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Effects of lactic acid bacteria and *Saccharomyces cerevisae* co-cultures used as starters on the nutritional contents and shelf life of cassava-wheat bread

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ABSTRACT
Objective: To determine the effects of co-cultures of lactic acid bacteria and yeast as starters on the nutritional content, physical properties and shelf life of bread produced using mixture of wheat and cassava flour.
Methodology and results: Lactic acid bacteria (LAB) isolated from retted cassava were screened for the production of antimicrobial compounds against bread spoilage moulds. Starter cultures of LAB in combination with *Saccharomyces cerevisae* were selected for bread production. The quantity of lactic acid produced by LAB species ranged between 2.25 and 10.7 g/l. *L. fermentum* produced the highest (12.7 g/l) diacetyl while the highest amount of hydrogen peroxide (51 mg/l) was produced by *Leuconostoc mesenteroides*. Composite flour of 10% cassava and 90% wheat compared favourably with pure wheat bread. Combined cultures of *Saccharomyces cereviceae* and *Lactobacillus* species produced the best bread in terms of sensory quality, nutritional contents and shelf life.
Conclusion and applications of findings: Lactobacillus species in combination with *Saccharomyces cerevisae* can be used as starter culture to improve the nutritional contents, physical properties and extend the shelf life of cassava-wheat bread. The mixture of 90% wheat and 10% cassava flour blend will reduce the cost of production and make bread more affordable. The increased shelf life of bread will reduce wastage especially among rural dwellers who may not have storage facilities.

Key words: Cassava-wheat bread, lactic acid bacteria, *Saccharomyces cerevisae,* shelf life

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INTRODUCTION

The consumption of bread in Africa is a well-established habit not only in the city but also in the rural areas (Bokanga, 1995). Bread requires little packaging and can be kept without spoiling for up to 4 days at room temperature. It is one of the most ancient of human food and is produced with the assistance of microorganisms such as yeast and lactic acid bacteria (Rehm & Reed, 1983).

The major problem with bread production in Africa is that wheat needed to make bread is largely imported because the crop gives poor yield in most consuming areas (Bokanga, 1995). Nigeria imports about 15 million metric tons of wheat flour annually for the production of bread (Abba, 2008). Wheat importation represents an immense drain on the economy (Edema *et al.,* 2005). In order to reduce foreign exchange expenditure, the Federal Government of Nigeria is working with bakers on ways to add cassava flour to the wheat flour for bread production. Since nutritional content of bread is an issue of concern, research done at the International Institute of Tropical Agriculture (IITA) and University of Ibadan led to the production of nutritious bread and other baked products such as cakes and biscuits using cassava starch/ maize starch, with soybean flour, margarine and eggs (Bokanga, 1995; Edema *et al,* 2005).

Another problem facing the baking industry is bread spoilage due to fungi, which shortens the shelf life (Samson *et al*., 2000). In addition to substantial economic losses associated with fungal spoilage, there are concerns regarding potential hazards of mycotoxin contamination, which could affect human health (Legan, 1993). This study investigated the use of co-cultures of lactic acid bacteria and *Saccharomyces cervisae* as starter to improve the nutritional content and shelf life of bread.

MATERIALS AND METHODS

Sample collection: A total of 48 strains of Lactic acid bacteria were isolated from retted cassava procured from local farmers in Ibadan, Oyo State, Nigeria using MRS agar incubated anaerobically at 30°C for 48h. The LAB isolates were then subjected to various morphological, physiological and biochemical tests including colonial and cell morphology, Gram staining, catalase, spore staining, growth at 15 and 45°C, motility test, oxidase, hydrogen sulphite production, nitrate reduction test, production of ammonia gas from arginine and fermentation of sugars, and identified using bacteriology manuals (Rogosa & Sharpe, 1959; Sneath, 1986) while moulds were isolated from bread obtained from a retail shop at Agbowo in Ibadan, Nigeria. The bread was tied in a black polyethylene bag for five days at ambient temperature (28 ±2 °C) and 45±2% relative humidity for the fungi to proliferate

Antifungal activity of some chemical preservatives: Thirty milliliters of sterile PDA broth containing different chemical preservatives, either acetic acid, propionic acid or ethanol at concentrations of 0 – 5% (v/v) was inoculated with 10^4 spores/ml of fungi inoculum prepared according to Krauss *et al*. (1988) and incubated at 30°C on a rotary shaker at 200rpm for 5 days. The mycelia produced in each flask was filtered using pre-weighed filter paper, and dried in an oven at 85°C for 24h, cooled in a desiccator and weighed, then re-dried in the oven as above until constant weight was obtained. The percentage reduction of mycelial growth of each mould was calculated by comparing with the growth of each mould in medium without any chemical preservative (control).

Extraction of antifungal compound: LAB strains selected as test organisms were propagated in MRS broth for 72h at 30°C. A cell-free solution was obtained by centrifuging at 10000rpm for 20 min at 4°C .

