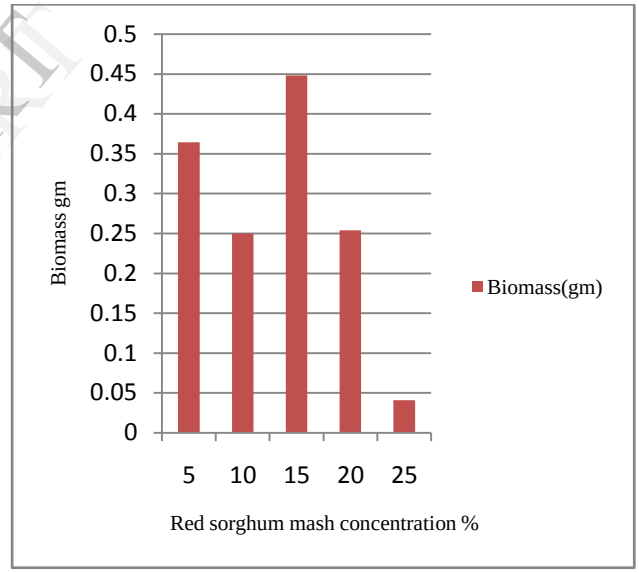

Figure 4. Effect of fermentation at 25^{0}C and time 24 hrs. on biomass, determined by filtration method. Values are mean of triplicate

Fig. no. 4 showing that the maximum biomass was produced at 15% mash concentration and minimum at 25% mash concentration.

Effect of yeast fermentation at 25^{0}C and 48 hrs. on sugar, ethanol, polyphenolic compound and biomass

Table 2. Effect of Yeast Fermentation at 25^{0}C and 48 Hrs. on Sugar, Ethanol, Polyphenolic Compound and Biomass Values are Mean of Triplicate

Mash conc. (%)	Initial sugar (gm)	Residual sugar (gm)	Alcohol (%)	Yps	SCR	Total polyphenolic compound (µgm/ml)	Biomass (gm)
5	3.5159	1.3868	0.9372	0.4401	0.6055	1.0905	0.3503
10	7.0318	1.2444	2.5823	0.4461	0.823	1.3851	0.5728
15	10.5477	0.7926	4.5281	0.4641	0.9248	1.8034	1.5189
20	14.0636	8.355	2.4935	0.4367	0.4059	2.0465	0.5614
25	17.5795	15.5964	0.8725	0.4399	0.1128	2.2131	0.2380

Table no. 2 showing that the maximum sugar was consumed, maximum alcohol production, high biomass was production and Maximum yield was at 15% mash concentration and maximum substrate conversion was at 15% mash concentration. The maximum polyphenolic compounds are observed at 25% mash concentration but the maximum changes in total polyphenolic compound were observed at 15% mash concentration.

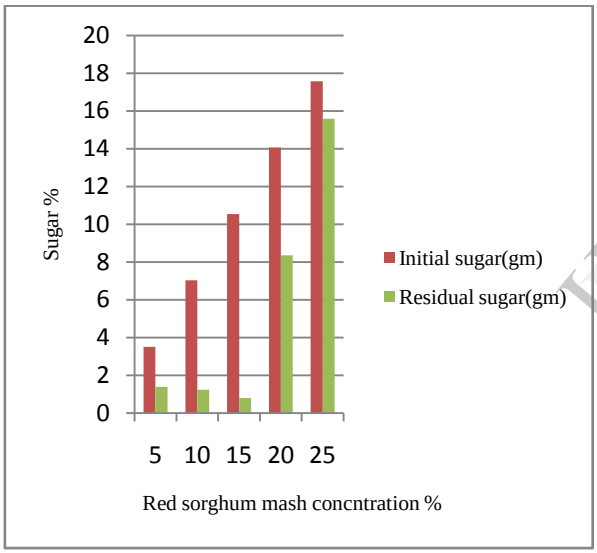

Figure 5 Effect of fermentation at 25^{0}C and time 48 hrs. on sugar, determined by phenol sulphuric method. Values are mean of triplicate

Fig. no. 5 showing that the maximum sugar was consumed at 15% mash concentration and minimum at 25% mash concentration

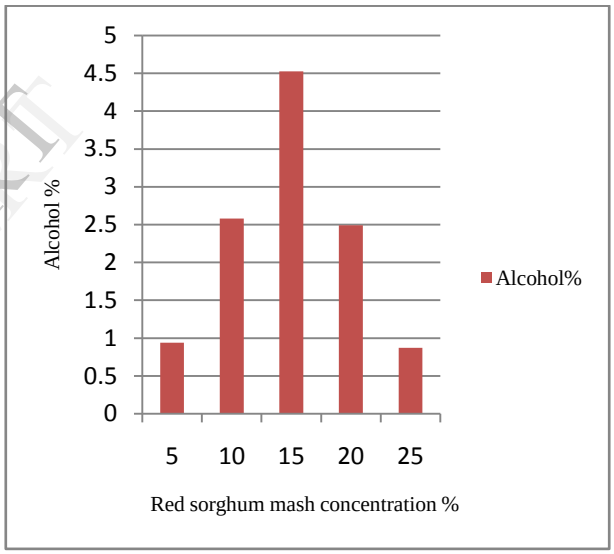

Figure 6 Effect of fermentation at 25^{0}C and time 48 hrs. on alcohol, determined by potassium dichromate method. Values are mean of triplicate

Fig. no. 6 showing that the maximum alcohol was produced at 15% mash concentration and minimum at 25% mash concentration.

