Original Research Article

Responses of *Lactiplantibacillusplantarum* and *Candida glabrata* isolated from retted cassava to acid stress and their influence on substrate fermentation for *Gari* production

ABSTRACT

Aim: Fermentation subject microbial cells to stress, such as, acid stress which can lead to inactivation and consequently death. To forestall this drawback, the physiological and proteomic response of *Lactiplantibacillusplantarum*LC03and *Candida glabrata*YC02 to acid and their influence on substrate fermentation for *Gari* production was studied.
Study Design:
Place and duration of study:
Methodology: Using the turbidimetry method and SDS-PAGE; LC-MS/MS, the physiological and proteomic responses of the LAB and yeastto acid stress were assessed. Analysis of the physiocochemical and organoleptic properties of the fermented cassava using the LAB and yeast alone and in combination was conducted by means of standard methods.
Results: *Lactiplantibacillusplantarum*LC03 and *Candida glabrata*YC02 had growth at pH 1, 2 and pH 2 respectively with an increased protein intensity of Type I glyceraldehyde-3-phosphate dehydrogenase and enolase 2 respectively. The lowest cyanide content (6.49^{d}), highest protein content (0.94^{c}) and organoleptic acceptability (7.92^{a}) was observed in *Gari* produced with thecombination ofLAB and yeast with significant differences in *Gari* produced with single starters and control.
Conclusion: Increased protein intensity during acid stress conditions enhanced the survival of *Lactiplantibacillusplantarum*LC03 and *Candida glabrata*YC02 (starters), thereby, improving the quality (improved sensory properties, nutritional and reduced anti-nutrient contents) of *Gari* produced.

Keywords: Lactiplantibacillusplantarum, Candida glabrata, Retted Cassava, Starters, Acid stress

1. INTRODUCTION

Cassava fermentation (retting) for the production of '*gari'* involves the spontaneous fermentation of grated or sliced pieces of cassava at room temperature for 4 to 6 days [1]. Lactic acid bacteria (species of *Lactobacillus, Lactococcus, Streptococcus, Corynebacterium*and*Leuconostoc*) [2, 3]and yeast (*Candida and Cryptococcus* species) are involved in fermentation of cassava for the production of *gari* resulting in the lowering of the pH of the fermenting medium [3]. *Lactiplantibacillusplantarum* have been reported as the most predominant lactic acid bacteria (LAB) in fermenting cassava [4]. Work done by [3] showed that *Candida* species were common occurrence in fermenting cassava for the production of *gari*. During fermentation, organic acids are produced by most microbes as either products or end-products [5]. Organic acids such as propanoic, lactic, butanoic and acetic acid amongst other acids are produced by fermenting LAB and yeasts in the course of fermentation, which are thought to bring about the reduction in pH and a distinctive aroma of fermented cassava foods [6].

The organic acid accumulated further acidifies the environment for microbial growth, thus having a negative impact on microbial productivity and volume of bioprocesses, with increasing concentration of the acids [7,5]. Increased intracellular acidity, as well as an accelerated metabolic disorders of the cells occurs as protonated acids enter the cell, dissociating into proton and corresponding ion [8, 9]. The reaction of microbes to vital changes in environmental conditions, natural stress and stress conditions experienced during food processing is known as microbial stress response [10].Microbial stress responses is described by the physiological changes and the transitory stimulation of general and specific proteins that are largely responsible for the organism's ability to survive the harsh conditions of the environment. Different metabolic pathways are commonly activated as an adaptation mechanism to the stress condition confronting the organism, which results in changes in the organism's protein profile [11].

The injury triggered by acidic environments is alleviated through cell membrane integrity and fluidity, pH homeostasis maintenance, macromolecule repair and regulation of metabolism. The methods of acid tolerance can be exploited in the protection of probiotics against acids in the course of food ingestion, as well as the development of biosynthesis of organic acids [12]. The method of acid stress response which relies on synthesis of protein has been reported in microbes [13]. Certain proteins are typically induced as a result of acid stress in order to protect or repair DNA and proteins. A number of chaperones are well-known and are essential for production, transfer, fold-up and breakdown of proteins [14]. Microbes are capable of changing their metabolic and energy fluxes, adjust growth rate and synthesis of enzymes and metabolites, so as to modify the carbon metabolism to the new environment [15].

Small membrane heat shock protein wascharacterized in *Oenococcusoeni* which improved acid tolerance by suppressing the clumping of protein [16]. Under acid stress conditions, *Lactobacillus casei* and *Streptococcus mutans* significantly decreased the production of phosphoenolpyruvatephosphotransferase system (PEP-PTS) for glucose [17, 18]. Similarly, at low pH, an increase in glucose phosphotransferase system was reported in *Streptococcoussobrinus* [19]and *Streptococcus macedonicus* [20]. An increased abundance of bifunctional acetaldehyde-CoA/alcohol dehydrogenase, 30S ribosomal protein S2 and 50S ribosomal protein L5 was observed in *Lactobacillus amylovorous* at low pH. Also, *Candida Kefyr* showed an increased abundance of 6-phosphogluconate dehydrogenase at low acid conditions [21]. High levels of these proteins may confer acid resistance.

In the course of fermentation of retted cassava to produce *Gari*, LAB and yeasts exposed to stress conditions which can bring about reduction in the viability and reproduction of the fermenting microbes, as well as the organoleptic and fermentative qualities. Responses of LAB and yeast to stress during the production of *Gari* is important in order to understand the physiological and proteomic response of the fermenting microbes to the stress conditions, as well as improve the utilization of these microbes for fermentation. This research studies the responses of *Lactiplantibacillusplantarum* and *Candida glabrata* and their influence on substrate fermentation (nutritional, anti-nutritional and organoleptic properties) for *Gari* production.

2. MATERIAL AND METHODS

2.1 Sample collection

Fresh cassava samples were collected from water area, University of Ibadan Road, Ibadan, Nigeria and were transferred to the laboratory for the laboratory preparation of '*Gari'* [22].

2.2 Isolation of lactic acid bacteria and yeast

In this study,*Lactiplantibacillusplantarum*and *Candida glabrata*which had formerly been isolated and characterized using morphological, physiological and biochemical tests, Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry, (MALDI TOF MS) by means of the formic acid extraction method were used [23].

2.3 Maintenance of lactic acid bacteria and yeast

The maintenance of *L. plantarum* and *C. glabrata* was done according to the method of [24]. Pure culture of *L. plantarum* was grown in new MRS (De Mann Rogosa Sharpe) agar slants micro-aerobically at 30^{o}C for 3 days. Also pure culture of *C. glabrata*wasgrown in new YPD (Yeast Extract Peptone Dextrose) agar slants at 25^{o}C for 2 days. After visible growth, the LAB and yeast were preserved at 4^{o}C for routine use, but sub-cultured every 4 weeks.

*2.4 Responses of L. plantarum*and *C. glabrata to acid stress at different pH concentrations*

2.4.1 Physiological response

Five (5) µL of fresh broth culture *L. plantarum*and *C. glabrata* were introduced into 250 µL of MRS and YPD broth respectively (Sigma Aldrich) adjusted to pH 1, 2, 3 and 4 in micro-plates. The micro-plates inoculated with *L. plantarum*were grown micro-aerobically at 30^{o}C for 24 h while the micro-plates inoculated with the *C. glabrata* were grown at 30^{o}C for 24 h. The experimental setup were repeated three times. With the use of microplate reader (Beck Coulter) at 620 nm, the growth of cultures were read and recorded at the end of 24 h [25].

