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Full Length Research Paper

Effect of controlled fermentation on the oligosaccharides content of two common Nigerian *Vigna unguiculata* beans (*drum* and *oloyin*)

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Two common local beans (*Vigna unguiculata*) varieties known as *drum* and *oloyin* were used for this study. These beans were fermented using *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Pediococcus acidilactici.* The fermentation was carried out for 24, 48, and 72 h at ambient temperature. The results showed that there was no significant difference in the temperature values of both *drum* and *oloyin* beans fermented with the three organisms, while there were significant differences in the pH and total titratable acidity (TTA) values. Fermentation for 72 h showed significant reduction in the stachyose content of *drum* beans slurry fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici* with 54.35, 53.06 and 57.84% reductions, respectively. Also, fermentation of *oloyin* beans slurry for the same period resulted in significant reduction in the stachyose content when fermented with *L. plantarum, L. fermentum* and *P. acidilactici* with 31.21, 65.38 and 67.63% reductions, respectively. Fermentation of *drum* beans for 72 h showed significant reduction in the raffinose content when fermented with *L. plantarum, L. fermentum* and *P. acidilactici* with 48.49, 79.09 and 74.81% reductions, respectively. For *oloyin* beans slurry, fermentation for 72 h also resulted in significant reduction in the raffinose content when fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici* with 53.68, 73.17 and 64.02% reductions, respectively. Sucrose content showed significant increase for both beans slurry fermented for 72 h with all of the organisms. This study has thus shown that lactic acid bacteria can effectively be used to reduce the flatulence-causing sugars present in beans.

Key words: Lactic acid bacteria, *Vigna unguiculata*, temperature, pH, total titratable acidity, flatulence-causing sugars.

INTRODUCTION

Grain legumes or pulses, although are rich and low-cost sources of dietary proteins and nutrients for a large part of the world's population (Egounlety and Aworh, 2003). But their nutritive value is limited by the presence of several antinutritional and toxic substances including oligosaccharides (especially raffinose, stachyose and verbascose which are contributory factors to the flatulence problem), phytates, polyphenols, lectins or haemagglutinins, cyanogens and saponins (Porzucek et al., 2002; Egounlety and Aworh, 2003).

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Oligosaccharides of the family raffinose are not digested due to lack of α-1,6-galactosidase in the intestinal mucosa (Porzucek et al., 2002). The absence of α-1,6-galactosidase capable of hydrolyzing the α-1,6-galactosidic linkage leads to accumulation of these saccharides in the lower intestine and these undergo anaerobic fermentation by bacteria, especially *Clostridium* spp. (Nowak, 1992). The latter process is accompanied by an emission of gases causing great discomfort to many consumers (Porzucek et al., 2002). This flatulence inducing property of leguminous plants may be accompanied by severe diarrhoea, headache and dyspepsia (Castilo et al., 1990).

Nutritional benefits are produced from legume fermen-

tation where microorganisms break down the flatulence-causing indigestible oligosaccharides, such as raffinose, stachyose and verbascose, into absorbable di- and monosaccharides, and then into organic acids (Granito et al., 2003). Odunfa (1982) found that the quantities of flatus-forming oligosaccharides decreased significantly during the first 24 h of fermentation of locust bean for *iru* production. This decrease was attributed to the activities of α- and β-galactosidase that hydrolysed the oligosaccharides to simple reducing sugars. Akinyele and Akinlosotu (1991) fermented cowpeas naturally for 24 h, and found a reduction of 79.7% in verbascose and 5.9% in stachyose. Frías et al. (1996) found 100 and 62% reductions in the content of raffinose and stachyose, respectively, after 24 h of natural fermentation of lentils. Czarnecka et al. (1998) studying the effect of lactic acid fermentation conditions as a pretreatment of bean and pea for extrusion confirmed a significant decrease in stachyose (86%) and verbascose (92%) in pea seeds. They also reported that lactic acid fermentation of bean seeds caused decrease of stachyose by 88.5% but not the reduction of raffinose and verbascose. Thus, the objective of this study is to investigate the role of lactic acid bacteria on the stachyose, raffinose and sucrose content of common Nigerian *Vigna unguiculata* beans; *drum* and *oloyin*.