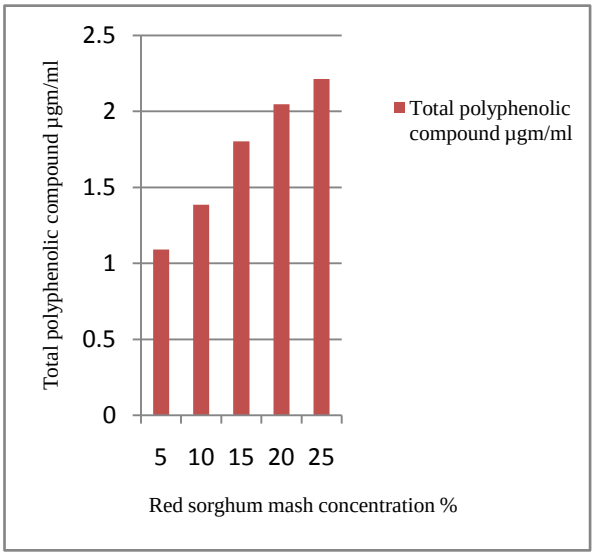

Figure 7 Effect of fermentation at 25^{0}C and time 48 hrs. on total polyphenolic compound, determined by Folin-Ciocalteu method. Values are mean of triplicate

Fig. no. 7 showing that the maximum total polyphenolic compound found at 25% mash concentration and minimum at 5% mash concentration. But as compared to fig. no. 3 the maximum effect on polyphenolic compound was at 15% mash concentration.

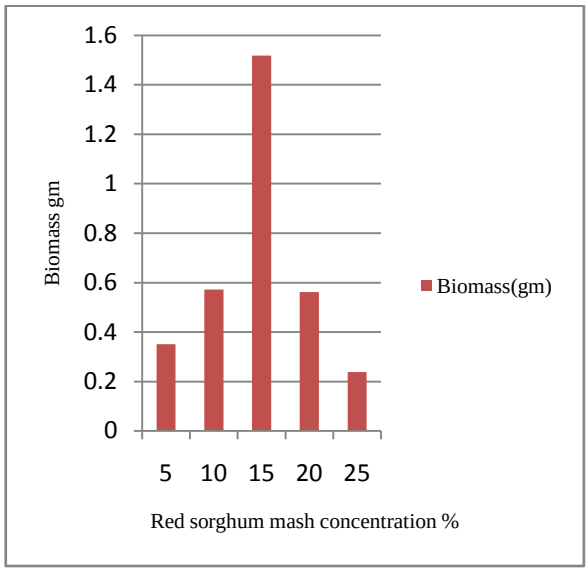

Figure 8 Effect of fermentation at 25^{0}C and time 48 hrs. on biomass, determined by filtration method. Values are mean of triplicate

Fig. no. 8 showing that the maximum biomass was produced at 15% mash concentration and minimum at 25% mash concentration.

Effect of yeast fermentation at 30^{0}C and 24 hrs. on sugar, ethanol, polyphenolic compound and biomass.

Table 3 Effect of yeast fermentation at 30^{0}C and 24 hrs. on sugar, ethanol, polyphenolic compound and biomass Values Are Mean Of Triplicate

Mash conc. (%)	Initial sugar (gm)	Residual sugar (gm)	Alcohol %	Yps	SCR	Total polyphenolic compound (µgm/ml)	Biomass (gm)
5	3.5159	1.9053	0.7075	0.4392	0.458	1.1188	0.1196
10	7.0318	4.8749	0.968	0.4487	0.3067	1.3336	0.3119
15	10.5477	7.6612	1.2758	0.4419	0.2736	1.3791	0.5375
20	14.0636	11.7036	1.0751	0.4555	0.1678	1.8498	0.267
25	17.5795	16.8267	0.3312	0.4399	0.0428	1.8701	0.0453

Table no. 3 showing that the maximum sugar was consumed; maximum alcohol production and high biomass were produced at 15% concentration. Maximum yield was at 20% mash concentration and maximum substrate conversion was at 5% mash concentration. The maximum polyphenolic compounds were observed at 25% mash concentration.

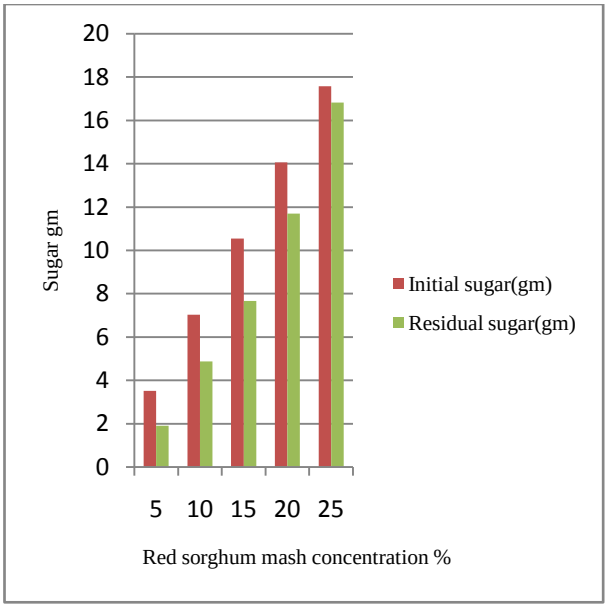

Figure 9 Effect of fermentation at 30^{0}c and time 24 hrs. on sugar, determined by phenol sulphuric method. Values are mean of triplicate.

