

## Technological properties of lactic acid bacteria and yeasts isolated from *ogi*, a West African fermented cereal gruel

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### Abstract

Lactic acid bacteria (LAB) and yeasts involved in *Ogi* production have been greatly studied. However, it is important to consider the technological roles of these organisms and their contribution to product quality and safety. LAB and yeasts were isolated from three varieties of *Ogi* and identified using biochemical characteristics and API 50CHL and API 20C AUX kits. Technological properties including acidification activity, production of  $\alpha$ -amylase, phytate degradation, tolerance to 2% bile salt and haemolytic activity were tested in the isolates. Fifteen isolates of LAB obtained included *Lactobacillus brevis*, *L. casei* and *L. plantarum* while the yeasts were *Candida famata*, *C. guilliermondii*, *C. lusitaniae* and *Cryptococcus humicola*. Isolates WML482 and YMY483 with  $\Delta$ pH of 1.62 and 2.10 respectively showed the most rapid acidification. Four isolates of LAB showed  $\alpha$ -amylase activity. The LAB and yeast isolates differed in their ability to degrade phytate but were all tolerant to 2% bile salt with the highest growth of 8.41 cfu/mL and 8.42 cfu/mL observed at 72 h in isolates RSL483 and YMY001 respectively. None of the isolates showed  $\beta$ -haemolysis. The isolates from this study possess notable technological properties which position them as good starters for production of *Ogi* with better and safe qualities.

**Keywords:** acidification; haemolysis; lab; *ogi*; yeasts

### Introduction

Cereals account for about 77% of the total food energy intake in many African countries. It also contributes significantly to dietary protein in many of these countries (Mitchell and Ingro, 1993; Owusu-Kwarteng, 2010) [20, 23]. Many types of traditional fermented foods made from cereals and consumed in Africa are processed using spontaneous fermentation through backslopping (Amoa and Jakobsen, 1996) [2]. *Ogi* is one of the indigenous fermented foods most commonly produced and consumed predominantly in West-Africa, particularly Nigeria. Processing of cereals into *Ogi* usually involves dehulling and washing of the cereal grains followed by wet milling. The flow-through from the sieved wet-milled cereal is then fermented over a period of about 48 – 72 h. *Ogi* has a mild sour taste owing to the reduction in pH resulting from the production of acids during the fermentation of the cereal (Owusu-Kwarteng *et al.*, 2010) [23]. It is taken as a staple and beverage by many adults. It is also consumed by infants as a weaning food. Furthermore, the production serves as a source of income for the indigenous women, and also offers links to local farmers as suppliers of raw materials for the production. Production of *Ogi* depends on spontaneous fermentation initiated by the indigenous microorganisms found on the cereals used, the processing utensils or equipment, normal flora of the hands of producers, and the atmosphere of the environment of production (Jespersen *et al.*, 1994) [16]. Lactic acid bacteria (LAB) and yeasts are the key players in the fermentation and intake of fermented foods and beverages (Wood and Holzappel, 1995; Caplice and Fitzgerald, 1999) [9]. This is because they are solely involved in the rapid acidification of the raw material, and production of organic acids, and several enzymes of importance (Omemu *et al.*, 2018) [21]. Moreover, the

ingestion of viable, non-pathogenic bacteria or yeasts that are able to reach the intestines in appropriate numbers to confer health benefits to the host, known as probiotics is Worth considering in the selection, and development of starter cultures (de Vrese and Schrezenmeir, 2008) [12]. In the attempt to develop starter cultures for controlled fermentation and production of *Ogi* with greater consistency in quality and safety, it is imperative to fully characterise the predominant LAB species and yeasts, and also determine the technological roles of these organisms in contributing to the safety and product quality. This work is therefore aimed at identifying the technological roles of the indigenous LAB and yeasts associated with *Ogi* production.

### Materials and Methods

#### Sample Collection

Raw samples of cereals including white maize, yellow maize, and red sorghum were obtained from Poka market located in Epe, Lagos State, Nigeria.