MATERIALS AND METHODS

Isolation of organisms

The *Lactobacillus plantarum*, *fermentum* and *Pediococcus acidilactici* used in this study were isolated from traditionally prepared *fufu, ogi* and *wara*. 10 g of each food item - *fufu*, *ogi* and *wara* was weighed aseptically into 90 ml of sterile diluent containing 0.1% peptone water and homogenized for 30 s in order to form a concentrated pulp. Serial dilutions were then made from the pulp and plated on de Man Rogosa and Sharpe (M.R.S.) agar (de Man et al., 1960) using the pour plate method (Harrigan and McCance, 1976). Experiments was conducted on the cultural, microscopic and biochemical/physiological characteristics of the various isolates, and they were identified according to Kandler and Weiss (1986) in Bergey's manual of systematic bacteriology Vol. II as *L. plantarum*, *L. fermentum* and *P. acidilactici*.

Inoculum preparation

L. plantarum, *fermentum* and *P. acidilactici* were grown in 25 ml sterile M.R.S. broth, and incubated in CO_2 - enriched jars for 24 h. They were then centrifuged at 3600-x g for 15 min (SELECTA s. a. Model J. P. Barcelona Spain). The recovered cells were rinsed using 10 ml sterile distilled water and spined twice at 3600-x g for 15 min (SELECTA s. a. Model J. P. Barcelona Spain). After this, 9 ml suspensions of the cells were made using sterile distilled water. The suspensions were serially diluted and plated out on plate count agar using the pour plate method (Harrigan and McCance, 1976). After 24 h incubation period in CO_2 - enriched jars, the colonies on each plate of dilution factor were counted and the plate with approximately 10^6 cfu/ml was noted. This was used at every inoculation of the beans slurries.

Preparation of beans slurries and inoculation

The two varieties of beans (*V. unguiculata*); *drum* and *oloyin* purchased at Bodija market, Ibadan, Nigeria were blown of dust, picked of broken seeds and other foreign materials, and then washed and soaked for 6 and 4 min respectively in distilled water. After this, the beans were dehulled by rubbing against the two palms. They were dehulled properly until all the outer coverings were totally removed. They were then spread on flat papers and dried in a cabinet dryer at 60°C for 12 - 14 h. The dried beans were milled into powder using a hammer mill. Twenty five grams each of the milled beans was reconstituted with 30 ml of distilled water in order to form slurries, and these were sterilized at 121°C for 15 min. After sterilization, 1 ml inoculum suspension of *L. plantarum*, *L. fermentum* and *P. acidilactici* containing approximately 10^6 cfu/ml were inoculated aseptically into the sterile beans slurries while uninoculated sterile beans served as controls. For each inoculation of beans with these organisms, fermentation was carried out at 24, 48 and 72 h. For analyses, triplicates of each sample were prepared.

Parameters determined

The temperatures of the fermented beans slurries were determined at 0, 24, 48 and 72 h with the aid of a thermometer. The pH was determined using a pH meter (Model 483219 Hanna Instruments Portugal). The total titratable acidity (TTA) was determined according to (A.O.A.C. 1990).

Determination of oligosaccharides – stachyose, raffinose and sucrose

This was carried out following the phenol-sulphuric acid method of Dubois et al. (1956).

RESULTS

The fermentation of *drum* beans slurry with *L. plantarum*, *L. fermentum* and *P. acidilactici* at 24, 48 and 72 h showed no significant difference ($p > 0.05$) in the values of the temperature (Table 1). There was significant decrease ($p < 0.05$) in the pH values when this slurry was fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici* as the fermentation period increased (Table 2). Increase in fermentation time resulted in significant increase ($p < 0.05$) in the TTA values when fermented with any of these organisms (Table 3). The stachyose content of fermented *drum* beans slurry showed significant reduction ($p < 0.05$) after 24 and 72 h when fermented with *L. plantarum*. This happened only after 72 h when fermented with *L. fermentum*, while it showed significant reduction after 24, 48 and 72 h when fermented with *P. acidilactici* (Table 4). The raffinose content showed significant reduction ($p < 0.05$) after 24 and 72 h when fermented with *L. plantarum*; after 72 h when fermented with *L. fermentum*, and after 48 and 72 h when fermented with *P. acidilactici* (Table 5). The sucrose content showed significant increase ($p < 0.05$) after 24, 48 and 72 h when fermented with *L. plantarum*. There was significant increase after 24 h when fermented with *L. fermentum* and *P. acidilactici* (Table 6).