Fig. no. 9 showing that the maximum sugar was consumed at 15% mash concentration and minimum at 25% mash concentration

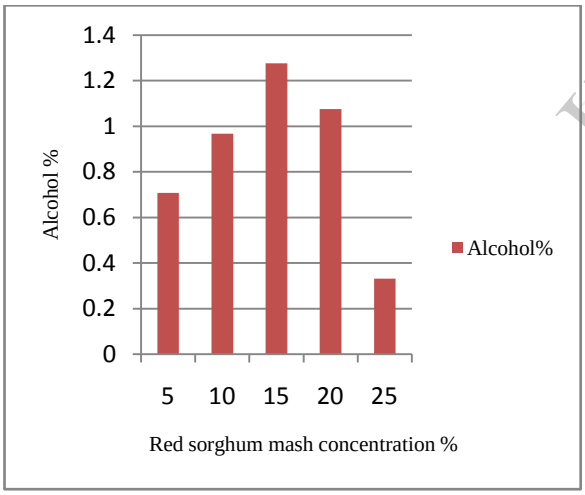

Figure 10 Effect of fermentation at 30^{0}c and time 24 hrs. on Alcohol, determined by potassium dichromate method. Values are mean of triplicate

Fig. no. 10 showing that the maximum alcohol was produced at 15% mash concentration and minimum at 25% mash concentration.

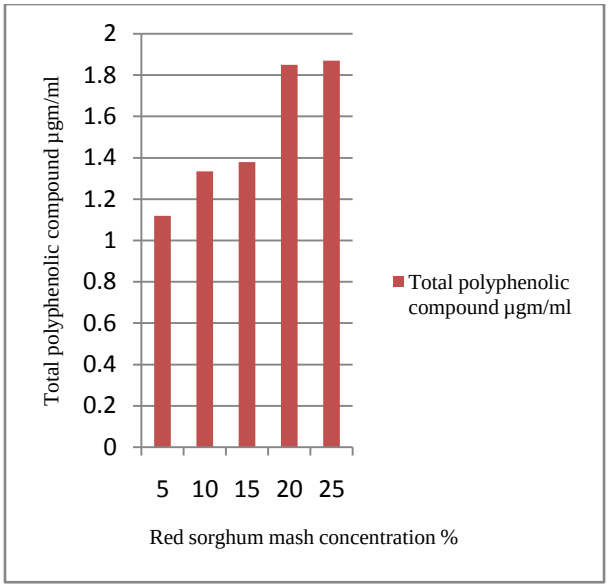

Figure 11 Effect of fermentation at 30^{0}c and time 24 hrs. on total polyphenolic compound, determined by Folin-Ciocalteu method. Values are mean of triplicate.

Fig. no. 11 showing that the maximum total polyphenolic compound found at 25% mash concentration and minimum at 5% mash concentration.

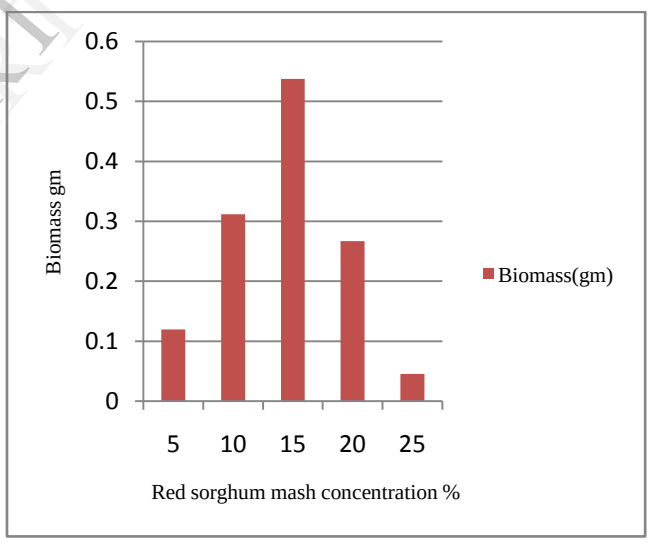

Figure 12 Effect of fermentation at 30^{0}c and time 24 hrs. on biomass, determined by Filtration method. Values are mean of triplicate.

Fig. no. 12 showing that the maximum biomass was produced at 15% mash concentration and minimum at 25% mash concentration.

Effect of yeast fermentation at 30^{0}C and 48 hrs. on sugar, ethanol, polyphenolic compound and biomass.

Table 4 Effect of yeast fermentation at 30^{0}C and 48 hrs. on sugar, ethanol, polyphenolic compound and biomass the values are mean of triplicate

Mash conc. (%)	Initial sugar (gm)	Residual sugar (gm)	Alcohol (%)	Yps	SCR	Total polyphenolic compound (µgm/ml)	Biomass (gm)
5	3.5159	0.9093	1.1684	0.4482	0.7413	1.2168	0.3663
10	7.0318	0.5256	2.9558	0.4543	0.9252	1.5324	0.6351
15	10.5477	0.4243	4.8891	0.4829	0.9597	1.7029	1.6495
20	14.0636	5.7663	3.6379	0.4384	0.5899	2.342	0.6283
25	17.5795	14.4677	1.2143	0.3902	0.177	2.3626	0.2648

Table no. 4 showing that the maximum sugar was consumed, maximum alcohol production and high biomass was produced at 15% concentration. Maximum yield and maximum substrate conversion was also at 15% mash concentration. The maximum polyphenolic compounds were observed at 25% mash concentration but maximum changes in polyphenolic compound was observed at 15% mash concentration.

