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PROTEIN ENRICHMENT OF SPENT SORGHUM RESIDUE USING *Candida* species and *Saccharomyces cerevisiae*.

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Abstract

Three different yeast isolates were used to improve the nutritional value of spent sorghum, a byproduct obtained during the production of a local drink "Akamo" in Nigeria. The yeast isolates used were *Saccharomyces cerevisiae* (dry yeast), *Candida* A1 isolated from locally processed milk "nono" and *Candida* A2 isolated from a local beverage "burukutu". Using *S. cerevisiae* and treated spent sorghum at 4.0g/100 ml, the optimum period of fermentation, 11th day of fermentation was determined. The optimum concentration of spent sorghum for protein enrichment with *S. cerevisiae* was 7.5g/100 ml. The protein content of unfermented sorghum (4.1-4.5% of dry matter) increased significantly on 11th day of fermentation for all the isolates (10.0 -12.5% of dry matter). The ether extract content of the unfermented spent sorghum also increased from 2.0% to 6.0% during fermentation by *S. cerevisiae, Candida* A1 and *Candida* A2 respectively. The crude protein and the ether extract contents of fermented spent sorghum are comparable to the levels found in most animal feeds available in the market. So inclusion of this nutritionally enriched byproduct in animal diet may be recommended preceded by animal trials.

Key words: *S.cerevisiae*, *Candida* sp., sorghum, protein enrichment, animal feed.

Introduction

Various yeasts strain are said to play a dominant role where biotechnology is applied to nutritional improvement of wastes. Yeast can rapidly grow on different wastes as substrates including sugar molasses, spent sulfite liquor, straw, wood waste, agricultural starchy food such as grains and potatoes fruit wastes, methanol, ethanol, alkanes and gas oil (Wainwright, 1992). Specific examples are production of single sell protein using *Candida utilis* and cassava starch effluent as substrate *Geotrichum candidum* and corn wastes as substrates, *Kleokera apiculata* and apple pomace as substrates *Saccharomyces cerevisiae* and molasses as substrates, *Schwaniomyces castelli* and molasses and starch wastes as substrates, *Kluyveromyces fragilis* and milk whey coconut water as substrate (Rahmat *et al* 1995; Dasilva *et al*., 1987; Wainwright,1992; Daniyan *et al*.,2000) . *Candida* yeast was cultivated from alkane with a growth rate comparable to that obtained from glucose and with a conversion factor of 100 dry yeast per 100g. of paraffin consumed (Dasilva *et al*.,1987).

Maize, (Zeamays), guinea corn (*Sorghum guinea*) and millet (*Penisatum Americana*) constitute the major crops in Nigeria (Oyenuga,1968). These are used for making akamo, a popular local drink. On dry matter basis millet has the highest protein content followed by sorghum and maize but the reverse is the situation for the soluble carbohydrate content or the total ash content. Millet grain has the highest oil content compared to maize and sorghum. An attempt was made here to improve the protein content of spent

crushed sorghum, the residue obtained during akamo production, using yeast isolates namely *Saccharomyces cerevisiae* and *Candida* sp.

Materials and Methods

Collection and Preparation of samples

Spent Sorghum residue

Sorghum (*Sorghum guineanse*) was purchased from Bosso Market, Minna, Nigeria. Five hundred grams of clean sorghum was soaked for 12 hours. The fermented sorghum after drainage was milled and sieved. After sieving, the filtrate was kept for akamo production and the residue was sundried and kept in oven for 6 hours at 60°C. The dried residue was kept under dry condition for further use.

Microbial strains used

Dried yeast (source: Vahine Professional, France) was used as the source of *S. cerevisiae*. Morphological and biochemical tests were also done to identify *S. cerevisiae*. *Candida* sp. A1 and *Candida* sp. A2 were isolated from two local drinks namely Burukutu and Nono using the procedure described by Hudson and Sherwood, (1997). *S. cerevisiae* and the isolated *Candida* strains were maintained on PDA slants for further use.

Preparation of fermentation sample

Five hundred milliliter of distilled water was added to 20 g of dried sorghum residue and boiled until the residue became semisolid and was autoclaved at 126°C for 10 minutes and allowed to cool at room temperature. After autoclaving, two portions slurry of the sorghum residue at concentration of 10g/100ml and 50g/100ml with water were made for fermentation processes.

Inoculum's preparation

5 ml of treated slurry (10g/100 ml) was added to 20 ml of sterile basal medium (composition: g/ l. $MgSO_4.7H_2O$- 0.2; NaCl- 0.1; NH_4Cl 2.5; KH_2PO_4 1.2; Na_2HPO_4 – 0.05; $FeCl_3$ –0.05; $MoSO_4$- 0.1, difco yeast extract- 0.1, $CuSO_4$-0.1. pH 5.1) in a 100 ml conical flask. A loopful of culture from a slant of *S. cerevisiae* was inoculated into the medium and incubated for 72-96 hours at 30°C.

