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Full Length Research Paper

Effect of supplementation of African breadfruit (*Treculia africana*) hulls with organic wastes on growth characteristics of *Saccharomyces cerevisiae*

Titus U. Nwabueze* and Ugochinyere Otunwa

Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria.

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African breadfruit (*Treculia africana*) hulls were supplemented at different levels with other organic food processing wastes (orange, plantain, cassava and soybean). Optimum supplementation of 40:60 (breadfruit hulls to each waste) was obtained. Proximate and mineral composition of the unsupplemented and the supplemented waste were determined. A synthetic medium with chemical nutrients was prepared. The growth performance and other fermenting parameters were monitored on test strain (*Saccharomyces cerevisiae*) incubated at predetermined pH (3.8) and temperature (27°C) conditions. Supplementation increased crude protein (4.38 – 8.31 g/100 g) and mineral content (Na, K and P) in the waste, and sugar concentration (4.44 – 4.56 g/l), sugar conversion efficiency (28.39 – 31.56%), biomass yield (11.0 – 30.1 g/l) and single cell protein (28.98- 34.26 g/100g) in the hydrolysate. Supplementation of the African breadfruit hulls with organic wastes was an improvement over the unsupplemented breadfruit medium and could promote use in the animal feed industry.

Key words: African breadfruit hulls, *Saccharomyces cerevisiae*, supplementation, *Treculia africana*.

INTRODUCTION

The agro-based industries generate a significant amount of solid wastes with appreciable potential problems. These wastes include peels from plantain, banana and oranges, bran and husk from rice, straw from cereals and hulls from African breadfruit (*Treculia africana*) and soybean. Such wastes if not appropriately handled, could pose disposal problems with consequent effects on the environment and harmonious relationship between the biotic and abiotic components of the ecosystem (Adoki and Adoki, 1993; Moo-Young, 1977; Ziino et al., 1999). The wastes could be disposed of by incineration or by other costly break-down systems or even by illegal dumping, all to the detriment of the environment (Scerra et al., 1999; Nwabueze and Nwabueze, 2001).

Many developing countries have nutritional problems and produce excess of waste materials rich in carbohydrates. In the wake of technological advancement, waste accumulation has assumed serious dimensions not only in the Western World but also in the Third World Countries. Over the years there have been considerable efforts to develop techniques for the recovery and utilization of the biopolymers in the wastes. This became necessary considering the fact that those wastes contain appreciable quantities of dry matter, crude protein, fibre, ether extract, minerals and high molecular weight cellulose and hemicelluloses and could be obtained at minimal cost.

This research was aimed at assessing the potentials of *Saccharomyces cerevisiae* cultured in African breadfruit (*Treculia africana*) hull hydrolysates. The objective was to upgrade the nutritional value of the breadfruit hulls with other food processing wastes from orange, plantain, cas-

*Corresponding authors E-mail: teeubueze@yahoo.co.uk.

sava and soybean processing. It was also to assess the improvement in cell yield and other growth performance characteristics in relation to the unsupplemented breadfruit hull and the more expensive synthetic medium. It was viewed that success of this objective could promote the use of African breadfruit hull supplements for animal feed.

MATERIALS AND METHODS

Raw materials

The raw materials used for this research were classified into three categories: unsupplemented African breadfruit hulls, supplemented African breadfruit wastes and synthetic medium. The unsupplemented African breadfruit hulls were obtained as wastes from breadfruit processing units in Umuahia main market, Abia State, Nigeria. It is composed of hulls mixed with some pieces of dehulled cotyledons. The supplemented African breadfruit waste consisted of African breadfruit and soybean hulls, and orange, plantain and cassava peels. These were obtained from their respective small scale processors in Umuahia. The synthetic medium consisted of calcium chloride ($CaCl_2$), magnesium sulphate ($MgSO_4$), zinc sulphate ($ZnSO_4$) and copper sulphate ($CuSO_4$). The test organism used was of the yeast strain, *Saccharomyces cerevisiae*, obtained from Golden Guinea Breweries Nigeria Plc., Umuahia.

