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Inhibitory Activity of Ogi on *Salmonella typhi*

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Abstract. The survival of two strains of *Salmonella typhi*, ATCC 14028 and SSH 41, were investigated in one uncooked and two cooked (one maintained at $\geq$ 25 °C, and second maintained at $\leq$ 35 °C) samples of Ogi, a fermented cereal porridge. Ogi was obtained by fermentation of maize for five days. The foods were inoculated with a known concentration of the cell suspension of the test organisms which showed a sharp decrease in their numbers after 5 h. The antibacterial effect of uncooked Ogi was more pronounced on the two strains of *Salmonella typhi* ATCC 14028 and *Salmonella typhi* SSH 41, with 4.57×10^3 cfu /h and 2.20×10^3 cfu /h decline in growth rate respectively within 5 h. *Staphylococcus* sp., *Aspergillus* sp., *Fusarium* sp., *Epholosporum* sp., *Pediococcus* sp., *Leuconostoc* sp., *Lactobacillus* sp., *Corynebacterium* sp., *Saccharomyces cerevisiae, Alkaligenes* sp., and *Candida mycoderma* were isolated from the fresh and fermented maize at different times of fermentation. It is interesting to note that the fermenting organisms depended much on calcium (67%) and sodium (30%) for their growth.

Keywords: Ogi, inhibition, *Salmonella typhi*

Introduction

Ogi is a porridge prepared from fermented maize (*Zea mays*) or other cereals in West Africa. It is staple food in this region of the world and also serves as weaning food for infants. Traditionally, Ogi is produced by first steeping the clean grains in water for 24-72 h followed by wet-milling and wet-sieving of the steeped grains (Steinkraus, 1983). The filtrate is fermented for 24-72 h to yield Ogi. Banigo and Muller (1972) and Steinkraus (1983), reported that the steeping of the grains and fermentation of the slurry are the two main stages in the production of Ogi. During the steeping stage, the micro-organisms responsible for fermentation of the slurry come into prominence by outgrowing other microorganisms (Banigo *et al.*, 1974).

Organisms such as *Lactobacillus plantarum*, *Streptococcus lactis* and *Saccharomyces cerevisae* are the most prominent microorganisms associated with Ogi fermentation (Akinrele, 1970). Usually, the fermenting organisms are initially present along with other organisms and colonize the food as a result of the low pH and increased acidity caused by steeping and fermentation of the grains and the slurry, respectively. It is however, important to note that the organisms involved in the fermentation process get there by chance and so the organisms could be seen as "chance inoculants."

Depending on the processor, Ogi might also get contaminated. The contaminating bacteria may multiply whenever, there is a delay for more than four hours between preparation and consumption of Ogi (Henry *et al.*, 1989). However, it has been discovered that inherent microorganisms in some fermented foods possess some antimicrobial properties (Kabara, 1980; Drassar and Hill, 1974), detected to be small molecular weight antibiotics, which inhibit coliforms. For example, *Lactobacillus lactis* produces lactobacillin which is inhibitory to a variety of organisms (Coventry *et al.* 1997; Vaughan *et al.*, 1994).

Antimicrobial compounds produced by most fermenting bacteria play an essential role in ensuring the safety and extending the shelf life of plant food products like Ogi. For example, *Streptococcus thermophilus* produces nisin, which has inhibitory effect on both gram positive and gram negative organisms (Fowler and Gasson, 1991). There are also bacteriocins which exhibit antimicrobial effect against many bacteria of different species and of the same species (Toba *et al.*, 1991). Lactobacillin is another group of small molecular weight compound, which inhibits coliforms like *Escherichia coli, Salmonella* spp. and *Shigella flexneri* (Garver and Muriana, 1993). This heat labile inhibitory product is usually released by *Lactobacillus brevis* (Coventry *et al.*, 1996).

