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INFLUENCE OF pH AND ACIDITY ON THE FERMENTATION OF FINGER MILLET SPICED OGI

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Abstract: *The purpose of this research work was to evaluate the effect of ginger and garlic on the fermentation dynamics of finger millet made ogi. It is important to allow cereals achieve acidification during fermentation in order to improve the organoleptic properties and safety of the product. Spiced finger millet (FM) ogi was produced in the ratios of 100% FM, 99% FM: 1% ginger, 95% FM: 5% ginger, 90% FM: 10%, 99% FM: 1% garlic, 95% FM: 5% garlic, 90% FM: 10% garlic. The data generated were subjected to statistical analysis and means were separated using Analysis of Variance. The results of microbial associations of spiced finger millet ogi samples investigated at 48 h during the secondary fermentation stages ranged from (5.0×10^3 – 43.0×10^3) cfu/ml for total bacteria count, (1.0×10^3 – 3.0×10^3) cfu/ml for total fungi count, (2.0×10^3 – 10.5×10^3) cfu/ml for total coliform count and (7.5×10^3 – 70×10^3) cfu/ml for total LAB count. All the isolates that were catalase negative, gram positive, non-spore forming were identified as lactic acid bacteria and sugar fermentation pattern revealed that they belong to the specie of Lactobacillus. During steeping the acidity increased with consequent drop in pH, the trend which was sustained during the souring stage, the pH decreased gradually ($p<0.05$) from 5.98 to 4.01 at 10% garlic inclusion, while it decreased significantly ($p<0.05$) from 5.54 to 3.42 at 10% inclusion of ginger.*

Keywords: *traditional, lactic acid bacteria, safety, dominate, acidification*

1. Introduction.

Finger millet (*Eleusine coracana*) locally known as ‘tamba’ in Nigeria, ‘ragi’ and ‘mandua’ in India belongs to the family *Poaceae* and genus *Eleusine*. It is consumed as one of the main staple food that supplies calories and protein to a relatively large population of rural dwellers and low income earners in these countries [1]. It is quite interesting that despite its excellent nutritional profile, finger millet is still being under-utilized as only a few people are aware of its health benefits and nutritional value [2]. The nutritional properties of the grains is outstandingly comparable with other cereals such as rye, barley and oats, being a rich source of riboflavin, thiamine, iron, methionine, leucine, isoleucine, phenylalanine and other essential amino acids [3].

Finger millet’s chemical composition consist of high content of protein (6%–13%), dietary fiber (18%), minerals (2.5%–3.5%), calcium (0.38%), carbohydrate (65%–75%), phytates (0.48%), tannins (0.61%), phenolic compounds (0.3–3%) and trypsin inhibitory factors [3]. It is recognized for its health benefits such as anti-diabetic, anti-diarrheal, anti-inflammatory, anti-ulcer, atherosclerogenic effects, antioxidant and antimicrobial properties [4, 5].

Ogi is a traditionally fermented food prepared by steeping cereal grains such as millet, maize, sorghum in water for 72 h, followed by wet milling and sieving using muslin clothe. The filtrate obtained is allowed to settle to give the slurry referred to as ogi and the supernatant discarded. The traditional processing of ogi is characterized with a lot of nutrient loss which include proteins, water soluble vitamins and minerals [6].

As a result several researchers have made attempts at improving the nutritional status of this unique food by enriching it with both plant and animal protein substrate **[**7-11]. However, this nutritional fortification has been reported to lower the pasting viscosity and also adversely affect the sensory attribute for which the product is desired [12]. The predominant organisms involved in the fermentation are mainly lactic acid bacteria and yeast which are also responsible for microbial stability, characteristic sour taste, flavour and aroma development of ogi.

The health benefit of some herbs and spices on humans has led to the gradual drifting towards natural, antimicrobial and traditional preservative techniques. Currently there is increased awareness in the use of spices such as ginger, garlic, conophor nuts etc in the production of ogi to improve its organoleptic properties, enhance shelf stability and provide other therapeutic benefits [13, 14]. In order to attain its desired sensorial quality and safety it is important to study and establish the fermentation kinetics of the fermenting grain and slurry as the degree of sourness which reflects its level of acidity varies with consumers and region. This study is therefore under-taken to evaluate the effect of ginger and garlic on the microbial load and fermentation dynamics of ogi produced from finger millet.