Preparation of waste materials

All the waste materials were pre-treated as described by Adoki and Adoki (1993) with slight modification. About 50 g of each waste material were sundried for 5 days. The dried samples were milled into flour in a commercial attrition mill. The flour from each sample was separately packaged in corked plastic container and stored at room temperature for later use.

Preparation of waste hydrolysates and synthetic medium

The unsupplemented African breadfruit flour was covered with enough HCl and heated for 20 min to boil through reflux. It was then left for 14 h after which it was washed with distilled water and filtered as described by Ferrer et al. (1996). Similar treatment was given to each waste to obtain various hydrolysates. Different supplemented African breadfruit hydrolysates were prepared by mixing African breadfruit hydrolysate with each waste hydrolysate in the ratio of 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10 (African breadfruit hydrolysate : waste hydrolysate).

The synthetic medium was obtained by mixing different concentrations (g/l) of $CaCl_2$ (0.5), $MgSO_4$ (0.5), $ZnSO_4$ (0.2) and $CuSO_4$ (0.15).

Determination of optimum growth conditions

A preliminary experiment to determine the optimum pH, temperature and incubation time for growth of *S. cerevisiae* was determined in the African breadfruit hydrolysate and used in all growth experiments. *S. cerevisiae* was grown under the pre-determined incubation conditions in the different supplemented breadfruit hydrolysates (10:90 to 90:10) to obtain optimum level of breadfruit hull supplementation. This level was adopted as the supplemented breadfruit hydrolysates (SBH).

The media (synthetic medium or hydrolysates) were dispensed into 500 ml capacity shake flasks. The pH was adjusted (Cruickshank et al, 1975) or left unadjusted depending on the original value. The entrances of the flasks were plugged with cotton wool to provide micro aerobic conditions (Konlani et al., 1996; Nwabueze and Nwabueze, 2001), autoclaved (Barnstead sterilizer) at 121°C and allowed to cool to room temperature. 5 g/l of *S. cerevisiae* was inoculated into each flask as seed culture. The flasks were incubated at pre-determined optimum growth conditions of temperature (27°C) and pH (Konlani et al., 1996; Ziino et al., 1999) for 8 h.

Proximate composition

The proximate and mineral composition of each waste and the optimum supplemented breadfruit (40:60) were determined. Proximate composition was determined for moisture, crude protein (N x 6.25) (Kjeldahl method), fat (soxhlet method), crude fibre and ash according to AOAC (1995). Total carbohydrate was determined by difference.

Mineral composition was determined after wet digesting with concentrated nitric acid and perchloric acid. Mg and Ca were determined with an atomic absorption spectrophotometer (Perkin Elmer, 2380, USA) as described by Attia et al. (1994). Na and K were determined by flame photometric method as reported in literature (Lawal, 1986). A flame photometer (Jenway, PFP7) was used to read off absorbance at 569 and 767 nm for Na and K, respectively. P was colorimetrically determined using Vanado-molybdate (yellow) method and reading off the absorbance in a Fisher Electrophotometer II at 400 nm.

Determination of fermentation parameters

Fermentation parameters such as ethanol, sugar and biomass yield were determined as fermentation progressed while single cell protein of pooled biomass was determined at the end of fermentation. Ethanol was measured by gas chromatography as described by Konlani et al. (1996). Residual sugar in the medium as carbon source for yeast was determined as glucose by the colorimetric method using 3,6-dinitrosalicylic acid (DNS). I ml of diluted supernatant left after the separation of the biomass was mixed with I ml of DNS and heated to 100°C for 5 min. The reaction was stopped in ice and optical density was read at 540 nm against a glucose standard graph.

The microbial profiles of African breadfruit hulls supplemented with each organic waste (orange, plantain, cassava peels and soybean hulls) were monitored for all the supplementations. 1 g of sample was serially diluted with saline. Serially diluted samples (0.1 ml) were plated on potato dextrose agar. The performance of each waste supplement with African breadfruit hulls hydrolysate was expressed as total viable counts and recorded as colony forming units (cfu).