2.4.2 Proteomic response

2.4.2.1 Protein extraction from L. plantarum and C. glabratasubjected to acid stress

*Lactiplantibacillusplantarum*was grown inMRS broth with acid stress conditions (MRS broth adjusted to pH 1, 2, 3, 4) and MRS broth without acid stress conditions (control) to examine the acid stress response and were then cultured at 30^{o}C for 24 h. Also, fresh culture of *C. glabrata* were grown in YPD broth subjected to acid stress at pH 1, 2, 3, 4 and YPD broth without acid stress conditions (control) and cultured at 25^{o}C for 24 h. At the end of incubation, proteins extraction from *L. plantarum*and *C. glabrata* was carried out. 100 ml of *L. plantarum*and *C. glabrata* cells at the different stress conditions and control were taken and centrifuged at 4200 rpm (Beckman Coulter, Allegra X-22 Centrifuge) for 20 mins, while the resulting liquid was thrown out. Collected pellets were washed thrice in phosphate buffer saline (PBS) solution (10 ml), centrifuged at 4200 rpm for 10 mins each. The weight of individual cell pellet in the eppendorf tubes was obtained and estimated to give the volume of thiourealysis buffer (4% Chaps, 2 M Thiourea, 1 % DTT, 7M Urea, 2% carrier ampholytes pH 3-10, 1 M Tris base, 10mg Protease inhibitor) to be added to each cell pellet. Lysing of cells were carried out using acid washed glass beads of about 212 to 300 µm diameter with thorough vortexing on table mixer (Maxi Mix II, Barnstead Thermolyne, USA) at a maximum speed approximately 2500 rpm with intermittent placement of the cell pellets on ice cubes for 1 min interval. The cells previously lysed were subsequently re-solubilized by ultra-sonication (Ultrasonic bath- Bio-equip 393 × 407) on ice for 6 rounds at 15 secs each. Samples were cooled on ice for a minute in between sonication to completely solubilize precipitated proteins. Removal of cell remains was done by centrifuging the cells at 4800 rpm for 10 mins at 4^{o}C. The resultant supernatantswhich contain the protein fraction was quantified according to the method of [26] and was then used for sodium dodecyl sulphate polyacrylamide gel electrophoresis(SDS PAGE).

2.4.2.2 SDS PAGE and protein detection

Protein samples extracted from *L. plantarum*and *C. glabrata* previously subjected to acid stress conditions was used to conduct the SDS PAGEusing 12% acrylamide resolving gel and stacking gels (Anderson and Anderson 1978). Staining and destaining of gels was carried out in order to detect the protein by usingCoomassie Brilliant Blue R-250 [27].

2.4.2.3 Peptide extraction, MS analysis and protein identification

Bands which showed higher or lower band intensity were excised from the gels and subjected to extraction using the method of [28], peptides were analyzed using the Liquid Chromatography

Mass Spectrometry (LC MS/MS) [29] and protein identification was done according to the method [30].

2.5 Preparation of laboratory produced Gari using *L. plantarum* and *C. glabrata* as starter cultures, fermentation and data analysis

Laboratory prepared '*Gari* using grated cassava was carried out and inoculated with pure cultures of *L. plantarum*, *C. glabrata* in singly and the combination of *L. plantarum* and *C. glabrata*following the method described by [31]. The grated cassava '*Gari'* inoculated with *L. plantarum*, *C. glabrata*and the combination of *L. plantarum* and *C. glabrata*were left to ferment at 28± 2^{o}C for a duration of 72 h. At the start and end of fermentation, the pH of inoculated grated *cassava* samples were recorded employing a Jenway pH meter; the proximate, mineral and anti-nutrient analysis of the samples were evaluated. According to the method reported by [32], the proximate composition (moisture content, neutral detergent fibre, acid detergent fibre, ash content, fat extract content, nitrogen free extract, crude fibre) and mineral content (potassium, manganese, iron, sodium, calcium, magnesium, zinc, phosphorous and copper) were evaluated. The tannin content, phytate and alkaloids content and the cyanide content of fermented samples were analyzed according the methods of [33, 34] and [35] respectively. Also, the organoleptic characteristics of the samples were examined to investigate the acceptability of the product. With the use of a panel group consisting of twenty members conversant with drinking *Ogi*, the organoleptic properties (including appearance, texture, flavour, and general acceptability) of fermented samples was accessed with the use of the 9- point hedonic scale method varying from 9 signifying "like exceptionally" to 1 indicating "dislike exceptionally. The laboratory produced *Ogi* was assessed by the panel members, thus indicating out the level of preference for each sample [36]. Results gotten from the pH determination, proximate, mineral, anti-nutrient content and organoleptic properties of laboratory produced *Ogi* using the starter cultures were analysed statistically using ANOVA and means were separated using Duncan multiple range test at $\alpha_{0.05.}$

3. RESULTS AND DISCUSSION

Figure 1 shows the physiological response of *L. plantarum* isolated from fermented cassava subjected to acid stress. An increase in growth of *L. plantarum* was observed as the pH of the growth medium increases from pH 1, 2, 3, to 4. The highest growth was recorded at pH 4 (0.659) while the lowest growth was observed at pH 1 (0.257).The physiological response of *L. plantarum* to acid stress showed that the growth of the LAB increased as the pH of the medium increased. The research on the response of *L. amylovorus* to different pH concentrations revealed an increase in the growth of *L. amylovorus* as the pH of the medium increased [21]. Research work on bile and acid tolerance of *L. plantarum* KCA-1 showed that *L. plantarum* KCA-1 was tolerant at pH 2.5 and a corresponding increase in the growth of *L. plantarum* KCA-1 was recordedat pH 4.5 [37].

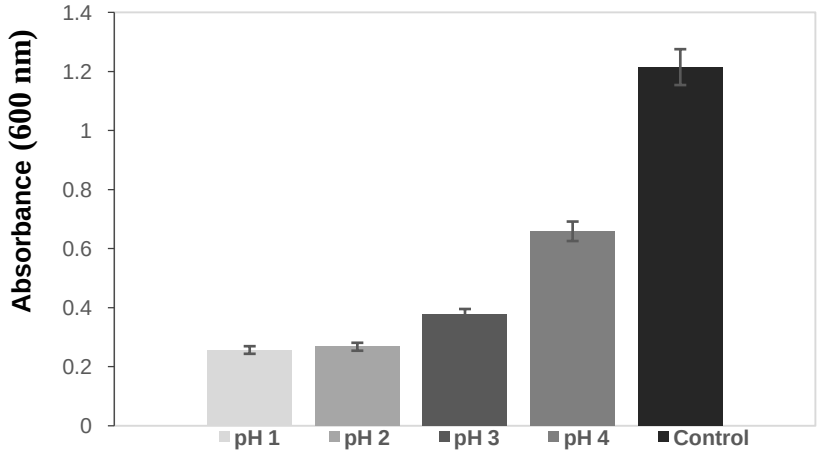

Fig. 1. Growth of *Lactiplantibacillusplantarum* subjected to acid stress at different pH concentrations.

X – axis label refers to the pH concentrations at pH 1, 2, 3, 4.

The absorbance at 600 nm (+ SD) of the growth of L. plantarumsubjected to acid stress at different pH concentration refers to Y – axis.

No pH adjustments were included in the control media.

The physiological response of C. glabrata isolated from fermented cassava subjected to acid stress at different acid concentrations is shown in Figure 2. *Candida glabrata* grew atpH 1, 2, 3, and 4 and recorded an increase in growth as the pH of the medium increased. At pH 4, *Candidaglabrata* recorded the highest growth (1.281) while at pH 1, the lowest growth (0.399) of the yeast was recorded. From this study, the physiological response of *Candida glabrata* to acid stress at pH 1, 2, 3 and 4 revealed an increase in the growth of the yeast as the pH increases, with an optimum growth at pH 4 and thereafter a decline in the growth concentration from pH 4 to 1. The effect of low pH on the growth of *Candida kefyr*isolated from *Ogi*, fermented sorghum gruel was studied and it was noted that the growth of *Candida kefyr*gradually decreased as the pH of the medium was lowered [21]. Similar observations reported the growth of *Candida glabrata* MTCC 3987 at low acidic pH [38].

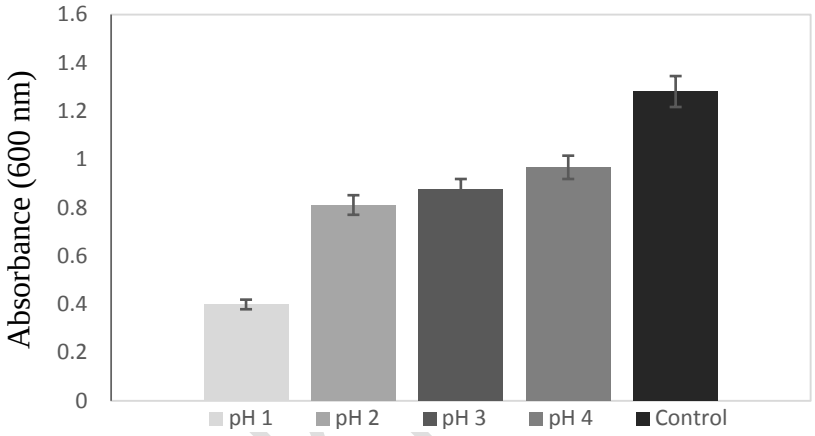

Fig. 2. Growth of *Candida glabrata* subjected to acid stress at different pH concentrations.