Antifungal activity of LAB isolates: Well diffusion assay method was employed (Schillinger & Lacke 1989). Potato dextrose agar plates inoculated with about 10^4 fungal spores per milliliter of agar were prepared. Wells with a diameter of 5mm were cut in the agar using a sterile cork-borer. A drop of molten agar was added to each well to seal the base in order to avoid leakage. A 50µl volume of the supernatant from each LAB culture was added to the well and allowed to diffuse into the agar during a 5h pre-incubation period at ambient temperature (25±1°C). This was followed by aerobic incubation at 30°C for 48 - 72h. The plates were examined for clear zones of inhibition around the wells.

Quantitative determination of antimicrobial compounds produced by LAB: The test organisms were grown in MRS broth for 72h with samples being aseptically collected at 12h intervals. Each sample was centrifuged at 3,000-x g for 15 min (Ogunbanwo *et al*., 2004) and the supernatant of each LAB was used for quantitative determination of lactic acid, hydrogen peroxide and diacetyl content (A.O.A.C. 1980).

Production of cassava flour: High quality cassava flour was produced as shown in Figure 1

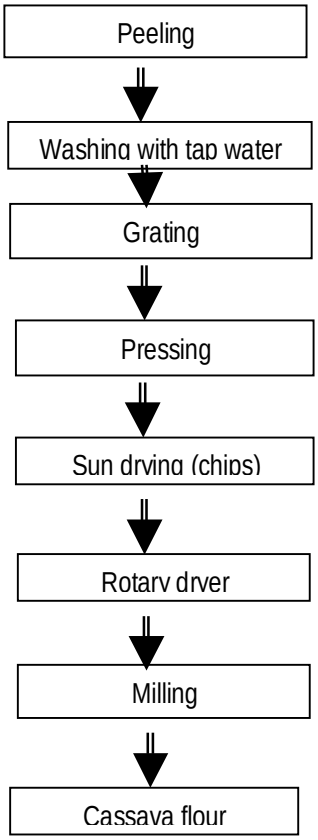

Figure 1: Flow diagram for the production of cassava flour.

Bread production: Bread dough was prepared using different blends of wheat/cassava flour, i.e. (a) 100g wheat flour alone, (b) 90g wheat/ 10g cassava flour, (c) 80g wheat/ 20g cassava flour, (d) 70g wheat/ 30g cassava flour. Other ingredients (per 100g) were 1g margarine, 10g sugar, 1.5g salt, 60ml tap water and Lactic acid bacteria /yeast (*S. cerevisiae*) starter culture. Two milliliters of inoculum containing approximately 10^9 cells per ml of Lactic acid bacteria / 1% of *S. cerevisiae* was used singly for the fermentation of dough while 0.5ml inoculum containing approximately 10^9 cells per ml of Lactic acid bacteria and 0.75% of *S. cerevisiae* was used in all cases of combined starters for dough fermentation using modified method of Halm *et al*. (1996). All ingredients were mixed to produce dough using a continuous high-speed mixer (60 xg) for 5min. The dough from each mixture was individually kneaded and placed in an aluminium pan (25cm x 10cm x 8cm) and incubated at 30°C for 150min to allow the dough to ferment before transferring to an oven. Baking was carried out in a batch oven at 220°C for 20min and the bread was cooled at room temperature for 90min before further analysis. Another batch of bread was prepared with propionic acid included in the dough at a concentration of 3000 parts per million to serve as control for shelf life determination.

Sensory analysis: Sensory evaluation of bread samples was carried out by a fifteen member trained panel of judges. The parameters used were colour, taste, texture, and overall acceptability. The rating was presented on a 9 point hedonic scale ranging from 9 = like extremely to 1 = dislike extremely.

Nutrient composition of bread: This was carried out on the loaves on dry matter basis according to conventional methods of A.O.A.C. (1980). Ash, crude protein, crude fiber, crude fat, as well as phosphorous, calcium, potassium and moisture content were determined.

Shelf life determination: The bread produced was packed in the conventional transparent polyethylene bags and stored at room temperature (27±2°C). The bread was observed daily through the transparent polythene bags to determine when spoilage would occur. The total mould load was determined at the beginning of spoilage on the 4^{th}, 8^{th}, and 12^{th} day by carefully and aseptically taking 10g of the moldy outer layer of the bread using sterile scalpel and homogenizing in 90ml of 0.1%(w/v) sterile peptone water for 3 min in a stomacher (A.J. Seward and Co. London). Ten fold serial dilutions were subsequently prepared by transferring 1ml of the homogenate into 0.1% (w/v) peptone water as diluents. Further serial dilutions were carried out and 1ml of appropriate dilution was aseptically plated using the pour plate technique on Potato dextrose agar supplemented with chloramphenicol. At the end of the incubation period the colonies were enumerated and expressed as colony forming units per gram (cfu/g) (Vanderzannt & Splittstoesser, 1992).

Data analysis: The data were subjected to ANOVA with $P<0.05$ considered significant.