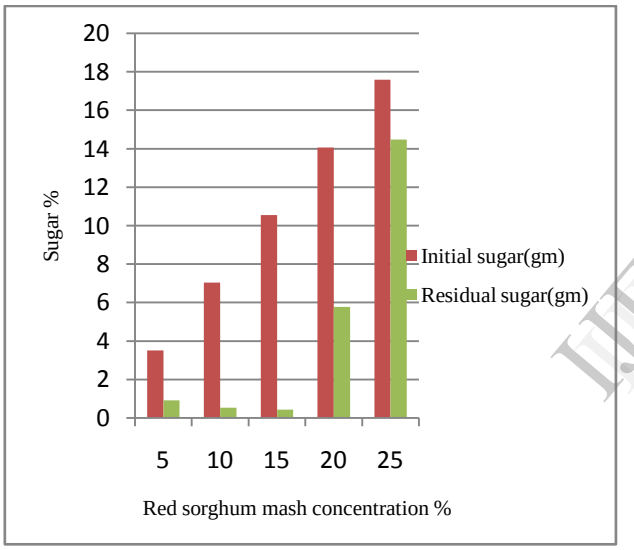

Figure 13 Effect of fermentation at 30^0 C and time 48 hrs. on sugar, determined by phenol sulphuric method. Values are mean of triplicate.

Fig. no. 13 showing that the maximum sugar was consumed at 15% mash concentration and minimum at 25% mash concentration.

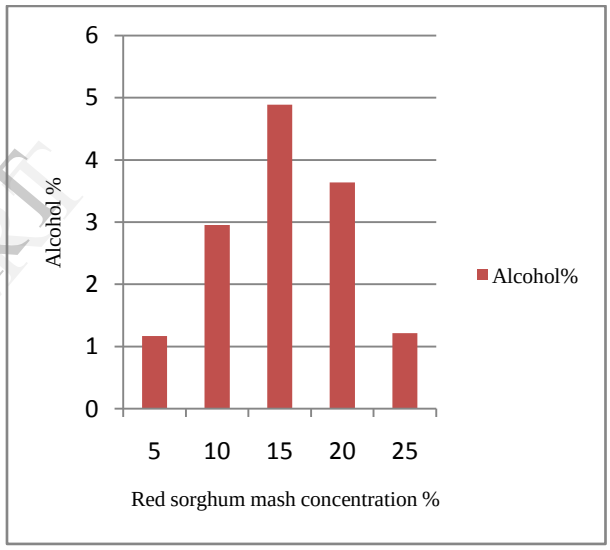

Figure 14 Effect of fermentation at 30^{0}C and time 48 hrs. on alcohol, determined by potassium dichromate method. Values are mean of triplicate.

Fig. no. 14 showing that the maximum alcohol was produced at 15% mash concentration and minimum at 25% mash concentration.

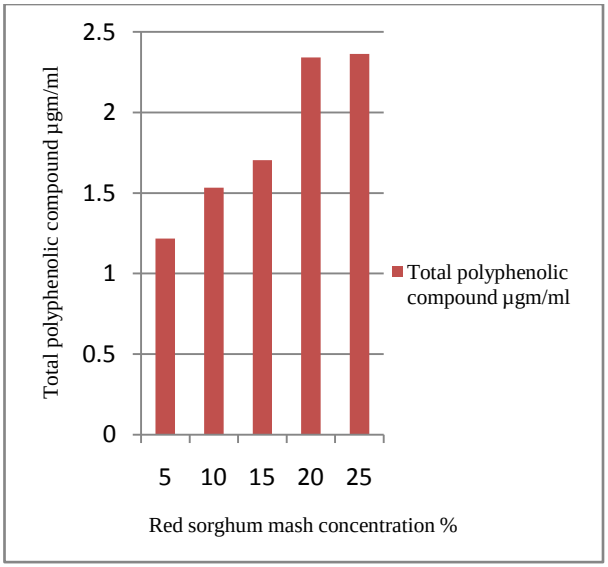

Figure 15 Effect of fermentation at 30^0c and time 48 hrs. on total polyphenolic compound, determined by Folin-Ciocalteu method. Values are mean of triplicate.

Fig. no. 15 showing that the maximum total polyphenolic compound found at 25% mash concentration and minimum at 5% mash concentration. But as compared to fig. no.11 the maximum effect on polyphenolic compound was at 15% mash concentration.

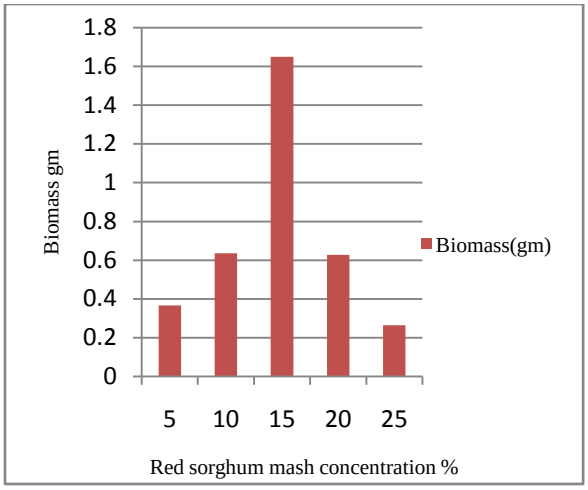

Figure 16 Effect of fermentation at 30^0c and time 48 hrs. on biomass, determined by Filtration method. Values are mean of triplicate.