X – axis label refers to the pH concentrations at pH 1, 2, 3, 4.

The absorbance at 600 nm (+ SD) of the growth of C. glabrata subjected to acid stress at different pH concentration refers to Y – axis. No pH adjustments were included in the control media.

Plate 1 represents the SDS PAGE of *L. plantarum* isolated from fermented cassava subjected to acid stress at different pH concentrations. Three gel bands, F6, G7 and H8 were cut, analyzed and identified as Type I glyceraldehyde-3-phosphate dehydrogenase, chitin-binding protein and elongation factor Tu respectively. It was observed that protein gel band F6 which recorded an approximate molecular weight of 43.3 kDa, showed more band intensity at pH 1 and 2 compared to acid stress conditions at pH 3 and 4 and control (Lane C). Also, protein gel band G7 obtained from the SDS PAGE of *L. plantarum* which had an approximate molecular weight, 36.6 kDa showed more intensity at pH 2 and 3 than at pH 1, 4 and control. More protein gel band intensity was observed in H8 (approximate molecular weight, 22.3 kDa) at pH 2 compared to pH 1 and 3 while low protein gel band intensity was noted at pH 4 and Control. Responses to acid stress in *L. plantarum* isolated from fermented cassava revealed a notable expression and repression of Type I glyceraldehyde-3-phosphate dehydrogenase, chitin-binding protein and elongation factor Tu. Expression of these proteins indicates the response of *L. plantarum* to stress. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is important in glycolysis as it results in the oxidative

production of 1, 3-diphosphoglycerate from phosphorylation of glyceraldehye 3-phosphate. GAPDH also function in apoptosis, duplication and management of DNA, endocytosis and control of messengerRNA [39]. The relationship of GAPDH to the cell envelope of Lactobacilli has been studied [40]. Remarkably, the connection of the membrane of *L. crispatus* at low pH, while at neutral pH of 7, GAPDH is quickly discharged into the medium which work together with lipoteichoic acid has been noted [41].Inactivation or reduced activity of GAPDH reduces glycolysis and metabolism in tricarboxylic acid cycle. Hence, there is a redirection of glucose to the pentose phosphate pathway, thereby producing additional NADPH, which is required by antioxidant enzymes [42].

The other differentially expressed protein in *L. plantarum* is elongation factor thermo unstable (EF-Tu). The low intensity of EF-Tu during acid stress is noted in this study. Previously, notable decrease in the intensity of 30 S ribosomal protein S2 and 50 S ribosomal L5 during acid stress in *L. amylovorus* isolated from fermented sorghum gruel was reported [21]. In several LAB, EF-Tu was localized to the cell wall and known as a moonlighting protein which is regarded as a protein with several unconnected roles at diverse location of the cell [43]. The function of EF Tu in the synthesis of protein is involved in the transportation of aminoacyl-transportRNA complex and facilitatesits binding to the ribosome (A site) [44]. Secondly, it protects other proteins from aggregating by attaching to the water-repelling areas of the altered protein; this shows the chaperone activity of elongation factor Tu [45]. Thirdly, isomerase activity (protein disulfide) is demonstrated by elongation factor Tu. When compared to other protein disulfide oxidoreductases, EF-Tu activates the establishment of disulfide bond, protein reduction and isomerization [46]. The possibility of EF-Tu involvement in protein folding as well as/otherwise defense of *E. coli* from stress plus its vital function in translation and elongation of protein [47]. The excessive expression of EF-Tu in the course of adaptation to acid stress has been described in *Propionibacteriumfreudenreichii*and *Streptococcus mutans* [48], also in adaptation of *Listeria monocytogenes* to osmotic stress [49]. Therefore, increased intensity of EF-Tuwith defensive ability on freshly formed protein noted in this work may be significant in folding and renaturation of protein which may confer tolerance to stress in *Lactiplantibacillusplantarum* subjected to acid and oxidative stress.

Furthermore, from this study, an increased intensity of chitin-binding protein (CBP) has been noted during acid stress at pH 1, 2 and 3 in *Lactiplantibacillusplantarum*. Previously, three *L. plantarum* strains produced some proteins extracellularly within the medium with unascertained roles, of which a few of the proteins were correspondingly found in the guts [50]. Additionally, more investigators have in recent times spent colossal attempts to discover surface-related proteins with a probable function as regards coherence of *Lactiplantibacillusplantarum* and its environs [30]. Chitinization process in *L. lactis* has been reported to be made up of chitinases and chitin binding proteins showing similarity to those from *Lactiplantibacillusplantarum* [51]. Chitin binding proteins are termed as complement proteins that do not produce hydrolysis and are important for the breakdown of chitin which attach to N-acetylglucosamine existing in varied polymers, in addition to chitin, also mucins [52, 53]. Hence, the increase in chitin binding protein in *L. plantarum* as seen in this work during acid stress may possibly participate in adapting and tolerating acid stress.

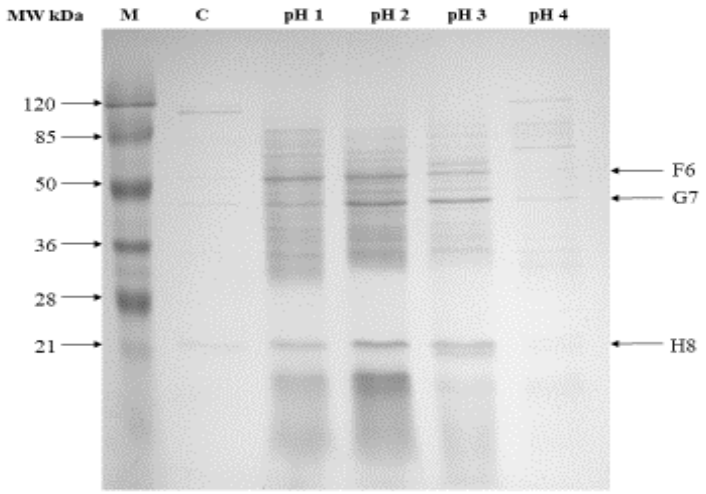

Plate 1. SDS PAGE of *Lactiplantibacillusplantarum* isolated from fermented cassava subjected to acid stress at different pH concentrations.

Key

**MW: Molecular weight *kDa: kilo Daltons *M: Molecular marker*

**C: Control *F6, G7, H8: Excised and identified bands.*

X – axis label refers to pH concentrations at pH 1, 2, 3, 4 at which proteins were extracted from Lactiplantibacillusplantarum after 24 h in growth medium. Y- axis label refers to molecular weight (MW) (kDa) of the molecular marker; which acts as the marker for the approximate molecular weight of protein gels. No pH adjustments were included in the control medium. With the use of LC ESI MS/MS analysis, protein gels F6, G7 and H8 were excised and identified.

The SDS PAGE of *C. glabrata* isolated from fermented cassava subjected to acid stress at different pH concentrations is represented in Plate 2. Protein gel bands I9 and J10 were cut, analyzed and identified. It was noted that the protein gel band I9 which recorded an approximate molecular weight of 46.3 kDa showed increased protein band intensity at pH 2, 3 and 4 compared to the control (no acid stress conditions included). Also, protein gel band J10 with an approximate molecular weight of 27.5 kDa showed increase in the protein band intensity at pH 2 compared to acid stress conditions at pH 3 and 4. However, no increase or decrease in protein band intensity was noted in protein gel band I9 and J10 at pH 1.The results obtained from the protein analysis of *Candida glabrata*isolated from fermented cassava in this study showed the identification of two proteins; enolase 2 and an uncharacterized protein. From this study, an increase in the abundance in enolase 2 was observed during acid stress. This possibly will bring about a more rapid level in glycolysis and tricarboxylic acid cycle as a glycolytic enzyme during encounter with acid stress conditions. Likewise, uncharacterized protein showed a lower level of abundance during acid stress compared to the glycolytic enzyme; enolase 2. Previous work reported an increase in the abundance of 6-phosphogluconate dehydrogenase and enolase in *Candida kefyr* isolated from fermented sorghum gruel during acid stress [21].