2. Materials and Methods

Collection of samples

For this study, Finger millet (*Eleusine coracana*), cloves of fresh garlic (*Allium sativum*) and ginger roots (*Zingiber officinale*) were all purchased from Owena market in Oriade local government area of Osun State, Nigeria.

Preparation of finger millet into ogi

This was done according to the methods described by 15 and [16] with slight modifications. Spiced finger millet ogi was produced in the ratios of (0:100, 1:99, 5:95, and 10:90 w/w) for both ginger and garlic. Prior to fermentation the dried finger millet grains were cleaned to remove pebbles and dirt, one thousand grams of the grain was weighed and washed thoroughly in clean water. Fresh ginger rhizomes and garlic cloves were washed peeled with a sharp knife and steeped together with the cleaned grains in 2000 ml of sterile water in a plastic container for 48 h. The steeped grains were wet-milled together with the steeping water using a sterile laboratory blender. The slurry was sieved using sterile muslin clothe and the filtrate allowed to settle for 48 h at 28±2 ^{o}C to undergo souring (secondary fermentation) and give a smooth starchy. Samples were aseptically taken 12 hourly for analyses during the primary and secondary fermentation period.

Microbiological analysis of spiced finger millet ogi samples

The samples were analyzed for total bacterial, fungal, coliform and Lactic acid bacteria [LAB] counts according to the methods stated in [17]. About 10 g of each composite mixture was homogenized with90 ml of sterile distilled water to give a ten-fold serial dilution of 10^{-1} level. From this, subsequent dilution levels were made

and cultured on appropriate agar medium using the pour plate technique. Pure cultures of isolated colonies of fermenting organisms (LAB) on de Man Rogosa and Sharpe (MRS) medium were obtained by incubating inoculated plates at 37 ^{o}C for 48 h under anaerobic conditions with successive streaking on plates and slants. The pure isolates were subjected to further characterization based on appropriate, cultural, morphological and biochemical tests to establish their identity.

Determination of pH and Titratable Acid

The pH of the finger millet ogi samples during the primary and the secondary fermentation stage were determined at 12 h intervals for 48 h as described by [18] using a digital pH meter (Hanna Instruments, Model HI 8314). The titratable acid (TA) of the fermenting finger millet grain during the primary and the secondary fermentation stage was determined by taking 10-ml sample and titrating against 0.1M NaOH, using 2 to 3 drops of phenolphthalein as indicator until a faint pink color was observed. This was carried out at 37 ^{o}C for 48 h, with a 12 hourly monitoring. The titratable acidity was calculated as follows:

$$\% \text{ acid (w/w)} = \frac{N \times E \times EQ}{W} \times 100$$

Where N= normality of titrant
E= volume of titrant
EQ= equivalent weight
W= mass of sample

Statistical Analysis

Data obtained from the study were subjected to analysis of variance (ANOVA) using a statistical package for the social sciences (SPSS). Differences among means were separated using Duncan's multiple range test and significances accepted at 5% level ($P \leq 0.05$). Total viable counts were expressed as means of three replicates.

3. Results and Discussion

Microbial Association of Spiced Finger Millet Ogi Samples

Table 1.

Microbial counts (cfu/ml) of Spiced Finger Millet Ogi Samples

Sample	TBC	TFC	TCC	TLC
FM	5.0 X 10^3	1.0 X 10^3	2.0 X 10^3	7.5 X 10^3
FGN1	8.0 X 10^3	18.0 X 10^3	12.5 X 10^3	T.N
FGN5	8.0 X 10^3	3.4 X 10^4	6.0 X 10^3	7.0 X 10^4
FGN10	6.0 X 10^4	5.6 X 10^4	4.1 X 10^4	T.N
FGR1	1.0 X 10^3	10.0 X 10^3	9.0 X 10^3	T.N
FGR5	2.0 X 10^4	3.0 X 10^3	10.5 X 10^3	T.N
FGR10	4.3 X 10^4	N.G	N.G	T.N