RESULTS

Eight species of Lactic acid bacteria (LAB) were isolated from retted cassava. They were identified as *Lactobacillus brevis*, *L. plantarum*, *L. casei*, *L. fermentum*, *L. reuteri*, *L. delbrueckii*, *L. acidophilus* and *Leuconostoc mesenteroides* (table 1). *L. brevis* was the most frequent at 18.75 %, followed by *L. plantarum* and *L. casei* while *L. reuteri* was least frequently isolated at 6.25 %.

Table 1: Lactic acid bacteria isolated from fermented food cassava at Ibadan, Nigeria.

Isolate	Number	%
[1]*L. brevis*	9	18.75
L. plantarum	8	16.67
L. fermentum	6	12.5
L. delbrueckii	4	8.33
L. reuteri	3	6.25
L. acidophilus	6	12.5
L. casei	8	16.67
[2]*Lc. mesenteroides*	4	8.33
Total	48	100

[1]Lactobacillus; [2]Lc.= Leuconostoc mesenteroides

Based on the cultural and microscopic appearance, the moulds isolated from spoiled bread were identified as *Aspergillus niger*, *A. flavus*, *Rhizopus oryzae*, *Penicillium sp* and *Fusarium oxysporum* (Plate 1- 5). Propionic acid had the highest antifungal activity and reducing mycelial growth of moulds by approximately 90% at 0.3% concentration (table 2). Acetic acid reduced mycelial growth by about 70% at 3% concentration except *A. flavus*, which was reduced by 58%. Ethanol had the least activity and required a minimum concentration of 5% to reduce the mycelia growth of the mould by over 80% except in the case *Fusarium oxysporum* where reduction of mycelial growth was just 43%.

L. plantarum had the highest antagonistic activity against all the tested moulds with 30 mm zone of inhibition against *Rhizopus oryzae*, *Penicillium sp* and *Fusarium oxysporum* (table 3). *L. delbrueckii* had the least antifungal activity with 5 mm zone of inhibition against *A. flavus.* The antagonistic activity of LAB metabolites against *Rhizopus oryzae* is shown in plate 6.

The quantity of lactic acid produced by LAB species ranged between 2.25 g/l to 10.7 g/l. *L. casei* produced the highest quantity (10.7 g/l) after 48h of incubation, closely followed by *L. plantarum* (10.1 g/l) (Fig. 1). *L. fermentum* produced the highest (12.7 g/l) diacetyl at 48 h of incubation while *L. casei* produced 3.7 g/l diacetyl as its maximum concentration at 48 h of incubation (Fig. 2). The highest amount of hydrogen peroxide (51 mg/l) was produced by *Leuconostoc mesenteroides*, closely followed by *L. brevis* and *L. acidophilus* with 50mg/l at 36 h and 40 mg/l at 72 h, respectively (Fig. 3).

Based on the quantity of antimicrobial compounds produced and the antagonistic activity against the moulds, bread was produced with mixture of different proportions of cassava flour and wheat flour using different starter cultures of *L. plantarum*, *L. brevis*, *L. acidophilus* and *Saccharomyces cervisae,* singly and in combination.

Bread produced with wheat flour alone was rated the best in texture, aroma, taste, crump colour and firmness, crust colour and overall acceptability (table 4). Bread produced with combination of 10% cassava flour and 90% wheat flour had no significant difference in aroma, crust colour and texture when compared to the bread produced with 100% wheat flour, although it was slightly different in taste, crump firmness, crump colour and overall acceptability. Bread produced with mixture of 30% cassava flour and 70% wheat flour was the least acceptable. .

Bread produced with mixture of 10% cassava flour and 90% wheat flour using combined starter cultures of *Saccharomyces cerevisae* and *L. acidophilus* was rated the best (table 5) followed by that produced with mixture of *Saccharomyces cerevisae* and *L. brevis* as starter culture. Bread produced with 100 % wheat flour using *L. plantarum* as starter culture was rated least acceptable.

Table 6 shows the height, weight, volume and weight to volume ratio parameters of bread produced from different flour blends using different starter cultures. Bread produced using *Lactobacillus brevis* and *Saccharomyces cerevisae* recorded the highest value for height and weight while bread produced with 100% wheat flour using *Saccharomyces cerevisae* as starter culture had the highest volume. Weight to volume ratio showed significant differences among the starter cultures used to ferment various flour blends. The use of *Lactobacillus brevis* in combination with yeast had a positive impact on bread volume.

Table 2: Reduction (%) of mycelial growth of mould in culture medium by chemical preservatives of bread.

