European Journal of Food Research & Review
1(1): 15-22, 2011

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African Breadfruit (*Treculia africana*) Seed as Adjunct in Ethanol Production

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Research Article

Received 15th December 2010
Accepted 22nd December 2010
Available online 13th January 2011

ABSTRACT

African breadfruit seeds have the potentials as carbon source for ethanol production with a carbohydrate value of 72.19%. On malting the seeds at 28±2°C for 9 days it yielded a 96% germination capacity and total malting loss of 25.70%. Grain dormancy was broken by the second day of malting. Malted breadfruit seeds were ground and defatted to 0.78% fat content. Full fat breadfruit and defatted breadfruit flours were used as adjuncts in the ratio of 3:5 (adjuncts: barley). Fermentation parameters such as wort fermentable sugar, specific gravity, extract yield and ethanol were measured over the 9 days of fermentation. Extract yields were 12.59, 9.66 and 11.23% while ethanol production was 5.79, 6.39 and 6.10% for wort from defatted breadfruit, full fat breadfruit and maize, respectively.

Keywords: [African breadfruit, Treculia africana, ethanol, adjunct in ethanol production, fungamyl and termamyl, Saccharomyces uvarrum, wort, fermentation, malting, mashing;]

1. INTRODUCTION

Traditionally, the raw materials for beer production are barley, hops, water, and yeast, but most brewers commonly supplement with alternative sources of soluble sugars in many countries. The cost of malting, the loss of weight of the original barley during malting, and the high enzyme levels in most malts have led to the wide spread use of comparatively inexpensive sources of starch. During the alcoholic fermentation, the contribution of aroma compounds from other ingredients to the final beer flavor depends on the wort composition, on the yeast strain, and mainly on the process conditions (Giovani et al., 2009).

Adjuncts are classified with reference to where they are used in brewing process, their origin and the extent to which processing is involved in their preparation. Adjuncts contribute virtually no enzymes to the wort so hydrolysis of their starch depends on those enzymes present in the malt to which they are added.

Numerous materials are used as adjuncts but the principal adjuncts are sorghum, rice, maize grits and cereal starches. Bvochora et al. (2000) reported use of malted and unmalted milled sorghum grain from sorghum cultivars DC-75 and SV-2 for ethanol fermentation. While deMancilha et al. (1984) reported increased ethanol production with three yeast strains from sweet sorghum, Tianwei et al. (2008)

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investigated the use of mixtures of sweet stem sorghum juice and sorghum grain in ethanol production under normal and very high gravity (VHG) fermentation conditions. Lakkana et al. (2009) also investigated various carbon adjuncts and nitrogen sources in ethanol production from sweet sorghum juice by *Saccharomyces cerevisiae* NP01 under very high gravity (VHG) fermentation.

Pyler and Thomas (1986) reported that 38% of total materials used in brewing (excluding hops) in the USA was contributed by adjuncts of this 46.5% was corn grits, 31.4% rice and 0.7% barley. Sugars and syrups accounted for the remaining 21.4%. Wudiri (1991) reported that only 25% of maize produced in Nigeria is consumed directly by human beings, 50-60% as livestock feeds and 20-50% as brewery raw materials, the balance being used in other industries such as textile and starch. Apart from the use of these sorghum adjuncts for ethanol production, Giovani et al. (2009) showed that banana can also be a raw material favorable to alcoholic fermentation being rich in carbohydrates and minerals and providing low acidity.

The problem associated with sorghum as an alternative to barley in beer production has geared into finding other alternative fermentable carbon sources capable of microbial transformation into ethanol. African breadfruit (*Treculia africana*) seed with about 40% carbohydrate as starch and 17% as protein (Okafor, 1980) offers a potential source of this fermentable carbon and a good base for biomass growth. African breadfruit belongs to the taxonomic family Moraceae, genus Treculia. It is a food item in parts of Southern West Africa. The seeds may be eaten after roasting or frying and many delicacies, including porridges are commonly produced from the seed. Moreover pastries, weaning foods, breakfast cereals and beverages can be developed for the production of pharmaceuticals, vegetable oils, soaps, perfumes and paints.

Okechukwu et al. (1984) investigated the malting characteristics of the seeds for possible use in alcohol fermentation and produced products with undesirable rancid odours. In this study, the African breadfruit seeds being rich in carbohydrates and minerals were investigated as raw material favorable to alcoholic fermentation. The objective was to evaluate the performance of wort adjusted with fullfat or defatted African breadfruit seed as malted or unmalted concentrations.