Majority of the small molecular weight antibiotics produced by bacteria generally termed bacteriocin, have been identified to effect bactericidal actions on coliforms and enteric bacteria isolated from person having diarrhoea and enteric fever (Barefoot and Klaenhammer, 1983). Typhoid fever is the most serious of the *Salmonella* diseases caused by *S. typhi* (Vaughan *et al.*, 1994). Initially, the organism survives the stomach acidity, enzymes and penetrates the epithelial lining of the

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small intestine, from where they enter into the lymphoid tissue of the lamina propria (Vaughan *et al.*, 1994). From the lymphatic system, the organisms enter the blood and infect various organs and tissues, including the liver, kidney, spleen, bone marrow, gall bladder and sometime, the heart. The patient manifests high fever (up to 40 °C) with occasional delirium and abdominal pain.

Nisin is the only antibiotic intentionally used as a preservative in fermented foods to date, but its success has served as a stimulus for further research on antimicrobial agent produced by lactic acid bacteria obtained from specific food source (Stoffels *et al.*, 1992; Barefoot and Klaenhammer, 1983).

This study was undertaken to examine the chemical and microbiological changes of maize grains during 5 days fermentation. The growth of *S. typhi* in raw and cooked samples of Ogi, stored at room temperature and elevated temperature of 37 °C was also monitored.

Materials and Methods

Source and treatment of samples: *Plant sample.* The maize (*Zea mays*) grains used for the research work were obtained from Oba's market in Ado-Ekiti, about 15 km from the laboratory of the Department of Microbiology, University of Ado-Ekiti, Nigeria. The grains were soaked in distilled water to ferment for 5 days. Following fermentation, the grains were subsequently milled with a sterile blender and sieved with a 0.2 ml sieve to remove the fibre and husks. The resulting filtrate, i.e. Ogi, was stored in a sterile beaker until required for chemical and microbiological analyses.

Preparation of Ogi for consumption. The method of Banigo and Muller (1972) was employed in the preparation of Ogi for consumption (subsequently designated as cooked samples). Ogi samples held at 37°± 2°C before consumption were referred to as 'cooked samples at elevated temperature'.

Source of test organism and preparation of stock culture. The inoculum, either *Salmonella typhi* ATCC 14028 or *S. typhi* SSH 41, used in this experiment as a contaminant was obtained from the stock culture unit of the Microbiology Department, University of Ado-Ekiti, Nigeria. The bacterial culture was usually reactivated by streaking a few cells of *S. typhi* onto the *Salmonella/Shigella* agar plates and MacConkey broth incubated for 18 h at 37 °C. One ml of the broth culture was serially diluted and later standardised for further use.

Microbiological analysis. About 1 ml of each Ogi sample (raw, cooked and at elevated temperature 37 °C) was serially diluted in 10 folds into sterile test tubes containing 9 ml distilled water as diluents. From fourth dilution 100 ml was inoculated into nutrient agar (LAB), potato dextrose agar (oxoid) plates, and later incubated at 37 °C for 24 h and at 25 °C for 48 h, respectively. The viable colonies of bacteria were counted while the incidence of fungi was determined by the weight of the mycelia.

Discrete bacteria colonies and fungi spores or propagules were sub cultured into test tubes containing nutrient broth (LAB) and potato dextrose agar, respectively. The tubes were incubated for 18 h at 37 °C, and between 24-72 h at 25 °C, for fungi. The bacteria colonies were identified using the methods of Elaine *et al.* (1994). The fungi besides employing the method of Beuchat (1987), were also identified through cross matching the organisms with standard stock cultures in the laboratory.

Proximate analysis. The AOAC (1990) methods were used in the determination of the ash, fat and protein contents of the maize grains. The glucose content was estimated as carried out by Dubois *et al.* (1956), while the carbohydrate content was determined by difference. The concentration of the mineral elements was determined using an atomic absorption spectrophotometer.