Values are means of triplicate measurement
TBC =Total bacterial count, TFC=Total fungal count
TCC=Total coliform count, TLC=Total LAB count
T.N = too numerous, N.G=no growth,
FM = 100% finger millet, FGN1 = 99% -1% ginger, FGN5 = 95%-5% ginger, FGN10 = 90% - 10% ginger, FGR1 = 99% - 1% garlic, FGR5 = 95%-5% garlic, FGR10 = 90%-10% garlic**.**

The growth kinetics of the microbial population investigated at 48 h of secondary fermentation stage as shown in Table 1 ranged from 5.0 x 10^3 to 4.3 x 10^4 cfu/ml for total bacteria counts and 1.0 x10^3 - 3.0 x 10^3 for total fungi counts, while the values obtained for total coliform and lactic acid bacteria (LAB) counts ranged from 2.0 x10^3 - 10.5 x 10^3 and 7.5 x10^3 - 70 x 10^3 respectively. This study revealed that the population of coliform and fungal organisms at this stage were relatively low compared to total bacteria and Lactic acid bacteria count due to low pH of the slurry which could be partly responsible for the inhibition of the latter because most coliform organism cannot survive under low pH [19]. Furthermore, the presence of gingerol and allicin in both ginger and garlic respectively could also contribute to the low coliform and total bacteria counts as also reported by [20]. Total bacterial count was lower than total LAB count possibly because LAB are anaerobic organisms requiring a more fastidious medium for growth and may not be able to grow on a general medium such as plate count agar used for total bacteria counts. Previous studies have reported the co-existence and symbiotic association of yeast and LAB in the fermentation of ogi where they contribute to the flavor and aroma development [15, 21 – 22].

The morphological and biochemical characteristic of the purified representative isolates on MRS agar is presented in Table 2. All the isolates were revealed to be catalase negative, gram positive, non-spore forming and rod shaped which characterizes lactic acid-producing bacteria. Sugar fermentation pattern revealed that the isolates belong to the species of facultative heterofermentative *Lactobacillus*. These were present throughout the fermentation process with their growth followed by simultaneous acidification of the product.

Table 2.

Morphological and Biochemical Characteristics of Lactic acid bacteria Isolates

Characteristics	Isolate 101	Isolate 102	Isolate 103
Colony/cell morphology	Large, white spreading colonies, non-sporing rods	Small whitish distinct colonies, non sporing rods	Small creamy shiny distinct colonies, short medium rods
Gram's reaction	+	+	+
Motility	Non-motile	Non-motile	Non-motile
Catalase test	–	–	–
Oxidase test	–	–	–
Vogue Proskauer	–	–	–
Methyl red test	–	–	–
Urease production	–	–	–
Nitrate reduction	–	–	–
Citrate utilization	+	+	+
Growth at 30 ºC	+	+	+
Growth at 37 ºC	+	+	+
Growth at 45 ºC			
Growth in FTM	F/A	F/A	F/A
CO_2 production	+	+	+
Mannitol utilization	+	+	+
Galactose utilization	+	+	+
Glucose utilization	+	+	+
Lactose utilization	+	+	+

Fructose utilization	+	+	+
Sucrose utilization	+	+	+
Maltose utilization	+	+	+
Probable identity	Lactobacillus spp	Lactobacillus spp	Lactobacillus spp

Notes: FTM, Fluid thioglycolate medium, +positive, - negative, F/A facultative anaerobic

This relates well with the work of [23] which confirmed the presence of heterofermentative LAB during ogi fermentation. The results obtained were also in accordance with the report of other authors [24 – 26] and were also referenced to Bergey's manual of modern bacteriology.

Changes in pH during fermentation of spiced finger millet ogi

Tables 3 to 6 show the changes in pH and TTA during fermentation of spiced finger millet ogi. There was a general steady reduction in pH and simultaneous significant increase in TTA during the 48 h steeping period.