Concentration (%) of preservative		Moulds				
		Rhizopus oryzae	*Aspergillus niger*	*A. flavus*	*Penicillium* sp	*F. oxysporum*
Propionic acid	0	0_a	0_a	0_a	0_a	0_a
	0.003	3_b	22_{bc}	32_{bd}	62_{be}	71_{bf}
	0.03	50_c	22_{bc}	52_{cd}	75_{ce}	89_{cf}
	0.3	90_d	56_{dc}	89_d	93_{de}	93_{de}
	3	92_e	96_{ec}	92_{ed}	94_{de}	94_{de}
	5	95_f	97_{ec}	96_{ec}	98_{fe}	94_{de}
Acetic aicd	0	0_a	0_a	0_a	0_a	0_a
	0.003	2_b	3_b	22_{bd}	3_b	0_a
	0.03	16_c	48_{cd}	33_{ce}	31_{cf}	4_{cg}
	0.3	55_d	64_{dd}	54_{de}	58_{df}	60_{dg}
	3	73_e	90_{ed}	58_{ee}	79_{ef}	79_{ef}
	5	81_f	93_{fd}	85_{fe}	94_{ff}	91_{fg}
Ethanol	0	0_a	0_a	0_a	0_a	0_a
	0.003	0_a	0_a	0_a	0_a	0_a
	0.03	06_b	06_{bc}	04_{bd}	35_{be}	0_a
	0.3	13_c	31_{cc}	14_c	40_{ce}	15_{cf}
	3	51_d	90_{de}	64_{df}	47_{dg}	40_{dh}
	5	93_e	91_{de}	90_{de}	86_{ef}	43_{eg}

Values in the same column followed by the same letter are not significantly different according to Duncans Multiple Range Test (P<0.05).

Table 3: Antagonistic activity of Lactic acid bacteria (LAB) metabolites against bread spoilage moulds.

LAB isolate	Mould				
	Rhizopus oryzae	*A.. niger*	*A.. flavus*	*Penicillium sp*	*Fusarium oxysporum*
Leuconostoc mesenteroides	5*	7	20	30	5
Lactobacillus delbrueckii	10	10	5	10	4
Lactobacillus acidophilus	30	15	20	30	10
Lactobacillus reuteri	25	10	5	10	25
Lactobacillus brevis	25	5	5	5	30
Lactobacillus fermentum	30	7	20	10	15
Lactobacillus casei	30	15	20	30	25
Lactobacillus plantarum	30	15	20	30	30

*data are inhibition zones (mm).

Table 4: Effects of cassava flour content on the sensory qualities of bread produced using *S. cerevisae*.

% Flour Composition	Sensory properties						
	Crust colour	Aroma	Taste	Texture	Crump colour	Crump firmness	Overall acceptability
0 Ca + 100 W	$7.33\pm0.3_a$	$70.47\pm0._a$	$7.80\pm0.35_a$	$7.53\pm0.27a$	$8.00\pm0.29_a$	$7.80\pm0.30_a$	$8.00\pm0.29_a$
10 Ca + 90 W	$7.47\pm0.3_a$	$6.93\pm0.3_a$	$6.47\pm0.29_b$	$6.93\pm0.21_a$	$6.87\pm0.35_b$	$6.33\pm0.27_b$	$6.73\pm0.40_b$
20 Ca + 80 W	$6.60\pm0.2_{ab}$	$6.67\pm0.2_a$	$5.27\pm0.40_b$	$5.87\pm0.35_b$	$6.53\pm0.29_b$	$5.80\pm0.44_{bc}$	$6.27\pm0.38_c$
30 Ca + 70 W	$5.93\pm0.4_b$	$5.27\pm0.4_b$	$4.67\pm0.36_c$	$5.20\pm0.46_b$	$5.20\pm0.37_c$	$5.13\pm0.38_c$	$5.47\pm0.34_c$

Values in the same column followed by the same letter are not significantly different according to Duncans Multiple Range Test (P<0.05). Ca = Cassava flour; W = Wheat flour

Bread produced using combination of *L. brevis* and *Saccharomyces cerevisae* as starter culture had the highest protein content of 9.43% while bread produced using only *L. brevis* as starter culture had the least protein content of 7.64% (table 7). Bread produced using *Lactobacillus brevis* and *Saccharomyces cerevisae* as starter culture had the highest carbohydrate content of 56.465 %. However, bread produced using mixture of *L. plantarum* and *Saccharomyces cerevisae* as starter cultures had the highest mineral content compared with other bread samples.

The bread produced using only *Saccharomyces cerevisae* as starter culture without addition of preservatives had a shelf life of 4 days (table 8). Bread produced using combination of *L. plantarum* and *Saccharomyces cerevisae* as starter culture had shelf life of 12 days while bread produced with chemical preservative (propionic acid at concentration of 3000 ppm) had a shelf life of 8 days. On the other hand, bread had a total microbial load of 1.5 x 10^7cfu/g when spoilage was assessed on day 4, while bread with addition of preservative had none on the same day while the microbial load of bread produced using only *Saccharomyces cerevisae* as starter culture without addition of preservatives increased to 3.2 x 10^9 cfu/ml at day 12.

Table 5: Influence of mono and combined starter culture of *Lactobacillus* species and *Saccharomyces cerevisae* on the sensory analysis of the bread samples.

