American Journal of Food Science and Health
Vol. 6, No. 1, 2020, pp. 32-42
http://www.aiscience.org/journal/ajfsh
ISSN: 2381-7216 (Print); ISSN: 2381-7224 (Online)

Evaluating the Functional Groups in a Novel Instant "Ogi" Produced from Maize Grains with Fermentation Starter Using Fourier Transform Infrared (FTIR) Technique

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Abstract

Rapid, sensitive and time saving features of infrared spectroscopy in assessing biological systems for important biochemical constituents has attracted much interest in the recent time. The proximate, nutritional and lactic acid bacteria (LAB) composition of five (5) different bioproducts; D60.1, C90.3, E90.3, E120.3 and community "Ogi" were accessed using standard techniques with their functional properties evaluated with Fourier Transform Infrared (FTIR) spectroscopy. The community "Ogi" exhibited the highest LAB load (1.0 x 10^5 CFU/ml) while sample C90.3 had the least LAB load (5.5 x 10^4 CFU/ml). There was an increase of 39.4% in the LAB load of D60.1 "Ogi" sample after 60min at 60°C (primary fermentation) while the LAB growth in C90.3 sample exhibited about 50.9% increase of its initial value (5.5 x 10^4 CFU/ml) within the same time. The growth rate of the LAB cells in E90.3 sample was however the highest (6.0 x 10^2 CFU/min) during the 1° fermentation. Subsequently, the microbial growth ranged between 9.3 x 10^4 CFU/ml in C90.3 and 1.38 x 10^5 CFU/ml after 180min at 28°C (secondary fermentation). A total of 19 strains of LAB were recorded with *Lactobacillus fermentum* having highest distribution of 48% and 16% of the strains were recovered from D60.1, while 21% were encountered in each of the four samples. Intensities of most functional groups found in the final product of E90.3 (after 2° fermentation for 180min at 28°C) were significantly higher than those of E120.3 treatment. The final product of D60.1 (fermented with 8g starter) after 270min, relatively retained most of the important functional groups at significant level. E90.3 maintained increase in functional groups with (21% for –OH, 24% and 85% for $–CH_2$, 81% for $–CH_3$, 14% for –C=O of amide and 14% and 28% for –C-O-C and C-O of CHO) increase after 2° fermentation for 180min at 28°C. Overall, E90.3 treatment was however found as the best and most promising bioprocess for the production of the instant "Ogi" hence is recommended for industrial production and commercialization of "Ogi".

Keywords

FTIR, LAB, Instant "Ogi", "Ogi" Sample, Fermentation, Functional Group, Bioproduct, Biotreatment

Received: January 1, 2020 / Accepted: February 13, 2020 / Published online: March 9, 2020

1. Introduction

Fermented maize (*Zea mays*) gruel, "Ogi" is the most consumed of the popular traditional staple cereal among the Yoruba in southwest Nigeria. Generally, "Ogi" exhibits such a smooth texture, distinct aroma and flavour and sour taste reminiscence of that of yoghurt [1], making it a meal of choice for infants during weaning, convalescence and the aged [2, 3, 4, 5].

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Every technique, including soaking, fermentation, milling (dry and wet milling) and sun-drying among others [6], involved in the traditional method of processing/producing "Ogi" is expected to improve the palatability and enrich the sensory quality of the food [6]. Especially that, functional microorganisms involved in the fermentation of the raw materials transform the chemical constituents thereby enhancing the bio-availability of nutrients, imparting bio-preservative properties, degrading toxic components and anti-nutritive factors, producing antioxidant and antimicrobial compounds, stimulating the probiotic functions and fortifying it with some health-promoting bioactive compounds [7, 8, 9, 10]. However, the time required (between 3 to 5 days) to produce this important maize gruel by traditional method has not only been the challenge, the deficiency in some amino acids; such as lysine and tryptophan, which are essential for growth, has also been reported [11].

For about two decades now, there have been several attempts to reduce the drudgery and time required to produce and improve the nutritional status of this staple "Ogi"; including dry and wet milling of the maize to improve bioavailability of nutrients [6], fortification with legumes (fermented and/or unfermented), vitamins and minerals to provide the deficient amino acids [12, 13], blending with germinated and ungerminated ground bean [11] among others. Recently, Aderiye *et al.* [14] reported pre-treatment of maize (*Zea mays*) grains before fermentation to produce "Ogi" in less than 5h, a process which hitherto lasts between 48 to 72h. This is the first report of pre-treatment of maize grains, which involved particle size reduction, hydrolysis of starch (steeping of maize grains) and addition of fermentation starter, with short term low heat exposure (1° fermentation). Thereafter, the desired product was obtained after blending, sieving and stabilizing the fermented maize gruel for another 1 to 2h at ambient temperature (2° fermentation).

Among the bacteria reportedly exploited for the fermentation of cereals for the production of "Ogi" are lactic acid bacteria (LAB) mostly the species of Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Weissella [15, 16]. The bacteria propagate in the maize by converting the sugars derived during fermentation into lactic acids, citric acids, butyric acids and many other probiotic compounds, which contribute to the characteristic sour taste and acid flavour [17, 1].

Although conventional methods used to analyze lactic acid bacteria and probiotics include plate counts, molecular biology and immunology based techniques [18] are very useful and reliable, but invasive and time-consuming and thus not suitable for increasing demands of real time and on-line analyses of large amounts of samples. Using infrared spectroscopy for the investigation of biological systems, including lactic acid bacteria and probiotics, is an increasing growing approach [19, 20]. This can be attributed to the rapid, sensitive, non-destructive and time saving features of the technique in detecting wide range of functional group and change in molecular structure and providing information on the basis of chemical composition and physical state of the sample [19, 20]. Measurements usually correspond to the infrared regions of the electromagnetic spectrum which are classified as; near (750–2500 nm), mid (2500–40,000 nm), and/or far-infrared (40,000–60,000 nm) [19].

We report here, important contributions and potentials of the Fourier Transform Infrared (FT-IR), a vibrational spectroscopy based technique in monitoring some of the biochemical attributes and the microbial components of our novel instant "Ogi" product.