The trend was also sustained during the souring stage, the pH decreased gradually ($p<0.05$) from 5.98 to 4.01 at 10% garlic inclusion, while it decreased significantly ($p<0.05$) from 5.54 to 3.42 at 10% inclusion of ginger This might be as a result of the utilization of sugars and subsequent production of lactic acid by the fermenting organisms responsible for the fermentation of ogi [18], [27]. This observation also agrees with the reports of [5, 15, 28, 29]. Low pH inhibits the growth and activities of many microorganisms and at the same time is a determinant of the type of microorganisms that grows and dominates the fermentation process for which lactic acid bacteria have been implicated to dominate [18. This activity which is also due to the high counts of lactic acid bacteria, accumulation of lactic acid and organic acids produced during the fermentation can contribute to the safety of finger millet ogi in addition to the activity of the added spices [18]. The degree of sourness attained is relative to individual consumer and determines the time of termination of the fermentation process.

Table 3.

Changes in pH during fermentation of spiced ogi at the steeping stage

	Period of fermentation (hours)				
Sample	**0**	**12**	**24**	**36**	**48**
FM	$6.38^{a} \pm 0.00$	$5.49^{ab} \pm 0.00$	$5.39^{a} \pm 0.00$	$5.19^{a} \pm 0.00$	$4.83^{ab} \pm 0.00$
FGN1	$6.23^{a} \pm 0.10$	$5.50^{ab} \pm 0.10$	$5.50^{a} \pm 0.10$	$5.22^{a} \pm 0.10$	$4.79^{abc} \pm 0.10$
FGN5	$5.86^{b} \pm 0.10$	$5.40^{a} \pm 0.58$	$5.45^{a} \pm 0.10$	$4.96^{ab} \pm 0.10$	$4.86^{a} \pm 0.10$
FGN10	$5.86^{b} \pm 0.10$	$5.33^{a} \pm 0.10$	$5.00^{a} \pm 1.00$	$4.89^{ab} \pm 0.10$	$4.62^{c} \pm 0.10$
FGR1	$5.55^{cd} \pm 0.10$	$5.56^{a} \pm 0.10$	$5.28^{a} \pm 0.10$	$4.63^{b} \pm 0.44$	$4.66^{bc} \pm 0.10$
FGR5	$5.49^{d} \pm 0.10$	$5.51^{ab} \pm 0.10$	$5.43^{a} \pm 0.10$	$5.02^{a} \pm 0.10$	$4.77^{abc} \pm 0.10$
FGR10	$5.70^{bc} \pm 0.10$	$5.49^{ab} \pm 0.10$	$5.41^{a} \pm 0.10$	$5.12^{a} \pm 0.10$	$4.79^{abc} \pm 0.10$

Notes: Means with different superscripts on the same column are significantly different ($p<0.05$)
Values are means ±SD of triplicate measurement
FM = 100% finger millet, FGN1 = 99% -1% ginger, FGN5 = 95%-5% ginger, FGN10 = 90% - 10% ginger, FGR1 = 99% - 1% garlic, FGR5 = 95%-5% garlic, FGR10 = 90%-10% garlic

Table 4.

Changes in TTA of spiced finger millet ogi at the steeping stage

	Period of fermentation (hours)				
Sample	**0**	**12**	**24**	**36**	**48**
FM	$0.20^{a} \pm 0.00$	$0.20^{a} \pm 0.00$	$0.30^{c} \pm 0.00$	$0.80^{b} \pm 0.00$	$0.40^{b} \pm 0.00$
FGN1	$0.20^{a} \pm 0.10$	$0.20^{a} \pm 0.10$	$0.30^{c} \pm 0.10$	$0.60^{c} \pm 0.10$	$0.50^{ab} \pm 0.10$
FGN5	$0.20^{a} \pm 0.10$	$0.20^{a} \pm 0.58$	$0.60^{a} \pm 0.10$	$1.00^{a} \pm 0.10$	$0.60^{a} \pm 0.10$
FGN10	$0.20^{a} \pm 0.10$	$0.20^{a} \pm 0.10$	$0.40^{bc} \pm 1.00$	$0.80^{b} \pm 0.10$	$0.60^{a} \pm 0.10$
FGR1	$0.20^{a} \pm 0.10$	0.40 ± 0.10	$0.50^{ab} \pm 0.10$	$0.60^{c} \pm 0.46$	$0.60^{a} \pm 0.10$
FGR5	$0.20^{a} \pm 0.10$	$1.50^{a} \pm 0.10$	$0.40^{bc} \pm 0.10$	$0.50^{c} \pm 0.10$	$0.60^{a} \pm 0.10$
FGR10	$0.20^{a} \pm 0.10$	$0.30^{a} \pm 0.10$	$0.40^{bc} \pm 0.10$	$0.80^{b} \pm 0.10$	$0.60^{a} \pm 0.10$