%Flour Culture	Sensory properties						
	Crust colour	**Aroma**	**Taste**	**Texture**	**Crump colour**	**Crump firmness**	**Overall acceptability**
100% W+ Y	7.33 ±0.31$_{abc}$	7.47 ±0.38$_{ab}$	7.80 ±0.35$_{a}$	7.53 ±0.27$_{ab}$	8.00 ±0.29$_{a}$	6.20 ±0.17$_{bc}$	8.00±0.29$_{ab}$
100% W+ C	6.53 ±0.25$_{bc}$	6.2 ±0.40$_{bcd}$	5.4 ±0.29$_{cd}$	3.53 ±0.49$_{d}$	5.4 ±0.29$_{cd}$	4.4 ±0.34$_{d}$	4.80±0.34$_{cd}$
100% W+ E	5.80 ±0.11$_{c}$	5.93 ±0.23$_{b}$	6.33 ±0.13$_{bc}$	6.27 ±0.12$_{bc}$	5.67 ±0.13$_{b}$	6.60 ±0.13$_{b}$	6.73±0.12$_{cd}$
100% W+ H	4.73 ±0.49$_{d}$	5.07 ±0.59$_{d}$	4.53 ±0.56$_{d}$	4.07 ±0.44$_{d}$	4.73 +0.56$_{d}$	4.73 ±0.62$_{d}$	4.73±0.56$_{d}$
100% W+ C + Y	8.00 ±0.22$_{a}$	6.80±0.39$_{abc}$	6.73±0.27$_{bc}$	6.87±0.31$_{ab}$	7.33±0.42$_{ab}$	6.80±0.40$_{bc}$	6.53±0.52$_{cd}$
100% W+ E + Y	6.53 ±0.57$_{c}$	6.53 ±0.40$_{bc}$	6.2 ±0.24$_{cd}$	6.73 ±0.52$_{b}$	6.13 ±0.48$_{cd}$	7.4 ±0.34$_{ab}$	6.47±0.58$_{cd}$
100% W+ H + Y	8.20 ±0.34$_{a}$	7.67 ±0.25$_{a}$	7.44 ±0.39$_{ab}$	7.80 ±0.26$_{a}$	7.60 ±0.35$_{ab}$	7.73 ±0.30$_{a}$	7.07±0.38$_{bc}$
10% Ca + 90% W + Y	7.47 ±0.36$_{abc}$	6.93 ±0.32$_{abc}$	6.47 ± 0.29$_{cd}$	6.93 +0.21$_{ab}$	6.87 ±0.35$_{bc}$	6.33 ±0.27$_{c}$	6.73±0.40$_{c}$
10% Ca +90% W + C	4.60 ±0.42$_{d}$	5.80 ±0.49$_{c}$	5.13 ±0.61$_{d}$	4.27 ±0.48$_{d}$	5.53 ±0.48$_{cd}$	5.4 ±0.49$_{cd}$	6.00±0.38$_{cd}$
10% Ca + 90% W + E	7.60 ±0.13$_{a}$	6.93 ±0.40$_{a}$	7.87 ±0.17$_{a}$	5.40 ±0.27$_{c}$	6.20 ±0.17$_{bc}$	6.07 ±0.15$_{bc}$	6.53±0.24$_{bc}$
10% Ca + 90% W + H	6.93 ±0.49$_{a}$	7.07 ±0.49$_{a}$	6.67 ±0.43$_{b}$	6.4 ±0.42$_{bc}$	6.27 ±0.33$_{bc}$	5.53 ±0.48$_{cd}$	6.80±0.47$_{bc}$
10% Ca + 90% W + C + Y	8.00±0.24$_{a}$	7.53±0.42$_{a}$	7.87±0.29$_{a}$	7.73±0.37$_{ab}$	7.53±0.39$_{ab}$	7.73±0.33$_{a}$	8.40±0.19$_{a}$
10% Ca + 90% W + E + Y	7.67±0.23$_{ab}$	7.20±0.26$_{abc}$	7.73±0.27$_{a}$	7.47±0.24$_{ab}$	7.40±0.27$_{ab}$	7.53±0.24$_{ab}$	8.00±0.20$_{ab}$
10% Ca + 90% W + H + Y	6.67±0.33$_{bc}$	6.07±0.27$_{c}$	5.80±0.26$_{d}$	5.07±0.27$_{c}$	5.40±0.35$_{d}$	4.67±0.33$_{d}$	5.60±0.36$_{d}$

Values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P<0.05). Ca = Cassava flour; W = Wheat flour; Y = *Saccharomyces cerevisae;* C = *Lactobacillus acidophilus;* E = *Lactobacillus brevis;* H = *Lactobacillus plantarum.*