Determination of optimum, fermentation period and concentration of spent sorghum residue

To 10 ml slurry of sorghum residue (10 g/100 ml) in a 100 ml capacity conical flask, 15 ml of sterile basal medium and 5 ml of inoculum were added. The flask was then incubated at 30°C for 15 days to for fermentation process to take place.

pH, dry matter and crude protein content determination:

pH, dry matter and protein content were determined at different time intervals of the fermentation. **pH** was determined, using a pH meter.
For the determination of **dry matter**, the samples at different time intervals were washed three times with sterile distilled water and filtered. The solid residue was dried to constant weight using an oven at 60°C and the weight of the dry matter was finally determined using a metler balance.
To determine the crude protein content, total nitrogen content was determined using Kjeldahl method and the **crude protein content** was determined by multiplying the value by 6.25 (Bermner, 1965).

Optimum slurry concentration
Optimum slurry concentration for protein enrichment was determined using different concentrations of spent sorghum slurry; 2.5 g/100 ml, 5.0 g/100 ml, 7.5g/ 100 ml and 10 g/100 ml in fermentation medium.

Fermentations of spent sorghum residues with *S. cerevisiae* and *Candida A1 and Candida A2.*
Fermentations were carried out in 250 ml Erlenmeyer flasks containing 50 ml of the fermentation medium inoculated with 5 ml inoculum. Duplicate flasks were used for each isolate. The flasks were then incubated at 27°C for 12 days.
At different time intervals, pH , dry matter, and crude protein content were determined. The moisture content (%), the fat content (%) and the ash content (%) were determined as described by A.O.A.C (1980).

Results
The results of the optimum cultural conditions for fermentation using *S. cerevisiae* is as shown in Table 1.

Table1: **Determination of optimum fermentation period using *S. cerevisiae***

Fermentation period (days)	pH, dry matter and protein content of slurry		
	pH	Dry matter(%)	Protein(%)
0	5.10	2.38	2.0
5	3.50	1.78	4.5
7	3.30	1.23	6.4
11	3.22	0.95	7.2
15	2.88	0.75	6.2

There is a decrease in pH and dry matter as fermentation proceeds. The crude protein content (%) increases to a maximum on the 11th day of fermentation and begin to decrease from the 12th day. The 11th day period was then chosen as the optimum cultural condition for fermentation. Statistical analysis using Pearson correlation coefficient (c) showed that protein concentration increases as dry matter concentration decreases and it is significant at p of F at 0.05 (c is -0.94 at p of F 0.05).
The optimum concentration of spent sorghum slurry for fermentation (see Table 2) was 7.5%.

Table 2: **Determination of optimum concentration of spent sorghum for protein enrichment by *S. cerevisiae***

*SP (%)	fermentation period (days)									
	#0th		5th		7th		11th		12th	
	D	P	D	P	D	P	D	P	D	P
0	-	2.0	-	1.9	-	1.9	-	2.2	-	2.5
2.5	2.38	4.0	2.29	4.5	2.20	5.8	2.10	6.4	2.00	6.0
5.0	3.08	4.5	2.89	7.5	2.65	5.4	2.40	8.5	2.40	9.0
7.5	3.21	5.0	2.95	8.6	2.80	10.2	2.38	12.0	2.34	12.2
10.0	3.48	5.6	3.00	7.5	2.75	9.0	2.45	10.2	2.02	10.8

* SP- spent sorghum #D- Dry matter (%) P- Protein content (%)

Protein production is significantly related to concentration of spent sorghum slurry (c is 0.88 for 5th day, 0.90 for 7th and 11th days, and 0.93 at 12th day at p of F 0.05).
The results of pH, dry matter, protein and fat contents of fermented spent sorghum residue with *S. cerevisiae, Candida* A1 and *Candida* A2 at different days of fermentation were as shown in Tables 3, 4 and 5).