Notes: Means with different superscripts on the same column are significantly different ($p<0.05$)
Values are means ±SD of triplicate measurement
FM = 100% finger millet, FGN1 = 99% -1% ginger, FGN5 = 95%-5% ginger, FGN10 = 90% -10% ginger,
FGR1 = 99% -1% garlic, FGR5 = 95%-5% garlic, FGR10 = 90%-10% garlic

Table 5.

Changes in pH during fermentation of spiced finger millet ogi at the souring stage

	Period of fermentation (hours)				
Sample	**0**	**12**	**24**	**36**	**48**
FM	$5.36^{d} \pm 0.10$	$4.82^{bc} \pm 0.10$	$4.31^{bcd} \pm 0.10$	$3.79^{c} \pm 0.10$	$3.40^{d} \pm 0.15$
FGN1	$5.70^{abc} \pm 0.10$	$4.66^{c} \pm 0.42$	$4.39^{abc} \pm 0.10$	$3.86^{c} \pm 0.10$	$3.64^{c} \pm 0.10$
FGN5	$5.60^{bcd} \pm 0.40$	$5.14^{a} \pm 0.01$	$4.26^{cd} \pm 0.10$	$3.82^{c} \pm 0.12$	$3.59^{c} \pm 0.11$
FGN10	$5.54^{cd} \pm 0.12$	$5.19^{a} \pm 0.01$	$4.17^{d} \pm 0.01$	$4.00^{c} \pm 0.05$	$3.42^{d} \pm 0.01$
FGR1	$5.89^{ab} \pm 0.00$	$4.58^{c} \pm 0.00$	$4.25^{cd} \pm 0.10$	$4.09^{b} \pm 0.10$	$3.89^{b} \pm 0.10$
FGR5	$5.92^{ab} \pm 0.01$	$4.78^{bc} \pm 0.02$	$4.42^{ab} \pm 0.01$	$4.10^{b} \pm 0.00$	$3.91^{b} \pm 0.12$
FGR10	5.98 ± 0.01	$5.07^{ab} \pm 0.01$	$4.50^{a} \pm 0.01$	$4.28^{c} \pm 0.10$	$4.09^{a} \pm 0.10$

Means with different superscripts on the same column are significantly different ($p<0.05$)
Values are means ± SD of triplicate measurement
FM = 100% finger millet, FGN1 = 99% -1% ginger, FGN5 = 95%-5% ginger, FGN10 = 90% - 10% ginger, FGR1 = 99% - 1% garlic, FGR5 = 95%-5% garlic, FGR10 = 90%-10% garlic

Table 6.