2. MATERIAL AND METHODS

2.1 SOURCE OF MATERIALS

Two kilograms of fresh whole breadfruit seeds were obtained from Umuahia main market. One hundred grams of maize grits were obtained from the Nigerian Breweries Plc Enugu while *Saccharomyces uvarrum*, barley grits and amyloglucosidase (AMG) (Nordisk, Denmark) were obtained from Golden Guinea Breweries Plc Umuahia.

2.2 MALTING

One and a half kilograms of African breadfruit seeds were washed and steeped in tap water at 28 ± 2^0C for 24h. The liquor was continually changed at 8h intervals to reduce microbial load and to prevent suffocation of the respiring embryo due to depletion of oxygen in the liquor.

At the end of the steeping period, the liquor was drained off and seeds cast on jute bag (previously sterilized), spread on laboratory bench for germination at room temperature (28 ± 2^0C). Kilning in a forced draught oven at 45^0C for 12h terminated malting which lasted for 9 days, which was later increased to 55^0C for 6h. The dry shelled sprouted seeds were ground to fine powder using an electric mill.

2.3 DEFATTING AND MASHING

Fifty grams of the malted breadfruit grit was defatted by the solvent extraction method using petroleum ether to a final fat content of 0.78%. The infusion method of mashing for wort production was adopted with minor modifications. About 30g each of full fat and defatted breadfruit and maize grits were mixed with 150ml of distilled water at 50^oC respectively in a three 250ml conical flasks to yield grit to water ratio of 1:5. Mashing procedure lasted for 10min. About 0.5ml each of *fungamyl* and *termamyl* was added to the mixtures and allowed to stand for another 30min with continuous stirring.

The resulting wort was heated up to 55^{o}C and held at that temperature for 30min. A gradual increase in temperature continued up to 65^{0}C and allowed for 45min with addition of 0.5ml amyloglycosidase (AMG). The wort was finally heated up to 80^{o}C and held for 10min. It was mixed with pre-mashed barley at 50^{o}C (grits to water ratio of 1:4). The temperature of the final mix rose to 60^{o}C while it was allowed to stand for 10min with continuous stirring. Saccharification test was performed for the three samples. The mash was than filtered through a 25cm Whatman filter paper to yield wort, which was made up to 450ml through sparging. About 2.60g of sucrose was added to the wort samples to beef up their original extract. The wort was further heated to 80^{o}C for 5 min and cooled.

A measure of 10ml each of yeast slurry was added to the wort samples and allowed to ferment for 9 days. Samples were drawn daily from the fermenter and analyzed for pH, titratable acidity, ethanol, specific gravity, brix and extract yield (Kunze, 1996).

2.4 CHEMICAL ANALYSIS

Proximate analysis of malted and unmalted African breadfruit seeds was carried out using the procedure described by AOAC (1995). The parameters were moisture content, protein (N x 0.25), ash, fat, crude fibre and carbohydrate (by difference).

2.5 DETERMINATION OF MALTING CHARACTERISTICS

The germination capacity of the malted grains was calculated by counting number of germinated seeds in an unbiased selection of 100 seeds and presented as

$$\frac{100 - \text{ungerminated seeds} \times 100}{100} \quad (1)$$

Malting loss was determined as $\frac{100(g_w - C_w)}{g_w}$ (2)

Where g_w is 100 grain weight of original seed and C is 1000 corn weight of the malt.

2.6 STATISTICAL ANALYSIS

All data were analyzed using analysis of variance and level of significance at 0.05% was adopted.

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3. RESULTS AND DISCUSSION

3.1 PROXIMATE COMPOSITION OF BREADFRUIT SEED

The higher protein value (15.75%) of the malted African breadfruit seeds compared to that of the unmalted (13.56%) (Table 1) could be explained from the activities of malting enzymes, which degrade bound hemicellulosic-protein-starch complex of the endosperm.

Table 1. Proximate composition of malted and unmalted breadfruit seed (%)

Sample	Moisture	Protein*	Fat	Fibre	Ash	Carb**
Malted Breadfruit	6.00^{a}	15.75^{a}	2.20^{a}	1.46^{a}	2.40^{a}	72.19^{a}
Unmalted Breadfruit	8.00^{b}	13.56^{b}	1.30^{b}	1.90^{a}	2.80^{a}	72.44^{a}

**N x 6.25, ** by difference, means in the same column with different superscripts are significantly (P > 0.05) different*.

This caused a release of protein, starch and other macromolecular compounds. These breakdown macro-products are further broken down to simpler materials such as glucose and amino acids by hydrolytic

enzymes for utilization by the respiring embryo (Hough, et al. 1981). The slight reduction in carbohydrate from 72.44-72.19% with malting can be attributed to respiration activities of the hydrolytic enzymes and depletion in carbohydrate content as germination progressed.

