



## Biochemical analysis of some fruit peels and comparison of Lactic acid production by autochthonous Lactic acid bacteria using fruit peels

Arekemase M.O., Adetitun D.O.\* & Ahmed M.I.

Department of Microbiology, Faculty of Life Sciences, University of Ilorin, P.M.B. 1515 Ilorin, Kwara State. Nigeria

### Abstract

Waste to wealth is a common trend in the world today. This work reports the utilization of waste fruit peels for production of lactic acid using autochthonous lactic acid bacteria. Peels of mango, orange and banana were prepared in powdered form, pretreated with HCl and added as carbon source for lactic acid production. The proximate analyses of the substrates were done. The organisms used for fermentation were isolated from spoiled banana and identified using biochemical and molecular tools. These organisms were grown using different growth factors over a period of eight days with initial pH of 6.0. Growth factors were then varied in order to optimize the yield of lactic acid. Percentage production of lactic acid was determined every 24 hours. The optimized conditions were then combined for further comparison. Isolated *Lactobacillus plantarum* and *Lactobacillus casei* were used for the production of lactic acid. The carbohydrate content of mango, banana and orange peels (62.48%, 55.34% and 57.06% respectively) showed that it was sufficient for use as a carbon source. The highest yield of lactic acid (27.10%) was at pH 6.0 with 2g of substrate and 1 ml inoculum at day six by *Lactobacillus casei* while the highest yield by *Lactobacillus plantarum* (23.85%) was at pH 6.0, with 4g of substrate and 2 ml inoculum at day six. The highest production of lactic acid was derived when mango peels were used. The results of this study showed that lactic acid can be produced using mango, orange and banana peels.

**Received:** 21 May 2019

**Accepted:** 24 Oct 2019

### Key words:

lactic acid;  
food waste;  
*Lactobacillus casei*;  
*Lactobacillus plantarum*;  
optimization

\*Corresponding author:

[adetitun.do@unilorin.edu.ng](mailto:adetitun.do@unilorin.edu.ng)

 <https://orcid.org/0000-0003-3814-8783>

Tel: +238036910988

### 1. Introduction

Fruit based industry produces a large volume of solid and liquid waste. These pose increasing disposal and pollution problems (high BOD or COD) and represents loss of valuable biomass and nutrients (Mridul & Preethi, 2014). Disposal of wastes from fruit-canning industries has been a problem due to high transportation costs and limited availability of landfills, as these by-products carry no commercial value, they are often discharged in places they ought not to be disposed (Pradeep et al., 2014). This particularly occurs where there is a lack of legislation and their enforcement on waste disposal (Omojola et al., 2009). These wastes directly affect environmental agencies and municipalities because food waste is a

primary source of methane gas in landfills (Gunders, 2012).

Recycling fruit-waste to develop new products has received much attention lately. Organic waste treatment processes (Purkayastha, 2012) and anaerobic digestion processes (Shin et al., 2010; Dai et al., 2013; Bernstad et al., 2013) are two promising technologies used in this regard. Presently, the main use of such domestic food waste is the production of valuable compounds by the controlled break down of the waste by microorganisms (Rounsefell et al., 2013).

Lactic acid has gained importance for its application in food, feed, chemical, pharmaceutical and beverage industries (Ray & Swain, 2011). Most important application of lactic acid is its use



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for the manufacture of biodegradable and biocompatible polylactate polymers (Khalaf, 2001). Microbial sources including many bacteria such as

*Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Clostridium* and *Weissella* have been reported to produce lactic acid (Bogaert&Coscach, 2000).

Due to the importance of this organic acid, there are ongoing research efforts related to its production (Hofvendahl& Hahn- Hägerdal, 2000). Variations in temperature, pH and nitrogen sources affect lactic acid production (Pavezzi et al., 2008; Jörissen et al., 2015).

The aim of this study was to evaluate the effect of physical, chemical and nutritional parameters on the production of lactic acid with the aid of two lactic acid bacteria.

## 2. Materials and Methods

Banana, orange and mango fruits were procured from a local market near Ilorin. The fruits were authenticated at the herbarium unit of the Department of Plant Biology, University of Ilorin, with voucher specimen numbers UILH/001/996, UILH/002/1080 and UILH/003/1249 respectively. These fruits were washed with clean water to remove dirt and then peeled. The peels were air dried at 50 °C in an oven to a constant weight. The peels were pounded with mortar and pestle and blended to derive fine particle sizes. The powdered product was stored in a cool, dry place to avoid uptake of moisture and then used as fermentation feedstock according to the protocol of Nandini et al. (2014).

### Determination of Ash Content and crude protein

Two gram each of each sample was weighed into small dry crucibles of known weight. Each sample was charred in the crucible on low flame. The charred materials were ashed in a furnace at red heat for 3 hours. Thereafter, each material was cooled, weighed and kept in a desiccator (Cullison, 1982; AOAC, 2000). The nitrogen concentration of the by-product sample was determined by employing the micro-Kjeldahl method. (AOAC, 2000).

