# Effect of starter cultures on the anti-nutrient contents, minerals and viscosity of ogwo, a fermented sorghum–Irish potato gruel

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Article history

#### <u>Abstract</u>

Received: 3 August 2014 Received in revised form: 9 November 2014 Accepted: 27 November 2014

<u>Keywords</u>

Ogwo Aanti-nutrient Fermentation This work investigated the effect of single starter cultures on the anti-nutrient, mineral contents and viscosity of fermenting 'ogwo', a fermented gruel from sorghum-Irish potato mixture. The organisms used were *Lactobacillus acidophilus*, *L. fermentum*, *L. plantarum*, *Geotrichum candidum* and *Saccharomyces cerevisiae*. There were significant reductions (P < 0.05) in the anti-nutrient contents of the samples fermented with starter cultures than the sample fermented naturally. The highest reductions were observed in phytic acid, saponin and flavonoid contents of *L. plantarum* fermented sample and in oxalate and tannin contents of sample fermented with *S. cerevisiae* and *L. acidophilus* respectively. Sodium, Calcium, Phosphorus, Copper and Manganese contents of 'ogwo' produced with starter cultures increased while fluctuations were observed in their Potassium, Magnesium and Zinc contents. The viscosities of the starter culture fermented samples reduced more than the naturally fermented sample with the highest and lowest reductions in *L. fermentum* and *L. plantarum* respectively. The results of this work showed that *Lactobacillus* and yeasts were good candidates for reducing the anti-nutrient contents and the viscosities as well as improving the mineral contents of the gruel.

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## Introduction

Fermented foods and beverages of cereal origin are widely consumed in southern and western African countries as traditional foodstuffs. They include the solid doughs and liquid gruels. The solid doughs include 'massa', 'kenke', 'agidi', 'kenkey' and 'koko' while the liquid gruels could be alcoholic ('burukutu', 'kiffer' beer, 'sekete', 'pito', 'Ikigage') and non-alcoholic ('ogi', 'ugi', 'kunu-saki', 'mawe', 'mahewu', 'ogwo') (Adegbehingbe and Fakoya, 2007; Kolawole et al., 2007; Agarry et al., 2010; Dike and Sanni, 2010; Wakil and Daodu, 2011; Oyelana and Coker, 2012). The commonly used cereals include maize, oat, wheat, millets and sorghum. More than 70% of cereal crops produced in developed countries is fed to livestock whereas in developing countries 68-69% of the crops is consumed by human (Chaven and Kadam, 1989).

Many of the fermented products consumed by different ethnic groups have therapeutic values. Some of the fermented foods contain high concentrations of probiotic bacteria that can lower the cholesterol level (Egbere, 2008). The slurries of carbohydrate based fermented Nigerian foods such as 'ogi' and 'fufu' have been known to exhibit health promoting properties such as control of gastroenteritis in animals and human (Aderiye *et al.*, 2007). Some advantages

of food fermentation include general improvement in the shelf life, texture, taste and aroma. Besides, fermentation helps to reduce the anti-nutritional and toxic factors in the raw materials by making the proteins and minerals which complex with these phytochemicals readily available. This action has been confirmed to be due to the enzymatic activity of the microorganisms which are responsible for their fermentation. This has significantly improved the nutritional value and digestibility of the meal (Okafor, 2009; Oyewole and Isah, 2012; Wakil and Kazeem, 2012).

'Ogwo' is a traditional beverage peculiar to some part of Benue State, Nigeria, particularly the Agatu people. It is a cloudy brown gruel with a sharp sweet to a sour taste which could be consumed at any time of the day particularly after returning from farm as a way of refreshment. Microorganisms associated with the fermentation of 'ogwo' include the fungal genera; Saccharomyces cerevisiae, S. chavaheri and Geotrichum candidum, and the bacterial genera; Bacillus subtilis, B. polymyxa, Lactobacillus plantarum, L. fermentum, L. brevis, L. acidophilus and Micrococcus luteus (Adegbehingbe and Odunfa, 2007). The gruel was reported to be dominated by lactic acid bacteria and the yeasts at the maturing stage (Adegbehingbe and Odunfa, 2007). Production of 'ogwo' using single cultures of L. plantarum, L.



