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Preselection of Yeast Strains with Probiotic Potential Isolated from Traditional Cassava Ferments (*Manihot esculenta* Crantz) for the Reduction of Anti-Nutritional Compounds in Foods: Case of Phytate

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ABSTRACT

The general objective of this work was to contribute to the improvement of the nutritional quality of food. Thus, the resistance of yeast strains isolated from cassava ferments to gastrointestinal conditions including pH (acid and basic), NaCl, bile salts, temperatures, digestive enzymes (pepsin and pancreatin) and adhesion (self-aggregation and hydrocarbons) as well that their degradation of phytates were evaluated. The results showed that the highest growth at acidic pH was obtained with strains S1fbvp at pH 2 (3.7±0.3) × 10^7 CFU/mL after 24 hours of incubation. The highest growth was obtained with the S4fbvp strains (6.7±0.8) × 10^7 CFU/mL at 42°C followed by the S4ff strain (5.2±0.4) × 10^7 CFU/mL at 37°C and of the S5fbs strain (5.1±2.7) × 10^7 CFU/mL at 30°C. The S4fbvp strain showed the best growth (4.7±0.4) × 10^7 CFU/mL at 1% NaCL and (5.3± 0.4) × 10^7 CFU/mL at 2% NaCL, (5.0±0.7) × 10^7 CFU/mL, (5.0±0.3) × 10^7 CFU/mL and (4.3±0.3) × 10^7 CFU/mL at 0.3%, 0, 6% and 0.9% bile salts respectively. The S3fbr strain had the highest adhesion to chloroform (85.06±2.74%) and viability in the presence of pancreatin (98.30±2.07%) unlike the S5fbs strains (52.94±1. 49%) to xylene and S4ff (95.93±0.40%) to pepsin. The strains S4fbvp (84.96±0.1%) and S5fbs (94.49±0.73%) had the highest rates of auto-aggregations at 4 hours and 24 hours of incubation respectively. The strains studied degraded phytates in rice and white corn flours from 47.07 ± 0.11% to 93.82 ± 0.32% with the highest degradation obtained with the S4fbvp strain.

Introduction

Cassava (*Manihot esculenta* Crantz) is an integral part of the diet of more than half a billion humans worldwide (Scaria *et al.,* 2024). It is a semi-shrub plant of the Euphorbiaceae family. It is cultivated on an area of 19.6 million hectares worldwide for a production of 302.7 million tonnes. Considered the second Ivorian food crop, cassava has experienced significant growth in recent years with a production of 6.4 MT Anonyme (2022).

In Côte d'Ivoire, cassava is used for the preparation of several local dishes including attiéké, placali, gari, foufou, atoupkou, cosette, starch, boiled pieces and fritters (fried) (Mendez *et al.,* 2017). The preparation process of most of these dishes requires the use of a cassava starter or leaven (Kouamé *et al.,* 2013). Cassava fermentation is a spontaneous fermentation which involves microbial activity (Boli *et al.,* 2020). The microorganisms involved in cassava fermentation are mainly lactic acid bacteria, *Bacillus*, yeasts and molds (Kakou *et al.,* 2017). In addition to their role in the natural preservation of food through the production of various compounds, notably organic acids and bacteriocins, greatly reducing the growth of pathogens, microorganisms sometimes have probiotic potential (Sawadogo *et al.,* 2016).

Nowadays, studies are turning to the search for probiotics of microbial origin. Probiotics are described as live microorganisms that confer a beneficial effect on the host when administered in adequate amounts (FAO/WHO, 2002). These are mainly bacteria and yeasts (Chen *et al.,* 2014) present in certain fermented foods or in food supplements in freeze-dried form. Several studies (Agarbati *et al.,* 2020; Sambrani *et al.,* 2021; Jeong *et al.,* 2023) have reported the beneficial effects of probiotic yeast strains, in particular the genera *Saccharomyces*, *Debaryomyces*, *Candida* and *Torulaspora* on human health. Probiotic yeasts are able to resist the extreme pH conditions of the stomach, strengthen the integrity of the intestinal barrier and improve digestive comfort (Menezes *et al.,* 2019). Nowadays, studies are turning to the search for probiotic yeasts from fermented foods (Leite *et al.,* 2015; Tamang and Lama, 2022; Dessalegna and Andualem, 2023). Numerous studies are currently being developed to test the technological capabilities of probiotic yeasts as a contribution to improving the formulation of feed for fry, rabbits and poultry.

With significant growth in the Ivorian population, the consumption of cereal-based foods is increasing over the years. Cereals represent a significant part of the human diet with 60% of total caloric intake (Schlemmer *et al.,* 2009). However, certain cereal products, notably rice and corn, widely consumed in West Africa (Traoré *et al.,* 2020) contain anti-nutritional compounds that can lead to an increase in the prevalence of mineral deficiencies (Foisnet, 2020). Antinutritional compounds are molecules that are sometimes indigestible for monogastric animals and cause a reduction in the absorption of minerals, such as iron, calcium, zinc and magnesium, and lead to nutritional deficiencies. These nutritional deficiencies can have serious impacts on health, such as impaired growth or reduced immunity and can in certain extreme cases lead to death (Kwon *et al.,* 2014). Furthermore, Kumar and Sinha (2018) reported that degradation of phytic acid allows an increase in the bioavailability of minerals and phosphorus as well as an improvement in the nutritional values of foods. Many sources of phytases, phytate degradation enzymes, exist but microbial sources are the most promising (Park *et al.,* 2011). Fungal phytases may be favored over bacterial phytases for their greater stability and higher activity (Song *et al.,* 2019).

However, to our knowledge in Côte d'Ivoire, no strain of yeast isolated from cassava ferments has yet been used in microbial cocktails, as a starter for trials aimed at its use as food ingredients with probiotic properties. Thus, the general objective of this work is to contribute to the improvement of the nutritional quality of foods by probiotic yeasts with a view to their use as potential starters.