### Determination of Crude Fibre

A known quantity (3.5g) of the defatted sample used for the lipid assay was transferred into a 500 ml conical flask ( $W_1$ ), 200 ml of boiling  $H_2SO_4$  (1.25%) was added, boiled within one minute and then allowed to boil gently for 30 minutes (Ibitoye,

2005). It was then filtered through filter paper using a Büchner funnel and rinsed well with hot distilled water. The residue obtained after filtration was dispensed into the flask with the aid of a spatula. To the same conical flask, 1.25% NaOH (200 ml) was added and boiled for 45 minutes and filtered. This procedure was repeated again, followed by addition of 200 ml of 10% HCl. After filtration, the resulting filtrate was diluted with 200 ml of water and filtered. This was repeated twice, allowed to dry and it was weighed. The % crude fibre content was calculated as follows:

$$\text{Percentage crude fibre} = W_2 - \frac{W_3}{W_1} * \frac{100}{1}$$

### Determination of Fat, Moisture and Dry Matter Content

This was determined using the Soxhlet method of extraction according to standard protocols (AOAC, 2000). The moisture content was determined by the loss in weight that occurred when the samples were dried to a constant weight in a regulated oven (Gallenkamp) according to the method used by Cullison (1982) and AOAC (2000).

### Carbohydrate content (Nitrogen Free Extract)

The Nitrogen Free Extract (N.F.E.) referred to as soluble carbohydrate was obtained as a fraction part (in percentage) of the sample after subtracting other components such as the ash content, crude lipid, crude protein and crude fibre.

$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ crude lipid} + \% \text{ crude fibre} + \% \text{ ash content} + \% \text{ crude protein})$ .

### Isolation and Identification of Lactic Acid Bacteria (LAB)

The lactic acid bacteria (LAB) used in the study were isolated from spoiled banana fruit using the spread plate method. Man Rogosa Sharpe (MRS) agar (Oxoid™) was used. The plates were incubated at  $35 \pm 2$  °C for 24 hours. The pure axenic cultures were stored at 4 °C. Identification of bacterial isolates was carried out by biochemical reactions as described in Bergey's Manual of Determinative Bacteriology (Holt, 1994). The bacterial isolates were identified based on morphological and biochemical characteristics. Identification was further confirmed by molecular tools using their 16S rRNA with 16SF (5'AGAGTTTGATCCTGGCTCAG3') and (5'TACCTTGTTACGACTT3') primers to get an amplicon size of 1500 bp., gel electrophoresis, sequencing and BLASTn on NCBI database based on percentage similarities with those on the database.

### Acid hydrolysis

The modified method of Puimputet *et al.*, (2008) was used for substrate hydrolysate preparation. Exactly 50 ml of 0.5 HCl was added to 2g each of the wastes and boiled at 80 °C for 30 min. The hydrolysate was recovered by filtration with Whatman filter paper no 1.

#### **Inoculum preparation**

The pure isolates were grown on Man Rogosa Sharpe (MRS) agar. They were sub-cultured in 50 ml liquid synthetic MRS medium instead of distilled water. The above medium was placed on a rotary incubator shaker at 35 °C and 120 rpm for 3 days as described by Mahanteshet *et al.* (2010).

#### **Media and fermentation conditions**

The fermentation medium was compounded as described by Quiroga *et al.* (2014). Measured inoculum was dispensed in an oven sterilized test tube after the third day of incubation. The supernatant was discarded after centrifugation (150 rpm for 10mins), 10 ml of sterile water was added to the pellet and mixed. Exactly 1 ml of the mixture was introduced into 50 ml of each of the sterile fermenting medium. The fermentation broth was incubated at 35 °C at 150 rpm for 8 days. Lactic acid production was monitored every 24 hrs. The final pH was adjusted using 2M NaOH or 1M HCl.

#### **Determination of optimal conditions for lactic acid production**

The optimization experiments were carried out to find out the effects of fermentation time, pH, inoculum size, quantity of substrate and nitrogen on the production of lactic acid. Fifty milliliters of the fermenting media in 250 ml Erlenmeyer flasks at initial pH 6.0 was inoculated by the test organisms, and incubated at 35 °C on a rotary shaker (150 rpm) for 8 days. Samples were withdrawn at 24-hour intervals for the estimation of lactic acid content. The initial pH of the fermentation medium was adjusted with 2M NaOH or 1M HCl to values ranging from 5.0-6.5. Thereafter the flasks were inoculated and incubated at 35 °C for 8 days on a rotary shaker (150 rpm). Fifty milliliters of fermentation medium (initial pH 6.0) in 250 ml Erlenmeyer flasks were inoculated with 3-day old culture, with different inoculum sizes (0.5, 1.0, 1.5 and 2.0 %) and incubated at 35 °C for 8 days with agitation at 150 rpm. The quantity of substrate added to 10 ml mineral salt medium was varied and ranged from 1g to 4g.

#### **Optimization experiments**

Optimization experiments were carried out by taking the pH values that produced the highest yield of lactic acid, the inoculum size that gave the highest yield and the substrate quantity that gave the highest yield. All these parameters were then

combined in a single experiment to determine the optimum conditions for lactic acid production.