3.2 FERMENTATION PARAMETERS

Fig. 1 shows percentage germination capacity and malting loss of the African breadfruit seeds at 28±2^{0}C. The low germination capacity of 0-4% observed in the first 2 days of malting indicates the difficulty of the breadfruit seed in breaking its dormancy. This could be attributed to the inability of the embryo to gain access to oxygen for malting. Once this dormancy was broken a linear increase in germination capacity was observed. Losses, which ranged from 2.60% on the first day to 25.70% on the last day of malting, could be attributed to rootlet growth, leaching of some materials from the grains into the steep liquor and respiration of the embryo.

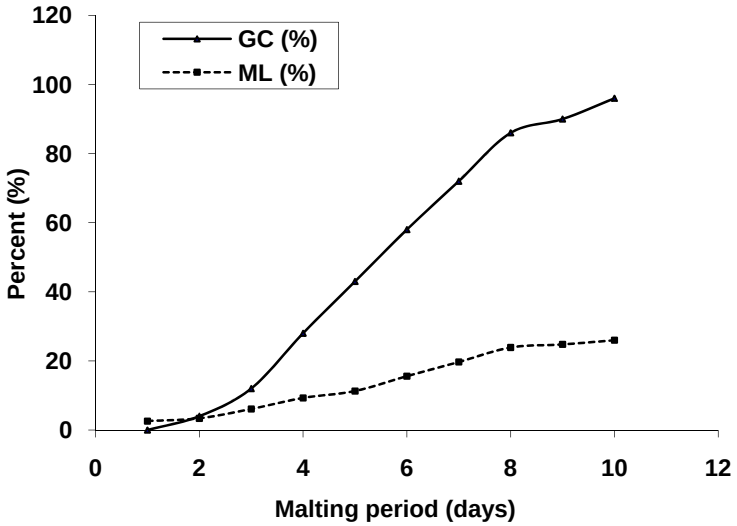

Fig 1. Germination capacity (GC) and malting loss (ML) of breadfruit seeds

The bulk of respiration losses and all the rootlets originate from the metabolic activity of the embryo. Increase in malting loss was therefore expected since rootlet development increased with days of germination. Excessive malting loss is however, disadvantageous to the malster but the values in this work are lower than 30.30% reported for sorghum malt (Jayatissa et al, 1980).

Table 2 shows the fermentation parameters of full fat and defatted breadfruit worts and maize grit. The trend in the fermentation parameters shows that while specific gravity ranged from 1.005 in the defatted sample to 0.066 in the maize, acidity of the worts became fairly constant after six days of malting ranging from pH 3.60 in defatted sample to 3.37 in maize sample.

Table 2. Parameters of wort as adjuncts in ethanol production at the end of 9 day fermentation period (%)

Sample	Extract	Ethanol	Specific gravity
Fullfat breadfruit	12.59^a	5.79^a	1.007^a
Defatted breadfruit	9.66^a	6.39^a	1.005^a
Fullfat maize	11.23^b	6.10^b	1.006^a

Means in the same column with different superscripts are significantly (P>0.05) different.

The specific gravity was higher in full fat than in defatted breadfruit and maize at the end of fermentation. This probably occurred because of the higher quantity of oil present in the full fat breadfruit wort. The gradual drop in the extract after the dormancy period (Fig. 2) indicated gradual nutrient utilization in the three wort samples by yeast. However, the higher remaining extract obtained in the full fat breadfruit wort showed that the activity of the yeast was hampered by the high fat. Extract utilization was found to be more in defatted (90.20%) than in other wort samples. Fat hampers yeast activity. Yeast activity and distribution throughout the fermentation depended amongst other factors on sedimentation, flocculation and yeast head formation.

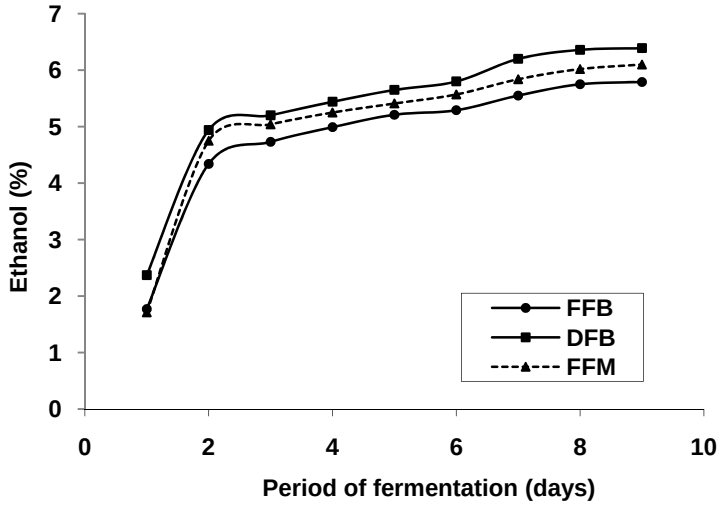

Fig. 2. Effect of fermentation on ethanol production from fullfat breadfruit (FFB), defatted breadfruit (DFB) and fullfat maize (FFM)