The biomass was harvested for the period of fermentation and estimated for dry cell weight. This was determined according to the method described by Nwabueze and Nwabueze (2001). At the end of fermentation, the culture medium was filtered through a funnel containing filter paper (Whatman No. 1) of known weight. The filter-retained biomass from consecutive batches was pooled together, lyophilized at 30°C and weighed. When the dry weights of three consecutive batches fell within ±5%, samples were obtained and stored at –25°C prior to analysis (Ziino et al., 1999) Biomass yield ($Y_{x/s}$) was calculated according to the method reported by Konlani et al. (1996) as $Y_{x/s}$ = g dry biomass/g glucose used. The protein content as single cell protein (SCP) was determined (AOAC, 1995) on cell biomass.

RESULTS AND DISCUSSION

Supplementation levels for African breadfruit hulls

S. cerevisiae, grown on PDA, developed creamy-white colonies which were elongated in shape. Optimal growth conditions were pH 3.8, 27°C and 4 h incubation time. Levels of supplementation of the African breadfruit hulls

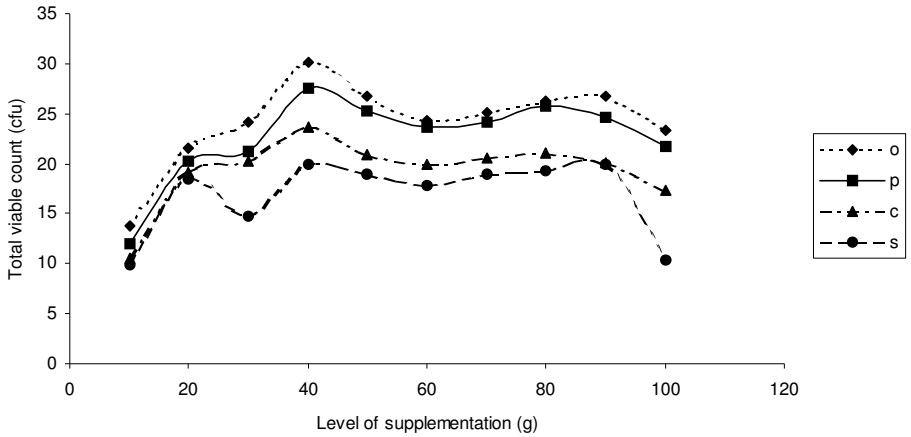

Figure 1. Levels of supplementation of the African breadfruit hulls with organic wastes (o = orange, p = plantain, c = cassava peels and s = soybean hulls). Optimum supplementations of African breadfruit hulls were obtained at 60 g African breadfruit hulls and 40 g (each waste).

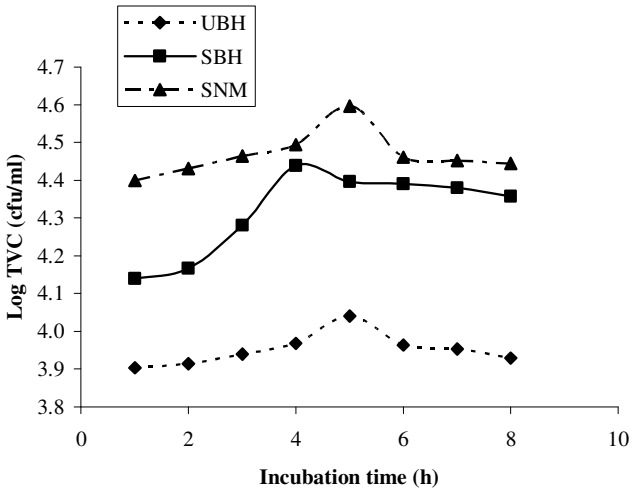

Figure 2. Growth of *S. cerevisiae* in substrate media (pH 3.5, 27°C and incubation time of 8 h). UBH = Unsupplemented breadfruit hydrolysate, SBH = supplemented breadfruit hydrolysate and SNM = synthetic medium.

with orange, plantain, cassava and soybean wastes were shown Figure 1. Optimum level of supplementation was obtained at 40 g African breadfruit hulls and 60 g other wastes. At this ratio optimum microbial cell count of 27.10, 26.50, 23.70 and 19.90 x 10^3 cfu for orange, plantain, cassava and soybean supplements, respectively. These values were higher than the optimum obtained with the unsupplemented breadfruit after 8 h of incubation by 2.5, 2.4, 2.2 and 1.8 times, respectively. The nutritional composition of these wastes could be responsible for the differences in optimal cell counts given the same test strain, culture conditions and supplemental base.