#### **Quantification of the lactic acid produced**

The production of lactic acid was detected by estimating the titratable acidity of the fermentation medium against 1N NaOH. Two milliliters of culture broth was taken from the fermented broth in a flask and 8 ml of distilled water was added to make up 10 ml and then boiled for 1 min to remove air. A drop of phenolphthalein indicator was added. Thereafter, 1N NaOH solution was added with continuous shaking till the formation of pink colour was observed. The quantity of NaOH utilized in the titration was measured (Quiroga *et al.*, 2014). The amount of Lactic acid was determined by using the formula.

$$\% \text{ of lactic acid} = \frac{\text{ml of NaOH} * \text{Conc. of NaOH} * 0.090 * 100}{\text{Weight of sample}}$$

0.090 is milliequivalent weight of lactic acid

#### **Lactic acid recovery and purification processes**

Various recovery and purification schemes used in industry were reported by Vickroy (1985) and Benninga (1990). The first step in the recovery processes was to raise the broth's temperature to 80-100 °C and increasing the pH to 10-11 [using Ca(OH)<sub>2</sub>] to inactivate the microorganisms, coagulate the protein, solubilize calcium lactate and degrade some of the residual sugars. The cells and coagulated protein were removed by filtration to produce a crude lactic acid extract. This crude extract was processed further by the following methods.

#### **Filtration, carbon treatment and evaporation**

Activated carbon was mixed with the crude extract to remove the coloured components. The spent carbon was then filtered out, and the filtrate was sent to the evaporator where excess water was evaporated under mild vacuum at moderate temperature (0.57 atm and 70 °C) to 37% calcium lactate concentration. This preparation was then acidified with 63% sulfuric acid to precipitate calcium sulfate, which was filtered out. The lactic acid was bleached a second time and then evaporated to 52 or 82% concentration. Food-grade lactic acid was treated with sodium sulfide to remove heavy metals and was bleached again before packaging. This process produced only food-grade and technical-grade lactic acid. It was also energy-intensive because the concentration process relied heavily on evaporation of excess water. Furthermore, large amounts of salt (CaSO<sub>4</sub>) were also produced, which created a waste disposal problem.

### Statistical Analysis

The data were statistically analyzed as a randomized complete block design. The means and standard deviation were compared by a one-way analysis of variance (ANOVA) (SPSS 15.0).

### 3. Results

#### Isolation and Identification of test organisms

The detected bacteria were *Lactobacillus plantarum* strain NM71-7, *Lactobacillus casei* strain AT4-518F, *Lactobacillus fermentum* strain CAU2928 and *Lactobacillus plantarum* strain B15.

#### DNA sequence of *Lactobacillus casei* strain NM717

CCGGAGTAGCGGGTTTTCCGAGACTCTATGGGCGTAA  
GCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCC  
CTCGGCTTAACCGAGGAAGCGCATCGGAACTGGGAA  
ACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGT  
AGCGGTGAAATGCGTAGATATATGGAAGAACCAGT  
GGCGAAGGCGGTGTCTGGTCTGTAAGTACGCTGAG  
GCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCC  
TGGTAGTCCATGCCGTAACGATGAATGCTAGGTGTTG  
GAGGGTTTTCCGCCCTCAGTGCCGACGTAACGCATTA  
AGCATTCCGCTGGGGAGTACGACCGCAAGGTTGAAA  
CTCAAAGGAATTGACGGGGGCCCGACAAGCGGTGGA  
GCATGTGGTTTAATTCGAAGCAACCGGAAGAACCCTTA  
CCAGGTCTTGACATCTTTTATCACCTGAGAGATCAGG  
TTTTCCCTTCGGGGGCAAAATGACAGGTGGTGCATGGT  
TGTCGTCAGCTCGTGTGCGTGAGATGTTGGGTTAAGTCC  
CGCAACGAGCGCAACCCCTATGACTAGTTGCCAGCATT  
TAGTTGGGCACTCTAGTAAGACTGCCGGTGACAAACC  
GGATGAAGGTGGGATGACGTCAAATCATCATGCCCC  
TTATGACCTGGGCTACACACGTGCTACAATGTATGGTA  
CACCGAGTTGCGAGACCGCGAGGTCAAGCTAATCTCA  
TAAGGCAGTTCTCAGTTCGGACTGTAAGCAGCAACCTC  
GGCCTACCGTAGTCGGAATCAGCTAGTAGATCGACG  
ATCGACCACCGCCCGCGGTGAATACGTTTTCCCGGGCTC  
TTAAAAGAACC

#### DNA sequence of *Lactobacillus plantarum* strain AT4-518F

TGTTGACTGATGGGGTACGTGACGGGTCTATTGGGCG  
TAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAA  
GCCTTCGGCTCAACCGAAGAAGTGCATCGGAACTGG  
GAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATG  
TGTAGCGGTGAAATGCGTAGATATATGGAAGAACC  
AGTGGCGAAGGCGGTGTCTGGTCTGTAAGTACGCT  
GAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATA  
CCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTG  
TTGGAGGGTTTTCCGCCCTCAGTGCTGACGTAACGCA  
TTAAGCATTCCGCTGGGGAGTACGGCCGCAAGGCTG  
AAACTCAAAGGAATTGACGGGGGCCCGACAAGCGGT  
GGAGCATGTGGTTAATTCGAAGCTACGCGAAGAACC  
TTACCAGGTCTTGACATACTATGCAAATCTAAGAGATT  
AGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCA  
TGGTTGTCGTCAGCTCGTGTGAGATGTTGGGTTAA