Materials and Methods

Study Material

The study material consists of, on the one hand, traditional cassava ferments (*Manihot esculenta* Crantz) (Figure.1-A, 1-B, 1-C and 1-D) and on the other hand, shelled grains of corn (*Zea mays*) (Figure.1-E) and rice (*Oryza sativa*) (Figure.1-F and 1-G).

Sampling of traditional cassava ferments and rice and corn grains

Samples of traditional ferments of fresh, braised, boiled cassava without the peel and boiled cassava with the peel were collected from producers in the commune of Adjamé. Three samples of approximately 500 g of each type of cassava ferment were collected from three different producers. A total of 36 cassava ferment samples were collected and each collected sample was put in a Stomacher bag then in a cooler and transported to the laboratory for microbiological analyses.

At the same time, cereal products including white corn grains and two varieties of rice grains (local rice and industrial rice) were collected in the main market of Adjamé, Abidjan (Côte d'Ivoire). Regarding white corn kernels, approximately 1 kg of hulled white corn was

collected randomly from three different traders. Which gives a total of 3 kg of white corn kernels. For rice grains, 1 kg of local rice was taken from three different sellers taken randomly, i.e. 3 kg in total and the same goes for industrial rice grains where 1 kg was purchased in three different shops, or 3 kg in total.

Production of corn and rice flour

The production diagram of corn and rice flour is shown in figure 2. The hulled corn and rice grains were sorted and winnowed to remove plant debris and other foreign bodies. The sorted and winnowed grains were washed with tap water then 1 kg of each sample was soaked for 6 hours in a basin containing 3 L of water, so that the grains were completely submerged. After the soaking time, the corn and rice grains were removed from the water, dried and then drained.

Then, the drained corn and rice grains were ground using a mill to obtain flour. The different flours obtained were sifted using 9 different sieves (mesh: 250 µm diameter). For each sample, 400 g of corn and rice flour were weighed and collected. In total, 9 samples consisting of 3 samples of white corn flour, 3 other samples of local rice flour and industrial rice each of approximately 400 g were obtained. These flours were individually packaged in sterile Stomarchers sachets, labeled, sealed and then analyzed.

Microbiological analyzes of traditional cassava ferments

Preparation of stock suspensions and decimal dilutions

The preparation of the stock suspensions and decimal dilutions was carried out according to standard ISO6887-1, 2017. At this level, 10 g of cassava ferment sample were taken under aseptic conditions and homogenized in a sterile "Stomacher" bag. (AES laboratory, France) marked and containing 90 mL of previously sterilized buffered peptone water.

The mixture obtained after homogenization corresponds to the stock suspension. Decimal dilutions were carried out through numbered test tubes, each containing 9 mL of sterilized buffered peptone water. A volume of 1 mL of the stock suspension was transferred aseptically using a sterile pipette into the tube marked No. 1. The well-homogenized mixture corresponds to the 10^{-2} dilution. Using a sterile pipette, this operation is repeated to achieve subsequent dilutions until well isolated colonies are obtained.

Yeast Enumeration

The yeasts were counted according to the NF ISO 6611 - 2004 standard. The count was carried out after the aseptic distribution of 0.1 mL of each of the respective dilutions on a Petri dish containing the previously prepared Sabouraud Chloramphenicol agar. Seeding was done by spreading. The plates were incubated at 30°C for 24 to 72 hours. After incubation, the whitish, creamy, ovoid, smooth, shiny and rounded yeast colonies with a bakery odor after 24 to 48 hours were counted by eye, then the charges were expressed as Colony Forming Unit per gram (CFU/g) using formula (1):

$$N\ (CFU/mL) = \frac{\sum C}{V(n_1 + 0{,}1 \times n_2) \times d} \ldots(1)$$

N: number of germs in CFU/g of product;

∑c: sum of colonies counted on the Petri dishes retained at the level of two successive dilutions;

V: volume of inoculum collected (0.1 mL);

n_1: number of boxes retained at the first dilution considered;

n_2: number of boxes retained at the second dilution considered;

d: dilution from which the first counts were retained.

Production of phytase by yeast strains isolated from cassava ferments

The demonstration of the phytase activity of yeast strains isolated from traditional cassava ferments was carried out according to the method of Ranjan and Sahay (2013). To do this, quantities of 1.5 g of glucose 1%, 0.45 g NH4NO3 0.3%, 0.075 g MgSO4 0.05%, 0.075 g KCl 0.05%, 0.015 g CaCl2 0.01% and 2.25 g of 1.5% agar were added to 150 mL of distilled water in a jar then adjusted to pH 6.8. Then, the mixture was homogenized using a stirrer for 3 minutes and then sterilized by autoclaving at 121°C for 15 minutes. After cooling, 0.075 g of 0.05% sodium phytate was added to the

medium then inoculated by spot with a suspension of yeast loading 10^7 CFU/mL and incubated for 48 to 72 hours at 30°C. A positive result results in a clear halo around the colony.

Study of the growth of yeast strains under gastrointestinal conditions

Preparation and adjustment of yeast strain loads

The adjustment of yeast loads was carried out according to the method of Biesta-Peters *et al.,* (2010). A study was carried out on each yeast strain reflecting the correspondence between optical density and microbial load. To do this, the yeast strains revivified on YPDA agar were cultured in YPD (yeast peptone dextrose) broth and incubated at 30°C in an oven (BJPX-B80II, China) for 24 h. The cultures obtained were then centrifuged at 5000 rpm for 5 min in a centrifuge (BKC-TH20R, China) at 4° C. The pellet obtained was then washed twice with sterile physiological water (NaCl 0.9 %, m/v) and suspended in the same saline solution. Then, a series of dilution (1/2, 1/3, 1/4, 1/6, 1/8, 1/12 and 1/16, v/v) of the yeast suspension with physiological water sterile (NaCl 0.9%, w/v) was carried out, then the optical density of each diluted yeast suspension was determined. After reading the optical density, a volume of 0.1 mL of each diluted yeast suspension was spread on YPDA agar, then the Petri dishes were incubated at 30°C for 72 h in an incubator (BJPX- B80II, China). After incubation, all characteristic yeast colonies (white, creamy, ovoid, smooth and shiny) were counted by eye and then the microbial loads were calculated from formula (1).