2. Materials and Methods

2.1. Pre-treatment and Fermentation [Primary (1°) and Secondary (2°) Fermentations] of Maize Grains

The modified method of Aderiye *et al*. (2017) was employed in the preparation of "Ogi" for this study. A known amount of the maize grain particles (≤ size 1.40mm) was soaked in an appropriate quantity of distilled water (1:5, w/v), inoculated with varying amounts of community "Ogi" (as fermentation starter). Table 1 shows the composition of the most palatable [14] "Ogi" samples obtained after fermentation at 60°C for an initial period of one to 3h (1° fermentation). Later, the resulting primary ferment was homogenized and allowed to stabilize for another 1 to 2h at ambient temperature (28^{o}±1°C) (2° fermentation). Following the secondary (2°) fermentation, the liquor was filtered through a 1µm sieve and the filtride de-watered, and either made into molds ready for packaging and storage or prepared for consumption as in Figure 1.

Table 1. Composition and treatment conditions of instant "Ogi" samples.

Sample Code*	Inoculation of pre-treated maize grains with fermentation starter**
C90.3	Six gram of fermentation starter with 50g of powered maize grain, placed in water bath at 60° for 90mins allowed to stabilize at room temperature for 3hrs
D60.1	Eight gram of fermentation starter with 50g of powered maize grain, placed in water bath at 60° for 60mins and allowed to stabilize at room temperature for 1hr.
E90.3	Ten gram of fermentation starter with 50g of powered maize grain, placed in water bath at 60° for 90mins allowed to stabilize at room temperature for 3hrs

Sample Code*	Inoculation of pre-treated maize grains with fermentation starter**
E120.3	Ten gram of fermentation starter with 50g of powered maize grain, placed in water bath at 60° for 120mins and allowed to stabilize at room temperature for 3hrs
Control	Community "Ogi" (fermentation starter**)

*The different sample codes denote the extent of acceptance by the sensory panellists [14].

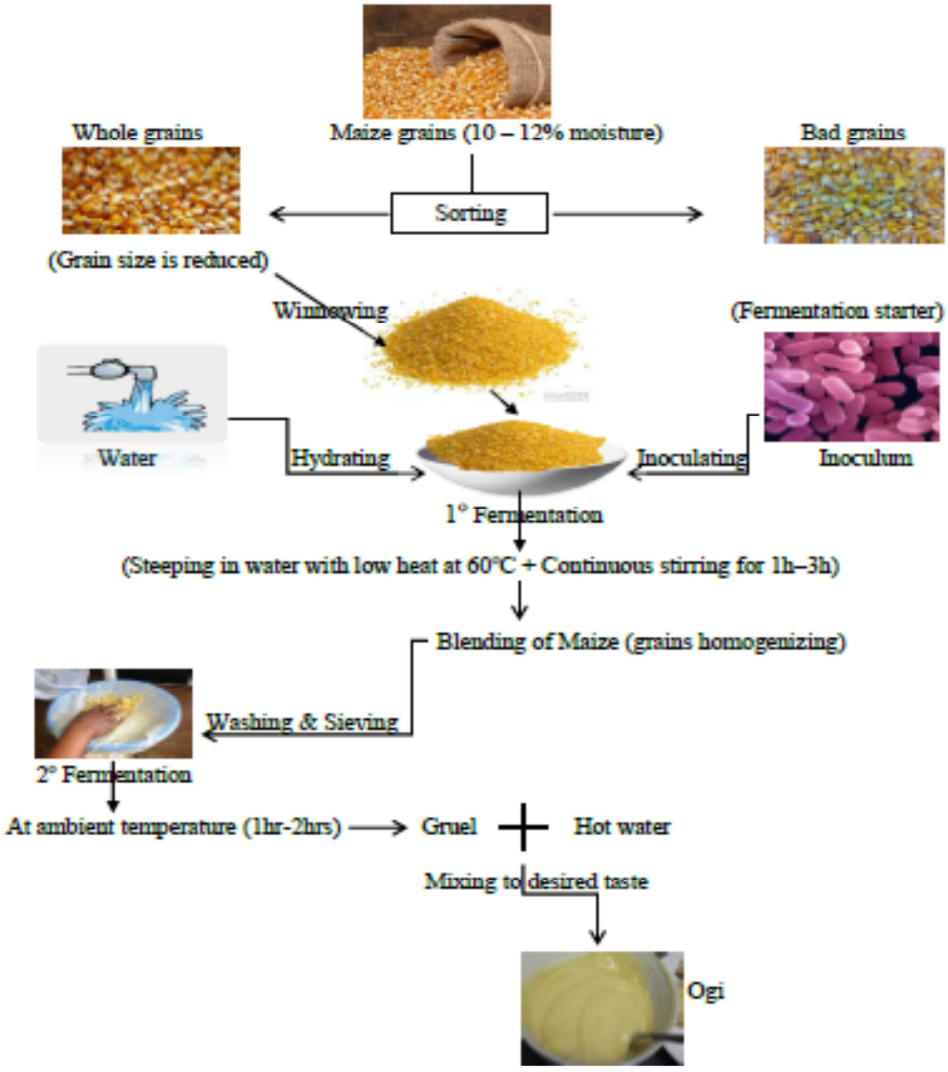

Fig. 1: Fermentation starter fermentation of maize (*Zea mays*) grains for ogi production

Figure 1. Flow chart for instant "Ogi" production.

2.2. Isolation and Enumeration of Microorganisms from Fermented Maize Gruel "Ogi"

One gram (1g) of each fermented sample was aseptically withdrawn and treated as described by Adebayo and Aderiye [21] using the spread plate method on MRS Agar [(de Mann Rogosa-Sharpe (MRS)] agar (Oxoid, UK). The incidence of the LAB colonies was monitored anaerobically on MRS agar culture plates at 30°C for 72 h.

2.3. Identification of Bacterial Strains

2.3.1. Cultural, Morphological and Biochemical Characterization

The cultural and morphological characteristics were examined as described by Cheesbrough [22] while the biochemical characteristics of the isolates were also

determined [23]. The results were interpreted in accordance with Bergey's Manual of Determinative Bacteriology Edition 8.0 [24].