3.3 YIELDS OF ETHANOL

Increase in quantity of ethanol (Fig 3) indicates utilization of fermentable extract and sugar by the added yeast. Ethanol yield was least in full fat breadfruit probably because of the inability of the yeast to perform effectively because of presence of oil. deMancilha et al.(1984) used three different mutants and wild types of yeast strains for ethanol production from sweet sorghum juice, and reported a final alcohol concentration of 13·28% (w/v) after 48h, corresponding to an SCE of 93·57%. The authors concluded that *Saccharomyces cerevisiae* IZ 1716 Mutant 10 produced a significantly greater yield of ethanol from sweet sorghum juice containing 26% sugar than the wild type.

In this study, *Saccharomyces uvarrum* was used to produce ethanol from fullfat and defatted African breadfruit seed worts. A final ethanol concentration of 6.395 for defatted breadfruit and 5.79% for fullfat breadfruit suggests the presence of much utilizable monosaccharide after yeast-enzyme breakdown. Under VHG conditions, maximum ethanol levels were about 16.8% (v/v) and 11% (v/v) for media containing malted and unmalted milled sorghum grain, respectively (Bvochora et al., 2000).

The authors carried out the fermentation for 96 h using malted and unmalted milled sorghum grain from sorghum cultivars DC-75 and SV-2. It was further observed that although fermentation did not occur to completion, levels of ethanol obtained under VHG conditions were three times higher than the levels obtained under normal fermentation conditions. Giovani et al. (2009) reported that addition of banana changed the concentration of all-malt wort or weight of the extract resulting to an increase in ethanol production, with approximately 0.4 g/g ethanol yield.

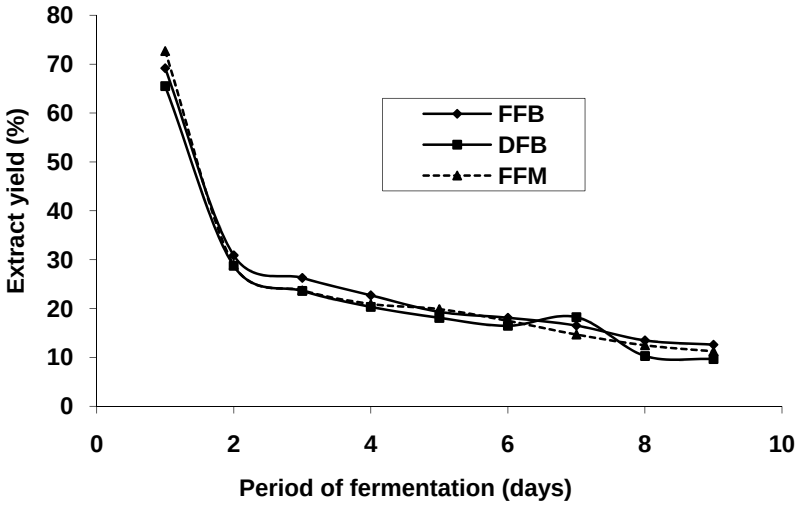

Fig. 3. Effect of fermentation on the extract yield of wort samples from fullfat breadfruit (FFB), defatted breadfruit (DFB) and fullfat maize (FFM)

The fall in pH (Table 3) from initial value of about 5.61 to 3.58 in fullfat breadfruit precludes the growth of acid intolerant pathogenic organisms such as *C. botulinum.* The implication of these trends is that ethanol production was significantly ($P \leq 0.05$) higher in defatted breadfruit sample (6.39%) than in either fullfat breadfruit (5.79%) or fullfat maize (6.0%) (Fig. 2), probably due to inability of the yeast to perform effectively in the fullfat samples because of presence of oil as fat extract.