Growth of yeast strains at different pH

The study of the growth of yeast strains at different pHs was carried out according to the method of Lohith and Anu-Appaiah (2014). YPD broths (1% yeast extract, 2% peptone, 2% glucose) were prepared at different pH (acidic and basic). For acidic pH (2 and 2.5), media were adjusted with 1M hydrochloric acid (HCl) and for basic pH (8 and 8.5), media were adjusted with sodium hydroxide (NaOH) 1M.

The medium at pH 6.8 served as a control. The medium was distributed into different test tubes at a rate of 3 mL per tube. The sterile medium contained in each tube is inoculated at 1% v/v with a pre-culture of yeast inoculated in YPD broth at 30°C for 24 hours and whose load was adjusted to 10^7 CFU/mL as previously described. The seeded media were incubated at 37°C for 24 hours. A tube is removed at each incubation time (4 and 24 hours) and the optical density is read at 600 nm using a spectrophotometer (BK-UV1000). The suspension, after reading the optical density, is placed on YPDA agar with a volume of 0.1 mL and incubated at 30°C for 24 to 72 hours. After incubation, all the characteristic yeast colonies (white, creamy, ovoid, smooth and shiny) were counted at each time (4 and 24 hours) then the microbial load was determined according to formula (1).

Growth of yeast strains at different temperatures

The study of the growth of yeast strains at different temperatures was carried out according to the method of Lohith and Anu-Appaiah (2014). YPD broths (1% yeast extract, 2% peptone, 2% glucose) were prepared and then distributed into different test tubes at a rate of 3 mL per tube. The sterile media contained in the tubes were then inoculated at 1% v/v with a pre-culture of yeast inoculated in YPD broth at 30°C for 24 hours. The load was adjusted to 10^7 CFU/mL. The seeded media were incubated at different temperatures including 30°C, 37°C and 42°C for 24 hours. A tube is removed at each incubation time (4 and 24 hours) and the optical density is read at 600 nm using the spectrophotometer (BK-UV1000). The suspension after reading the optical density is placed on YPDA agar and incubated for 24 to 72 hours. The number of colonies is then counted.

Growth of yeast strains at different NaCl concentrations

The study of the growth of yeast strains at different concentrations of NaCl was carried out according to the method of Mangala and Nilanjana (2017). To do this, YPD broths (1% yeast extract, 2% peptone, 2% glucose) were prepared and distributed in different test tubes at a rate of 3 mL per tube to which concentrations of NaCl (1% and 2%) then everything was sterilized in an autoclave at 121°C for 15 min. Then, the media were inoculated at 1% v/v with a pre-culture of yeast inoculated in YPD broth at 30°C for 24 h, the load was adjusted to 10^7 CFU/mL. These media were incubated at 37°C for 24 hours. One tube is removed at each incubation time (4 and 24 hours) and yeast growth is determined by measuring yeast turbidity at 600 nm. The suspension after reading the optical density is placed on

YPDA agar as previously described and incubated at 37°C for 24 to 72 hours then the number of colonies is counted.

Growth of yeast strains at different concentrations of bile salts

The study of the growth of yeast strains at different concentrations of bile salts was carried out according to the method of Lohith and Anu-Appaiah (2014). YPD broths (1% yeast extract, 2% peptone, 2% glucose) were prepared and different concentrations of Ox-bile including 0.3%, 0.6% and 0.9% were added. Broth without Ox-bile served as a control. The medium was distributed in different test tubes at a rate of 3 mL per tube then sterilized in an autoclave at 121°C for 15 min. Each medium contained in a tube was inoculated at 1% v/v with a pre-culture of yeast inoculated in YPD broth at 30°C for 24 hours, the load was adjusted to 10^7 CFU/mL. Then, these media were incubated at 37°C for 24 hours. A tube is removed at each incubation time (4 and 24 hours) and the optical density is read at 600 nm. After reading the optical density, the suspension is placed on YPDA agar and incubated at 37°C for 72 hours then counted.

Growth of yeast strains in the presence of digestive enzymes

The study of the growth of yeast strains in the presence of digestive enzymes such as pepsin and pancreatin was carried out according to the modified method of Lohith and Anu-Appaiah (2014). The selected yeast strains were subcultured on YPDA agar (Yeast 1%, Peptone 2%, Glucose 2% and Agar 1.5%), incubated at 30°C for 24 h then placed in YPD broth and incubated at 30 °C for 24 hours.

The broths were then centrifuged at 5000 rpm for 5 min and washed twice with PBS buffer (Phosphate Buffer Saline, pH 6.8). The cell load of the cultures was then adjusted to 10^7 CFU/mL then the yeast suspensions obtained were used for the various viability tests subsequently.

Growth of yeast strains in the presence of pepsin

A 50 mL solution of pepsin was prepared by dissolving pepsin in saline solution (sodium chloride, 0.5%, w/v) to obtain a 0.3% (w/v) enzyme solution. Then, the pH of the reaction medium was adjusted to 2 with a hydrochloric acid solution (5 M). The medium obtained was filtered with a millipore filter with a diameter of 0.20 µm. The filtrate was inoculated (25%, v/v) with a yeast suspension previously adjusted to 10^7 CFU/mL, then incubated at 37°C for 4 h in an oven (BJPX-H64II, China). Subsequently, the inoculated medium was considered as a stock solution and successive decimal dilutions (10^{-1} to 10^{-6}) were made with sterile buffered peptone water.