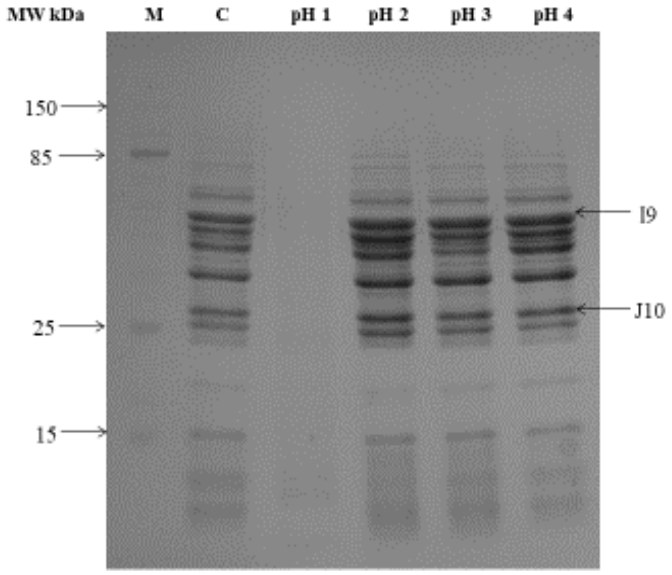

Plate. 2. SDS PAGE of *Candidaglabrata* isolated from fermented cassava subjected to acid stress at different pH concentrations

Key

**MW: Molecular weight *kDa: kilo Daltons *M: Molecular marker *C: Control *I9,J10: Excised and identified bands*

X – axis label refers to pH concentrations at pH 1, 2, 3, 4 at which proteins were extracted from Candida glabrata after 24 h in growth medium. Y- axis label refers to molecular weight (MW) (kDa) of the molecular marker; which acts as the marker for the approximate molecular weight of protein gels. No pH adjustments were included in the control medium. With the use of LC ESI MS/MS analysis, protein gels I9 and J10 were excised and identified.

Table 1 shows the identification of protein using the LC ESI MS/ MS analysis from protein gel bands of *Lactiplantibacillusplantarum* and *Candida glabrata* isolated from fermented cassava. Proteins from gel bands F6, G7 and H8 obtained from *L. plantarum* recorded the number of peptide matches as 652, 542 and 99 respectively and a corresponding emPAI of 77.07, 23.48 and 33.27 respectively with a score range of 4992 and 41098. The proteins obtained from gel bands D4, E5 and F6 were identified as type 1 glyceraldehyde 3 phosphate dehydrogenase, elongation factor Tu and chitin-binding protein respectively with a reference organism from *L. plantarum*. Meanwhile, the proteins from gel band I9 and J10 obtained from *C. glabrata* were identified as enolase 2, uncharacterized protein, with reference organism from *C. glabrata*. The number of peptide matches obtained from I9 and J10 using the LC-ESI-MS/ MS analysis were 834 and 393 and an emPAI of 76.77 and 81.10 respectively.

Table 1. Identification of protein characterized by LC ESI MS/MS analysis from *Lactiplantibacillusplantarum* and *Candida glabrata* isolated from fermented cassava to acid stress.

Band/ spot code	Organism origin	Data base	Accession number	Mass (kDa)	pI	No of peptide matches	No of sequences	emPAI	Score	Protein identity	Organism/Reference
Band-F6	*Lactiplantibacillusplantarum*	NCBIprot	WP_024521321.1	36.641	-	652	36	77.07	410918	Type I glyceraldehyde-3-phosphate dehydrogenase	*Lactiplanti-bacillusplantarum*
Band-G7	*Lactiplantibacillusplantarum*	NCBIprot	WP_044431076.1	43.364	-	542	25	23.48	29526	Elongation factor Tu	*Lactiplanti-bacillusplantarum*
Band-H8	*Lactiplantibacillusplantarum*	NCBIprot	WP_076633771.1	22.368	-	99	15	33.27	4992	Chitin-binding protein	*Lactiplanti-bacillusplantarum*
Band-I9	*Candida glabrata*	NCBIprot	SCV15549.1	46.362	-	834	42	76.77	27747	Enolase 2	*Candida glabrata*
Band-J10	*Candida glabrata*	NCBIprot	XP_445966.1	27.574	-	393	28	81.10	226119	Uncharacterized protein	*Candida glabrata*

Key:
NCBI- National center for biotechnology information
NCBIprot- database for protein, a pool of sequences from some resources comprising translation from noted coding segment in GenBank and TPA, along with SwissProt, PIR, PRF and PDB
Accession number: chains of figures that are allocated successively to every single sequence data sorted by NCBI.
Mass (kDa): theoretic mass expected from the amino acid series of the known protein.
pI: theoretic isoelectric point expected from the amino acid series of the known protein
Number of sequence: analysis of the amino acid series of the known protein
Number of peptide matches: total of corresponding peptides centered on MS/MS data searching, without the replica corresponds.
emPAI is the Exponentially Modified Protein Abundance Index
Organism reference: organism discovered after alignment of the known protein series.

Table 2 represents the pH changes in laboratory prepared Gari using *Lactiplantibacillusplantarum* and *Candida glabrata* and the combination of *Lactiplantibacillusplantarum*and *Candida glabrata* as starter cultures in the course of fermenting cassava. Decreased pH were recorded in fermented cassava prepared using the starter cultures and the control (spontaneous fermentation). The changes observed in the pH of fermented cassava made using the single starter culture *L. plantarum* recorded the lowest pH (3.81^{d}) compared to fermented cassava produced using the single starter culture *C. glabrata* (4.01^{b}) and the combination of *L. plantarum*and *C. glabrata* (3.96^{c}) and the control (spontaneous fermentation) (4.46^{a}) (Table 2). Previous works have reported the use of lactic acid bacteria and yeasts as starter cultures in the production of fermented foods [54, 55, 56]. Fermentation of grated cassava using *L. plantarum* and *C. glabrata* as starter cultures was characterized by a fall in pH which was noticed during the course of fermentation. The lowering of pH through application of starters has been described in some fermented cereal beverages [57, 58, 55]. Reduction in pH arose from rising hydrogen ion content, perhaps owing to the activities of microorganisms in breaking down sugar and more existing food items to yield organic acids [59].

Table 2. Changes in pH of laboratory prepared *Gari*using starter cultures

pH	Ctrl	Ogi produced with Starter cultures			P VALVE	SEM
		C. gla	*L. pla*	*C. gla*+ *L. pla*		
0 h	6.74^{a}	6.67^{b}	6.41^{d}	6.59^{c}	0.001	0.03
72 h	4.46^{a}	4.01^{b}	3.81^{d}	3.96^{c}	0.001	0.09

Values are the means of triplicate determinations. Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05}$.

***Key**:*

**Ctrl-Control (spontaneous fermentation) *C. gla - Candida glabrata *L. pla - Lactiplantibacillusplantarum *C. gla + L. pla - Candida glabrata + Lactiplantibacillusplantarum *P value- Probability value *SEM-Standard Error Mean*

The proximate composition of laboratory prepared *Gari* using starter cultures is represented in Table 3. The results obtained from the proximate composition of laboratory prepared *Gari* showed substantial variations as regards the protein content, ash content ADF and NDF *Gari* produced using the combined starters of *Lactiplantibacillusplantarum*and *Candida glabrata*compared to *Gari* produced with single starter cultures of*Lactiplantibacillusplantarum*and *Candida glabrata.* The highest value of protein content (0.94^{c} %) and ash content (2.80^{a} %) was observed in fermented cassava made with combined starters of *Lactiplantibacillusplantarum*and *Candida glabrata* compared to the single starters of *Candida glabrata*(0.85^{b} % and 2.56^{c} % respectively) and *Lactiplantibacillusplantarum* (0.90^{a} % and 2.64^{b} %). *Gari* produced with single starter *Candida glabrata*recorded the highest moisture content (8.55^{a} %), while *Gari* produced with combined use of the starter cultures *Lactiplantibacillusplantarum*and *Candida glabrata*recorded the lowest moisture content (6.08^{b} %).It was observed that no significant variation was noted in the fat content and NFE of *Gari* produced with single starter *Lactiplantibacillusplantarum*and the combined use of *Lactiplantibacillusplantarum*and *Candida glabrata*(Table 3). From this work, notable increase in protein content of *Gari* produced using combined starters of *Lactiplantibacillusplantarum and Candida glabrata*during fermentation of grated cassava compared to using single starters and the control (spontaneous fermentation) is in line with the report of [21] that recorded an improvement in the protein content of *Ogi* (a fermented sorghum gruel) produced using combined starters of *Lactobacillus amylovorus and Candida kefyr.* Also, a three-fold increase in the crude protein content was recorded in fermented cassava after a fermentation period of 48 h [60]. Similar work reported an improvement in the crude protein content (from 2% to 34.5 %) of cassava chip fermented with *Saccharomyces cerevisiae*as single starter culture [61]. The secretion of extracellular enzymes and the action of microbial growth may account for the increase in protein content during fermentation using starter cultures [62]. In the same vein, increased ash content observed in *Gari* produced using combined starters of *Lactiplantibacillusplantarum and Candida glabrata*during fermentation of grated cassava compared to using single starters and the control (spontaneous fermentation) may perhaps be as a result of the activities of enzymes and microbes during fermentation resulting in the dissipation of dry matter [63].Similarly, increased fat content was observed in *Ogi* produced with the combined starters of *Lactobacillus amylovorus and Candida kefyr* during fermentation of sorghum gruel compared to the *Ogi* produced using single starters and control [21].

