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Development of Starter Cultures for Enriching Ogi, A West African Fermented Cereal Gruel

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Fermented corn gruel is often used as a dietary staple in West Africa. The fermented gruel, called "ogi" in Nigeria, is popular across all age, ethnic and income groups. When used as a weaning food it becomes a primary source of nutrients to the infant. Unfortunately, corn, like other grains, is relatively low in protein value and in other nutrients. In addition, ogi preparation may cause a considerable loss in the original food value of corn (1-3).

Benefits from lactic-fermented cereals for infants are reported to include increased digestibility of energy and protein, and the improved availability of iron due to hydrolysis of phytates (4). Also, fermented corn gruel has been recommended for weanling infants in Ghana to help control diarrhea. In fact, fermented cereal gruel was found to be as effective as standard salt solutions for oral rehydration therapy (5).

Although the overall population is likely to suffer when nutritional deficiencies are common, preschool children are at greatest risk because of their critical needs for growth, including physical and mental development. Widespread malnutrition has been reported among children in Nigeria as evidenced by retarded growth and inadequate intakes of nutrients. Protein is often deficient in both quantity and quality, and B vitamins may be in short supply (1-3).

The goal of this research was to improve the protein quality and B vitamin content of ogi through selective fermentation. The technology developed in this project should have ready application in many countries of Africa and Asia where the staple food is fermented corn.

Ogi is still manufactured in Nigeria by the traditional wet milling of maize, sorghum or millet (6,7). Efforts to use industrial methods for production of instant ogi have not been successful commercially. Some work has been done on the biochemistry, microbiology and production methods of ogi (8). Many attempts have been made to modify the processing of ogi to enhance its nutritive quality. The influence of starting pH, fermentation time, and temperature on the nutritive value of cornmeal has been studied (9,10). Attempts were also made to use sprouted grains and proteolytic bacteria to improve the nutritive value of naturally fermented grains (11,12). Increases in relative nutritive value of cornmeal fermented by single organisms or their mixtures have been observed (13).

The potential benefit from modifying the amino acid content of cereals was demonstrated with opaque-2 corn. Lysine, the most limiting amino acid in corn, was found to be 25% higher and tryptophan 100% higher in the protein of opaque-2 compared to regular corn. Opaque-2 corn produced five times as much growth in weanling rats as regular Guatemalan corn with a 47% increase in protein efficiency ratio (PER). More significantly, nitrogen retention was found to be essentially equal when either opaque-2

corn or milk was fed to preschool children as the only source of protein (14). Children suffering from kwashiorkor, a severe protein deficiency disease, recovered when opaque-2 corn was given as their sole source of protein (15).

Materials and Methods

In the initial research for this project, Lactobacillus and yeast strains were checked for lysine production. However, ogi produced by these starter cultures had less lysine than unfermented corn. In the second phase of work, Lactobacillus plantarum and yeast strains were mutated to obtain increased lysine productivity. The mutants were used as starter cultures for ogi production and the lysine content was again measured. Other qualities such as riboflavin, niacin and thiamine concentrations of the ogi were also assessed. Enzyme production by mutants such as phytase and a-galactosidase activities were assayed and finally an attempt was made at producing dehydrated starter cultures using corn meal as carriers.

1. Mutagenesis for Lysine Production

One strain each of Lactobacillus X043 and yeast OY4 were grown up overnight in MRS broth. The cells were washed twice in 0.1M citrate buffer (pH 5.5) and resuspended in 4.0mls of the same buffer. A 1.0mg/ml solution of N-methyl-N-Nitrosoquanire was added to give a final concentration of about 50ug/ml. The tubes were incubated at 37°C for 30 minutes in a water bath. The cells were then removed by centrifugation and washed twice in 0.1M phosphate buffer (pH 7.0). The mutagenised cells were finally suspended in

1.0ml buffer and 0.1ml portions spread on a selective medium made up of yeast nitrogen base (without amino acids) agar containing 500ug/ml thialysine. Plates were incubated at 35°C for 2-5 days in a candle jar and thialysine resistant mutants were picked as lysine producers.

2. Evaluation of Lysine Producing Mutants

The mutants obtained from both Lactobacillus and yeast strains were then evaluated for lysine production, phytase activity and a-galactosidase activity.

(a) Lysine Production by Mutants

Lactobacillus and yeast mutants were grown up in 5.0mls of yeast nitrogen base without amino acids (17) containing 0.6% glucose at 35°C for 24 hours. The cells were removed by centrifugation. The supernatant was then used to determine the amount lysine secreted by the microorganism. The intracellular pool of lysine was obtained by resuspending bacterial pellets in 5mls distilled water and boiling in a water bath for 10 minutes. The resulting supernatant was used to determine the intracellular lysine concentration.