Aliquots of 0.1 mL of the stock solution and dilutions were spread in Petri dishes containing YPDA agar then incubated at 30°C for 72 h in an incubator (BJPX-B80II, China). After incubation, all characteristic yeast colonies (white, creamy, ovoid, smooth and shiny) were counted by eye, then the microbial loads were calculated according to formula (1). A control was carried out under the same conditions with a solution without pepsin.

$$\textit{Viability log}\ (\%) = \frac{Log\ N}{Log\ N_0} \times 100 \quad \ldots(2)$$

N: microbial load after incubation
N_0: microbial load before incubation

Growth of yeast strains in the presence of pancreatin

A 50 mL solution of pancreatin was prepared by dissolving pancreatin in saline (sodium chloride, 0.5%, w/v) to obtain a 0.1% (w/v) enzyme solution. Then the pH of the reaction medium was adjusted to 8 with a sodium hydroxide solution (5 M). The medium obtained was filtered with a millipore filter with a diameter of 0.20 µm. The filtrate was inoculated (25%, v/v) with a yeast suspension previously adjusted to 10^7 CFU/mL, then incubated at 37°C for 4 h in an incubator (BJPX-H64II, China).

Subsequently, the inoculated medium was considered as a stock solution and successive decimal dilutions (10^{-1} to 10^{-6}) were made with sterile buffered peptone water. Aliquots of 0.1 mL of the stock solution and dilutions were then spread in Petri dishes containing YPDA agar and then incubated at 30°C for 72 h in an incubator (BJPX-B80II, China). After incubation, all characteristic yeast colonies (white, creamy, ovoid, smooth and shiny) were counted by eye and the yeast loads were determined according to formula (1). A control was carried out under the same conditions with a solution without pancreatin.

Yeast strain adhesion tests

Self-aggregation capacity of yeast strains

The self-aggregation test of the yeast strains was carried out according to the method used by Collado *et al.,* (2008). The yeast strains were first cultured at 30°C for 24 h in an incubator in YPD broth. They were then harvested by centrifugation at 5000 rpm for 5 min at 4°C and washed twice with phosphate buffer (11.76 mM, pH 6.8) containing sodium chloride (137 mM) and potassium chloride (2.7 mM). The pellet was suspended in the same sterile phosphate buffer and the cell load was then adjusted to 10^7 CFU/mL. Approximately 10 mL of each suspension contained in different test tubes were incubated at 37°C for 4 h or 24 h in the incubator (BJPX-H64II, China). Then, the absorbances were determined at 600 nm against a blank consisting of sterile phosphate buffer. The self-aggregation percentage was calculated using the following formula:

$$\text{Self} - \text{aggregation}\ (\%) = [1 - (A_t/A_0)] \times 100 \quad \ldots(3)$$

A_t is the absorbance of the yeast suspension at 4 or 24 hours of incubation

A_0 is the absorbance of the yeast suspension before incubation

Adhesion tests of yeast strains in the presence of hydrocarbon

The test for adhesion of yeast strains to hydrocarbons was carried out according to the method of Bellon-Fontaine *et al.,* (1996). The yeast strains were first cultured at 30°C for 24 h in an incubator in YPD broth. They were then harvested by centrifugation at 5000 rpm for 5 min at 4°C and washed twice with phosphate buffer (11.76 mM, pH 6.8) containing sodium chloride (137 mM) and potassium chloride. (2.7 mM). The pellet was suspended in the same sterile phosphate buffer and the cell load was then adjusted to 10^7 CFU/mL. Then, 6 mL of cell suspension and 1.2 mL of each solvent (xylene and chloroform) were mixed and homogenized by mechanical stirring using a vortex (MX-S, Mexico) for 1 min, then the mixtures were incubated at 37°C for 1 h in an incubator (BJPX-H64II, China). After this incubation, the aqueous suspensions of the mixtures were recovered and the absorbances were determined at 600 nm against a blank consisting of sterile phosphate buffer. The membership percentage was calculated using the following mathematical formula:

$$\text{AH}\ (\%) = [1 - A^*/A_0] \times 100 \quad \ldots(4)$$

AH: Adhesion to hydrocarbon

A^*: Absorbance of the aqueous suspension of the mixture after 1 hour of incubation,

A_0: Absorbance of the yeast suspension before mixing.

Determination of the concentration of phytates in corn and rice flours

The concentration of residual phytates contained in cereal flours was measured using the method of Latta and Eskin (1980). To do this, 5 g of sample (corn and rice flour) were dissolved in 100 mL of HCl (0.65 N) then homogenized with stirring at 28°C for 12 hours. The mixture was centrifuged at 3000 rpm for 30 min and 3 mL of the collected supernatant was mixed with 1 mL of Wade's reagent (0.03% FeCl3 and 0.3% sulfosalicylic acid in distilled water). The whole thing was vortexed for 5 seconds and left to stand for 10 minutes. The optical density of the mixture was read at 490 nm on the spectrophotometer against a control containing supernatant without Wade reagent. And the quantity of phytates corresponding to each optical density read was determined using a calibration line established from a stock solution of sodium phytate at different concentrations ranging from 0 to 40µg/mL.

Degradation of phytates in corn and rice flours by yeast strains

The preparation of corn and rice flours for the fermentation test was carried out according to the method of Reale *et al.,* (2004). To do this, 15 g of sample (rice and corn flour) were dissolved in 100 mL of sterile distilled water then the mixture was pasteurized in a water bath at 75°C for 30 minutes. Then, the media were inoculated at 1% v/v with a pre-culture of yeast strains of interest (S3fbs and S4fbvp) whose load was previously adjusted to 10^7 CFU/mL then everything was incubated in a rotary shaker (150 rpm) for 48 hours at 30°C. Subsequently, 3 mL of the supernatant was collected in a test tube and mixed with 1 mL of Wade's reagent (0.03% FeCl3 and 0.3% sulfosalicylic acid in distilled water).

The optical density of the mixture was read at 490 nm on the spectrophotometer against a control containing supernatant without Wade reagent. And the quantity of phytates corresponding to each optical density read was determined using a calibration line established from a stock solution of sodium phytate at different concentrations ranging from 0 to 40 µg/mL.

Statistical analysis

The statistical analysis of the results was carried out with the R software version 4.1.1. The different parameters analyzed were then subjected to an analysis of variance (ANOVA). For this purpose, a repeated measures ANOVA and Tukey's multi-range tests at the 0.5% significance level were used. In the event of a significant difference between the parameters studied, the classification of the means was done according to the Tukey's test.