Also, reduced fat content noted in *Gari* produced using the combined starter cultures of *Lactiplantibacillusplantarum and Candida glabrata*compared to the use of single starters and the control (spontaneous fermentation) is in line with work done by [64, 65, 21]. The action of enzymes (lipolytic) during the process of fermentation could be accredited to the notable decrease in the fat content.

Furthermore, improved crude fibre content (neutral detergent fibre and acid detergent fibre) of *Gari* produced using the single starter cultures and the combined starter cultures with

significant differences was noted compared to the control. Higher crude content was reported in *Ogi* produced using combined starter cultures of *Lactobacillus amylovorus and Candida kefyr* [21]. Precious report have stated the important benefits of consuming dietary fibre in diet [66, 67, 68, 69].

Table 3. Proximate composition of laboratory prepared *Gari* using starter cultures

		Starter cultures				
Proximate composition (%)	**Ctrl**	***C. gla***	***L. pla***	***C. gla + L. pla***	**PVALUE**	**SEM**
Moisture	6.32^{d}	8.55^{a}	6.45^{c}	6.08^{b}	0.0001	0.362
Protein	0.91^{a}	0.85^{b}	0.90^{a}	0.94^{c}	0.0001	0.019
Ash	2.31^{d}	2.56^{c}	2.64^{b}	2.80^{a}	0.0001	0.267
Fat	0.39^{c}	0.41^{c}	0.66^{a}	0.54^{b}	0.0001	0.360
NFE	0.15^{a}	0.14^{a}	0.63^{a}	0.13^{a}	0.055	0.122
ADF	5.87^{d}	6.77^{b}	7.47^{a}	7.66^{c}	0.001	0.218
NDF	7.64^{d}	11.15^{b}	12.90^{a}	13.84^{c}	0.001	0.840

Values are the means of triplicate determinations. Means along the rows with distinct superscript are substantially distinct from each other at α 0.05.
Key:
**Ctrl-Control (spontaneous fermentation) *C. gla- Candida glabrata *L. pla - Lactiplantibacillusplantarum *C. gla + L. pla - Candida glabrata + Lactiplantibacillusplantarum *P value- Probability value *SEM-Standard Error Mean *NFE - Nitrogen Free Extract *ADF – Acid Detergent Fibre *NDF - Neutral Detergent Fibre*

Table 4 shows the mineral composition of laboratory prepared *Gari* using starter cultures. *Gari* produced using joined starters of *Lactiplantibacillusplantarum* as well as *Candidaglabrata* recorded the utmost mineral content in calcium (2.06^{a} %) and zinc (0.00075^{a} %) which showed significant differences compared to the *Gari* produced using single starter cultures *Lactiplantibacillusplantarum*; calcium (0.08^{b} %) and zinc (0.0004^{b} %) and *Candidaglabrata*; calcium (0.09^{b} %) and zinc (0.00035^{b} %) following the results of the analysis of variance. The highest potassium content was observed in *Gari* produced using single starter culture of *Lactiplantibacillusplantarum* (1.05^{a} %) which showed no significant difference compared to *Gari* made with single starter of *Candida glabrata* (0.97^{b} %) and joined starters of *Lactiplantibacillusplantarum* as well as *Candida glabrata* (0.98^{b} %) and control (0.95^{b} %) (Table 4).Increased mineral composition of *Gari* produced with the starter cultures were noted in the study. Results obtained from this research disclosed that the produced *Gari* with the use of *Lactiplantibacillusplantarum* and *Candida glabrata* recorded better and more improved mineral composition compared with *Gari* made with single starter cultures. Precious research reported improved mineral content with the use of starter cultures in the production of Burukutu[55]and Ogi[21]. Improved mineral composition in *Gari* produced with starter cultures significantly implies an improvement in the nutritional composition of the product.

Table 4. Mineral composition of laboratory prepared *Gari*using starter cultures

Mineral composition (%)	Ctrl	Starter cultures			PVALUE	SEM
		C. gla	*L. pla*	*C. gla* + *L. pla*		
Calcium (Ca)	0.06^{b}	0.09^{b}	0.08^{b}	2.06^{a}	0.0001	0.170
Magnesium (Mg)	0.06^{a}	0.07^{a}	0.07^{a}	0.07^{a}	0.242	0.043
Potassium (K)	0.95^{b}	0.97^{b}	1.05^{a}	0.98^{b}	0.0001	0.110
Sodium (Na)	0.11^{a}	0.11^{a}	0.09^{a}	0.05^{b}	0.0001	0.010
Phosphorous (P)	0.05^{d}	0.05^{d}	0.05^{d}	0.06^{d}	0.381	0.001
Zinc (Zn)	0.0000^{c}	0.00035^{b}	0.0004^{b}	0.00075^{a}	0.0001	1.013
Copper (Cu)	0.0002^{a}	0.0003^{a}	0.00025^{a}	0.00025^{a}	0.381	0.189
Manganese (Mn)	0.0011^{b}	0.0017^{a}	0.00125^{b}	0.00165^{a}	0.0001	0.944
Iron (Fe)	0.0017^{d}	0.00155^{d}	0.00145^{d}	0.00165^{d}	0.173	0.441

Values are the means of triplicate determinations. Means along the rows with distinct superscript are substantially distinct from each other at α 0.05.

***Key**:*

**Ctrl-Control (spontaneous fermentation) *C. gla- Candida glabrata *L. pla-Lactiplantibacillusplantarum *C. gla + L. pla- Candida glabrata + Lactiplantibacillusplantarum *P value- Probability value *SEM-Standard Error Mean*

The anti-nutrient composition of laboratory prepared *Gari* using starters is represented in Table 5. In correspondence to the outcome of the analysis of variance, the anti-nutrient composition of *Gari* produced without starter culture (control) recorded the highest anti-nutrient components which showed significant differences to anti-nutrients composition of *Gari* produced with starter cultures. The lowest tannin (0.0007^{d} %), phytate (0.0078^{c} %), alkaloids (0.14^{c} %) and cyanide (6.49^{d} mg/kg) was observed in *Gari* made using joined starters of *Lactiplantibacillusplantarum* and *Candidaglabrata* compared to *Gari* made using single starters of *Lactiplantibacillusplantarum* or *Candidaglabrata* (Table 5).A notable decrease in the anti-nutrient components including phytate, tannin, total alkaloids and cyanide was observed in *Gari* produced using both the single starter cultures and the combined starter cultures. Anti-nutrients are capable of interfering with the assimilation of important food nutrients and minerals during digestion [70], although, some studies have reported the positive attributes of some anti-nutrients [71, 72, 73]. Reduced anti-nutrient composition of tannins, polyphenols and phytate was reported in burkutu produced using combined starter cultures of *L.fermentum* I and *S. cerevisiae* [55]. Other research findings have reported that *Ogi* produced using single and combined starter cultures of *Lactobacillusamylovorus* and *Candidakefyr*recorded reduced anti-nutrient content compared with *Ogi* produced without the use of starter cultures [21]. Reductionsin phytate and tannin content during and at the end of fermentation of cassava for the production of Ikivunde and Inyanga have also been investigated [74].

Also, reports have shown that fermentation of cassava may result in the reduction of cyanide [60, 75]. The reduction of cyanide observed in *Gari*produced using both the single starters and the combined starter cultures in this work is in line with the report of [76, 60, 75]. The notable reduced cyanide content may be as a result of the ability of the starter cultures to secrete the enzyme linamarase which subsequently hydrolyze linamarin, resulting in the degradation of cyanogenic glycosides to HCN which is further broken down to formamide and is utilized as source of carbon and nitrogen [76, 75].