Supernatant for both extracellular and intracellular lysine concentrations were added to lysine assay medium and inoculated with P. acidilactici ATCC 8042. Absorbance values were obtained at 550 nM after 24 hrs incubation at 30°C. Lysine concentrations were extrapolated from a standard curve obtained by using standard lysine in place of culture supernatants.

(b) Phytase Production by Selected Mutants

Three mutants, M4, M5 and YM7, were selected for ogi production and screened for phytase activity.

MRS broth was supplemented with 1.55 g/L sodium phytate. The mixture was added to test tubes and autoclaved at 121°C for 15 mns. and inoculated with the mutant strains. The tubes were incubated for 5 days after which the phytate phosphorus was determined by the method of Lopez et al (16). The percentage digestion of phytate was calculated by comparing with uninoculated controls.

(c) Alpha-galactosidase Activity of Selected Mutants

Selected mutants were grown in a modified MRS broth (in which glucose is replaced by galactose) for 24 hours. The cells were removed by centrifugation and the supernatant used as crude enzyme extract. Two milliliters of crude enzyme extract were mixed with 1.0 ml of 1.0% (wt/vol) raffinose and incubated for 2 hours at 40°C. The reaction was terminated by adding 3 ml dinitrosalicylic acid reagent (DNS) and boiling in a water bath for 5 min. The tubes were cooled in cold water and then diluted with distilled water. The absorbance of the resultant solution was measured at 550 nm in a spectrophotometer. The blank was treated as for the test samples except that DNS was added before the raffinose solution. The amount of reducing sugar formed was calculated from a standard curve with known concentrations and glucose.

3. <u>Production of Ogi with Single and Mixed Bacterial Cultures</u>

To prevent the loss of nutrients as reported by Akinele (6) Ogi was produced using a modified method. The maize grains were

picked clean of debris, dehulled in a cereal-dehuller and milled into a fine flour. Two hundred grams of dehulled milled maize was mixed with 400 mls of sterile distilled water. The resulting slurry was then mixed with 10 mls of bacteria suspended in sterile distilled water. The ogi was then allowed to ferment for 72 hours at ambient temperature.

Mutants of Lactobacillus (M4 & M5) and yeast (YM7) were used singly and in combination with each other. The wild type organisms X043 and OY4 were also used to inoculate the slurries while an uninoculated control was also fermented along with the others. The ogi samples were than freeze-dried and stored at 4°C for analysis.

4. Analysis of Ogi Samples

The ogi samples thus obtained were analyzed for lysine, riboflavin, niacin, thiamine and phytate content.

(a) Preparation of samples for lysine and vitamin assay

A 1% (w/v) of freeze-dried ogi suspension was shaken overnight in a Gallen kamp shaker incubator. The extract was filtered through Whatman No. 1 filter paper and the filtrate stored in a freezer until used for lysine and vitamin assays.

(b) Lysine content of Ogi samples

1.0 ml of ogi extract was used with lysine assay medium and inoculated with P. acidilactici ATCC 8042. Lysine content was obtained as described previously.

(c) Riboflavin content of Ogi samples

1.0 ml of ogi extract was used to supplement the

riboflavin assay medium (17). This was then inoculated with Lactobacillus casei ATCC 7469 and the riboflavin content was obtained as described in Difco (17).

(d) Niacin content and ogi samples

1.0 ml of ogi extract was used to supplement the niacin assay medium. This was then inoculated with L. plantarum ATCC 8014 and the niacin content was obtained as described in the Difco Manual (17).

(e) Thiamine content of ogi samples

1.0 of ogi extract was used to supplement the thiamine assay medium. This was inoculated with L. fermentum ATCC 9338 and the thiamine content was obtained as described by Difco (17).

(f) Phytate content of ogi samples

2.0 g samples of ogi were added to 50 ml of phytate extract solution (100 g Na2SO4 + 32.5 ml conc. HCl/L) in a flask. The flask was shaken overnight in a shaker, and the extract was filtered through Whatman No. 1 filter paper.

5.0 ml of the filtrate was pipetted into each test tube, followed by 2.5 ml of FeCl3 solution (6.66 g FeCl3 .6H20 + 16.5 ml conc. HCl/L) and 5.0 ml distilled water. The tubes were shaken and heated for 15 mn. in a boiling water bath and cooled for another 15 mn. in a beaker with ice. After precipitation was complete, the tubes were centrifuged for 10 mn. and the supernatant discarded.

The precipitate was washed twice with 10 mls of wash solution (50 g $NaSo_4$ + 16.2 g conc. HCl/L).