The yeast strains of interest with regard to their probiotic potential and their ability to degrade phytates were selected using a pheatmap multivariate analysis.

Results and Discussion

Average loads of yeast strains isolated from cassava ferments

The average loads of the yeast strains isolated from the different cassava ferments are presented in Table 1. Analysis of the table indicates that the different average yeast loads vary from $1.6\pm0.2 \times 10^6$ CFU/g to $1.7\pm0.0 \times 10^7$ CFU/g. The highest average load ($1.7\pm0.0 \times 10^7$ CFU/g) was obtained in the samples of cassava ferments boiled without the peel, followed by those of cassava ferments boiled with the peel ($5.6 \pm0.3 \times 10^6$ CFU/g) and braised cassava ferment ($2.9\pm0.8 \times 10^6$ CFU/g). The lowest average load ($1.6\pm0.2 \times 10^6$ CFU/g) was obtained in fresh cassava ferments. The analysis of the different average loads showed a significant difference ($P<0.05$) between the loads of the different cassava ferments.

Ability of yeast strains to produce phytase

The ability of different yeast strains to produce phytase made it possible to highlight areas of phytate hydrolysis depending on the capacity of each yeast strain to degrade the phytate available in the medium. A positive reaction is reflected by a hydrolysis zone around the colony. The intensity of the phytase activity is proportional to the diameter of the hydrolysis zone. The highest hydrolysis diameter (2.25±0.07 cm) was obtained with strain 4 of boiled cassava ferment with peel (S4fbvp) while the lowest hydrolysis diameter (0.5± 0.14 cm) was obtained with strain 2 of fresh cassava ferment (S2ff). The hydrolysis diameters obtained from the yeast strains of the different ferments are significantly different ($P<0.05$) (Table 2).

Probiotic potential of yeast strains

Eight yeast strains having at least a hydrolysis diameter greater than or equal to 2 cm were selected for further work.

Capacity of yeast strains at different pH

The ability of yeast strains to grow at different acidic and alkaline pH after 4 and 24 hours of incubation is illustrated in Table 3. It appears from the analysis of the table that certain yeast strains are both acidophilic and basophilic after 4 hours of incubation. This is the case of the S1fbvp strain which has the highest growth at both pH 2 $(3.3\pm0.2) \times 10^6$ CFU/mL, pH 2.5 $(3.4\pm0.5) \times 10^6$ CFU/mL, and at pH 8 and pH 8.5 with a similar loading of $1.6\pm0.1 \times 10^7$ CFU/mL. After 24 hours of incubation, the S1fbvp and S3fbs strains were the most acidophilic with loads $3.7\pm0.3 \times 10^7$ CFU/mL and $4.5\pm0.0 \times 10^7$ CFU/mL respectively at pH 2 and pH 2.5 on the other hand, the strains S3fbs and S4ff were basophilic respectively with a load of $4.9\pm0.2 \times 10^7$ CFU/mL at pH 8 and $4.0\pm0.1 \times 10^7$ CFU/mL at pH 8, 5. However, a significant difference ($P<0.05$) was observed between the fillers at different pH.

Capacity of yeast strains at different temperatures

The ability of yeasts to develop at different temperatures, namely 30, 37 and 42°C, is presented in table 4. It emerges from the analysis of this table that certain yeast strains are mesophilic after 4 hours of incubation, in particular the strain S1fbvp with loads of $2.2\pm0.6 \times 10^7$ CFU/mL at 30°C and $2.4\pm0.1 \times 10^7$ CFU/mL at 37°C. On the other hand, strain S4ff was thermophilic with a higher load of $2.2\pm0.3 \times 10^7$ CFU/mL at 42°C. After 24 hours of incubation, the S5fbs and S4ff strains were mesophilic with the highest loads of $5.1\pm2.7 \times 10^7$ CFU/mL at 30°C and $5.2\pm0.4 \times 10^7$ CFU/mL,

respectively at 37°C while the S4fbvp strain was thermophilic with the highest loading of 6.7±0.8 × 10^7 CFU/mL at 42°C. However, a significant difference (P<0.05) was observed between the strains at the temperatures studied.

Capacity of yeast strains at different NaCl concentrations

The growth of yeast strains at different NaCl concentrations is presented in Table 5. Analysis of the table shows that the yeast strains tested are halophilic. After 4 hours of incubation, the S1fbvp strain showed the best growth with a load of 1.9±0.2 × 10^6 CFU/mL at 1% NaCl while at 2% NaCl the loads remained substantially the same at the initial load 10^5 CFU/mL.

After 24 hours of incubation, the highest loads including 4.7±0.4 × 10^7 CFU/mL and 5.3± 0.4 × 10^7 CFU/mL were observed with the S4fbvp strain at 1% NaCl and 2% Nacl. In the absence of NaCl, the S1fbvp strain showed the strongest growth at 4 hours and 24 hours of incubation, respectively 1.6±0.0 × 10^7 CFU/mL and 4.7±0.3 × 10^7 CFU/mL. However, a significant difference (P>0.05) was observed in the absence and presence of 1% and 2% NaCl between the different yeast strains.

Ability of yeast strains to different concentrations of bile salts

Table 6 shows the growth of yeast strains at different concentrations of bile salts. Generally, the yeast strains showed good growth ability in the presence of bile salt concentrations of 0.3%, 0.6% and 0.9%. The S1fbvp strain showed the strongest growth at all bile salt concentrations including 0.3%, 0.6% and 0.9% with respective loads of 1.7±0.4 × 10^7 CFU/mL, 1.2±0.2 × 10^7 CFU/mL and 2.1±0.1 × 10^7 CFU/mL after 4 hours of incubation. However, a significant difference (P>0.05) was observed between the loads of the different yeast strains tested after 4 hours of incubation.