Table 5. Anti-nutrient composition of laboratory prepared *Gari* using starter cultures

	Starter cultures					
Anti-nutrient composition (%)	**Ctrl**	***C. gla***	***L. pla***	***C. gla + L. pla***	**PVALUE**	**SEM**
Tannin	0.0018[a]	0.0014[b]	0.0011[c]	0.0007[d]	0.005	0.00015
Phytate	0.0093[a]	0.0089[b]	0.0086[b]	0.0078[c]	0.002	0.00019
Alkaloids	0.17[a]	0.15[b]	0.14[c]	0.14[c]	0.0001	0.00235
Cyanide (mg/kg)	7.11[a]	6.59[b]	6.53[c]	6.49[d]	0.0001	0.44015

Values are the means of triplicate determinations. Means along the rows with distinct superscript at each starter cultures are substantially distinct from one other at P< 0.05.
Key:
**Ctrl-Control (spontaneous fermentation) *C. gla- Candida glabrata *L. pla- Lactiplantibacillusplantarum *C. gla + L. pla- Candida glabrata + Lactiplantibacillusplantarum *P value- Probability value *SEM-Standard Error Mean*

Table 6 shows the organoleptic properties of laboratory prepared *Gari* produced using starters. The outcome of the variance analysis disclosed remarkable changes of mean scores of preference (flavour, texture, appearance and general overall acceptability) in *Gari* made using joined starters of *Lactiplantibacillusplantarum* and *Candida glabrata*. The least mean score of preference (flavour 6.94[c], texture 7.63[c], appearance 6.29[d], general overall acceptability 7.13[d]) was observed in *Gari* produced with no starter culture (control) compared to *Gari* produced with combined and single starter cultures.In this study, the organoleptic attributes of *Gari* produced using the combined starter cultures of *Lactiplantibacillusplantarum* and *Candida glabrata* showed an improvement in the texture, flavor, appearance and general acceptability of the product. This agrees with the findings of [77] and [78] who proposed that the use of starters for fermentation enhances the flavour of the products. Also, improvement in the flavor, appearance texture and general acceptability was recorded in fermented foods produced using starter cultures [55, 21].

Table 6. Organoleptic properties of laboratory produced *Gari* using starter cultures

Organoleptic properties	Ctrl	*Starter cultures*			P VALUE	SEM
		C. gla	*L. pla*	*C. gla+ L. pla*		
Flavour	6.94^{c}	8.18^{b}	7.87^{b}	8.82^{a}	0.001	0.258
Texture	7.63^{c}	7.72^{ab}	7.69^{bc}	7.84^{a}	0.013	0.022
Appearance	6.29^{d}	7.85^{b}	6.90^{c}	7.93^{a}	0.001	0.258
General overall acceptability	7.13^{d}	7.73^{b}	7.63^{c}	7.92^{a}	0.001	0.117

Values are the means of triplicate determinations. Means along the rows with distinct superscript are substantially distinct from each other at $\alpha_{0.05}$.

***Key**:*

**Ctrl-Control (spontaneous fermentation) *C. gla- Candida glabrata *L. pla-Lactiplantibacillusplantarum *C. gla + L. pla- Candida glabrata + Lactiplantibacillusplantarum *P value- Probability value *SEM-Standard Error Mean*

4. CONCLUSIONS

The report on the physiological and proteomic response of *Lactiplantibacillus plantarum*LC03 and *Candida glabrata*YC02 isolated from fermented retted cassava to acid stress conditions at different pH concentration is presented. *Lactiplantibacillus plantarum*LC03 and *Candida glabrata*YC02 were able to survive the different acid stress conditions as wellas show the expression of induced and repressed proteins. The identified proteins could be involved in the survival of the LAB and yeast to acid stress conditions, thereby enhancing the improvement of mineral content and organoleptic properties of *Gari*, as well as a reduction of anti-nutrient contents of the product produced using *Lactiplantibacillus plantarum*LC03 and *Candida glabrata*YC02 as single and combined starters. Increased abundance of protein revealed the proteomic response of the LAB and yeast to acid stress conditions. Therefore, cassava fermentation can be improved by using stress-adapted organisms as starters.

REFERENCES

1. Haakuria VM. Towards the development of a starter culture for gari production. A Research Report Submitted to the Faculty of Science. University of the Witwatersrand, in partial fulfillment of the requirements for the degree of Master of Science. 2005:39. http://hdl.handle.net/10539/1782

2. Meraz M, Shirai K, Larralde P, Revah S. Studies on the bacterial acidification process of cassava (*Manihotesculenta*). J. Sci. Food Agric.1992:60(3):457-463. DOI:10.1002/JSFA.2740600409.

3. Edward VA, Egounlety M, Huch M, Van Zyl PJ, Singh S, Nesengani ND et al. Isolation and screening of microorganisms from a gari fermentation process for starter culture development. Afr. J. Biotechnol. 2012:11(60):12865-12877. DOI:10.5897/AJB12.3672.

4. Oyewole OB, Odunfa SA. Characterization and distribution of lactic acid bacteria in cassava fermentation during fufu production. J. Appl. Bacteriol. 1990:68(2):145-152. DOI:10.1111/j.1365-2672.1990.tb02559.x

5. Jiang L, Cui H, Zhu L, Hu Y, Xu X, Li S et al. Enhanced propionic acid production from whey lactose with immobilized *Propionibacteriumacidipropionici* and the role of trehalose synthesis in acid tolerance. Green Chem. 2015:17(1):250-259. DOI:10.1039/C4GC01256A

6. Oyewole, O.B. National Research Council (US) Panel on the Application of Biotechnology to Traditional Fermented Foods. Applications of Biotechnology to Fermented Foods: Reports of an Ad Hoc Panel of the Board of Science and Technology for International Development: Cassava Processing in Africa. Washington (DC): National Academies Press (US). 1992:12.

7. Ghaffar T, Irshad M, Anwar Z, Aqil T, Zulifqar Z, Tariq A. et al. Recent trends in lactic acid biotechnology: a brief review on production to purification. J. Radiat. Res. Appl. Sci. 2014:7(2):222-229. DOI:10.1016/j.jrras.2014.03.002.

8. Trcek J, Mira NP, Jarboe LR. Adaptation and tolerance of bacteria against acetic acid. Appl. Microbiol. Biotechnol. 2015:99(15):6215-6229.DOI:10.1007/s00253-015-6762-3

9. Geng P, Zhang L, Shi GY. Omics analysis of acetic acid tolerance in *Saccharomyces cerevisiae*. World J. Microbiol. Biotechnol.2017:33(5):94. DOI: 10.1007/s11274-017-2259-9

10. Yousef AE, Juneja VKeditors. Microbial Stress Adaptation and Food Safety. 1st ed. Boca Raton: CRC Press. 2003. DOI:10.1201/9781420012828.

11. Heim S, Lleo MDM, Bonato B, Guzman CA, Canepari P. The viable but nonculturable state and starvation are different stress responses of *Enterococcus faecalis*as determined by proteome analysis. J. Bacteriol. 2002:184(23):6739-6745. DOI:10.1128/JB.184.23.6739-6745.2002.

12. Guan N, Liu L. Microbial response to acid stress: mechanisms and application. Appl. Microbiol. Biotechnol.2020:104:(1):51-65.DOI:10.1007/s00253-019-10226-1.

13. Liu, Y, Tang H, Lin Z, Xu P. Mechanisms of acid tolerance in bacteria and prospects in biotechnology and bioremediation. *Biotechnol. Adv*.2015c:**33**(7)1484–1492. DOI: 10.1016/j.biotechadv.2015.06.001.

14. Nicolaou SA, Gaida SM, Papoutsakis ETA. Comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation. Metab Eng.2010:12(4):307–331.DOI:10.1016/j.ymben.2010.03.004

15. Koponen J,Laakso K, Koskenniemi K, Kankainen M, Savijoki K, Nyman TA et al. Effect of acid stress on protein expression and phosphorylation in *Lactobacillus rhamnosus* GG. J. Prot.2012:75(4):1357–1374. DOI: 10.1016/j.prot.2011.11.009.