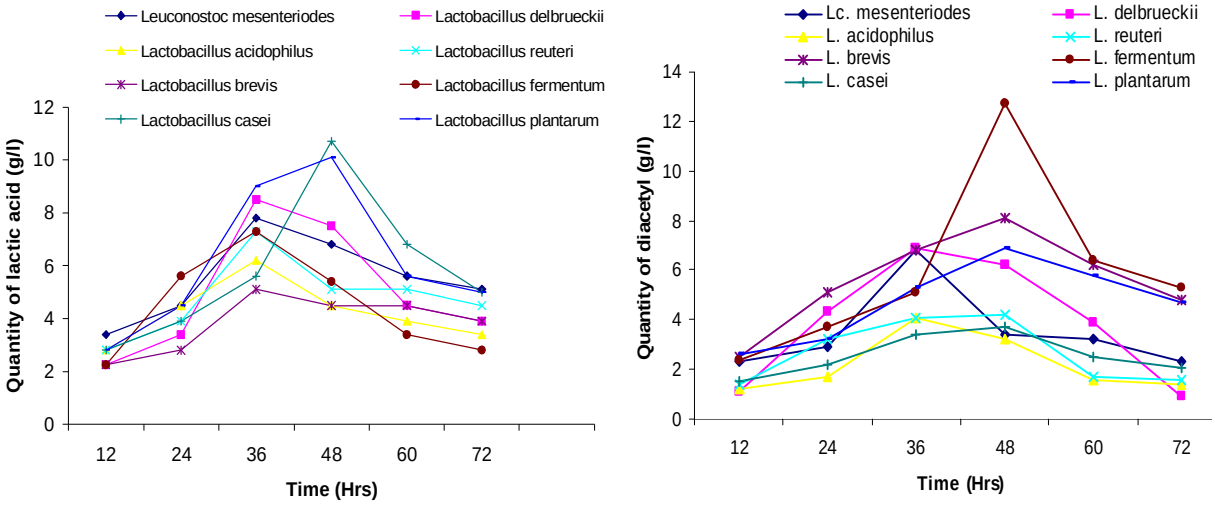

Figure 1 and 2: Quantity of lactic acid and diacetyl (g/L) produced by lactic acid bacterial isolates.

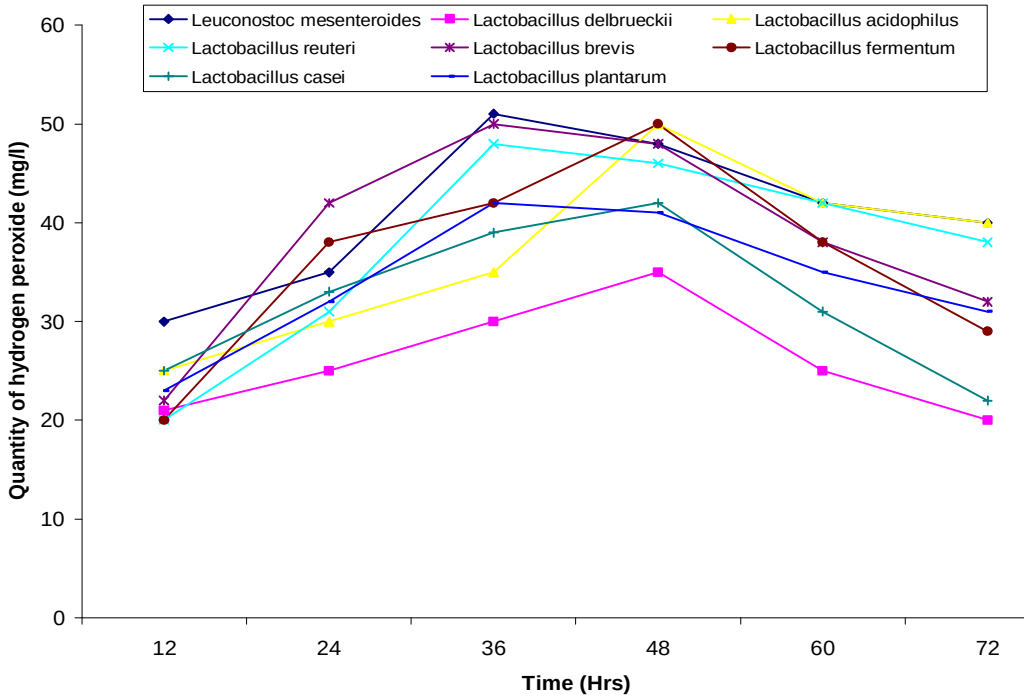

Figure 3: Quantity of hydrogen peroxide (mg/L) produced by lactic acid bacterial isolates.

Plate 1: *Aspergillus flavus* (2) *Aspergillus niger* (3) *Penicillium sp* (4) *Rhizopus oryzae*

Table 6: Properties of bread samples of 100% wheat flour and 90% wheat: 10% cassava flour blends.