#### Spontaneous Fermentation of *Ogi*

Five hundred grams (500 g) of each cereal type (white maize, yellow maize, and red sorghum) was soaked (steeping) for 48 h. The samples were wet-milled using a blender (Sapphire) sterilized with 70% ethanol in-between use. The blends obtained were sieved and the flow-through obtained was allowed to ferment for 72 h (Wakil and Daodu, 2011) [32].

#### Determination of pH and Total Titratable Acidity (TTA) of *Ogi* during Fermentation

Five mL of each of the fermenting batch of *Ogi* was pipetted into a beaker and the pH taken using (Oarsman PHS-3C) digital pH meter. The pH determination was done 24 hourly

(AOAC, 2005) [3]. The TTA was determined by titrating 0.1M NaOH against 25 mL of each of the fermenting *Ogi* samples using phenolphthalein as the indicator. Samples were drawn at the start of fermentation and 24 hourly till the end of the 72 h fermentation period. Each mL of NaOH used was taken to be equivalent to 0.9008g of lactic acid (AOAC, 2005) [3].

### Isolation of Lactic Acid Bacteria (LAB) and Yeasts from the Fermenting *Ogi*

#### Homogenization, Serial Dilution and Pour Plate Technique

One mL of each of the fermenting *Ogi* was carefully mixed with 9 mL of sterile peptone. Each homogenate was serially diluted up to  $10^{-10}$ . One mL aliquot of the dilutions was directly inoculated into duplicate Petri plates. About 15 mL each of appropriate isolation media for both LAB and yeasts was then aseptically poured into each plate. MRS agar (Oxoid) was used for the isolation of lactic acid bacteria while Malt Extract Agar (MEA) (Oxoid) was used for the yeasts. The plates were allowed to solidify before incubation in an inverted position under anaerobic conditions at 35 °C for 24 – 48 h for LAB, and 25 °C for 3 – 5 days for yeasts.

#### Pure Culturing and Storage of Isolates

Pure cultures of the LAB and yeast isolates were obtained by selecting distinct colonies from the culture plates and inoculated on MRS agar and MEA respectively. The streaked MRS agar plates were incubated at 35 °C under anaerobic conditions for 18 – 24 h while the MEA plates were incubated at 25 °C for 3 – 5 days and stored in MRS broth and Malt Extract broth (MEB) at appropriate temperature and time. For long-term storage, purified isolates were stored in MRS broth and MEB containing 20% (v/v) glycerol at – 20 °C.

### Characterisation of the LAB and Yeast Isolates

#### Gram's Reaction and Biochemical Characterisation of LAB Isolates

The Gram's reaction was done as described by Powers (1995) [25] and catalase activity of the LAB isolates carried out according to the method described by Cheesbrough (2006) [10]. Citrate utilization test was done using the method of Kiiyukic (2003) [18], gelatin liquefaction test was done using the method of Sagar Aryal (2018) [26] while the production of CO<sub>2</sub> from glucose was assessed using the method of Fawole and Oso (2004) [14].

#### Germ Tube Test for the Yeast Isolates

This was done using the method described by Sagar Aryal (2018) [26]. 0.5 mL of human serum was placed in a test tube. A colony each of the yeast isolates was gently emulsified in the serum. The test tubes were incubated at 37 °C for 2 – 4 h. After the incubation, a drop each of the serum was pick and placed on a grease-free glass slide and examined microscopically under the low and high power objective lenses.

#### Analytical Profile Index (API) Identification of the LAB and Yeast Isolates

Analytical profile index kits were used in assigning an identity to the isolates. The API kits used were API 50 CHL for the lactic acid bacteria (LAB) and API 20C AUX for the yeasts. The kits were used according to the protocols

provided by the manufacturer (Biomerieux, France).

### Determination of Technological Properties of the LAB and Yeast Isolates

#### Acidification Activity of the LAB and Yeast Isolates

This was done according to the method described by Ayad *et al.* (2004) [5] using MRS broth and Malt extract broth for the LAB and yeast isolates respectively. The rate of acidification was calculated as change in pH ( $\Delta$ pH):

$$\Delta pH = pH (\text{zero hour}) - pH (\text{given hour})$$

#### Production of $\alpha$ -Amylase by LAB

The method described by Sanni *et al.* (2002) [28] was used to assess the ability of the LAB isolates to produce  $\alpha$ -amylase.