*fermentum, L. acidophilus, B. subtilis S. cerevisiae, G. candidum,* and their various combinations had been reported to increase their protein and fat contents. Besides they were more acceptable than 'ogwo' produced by natural fermentation (Adegbehingbe and Fakoya, 2007).

Sorghum has been, for centuries, one of the most important staple foods for millions of poor rural people in the semi-arid tropics of Asia and Africa. For some impoverished regions of the world, sorghum remains a principal source of energy, protein, vitamins and minerals. Sorghum is used in the same way as barley to produce a 'malt' that can form the basis of a mash in beer production (Murwan and Ali, 2011). Sorghum contains resistant starch which impairs digestibility.

The white or Irish potato (Solanum tuberosum), is nutritionally very good, high in starch (8-28%) but with 1-4% protein. Irish potato contains vitamins and minerals, as well as an assortment of phytochemicals, such as carotenoids and natural phenols. The potato is best known for its carbohydrate content. The predominant form of this carbohydrate is starch. A small but significant portion of this starch is resistant to digestion by enzymes in the stomach and small intestine (Hijmans and Spooner, 2001). In addition to the usual eating of the tuber, Irish potato is used in the fermentation of an alcoholic beer known as vodka (Jai Gopal and Khurana, 2006).

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. One common example is phytic acid, which forms insoluble complexes with Calcium, Zinc, Iron and Copper. Some proteins can also be anti-nutrients, such as the trypsin inhibitors and lectins found in legumes. These enzyme inhibitors interfere with digestion. Another particularly widespread form of anti-nutrients is the flavonoids, which are a group of polyphenolic compounds that include tannins. Antinutrients are found at some level in almost all foods for a variety of reasons (Beecher, 2003).

Many traditional methods of food preparation such as fermentation, cooking, and malting increase the nutritive quality of plant foods through reducing certain anti-nutrients such as phytic acid, polyphenols, and oxalic acid. Such processing methods are widely used in societies where cereals and legumes form a major part of the diet (Hotz and Gibson, 2007; Oboh and Oladunmoye, 2007). Apart from poor nutritional qualities of cereal grains, they have been reported to contain anti-nutrient contents which bind some of the minerals and protein present in the grains. Besides, traditional African cerealbased gruels used as complementary foods have high hot paste viscosity and require considerable dilution before feeding; a factor that further reduces energy and nutrient density. Therefore this work investigated the effect of fermenting 'ogwo' with single starters of dominant microorganisms isolated from ogwo on the anti-nutrient contents, mineral contents and the viscosity of the products.

## **Materials and Methods**

# Preparation of 'Ogwo'

'Ogwo' was prepared in the traditional way as follows; 300 g of sorghum grains were washed in tap water, drained using plastic sieves and steeped for 6 h. The water was drained again using a clean plastic sieve. The grains were spread on a plastic tray and covered with clean, black cellophane bag for about 4 days to induce sprouting. During malting, the grains were turned occasionally and watered two times daily until the plumule attained a length of about 2 cm. One hundred grams of peeled Irish potato were wet-milled with the malted grains in 500 ml of sterile water. Another batch of dry, clean and healthy sorghum grains was dry-milled into a powdery form. Then 150 g of the powder was mixed with 500 ml of boiling water to form slurry similar to a pap or paste but coarser in texture. The slurry was added to the malted sorghum-potato mixture in a sterile translucent plastic bucket and stirred thoroughly with a clean wooden rod. The resulting mixture was further diluted with 500 ml of sterile water. The gruel was allowed to ferment at room temperatures (28°C±0.5) for 48 hours.

## Preparation and inoculation of starter cultures:

Pure cultures of six dominant microorganisms that have been earlier isolated from 'ogwo' sample (Adegbehingbe and Odunfa, 2007) were subcultured into appropriate slants. The isolates were Lactobacillus acidophilus, L. fermentum, L. plantarum, Geotrichum candidum and Saccharomyces cerevisiae. Ten milliliters of sterile peptone water was added to 18-24 h old cultures on slants and shaken to dislodge the microbial cells and make a suspension. Two millilitres was transferred from the suspension and inoculated into 100 ml of sterile saline water and incubated at 37°C for 24 hours. Ten milliliters of the cell suspension of each of the starter cultures was inoculated to 400 ml of prepared gruel sample that had been previously sterilized in an autoclave at 110°C for 10 minutes. The inoculated gruels were allowed to ferment at room temperatures  $(28^{\circ}C\pm 2)$ for 48 hours. 'Ogwo' which was produced by natural fermentation served as control.