Furthermore, the S4fbvp strain presented both the best growth at 0.3%, 0.6% and 0.9% of bile salts after 24 hours of incubation with respective loads of 5.0±0.7 × 10^7 CFU/mL, 5.0±0.3 × 10^7 CFU/mL and 4.3±0.3 × 10^7 CFU/mL. A significant difference (P>0.05) was observed between the loads of the different yeast strains tested after 24 hours of incubation at bile salt concentrations.

Growth capacity of yeast strains in the presence of digestive enzymes

Table 7 presents the viability rates of yeast strains in the presence of pepsin and pancreatin. The viability rates of yeast strains in the presence of pepsin vary from 80.61±1.95 to 95.93±0.40% after 4 hours of incubation. The highest viability rate (95.93±0.40%) was observed with the S4ff strain, however, the lowest viability rate (80.61±1.95%) was obtained with the S1fbvp strain. However, the S3fbs and S4ff strains showed a higher viability rate in the absence of pepsin. No significant difference (P>0.05) was observed between the different yeast strains in the presence of pepsin.

As for the viability rate of yeast strains in the presence of pancreatin, the results obtained vary from 83.03±0.33 to 98.30±2.07%. The highest viability rate (98.30±2.07%) was obtained with the S3fbr strain unlike the S4fbvp strain which obtained the lowest rate (83.03±0.33%). A significant difference (P>0.05) was observed between the different yeast strains in the presence of pancreatin.

Adhesion capacity of yeast strains

Self-aggregation capacity of yeast strains

The different yeast strains showed a self-aggregation capacity with values varying from 50.61±1.96 to 84.96±0.1% and from 79.68±0.5 to 94.49±0.73% after 4 hours and 24 hours of incubation respectively. The highest auto-aggregation rates, 84.96±0.1% and 94.49±0.73%, were observed with the S4fbvp and S5fbs strains after 4 hours and 24 hours of incubation, respectively. On the other hand, the lowest auto-aggregation rates were obtained with the strains S5ff (50.61±1.96%) and S3fbr (85.38±0.88%) respectively after 4 hours and 24 hours of incubation. However, a significant difference (P<0.05) was observed between the self-aggregation rates of the different yeast strains (Table 8).

Adhesion capacity of yeast strains to hydrocarbons

The rates of adhesion of yeast strains to hydrocarbons are presented in Table 9. The results indicate adhesion of yeast strains to hydrocarbons with values varying from 32.10± 1.3% to 85.06±2.74% for chloroform and from 14.81±1.67 to 52.94±1.49% for xylene. The highest

adhesion rate of chloroform (85.06±2.74%) was observed with the S3fbr strain while that of xylene (52.94±1.49%) was obtained with the S5fbs strain. On the other hand, the lowest adhesion rates of chloroform (32.10±1.3%) and xylene (14.81±1.67%) were respectively obtained with the S1fbr and S5ff strains. Statistical analysis of the results showed a significant difference (P<0.05) between the different rates of adhesion to hydrocarbons.

Probiotic profile of the yeast strains studied

The pheatmap multivariate statistical analysis carried out using the probiotic potentialities of the yeast strains showed differences in the potentialities of the yeasts represented by a color key with a decreasing gradient from red to blue (Figure 3). This pheatmap analysis made it possible to classify the 8 yeast strains studied into two main groups noted I and II. Group I is made up of 4 strains, namely S3fbr, S5ff, S1fbr and S1fbvp, presenting sensitivity to low pH and bile salts, and low phytase activities. As for group II strains, they are subdivided into two subgroups noted IIa and IIb. The strains of subgroup IIa, consisting of the S3fbs and S4fbvp strains, are characterized by strong growth at low pH, at concentrations of bile salts and NaCl, high phytase production, strong self-aggregation capacity and adhesion as well as a high rate of viability in the presence of digestive enzymes pepsin and pancreatin. Furthermore, subgroup IIb, composed of strains S5fbs and S5ff, has poor growth at low pH and strong growth in the presence of bile salts. Thus, from this analysis, it clearly appears that the yeast strains which would have the best probiotic potential and which would therefore be of interest for food applications were the S3fbs and S4fbvp strains.

Degradation of phytates by yeast strains in corn and rice flours

The ability of yeast strains to degrade phytate is illustrated in Table 10. The initial concentration of phytates in the different flour samples ranges from 75.41 ± 3.86 µg/mL to 164.21 ± 9.66 µg. /mL respectively in industrial rice flour and local rice flour. After fermentation with the selected yeast strains, phytate concentrations in the fermented flours varied from 5.08 ± 0.39 µg/mL to 157.71 ± 10.37 µg/mL. The highest concentration (157.71 ± 10.37 µg/mL) was obtained in local rice flour with the control sample while the lowest concentration (5.08 ± 0.39 µg/mL) was obtained in white corn flour incorporated by the S4fbvp strain. However, a significant difference (P>0.05) was observed between the concentrations of phytates obtained in the different samples of corn and rice flours. The phytate degradation rates of the yeast strains varied from 47.07 ± 0.11 to 93.82 ± 0.32%. The rate of phytate degradation by yeast strains ranges from 47.07 ± 0.11% to 93.82 ± 0.32%. The highest degradation (93.82 ± 0.32%) was recorded in white corn flour with the S4fbvp strain while the lowest degradation (47.07 ± 0.11%) was obtained in corn flour. industrial rice with the same strain S4fbvp. In generally, yeast strains have degraded more than 50% of the phytates contained in different types of flour. A significant difference (P>0.05) was observed between the different degradation rates of the yeast strains.

Cassava ferments are products rich in microorganisms, particularly yeasts. During this study, the yeast loads varied from one ferment to another. Our results corroborate those of Konan *et al.,* (2019) and Guira *et al.,* (2021) who reported a diversity of yeast strains in traditional cassava ferments. The differences in load observed could be due either to the type of ferment, to the production environment or to the personnel involved in the production process (Ramos *et al.,* 2015; Jara *et al.,* 2016). Furthermore, Oguntoyinbo *et al.,* (2016) reported that the production of traditional ferments is generally carried out in Africa through the use of simple non-sterile equipment, random or natural inocula and unregulated conditions. Also, Guira *et al.,* (2021) reported that the traditional starter production process may be the main factor in the variation in yeast load diversity.