The ferric phytate precipitate was then mixed with 3 ml conc. HNO_3 and 1.0 ml conc. H_2SO_4. The contents were transferred to a boiling tube and placed on a hot plate. Digestion was continued until only the H_2SO_4 remained containing phosphorus. About 1.0 ml distilled water was added to the boiling tube and the tube reheated to boiling for about 2 mn. The tube was allowed to cool and contents transferred to a 50 ml volumetric flask. Phosphorus was then determined spectrophotometrically by the method of Hanson (16).

5. Preparation of Dehydrated Starter Cultures

Bacterial strains were grown on MRS slants at 35°C for 48 hrs. after which 3.0 mls of sterilized distilled water was added. The slants were shaken rapidly for 60 sec. The resultant suspension was used to produce the dehydrated starter culture. Corn flour (20 g) was put into conical flasks and sterilized at 121°C for 15 min. The sterilized flour was then hydrated with 50 mls of sterile distilled water and inoculated with 0.5 ml of the respective bacterial suspension.

The inoculated flasks were incubated at 35°C for three days, after which the samples were freeze-dried. The bacteria count in each freeze-dried sample was then evaluated.

6. Testing for aflatoxins

Ogi samples produced by selective fermentation were sent to a

commercial laboratory (Romer Labs, Inc., Union, MO) for determination of aflatoxins.

7. Measurement of protein and energy

Standard methods were used for energy (Parr adiabatic oxygen bomb calorimeter) and protein assays (micro Kjeldahl) of the samples (20).

RESULTS

1. Mutagenesis for Lysine Production

Fifty mutants from *Lactobacillus plantarum* X043 and seven mutants from yeast OY4 were analyzed for lysine production.

2. Evaluation of Lysine Producing Mutants

(a) Lysine production

The lysine production by the *Lactobacillus* and yeasts mutants are shown in Tables I and II, respectively.

Lysine production by mutants of the *Lactobacillus* (Table I) show a 12 fold increase in M47 to a 41 fold increase in M4. Two mutants with the highest lysine production (M4 and M5) were selected for use as starter cultures for Ogi production.

The yeast mutants showed a 3 fold (for YM3) to 4 fold increase (for YM7) in lysine production. The mutant YM7 was thus selected for use as starter for ogi production.

(b) Phytase production in selected mutants

The levels of phytase produced in selected mutants are shown by the amount of phytate left after incubation with test organisms for 5 days. This is shown in Table III.

(c) Alpha-galactosidase activity in selected mutants

No activity was detected for a-galactosidase in the selected mutants.

3. Evaluation of Ogi Produced With Single and Mixed Bacterial Starter Cultures

(a) Vitamin content of ogi produced with starter cultures.

The thiamine, niacin and riboflavin levels of ogi produced using different starter cultures are shown in Tables IV, V and VI.

Thiamine content (Table IV) increased with M4, M5 had the same value as unfermented cornmeal and all others had less or no thiamine at all.

Niacin concentration (Table V) was increased slightly in M4, M5 and X043. All others had approximately the same niacin concentration as unfermented corn meal.

Riboflavin content was increased in ogi produced by M4 and M5 (Table VI).

(b) Lysine content

The amount of lysine in ogi produced by different starter cultures is shown in Table VII.

A three fold increase in lysine was found in ogi produced by M4. A two fold increase was measured for M5 while other samples had slight increases.

(d) Phytic acid content

The phytic acid content of ogi produced by different starter cultures is shown in Table VIII.

4. Production of Dehydrated Starter Cultures

The bacterial count of dehydrated starter cultures is shown in Table XI.

5. Aflatoxin Contamination

Results (from a commercial lab using HPLC methods) indicated that aflatoxins ranged from 4.9 to 7.7 ppb in the samples tested. These levels were well below the U.S. Food and Drug Administration limits of 20 ppb for human food.

6. Crude Protein and Energy Content

Total protein was lower in two and higher in six of the fermented samples, compared to the nonfermented corn meal. Small decreases in energy content occurred in all but one fermented sample, reflecting carbohydrate utilization during fermentation.

Research Publication

Two reports were given on preliminary results of this research obtained during the past year (18, 19).

Summary

Selective fermentation was utilized to improve protein value of corn gruel (ogi) by increasing lysine, the most limiting amino acid in corn for growth. Some of the B vitamins were also shown to increase, while phytate was decreased. Modified techniques of ogi preparation to save most of the germ and seed coat helped retain nutrients and energy value that would otherwise be lost when these components are largely removed in traditional methods of ogi production.

Lactic fermentation of cereals has previously been reported to improve digestibility of protein, energy and iron for infants. Diarrhea was also found to be less of a problem when fermented

grain was fed. When these benefits are combined with improvements in protein quality and energy value demonstrated in this research, the potential benefits to infants who must rely on ogi as their primary source of nutrients are considerable.