Fig. no.16 showing that the maximum biomass was produced at 15% mash concentration and minimum at 25% mash concentration.

Effect of yeast fermentation at 35^0C and 24 hrs. on sugar, ethanol, polyphenolic compound and biomass

Table 5 Effect of yeast fermentation at 35^0C and 24 hrs. on sugar, ethanol, polyphenolic compound and biomass the values are mean of triplicate

Mash conc. (%)	Initial sugar (gm)	Residual sugar (gm)	Alcohol (%)	Yps	SCR	Total polyphenolic compound (µgm/ml)	Biomass (gm)
5	3.5159	2.7652	0.3094	0.4121	0.2135	1.1352	0.0142
10	7.0318	6.4445	0.3225	0.4543	0.0835	1.2743	0.0165
15	10.5477	9.275	0.5292	0.4158	0.1206	1.331	0.0334
20	14.0636	13.3227	0.3247	0.4382	0.0526	1.5787	0.0154
25	17.5795	16.9156	0.2961	0.446	0.0377	1.7287	0.0061

Table no. 5 showing that the maximum sugar was consumed; maximum alcohol production and high biomass were produced at 15% concentration. Maximum yield was at 10% mash concentration and maximum substrate conversion was at 5% mash concentration. The maximum polyphenolic compounds were observed at 25% mash concentration.

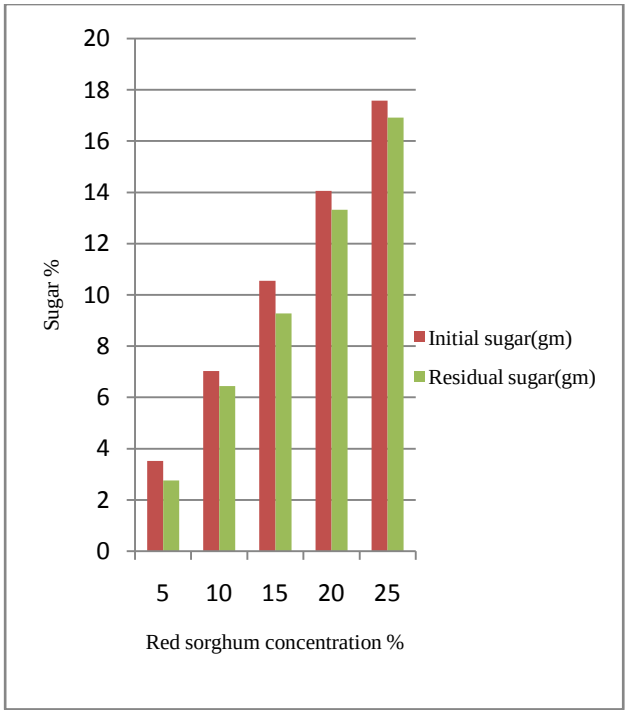

Figure 17 Effect of fermentation at 35^{0}C and time 24 hrs. on sugar, determined by phenol sulphuric method. Values are mean of triplicate

Fig. no. 17 showing that the maximum sugar was consumed at 15% mash concentration and minimum at 25% mash concentration.

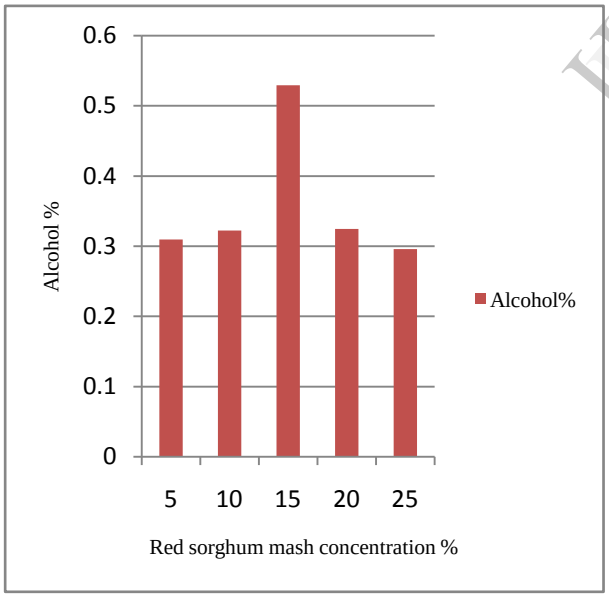

Figure 18 Effect of fermentation at 35^{0}C and time 24 hrs. on Alcohol, determined by potassium dichromate method. Values are mean of triplicate

Fig. no. 18 showing that the maximum alcohol was produced at 15% mash concentration and minimum at 25% mash concentration.