The isolated yeast strains were tested for their probiotic abilities. Survival and proliferation in the host intestine are important criteria for probiotic selection. During their passage through the gastrointestinal tract, probiotics are exposed to different unfavorable conditions, such as an acidic environment (pH between 1.5 and 2.5) (Lacour and Belon, 2015), as well as to toxic nature of bile salts and digestive enzymes in the host intestine. Therefore, several selection criteria are used for the study of probiotics. pH is one of the parameters that influences the growth of probiotics in the gastrointestinal tract. Thus, in this study, the yeast strains studied were exposed to acidic (pH2) and alkaline (pH8) conditions and to temperature conditions similar to those encountered in the stomach. All these yeast strains were able to tolerate these different environments and show good growth. This indicates that these yeast strains are resistant to the acid that would be encountered in the gastrointestinal system and could maintain optimal cell growth there. The

growth of yeast strains under these conditions could allow them to survive in the unfavorable conditions of the gastrointestinal barrier. Indeed, the stomach is governed by an acidic pH (pH 2 to 3) and the intestine by a basic pH (pH 7.5 to 8) which would not constitute a limiting factor for the survival of the strains tested if they were found in such an environment. Also, the strong growth capacity of yeast strains at acidic pH would be related to their isolation medium. Indeed, the yeasts tested were isolated from cassava ferments, which is a spontaneously fermented food with an acidic pH of 3.9 (Egbune *et al.,* 2023). Due to their catabolism, yeasts are capable of acidifying their environment through the fermentation of sugars by the production of organic acids (Tran *et al.,* 2022), which would give them this resistance to acidity. The present study results are in agreement with those of Helmy *et al.,* (2019) and Wulan *et al.,* (2021) who reported that yeast isolates are able to withstand and survive acidic pH.

The results of this study also showed that all the yeast strains had good growth ability at 37°C and 42°C, knowing that 30°C is their optimal growth temperature. These results could be due to the fact that yeasts have a capacity to survive and adapt depending on the environment (Kumura *et al.,* 2004). The growth of yeast strains at a temperature of 37°C indicates that they could grow at a temperature of the digestive tract around 37°C. These results are similar to those of Wang *et al.,* (2024) who indicated the growth or tolerance of probiotic yeasts at the same temperatures studied, in particular 30°C, 37°C and 42°C. These results could be due to the fact that probiotic yeasts are able to grow at human body temperature and confer health benefits (Hossain *et al.,* 2020; Hsu and Chou, 2021).

Bile flow plays a very important physiological role since it facilitates the digestion of lipophilic compounds from food. It is also an antimicrobial agent influencing the establishment of the intestinal microbiota. During passage through the gastrointestinal tract, microorganisms resistant to biliary stress can colonize the intestine. Bile mainly affects the lipid and protein composition of the membrane thus disrupting its functionality. It also generates oxidative stress at the DNA level and impacts sugar metabolism (Begley *et al.,* 2005). Indeed, the optimal concentration of bile in the human intestinal environment varies from 0.3% to 0.9%. In this study, all yeast strains studied showed strong resistance to these concentrations of bile salts for their growth. This implies that the yeast strains studied can withstand the concentrations of bile in the human intestine. Bile tolerance is another essential criterion in the characterization of yeast strains as probiotics because it could allow their growth in the intestinal tract (Diguta *et al.,* 2023). The present study results corroborate those of Helmy *et al.,* (2019) and Maione *et al.,* (2024) who reported the resistance of yeast strains to bile salts as probiotics. These results could also be due to an exo-hydrolasic activity which allows them to resist the detergent action of bile salts by transforming them into harmless derivatives (Cotton *et al.,* 2016).

Growth or tolerance at different temperatures, low pH, in the presence of bile salts and sodium chloride (NaCl) solutions is considered an important selection criterion in the selection of probiotic strains that will likely survive in the intestine human (Menezes *et al.,* 2020). This tolerance is generally considered necessary to evaluate the ability of strains to resist the effects of NaCl.

Yeasts have a number of very important advantages, notably NaCl tolerance. The present study yeast strains were able to grow and tolerate concentrations of 1% NaCl and 2% NaCl, implying their use as a potential probiotic that can survive different NaCl concentrations of the digestive tract for pathogen inhibition. The present study results are consistent with those of Andrade *et al.,* (2019) and Wang *et al.,* (2024) who reported the tolerance of probiotic yeasts to NaCl concentrations.

The multivariate analysis carried out to group the yeast strains tested according to the different probiotic properties evaluated led to two large distinct groups. It appears that the S3fbs and S4fbvp strains presented the best probiotic profiles. These results corroborate those of several authors (Lazo-Vélez *et al.,* 2018; Hossain *et al.,* 2020; Hsu and Chou, 2021) who have reported the probiotic potential of several globally recognized yeast strains including *S. cerevisiae* and *S. cerevisiae* var. boulardii.

Yeast strains selected for their ability to degrade phytate and their probiotic aptitude were tested by their incorporation into rice and corn flours containing high concentrations of phytates with a view to potential reduction. Indeed, phytates represent the main form of phosphorus storage in plants and mainly in cereal grains which are also the main ingredients used in food. Thus, in general, the selected yeast strains made it possible to reduce more than 50% of phytates in rice and corn flours. These results relate to those of Karaman *et al.,* (2018)

and Menezes *et al.,* (2020) who indicated the capacity of yeast isolates to solubilize phytate, an abundant antinutritional factor in cereals. Furthermore, Fang *et al.,* (2023) reported that mixed sourdough fermentation of *S. cerevisiae* yeasts and lactic acid bacteria promotes much greater degradation of phytate in the breadmaking of fermented wheat dough. This degradation would be due to a high production of phytases, notably cellulolytic enzymes such as β-glucanase, mannanase and xylanases of selected yeast strains. Indeed, Fekri *et al.,* (2020) reported that phytate reduction is linked to synthesized microbial phytase. However, the highest phytate degradation rate obtained in this study would be lower than that obtained (96.6%) by Fang *et al.,* (2023). This difference could be due to a lower phytase activity of the selected yeast strains.