GTCCCCCAACGAGCGCAACCCTTATTATCAGTTGCCAG  
CATTAAGTTGGGCACTCTGGTGTGACTGCCGGTGACA  
AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATG  
CCCCCTATGACCTGGCTACACACGTGCTACAATGGATG  
GTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATC  
TCTTAAAGCAATTCTCAGTTCCGATTGTAGCTGCAACT  
CGCCTACATGAAGTCCGCATCACTAGTAATCGACGATC  
GAGCATGCCCGCGGTGAATAACGTTCTCCCCGGGGCTC  
TTAAAAAAGGG

#### DNA sequence of *Lactobacillus fermentum* strain CAU2928

CGCAACAGAAGGCCCAAGGGTTTCTTTGGGCGTAA  
GAGAGTGCAGGCGGTTTTCTAAGTCTGATGTGAAAGC  
CTTCGGCTTAACCGGAGAAGTGCATCGGAACTGGAT  
AACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTG  
TAGCGGTGGAATGCGTAGATATATGGAAGAACCACG  
TGGCGAAGGCGGCTACCTGGTCTGCAACTGACGCTGA  
GACTCGAAAGCATGGGTAGCGAACAGGATTAGATACC  
CTGGTAGTCCATGCCGTAACGATGAGTGTAGGTGTT  
GGAGGGTTTTCCGCCCTCAGTGCCGGAGCTAACGCATT  
AAGCACTCCGCTGGGGAGTACGACCGCAAGGTTGAA  
ACTCAAAGGAATTGACGGGGGCCCGACAAGCGGTGG  
AGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCCTTA  
CCAGGTCTTGACATCTTGCGCCAACCCTAGAGATAGGG  
CGTTTCTTCGGGAACGCAATGACAGGTGGTGCATGGT  
CGTCGTCAGCTCGTGTGCGTGAGATGTTGGGTTAAGTCC  
CGCAACGAGCGCAACCCCTTGTACTAGTTGCCAGCATT  
AAGTTGGGCACTCTAGTAAGACTGCCGGTGACAAACC  
GGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCC  
CTTATGACCTGGGCTACACACGTGCTACAAATGGGAC  
GGTACAACGAGTCCGGAACCTCGCGAGGGGCAAGCAA  
TCCTCTTAAAAACCCTTCTCAGTTCGGACTGCAGGCTG  
GCAACTCGCTGCTCGAAGTCCGGAATCGCTAGTATCCC  
CGGATCAGCATGCCCGCGGTGAAATACGTTTTCCCGG  
GTCTAAAAA

#### DNA sequence of *Lactobacillus plantarum* strain B15

CCCATTTCGAAGGGGCCGATTGTTGGTCTCGATGGGCAAT  
AAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTG  
AAAGCCTTCGGCTCAACCGAAGAAGTGCAT  
CGGAACTGGGAACTTGAGTGCAGAAGA  
GGACAGTGGAACTCCATGTGTAGCGGTGAA  
ATGCGTAGATATATGGAAGAACCAGTGG  
CGAAGGCGGCTGTCTGGTCTGTAAGTACG  
CTGAGGCTCGAAAGTATGGGTAGCAAACA  
GGATTAGATACCCTGGTAGTCCATACCCTTA  
AACGATGAATGCTAAGTGTGGAGGGTTTC  
CGCCCTCAGTGCTGACGCTAACGCATTAA  
GCATTCCGCTGGGGAGTACGGCCGCAAGG  
CTGAAACTCAAAGGAATTGACGGGGGCC  
GCACAAGCGGTGGAGCATGTGGTTTAATTC  
GAAGCTACGCGAAGAACCCTTACCAGGTCTT  
GACATACTATGCAAATCTAAGAGATTAGAC  
GTTCCCTTCGGGGACATGGATACAGGTGGT  
GCATGGTTGTCGTCAGCTCGTGTGAGTA  
TGTTGGGTTAAGTCCCGCAACGAGCGCAAC

CCTTATTATCAGTTGCCAGCATTAAGTTGG  
 GCACTCTGGTGAGACTGCCGGTGACAAACC  
 GGAGGAAGGTGGGGATGACGTCAAATCAT  
 CATGCCCTTATGACCTGGGCTACACACGT  
 GCTACAATGGATGGTACAACGAGTTGCGAA  
 CTCGCGAGAGTAAGCTAATCTCTTAAAGCA  
 TTCTCAGTTCGGATTGTAGGCTGCAACTCG  
 CCTACATGAAGTCGAATCGCTAGTAATCGC  
 GATCAGCATGCGCGGTGATACGTTTCCCGG  
 TCCTAAAAAGAA

**Properties of substrates used**

The proximate composition of the substrates used is presented in Table 2. The production of lactic acid gave a maximum yield of 10.53%, 6.75% and

5.40% on day 6 with mango, banana and orange peels respectively using *L. plantarum*. This is presented in Table 3, while *L. casei* gave a maximum yield of 12.03%, 8.10% and 7.20% on day 6 with mango, banana and orange peels respectively as presented in Table 4.