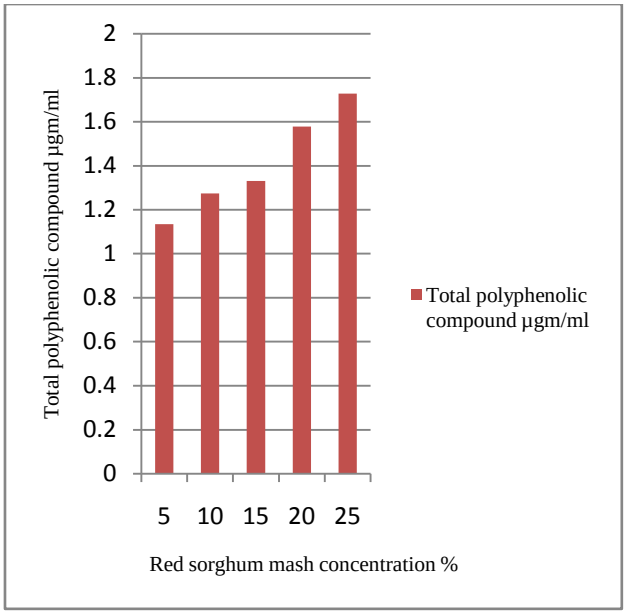

Figure 19 Effect of fermentation at 35^{0}C and time 24 hrs. on total polyphenolic compound, determined by Folin-Ciocalteu method. Values are mean of triplicate.

Fig. no. 19 showing that the maximum total polyphenolic compound found at 25% mash concentration and minimum at 5% mash concentration.

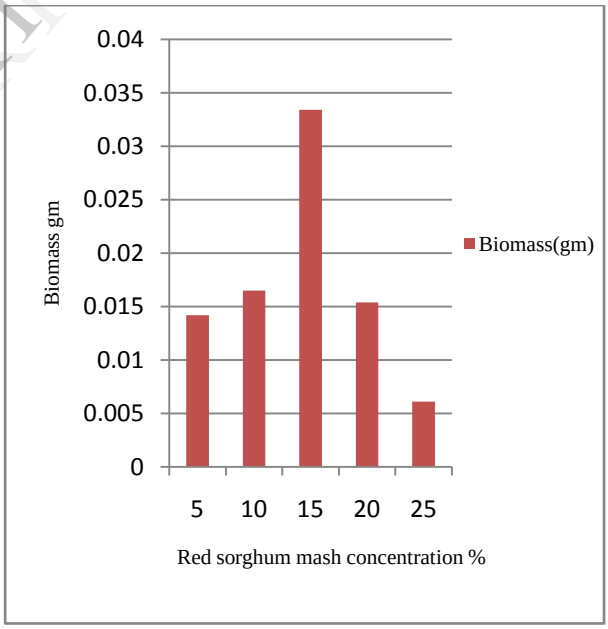

Figure 20 Effect of fermentation at 35^{0}C and time 24 hrs. on biomass, determined by filtration method. Values are mean of triplicate.

Fig. no.20 showing that the maximum biomass was produced at 15% mash concentration and minimum at 25% mash concentration.

Effect of yeast fermentation at 35^{0}C and 48 hrs. on sugar, ethanol, polyphenolic compound and biomass

Table 6 Effect of yeast fermentation at 35^{0}C and 48 hrs. on sugar, ethanol, polyphenolic compound and biomass the values are mean of triplicate

Mash conc. (%)	Initial sugar (gm)	Residual sugar (gm)	Alcohol (%)	Yps	SCR	Total polyphenolic compound (µgm/ml)	Biomass (gm)
5	3.5159	2.6941	0.3338	0.4061	0.2337	1.1644	0.0267
10	7.0318	6.1207	0.4028	0.4421	0.0911	1.2881	0.0238
15	10.5477	9.1781	0.5799	0.4234	0.1298	1.3516	0.1078
20	14.0636	13.2004	0.379	0.439	0.0653	1.5999	0.0193
25	17.5795	16.5758	0.323	0.3218	0.057	1.7536	0.0033

Table no. 6 showing that the maximum sugar was consumed; maximum alcohol production and high biomass was produced at 15% concentration. Maximum yield was at 10% mash concentration and maximum substrate conversion was at 5% mash concentration. The maximum polyphenolic compounds were observed at 25% mash concentration but maximum changes in total polyphenolic compound was observed at 15% mash concentration

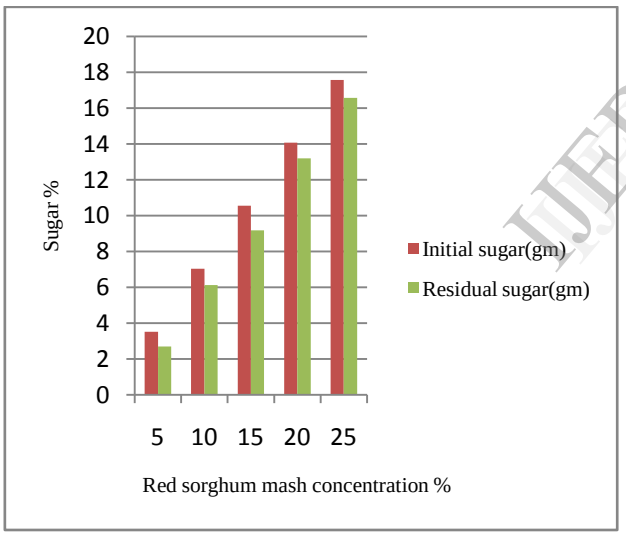

Figure 21 Effect of fermentation at 35^0 C and time 48 hrs. on sugar, determined by phenol sulphuric method. Values are mean of triplicate.

Fig. no. 21 showing that the maximum sugar was consumed at 15% mash concentration and minimum at 25% mash concentration.