16. Delmas F, Pierre F, Coucheney F, Divies C, GuzzoJ. Biochemical and physiological studies of the small heat shock protein Lo18 from the lactic acid bacterium *Oenococcusoeni*. J. Mol. Microbiol. Biotechnol2001:3(4):601–610. PMID:11545277

17. Len AC, Harty DW, Jacques NA. Proteome analysis of *Streptococcus mutans* metabolic phenotype during acid tolerance. Microbiology. 2004:150(5)1353-1366. DOI:10.1099/mic.0.26888-0.

18. Wu R, Zhang W, Sun T, Wu J, Yue X, Meng H, et al. Proteomic analysis of responses of a new probiotic bacterium *Lactobacillus casei* Zhang to low acid stress. Int. J. Food Microbiol.2011:147(3)181–187.DOI:101016/j.ijfoodmicro.2011.04.003

19. Nascimento MM, Lemos JA Abranches J. Goncalves, RB, Burne RA. Adaptive acid tolerance response of *Streptococcus sobrinus*. J. Bacteriol.*2004:*186(19):6383–6390.DOI:10.1128/JB.186.19.6383-6390.2004.

20. Papadimitriou K, Pratsinis H, Nebe-von Caron G, Kletsas D, Tsakalidou E. Acid tolerance of *Streptococcus macedonicus* as assessed by flow cytometry and single-cell sorting. *Appl. Environ.Microbiol*2007:73(2):465–476. DOI: 10.1128/AEM.01244-06.

21. Adewara OA, Ogunbanwo ST. Acid stress responses of *Lactobacillus amylovorus* and *Candida kefyr* isolated from fermented sorghum gruel and their application in food fermentation. Can. J. Microbiol.2022:69(4):269-280. DOI: 10.1139/cjm-2021-0118.

22. Oboh G,Akindahunsi AA. Biochemical changes in cassava products (flour &gari) subjected to *Saccharomyces cerevisae* solid media fermentation. Food Chem.2003:82(4):599-602. DOI: 10.1016/S0308-8146(03)00016-5.

23. Elbehiry A, Marzouk E, Hamada M, Al-Dubaib M, Alyamani E, Moussa IM et al. Application of MALDI-TOF MS fingerprinting as a quick tool for identification and clustering of foodborne pathogens isolated from food products. New Microbiol.2017:40(4):269-278. PMID: 28825446.

24. Lammert J. Techniques in Microbiology: a student handbook. 1sted. Pearson education Inc. Upper Saddle River, NJ. 2007.

25. Brandao RL, Rosa JCC, Nicoli JR, Almeida MVS, doCarmo AP, Queiros HT et al. Investigating acid stress response in different *Saccharomyces* strains. Journal of Medical Mycology2014:2014:1-9.DOI: 10.1155/2014/178274

26. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities in protein utilizing the principles of protein-dye binding. Anal. Biochem.1976:72(1-2):248-254.DOI: 10.1016/0003-2697(76)90527-3.

27. Westermeier R. Sensitive, quantitative and fast modifications for Coomassie Blue staining of polyacrylamide gels. Proteomics. 2006:6(1-2):61-64.DOI: 10.1002/pmic.200690121.

28. Shevchenko A, Tomas H,Havlis J, Olsen JV,Mann M. In gel digestion for mass spectrometric characterization of protein and proteomes. Nat. Protoc. 2006:1(6):2856-2860.DOI: 10.1038/nprot.2006.468.

29. Wu R, Wang W, Yu D, Zhang W, Li Y, Sun Z et al. Proteomics analysis of *Lactobacilluscasei* Zhang, a new probiotic bacteria isolated from traditional home-made Koumiss in Inner Mongolia of China. Mol. Cell Proteomics.2009:8(10):2321-2338.DOI: 10.1074/mcp.M800483-MCP200.

30. Beck HC, Madsen SM, Glenting J, Petersen J, Israelsen H,Norelykke MR. Proteomic analysis of cell surface-associated proteins from probiotic *Lactobacillusplantarum.*FEMS Microbiol. Lett.2009:297(1):61-66.DOI: 10.1111/j.1574-6968.2009.01662.x.

31. Okafor N, Umeh C,Ibenegbu CI. Amelioration of Gari, a fermented food derived from cassava *ManihotesculentaCrantz* by microbial inoculation. *World J. Microb.Biot.*1998:14(6): 835-838.DOI: 10.1023/A:1008897711947.

32. Association of Official Analytical Chemists AOAC. Official methods of analysis Association of Analytical Chemists. 17th edition. Gaithersburg, MD; 2006.

33. Association of Official Analytical Chemists AOAC. Official methods of analysis Association of Analytical Chemists. 13th edition. Arlington, VA, USA; 1980.

34. Association of Official Analytical Chemists AOAC. Official methods of analysis Association of Analytical Chemists. 15th edition. Washington DC, USA; 1990.

35. Bradbury MG, Egan SV, Bradbury JH. Picrate paper kits for determination of all forms of total cyanogens in cassava roots and all forms of cyanogens in cassava products. *J. Sci. Food Agr.* 1999:79(4):593-601.DOI:10.1002/(SICI)1097-0010(19990315)79:4<593::AID-JSFA222>3.0.CO;2-2.

36. Lim J. Hedonic scaling: a review of methods and theory. *Food Qual. Prefer* 2011:22(8):733-747.DOI:10.1016/j.foodqual.2011.05.008.

37. Anukam KC,Koyama TE. Bile and Acid tolerance of *Lactobacillus plantarum* KCA-1: A Potential Probiotical Agent. *Int. J. Diary Sci.* 2007:2(3):275-280.DOI:10.3923/ijds.2007.275.280.

38. Buragohain AK, Tanti B, Sarma HK, Burman P, Das K. Characterization of yeast starter cultures used in household alcoholic beverage preparation by a few ethnic communities of Northeast India. *Ann. Microbiol*2013:63(3):863-869.DOI:10.1007/s13213-012-0537-1.

39. Bonafe N, Gilmore-Hebert M, Folk NL, Azodi M, Zhou Y, Chambers SK. Glyceraldehyde-3-phosphate dehydrogenase binds to the AU-rich 3′ untranslated region of colony-stimulating factor-1 (CSF-1) messenger RNA in human ovarian cancer cells: possible role in CSF-1 posttranscriptional regulation and tumor phenotype. *Cancer Res.* 2005:65(9):3762-3771.DOI:10.1158/0008-5472.CAN-04-3954.

40. Ruiz L, Coute Y, Sanchez B, de Los Reyes-Gavilan CG, Sanchez C, Margolles, A. The cell envelope proteome of *Bifidobacteriumlongum* in an invitro bile environment. *Microbiology*2009:155(3):957-967.DOI:10.1099/mic.0.024273-0.

41. Anikainen J, Kuparinen V, Lahteenmaki K, Korhonen TK. pH-dependent association of enolase and glyceraldehyde-3-phosphate dehydrogenase of *Lactobacillus crispatus*with the cell wall the cell wall and lipoteichoic acids. *J. Bacteriol*2007:189(12):4539-4543.DOI: 10.1128/JB.00378-07

42. Godon C, Lagniel G, Lee J, Buhler JM, Kieffer S, Perrot M et al.The H_2O_2stimulon in *Saccharomycescerevisiae.J. Biol. Chem*1998:273(35):22480-22489.DOI: 10.1074/jbc.273.35.22480.

43. Siciliano RA, Cacace G, Mazzeo MF, Morelli L, Elli M, Rossi M et al. Proteomics investigation of the aggregation phenomenon in *Lactobacillus crispatus.BiochimBiophys. Acta Protein Proteom.* 2008:1784(2):335-342.DOI:10.1016/j.bbapap.2007.11.007.

44. Riis B, Rattan SIS, Clark BFC, Merrick WC. Eukaryotic protein elongation factors. *Trends Biochem. Sci*. 1990:**15**:420-424.DOI:10.1016/0968-0004(90)90279-K

45. Rao D, Momcilovic I, Kobayashi S, Callegari E,Ristic Z. Chaperone activity of recombinant maize chloroplast protein synthesis elongation factor, EF-Tu. *Eur. J. Biochem.*2004:271(18)**:**3684–3692.DOI: 10.1111/j.1432-1033.2004.04309.x.

46. RicharmeG. Protein-disulfide isomerase activity of elongation factor EF-Tu. *Biochem. Biophys. Res. Commun.*1998:252(1):156–161.DOI: 10.1006/bbrc.1998.9591.

47. Caldas TD, El Yaagoubi A,Richarme G. Cheperone properties of bacterial elongation factor EF-Tu. *J. Biol. Chem.*1998:273(19):11478-11482.DOI: 10.1074/jbc.273.19.11478.