2.3.2. Molecular Characterization

Bacterial isolates recovered during the production of instant "Ogi" were characterised by amplification and sequencing of the 16S rRNA gene (~ 1500 bp). Genomic DNA of the isolates was extracted using Bacterial Genomic DNA Isolation Kit (Jena Bioscience, Germany) following manufacturer's instructions. Subsequently, the 16S rRNA gene was amplified from the extracted genomic DNA by polymerase chain reaction (PCR) using universal bacterial primers; 27F (5'-AGAGTTTGATYMTGGCTCAG-3') and 1390R (5'-ACGGGCGGTGTGTRCAA-3') [25]. The PCR reaction solution (30µL) consisted of 6µL of RedLoad (Jena Bioscience, Germany) PCR mix, 0.3µL of each primer, 18.4µL of PCR grade water and 5µL of DNA template. PCR was done with GeneAmp 9700 (Applied Biosystems, USA) thermal cycler as follows; 94°C for 3min, 30 cycles of 94°C for 30s, 54°C for 30s, 60°C for 90seconds and a final 7min extension at 72°C, after which product was held at 4°C till terminated. The PCR products were subsequently resolved on a 1% gel stained with ethidium bromide and viewed using a UV transilluminator (Fotodyne Incorporated, USA). Commercial facility (Macrogen Inc., South-Korea) was patronized for purification and Sanger sequencing of the amplicon.

To identify the bacterial isolates, the sequences were compared (using the BLASTn programme) to other publicly available nucleotide sequences in GenBank.

2.4. FT-IR Spectroscopy of "Ogi" Product

Formation and/or disappearance of new functional groups in the instant "Ogi" during fermentation was determined using Fourier Transform-Infrared (FT-IR) spectrophotometer [8400 Shimadzu, Japan, with Hyper IR-1.7 software for Windows] [26]. "Ogi" samples were completely dried and the powder used for analysis was recorded from frequency of 400-4000 cm^{-1} at a resolution of 4 cm^{-1} at room temperature with a helium–neon laser lamp as a source of IR radiation [27]. Each spectrum was rationed against a fresh background spectrum recorded from the bare crystal that has been cleaned with absolute ethanol to remove any residue [20]. Each sample was scanned in triplicate.

3. Results

The fermentation starter (i.e. community "Ogi" which served as the control) had the highest lactic acid bacterial load (1.0 x 10^5 CFU/ml) while "Ogi" sample prepared from 6g fermentation starter at 60°C for 90min and allowed to stabilize at 28°C (2° fermentation) for 180min (C90.3) exhibited the least LAB load (5.5 x 10^4 CFU/ml) (Table 2). Table 2 also reveals the trend of succession of the LAB cells during fermentation. During the 1° fermentation (at 60°C), the LAB concentration of the gruel samples increased when exposed to longer fermentation period (i.e. 30 to 90 min). For instance, there was an increase of about 2.8 x 10^4 CFU/ml (i.e. 39.4%) over the initial LAB load of D60.1 "Ogi" sample after 60min at 60°C while the LAB growth in C90.3 sample was about 50.9% over its initial value (5.5 x 10^4 CFU/ml) within the same time. The growth rate of the LAB cells in E90.3 sample was the highest (6.0 x 10^2 CFU/min) during the 1° fermentation.

After the primary fermentation at 60°C, the maize granules were mashed and sieved, which resulted into reduced LAB load (ranged between 8.80 x 10^4 and 1.32 x 10^5 CFU/ml in C90.3 and E90.3 respectively). Thereafter, the "Ogi" samples were allowed to undergo secondary (2°) fermentation at 28°C. Subsequently, the microbial growth ranged between 9.3 x 10^4 CFU/ml in C90.3 and 1.38 x 10^5 CFU/ml in E120.3, even after 3h of fermentation at 28°C. At this stage, the microbial growth rate in the samples was very low (ranged between 2.8 x 10^1 CFU/min in E120.3 and 1.2 x 10^2 CFU/min in E90.3). The LAB growth rate in the control sample (i.e. community "Ogi" wrapped in polythene bag) at 28°C was also low (1.2 x 10^2 CFU/min), even though the LAB load increased by 37% of its initial value.

Table 2. Lactic acid bacteria load (x 10^5 CFU/ml) of the fermented "Ogi".

"Ogi" sample	#Fresh maize granule	Fermentation conditions and periods (min)							
		@60°C (1° fermentation)			Mashing /Sieving*	@28°C (2° fermentation)			
		30	60	90		120	150	270	300
D60.1	0.71	0.88	0.99 (0.93)	-	0.93	1.11	1.14	-	-
C90.3	0.55	0.71	0.83	1.04 (0.88)	0.88	0.93	0.95	-	-
E90.3	0.82	0.87	0.98	1.36 (1.15)	1.15	1.32	1.20	1.36	-
E120.3	0.87	0.99	1.15	1.31	1.32	1.15*	1.27	1.37	1.38
Control	1.00	-	-	-	-	-	-	-	1.37

#partially grated maize grain with known amount of fermentation starter
*mashed sample was analyzed for LAB load after respective fermentation period at 60°C
-No LAB count

Table 3 shows the distribution of nineteen (19) strains of lactic acid bacteria (LAB) isolated from the "Ogi" samples.

The LAB isolates comprised eight species belonging to either genus; *Lactobacillus* or *Lactococcus*. *Lactobacillus fermentum* had the highest distribution of 48%, followed by *Lactococcus lactis* (22%) and others; *Lactobacillus acidophilus*, *L. helviticus*, *L. brevis*, *L. cellobiosus*, *L. plantarum* and *L. casei* with 5% occurrence each.

Table 3. Distribution of lactic acid bacterial (LAB) species in instant "Ogi" samples.