Table 1. Temperature (°C) of *drum* and *oloyin* beans slurries fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici*.

Time (h)	*L. plantarum*		*L. fermentum*		*P. acidilactici*	
	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *oloyin*
0	31.0 ± 1.00^{a}	32.0 ± 1.00^{a}	32.0 ± 3.00^{a}	32.0 ± 1.00^{a}	32.0 ± 2.00^{a}	31.0 ± 2.00^{a}
24	32.0 ± 1.00^{a}	33.0 ± 1.00^{a}	31.0 ± 2.00^{a}	33.0 ± 2.00^{a}	32.0 ± 1.00^{a}	33.0 ± 1.00^{a}
48	32.0 ± 1.00^{a}	31.0 ± 1.00^{a}	32.0 ± 2.00^{a}	31.0 ± 2.00^{a}	31.0 ± 2.00^{a}	32.0 ± 0.50^{a}
72	33.0 ± 1.00^{a}	32.0 ± 1.00^{a}	34.0 ± 4.00^{a}	32.0 ± 2.00^{a}	33.0 ± 2.00^{a}	33.0 ± 0.00^{a}

Values are averages of three independent determinations ± standard deviation.
Significant differences ($p < 0.05$) are indicated by different letters in the same column.

Table 2. pH of *drum* and *oloyin* beans slurries fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici*.

Time (h)	*L. plantarum*		*L. fermentum*		*P. acidilactici*	
	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *drum*	Treated *oloyin*	Treated *oloyin*
0	6.80 ± 0.08^{a}	5.70 ± 0.06^{a}	5.90 ± 0.01^{a}	5.80 ± 0.06^{a}	6.10 ± 0.40^{a}	6.10 ± 0.14^{a}
24	5.50 ± 0.50^{b}	5.60 ± 0.08^{a}	5.30 ± 0.38^{a}	5.10 ± 0.12^{ab}	5.40 ± 0.20^{ab}	6.05 ± 0.13^{a}
48	4.30 ± 0.30^{bc}	4.20 ± 0.30^{b}	4.00 ± 0.00^{b}	4.40 ± 0.50^{bc}	4.70 ± 0.13^{b}	5.20 ± 0.15^{b}
72	3.20 ± 0.30^{c}	3.20 ± 0.06^{c}	3.30 ± 0.20^{b}	3.60 ± 0.40^{c}	3.50 ± 0.15^{c}	4.60 ± 0.06^{c}

Values are averages of three independent determinations ± standard deviation.
Significant differences ($p < 0.05$) are indicated by different letters in the same column.

Table 3. Total titratable acidity (TTA) in (g/l) of *drum* and *oloyin* beans slurries fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici*.

Time (h)	*L. plantarum*		*L. fermentum*		*P. acidilactici*	
	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *drum*	Treated *oloyin*	Treated *drum*
0	0.27 ± 0.03^{c}	0.18 ± 0.04^{c}	0.45 ± 0.04^{c}	0.27 ± 0.03^{c}	0.09 ± 0.02^{c}	0.27 ± 0.02^{c}
24	0.32 ± 0.03^{c}	0.63 ± 0.00^{b}	0.63 ± 0.03^{b}	0.54 ± 0.03^{b}	0.18 ± 0.02^{c}	0.29 ± 0.02^{c}
48	1.44 ± 0.04^{b}	1.71 ± 0.11^{a}	0.27 ± 0.02^{d}	1.44 ± 0.03^{a}	0.72 ± 0.03^{b}	1.08 ± 0.02^{b}
72	2.52 ± 0.06^{a}	1.89 ± 0.03^{a}	3.24 ± 0.04^{a}	1.53 ± 0.05^{a}	2.34 ± 0.03^{a}	1.89 ± 0.02^{a}

Values are averages of three independent determinations ± standard deviation.
Significant differences ($p < 0.05$) are indicated by different letters in the same column.

Table 4. Stachyose content in (mg/g) dry weight basis of *drum* and *oloyin* beans slurries fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici*.