Flour Composition and starter culture	Property			
	Height(cm)	Weight (g)	Volume (ml)	Weight: Volume ratio
100% W+ Y	6.60	155	129	1.20
100% W+ C	3.40	134	52	2.58
100% W+ E	4.20	136	59	2.31
100% W+ H	3.50	132	53	2.50
100% W+ C + Y	6.40	147	108	1.36
100% W+ E + Y	6.50	156	128	1.22
100% W+ H + Y	6.30	145	107	1.36
10% Ca + 90% W+ Y	6.30	149	126	1.18
10% Ca+90% W+ C	3.50	138	51	2.71
10% Ca+ 90% W+ E	4.10	140	57	2.46
10% Ca+ 90% W + H	3.60	137	50	2.74
10% Ca+ 90% W+ C + Y	6.60	157	106	1.48
10% Ca+ 90% W+ E + Y	6.90	157	126	1.25
10% Ca+ 90% W+ H + Y	6.40	148	105	1.41

Key: Ca = Cassava flour; W = Wheat flour; Y = *Saccharomyces cerevisae;* C = *Lactobacillus acidophilus;* E = *Lactobacillus brevis;* H = *Lactobacillus plantarum*

Table 7: Nutritional analysis of bread samples produced using a mixture of 90 % wheat flour and 10% cassava flour and different starter cultures.

Sample	Fat	Ash	Moisture	CF	protein	carbohydrate	*Ca	Mg	K	p	Zn	Fe
B	1.4	0.784	33.79	3.1	8.63	51.996	259	395	2804	2544	14	35
Bi	0.5	0.792	32.33	2.78	8.89	53.431	266	415	2905	2548	16	39
Bii	0.7	0.815	36.16	2.71	7.64	50.815	275	468	2990	2565	15	40
Biii	0.6	0.77	31.57	2.75	8.36	55.951	258	410	2850	2548	12	38
Biv	0.8	0.827	32. 06	2.98	9.24	55.632	279	430	2930	2550	17	41
Bv	1.1	0.825	31.03	2.94	9.43	56.465	279	464	3001	2560	18	45
Bvi	1.0	0.856	35.13	2.82	8.63	51.564	285	470	3015	2580	20	48

Key: b = bread produced using *Saccharomyces cerevisae;* bi= bread produced using *Lactobacillus acidophilus;* bii= bread produced using *Lactobacillus brevis;* biii = bread produced using *Lactobacillus plantarum;* biv= bead produced using *Lactobacillus acidophilus* and *Saccharomyces cerevisae;* bv = bread produced using *Lactobacillus brevis* and *Saccharomyces cerevisae*; bvi = bread produced using *Lactobacillus plantarum* and *Saccharomyces cerevisae;*CF = crude fiber; * *elements assayed in* parts per million.

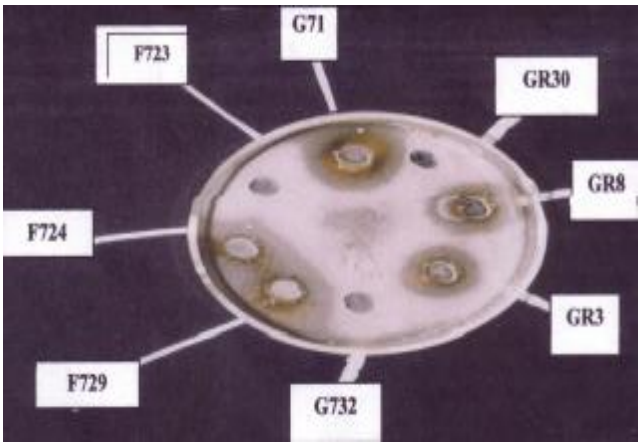

Plate 6: Antagonistic activity of LAB metabolites against *Rhizopus oryzae.* LAB code: G71 = *Lactobacillus plantarum;* GR30 = *Lactobacillus reuteri;* GR8 = *Lactobacillus brevis;* GR3 = *Lactobacillus acidophilus;* G732 = *Leuconostoc mesenteroides;* F729 = *Lactobacillus casei;* F724 = *Lactobacillus fermentum;* F723 = *Lactobacillus delbrueckii*

Table 8: Mould count of bread samples (cfu/g) at different storage intervals.

Bread code	Time (Days)		
	4	8	12
A	8.0×10^6	1.8×10^7	2.4×10^9
Aiv	ND	5.2×10^5	3.4×10^7
Av	ND	8.0×10^5	2.8×10^7
Avi	ND	ND	3.0×10^5
Avii	ND	5.0×10^6	3.1×10^8
B	1.5×10^7	2.8×10^8	3.2×10^9
Biv	ND	3.0×10^6	3.0×10^7
Bv	ND	5.2×10^6	3.0×10^7
Bvi	ND	ND	ND
Bvii	ND	5.6×10^6	1.1×10^8

Key: a- 100% Wheat flour + *Saccharomyces cerevisae* ; aiv- 100% Wheat flour + *Saccharomyces cerevisae* + *Lactobacillus acidophilus;* av- 100% Wheat flour + *Saccharomyces cerevisae* + *Lactobacillus brevis;* avi- 100% Wheat flour + *Saccharomyces cerevisae* + *Lactobacillus plantarum;* avii- 100% Wheat flour + *Saccharomyces cerevisae* + Propionic acid ; b- 10% Cassava+90% Wheat flour + *Saccharomyces cerevisae;* biv- 10% Cassava+90% Wheat flour + *Saccharomyces cerevisae* + *Lactobacillus acidophilus;* bv- 10% Cassava+90% Wheat flour + *Saccharomyces cerevisae* + *Lactobacillus brevis;* bvi- 10% Cassava+90% Wheat flour + *Saccharomyces cerevisae* + *Lactobacillus plantarum;* bvii- 10% Cassava+90% Wheat flour + *Saccharomyces cerevisae* + Propionic acid; ND- Not detected