Table 3. Fermentation parameters of breadfruit, and maize adjuncts

FP* (Days)	Specific gravity			Titratable acidity (mg ml^{-1})			pH		
	FFB**	DFB***	FFM****	FFB	DFB	FFM	FFB	DFB	FFM
0	1.064	1.068	1.066	0.024	0.024	0.019	5.61	5.62	5.33
1	1.047	1.044	1.049	0.062	0.058	0.048	4.22	4.06	3.96
2	1.022	1.019	1.020	0.092	0.089	0.077	3.89	3.93	3.78
3	1.018	1.017	1.017	0.140	0.110	0.090	3.69	3.72	3.58
4	1.015	1.014	1.015	0.189	0.156	0.100	3.63	3.67	3.46
5	1.013	1.012	1.013	0.190	0.158	0.120	3.60	3.62	3.40
6	1.012	1.011	1.017	0.194	0.162	0.138	3.59	3.60	3.38
7	1.010	1.007	1.009	0.198	0.168	0.144	3.58	3.60	3.37
8	1.008	1.005	1.007	0.120	0.169	0.146	3.58	3.59	3.37
9	1.007	1.005	1.006	0.120	0.169	0.146	3.58	3.59	3.37

**FP is fermentation period, **FFB is full fat breadfruit, ***DFB is defatted breadfruit and ****FFM is full fat maize*

Increase in quantity of ethanol with malting (Fig. 2) in all the samples indicates utilization of fermentable extract and sugar such as utilization of monosaccharide after yeast-enzyme breakdown by the added yeast. Lakkana et al. (2009) reported that when sugarcane molasses was used as an adjunct, the juice under the same conditions gave the maximum ethanol concentration, productivity and yield with the values of 109.34g g^{-1}, 1.52g g^{-1} and 0.45g g^{-1}, respectively. In addition, ammonium sulphate was not suitable for use as a nitrogen supplement in the sweet sorghum juice for ethanol production since it caused the reduction in ethanol concentration and yield for approximately 14% when compared to those of the unsupplemented juices.

A reverse trend in extract yield was observed in Fig. 3. From this figure it could be seen that defatted breadfruit sample had the least extract yield (10.0%) at the end of malting compared to fullfat (11.0%) and maize (13.0%) worts. The gradual drops in the extract yield after the dormancy period (Fig. 2) indicated gradual nutrient utilization in the three wort samples by yeast. Similarly, the higher remaining extract obtained in the fullfat breadfruit and maize worts showed that oil hampered the activity of the yeast in these samples. The yeast utilized between 87.83% fermentatable sugar in fullfat breadfruit to 91.59% in the defatted breadfruit worts.

Fig. 4 shows the sharp utilization of fermentatable sugar from initial values of 16.42% (defatted breadfruit), 15.84% (fullfat breadfruit) and 16.12% (fullfat maize) to 1.38, 1.93 and 1.78%, respectively at the end of fermentation. Defatting breadfruit seed prior to malting therefore is beneficial in ethanol production from African breadfruit seeds.

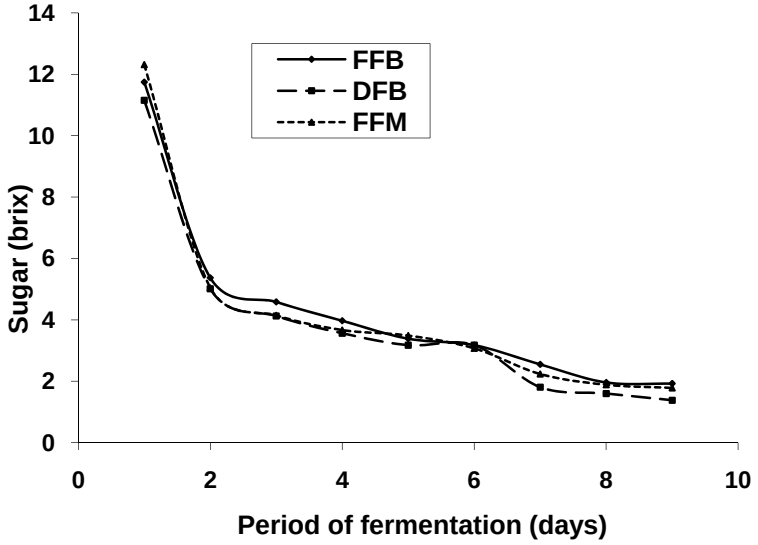

Fig. 4. Sugar utilization during fermentation of wort samples of fullfat breadfruit (FFB), defatted breadfruit (DFB) and fullfat maize (FFM)

4. CONCLUSION

With more than 70% carbohydrate as indicated in this study, the potentials of African breadfruit seeds as source of fermentable sugar could be efficiently utilized as an adjunct in brewing. Moreover, defatting the seeds prior to malting will enhance better sugar utilization and hence ethanol yields. Thus, it can be concluded that African breadfruit seed can be used as an adjunct in brewing methods, helping in the development of new products as well as in obtaining concentrated worts.

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