#### Phytate Degradation by the LAB and Yeast Isolates

This was carried out according to the method described by Onipede *et al.* (2014) [22]. The LAB and yeast isolates were qualitatively screened for their ability to degrade phytate through phytase production using phytase screening agar (PSA).

#### Bile Tolerance of the LAB and Yeast Isolates

The ability to survive gastrointestinal conditions was investigated by screening for bile salt tolerance as described by Minelli *et al.* (2004) [19].

#### Haemolytic Activity of the LAB and Yeasts Isolates

Twenty-four-hour LAB and yeast cultures pre-cultivated on their appropriate media inoculated on blood agar plates containing 2.5% (v/v) defibrinated human blood and incubated at 37 °C for 48 hours. Clear zones and green-hued zones around the blood agar plates is indicative of  $\beta$ -haemolysis and  $\alpha$ -haemolysis respectively while no zone around colonies indicates  $\gamma$ -haemolysis (Yoon *et al.*, 2008) [35].

### Results and Discussion

The changes in pH during the fermentation of the three varieties of *Ogi* are represented in Figure 1. The pH of white maize *Ogi* decreased from 6.11 at the start of the fermentation to 4.39 at the end of the 72 h fermentation time. The pH of red sorghum *Ogi* decreased from 6.48 at the start of fermentation to 4.79 at the end of the 72 h fermentation time. The pH of yellow maize *Ogi* decreased from 6.02 at the start of the fermentation time to 4.15 at the end of the fermentation time. The total titratable acidity (TTA) during the fermentation of the three varieties of *Ogi* are presented in Figure 2. The TTA of white maize *Ogi* increased from 6.00 at the start of the fermentation to 13.5 at the end of the 72 h fermentation time. The TTA of red sorghum *Ogi* increased from 4.58 at the start of the fermentation to 19.3 at the end of the 72 h fermentation time. The TTA of yellow maize *Ogi* increased from 5.50 at the start of the fermentation to 16.3 at the end of the 72 h fermentation time. In this study, the pH during the fermentation of the maize and sorghum to produce *Ogi* dropped significantly from pH above 6 to pH below 5 while the total titratable acidity (TTA) obtained during the fermentation differed among the three varieties of *Ogi* having highest value of 17.39 for red sorghum *Ogi* obtained at 72 h of fermentation. Similar trends of drop in pH have been associated with fermented cereal-based foods

(Sawadogo-Lingani *et al.*, 2007, Omemu *et al.*, 2018) [29, 21]. The general reduction in pH and increase in TTA during fermentation results from the various organic acids

produced by the fermenting lactic acid bacteria and yeasts present during the fermentation (Basinskiene *et al.*, 2016) [7].