Probable Isolate	Occurrence by "Ogi" sample					Total number of isolates	Distribution (%)
	Control	D60.1	C90.3	E90.3	E120.3		
Lactobacillus fermentum	2	Nil	1	3	3	9	48
Lactobacillus acidophilus	Nil	Nil	1	Nil	Nil	1	5
Lactobacillus lactis	1	1	2	Nil	Nil	4	22
Lactobacillus helviticus	Nil	Nil	Nil	1	Nil	1	5
Lactobacillus brevis	1	Nil	Nil	Nil	Nil	1	5
Lactobacillus cellobiosus	Nil	1	Nil	Nil	Nil	1	5
Lactobacillus plantarum	Nil	1	Nil	Nil	Nil	1	5
Lactobacillus casei	Nil	Nil	Nil	Nil	1	1	5
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Total	3	3	4	4	4	19	

Table 4 reveals the nomenclature of the LAB isolates based on cultural, morphological, biochemical and molecular techniques. Nine of the isolates (47%) recovered from the samples were identified based on the highest similarity score of their 16S rDNA with other strains available in GenBank while the remaining 10 (53%) were identified in accordance with Bergey's Manual of Determinative Bacteriology Edition 9.0 [24]. The incidence of these LAB isolates in each of the "Ogi" treatment is also presented in Table 4. Three (16%) of the LAB strains (*Lactococcus lactis* LAB 16, *Lactobacillus plantarum* LAB 17, *Lactobacillus cellobiosus* LAB 12) were recovered from D60.1, while four (21%) of these LAB strains were encountered in the other 4 samples; C90.3, E90.3, E120.3 and the control "Ogi" samples respectively (Table 4).

Table 4. Incidence and characterization of LAB isolates from "Ogi" samples.

S/N	Sample Code	Isolate Code	[1]Probable Isolate	[2]Closest Strain Type in NBCI Data Base	Ascession Number	16S rDNA Identity (%)
1	D60.1	LAB 12	*Lactobacillus cellobiosus*			
		LAB 16	*Lactococcus lactis*			
		LAB 17	*Lactobacillus plantarum*			
2	C90.3	LAB 1	*Lactobacillus fermentum*	*L. fermentum* IFO3956	NC010610	88
		LAB 3	*Lactobacillus acidophilus*			
		LAB 13	*Lactobacillus lactis*	*Lactobacillus lactis*	KM822722	74
		LAB 19	*Lactococcus lactis*			
3	E90.3	LAB 2	*Lactobacillus fermentum*	*L. fermentum* TCD45.2	KU851162	88
		LAB 7	*Lactobacillus helviticus*			
		LAB 9	*Lactobacillus fermentum*	*Lactobacillus* sp. MJM60420	KM485568	94
		LAB 10	*Lactobacillus fermentum*	*L. fermentum* 3872	CP011536	99
4	E120.3	LAB 4	*Lactobacillus fermentum*	*L. fermentum* CIP102980	NR104927	84
		LAB 6	*Lactobacillus fermentum*			
		LAB 8	*Lactobacillus fermentum*	*Lactobacillus* sp. A-1-40B	KT583338	82
		LAB 14	*Lactococcus casei*			
5	*Control	LAB 5	*Lactococcus lactis*			
		LAB 11	*Lactobacillus brevis*			
		LAB 15	*Lactobacillus fermentum*	*Lactobacillus fermentum*	KJ003857	84
		LAB 18	*Lactobacillus fermentum*	*L. fermentum* KSBT28	JQ669802	94

[1] Identification of isolates as described by Holt *et al*. (1994) and Olutiola *et al*. (2000)
[2] Identification of isolates as described by Mao *et al*. (2012)
*Fermentation starter

Figure 2 presents comparative FTIR spectra of fifty gram of partially grated maize inoculated with 10g of fermentation starter subjected to two treatments (E90.3 and E120.3) showing the effect of fermentation time on the instant "Ogi" with the summary of wavelength of identifiable bands associated with each set in Table 5. The FTIR revealed strong broad absorption in the two treatments with range of 526.58cm^{-1} to 3414.12cm^{-1} for E90.3 and 438.78cm^{-1} to 3396.76cm^{-1} for E120.3. Another absorption band of lower importance, but often characteristic of bending vibration, out-of-plane bend or wagging vibration of the O-H group appeared at 1460.16cm^{-1}. However, after cooling and stabilization (2° fermentation) of the product at 28°C for 180 min, the intensity of the –OH bond in E90.3 1 treatment increased to 45%, which is much higher than 26% found in E120.3 treatment. As in Table 5, the biochemical compositions of our instant "Ogi" samples produced with these treatments at different 1° fermentation time (90min and

120min) at 60°C varied from each other and also from respective control at 0min. Although, there was no indication of the presence of $–CH_2$ bond (from 2934-2921 cm^{-1}), an emerging band at 2926 cm^{-1} with 25% intensity appeared after 1° fermentation for 90min at 60°C which became very intense after 2° fermentation for 180min at 28°C with band at 2928 cm^{-1} with 75% intensity.

In contrast, except the bonds of $–CH_2$, -C=O of amide and –C-O-C and C-O of carbohydrates that showed slight increase in intensity (from 56 to 57, 64 to 71 and 43 to 46 respectively) after cooling and stabilization, there was no significant improvement in the biochemical composition of the E120.3 product. The –OH bond (at 3412cm^{-1}) with 29% intensity in the control for treatment 2 showed a variance of 16 to the final product after been subjected to heat at 60°C for 120min and stabilized at 28°C for 180min. There was drastic reduction of 18%, 15% and 6% in the concentration of the $–CH_2$, $-CH_3$ and –C=O groups respectively when subjected to heat treatment at 60°C for 120min, their intensities in the final product after 2° fermentation however increased by 19%, 15% and 13% respectively.