Growth of *S. typhi* in Ogi. About 1 g of fresh or cooked Ogi (both samples held at 28 ° ± 2 °C and 37 ° ±2 °C was dispensed into a sterile 100 ml beaker containing 10 ml distilled water, respectively, and later inoculated with 1 ml of 18 h broth culture containing 10^6 cells/ml of *S. typhi*. One millilitre of the mixture of Ogi was later mixed thoroughly with 5 ml of MacConkey broth (oxoid) in sterile test tubes which were later incubated at 37 °C. After incubation for 1, 2, 3, 4 and 5 h, respectively, 1 ml each of the samples was withdrawn and cultured on *Salmonella/Shigella* agar (oxoid) plates which were eventually incubated at 37 °C for 18 h. Following incubation, the viable counts were estimated using the colony counter.

All the experiments were carried out in triplicate and repeated twice. The control experiments were conducted by sterilizing the Ogi samples through a membrane filter before inoculating it with the test bacterium.

Results and Discussion

Microbial load of fresh and fermented maize grains. The microbial load of fresh maize reduced to 4.7×10^3 cfu/g after 4 days fermentation (Table 1). A further decrease in the population of the bacteria observed on the 5th day (3.0×10^3 cfu/g) was attributed to the presence of lactic acid bacteria, which are usually the predominating fermenting organisms at this stage (Odugbemi *et al.*, 1991).

Table 1. The bacterial load and fungal mycelia mass of maize

Days of fermentation	No. of colony ($\times 10^3$ cfu/g)	Wt. of mycelia (mg/ml)
Fresh	4.9	12
4th	4.7	64
5th	3.0	21

The mycelia mass reduced drastically to about 21 mg/gm of the maize gruel. The following microorganisms were isolated from the fresh maize grains *Staphylococcus, Streptococcus, Aspergillus, Epholosporum* and *Fusarium*. Meanwhile, on the 4th day of fermentation, *Staphylococcus, Pediococcus* and *Leuconostoc* were isolated. Some species of the following genera *Staphylococcus, Lactobacillus, Corynebacterium, Saccharomyces cerevisiae and Candida mycoderma* were also obtained on the 5th day of fermentation. Bacteria persisted in detectable numbers throughout the fermentation period. The occurrence of fungi was however, restricted to the first half of the fermentation period. This may be due to the anaerobic condition of the medium which usually existed at the latter stage of fermentation (Akingbala *et al.*, 1981). Fernandez *et al.* (1992) reported that fungi generally prefer anaerobic environment and their growth is restricted by high concentration of carbon dioxide as produced during anaerobic fermentation. The production of toxic compounds such as organic acid, other metabolites and the anaerobic condition under which fermentation proceeded are likely to inhibit the continuous proliferation of this group of organisms (Martin *et al.*, 1998).

The sequence of microbial proliferation may be described as follows: aerobic organisms predominate at the onset of fermentation, then the aerophiles and the aerobic organisms hence the little available oxygen was used up rapidly (Aderiye and Ojo, 1997). With more carbon dioxide and acids produced during lactic acid fermentation as in the processing of Ogi, only aciduric, and facultative anaerobes grow better (Aderiye and Ojo, 1997). This of course is desirable because the aerobic bacteria, if not suppressed could produce gas and unpleasant flavour in the fermented maize product (Nout *et al.*, 1988).

The species of genera *Corynebacterium, Lactobacillus, Pediococcus* and *Leuconostoc* were the major microbial isolates obtained in this work. These findings correlate with those of Henry *et al.* (1989). The occurrence of *Staphylococcus aureus* could be as a result of contamination from utensils, hands of processors, and water used during processing. The persistent occurrence of *Lactobacillus* sp. in the fermented Ogi has been demonstrated to produce antagonistic activity against food spoilage and pathogenic bacteria (Vaughan *et al.*, 1994). Moulds isolated during the early stage of fermentation of the maize gruel include *Epholosporium, Fusarium* and *Aspergillus* species. The presence of yeasts *Candida mycoderma* and *Saccharomyces cerevisae* was also prominent on the 5th day of fermentation. These findings correlate with an earlier investigation by Leistner (1990).