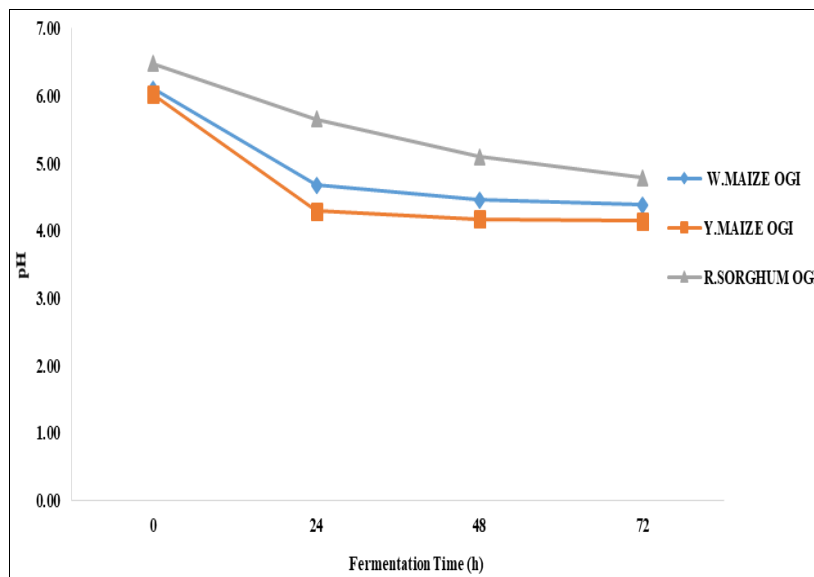


Fig 1: pH of fermenting Ogi

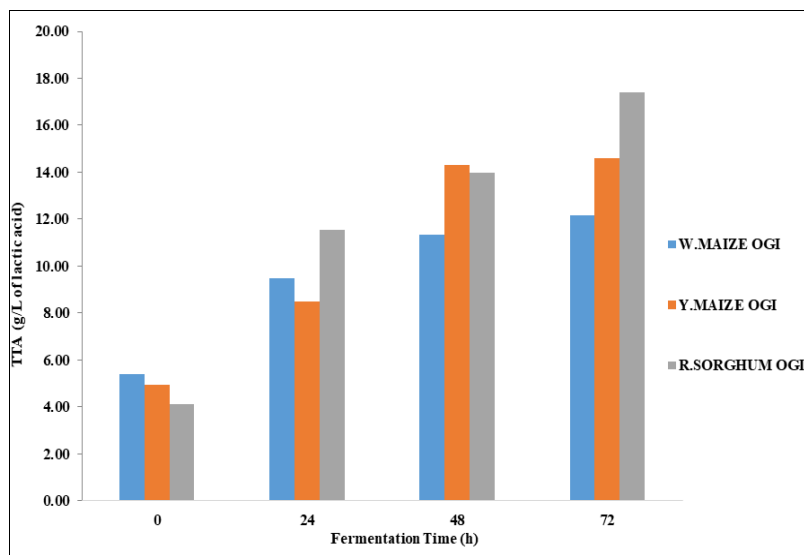


Fig 2: Total titratable acidity (TTA) of fermenting Ogi

Fifteen presumptive lactic acid bacteria and 15 yeasts were isolated from white maize Ogi, red sorghum Ogi and yellow maize Ogi. The Gram's reaction, morphology and biochemical characteristics of the LAB isolates are presented in Table 1. The fifteen isolated lactic acid bacteria were gram-positive. They were all negative for catalase activity and citrate utilization. Eleven of the LAB isolates were positive while 4 showed negative reaction for gelatin liquefaction. Only 2 of the LAB isolates produces CO<sub>2</sub> from glucose. Five of the yeast isolates were negative while 10 showed positive reaction for gelatin liquefaction. All the yeast isolates were negative for the germ tube test (Table 2). The API 50CHL kit identified the LAB isolates as *Lactobacillus brevis* (2), *L. casei* (3) and *L. plantarum* (10). Table 3 shows the fermentation pattern of some of the identified LAB. The species of LAB isolated from this study correlates the species that have been reported to be associated with Ogi fermentation with *L. plantarum* being the most frequently occurring species (Omemu *et al.*, 2018)

[21]. The API 20C AUX kit identified the yeasts as *Candida famata* (7), *C. guilliermondii* (4), *C. lusitaniae* (3) and *Cryptococcus humicola* (1) with the representatives shown in Table 4. Although the species of yeasts obtained in this study are not frequently encountered during Ogi fermentation, *Candida famata* has been recently reported in related fermented products (Arifin *et al.*, 2014) [4]. The association of yeasts and lactic acid bacteria is known in a variety of traditional fermented foods and beverages (Oyewole, 1992; Sanni, 1993) [24, 27]. A co-metabolism between yeasts and lactic acid bacteria has been suggested, whereby the bacteria provides the acidic environment, which select for the growth of yeasts, while other growth factors and vitamins are made available to the bacteria by the yeasts (Steinkraus, 1996) [30]. The symbiotic relationship between yeasts and lactic acid bacteria due to a mutual growth stimulation based on their amino acids and carbohydrates metabolisms during the

production of fermented foods have been proposed (Gobbetti *et al.*, 1994) [15].