Time (h)	*L. plantarum*		*L. fermentum*		*P. acidilactici*	
	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *drum*	Treated *oloyin*	Treated *drum*
0	1.38 ± 0.004^{a}	1.54 ± 0.003^{a}	1.47 ± 0.002^{a}	1.82 ± 0.004^{a}	1.85 ± 0.003^{a}	1.73 ± 0.005^{a}
24	0.85 ± 0.004^{b}	1.41 ± 0.004^{b}	1.35 ± 0.003^{ab}	1.63 ± 0.002^{b}	1.54 ± 0.004^{b}	1.51 ± 0.003^{b}
48	0.78 ± 0.003^{b}	1.29 ± 0.003^{b}	1.32 ± 0.003^{b}	1.47 ± 0.001^{c}	1.29 ± 0.003^{c}	1.22 ± 0.004^{c}
72	0.63 ± 0.003^{c}	0.97 ± 0.003^{c}	0.69 ± 0.004^{c}	0.63 ± 0.003^{d}	0.78 ± 0.003^{d}	0.56 ± 0.003^{d}

Values are averages of three independent determinations ± standard deviation.
Significant differences ($p < 0.05$) are indicated by different letters in the same column.

The fermentation of *oloyin* beans slurry with either of *L. plantarum*, *L. fermentum* and *P. acidilactici* at 24, 48 and 72 h showed no significant difference ($p > 0.05$) in the values of the temperature as seen in Table 1. Significant decrease ($p < 0.05$) was observed in the pH values of this beans slurry when fermented with *L. plantarum*, *L. fer-*

Table 5. Raffinose content in (mg/g) dry weight basis of *drum* and *oloyin* beans slurries fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici*.

Time (h)	*L. plantarum*		*L. fermentum*		*P. acidilactici*	
	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *oloyin*
0	0.796 ± 0.0001^{a}	0.95 ± 0.003^{a}	1.10 ± 0.00^{a}	1.23 ± 0.002^{a}	1.31 ± 0.004^{a}	1.64 ± 0.0004^{a}
24	0.59 ± 0.004^{b}	0.74 ± 0.003^{b}	1.00 ± 0.01^{a}	1.16 ± 0.003^{a}	1.21 ± 0.004^{a}	1.41 ± 0.0003^{b}
48	0.59 ± 0.004^{b}	0.616 ± 0.0003^{c}	1.00 ± 0.0385^{a}	0.462 ± 0.0004^{b}	0.976 ± 0.0003^{b}	0.694 ± 0.0005^{c}
72	0.41 ± 0.002^{c}	0.44 ± 0.003^{d}	0.23 ± 0.004^{b}	0.33 ± 0.003^{c}	0.33 ± 0.0004^{c}	0.59 ± 0.004^{c}

Values are averages of three independent determinations ± standard deviation.
Significant differences ($p < 0.05$) are indicated by different letters in the same column.

Table 6. Sucrose content in (mg/g) dry weight basis of *drum* and *oloyin* beans slurries fermented with *L. plantarum*, *L. fermentum* and *P. acidilactici*.

Time (h)	*L. plantarum*		*L. fermentum*		*P. acidilactici*	
	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *oloyin*	Treated *drum*	Treated *oloyin*
0	0.56 ± 0.03^{d}	0.97 ± 0.03^{d}	1.05 ± 0.04^{b}	1.23 ± 0.04^{d}	1.72 ± 0.04^{b}	1.79 ± 0.02^{ab}
24	1.31 ± 0.03^{c}	1.53 ± 0.05^{c}	1.605 ± 0.04^{a}	1.53 ± 0.03^{c}	1.90 ± 0.04^{a}	1.269 ± 0.04^{b}
48	1.64 ± 0.04^{b}	1.79 ± 0.04^{b}	1.61 ± 0.04^{a}	1.754 ± 0.04^{b}	2.016 ± 0.05^{a}	2.05 ± 0.04^{ab}
72	2.17 ± 0.03^{a}	2.28 ± 0.05^{a}	1.68 ± 0.04^{a}	2.50 ± 0.00^{a}	2.02 ± 0.03^{a}	2.50 ± 0.50^{a}

Values are averages of three independent determinations ± standard deviation.
Significant differences ($p < 0.05$) are indicated by different letters in the same column.