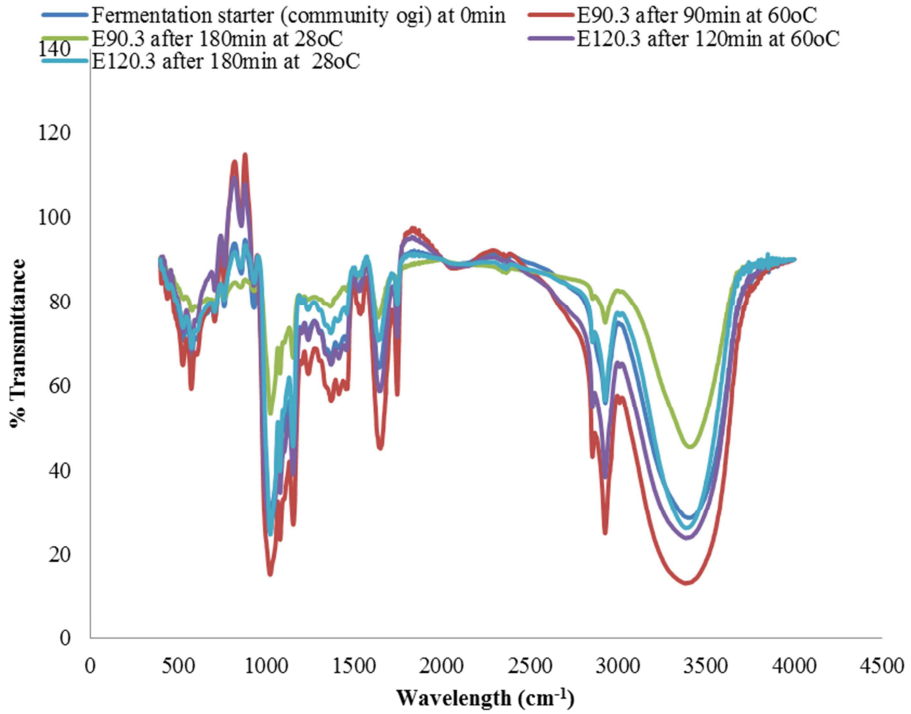

Figure 2. FTIR spectra of E90.3 (Figure 2A) and E120.3 (Figure 2B) "Ogi" treatments at different fermentation time; 0min, 90min (60°C), 120min (60°C) and 180min (28°C).

Table 5. Effect of fermentation time on FTIR spectra of instant "Ogi" treatments (E90.3 and E120.3).

Vibrational freq. (Vfh) cm^{-1}	Treatment 1 [E90.3 for 0min, 90min at 60°C and 180min at 28°C] cm^{-1} (% Intensity)				Treatment 2 [E120.3 for 0min, 120min at 60°C and 180min at 28°C] cm^{-1} (% Intensity)				Functional groups/Bands
	Control	E90.3-A	E90.3-B	Variance	Control	E120.3-A	E120.3-B	Variance	
~3500 v	3396 (29)	3408 (13)	3404 (45)	12	3396 (29)	3396 (24)	3396 (26)	16	(-OH)
2934–2921 v	2928 (56)	2926 (25)	2928 (75)	2	2928 (56)	2928 (38)	2928 (57)	0	($-CH_2$)
2872 v	2854 (70)	2854 (43)	2854 (81)	0	2854 (70)	2854 (55)	2854 (70)	2	($-CH_3$)
2340 v	2360 (89)	2364 (91)	2364 (86)	4	2360 (89)	2360 (89)	2364 (88)	6	($-CO_2$)
1741–1715 v	1745 (82)	1745 (57)	1745 (85)	0	1745 (82)	1745 (71)	1745 (79)	2	($-CH_2$)
~1637 v	1637 (64)	1643 (45)	1641 (77)	6	1637 (64)	1643 (58)	1641 (71)	4	(-C=O) of amide
1200–900 v	1155 (43)	1155 (27)	1153 (67)	2	1155 (43)	1155 (39)	1153 (46)	2	(-C-O-C, C-O) of CHO
1200–900 v	1078 (40)	Absent	Absent	-	1078 (40)	1080 (35)	1080 (40)	2	(-C-O-C, C-O) of CHO
1200–900 v	1022 (28)	1024 (15)	1024 (53)	2	1022 (28)	1020 (25)	1024 (25)	4	(-C-O-C, C-O) of CHO

Keys: Control-1 = Fermentation starter (community "Ogi") at 0min
E90.3-A = after 90min at 60°C
E90.3-B = Finished product after 180min at 28°C
E120.3-A = after 90min at 60°C
E120.3-B = Finished product after 180min at 28°C

Figure 3 shows the comparative FTIR spectra of two treatments, C90.3 fermented with 6g of starter and D60.1 with 8g of starter showing the effect of starter concentration on the instant "Ogi" with the summary of wavelength of

identifiable bands associated with each set in Table 6. The FTIR revealed strong broad absorption in the two treatments with range of 526.51cm^{-1} to 3381.33cm^{-1} for C90.3 and 526.58cm^{-1} to 3404.47cm^{-1} for D60.1.

There was high concentrations of –CH_3 (70%) and –CH_2 (82%) at 2854cm^{-1} and 1743cm^{-1} respectively in the control (Table 6). After fermentation for 90min at 60°C, the sample lost appreciable amount of some of the biochemical constituents: -OH (17%) at 3396cm^{-1}, -CH_2 (33%) at 2926cm^{-1}, -CH_3 (24%) at 2854cm^{-1}, -CH_2 (29%) at 1745cm^{-1}, C-O-C and –C-O of carbohydrate (22%) at 1155cm^{-1} and 17% at 1024cm^{-1}, and 22% at 1080cm^{-1}. After this product was allowed to cool and stabilize for 180min at 28°C, increase of about 10% of the –OH at 3385cm^{-1}, 22% of –CH_2 at 2926cm^{-1}, -CH_3 (19%) at 2854cm^{-1}, -CH_2 (24%), and the C-O-C, C-O of CHO ranging between 8% at 1024cm^{-1} and 21% at 1153cm^{-1} were however recorded. Similarly, D60.1 bioproduct was not able to retain sufficient amount of –OH, -CH_2, -C=O of amide found in the raw substrate, losing 9%, 15% and 17%, and 15% at 3396, 2926 and 1745, and 1643cm^{-1} respectively. Eventually, the final product after 2° fermentation invariably retained the integrity of –CO_2 at 2360cm^{-1} and C-O-C, C-O of CHO at 1153cm^{-1}, 1078cm^{-1} and 1024cm^{-1}.

