* DAMISA D., ¹AMEH, J.B., AND ²OGBADU, L.J.

Dept. of Applied Science, C.S.T., Kaduna Polytechnic, Nigeria.

> ¹.Dept. of Microbiology, Ahmadu Bello University, Zaria.

> ²Dept. of Microbiology, **20** Benue State University, **20** Makurdi.

*To whom correspondence may be made.

ABSTRACT

The effect of Pear Millet, *Pennisetum americanum* (L), malt wort obtained by modified infusion method of mashing was investigated on the brewers yeast, *Saccharomyces uvarum*, growth and fermentation performance. Bud formation in the yeast was observed nine hours into the initiation of the fermentation process which slowed down towards the termination of the fermentation period with cells looking elongated and with irregular buds. Yeast generation time was high (32 to 40 hours) with low number of generation (5 to 8) and low growth rate 5.1×10^4). The percent acidity as acetic acid was low (50%) however, the wort pH was satisfactory (3.8). Malting regime was found to have direct correlation with yeast viable counts as the wort obtained from the 3 day regime malt gave the highest viable counts. Yeast viability was maintained for the three pitching cycles. Erythrodextrin and amylodextrin were largely the products of the unconverted starch in the 3 - day and 2 - day regime wort - derived malt respectively. Yeast flocculence in the wort. Alcohol percent by volume obtained was low (1.3% V.V). This cereal appears inadequate for beer production due to the low extract content of the wort.

INTRODUCTION and bus shows been explored and a nonpul solution

ACC control of the beer brewing industry in Nigeria represents a vital industry in the generation of income for the country. Barely malt has been the brewers choice for beer fermentation (MacGregor *et al.*, 1988). In recent times, it has become imperative to examine the suitability of worts derived from other cereals, such as Millets because the barley malt, from which wort was derived by the brewing industry, was banned by the Federal government in 1988.

The consequence of such ban in that majority of the breweries in the country are now operating below their total installed capacity (Odeyemi, 1984). Traditional brews have been made using worts derived from other cereals (Ekundayo, 1969). A one-hundred per-cent sorghum based (wort) beer has been produced (Aisien and Ghosh, 1978). Malting of Millets to derive worts for beer fermentation will obviously interest brewers in regions such as the sahelain zone where the crop find its main agricultural niche (Guillaument, 1980).

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In the traditional fermentation, the dregs of a previous brew are inoculated into the new wort to initiate the process - and since the fermentation process is uncontrolled, variation in the quality and stability of the products do exist (Sanni, 1993). Such brews normally contain unusually large populations of fermentative and beneficial organisms and very likely, some pathogens too (Odeyemi, 1984). An aqueous extract of malted cereal is an excellent substrate for many yeast (Crueger and Crueger, 1984).

Therefore the objective of this work was to ferment wort obtained from Millet malts that have been variously treated so as to assess its effect on growth and fermentative properties of brewer's yeast, *Saccharomyces uvarum*. This yeast is a bottom yeast that settle to the bottom of the fermentor at the end of the alcohol generating process, leaving the lager beer as a clear liquid (Hardwick, 1983). Recovered yeast after fermentation can be recycled up to twenty times before it needs to be replaced by a pure culture. There are several measures through which the composition of wort can be varied with respect to its malt so as to favour the colloidal stability of the beer (Moll, 1987). Once a successful brew has been made with yeast, the clean yeast drop from the final racking can be stored under beer in a sealed bottle and kept at 3°C to 5°C in a refrigerator (Harrison, *et al.*, 1987). Acidification power test (Kara, *et al.*, 1983) has been used to measure the fermentation performance (wort attenuation) of *S. cerevisiae*. The quantity of yeast in suspension depends on its flocculence, largering time and fermenter type (Moll, 1987).

MATERIALS AND METHODS

The yeast, *Saccharomyces uvarum* was obtained from Nigeria Breweries Plc Kaduna. Peptone yeast extract glucose broth prepared according to the methods of Demain and Solomon (1986) but with slight modifications was used to grow and assay the yeast cell to a population of 1.8 x 10^7 cells/ml. Each wort prepared from Millets that have been malted for 2 days and 3 days and subsequently Kilned at either 40°C, 45°C, 50°C and 60°C were inoculated with the yeast organism grown to this population. The wort was made using standard wort preparation procedure of infusion mashing (Analytical EBC III 1975), and fermented for nine days at room temperature. Fermented wort was filtered using Whatman No. 1 type filter paper.

The wort pH was determined using PYE UNICAM model pH meter. Extract yield was determined using the specific gravity values according to AOAC (1980). Yeast maphology was observed by wet mount using methylene blue stain. The yeast viable count was determined by plating an aliquot of the 10⁻⁶ dilution on Malt Extract Agar. The yeast dead cells was counted by adopting the improved Neubauer counting chamber method (Baker and Silverton, 1985) using WBC pipette. The yeast flocculence was determined by suspending the cell in acetate buffer according to Helm *et al.*, (1953). The iodine reaction test of the unconverted starch was determined using AOAC method (1980) by addition of 0.2M iodine solution to the liquor. Total acidity measured as acetic acid was determined by titrating the wort to end point with sodium hydroxide using phenolphthalein indicator (Pearson, 1976). The alcohol percent (V/V) was determined by the specific gravity values of the distillate (AOAC, 1980).