**Table 1:** Gram's Reaction, Morphology and Biochemical Characteristics of the LAB Isolates

Isolate code	Gram's Reaction	Morphology	Biochemical Characteristics			
			Catalase	Citrate Utilisation	Gelatin liquefaction	Production of CO <sub>2</sub> from glucose
WML001	+	Rod	-	-	+	-
WML241	+	Rod	-	-	+	-
WML242	+	Rod	-	-	+	-
WML482	+	Rod	-	-	-	+
WML722	+	Rod	-	-	-	-
WML725	+	Rod	-	-	+	-
YML721	+	Rod	-	-	+	-
YML722	+	Rod	-	-	+	-
YML723	+	Rod	-	-	+	+
RSL001	+	Rod	-	-	+	-
RSL003	+	Rod	-	-	+	-
RSL004	+	Rod	-	-	+	-
RSL242	+	Rod	-	-	+	-
RSL483	+	Rod	-	-	-	-
RSL485	+	Rod	-	-	-	-

+ = Positive reaction; - = Negative reaction

**Table 2:** Gelatin liquefaction and germ tube test for the yeast isolates

Isolate code	Gelatin liquefaction	Germ tube test
WMY001	+	-
WMY241	+	-
WMY242	+	-
WMY481	-	-
WMY482	+	-
WMY721	-	-
YMY001	+	-
YMY241	+	-
YMY481	-	-
YMY482	-	-
YMY483	-	-
RSY001	+	-
RSY002	+	-
RSY241	+	-
RSY242	+	-

+ = Positive reaction; - = Negative reaction

**Table 3:** API 50CHL Kit Fermentation Pattern for Selected LAB Strains

Substrate	Isolates and their fermentation pattern				
	WML482	WML722	RSL004	RSL242	RSL483
Glycerol	-	-	-	-	-
Erythritol	-	-	-	-	-
D-Arabinose	-	-	-	-	-
L-Arabinose	+	+	+	+	+
D-Ribose	+	+	+	+	+
D-Xylose	+	+	+	+	+
L-Xylose	-	-	-	-	-
D-Adonitol	-	-	-	-	-
Methy-β-D-Xylopyranoside	-	-	-	-	-
D-Galactose	+	+	+	+	+
D-Glucose	+	+	+	+	+
D-Fructose	+	+	+	+	+
D-Mannose	+	+	+	+	+
L-Sorbose	-	-	-	-	-
L-Rhamnose	-	+	-	-	-
Dulcitol	-	-	-	-	-
Inositol	-	-	-	-	-
D-Mannitol	-	-	-	+	+
D-Sorbitol	-	-	-	-	-
Methy-α-D-Mannopyranoside	-	-	-	-	-
Methy-α-D-Glucopyranoside	-	-	-	-	-
N-AcetylGlucosamine	-	+	+	-	-

Amygdalin	-	+	-	-	-
Arbutin	+	+	+	+	+
Esculin	+	+	+	+	+
Salicin	+	+	+	+	+
D-Cellobiose	+	+	+	+	+
D-Maltose	+	+	+	-	-
D-Lactose	-	-	-	+	+
D-Melibiose	+	+	+	-	-
D-Saccharose	+	+	+	+	+
D-Trehalose	+	+	+	-	-
Insulin	-	-	-	-	-
D-Melezitose	-	-	-	-	-
D-Raffinose	+	+	+	+	+
Amidon	-	-	-	-	-
Glycogen	-	-	-	-	-
Xylitol	-	-	-	-	-
Gentibiose	-	-	+	-	-
D-Turanose	-	-	-	-	-
D-Lyxose	-	-	-	-	-
D-Tagatose	+	+	+	-	-
D-Fucose	-	-	-	-	-
L-Fucose	-	-	-	-	-
D-Arabitol	-	-	-	-	-
L-Arabitol	-	-	-	-	-
Potassium Gluconate	-	-	-	-	-
Potassium 2-Ketogluconate	-	-	-	-	-
Potassium 5-Ketogluconate	-	-	-	-	-
Identity	<i>Lactobacillus brevis</i>	<i>L. brevis</i>	<i>L. casei</i>	<i>L. plantarum</i>	<i>L. plantarum</i>