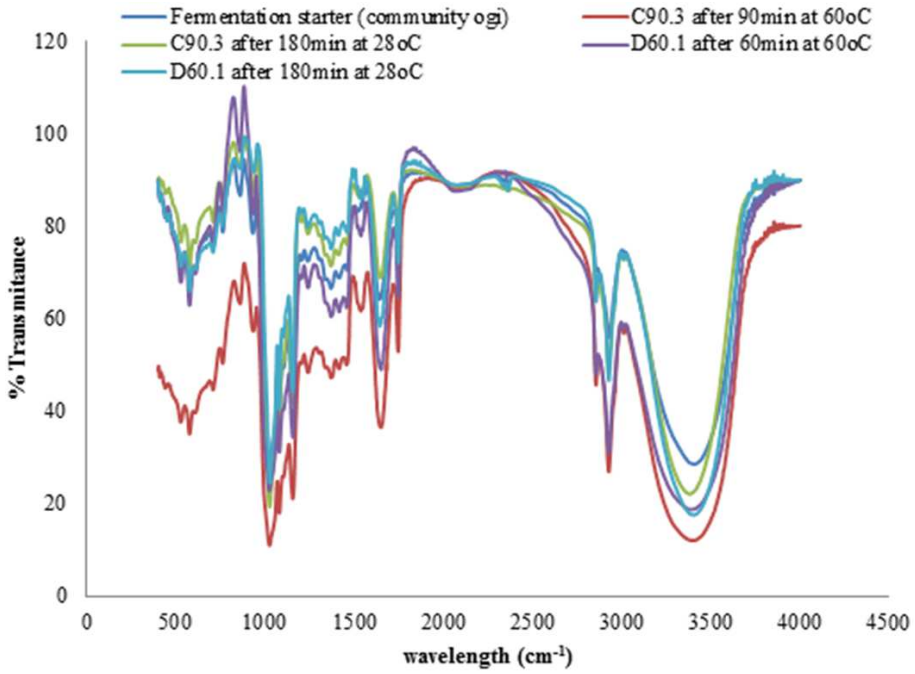

Figure 3. FTIR spectra of C90.3 "Ogi" fermented with 6g fermentation starter and D60.1 "Ogi" fermented with 8g fermentation starter (Figure 3B); at 0min, 90min (60°C) and 180min (28°C).

Table 6. Effect of starter concentration on FTIR spectra of instant "Ogi" treatments (C90.3 and D60.1).

Vibrational freq. (Vfh) cm^{-1}	Treatment 1 cm^{-1} (% Intensity)				Treatment 2 cm^{-1} (% Intensity)				Functional groups/Bands
	Control	C90.3-A	C90.3-B	Variance	Control	D60.1-A	D60.1-B	Variance	
~3500 v	3398 (29)	3396 (12)	3385 (22)	13	3398 (29)	3396 (19)	3396 (18)	0	(-OH)
2934–2921 v	2928 (60)	2926 (27)	2926 (49)	2	2928 (60)	2926 (41)	2926 (47)	2	(-CH_2)
2872 v	2854 (70)	2854 (46)	2854 (65)	0	2854 (70)	Absent	Absent	-	(-CH_3)
2340 v	2345 (90)	Absent	Absent	-	2345 (90)	2359 (92)	2360 (87)	15	(-CO_2)
1741–1715 v	1743 (82)	1745 (53)	1745 (77)	2	1743 (82)	1745 (65)	1745 (72)	2	(-CH_2)
~1637 v	1637 (64)	Absent	Absent	-	1637 (64)	1643 (49)	1643 (58)	6	(-C=O) of amide
1200–900 v	1155 (43)	1155 (21)	1153 (42)	2	1155 (43)	1153 (35)	1153 (44)	0	(-C-O-C, C-O) of CHO
1200–900 v	1022 (28)	1024 (11)	1024 (19)	2	1022 (28)	1080 (31)	1078 (40)	2	(-C-O-C, C-O) of CHO
1200–900 v	1080 (40)	1080 (18)	1078 (35)	2	1080 (40)	1022 (30)	1024 (25)	2	(-C-O-C, C-O) of CHO

Keys: Control = Fermentation starter (community "Ogi") at 0min
C90.3-A = after 90min at 60°C with 6g fermentation starter
C90.3-B = Finished product after 180min at 28°C with 6g fermentation starter
D60.1-A = after 90min at 60°C with 8g fermentation starter
D60.1-B = Finished product after 180min at 28°C with 8g fermentation starter

The band wavelength and concentration of biochemical components of our novel instant "Ogi" products of four (4) different treatments compared to the commercial "Ogi" product at different fermentation conditions are depicted in Table 7; with the FTIR spectra presented in Figure 4. The community "Ogi" (control) exposed to 60°C for 60min,

90min, 120min; and 28°C for 180min was analysed using FTIR spectroscopy and compared to the profile of our four treatments of instant "Ogi". Comparatively, in the 1° fermentation at 60°C, C90.3 and E90.3 gruels fermented for 90min showed appreciable increase in the concentration of most functional groups; from 1% to 13% for –OH at 3408cm^{-1}, 3% and 6% to 27% and 57% for CH_2 at 2926cm^{-1} and 1745cm^{-1} respectively, 4% to 46% for $–CH_3$ at 2854cm^{-1}, 9% to 91% for $–CO_2$ at 2364cm^{-1}, 4% to 45% for –C=O of amide and 0% to 15% for –C-O-C and C-O of carbohydrate (CHO). Similarly, after 2° fermentation at 28°C for 180min, the treatment fermented for 90min particularly (E90.3) retained significant increase in most functional groups (with 21% for –OH at 3405cm^{-1}, 24% and 85% for $–CH_2$ at 2928cm^{-1} and 1745cm^{-1} respectively, 81% for $–CH_3$ at 2854cm^{-1}, 14% for –C=O of amide at1641cm^{-1} and 14% and 28% for –C-O-C and C-O of CHO at 1153 cm^{-1} and 1024 respectively) of biochemical importance, compared to the community "Ogi".