mentum and *P. acidilactici* as the fermentation period increased (Table 2). Increase in fermentation time resulted in significant increase ($p < 0.05$) in the TTA values when fermented with any of these organisms (Table 3). The stachyose content of fermented *oloyin* beans slurry showed significant reduction ($p < 0.05$) after 24 and 72 h when fermented with *L. plantarum*. It showed significant reduction after 24, 48 and 72 h when fermented with *L. fermentum* and *P. acidilactici* (Table 4). There was significant reduction ($p < 0.05$) in the raffinose content after 24, 48 and 72 h when fermented with *L. plantarum* and *P. acidilactici* (Table 5). When fermented with *L. fermentum*, significant reduction was observed after 48 and 72 h (Table 5). There was significant increase in the sucrose content after 24, 48 and 72 h when fermented with *L. plantarum* and *L. fermentum*. Significant increase was only observed after 72 h when fermented with *P. acidilactici* (Table 6).

DISCUSSION

The reduction in pH is in comparison with the findings of Granito et al. (2003) who found a dramatic decrease in the pH of beans (*Phaseolus vulgaris*) fermented from 24 to 72 h with *L. acidophilus*, *Bifidobacterium* and *Streptococcus thermophilus*. Barampama and Simard (1995) also reported a rapid decrease in pH from 6.6 to 5.4 for the first 20 h of beans fermentation (*Phaseolus vulgaris* variety Dore di Kirundo) using *L. fermentum* as starter.

The total titratable acidity (TTA) increased in both beans slurries, and this can be related to the fall in pH. The increase in TTA can be compared with the results of Granito et al. (2003) who found increase in the TTA during the first 24 h of beans (*Phaseolus vulgaris*) fermented with *L. acidophilus*, *Bifido-bacterium* and *S. thermophilus.* A similar increase was reported by Ragaee et al. (1985)

who carried out natural lactic acid fermentation on lentils. Frías et al. (1996) found high lactic acid production during the natural fermentation of lentils at 43°C for 96 h. The selected LAB seem to produce more lactic acid at 72 h in the *drum* beans slurry compared to the *oloyin* beans slurry with *L. fermentum* producing the highest.

Longer incubation period was found to significantly reduce the stachyose content of the fermented beans slurries inoculated with *L. plantarum*, *L. fermentum* and *P. acidilactici*, and this can be attributed to the ability of the organisms to produce α-galactosidase enzyme that breaks down the α-1,6-glycosidic bonds. These results compared well with that of Duszkiewicz-Reinhard et al. (1994) who found decrease in stachyose content of pinto bean flour inoculated with *L. fermentum* or *L. plantarum*. Akinyele and Akinlosotu (1991) on fermenting cowpeas naturally also found a reduction of 5.9% in the stachyose content while Tewary and Muller (1992) found that the total concentration of raffinose and stachyose decreased from 4.4 to 0.6% on fermenting black bean and soybean with *Lactobacillus bulgaricus* and *S. thermophilus*. Similar reports were given by Granito et al. (2003) who found 11% reduction in the stachyose content of fermented beans (*Phaseolus vulgaris*) and Frías et al. (1996) who found 100 and 62% reductions in the content of raffinose and stachyose respectively after 24 h of natural fermentation of lentils. Tongnual and Fields (1984) also found a notable reduction in the concentration of flatus-producing oligosaccharides after natural fermentation of soybean-corn blends. Stachyose was more reduced in *oloyin* beans slurry compared to *drum* with 34.05% difference when both were inoculated with *P. acidilactici* at 48 h.

The *drum* beans slurry did not have any reduction in its raffinose content when inoculated with *L. plantarum* and *L. fermentum* at 48 h, and this can be compared with the report of Czarnecka et al. (1998) who found no reduction in the raffinose content of bean seeds fermented with lactic acid bacteria. However, raffinose was more effectively reduced in *oloyin* beans slurry inoculated with *L. plantarum*, *L. fermentum* and *P. acidilactici* at 48 h. The general increase in sucrose content of the fermented beans slurries probably may be that sucrose was produced during the breaking down of stachyose and raffinose by α-galactosidase enzyme produced by the lactic acid bacteria.

The fermented beans product obtained can be marketed as dried beans powder for weanlers or extruded products that can be sold at eateries. Meanwhile, *P. acidilactici* reduced the stachyose content of *drum* and *oloyin* beans than any of the organisms when fermented for 72 h. In like manner, *L. fermentum* reduced the raffinose content of both beans than any of the organisms when fermented for 72 h.

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