Changes in TTA of spiced finger millet ogi at the souring stage

	Period of fermentation (hours)				
Sample	**0**	**12**	**24**	**36**	**48**
FM	$1.90^{a} \pm 0.10$	$1.80^{cd} \pm 0.10$	$3.77^{e} \pm 0.15$	$6.20^{a} \pm 0.00$	$7.90^{b} \pm 0.10$
FGN1	$2.03^{a} \pm 0.06$	$1.73^{d} \pm 0.53$	$3.90^{de} \pm 0.10$	$4.90^{c} \pm 0.10$	$8.40^{a} \pm 0.10$
FGN5	$1.07^{b} \pm 0.15$	$1.70^{d} \pm 0.10$	$4.30^{c} \pm 0.10$	$6.20^{a} \pm 0.10$	$7.90^{b} \pm 0.10$
FGN10	$2.00^{a} \pm 0.10$	$1.90^{cd} \pm 0.10$	$4.70^{a} \pm 0.10$	$4.60^{d} \pm 0.10$	$8.40^{a} \pm 0.10$
FGR1	$1.20^{b} \pm 0.00$	$2.00^{c} \pm 0.10$	$4.10^{cd} \pm 0.10$	$5.00^{c} \pm 0.10$	$6.70^{c} \pm 0.10$
FGR5	$1.20^{b} \pm 0.10$	$3.10^{b} \pm 0.10$	$4.10^{cd} \pm 0.10$	$5.20^{b} \pm 0.10$	$5.60^{d} \pm 0.10$
FGR10	$1.10^{b} \pm 0.00$	$1.10^{b} \pm 0.00$	$4.50^{b} \pm 0.10$	$5.30^{b} \pm 0.10$	$5.60^{d} \pm 0.10$

Means with different superscripts on the same column are significantly different ($p<0.05$)
Values are means ± SD of triplicate measurement
FM = 100% finger millet, FGN1 = 99% -1% ginger, FGN5 = 95%-5% ginger, FGN10 = 90% - 10% ginger, FGR1 = 99% - 1% garlic, FGR5 = 95%-5% garlic, FGR10 = 90%-10% garlic.

Antimicrobial Effect of Ogi samples against Pathogens.

Traditionally fermented foods are known to exhibit antimicrobial and health promoting properties which has been demonstrated indigenously by rural dwellers who administer raw ogi slurry to diarrhea patient [26, 29]. The ability of LAB to produce organic acids, hydrogen peroxide and bacteriocins and other metabolites confer on them the potential to inhibit the growth of food-borne pathogens [29]. All the ogi samples showed clear zones of inhibition against the tested pathogenic organisms. The highest inhibition zone of 20.0 mm against *E. coli* was observed by 10% garlic spiced ogi (FGR10), while the control FM which is the un-spiced ogi gave the lowest inhibition zone of 5 mm against *E.coli*. Incorporation of some spices to groundnut products significantly reduced the microbial load [30]. This result also corresponds with the findings of several authors who have reported antibacterial activities of garlic, ginger and LAB isolates against pathogenic organisms [5, 25, 31]. The large clear zones of inhibitions observed for the spiced ogi is due to the result of the synergistic effect of the antimicrobial activity of the isolates and antibacterial properties present in the spices as spices have also been reported to inhibit microbial growth [32].

Table 7.

Inhibitory effect of samples against selected pathogenic organisms
Diameter of inhibition zones in mm

Isolate code	*Escherichia coli*	*Klebsiella pneumonia*	*Proteus mirabilis*
FM	5.0	5.0	10,
FGN1	16.0	9.0	13.0
FGN5	18.0	9.0	12.5
FGN10	14.0	15.0	7.0
FGR1	18.0	11.0	6.0
FGR5	14.5	6.0	9.0
FGR10	20.5	9.0	13.0

FM = 100% finger millet, FGN1 = 99% -1% ginger, FGN5 = 95%-5% ginger, FGN10 = 90% - 10% ginger, FGR1 = 99% - 1% garlic, FGR5 = 95%-5% garlic, FGR10 = 90%-10% garlic

4. Conclusion

This study revealed that the inclusion of ginger and garlic contributed to the improvement of the organoleptic quality of finger millet ogi and also able to inhibit pathogens and spoilage microorganisms, thus ensuring safety of the product. The addition of spice in ogi and probably other food items would reduce the risk of food contamination, protect the consumer from

different food- borne diseases and improve health status by using small quantities as low as 1%. The study of the fermentation dynamics of ogi will further facilitate the use of starter culture for a controlled fermentation and more so, enhance its process optimization for improved ogi. It is therefore important to allow cereals to achieve the right fermentation by attaining a considerable low pH in order to enhance the organoleptic qualities and also improve the safety of the fermented food.

5. Conflict of interests

The author(s) did not declare any conflict of interest.

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