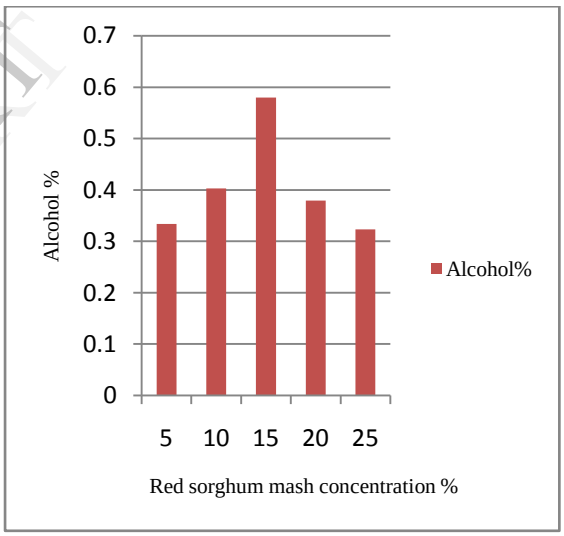

Figure 22 Effect of fermentation at 35^{0}C and time 24 hrs. on Alcohol, determined by potassium dichromate method. Values are mean of triplicate.

Fig. no. 22 showing that the maximum alcohol was produced at 15% mash concentration and minimum at 25% mash concentration.

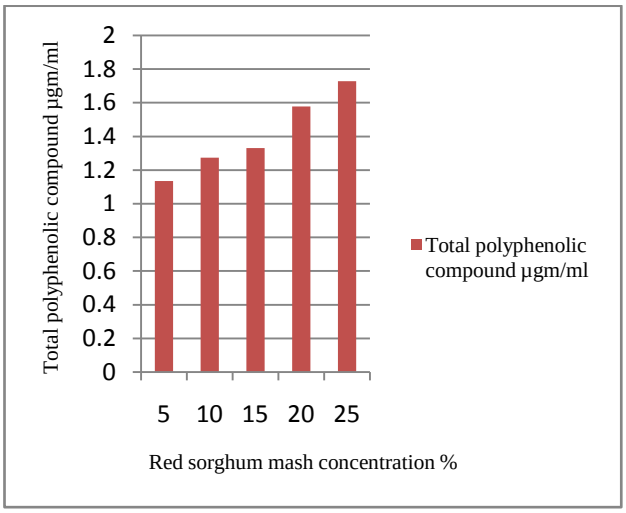

Figure 23 Effect of fermentation at 35^{0}C and time 48hrs. on total polyphenolic compound, determined by Folin-Ciocalteu method. Values are mean of triplicate.

Fig. no. 23 showing that the maximum total polyphenolic compound found at 25% mash concentration and minimum at 5% mash concentration. But as compared to fig. no. 19 the maximum effect on polyphenolic compound was at 15% mash concentration.

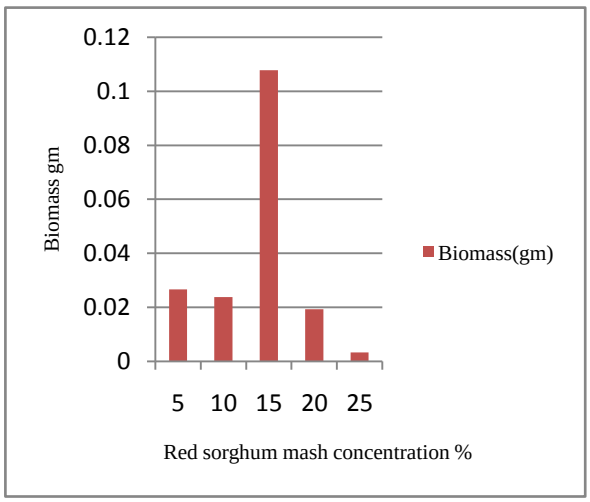

Figure 24 Effect of fermentation at 35^{0}C and time 48 hrs. on biomass, determined by filtration method. Values are mean of triplicate.

Fig. no.24 showing that the maximum biomass was produced at 15% mash concentration and minimum at 25% mash concentration.

IV. CONCLUSION

From the above study it was conclude that maximum sugar consumption, alcohol production, biomass production was occurred at 30^{0}C and 15% mash concentration. Maximum changes in total polyphenolic compound were observed at 30^{0}C temperature and 15% mash concentration. The same results were followed by fermentation. At 25^{0}C the consumption of sugar and production of alcohol and biomass was decreased as compared to 30^{0}C.At 35^{0}C there was no adequate consumption of sugar and production of alcohol and biomass.