+ = Positive reaction;- = Negative reaction

**Table 4:** API 20C AUX Kit Fermentation Pattern for the Yeast Isolates

Substrate	Isolates and their fermentation pattern				
	WMY241	WMY721	YMY001	RSY001	RSY242
Glucose	+	+	+	+	+
Glycerol	+	+	+	+	+
Calcium 2-Keto-Gluconate	+	+	+	+	+
L-Arabinose	+	+	+	+	+
D-Xylose	+	+	+	+	+
Adonitol	+	+	+	+	+
Xylitol	+	+	+	+	+
D-Galactose	+	+	+	+	+
Inositol	-	-	-	+	-
D-Sorbitol	+	+	+	+	+
Methyl- $\alpha$ -D-Glucopyranoside	+	+	+	+	+
N-Acetyl-Glucosamine	+	+	+	+	+
D-Cellobiose	+	+	+	+	+
D-Lactose	-	+	+	+	+
D-Maltose	+	+	+	+	+
D-Saccharose	+	+	+	+	+
D-Trehalose	+	+	+	+	+
D-Melezitose	+	+	+	+	+
D-Raffinose	+	+	-	+	+
Identity	<i>Candida guilliermondii</i>	<i>Candida famata</i>	<i>Candida lusitanae</i>	<i>Cryptococcus humicola</i>	<i>Candida famata</i>

+ = Positive reaction;- = Negative reaction

The acidification activity for the LAB and yeast isolates are presented in Figure 3 and Figure 4. The isolates differed in their ability to rapidly produce acid as shown by the reduction in pH of the MRS and MEB over a 36-hour period. The most rapid acid-producing LAB was WML482 with  $\Delta$ pH of 1.62 while the least producer was WML001 with  $\Delta$ pH of 1.27. The most rapid acid-producing yeast was YMY483 with  $\Delta$ pH of 2.10 while WMY001 had the least value of 1.86.

Majority of the LAB and yeasts isolated from the three varieties of *Ogi* demonstrated a faster acidification property

in the medium having a  $\Delta$ pH up to 1.7 and 2.1 at 36 hours, and this agreed with previous reports of Badis *et al.* (2004) [6] and Dagdemir and Ozdemir, (2008) [11]. Thus, these isolates can be considered for the development of starter cultures for controlled fermentation processes as faster acidification is necessary for reducing fermentation time and reducing contamination by spoilage or pathogenic microorganisms. In general, the required characteristics for industrial LAB or yeast starters are the abilities to rapidly and totally transform raw materials into lactic acid with the least nutritional requirements. Lactic acid bacteria and

yeasts are generally fastidious on artificial media, but they grow readily in most food substrates and rapidly lower the

pH to a point where competing organisms are no longer able to grow (Badis *et al.*, 2004) [6].