DISCUSSION

Lactic acid bacteria (LAB) were isolated from fermented retted cassava. The predominant species isolated were *L. brevis* and *L. plantarum*, confirming the reports of Odunfa & Oyewole (1998) and Ogunbanwo *et al*., (2003). In these previous studies these organisms were reported to be dominant in fermented foods. The fungi isolated from mouldy bread included *Fusarium oxysporum, Aspergillus niger*, *A. flavus*, *Penicillium* species and *Rhizopus oryzae*. The cultural and microscopic appearance of these fungi were similar to those described by Moon (1983), Filtenborg *et al.,* (1996), Corsetti *et al*., (1998) and Pitt & Hocking (1999).

Although the chemical preservatives have antifungal activity, our findings suggest that the fungi are becoming resistant. For instance, about 3% acetic acid was required to inhibit fungal growth, which is significantly higher than the 0.15 – 0.8 % concentration that is usually used in food preservation (Davidson, 1994).

All the LAB isolates evaluated in this study produced metabolites that were antagonistic to bread spoilage mould. The metabolites produced by the LAB include lactic acid, hydrogen peroxide and diacetyl which have been documented previously (Earnshaw, 1992; Sanni *et al*., 1995; Ogunbanwo *et al.,* 2004). Magnusson & Schnurer (2001) reported *Lactobacillus coryniformis* sub sp *coryniformis* to have strong inhibitory activity in dual – culture agar plate assay against *Aspergillus fumigatus, A. nidulans*, *Penicillium roqueforti*, *Mucor heimalis*, *Talaromyces flavus*, *Fusarium poae, F. graminearum*, *F. culmorum* and *F. sporotricoides*.

In this study, bread was produced with a mixture of cassava and wheat flour. Increasing the ratio of cassava flour adversely affected bread quality, probably due to the absence of gluten protein in cassava flour which is necessary for the framework of bread and also the cell structure of the interior of the loaf, and normally abundant in wheat flour.

Yeast and lactic acid bacteria usually co-exist in the natural ecosystem of traditional fermenting food or beverages (Alexander, 1971; Bull & Slater, 1982; Kenns *et al*., 1991). Our results show that the use of *Saccharomyces cerevisae* and *Lactobacillus species* as mixed starter culture brings about improvement in the quality of the bread produced, especially when using 10% cassava flour. Salim-ur-Rehman *et al*., (2007) fermented bread dough using lactic acid bacteria (*L. bulgaricus*) alone and in combination with *Saccharomyces cerevisiae*. The reason for the wide spread use of these organisms in food fermentation have been documented by Clossy (1985). These include their ability to produce desired flavour,

discourage spoilage and contamination by other microorganisms and improve the nutritional content of food.

Bread produced using combination of *L. brevis* and *Saccharomyces cerevisae* as starter culture had increased protein content while bread produced using combination of *L. plantarum* and *S. cerevisae* had more mineral content, e.g. calcium, magnesium, potassium and iron, compared to bread fermented with either *Saccharomyces cerevisae* or *Lactobacillus species* alone. According to Oboh and Akindahunsi (2003), use of *Saccharomyces cerevisae* in the fermentation of cassava pulp resulted in significant increase in the protein content of the flour. In addition, lactic acid bacteria contain proteinases and peptidases, which can be found in variable concentrations in species of sour dough organisms. Only 30% of the protein is soluble in flour-water dough which has not been acidified. After 17h fermentation, 62.1% of the protein was soluble and with "strong" acidification the solubility increases to 82.7% (Lemmerzahl, 1937). The minerals and trace elements in bread are important for the health and well being of man (Rehm & Reed, 1983).

Bread produced using *Saccharomyces cerevisae* only as starter culture without addition of preservative had a shelf life of 4 days while bread produced with addition of chemical preservative using yeast as starter culture had shelf life of 8 days. However, the combination of *L. plantarum* and *Saccharomyces cerevisae* extended the shelf life of bread up to 12 days, which may be due to the production of lactic acid by these organisms from carbohydrate and the resultant decrease in pH (Schillinger & Lucke, 1989). Apart from lactic acid produced by these organisms, other metabolites such as diacety and, hydrogen peroxide were also produced as reported by Ogunbanwo *et al*., (2004), which could act in synergy to prolong the shelf life of the bread.

Based on our findings it can be concluded that *Lactobacillus species* in combination with *Saccharomyces cerevisae* can be used as starter culture to improve the nutritional contents, physical properties and extend the shelf life of cassava-wheat bread.

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