The effects of varying fermentation time on the production of lactic acid for *L. plantarum* and *L. casei* are shown in Table 3 and 4 respectively. The production of lactic acid by the two test organisms using mango, banana and orange peels increased progressively up to day 6 of fermentation, and then dropped sharply on day 7.

**Table 1. Colonial, Morphological and Biochemical Characteristics of Bacterial Isolates**

Isolate	B1	B2	B3	B4
<b>Colonial characteristics</b>	Circular, convex, entire, cream, smooth, opaque	Irregular, raised, undulate, cream, moist, opaque	Circular, raised, entire, cream, smooth, opaque	Circular, convex, entire, cream, smooth, opaque
<b>Morphological characteristics</b>	Rods in cluster	Rods in chain	Rods in chain	Rods in cluster
<b>Gram reaction</b>	+	+	+	+
<b>Spore staining</b>	-	-	-	-
<b>Motility</b>	-	-	-	-
<b>Catalase</b>	-	-	-	-
<b>Oxidase</b>	+	+	+	+
<b>Citrate</b>	+	-	-	+
<b>VogesProskauer</b>	-	+	+	-
<b>Methyl Red</b>	+	+	+	+
<b>Indole</b>	-	-	-	-
<b>Urease</b>	-	-	-	-
<b>Starch hydrolysis</b>	-	-	-	-
<b>Tentative identity</b>	<i>Lactobacillus Plantarum</i>	<i>Lactobacillus Casei</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus Plantarum</i>

**KEY:**+ = Positive, - = Negative

**Table 2. The proximate analysis of mango, banana and orange peels**

S/No	Parameters Investigated	Mango (%)	Banana (%)	Orange (%)
1	Moisture Content	10.28	9.80	10.36
2	Total Ash	4.68	8.52	5.14
3	Total Fat	4.92	6.29	6.84
4	Crude Fibre	12.46	10.42	12.18
5	Crude Protein	5.18	9.63	8.42
6	Carbohydrate content	62.48	55.34	57.06
6	Sugar mg/100g	6.24	6.82	6.56
7	TTA mg/100g	0.31	0.28	0.26
8	pH	4.30	6.00	4.70

**Key:** % = Percentage, TTA =Titratable acidity.

The effect of pH variations on the production of lactic acid are shown in Figures G and H respectively

and pH optimization experiments on *L. plantarum* showed that pH 5.0 and 6.0 were best.

Banana produced its highest yield (5.40%) at pH 5.0 while pH 6.5 produced the lowest yield (2.25%). Orange peels produced its highest yield (4.05%) at pH 5.0 p and lowest (1.80%). at pH 6.5. When *Lactobacillus casei* was used, mango peels also produced the highest lactic acid of (7.9%) at pH 6.0, as did banana peels (7.40%) and orange peels (5.00%). The lowest yields were observed at pH 5.0 as 4.50%, 3.60% and 1.35% for mango, banana and orange peels respectively.

The effects of inoculum size variation on the yield of lactic acid are shown in Figure A and Figure B. During the inoculum size optimization test for *L. plantarum* using mango, banana and orange peels, inoculum size of 2 ml produced the highest yield of lactic acid. The production was 23.85%, 26.7% and 18.9% for mango, banana and orange peels respectively, while *Lactobacillus casei* had the unusual high production of lactic acid at inoculum size of 1.0 ml which was 17.10% for mango peels, 8.10% for banana peels and 6.30% for orange peels.

The impact of substrate variation for *L. casei* and *L. plantarum* are shown in Figure C and Figure D respectively. *Lactobacillus casei* and *Lactobacillus plantarum* produced the highest amount of lactic acid with 4g of substrate. The ranges of lactic acid produced by *L. casei* for the three substrates were 5.90-13.50% for mango peels, 5.20-9.90% for banana peels and 3.80-9.00% for orange peels, while *L. plantarum* produced 4.10-9.00% for mango peels, 2.50-6.30% for banana peels and 1.80-5.40% for orange peels.

The optimized process for the production of lactic acid resulted from variation of the above named parameters. These were combined in a single fermentation for each of the organisms and different substrates to get optimum yields. The optimized conditions (Table 5) for *L. plantarum* were pH 5.0, 4g substrate, 2 ml inoculum, 10g of nitrogen source for 8 days resulting in 20.70%, 12.97% and 10.83% yield for mango, banana and orange peels respectively. For *L. casei* (Table 6), they were pH 6.0, 4g of substrate, 1 ml inocula size, 10g of nitrogen source for 8 days resulting in 23.28%, 18.30% and 12.90% yield for mango, banana and orange peels respectively.

*L. plantarum* was inoculated in various wastes and incubated at uniform pH (6.0). Among the substrate, lactic acid production was found to be highest in mango peels (5.94%), followed by banana peels (3.60%) and orange peels (3.40%).