Effect of supplementation of African breadfruit hulls with organic wastes on the growth of *S. cerevisiae* is shown in Figure 2. Generally growth was higher in the supplemented hydrolysate than in the unsupplemented breadfruit hydrolysate. Optimum cell count of 11 x 10^3 cfu/ml was obtained at 5 h period of incubation in the unsupplemented breadfruit hydrolysate. Higher optimal value of 27.50 x 10^3 cfu/ml for the supplemented breadfruit hydrolysate was obtained at a shorter period of incubation (4 h).

Supplementation with organic wastes improved optimum *S. cerevisiae* performance by 43.64%. This suggests that supplementing the African breadfruit hulls with organic wastes have some benefits of utilizing more nutrients and energy sources for microbial growth. Similar observation had been reported in literature for wastes derived from orange (*Citrus sinensis*), banana (*Musa* spp) and plantain (*Musa sapientum*)(Adoki and Adoki, 1993). Waste supplementation has other environmental benefit of catering for the disposal of a number of agro-wastes while at the same time providing high microbial cell growth that can be of use in animal feeding.

Proximate and mineral composition

The proximate and mineral composition of dry hulls and peels of organic wastes are shown in Table 1. The hulls had lower moisture content (8.60 – 9.30 g/100 g) than the peels (10.30 – 13.70 g/100 g). Crude protein values of the wastes ranged from 4.38 g/100 g in the unsupplemented African breadfruit to 13.56 g/100 g in soybean

Table 1. Proximate and mineral composition of food processing wastes (hulls and peels).

Parameter g/100g	Hulls		Peels			(40:60 wastes)
	UB	SB	PT	CA	OR	SBW
Moisture	9.30	8.60	13.70	10.30	12.70	12.00
*Crude protein	4.38	13.56	5.06	5.25	10.09	8.31
Crude fibre	6.36	5.96	8.44	5.84	6.84	6.28
Fat	2.16	4.32	8.63	1.74	3.84	6.08
Ash	5.20	6.0	8.65	6.30	5.80	7.90
**Carbohydrate	72.60	61.83	55.52	70.57	60.73	59.43
Mg (mg/100g)	0.41	0.49	0.34	0.54	0.07	0.22
Ca (mg/100g)	0.64	0.32	0.28	0.84	0.88	0.44
Na (mg/100g)	0.03	0.08	0.12	0.04	0.10	0.10
K (mg/100g)	0.05	0.10	0.15	0.06	0.07	0.10
P (mg/100g)	0.37	0.60	0.51	0.44	0.98	0.99

*N x 6.25.
**By difference.
UB= unsupplimented breadfruit, SB = soybean, PT = plantain, CA = cassava OR = orange and SBW = supplimented breadfruit waste.

hulls. This protein content of the hulls of breadfruit is about 70% lower than the range in the dehulled seed. The wastes exhibited high carbohydrate composition ranging from 55.52 g/100 g in plantain peels to 72.60 g/100 g in the breadfruit hulls. The high carbohydrate content of the African breadfruit hull constitutes an important carbon source for microbial growth. Ca content ranges from 0.28 mg/100 g in plantain peels to 0.88 mg/100 g in orange peels, while P ranges from 0.37 mg/100 g in African breadfruit to 0.98 mg/100 g in orange peels. Supplementing the African breadfruit hulls with 60 g/100 g organic wastes increased crude protein from 4.38 – 8.31 g/100 g, and Na, K and P from 0.03 – 0.10, 0.05 – 0.10 and 0.37 – 0.99 mg/100 g, respectively.

Substrate media and cell biomass parameters

Analysis of the supplemented and unsupplemented hydrolysates at the end of 8 h period of incubation is shown in Table 2. Initial substrate crude protein values were 4.38 and 8.31 g/100 g while sugar concentrations as glucose were 4.44 and 4.56 g/l for unsupplemented and supplemented breadfruit hydrosates, respectively. The sugar conversion efficiency was 27.48, 31.58 and 44.41% in the unsupplemented, supplemented hydrolysates and synthetic medium, respectively.