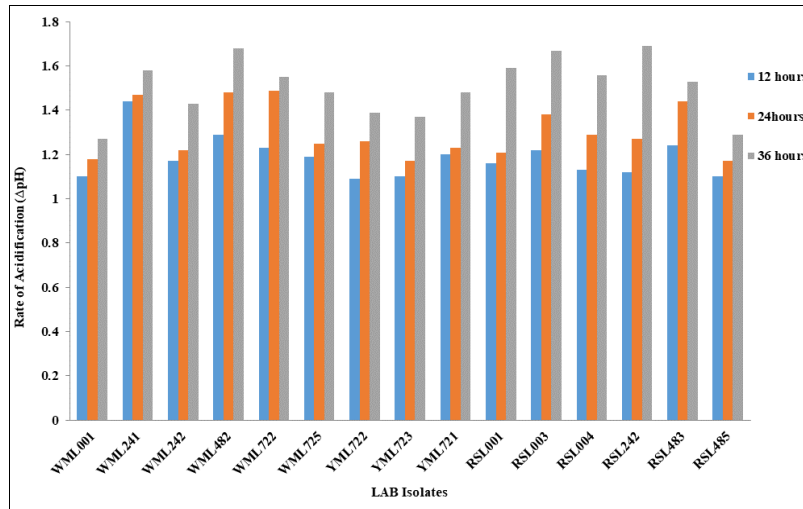


Fig 3: Acidification activity of LAB isolates

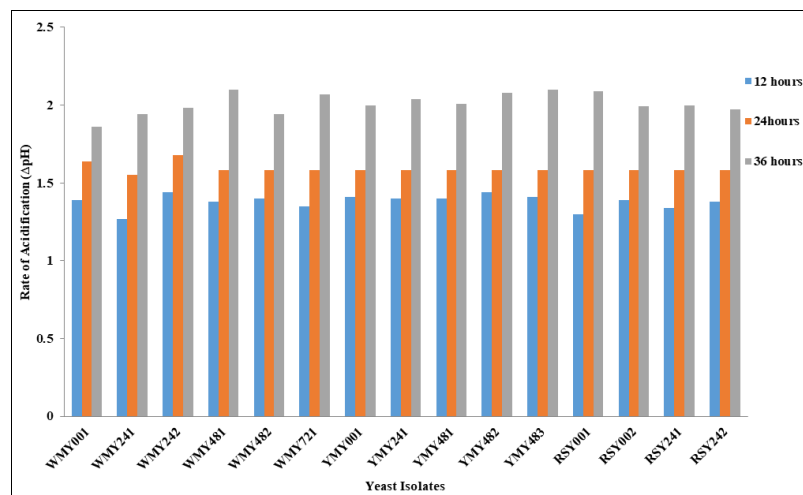


Fig 4: Acidification activities for yeast isolates

The production of  $\alpha$ -amylase, phytate degradation, tolerance to 2% bile salt and haemolytic activity are presented in Table 5. Four of the isolates showed a clear halo zone of degradation which is indicative of  $\alpha$ -amylase production. Isolate WML722 had the highest zone of 6 mm diameter, WML482 and RSL001 had 5 mm each while YML721 had the least zone of 4 mm. The predominant LAB from the three varieties of *Ogi* showed very weak or no amylase activities as only four of the isolates were positive. According to other studies, high prevalence of amylase producing LAB has not been reported. However, few strains like *L. fermentum* isolated from African maize products have been reported as amylase producers (Agati *et al.*, 1998; Sanni *et al.*, 2002) [1, 28]. Amylolytic lactic acid bacteria from traditional fermented foods could be of economic interest in the production of lactic acid from direct fermentation of starchy products (Xiaodong *et al.*, 1997) [34]. They also present the potential for decreasing the bulk viscosity of starchy weaning foods for young children in developing countries, leading to the improvement in their nutrient density while maintaining an acceptable thickness (FAO/WHO 1995) [13].

Twelve of the 15 LAB isolates showed the ability to degrade phytate with the highest activity observed in isolate

RSL004 with 26 mm zone of degradation. All the 15 isolates showed tolerance to the 2% bile salt. The results showed that bile salt at 2% did not affect the viability of the LAB isolates at the end of the 72-h period. The highest growth of 8.41 cfu/mL was observed in RSL483 and the least growth of 7.30 cfu/mL in RSL242. Green-hued zones which indicate  $\alpha$ -haemolysis was observed in 5 of the 15 LAB isolates while the remaining 10 isolates had  $\gamma$ -haemolysis with no zones around the colonies. None of the isolates had  $\beta$ -haemolysis (Table 5). The ability to degrade phytate was observed in only 3 of the 15 yeast isolates. The highest zone of degradation (11 mm) was observed in isolate WMY241 while the least zone of 4 mm was observed in WMY721. All the 15 yeast isolates were able to survive the 2% bile salt for the 72-h period with the highest growth of 8.42 cfu/mL observed in isolate YMY001. The least growth of 6.00 cfu/mL was observed in YMY482 which showed no growth during the first 12 h of incubation. Similarly, no growth was observed in isolate RSY002 until 72 h. The haemolysis test revealed  $\alpha$ -haemolysis in isolates WMY001 and RSY001. The remaining 10 isolates had  $\gamma$ -haemolysis (Table 6).