V. REFERENCES

1. T. Sivakumar, P. Thangamathi, A. Mariashobana, T. Rathimeena, T. Shankar, 2014. Optimization of Invertase production using Saccharomyces Cerevisiae MTCC 170 under varying cultural conditions. Journal Of Advancement In Medical And Life Sciences, Volume1/Issue2 ISSN: 2348-294X, 2014, pp 1-8.
2. Sheorain V, Banka R, and Chavan M., 2000. Ethanol production from sorghum. Technical and institutional options for sorghum grain mold management: proceedings of an international consultation, 228-239.
3. Louise Svensson,Bonno Sekwati-Monang,Daise Lopes Lutz,Andreas Schieber, And Michael G. Ganzle, 2010 .Phenolics Acids and Flavonoids in Nonfermented and Fermented Red Sorghum (Sorghum bicolor (L.) Moench) J. Agric. Food Chem., 58, 9214–9220.
4. Van Sumere, C.F., Cottenie, J., De Greef, and Kint, J. 1972. Biochemical studies in relation to the possible germination regulatory role of naturally occuring coumarin and phenolics. RecentAdv.Phytochem., 4:165–221.
5. Shahidi, F. 2000. Antioxidants in food and food antioxidants. Nahrung, 44, 158–163.
6. Shahidi, F. 2002. Antioxidants in plants and oleaginous seeds, in Free Radicals in Food: Chemistry, Nutrition, and Health Effects, Morello, M.J., Shahidi, F., and Ho, C-T.,Eds., ACS Symposium Series 807. American Chemical Society. Washington, D.C., 162–175.
7. Ribereau-Gayon, P. 1972. Plant Phenolics, Oliver and Boyd, Edinburgh, U.K.
8. Deshpande, S.S., Sathe, S.K., Salunkhe, D.K., and Cornforth, D.P. 1982. Effects of dehullingon phytic acid, polyphenols, and enzyme inhbitors of dry beans (Phaseolus vulgaris L.). J. Food Sci., 47:1846–1850.
9. Strumeyer, D.H. and Malin, M.J. 1975. Condensed tannins in grain sorghum: isolation, fractionation, and characterizations. J. Agric. Food Chem., 23:909–914.
10. Charalambous, G. 1974. Progress report of flavor influence and determination of beer polyphenols. MBAA Tech. Q., 11:146–147.
11. Dadic, M., Van Gheluwe, J.E.A., and Valyi, Z. 1974. Formation of volatile carbonyls through the action of phenolics. MBAA Tech. Q., 11:164–165.
12. Gramshaw, J.W. 1969. Phenolics constituents of beer and brewing materials. IV. Anthocyanogens and catechins as haze precursors in beer. J. Inst. Brew., 75:61–83.
13. Fantozzi, P., Montanari, L., Mancini, F., Gasbarrini, A., Simoncini, M., Nardini, M., Ghiselli,A., and Scaccini, C. 1998. In vitro antioxidant capacity from wort to beer. Lebensm.Wiss. u-Technol., 31:221–227.
14. Dadic, M., Van Gheluwe, J.E.A., and Valyi, Z. 1971. Chemical constituents of nylon-66 beer adsorbate. II. Thin-layer chromatography of acidic and alkanoline hydrolysis products. J.Inst.Brewing,77:48–56.
15. Dadic, M. 1974b. Phenolics and beer staling. MBAA Tech. Q., 11:140–145.
16. Bohm, B.A. 1989. Chalcones and aurones, in Methods in Plant Biochemistry, vol.1, Plant Phenolics, Harborne, J.B., Ed., Academic Press, London, 237–281.
17. Drawert, F., Leupold, G., and Lessing, V. 1977. Gaschromatographische Analyse von phenolischen Verbindugenim Bier. Brauwissenschaft, 30:13–18.

18. Stevens, J.F., Taylor, A.W., Clawson, J.E., and Deinzer, M.L. 1999. Fate of xanthohumol and related prenylflavonoids from hops to beer. J. Agric. Food Chem., 47:2421–2428.
19. Stevens, J.F., Taylor, A.W., and Deinzer, M.L. 1999. Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. J. Chromatogr., 832:97–107.
20. Chikezie I. Ovvuama,1999, Brewing Beer with Sorghum,journal of institute of brewing, vol.1,23-34.
21. Beta, T, Rooney, L.W. & Waniska, R.D., Cereal Chemistry, 1995, 72,533.
22. Taylor, J.R.N. & Robbins, D.J., Journal of the Institute of Braving,1993, 99,413.
23. Manners, D.J., Brewers Digest, 1974,49, 56.
24. St Angelo, AJ. & Ory, R.L., 1975, Journal of Agriculture and Food Chemistry, 23,150.
25. Lin, Y., Wimer, L.T. & Huang, A.H.C., Plant Physiology, 1983, 73,460.
26. Nwanguma, B.C., Eze, M.O. & Ezenwa, O.O., journal of the Institute of Brewing, 1996,102, 39.
27. Findlay,W.P.K, , "Wainwright, T, 1971, Modern Brewing Technology," The Macmillan Press London, l21.
28. Solomon, B.O., Layokun, S.K., Idowu, A.O. & Ilori, M.O., 1994, Food Biotechnology, 8, 243.
29. Williams, M. B. and Reese, Darwin, "Colorimetric determination of ethyl alcohol," 1950. Anal. Chem., 22, 1556.
30. S. Suzanne Nielsen, 2010, food analysis laboratory manual, springer, 2nd edition, 47-55.
31. A. Sluiter, D. Hyman, C. Payne, and J. Wolfe,2008, Determination of Insoluble Solids in Pretreated Biomass Material, national renewable energy laboratory,4-6.
32. A. Sluiter, D. Hyman, C. Payne, and J. Wolfe,2008, Determination of Insoluble Solids in Pretreated Biomass Material, national renewable energy laboratory,4-6.
33. Folin, O. and Denis, W, 1912 on phosphotungstic-phosphomolybdic compounds as color reagents. J. Biol. Chem., 12:239–243.
34. Singleton, V.L. and Rossi, J.A. 1965, Colorimetry of total phenolics with phosphor molybdic phosphor tungstic acid, Am. J. Enol. Vitic., 16:155–158.
35. Hoff, J.F. and Singleton, K.I., 1977 A method for determination of tannin in foods by means of immobilized enzymes, J. Food Sci., 42:1566–1569.