Biomass yield increased with supplementation from 11.0 g/l in the unsupplemented to 30.10 g/l in the supplemented hydrolysates. Similarly SCP increased from 28.98 g/100 g in the unsupplemented to 34.26 g/100 g in the supplemented hydrolysate. The biomass yield and SCP from these hydrolysates are lower than those from synthetic medium by 42 and 20%, respectively. This could be due to the differences in composition between organic hydrolysates and chemical medium. When similar observation was reported for sorghum hydrolysate, Rose (1987) attributed the difference to the presence of tannins or some mineral salts in excess in the hydrolysate which could have inhibited yeast growth. In this research, the increased nutrient value in the supplemented hydrolysate particularly the crude protein could be responsible for the better yeast growth over the unsupplemented hydrolysate in addition to possible intense microbial activity as explained by Scerra et al. (1999).

Fermentation parameters

Fermentation parameters such as glucose concentration and ethanol production from the hydrolysates and synthetic medium are also shown in Table 2. A portion of the sugar was converted into ethanol in all the substrates. This portion increased with increased sugar concentration as glucose. Glucose concentration declined sharply at optimum incubation time from 4.440 to 1.007, 4.550 to 3.114 and 5.726 to 4.438 g/l in the unsupplemented hydroysate, supplemented hydrolysate and synthetic medium, respectively. This corresponded to equally increase in ethanol production from 0.304 to 1.326, 1.222 to 1.326 and 1.224 to 1.530 g/l for unsupplemented hydrolysate, supplemented hydrolysates and synthetic medium, respectively. At the end of the 8 h incubation at pH 3.8 and 27°C, it was observed that glucose concentration fell to a minimum of 1.005, 3.114 and 3.10 g/l, while ethanol reached a maximum value of 1.428, 1.428 and 1.734 g/l for unsupplemented, supplemented hydrolysates and synthetic medium, respectively.

Table 2. Analysis of substrate media and cell biomass of *S. cerevisiae*[a].

Substrate media	SNM	UBH	SBH
*Crude protein (g/100 g)	4.38	8.31	ND
Initial sugar concentration (g/l)	4.44	4.56	5.72
Utilized sugar (g/l)	1.22	1.44	2.54
Sugar conversion efficiency (%)	27.48	31.58	44.41
Mean specific gravity	1.01	1.02	1.02
Ethanol production (g/l)	0.34	1.22	1.22
Biomass yield (x 10^3 cfu/ml)	11.00	30.10	31.20
Biomass volumetric weight (g/l)	1.20	1.23	1.53
*Single cell protein (g/100 g)	28.98	34.26	41.21

[a]Analysis at the end of 8 h fermentation, pH 3.8, 27°C.
*N x 6.25.
ND = not determined, UBH = unsupplemented breadfruit hydroylsate, SBH = supplemented breadfruit hydrolysate and SNM = synthetic medium.

Biomass volumetric weight across the fermenting broth was highest in synthetic medium, ranging from 1.201 in unsupplemented hydrolysates to 1.530 g/l in the synthetic medium.

Since most saprophytic bacteria could grow at pH as low as 4.4 (Cruickshank et al., 1975) the pH (Ziino et al., 1999; Konlani et al., 1996) obtained in this research coupled with increasing level of ethanol production in the fermentation broth could limit or reduce bacteria contamination. Konlani et al. (1996) who made similar observation reported this situation as an advantage in rural settings where total asepsis is difficult to realize.

Conclusion

Adaptation of wastes from African breadfruit processing indicated good potentials as source of carbon for growth of *S. cerevisiae*. The study showed that supplementation with other organic wastes from orange, plantain, cassava and soybean processing upgraded the nutritional value of African breadfruit hull hydrolysate. It improved growth performance of the *S. cerevisiae*, sugar conversion into biomass and SCP as well as increased the potential for ethanol production. The supplemented African breadfruit waste can serve as a cheap alternative compared to the more expensive synthetic medium for biomass and SCP production in animal feed.

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