48. Leverrier P, Vissers JP, Rouault A, Boyaval P,Jan G. Mass spectrometry proteomic analysis of stress adaptation reveals both common and distinct response pathways in *Propionibacteriumfreudenreichii.Arch. Microbiol.*2004:181(3):215-230.DOI: 10.1007/s00203-003-0646-0.

49. Duche O, Tremoulet F, Glaser P, Labadie J. Salt stress proteins induced in *Listeria monocytogenes.Appl. Environ. Microbiol.*2002:68(4):1491-1498.DOI: 10.1128/AEM.68.4.1491-1498.2002.

718 50. Marco ML, de Vries MC, Wels M, Molenaar D, Mangell P, Ahrne S et al. Convergence
719 in probiotic *Lactobacillus* guts adaptive responses in humans and mice. *ISME*
720 *J.*2010:4(11):1481-1484.DOI: 10.1038/ismej.2010.61

721 51. Kleerebezem M, Boekhorst J, van Kranenburg R, Molenaar D, Kuipers OP, Leer R et
722 al. Complete genome sequence of *Lactobacillus plantarum* WCFS1 *Proc Natl. Acad. Sci.*
723 *U. S. A.* 2003:100**(4):**1990-1995.DOI: 10.1073/pnas.0337704100.

724 52. Kirn TJ, Jude BA, Taylor RK. A colonization factor links *Vibrio cholerae* environmental
725 survival and human infection. *Nature* 2005:438(7069):863-866.DOI: 10.1038/nature04249.

726 53. Kawada M, Chen CC, Arihiro A, Nagatani K, Watanabe T,Mizoguchi E. Chitinase 3-like-
727 1 enhances bacterial adhesion to colonic epithelial cells through the interaction with
728 bacterial chitin-binding protein. *Lab. Invest.*2008:88(8):883-889.DOI:
729 10.1038/labinvest.2008.47.

730 54. Steinkraus K. Handbook of indigenous fermented foods, Revised and Expanded.2nd ed.
731 Boca Raton: CRC Press. 1995 .DOI: 10.1201/9780203752821.

732 55. Ogunbanwo ST, Adewara OA, Fowoyo PI. Effect of fermentation by pure cultures of
733 *Lactobacillus fermentum* I and *Saccharomyces cerevisiae* as starter culture in the
734 production of burukutu. *N. Y. Sci. J.* 2013:6(1):73-81.ISSN: 1554-0200.

735 56. Sidari R, Martorana A, Zappia C, Mincione A, Giuffre AM. Persistence and effect of a
736 multistrain starter culture on antioxidant and rheological properties of novel wheat
737 sourdoughs and bread. *Foods*2020:**9**(9):1258 – 1281.DOI: 10.3390/foods9091258.

738 57. Gassem MAA. Study of microorganisms associated with the fermented bread (Khamir)
739 produced from sorghum in Gizan region Saudi Arabia. *Int. J. Food Microbiol.*
740 1999:86(2):221-225.DOI: 10.1046/j.1365-2672.1999.00648.x.

741 58. Agarry O, Nkama I, Akoma O. Production of Kunun-Zaki, a Nigerian fermented cereal
742 beverage using starter culture. *Int. Res. J. Microbiol.*2010:1:18-25.

743 59. Adeyemi IA, Umar S. Effects of method of manufacture on quality characteristic of
744 Kunun-zaki, a millet based beverage. *Niger. Food J.*1994:12:34-41.

745 60. Tefera T, Ameha K, Bruhtesfa A. Cassava based food: Microbial fermentation by single
746 starter culture towards cyanide reduction, protein enrichment and palatability. *Int. Food*
747 *Res.J.*2014:21(5):1751-1756.ISSN:1985-4668.

748 61. Boonnop K, Wanapat M, Nontaso N,Wanapat S. Enriching nutritive value of cassava
749 root by yeast fermentation. *Sci. Agric.*2009:66(5):616-620.DOI: 10.1590/S0103-
750 90162009000500007.

751 62. Bala JD, Ijah UJJ, Abioye OP, Emele LC. Protein enrichment of cassava with yeast for
752 gari production. *Biotechnology, An Indian Journal* 2012:6(4):120-126.

753 63. Uvere PO, Onyekwere EU,Ngoddy PO. Production of maize-bambara groundnut
754 complementary foods prefermentation with processed foods rich in calcium, iron, zinc and
755 provitamin A. *J. Sci. Food Agri.*2010:90(4):566-573.DOI: 10.1002/jsfa.3846.

756 64. Saleh AS, Zhang Q, Chen J,Shen Q. Millet grains: nutritional quality, processing, and
757 potential health benefits. *Compr. Rev. Food Sci. F.* 2013:12(3)**:**281- 295.DOI:
758 10.1111/1541-4337.12012.

759 65. Apaliya MT, Kwawbc E, Tchaboc W, Sackeyb AS, Boateng NAS. The use of lactic acid
760 bacteria as starter culture and its effect on the proximate composition and sensory
761 acceptability of millet beverage. *Int. J. Innov. Food Sci. Technol.*2017:1:1-8.DOI:
762 10.25218/ijifst.2017.01.001.01.

66. Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM,Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabeties mellitus. *N. Engl. J. Med.*2000:342(19):1392-1398.DOI: 10.1056/NEJM200005113421903.

67. Elleuch M., Bedigian D, Roiseux O, Besbes S, Blecker C, Attia H. Dietary fibre and fibre rich by-products of food processing: characterization, technological functionality and commercial applications: A review. *Food Chem.*2011:124(2):411-421.DOI: 10.1016/j.foodchem.2010.06.077.

68. Rosoamanana R, Even PC, Darcel N, Tome D, Fromentin G. Dietary fibers reduce food intake by satiation without conditioned taste aversion in mice. *Physiol. Behav.* 2013:110-111:13-19.DOI: 10.1016/j.physbeh.2012.12.008.

69. Roberts CL, Keita AV, Parsons BN, Prorok-Hamon M, Knight P, Winstanley C et al. Soluble plantainfibre blocks adhesion and M-cell translocation of intestinal pathogens. *The J. Nutr. Biochem.*2013:24(1):97-103.DOI: 10.1016/j.jnutbio.2012.02.013.

70. Hambridge KM, Miller LV, Westcott JE,Krebs NF. Dietary reference intakes for zinc may require adjustment for phytate uptake based upon model predictions. *J. Nutr.* 2008:138(12):2363-2366.DOI: 10.3945/jn.108.093823.

71. McGee H. On food and cooking: the science and lore of the kitchen. New York: Scribner. 2004:714.

72. Kolodziej H,Kiderlen AF. Antileshmanial activity and immune modulatory effects of tannins and related compounds on *Leismania* parasitized RAW 264.7 cells.*Phytochemistry*. 2005:66(17)**:**2056–2071.DOI: 10.1016/j.phytochem.2005.01.011.

73. Miglio C, Chiavaro E, Visconti A, Fogliano V, Pellegrini N. Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *J. Agric. Food Chem.*2008:56(1):139–147.DOI: 10.1021/jf072304b.

74. Taiwo O. Physical and nutritive properties of fermented cereal food. *Afri. J. Food Sci.*2009:3(2):023-027. ISSN: 1996-0794.

75. Halake NH, Chinthapalli B, Chitra DSV. Role of selected fermentative microorganisms on Cyanide reduction, Protein enhancement and Palatability of cassava based food. *IJRAF* 2019:6(12):1-12.

76. KobawilaSC, Louembe D, Keleke S, Hounhoungan J, Gamba C. Reduction of cyanide content during fermentation of cassava roots and leaves to produce Bikende and NtobaMbodi, two food products from Congo. *Afr. J. Biotechnol.*2005:4(7):689-696.DOI: 10.5897/AJB2005.000-3128.

77. Omemu AM, Oyewole OB,Bankole OM. Significance of yeasts in the fermentation of maize for Ogi production. *Food Microbiol.*2007:24(6):571-576.DOI: 10.1016/j.fm.2007.01.006.

78. Mukisa IM, Porcellato D, Byaruhanga YB, Muyanja CM, Rudi, Langsrud T. et al. The dominant microbial community associated with fermentation of Obushera (sorghum and millet beverages) determined by culture-dependent and culture-independent methods. *Int. J. Food Microbiol.* 2012:160(1):1–10.DOI: 10.1016/j.ijfoodmicro.2012.09.023.