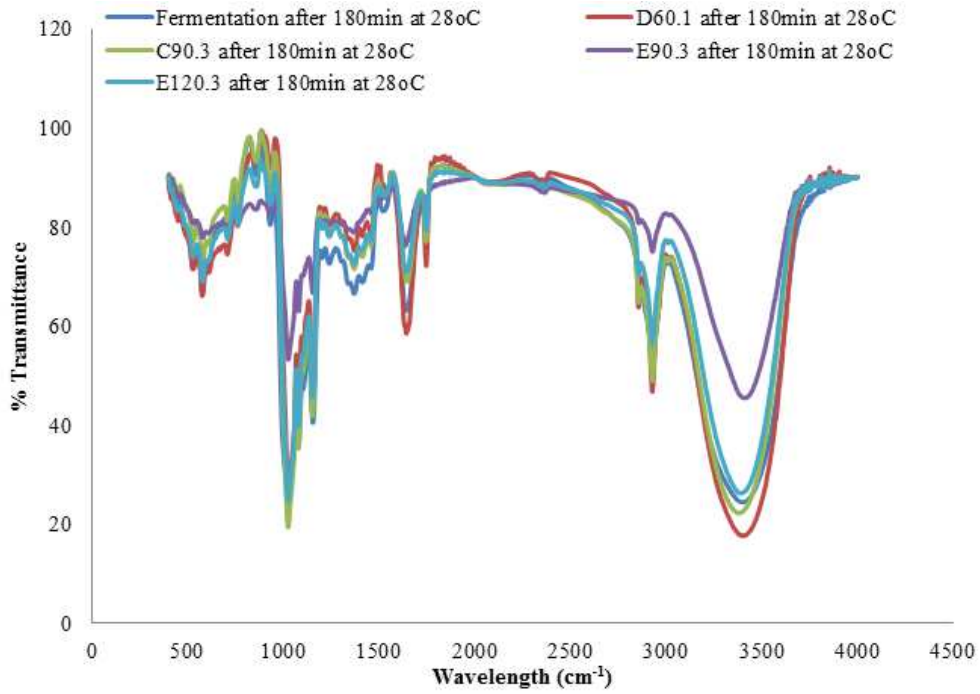

Figure 4. FTIR spectra of commercial "Ogi" (fermentation starter) at 60°C for different fermentation time; 0min, 60min, 90min and 120min (Figure 4A); and at 28°C for 90min and 180min (Figure 4B).

Table 7. Band wavelength and concentration of biochemical constituent in novel instant "Ogi" products in comparison with commercial (control) samples after fermentation.

Vibrational freq. (Vfh) cm^{-1}	1° Fermentation @60°C by time								2° Fermentation @28°C for 180min					Functional group/Bands
	Wavelength (cm^{-1}) (% Intensity)								Wavelength (cm^{-1}) (% Intensity)					
	60min		90min				120min		180min					
	FS	D60.1	FS	C90.3	FS	E90.3	FS	E120.3	FS	D60.1	C90.3	E90.3	E120.3	
~3500 v	3396 (35)	3396 (19)	3396 (1)	3396 (12)	3396 (1)	3408 (13)	3396 (23)	3396 (24)	3396 (24)	3396 (18)	3385 (22)	3404 (45)	3396 (26)	(-OH)
2934–2921 v	2928 (60)	2926 (41)	2928 (3)	2926 (27)	2928 (3)	2926 (25)	2928 (48)	2928 (38)	2928 (51)	2926 (47)	2926 (49)	2928 (75)	2928 (57)	(-CH_2)
2872 v	2854 (72)	Nil	2854 (4)	2854 (46)	2854 (4)	2854 (43)	2854 (65)	2854 (55)	Nil	Nil	2854 (65)	2854 (81)	2854 (70)	(-CH_3)
2340 v	2360 (87)	2359 (92)	2360 (9)	Nil	2360 (9)	2364 (91)	2360 (88)	2360 (89)	2360 (89)	2360 (87)	Nil	2364 (86)	2364 (88)	(-CO_2)
1741–1715 v	1743 (86)	1745 (65)	1745 (6)	1745 (53)	1745 (6)	1745 (57)	1745 (81)	1745 (71)	Nil	1745 (72)	1745 (77)	1745 (85)	1745 (79)	(-CH_2)
~1637 v	1641 (69)	1643 (49)	1643 (4)	Nil	1643 (4)	1643 (45)	1643 (62)	1643 (58)	1641 (63)	1643 (58)	Nil	1641 (77)	1641 (71)	(-C=O) of amide
1200–900 v	1155 (49)	1153 (35)	1155 (30)	1155 (21)	1155 (30)	1155 (27)	1155 (39)	1155 (39)	1018 (24)	1153 (44)	1153 (42)	1153 (67)	1153 (46)	(-C-O-C, C-O) of CHO
1200–900 v	1022 (32)	1080 (31)	1022 (15)	1024 (11)	1022 (15)	Nil	1020 (23)	1080 (35)	1153 (40)	1078 (40)	1024 (19)	Nil	1080 (40)	(-C-O-C, C-O) of CHO
1200–900 v	Nil	1022 (30)	Nil	1080 (18)	Nil	1024 (15)	Nil	1020 (25)	Nil	1024 (25)	1078 (35)	1024 (53)	1024 (25)	(-C-O-C, C-O) of CHO

As in Table 1

4. Discussion

The lactic acid bacteria count recorded in this study was lower than 7.05 x 10^6 CFU/ml reported by Nwachukwu and Ijeoma [28], but is in line with the previous report of Aderiye *et al*. [14]. Aderiye *et al*. [14] also reported almost double (1: 1.88) the number of LAB cells in "Ogi" sample after primary fermentation at 60°C for 90min. Meanwhile, the decrease in the growth rate of LAB count after 2° fermentation could be attributed to depletion of nutrient coupled with low temperature (28°C) which probably reduced the optimal activity of the microorganisms [29, 28].

The distribution of LAB isolates in the "Ogi" samples conforms with different reports of several lactic acid bacteria belonging to *Lactobacillus* and *Lactococcus* as predominant among other bacteria reportedly encountered in fermented "Ogi" [30, 28, 31, 32, 14]. Perhaps, changes in the occurrence of LAB strains in each of the "Ogi" treatments, including the community "Ogi" sample could be attributed to different ability of each strain to cope with respective condition of the treatments. Fermentation is usually a result of competitive activities of different microorganisms, where strains with highest growth rate will dominate at different stages of fermentation [33, 28]. In accordance with the work of Adegbehingbe [34] and Izah *et al*. [32], this study also suggests that some strains of *Lactobacillus fermentum* among others mainly play essential role in the fermentation of "Ogi".