#### Anti-nutrient Determination

The saponin, tannin, phytic acid, oxalate, saponin and cyanide contents of the raw and the fermented samples were determined (Bradbury *et al.*, 1999; Egwaikhide *et al.*, 2009).

#### Minerals analysis

For each formula, Potassium and Sodium contents were assessed by flame spectrophotometer (Sena *et al.*, 1998) while Calcium, Magnesium, Manganese, Iron, Zinc and Copper were analyzed using an atomic absorption spectrophotometer (Sena *et al.*, 1998). Phosphorus was analyzed using Technicon Autoanalyzer methodology (Lockett *et al.*, 2000).

## Viscosity

One hundred millilitres each of the samples was mixed with 1000 ml of clean water and heated at 100°C for 10 min. After cooking the porridge, temperature was followed until freezing around 45°C and the measurement by Bostwick consistometer was done in triplicate with three different preparations (Mouquet *et al.*, 2006).

Data obtained were analyzed by ANOVA and significant differences between means were compared using Duncan (Duncan, 1955) multiple range test with the aid of SAS/STAT program).

## Results

Anti-nutrient contents observed were to decrease significantly after natural fermentation of the gruel (Table 1). Anti-nutrient contents of the gruel fermented with starter cultures were also significantly lower than the naturally fermented sample. Significant reductions were also observed. The highest reductions in oxalate content was observed in S. cerevisiae fermented sample (0.19 mg/kg) while L. plantarum fermented sample had the lowest reduction in phytic acid (10.36), saponin (0.94%) and flavonoid (2.19%) contents. The highest reduction in tannin contents was determined in the gruel fermented with L. acidophilus (5.65 mg/kg).

Potassium was the highest mineral contents before and after fermentation of the samples (Table 2). This was followed by Magnesium and Calcium contents while zinc was observed to be the lowest in the samples. Fluctuations were observed in the mineral contents of the gruels after fermentation. Sodium, Phosphorus, Copper, Iron and Manganese contents increased after fermentation in all the samples. All the mineral contents were observed to increase in *L. plantarum* fermented sample except Calcium and

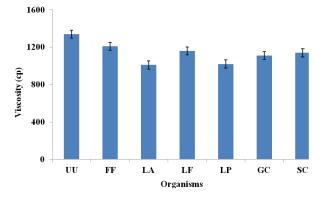


Figure 1. Viscosity of Ogwo produced with starter cultures Legend: NN= unfermented sample; FF= Naturally fermented sample; LA=*L. acidophilus* fermented sample; LF=*L. fermentum* fermented sample; LP=*L. plantarum* fermented sample; GC=G. candidum fermented sample; SC=*S. cerevisiae* fermented sample

Zinc contents. *Saccharomyces cerevisiae* fermented sample had the highest Potassium, Phosphorus and Iron contents among the starter culture fermented samples. Copper contents of the unfermented and the fermented samples were not significantly different from one another and it ranged from 1.12 ppm to 1.16 ppm. Viscosities of the starter culture fermented samples were lower than the naturally fermented samples (Figure 1). The lowest and the highest viscosities were observed in *L. fermentum* fermented sample (1210 cp) and *L. plantarum* fermented sample (1020 cp) respectively.

## Discussion

The reduction in the anti-nutrient contents after fermentation could be due to leaching of the antinutrients into the soaking. This was in agreement with Gernah et al. (2011) while studying the effect of fermentation on some chemical and physical properties of maize. Reduction in tannin contents reduced the risk of bowel irritation, kidney irritation, liver damage, irritation of the stomach and gastrointestinal pain which are associated foods containing high level of tannin. The fermenting microorganisms were responsible for the cleavages of tannin-protein, tannic acid-starch and tanniniron complexes thereby releasing the free nutrients which will invariably improve the availability of the nutrients (Obizoba and Atii, 1991; Onweluzo and Nwabugwu, 2009).