Table 3: **Dry matter and protein content of spent sorghum at different time of fermentation using different yeast isolates**

Yeast strain used	*fermentation period (days)									
	#0		5th		7th		11th		12th	
	D	P	D	P	D	P	D	P	D	P
S. cerevisiae	3.12	4.10	2.75	9.2	2.39	11.0	1.40	12.5	1.30	12.7
Candida A1	3.38	4.30	2.96	5.9	2.50	9.0	1.85	10.0	1.70	10.0
Candida A2	3.48	4.50	2.83	7.5	2.42	8.5	1.50	9.0	1.42	9.1

* D- dry matter (%), P- protein content (%)

Table 4: **Statistical analysis showing the relationship of organisms in case of protein production.**

*Organism	*PrLsmean	Days	Pr LSmean
Sc	9.90	0	4.30
Ca1	7.84	5	7.53
Ca2	7.72	7	9.50
		11	10.50
		12	10.60
Prob. of F	0.05		0.05
S.E	0.44		0.56
L.S.D	1.8266		1.4149

*Sc- *Saccharomyces cerevisiae,* Ca1 – *Candida* A1 , Ca2- *Candida* A2
PrLsmean – Protein LSmean

The results of the ash content, pH and fat content of fermented sorghum residue with the different isolates are as shown in Table 5.

Table 5: **pH, fat and ash content of spent sorghum at different time of fermentation using different yeast isolates**

Yeast isolate	!* fermentation period (days)											
	5th			7th			11th			12th		
	p	f	a	p	f	a	p	f	a	p	f	a
S. cerevisiae	3.88	4.0	1.5	3.95	6.0	1.4	3.22	6.0	1.4	3.20	6.2	1.3
Candida A1	2.96	4.0	1.5	3.34	5.0	1.4	3.30	5.8	1.3	3.33	6.0	1.2
Candida A2	2.88	4.0	1.5	2.75	6.0	1.5	2.65	6.2	1.4	2.60	6.6	1.4

* p, f and a represent pH, fat content (%) and ash content (%) .

The values of pH, fat (%) and ash content (%) of spent sorghum on 0 day of fermentation are 5.10, 2.0 and 1.7 respectively.

There was a general decrease in the, pH, ash and fat contents in all the fermentation media.

Discussion

Yeasts are nutritionally good sources of protein and B vitamins. Production of protein using different Yeast strains, e.g. *S. cerevisiae and Candida utilis* fermentation on wastes may be associated with better economics because of their ability to assimilate a wide variety of carbon and nitrogen compounds under relatively simple fermentation conditions. *Candida arborea* and *Oidium lactis* also have been utilized to produce feed yeast on a commercial scale (Casida, 1984). By applying recycling method biomass of *Pleurotus ostreatus* have been produced using refinery effluent as substrate and the preliminary result has certified the high nutritional value (22%) and the complete safety when tested on animals (De and Ayeleke,2000).

Optimal conditions for yeast production vary with the yeast employed and the substrates used. Grain wastes like rice wastes, sorghum, millet wastes generally are sources of carbohydrate which literally mean simple sugars and disaccharides, maltose and hexose. Nitrogen is added in the form of ammonium salts.

The optimum pH should be kept on the acid side usually 4.5 and 6.0. In this study, using *S. cerevisiae*, the optimal conditions for fermentation were determined. The pH range was found to be between 5.0 – 5.1 and the optimum period of fermentation was on the 11th day of fermentation. There is decrease in protein content of spent sorghum on 15th day of fermentation. This decrease in protein content may due to cell lysis. The concentration of fermentable sugar is maintained at a level not higher than that necessary for good yield of cells. Too little substrates encourage alcohol production rather than growth, and too much favors increased respiration and heat reduction and hence lowered yield f the yeast cells. The optimum concentration of spent sorghum residue was 7.5 gm/100 ml of fermentation medium. The dry matter content of spent sorghum residue was decreasing during fermentation by *S. cerevisiae, Candida* A1 and *Candida* A2. This might be due to the constant utilization of nutritional materials (mainly carbohydrate) by the yeast isolates and also probably as a result of losses of organic materials in gaseous forms during fermentation (Edward, 1990). Crude protein content of unfermented spent sorghum residue was 4.1-4.5% of dry matter. This value increases significantly during the fermentation process with all the three isolates. Noteworthy, however, is the high value of protein content (12.5% of dry matter) obtained from fermentation with *S. cerevisiae*. This level of protein is adequate for normal growth and production of both layers and broilers' chicken (Oyenuga, V.A. (1968). Using *Candida* A1 and *Candida* A 2, the level of protein is 10% and 9.1% of dry matter respectively. However, modifying the physio-chemical parameters for fermentation and proper nitrogen supplement may increase the protein content of fermented spent sorghum residue. Ether extract is 2% of dry matter for the unfermented spent sorghum residue. This value increased from 2 % to 6 % during fermentation with, S. *cerevisiae, Candida* A 1 and *Candida* A 2. This value was comparable to ether extract content of most animal feed stuffs except those of oil seeds and animal origin (Oyengua, 1968). There was a general decrease in the ash content of unfermented spent sorghum residue during fermentation with the isolates.

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