Chemical and nutritional changes in maize during fermentation. The chemical and mineral components of the fresh and fermented maize grain determined on dry weight basis are shown in Tables 2 and 3, respectively. The concentration of the ash content decreased from 3.41% (fresh) to 0.42% (fermented), crude protein from 10.65% (fresh) to 6.14% (fermented), fats and oil from 4.12% (fresh) to 3.68% (fermented) and carbohydrate from 72.33% to 1.3% (fermented) (Table 2). Same trend of reduction was observed in the mineral components. For example, in the fresh maize grain the concentration of potassium, sodium, calcium, phosphorous and magnesium were 400 mg, 47 mg, 6 mg, 298 mg and 160 mg/ml, respectively, decreased in the fermented maize grain to 343 mg, 33 mg, 2 mg, 241 mg and 146 mg/ml after 5 days (Table 3). Therefore, the percentage amount of mineral nutrients utilized by the fermenting organisms ranged from 9% (magnesium) to 67% (calcium) of the initial concentration in the fresh maize gruel. It is interesting to note that the

Table 2. Proximate composition and pH value of fresh and fermented maize gruel

Chemical component	Concentration % (fresh)	Concentration % (5th day of fermentation)
Ash	3.41	0.42
Fat (oil)	4.12	3.68
Crude protein	10.65	6.14
Crude fibre	9.60	-
Carbohydrate	*66.21	13 g (as glucose)
pH	6.5	4.0

* = obtained by difference

Table 3. Mineral component of fresh and fermented maize gruel

Minerals	Concentration (mg/ml)	
	(fresh)	(5th day of fermentation)
Potassium	400.0	343.0
Sodium	47.0	33.0
Calcium	6.0	2.0
Phosphorus	298.0	241.0
Magnesium	160.0	146.0

fermenting organisms depended much on calcium (67%) and sodium (30%) for growth.

The fermentation process induced changes in the chemical composition and nutritive value of the final product. Table 2 shows that protein was degraded to about two-thirds of its initial value by the bacterial species involved in the fermentation. This may be due to the significance of amino acid as nitrogen source for bacterial growth.

The proximate composition of Ogi (uncooked or cooked) obtained from fermentation of maize for five days, showed changes in the major nutrients at relatively small amount with a tremendous decrease in the fibre and ash contents when compared to the whole maize. This together with the elimination of the germ from kernels explains the very low protein quality of Ogi (Adeniji and Potter, 1978).

Growth of *Salmonella typhi* in Ogi. The growth of *S. typhi* in different forms of processed Ogi on the 5th day of fermentation is shown in Fig. 1. *S. typhi* exhibited very poor growth in the different Ogi samples, with the raw uncooked samples showing a tremendous inhibitory effect on the organism even after 5 h of incubation. There was a significant difference when the processed Ogi samples, stored either at room temperature or at an elevated temperature of 37 °C, were inoculated with *S. typhi*.

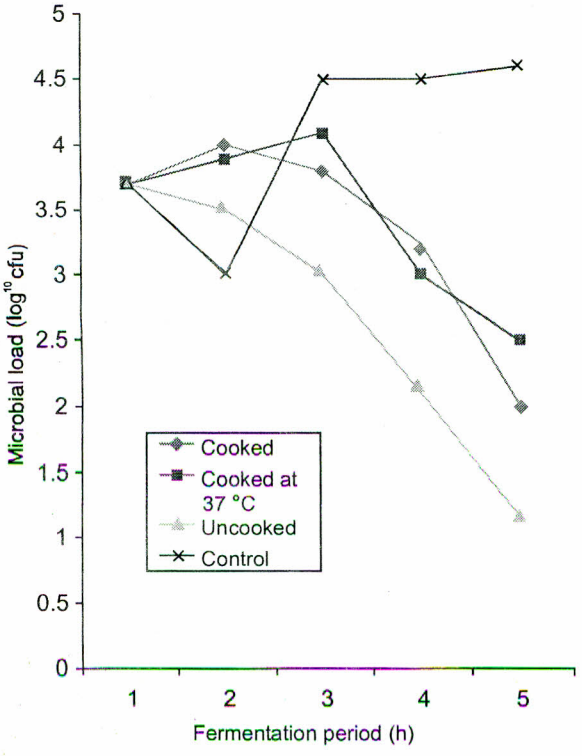

Fig. 1. The growth of *S. typhi* AT CC 14028 in cooked, uncooked and cooked (maintained at temperature greater than or equal to 37 °C) Ogi on the 5th day of fermentation.