The decrease in phytate levels in all fermented samples could be due to the activities of phytase during fermentation. Usha and Chandra (1997) observed that fermentation of finger millet (Eleusine coracane) with starter cultures from previously fermented finger millet resulted in the reduction in

Table 1. Anti-nutrient contents of Ogwo produced with starter cultures

Anti-nutrient	UU	FF	LA	LE	LP	GC	SC
		••					
Oxalate (mg/kg)	0.79 <sup>a</sup>	0.35 <sup>b</sup>	0.21 <sup>d</sup>	0.22 <sup>d</sup>	0.26 <sup>c</sup>	0.28 <sup>c</sup>	0.19 <sup>d</sup>
Phytic acid (mg/kg)	26.75 <sup>a</sup>	14.13 <sup>b</sup>	11.34 <sup>d</sup>	13.23 <sup>c</sup>	10.36 <sup>e</sup>	12.34 <sup>cd</sup>	11.37 <sup>d</sup>
Tannin (mg/kg)	17.29 <sup>a</sup>	9.45 <sup>b</sup>	5.65 <sup>f</sup>	7.34 <sup>d</sup>	6.94 <sup>e</sup>	7.38 <sup>d</sup>	8.39 <sup>c</sup>
Saponin (%)	3.14 <sup>a</sup>	1.63 <sup>b</sup>	1.16 <sup>d</sup>	1.34 <sup>c</sup>	0.94 <sup>e</sup>	1.11 <sup>d</sup>	0.98 <sup>e</sup>
Flavonoids (%)	5.23 <sup>a</sup>	3.21 <sup>b</sup>	2.54 <sup>cd</sup>	2.40 <sup>d</sup>	2.19 <sup>e</sup>	2.73 <sup>c</sup>	2.27 <sup>e</sup>

Samples with the same superscripts across the row are not significantly different (P>.05).

Legend: NN= unfermented sample; FF= Naturally fermented sample; LA=L. acidophilus fermented sample;

LF=L. fermentum fermented sample; LP=L. plantarum fermented sample;

GC=G. candidum fermented sample; SC=S. cerevisiae fermented sample

Table 2. Mineral contents of Ogwo produced with starter cultures

Minerals	UU	FF	LA	LF	LP	GC	SC
Potassium (mg/g)	142.00 <sup>b</sup>	43.43 <sup>b</sup>	41.87 <sup>b</sup>	38.45 <sup>c</sup>	44.65 <sup>a</sup>	42.34 <sup>ab</sup>	44.45ª
Sodium (mg/g)	2.32 <sup>c</sup>	2.48 <sup>b</sup>	2.44 <sup>b</sup>	2.45 <sup>b</sup>	2.68 <sup>a</sup>	2.38 <sup>c</sup>	2.14 <sup>d</sup>
Magnesium (mg/g)	9.17 <sup>c</sup>	8.16 <sup>d</sup>	10.45 <sup>a</sup>	9.34 <sup>b</sup>	10.34 <sup>a</sup>	7.46 <sup>e</sup>	9.39 <sup>b</sup>
Phosphorus (mg/g)	0.92 <sup>d</sup>	0.98 <sup>d</sup>	1.02 <sup>cd</sup>	2.13 <sup>b</sup>	2.13 <sup>b</sup>	2.01 <sup>c</sup>	2.34 <sup>a</sup>
Calcium (mg/g)	14.34 <sup>c</sup>	9.37 <sup>e</sup>	14.94 <sup>c</sup>	19.17 <sup>a</sup>	17.04 <sup>ab</sup>	12.43 <sup>d</sup>	14.26 <sup>c</sup>
Copper (ppm)	1.12 <sup>a</sup>	1.16 <sup>a</sup>	1.14 <sup>a</sup>	1.15 <sup>a</sup>	1.13 <sup>a</sup>	1.15 <sup>ª</sup>	1.15 <sup>a</sup>
Iron (ppm)	3.26 <sup>d</sup>	3.60 <sup>a</sup>	3.5 <sup>ab</sup>	3.44 <sup>b</sup>	3.38 <sup>b</sup>	3.32 <sup>c</sup>	3.49 <sup>ab</sup>
Zinc (ppm)	0.34ª	0.14 <sup>c</sup>	0.34ª	0.32ª	0.23 <sup>b</sup>	0.28 <sup>b</sup>	0.12 <sup>c</sup>
Manganese (ppm)	0.42 <sup>c</sup>	0.45 <sup>b</sup>	0.54 <sup>a</sup>	0.47 <sup>b</sup>	0.52 <sup>a</sup>	0.43 <sup>c</sup>	0.47 <sup>b</sup>