The peak bands between 526.58 and 576.74 cm^{-1} in the FTIR spectra of E90.3 after 90min at 60°C revealed the presence of alkyl halides which arose as a result of halides and its stretching vibration from carboxylic groups. The OH bending vibrations are broadened by hydrogen bonding as is the stretching absorption, but often to a lesser extent. These represent the primary and secondary alcohols which are the spectra of primary 1-octanol. The other most important set of bands are the aromatic ring vibrations around 1600 and 1500cm^{-1}, (1531.53cm^{-1}) which usually appear as a pair of band structures, often with some splitting [19]. Apart from –CO_2 bond that showed slightly decreased intensity (86%) in E90.3 treatment when compared to the control (89%), the intensities of all functional groups found in the final product of E90.3 (after fermentation for 180min at 28°C) were significantly higher than those of E120.3. This could be taken as an indication that there was a slowdown, if not a total stop, in the metabolic activities of the fermenting LAB that typically breakdown complex molecules in the "Ogi" sample into carbon dioxide and water [31].

In E90.3, the variance of 12 in the hydroxyl (–OH) bond (at around 3500cm^{-1}) between the control (at 0min) (3396 cm^{-1}) and when the sample was exposed to 60°C for 90min (3408 cm^{-1}), where the intensity of the –OH bond decreased to 13%, could be related to the release and abundance of alcohol through the bioprocess, which will likely act as preservative as well as inhibit spoilage organisms, thereby increase the shelf life of the product [35, 36]. In addition, IR absorptions (2364.81cm^{-1} and 1508.38cm^{-1}) found in E90.3 bioproduct representing -C N- stretch and N-H revealed the presence of nitriles and amines respectively where –C-N- which is a characteristic of double bond structures showing the presence of oleifinic unsaturated and aromatic ring. The absorption peak at 858-934cm^{-1} is indicative of polysaccharide compounds of sugar derivative. Another absorption peak at 578.66 and 530.44cm^{-1} are also known to be sugar derivatives, suggesting that the component of the sample includes glycolipopeptide in nature [19].

The maximum peak of 3381.33^{-1} and 3404.47cm^{-1} shown in C90.3 and D60.1 respectively was relatively broad with strong absorption, centered around 3400 cm^{-1} representing –OH and –NH stretching vibrations, which is characteristic of carbon containing compounds with unsaturated amino bonds of protein. Lower frequency of the band at 1529.60cm^{-1} in D60.1 bioproduct is characteristic of double bond structures showing the presence of oleifinic unsaturated and aromatic ring [19]. The final product of D60.1 (fermented with 8g starter) after 270min, however relatively retained most of the important functional groups at significant level. According to Izah *et al*. [32], this indicates that the higher the fermentation starter the better the biochemical composition of the products.

It is also notable that the band wavelengths recorded for our instant "Ogi" with the four treatments, both in 1° and 2° fermentation in this study, are closely similar to those earlier reported for sweet potato flour by Ajayi *et al*. [20] and flour sample by Supriya and Rajinder [37]. Significant increase (90%) in the –CO_2 recorded for treatment E90.3 during 1° fermentation could be as a result of massive degradation of the substrate by the fermenting LAB resulting in high concentration of carbon dioxide (CO_2). Consequently, the minimal or zero level of the bacterial metabolisms could also be the reason for 2% decrease in the –CO_2 recorded after cooling (2° fermentation) at 28°C for 180min [20].

The FTIR spectra with wavelength range of 4000 to 400cm^{-1} identified in this study affirm that bands between 900 - 600 cm^{-1} are the finger print region associated with biological samples [38]. The functional groups in biological parlance associated with characteristic bands identified include the C-O-C, C-O ring vibrations of carbohydrates in the media with bands at varying wavelengths between 1200cm^{-1} and 900cm^{-1} and characteristic vibrational O-H bonds with bands at around 3400cm^{-1}. Although, water absorbs strongly in this infrared region of the spectrum due to its O-H stretching and H bending vibrations, but its quantization is frequently

complicated by spectral interferences from other OH containing constituents like alcohol and confounded further by hydrogen bonding effects [35].

The bands at 1310-1240cm^{-1} of Figure 4 depict vibrational C-N bonds and bending N-H bonds indicating the presence of amides of proteins (Amide III) in the sample. Other amides with vibrational N-H bonds, with bands around 1548cm^{-1} (Amide II), 1655cm^{-1} (Amide I) and 3200cm^{-1} are traceable to other protein derivatives [38, 19]. The bands at 2340cm^{-1} indicating the formation of CO_2 could be as a result of metabolic activities leading to biochemical changes in the sample [38]. However, the vibrational C-H_3 bands around 2959cm^{-1} and/or 2872cm^{-1} and the vibrational CH_2 band at 2934cm^{-1} could also be attributed to the organic skeleton of the substrate. According to Aderiye *et al*. [27], any alteration of these functional groups either by band shift, appearance or disappearance of characteristic bands due to any of these functional groups in any of the bioproducts (D60.1, C90.3, E.90.3 and E120.3) in relation to the fermentation starter is an indication of LAB fermentation.

5. Conclusion

Fourier Transform Infrared (FTIR) spectroscopy has shown convincing proofs and promising potentials as an essential tool to monitor the functional properties (which constitute the palatability, suitability and biochemical features) of food products, particularly "Ogi" sample as in this study. Comparatively, each of the bioproducts (Instant "Ogi") made from specific bioprocess (treatment) in this study has shown some biochemical characteristics that prove their suitability and betterment over community "Ogi" sample. Overall, this study also reveals that E90.3 treatment is however the best and most promising bioprocess for the production of the novel instant "Ogi", hence is recommended for industrial production and commercialization of "Ogi".

Conflict of Interest Statement

The authors declare no competing interest

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