**Table 3. Percentage Production of Lactic acid by fermentation from mango, banana and orange peels using *Lactobacillus plantarum***

Fermentation time (Days)	Lactic acid production (%)		
	Mango peels	Banana peels	Orange
1	1.650±0.150 <sup>a</sup>	1.050±0.150 <sup>a</sup>	1.200±0.150 <sup>a</sup>
2	2.400±0.150 <sup>a</sup>	1.500±0.150 <sup>b</sup>	1.650±0.150 <sup>a</sup>
3	3.150±0.260 <sup>b</sup>	2.100±0.150 <sup>a</sup>	2.250±0.260 <sup>b</sup>
4	4.200±0.300 <sup>c</sup>	2.550±0.150 <sup>a</sup>	3.000±0.300 <sup>c</sup>
5	5.70±0.912 <sup>ab</sup>	3.600±0.000 <sup>a</sup>	3.900±0.150 <sup>a</sup>
6	10.53±1.114 <sup>d</sup>	5.400±0.687 <sup>b</sup>	6.750±0.450 <sup>d</sup>
7	7.650±0.260 <sup>b</sup>	4.200±0.397 <sup>c</sup>	5.850±0.260 <sup>b</sup>
8	6.300±0.520 <sup>d</sup>	3.300±0.150 <sup>a</sup>	4.200±0.397 <sup>c</sup>

**Key:** Each value is a mean of three determinations ± standard error of mean. Different superscripts across the columns are significantly different (p<0.05).

**Table 4. Percentage Production of Lactic acid by fermentation from mango, banana and orange peels using *Lactobacillus casei***

Fermentation time (Days)	Lactic acid production (%)		
	Mango peels	Banana peels	Orange peels
1	2.100±0.150 <sup>a</sup>	1.500±0.150 <sup>a</sup>	1.050±0.150 <sup>a</sup>
2	2.850±0.397 <sup>c</sup>	1.950±0.150 <sup>a</sup>	2.025±0.225 <sup>b</sup>
3	3.450±0.397 <sup>c</sup>	2.700±0.520 <sup>d</sup>	2.400±0.150 <sup>a</sup>
4	4.650±0.300 <sup>c</sup>	3.450±0.397 <sup>c</sup>	3.300±0.397 <sup>c</sup>
5	6.150±0.150 <sup>a</sup>	4.200±0.397 <sup>c</sup>	3.900±0.300 <sup>c</sup>
6	12.03±1.091 <sup>d</sup>	8.10±0.520 <sup>ab</sup>	7.200±0.520 <sup>ab</sup>
7	8.10±0.520 <sup>ab</sup>	5.700±0.300 <sup>c</sup>	4.800±0.300 <sup>c</sup>
8	6.600±0.300 <sup>c</sup>	4.667±0.167 <sup>b</sup>	4.067±0.467 <sup>ab</sup>

**Key:** Each value is a mean of three determinations ± standard error of mean. Different superscripts across the columns are significantly different (p<0.05).

Nitrogen concentration was varied with yeast extract (2.5g/l, 5g/l, 7.5g/l and 10g/l) used for the two organisms (Figures E and F). The results of these experiments showed that yield increased with increase in concentration of nitrogen. This implied that addition of nitrogen to fermentation media, at least at the concentrations tested influenced the production of lactic acid from agro-wastes.

All values giving the highest yields were put together in a flask to obtain an optimized yield (Tables 5 and 6). A yield of 22.50% was obtained

on Day 6 at a pH of 5.0, substrate amount of 4g/50 ml, nitrogen concentration of 10g/l and inoculum size of 2 ml with *L. plantarum*, while with *L. casei*, a yield of 27.10% was obtained on Day 6 at a pH of 6.0, substrate amount of 4g/50 ml, nitrogen source of 10g/l and an inoculum size of 1.0 ml.

**Table 5. Optimization Production of Lactic acid from mango, banana and orange peels using *Lactobacillus plantarum***

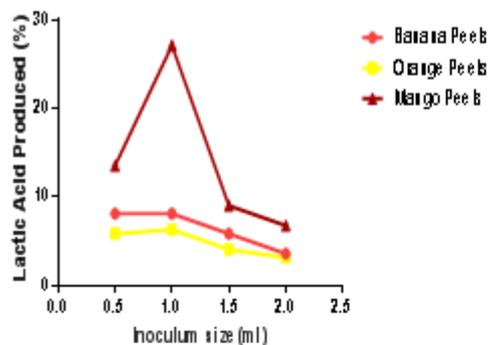
Fermentation time (Days)	Lactic acid production (%)		
	Mango peels	Banana peels	Orange peels
1	4.200±0.397 <sup>c</sup>	3.750±0.150 <sup>a</sup>	3.000±0.300 <sup>b</sup>
2	5.100±0.397 <sup>c</sup>	4.667±0.309 <sup>b</sup>	3.900±0.150 <sup>a</sup>
3	6.600±0.300 <sup>b</sup>	5.700±0.150 <sup>a</sup>	4.967±0.017 <sup>a</sup>
4	8.700±0.600 <sup>c</sup>	6.600±0.300 <sup>b</sup>	6.000±0.150 <sup>a</sup>
5	12.96±0.267 <sup>b</sup>	8.400±0.300 <sup>b</sup>	6.900±0.300 <sup>b</sup>
6	20.700±1.039 <sup>d</sup>	12.97±0.267 <sup>ab</sup>	10.83±0.934 <sup>cd</sup>
7	16.50±2.163 <sup>cd</sup>	10.833±0.933 <sup>cd</sup>	7.500±0.300 <sup>b</sup>
8	12.03±1.091 <sup>cd</sup>	8.400±0.300 <sup>b</sup>	6.600±0.300 <sup>b</sup>

**Key:** Each value is a mean of three determinations ± standard error of mean. Different superscripts across the columns are significantly different (p<0.05).