RESULTS and a second seco

The pH values was satisfactory for all the worts intended for fermentation and falls with the range of 5.0 to 5.3 (Tables 1 and 2). Kilning temperature and germination regime of malts from which the wort was derived has no effect on the pH values obtained. Extract yield of the wort was low in comparison to barley wort. At the time of pitching the wort (time, T=0), the yeast cells were ovoid in shape. Formation of bud was observed nine hours later;

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Table 1:

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which slowed tremendously towards the end of fermentation and cells now looking elongated with irregular buds. The wort obtained from the 3 - day germinated Millet (Kilned at 55° C) gave the highest percentage of increase in yeast viable count (Table 2). Generation number of the yeast was generally low (5 to 7) and generation time was high (32 to 40 hours, Tables 1 and 2). Percentage of yeast dead cell for each wort sample was low per litre (2% to 6%). The yeast cells showed satisfactory flocculence although the degree of flocculation was influence by the wort constituents which is a factor of malting regime and kilning temperature. Unconverted starch test using iodine showed the presence of amylodextrin (Table 1) and crythrodextrin (Table 2). Total acetic acid value was low (0.002% to 0.003%). Highest percentage of alcohol yield after distilling the wort was 0.53 V/V for the 2 - day regime (Table 1) and 1.34 V/V for the 3 - day regime (Table 2). There is a positive correlation between kilning temperature of malt, malting regime and the ethanol content of worts derived from the malt. The fermentative ability of the cells was maintained for the three pitching cycles.

Wort Derived from 2 Day Regime Malt. blos blos blos blos blos blos

Wort Properties	Kilning Temperature (°C)				
Balas, P.J. and Diletters, R.G., (17)	40	45	50	55	60
Wort pH	4,4	5.0	4.6 /	4.0	4.2
Wort pH (after fermentation)	3.6	3.6	3.5	3.4	3.8
Yeast viable count (cfu/ml)	7.5x10 ⁸	7.9x10 ⁸	7.7×10^{8}	9.5x10 ⁸	1.6x10 ⁹
Number of Generation (n)	5.4	5.5	5.4	5.7	6.5
Generation Time (hrs)	40.1	40.0	40.0	38	33.4
Growth rate (k) $(x10^{-4})$	4.2	4.2	4.2	4.4	5.0
Yeast dead cell/L (%)	6	4	5	4	5
Flocculence (%)	55	60	56	65	54
Iodine reaction for unconverted starch	AMYLODEXTRIN AMYLODEXTRIN				
Percent acidity (acetic acid	0.002	0.002	0.002	0.002	0.002
Hot water extract (%)	11.6	12.0	12.5	14.2	12.1
Percent alcohol (V/V)	0.53	0.55	0.54	0.88	0.68

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Wort Properties	Kilning Temperature (°C)					
	40	45	50	55	60	
Wort pH Wort pH (after fermentation) Yeast viable count (cfu/ml) Number of Generation (n) Generation Time (hrs) Growth rate (k) (x10 ⁻⁴) Yeast dead cell/L (%) Flocculence (%)	5.1 3.3 1.7x10 ⁹ 6.6 33.0 5.1 3 70	5.2 3.7 1.8x10 ⁹ 6.6 33.0 5.1 3 74	5.0 3.5 1.8x10 ⁹ 6.6 33.0 5.1 2 75	5.2 3.8 1.9x10 ⁹ 6.7 32.1 5.2 2 79	5.1 3.6 1.1x10 ⁵ 5.9 36.4 4.6 3 65	
Iodine reaction for unconverted starch	ERYTHRODEXTRIN ERYTHRODEXTRIN					
Percent acidity (acetic acid Hot water extract (%) Percent alcohol (V/V)	0.003 12.7 0.94	0.003 13.2 1.01	0.003 14.7 1.34	0.003 14.6 1.09	0.003 12.2 1.09	

Table 2: Wort Derived from 3 Day Regime Malt.

DISCUSSION

The wort pH was satisfactory and in the acidic region and this is to be expected because of the acidic nature of the enzymes elaborated by the grain during the germination process (Mallesh and Desikachar, 1986). Lower pH values would have had a deleterious effect on sugar formation particularly for the low diastatic power malts (Novellie, 1966). Formation of buds by the yeast cells significantly decreased with prolonged fermentation. This may be due to the inhibitory effects of certain organic acids produced into the medium in the course of fermentation (Maiorella et al., 1983). Fast maltose production from starch is principally the action of B-amylase (Nout and Davies, 1982). In this study, wort obtained from the 3 - day regime malt, kilned at between 50°C and 55°C gave the maximum yeast yield in terms of viable count. They also demonstrated increased recovery on Yeast Extract Agar. Difficulty in obtaining reasonable counts of the yeast cells is likely to be the clumping problem due to flocculation. Yeast generation number was low probably due to the nutrient composition of the wort which does not support fast growth, even though the composition is assured for yeast nutrition due to the low dead cell number recorded. The degree of yeast flocculence was affected by wort compostion because the wort derived from the 3 - day regime malt flocculated better than the wort obtained from the 2 - day regime malt. The presence of amyloderxtins and eythrodextrins in the wort may be due to the enzymatic composition of the malt. Millet Malt contains more x-amylase than B-amylase (Malleshi and Desikachara, 1986) and maltose production is largely the influence of B-amylase. The acetic acid content of the fermented wort was lower than that for beer from barley wort. There is a direct relationship between the malting regime and alcohol content. Low alcohol yield is likely to be due to the low extract of the wort.

CONCLUSION

Use of 100% wort alone obtained from the malted Millet for the production of lager beer does not appear adequate due to the low extract content. Wort composition was satisfactory as the crop of yeast recovered was high and responded well in fermentation to the

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three pitching cycles. The low generation number, high generation time and low growth rate suggests that even though the wort composition was satisfactory, it does not enhance the vigorous multiplication of cell within a short time. This is a disadvantage to the brewer since fermentation are operated for maximum efficiency and minimum production cost. Factors such as total soluble nitrogen, colour flavour and filterability and clarity are also important to be determined if worts obtained from Pearl Millet malts are to be used alone for conventional larger beers. In the second se

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