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Table I. Lysine Production By Lactobaccilus Mutants (ug/l)

Mutant Code	External	Internal	Total
1	310	65	375
2	240	37	277
3	240	110	350
4	420	180	600
5	285	165	450
6	210	175	385
7	260	140	400
8	350	90	440
9	185	105	290
10	350	45	395
11	285	60	330
12	285	110	395
13	190	140	330
14	140	45	185
15	210	65	275
16	210	50	260
17	140	90	230
18	165	110	275
19	260	110	375
20	285	165	450
21	330	90	420
22	240	115	355
23	285	165	450
24	165	165	330
25	260	0	260
26	260	0	260
27	310	0	310
28	370	15	385
29	260	45	305
30	260	20	280
31	310	0	310
32	185	20	205
33	240	0	240
34	260	30	290
35	210	30	240
36	285	140	425
37	240	0	240
38	285	37	327
39	285	60	345
40	140	75	215
41	185	75	260
42	240	20	260
43	185	130	315
44	210	100	310
45	185	20	205
46	260	50	310
47	115	60	175
48	185	120	305
49	285	165	450
50	240	120	360

Table II. Lysine Production by Yeast Mutants (ug/ml)

Mutant Code	External	Internal	Total
1	140	140	280
2	310	37	347
3	210	50	260
5	310	20	330
7	240	140	380
8	240	60	300
9	285	45	330

Table III. Phytase Production by selected Lactobacillus and yeast mutants

Code	% Phytate Digested
M_4	7.097
M_5	17.42
M_8	22.59
YM_7	0.00

Table IV. Thiamine Concentration in Ogi Fermented with Starter Cultures

Sample Code	Thiamine Concentration (ug/g)
M_4	31.66
M_5	14.66
$YM_7 + M_5$	3.3
YM_7	0.00
$YM_7 + M_4$	0.00
X043	9.0
OY_4	0.00
Natural[1]	0.00
Corn meal[2]	14.66

[1]Natural fermentation
[2]Not fermented

Tabel V. Niacin Concentration in Ogi Fermented with Starter Cultures

Sample Code	Niacin Concentration (ug/g)
M_4	14.8
M_5	12.16
YM_7 M_5	11.0
YM_7	11.0
YM_7 M_4	11.6
X043	13.5
OY_4	12.66
Natural	11.8
Corn meal	11.16

Table VI. Riboflavin Concentration in Ogi Samples from Starter Cultures

Sample Code	Riboflavin (ug/g)
M_4	25.3
M_5	12.66
YM_7 M_5	10.0
YM_7	8.0
YM_7 M_4	10.75
X043	10.3
OY_4	14.0
Natural	13.6
Corn meal	12.5

Table VII. Lysine Concentration of Ogi Samples Produced by Starter Cultures

Sample Code	Lysine Concentration (mg/g)
M_4	10.17
M_5	7.13
YM_7 + M_5	3.75
YM_7	4.92
YM_7 + M_4	6.23
X043	4.75
OY_4	4.82
Natural	4.6
Corn meal	3.35

Table VIII. Phytic Acid Content of Ogi Produced by Starter Cultures

Sample Code	Phytic Acid* (ug/g)
M_4	
M_5	
YM_7 + M_5	0.00
YM_7	600.00
YM_7 + M_4	0.00
X043	600.00
OY_4	600.00
Natural	0.00
Corn meal	666.6

*Phytic Acid is determined as Phytate Phosphorus

Table IX. Microbial Content of Strains on Maize Carrier

		Bacterial count $x10^6$		
Sample Code	Organism	1	2	3
OY_4	Yeast	81.2	780	106
X043	Lactobaccilus	42	47	41

Table X. Aflatoxin Content of Ogi Samples

Sample Code	**Aflatoxins (ppb)**			
	B1	**B2**	**G1**	**G2**
M_5	5.2	ND*	2.1	ND
YM_7 M_4	5.2	ND	2.3	ND
YM_7 M_5	6.5	ND	3.1	ND
X043	5.3	ND	2.9	ND
OGP_5	7.7	0.74	1.1	ND
Natural	4.9	ND	0.97	ND

*None detected

Table XI. Crude Protein and Energy in Selected Ogi Samples

Sample code	Total protein* (%)	Total energy* (Kcal/g)
M_4	7.92	4074
M_5	7.03	3871
YM_7 M_5	8.54	3827
YM_7	7.61	3878
YM_7 M_4	8.46	3827
X043	7.83	3922
OY_4	7.39	3940
Natural	8.46	3922
Corn meal	7.58	3990

*10% moisture basis