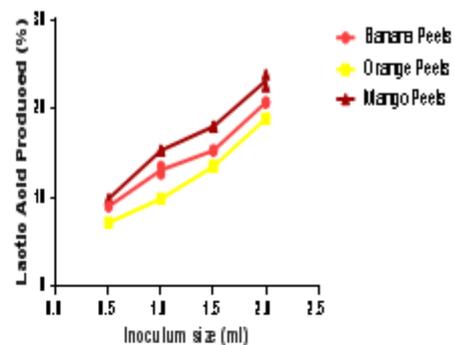
**Table 6. Optimization Production of Lactic acid from mango, banana and orange peels using *Lactobacillus casei***

Fermentation time (Days)	Percentage Lactic acid production (%)		
	Mango peels	Banana peels	Orange peels
1	4.667±0.309 <sup>c</sup>	3.750±0.150 <sup>a</sup>	4.200±0.397 <sup>c</sup>
2	5.717±0.381 <sup>c</sup>	4.350±0.300 <sup>b</sup>	4.667±0.309 <sup>c</sup>
3	6.450±0.397 <sup>c</sup>	4.983±0.017 <sup>a</sup>	5.267±0.292 <sup>ab</sup>
4	9.93±1.588 <sup>cd</sup>	5.867±0.433 <sup>d</sup>	7.20±0.520 <sup>d</sup>
5	18.48±3.225 <sup>cd</sup>	10.23±1.338 <sup>d</sup>	12.90±1.587 <sup>ab</sup>
6	23.28±2.384 <sup>cd</sup>	12.90±1.587 <sup>ab</sup>	18.30±1.587 <sup>ab</sup>
7	14.77±2.067 <sup>cd</sup>	9.000±0.520 <sup>e</sup>	12.03±1.091 <sup>ab</sup>
8	8.700±0.794 <sup>c</sup>	6.600±0.300 <sup>e</sup>	7.200±0.520 <sup>d</sup>

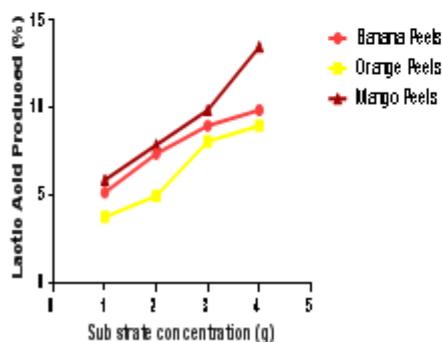
**Key:** Each value is a mean of three determinations ± standard error of mean. Different superscripts across the columns are significantly different (p<0.05).



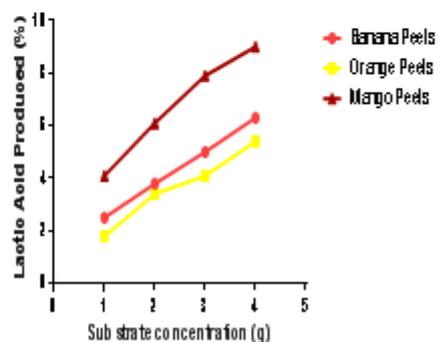
**Figure A: Percentage of lactic acid produced against inoculum size of *Lactobacillus casei***



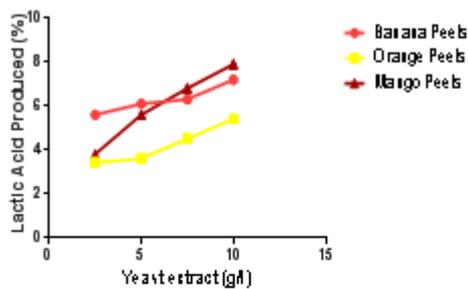
**Figure B: Percentage of lactic acid produced against inoculum size of *Lactobacillus plantarum***



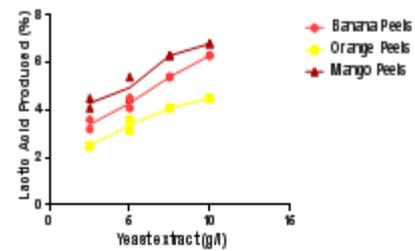
**Figure C: Percentage of lactic acid produced with substrate using *Lactobacillus casei***



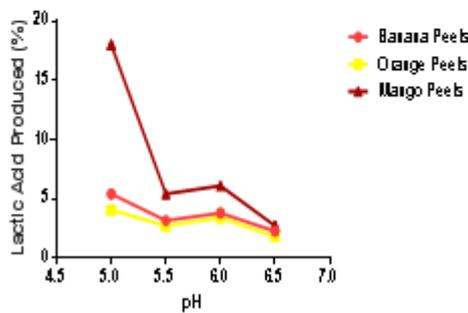
**Figure D: Percentage of lactic acid produced with substrate using *Lactobacillus plantarum***