The probiotic properties of the predominant LAB and yeasts isolated from *Ogi* suggest their potential for the

development of starter cultures with probiotic effects due to their ability to survive and grow in low pH, bile salt and at 37 °C which is the normal human body temperature and also a requirement for probiotic. All organisms obtained in this study were able to survive and show exponential growth at 2% bile salt. As a requirement, probiotic microorganisms should reach the site of action in a viable state (Terpou *et al.*, 2019) [31]. The survival ability of bacteria through the upper digestive tract to reach the colon, where their beneficial role are expected to be observed is crucial for their selection as a probiotic (Bezkorovainy, 2001) [8]. Testing for the presence of haemolysis in the LAB and yeast isolates is also crucial for safety. This is because the probiotic microorganisms must be safe, i.e., the probiotic bacteria like lactobacilli, essentially be incompetent to cause haemolysis. In this study, there was no β-haemolysis for the LAB and yeasts. Although α-hemolysis among lactobacilli from foods and dairy products is not uncommon (Kaktcham *et al.*, 2012) [17]. The isolates WML242, WML722, YML723, RSL004, RSL485, WMY001 and RSY001 for LAB and yeasts however showed α-haemolysis which indicates partial or no haemolysis. This indicates their safety in terms of haemolysin, supporting their potential to be used as starter culture for controlled fermentation. Haemolysin plays an important role in virulence as it may increase chance of infection.

**Table 5:** Technological properties of the LAB isolates

Isolate code	Production of α-amylase (mm)	Phytate degradation (mm)	2% bile salt tolerance (Growth in log <sub>10</sub> cfu/mL)			Haemolytic activity
			24h	48h	72h	
WML001	-	16	0.00	5.58	8.28	γ
WML241	-	15	0.00	6.12	8.32	γ
WML242	-	-	5.35	6.10	7.94	α
WML482	5	23	4.48	5.11	8.21	γ
WML722	6	8	0.00	4.00	7.49	α
WML725	-	16	0.00	6.03	8.29	γ
YML721	4	-	0.00	5.81	8.21	γ
YML722	-	17	3.39	5.87	8.00	γ
YML723	-	-	4.00	5.11	7.32	α
RSL001	5	6	4.39	6.34	7.56	γ
RSL003	-	11	5.51	6.20	8.34	γ
RSL004	-	26	5.44	5.39	8.13	α
RSL242	-	22	3.70	6.37	7.30	γ
RSL483	-	18	5.30	6.29	8.41	γ
RSL485	-	8	3.70	6.23	7.53	α

**Table 6:** Technological properties of yeast isolates

Isolate code	Phytate degradation (mm)	2% bile salt tolerance			Haemolytic activity
		24h	48h	72h	
WMY001	-	5.49	6.35	8.32	α
WMY241	11	5.61	6.09	8.37	γ
WMY242	-	6.67	6.00	8.39	γ
WMY481	-	5.56	6.18	8.21	γ
WMY482	-	5.89	5.99	7.92	γ
WMY721	4	5.70	6.13	8.29	γ
YMY001	-	5.81	6.18	8.42	γ
YMY241	-	5.33	5.89	7.54	γ
YMY481	-	5.75	6.15	8.30	γ
YMY482	-	0.00	5.38	6.00	γ
YMY483	-	6.13	6.30	8.35	γ
RSY001	7	5.90	6.31	8.25	α
RSY002	-	0.00	0.00	7.48	γ
RSY241	-	5.69	6.28	8.18	γ
RSY242	-	4.54	6.16	8.40	γ

**Conclusion**

The predominant LAB and yeasts obtained in this study possess technological properties with potentials for the development of starter cultures with consistent quality and safety. Further investigations of these organisms as starter cultures based on their performance under controlled fermentation conditions, and their contributions to the development of flavours and organoleptic properties is recommended.

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