Samples with the same superscripts across the row are not significantly different (P>.05).

Legend: NN= unfermented sample; FF= Naturally fermented sample; LA=L. acidophilus fermented sample;

LF=*L. fermentum* fermented sample; LP=*L. plantarum* fermented sample;

GC=G. candidum fermented sample; SC=S. cerevisiae fermented sample

the phytate and tannin contents when compared to uncontrolled fermentation. Phosphorus which is one of the constituents of phytate as well as other metals such as calcium, magnesium, iron, and zinc which bind to the substrate were released during the process thus increasing their availability in a form that could be readily available in the intestine (Hurrell, 2003). Mulimani *et al.* (2003) also reported a 1/3 reduction in the phytic acid content of soybean due to fermentation. It had earlier been reported that lactic acid bacteria could hydrolyse phytate thereby releasing minerals which are bound to it (Lopez *et al.*, 1983). *Saccharomyces cerevisiae* has been reported to reduce phytic acid in water melon rinds (Erukainure *et al.*, 2010).

Besides, combination of *Lactobacillus* and *Leuconostoc* strains with the yeast strains had been reported to enhance phytate reduction in fermented Soughdough bread (Chaoui *et al.*, 2003), The reduction in oxalate levels in the starter fermented samples alleviates the fear of renal calcium absorption which results into kidney stones which is

characteristic of food with high oxalate contents (Chai and Liebwan, 2004). Alkaloids have been associated with convulsing and nerve poison activities (Okwu and Ndu, 2006). Saponins form foams in aqueous solution, haemolytic activity, cholesterol binding and bitterness (Sodipo *et al.*, 2000).

The fluctuations which were observed in the mineral contents in this work were similar to Kayode and Sani (2010) while fermenting mango kernel. The increased Iron contents in all the samples may improve the health condition of consumers since Iron is an important substance in the haem group of oxygen-carrying protein and myoglobin in the muscles (Thomas, 2002). The significant increase in the Potassium, Calcium, Magnesium and Iron content in some of the starter culture fermented samples may be adduced to the degradation of antinutritional factors like phytate, oxalate and tannin. The increase observed in Potassium contents in some of the starter culture fermented samples is of significant importance. Potassium is important in normal muscular activity, prevention of mental disorientation and cardiac irregularities which are caused by inadequate level of Potassium in the plasma. Lower mineral contents in some of the starter fermented samples could be due to the utilisation of these metals by the microorganisms for their metabolisms after they might have been cleaved from some of the anti-nutrient compounds which previously bound them.

There was a significant reduction in the viscosity from unfermented gruel to naturally fermented sample and starter culture fermented sample. Ariahu et al. (1999) attributed the reduction in viscosity to the fermenting organisms. The reduction in the viscosity could also be attributed to the hydrolytic enzymes which resulted from sprouting process of the sorghum grains. This was in agreement with the findings of Gernah et al. (2011) malting and lactic acid fermentation of maize. The lower viscosities in the sample fermented with starter culture could be attributed to the ability of the starter to metabolise carbohydrate content and other nutrients. The reduction in viscosity is beneficial in the sense that less viscous gruel digests faster than the more viscous ones. Most people prefer thin gruels with low viscosities as it could encourage higher intake of the gruel and consequent higher energy intake (Zakari et al., 2010).

## Conclusion

Results from this research work shows that microorganisms play an important role in reducing the anti-nutrient contents of the gruel making the nutrients bound to them readily available in a utilisable form. The starter culture fermentation also reduced the viscosity of the gruel. Further studies on the anti-nutrient contents of the gruel should be focused on fermenting the gruel with mixed cultures.

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