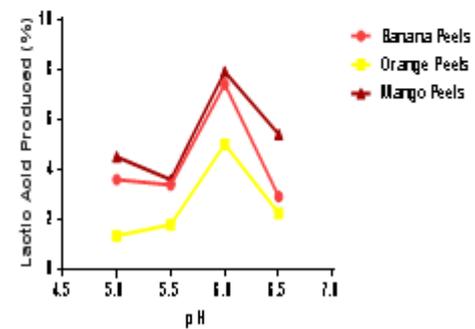
**Figure E:** The effect of yeast extract as a nitrogen source on the percentage of lactic acid produced by *Lactobacillus casei*



**Figure F:** The effect of yeast extract as a nitrogen source on the percentage of lactic acid produced by *Lactobacillus plantarum*



**Figure G:** pH optimization and percentage of lactic acid produced by *Lactobacillus plantarum*



**Figure H:** pH optimization and percentage of lactic acid produced by *Lactobacillus casei*

With both organisms, the 10g yeast extract gave the highest yield for lactic acid production. *L. casei* gave 7.90%, 7.20% and 5.40% for mango, banana and orange peels respectively (Figure E), while *L. plantarum* gave the highest yield of 6.80%, 6.30% and 4.50% on day 6 for mango, banana and orange peels respectively (Figure F).

#### 4. Discussion

##### Isolation and Identification of test organisms

The use of autochthonous lactic acid bacteria (LAB) for the production of cheese and organic acid was reported by Renes *et al.*, (2019). This is in agreement with the indigenous bacteria used in this work. In a report by Yang *et al.*, (2016), eighty-two strains of LAB were isolated from fruit residues such as banana leaf, pineapple peel and papaya peel. In another study on lactic acid production, the organisms were isolated from spent coffee mucilage (Bretón-Toralet *et al.*, 2017). In a study by Palsaniya *et al.* (2012) on production of bacterial alkaline protease, the bacteria used were isolated from the soil. The pH range used in this work was 5 to 6.5 with highest yield obtained at pH 5 and 6 respectively for *L. plantarum* and *L. casei*. This is not in concord with the report of Couriet *et al.* (2000) who reported a pH of 3 for orange peels and a pH of 3.9 for mango. Wilkins *et al.* (2007) reported a pH range of 4.3 – 4.4 for lactic acid production

from banana peels. Tang *et al.* (2017) reported that the highest lactic acid was generated at pH 5 using fresh food waste as the inoculum. Tang *et al.* (2016) also reported the highest production of lactic acid at pH 6. In the work of Wu *et al.* (2015), lactic acid was produced at pH 4.0 with improvement at pH 5.0.

The inocula applied by Tang *et al.* (2017) were methanogenic sludge, fresh food waste and anaerobic activated sludge. These inocula varied from that used in this study showing that there are no limitations to inocula source. Lactic acid production increased progressively with increase of inoculum size for *L. plantarum* with the highest production observed when 2 ml of the prepared inoculum was used for all the substrates with yields of 23.85%, 26.7% and 18.9% for mango, banana and orange peels respectively. However, the reversed production was observed when *L. casei* was used; 1.0 ml of the prepared inoculum gave the highest production of lactic acid with 17.10%, 8.10% and 6.30% for mango, banana and orange peel, while 0.5, 1.5 ml and 2.0 ml produced lower amount of lactic acid (Figures C and D). It was important to monitor the optimum inoculum size for the production of lactic acid because low inoculum might be insufficient to utilize the available substrate and that might lead to reduction in the quantity of lactic acid produced while

excessive inoculum sizes might form superfluous biomass and cause a significant exhaustion of the nutrients that should otherwise have been utilized for the production of lactic acid (Shin *et al.*, 2010; Pradeep *et al.*, 2014).

The quantity of lactic acid produced increased progressively from day 1 to 6 and then dropped sharply (Table 3). This might be due to the depletion of nutrient and accumulation of toxic metabolites which resulted in reduced production of lactic acid on day seven (Quiroga *et al.*, 2014). The growth pattern observed with *L. casei* (Table 4) which gave the higher yields agreed with the work of John *et al.* (2007) and Rutuja *et al.* (2016) on lactic acid production by *Lactobacillus* sp. using vegetable waste who also reported highest production by day 5 and day 6.

Increase in nitrogen concentration caused an increase in lactic acid production. This agrees with the findings of Chinedu *et al.* (2011) where the concentration of nitrogen was varied for improved production of cellulase by *Aspergillus niger*, *Penicilliumchrysogenum* and *Trichodermaharzianum*. Arakaki *et al.* (2001) also found improved biomass production in yeasts grown under submerged fermentation when nitrogen concentration was increased. The effectiveness of nitrogen in this study might be because nitrogen was one of the major elements required for nucleic acid synthesis in microorganisms during the biosynthesis of lactic acid. The study of Wee *et al.* (2006) also lends further credence to this observation. After the optimization process, *L. casei* showed a higher lactic acid producing ability than *L. plantarum*.

## 5. Conclusions

Mango peels produced the largest amount of lactic acid, while orange peels were found to produce the lowest amount. Further studies are required on mango peels in order to improve its use in production of lactic acid.

## Acknowledgement

The authors acknowledge the Department of Microbiology, University of Ilorin for providing enabling environment for this research

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