Growth experiments showed that the strains of *S. typhi* used as the test organisms experienced reduction in bacteria population in the Ogi samples over a period of 5 h at 30 °C.

The antibacterial effect of uncooked Ogi was more pronounced on the two strains of *Salmonella typhi* ATCC 14028 and *S. typhi* SSH 41 with 4.57 x 10^3 cfu /h and 2.20 × 10^3 cfu /h decline in growth rate, respectively, within 5 h. It is interesting to note that both strains exhibited the same growth pattern. Similar findings were reported by Svanberg *et al.* (1992). Recent research reports in this field indicate that lactic acid fermented cereals significantly suppressed the growth of food-borne pathogens (Coventry *et al.*, 1997; Olukoya *et al.*, 1994). Studies by Odugbemi *et al.* (1991) showed that the reduction in the population of bacteria may be due to the presence of native organisms, which are directly or indirectly responsible for fermentation and also play an additional inhibitory role.

Many investigators (Olukoya *et al.*, 1994; Odugbemi *et al.*, 1991) had shown that traditional fermented foods showed antimicrobial effects against many pathogenic organisms. Specific inhibitory activities of fermented cereals have been shown against bacteria like *Escherichia coli*, *Staphylococcus aureus* 3601 and *S. typhimurium* (Nout *et al.*, 1988). It was observed that in fermented and acidified samples of gruels of maize, the numbers of inoculated pathogens were reduced over the incubation period of 24 h at 30 °C. Similar findings have been reported for *Escherichia, Shigella*, and *Salmonella* (Ahn and Stiles, 1990). This result also showed that Ogi at different processing procedures and temperature exposure exhibited some antibacterial effects on *S. typhi*.

It was evident from this work that Ogi fermentation followed the typical lactic acid fermentation in which a variety of organisms are involved. *Corynebacterium* sp. was also isolated during maize fermentation. This organism had been reported to ferment maize with the production of acid, thus making the substrate more favourable for the growth of the other lactic acid bacteria (Odugbemi *et al.*, 1991). The lactic acid bacteria identified in this work are *Lactobacillus* sp., *Pediococcus* sp., and *Streptococcus* sp. Similar isolation and identification of the organisms have been reported by Nout *et al.* (1988).

Lactic acid bacteria isolated predominantly during fermentation have been the subject of intense interest with respect to the production of growth inhibitory compounds, including bacteriocin (Coventry *et al.*, 1997; Vaughan *et al.*, 1994; Garver and Muriana, 1993; Anderson and Mckay, 1983). Other mechanisms of the antagonistic effects of lactic acid bacteria that have been proposed are, organic acid production,

competition for nutrients, hydrogen peroxide formation, and production of antibiotic like substances (Odugbemi *et al.*, 1991).

In conclusion, this study demonstrated that the fermenting organisms in Ogi possess the ability to inhibit the growth of *S. typhi* the etiologic agent of typhoid fever. Though the reduction in cell population of the bacterium was demonstrated in all the Ogi samples, the efficiency of the inhibitory activity was more pronounced in the uncooked Ogi. This finding correlates with those of Odugbemi *et al.* (1991), where temperature and pH affected the various antimicrobial agents in fermented foods.

However, research is continuing on the extraction and isolation of these various inhibitory (antimicrobial) substances from the native bacteria and their effects on pathogenic micro-organisms. The applicability of these substances in natural food preservation system and the utilization of uncooked Ogi as a preventive agent for minor abdominal ailments in Nigeria are also being explored.

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