Table.1 Average loads of yeast strains of different cassava ferments

Cassava ferments	Average loads of yeast strains (CFU/g)
Fresh cassava ferments	$(1,6\pm0,2) \times 10^{6d}$
Braised cassava ferments	$(2,9\pm0,8) \times 10^{6c}$
Cassava ferments boiled without the peel	$(1,7\pm0,0) \times 10^{7a}$
Cassava ferments boiled with the peel	$(5,6\pm0,3) \times 10^{6b}$

In the same column, values bearing the same letters are not significantly different at the 5% threshold according to Tukey's test.

Table.2 Phytate hydrolysis diameters of yeast strains isolated from cassava ferments

Yeast strains	Phytate hydrolysis diameters (cm)
S1ff	$0,65 \pm 0,07^{c}$
S2ff	$0,5 \pm 0,14^{c}$
S3ff	$0,75 \pm 0,07^{c}$
S4ff	$2,05 \pm 0,01^{a}$
S5ff	$2,15 \pm 0,07^{a}$
S1fbr	$2,07 \pm 0,14^{a}$
S2fbr	$1,3 \pm 0,00^{b}$
S3fbr	$2,08 \pm 0,07^{a}$
S4fbr	$1,05 \pm 0,07^{bc}$
S5fbr	$1,05 \pm 0,2^{bc}$
S1fbs	$1,25 \pm 0,07^{bc}$
S2fbs	$1,15 \pm 0,07^{bc}$
S3fbs	$2,20 \pm 0,00^{a}$
S4fbs	$1,6 \pm 0,14^{ab}$
S5fbs	$2 \pm 0,14^{a}$
S1fbvp	$2,15 \pm 0,07^{a}$
S2fbvp	$1,65 \pm 0,07^{ab}$
S3fbvp	$1,7 \pm 0,14^{ab}$
S4fbvp	$2,25 \pm 0,07^{a}$
S5fbvp	$1,45 \pm 0,07^{b}$

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel.

Table.3 Growth of yeast strains at acidic and basic pH after 4 hours and 24 hours of incubation

Yeast	Time	Yeast load (CFU/mL)				
		pH 2	pH 2,5	pH control	pH 8	pH 8,5
S1fbr	4h	$(1,5\pm0,2)10^{5e}$	$(5,4\pm1,7)10^{5d}$	$(2,8\pm0,0)10^{6C}$	$(1,7\pm0,4)10^{5d}$	$(9,8\pm1,2)10^{5d}$
	24h	$(1,9\pm0,2)10^{6c}$	$(7,2\pm 1,0)10^{6c}$	$(3,2\pm0,4)10^{7a}$	$(1,9\pm0,0)10^{7bc}$	$(1,6\pm0,0)10^{7b}$
S3fbr	4h	$(4,1\pm0,6)10^{5d}$	$(2,0\pm0,2)10^{6ab}$	$(8,1\pm1,2)10^{5e}$	$(3,7\pm0,3)10^{6b}$	$(1,8\pm0,2)10^{6c}$
	24h	$(7,8\pm0,0)10^{5de}$	$(4,2\pm0,8)10^{6d}$	$(4,1\pm0,1)10^{7a}$	$(4,0\pm0,5)10^{7a}$	$(3,9\pm0,4)10^{7a}$
S3fbs	4h	$(2,2\pm0,4)10^{6e}$	$(1,2\pm0,1)10^{6bc}$	$(4,1\pm0,3)10^{6b}$	$(7,9\pm0,7)10^{6ab}$	$(3,4\pm0,3)10^{6b}$
	24h	$(2,3\pm0,0)10^{7a}$	$(4,5\pm0,0)10^{7a}$	$(3,6\pm0,0)\ 10^{7a}$	$(4,9\pm0,2)10^{7a}$	$(3,9\pm0,2)10^{7a}$
S5fbs	4h	$(1,9\pm1,0)10^{6bc}$	$(9,1\pm4,9)10^{5cd}$	$(4,2\pm2,2)10^{6b}$	$(5,4\pm2,9)10^{6b}$	$(1,7\pm0,9)10^{6c}$
	24h	$(2,3\pm1,2)10^{6bc}$	$(1,6\pm0,9)10^{7b}$	$(4,3\pm2,3)10^{7a}$	$(3,9\pm2,1)10^{7a}$	$(3,9\pm2,0)10^{7a}$
S4ff	4h	$(1,2\pm0,1)10^{6c}$	$(1,7\pm0,4)10^{5e}$	$(4,0\pm0,4)10^{6b}$	$(9,0\pm1,2)10^{5c}$	$(3,7\pm0,3)10^{6b}$
	24h	$(5,2\pm0,2)10^{5e}$	$(1,6\pm0,4)10^{5e}$	$(4,2\pm0,3)10^{7a}$	$(1,1\pm0,2)10^{7c}$	$(4,0\pm0,1)10^{7a}$
S5ff	4h	$(2,1\pm0,2)10^{6ab}$	$(1,0\pm0,0)10^{6cd}$	$(1,7\pm0,2)10^{6d}$	$(8,9\pm1,2)10^{5c}$	$(3,3\pm0,8)10^{5e}$
	24h	$(9,5\ \pm2,4)10^{5d}$	$(1,5\pm0,2)10^{5f}$	$(1,1\pm0,2)10^{7b}$	$(1,8\pm0,5)10^{7c}$	$(1,5\pm0,2)10^{7b}$
S1fbvp	4h	$(3,3\pm0,2)10^{6a}$	$(3,4\pm0,5)10^{6a}$	$(1,5\pm0,0)10^{7a}$	$(1,6\pm0,1)10^{7a}$	$(1,6\pm0,1)10^{7a}$
	24h	$(3,7\pm0,3)10^{7a}$	$(4,3\pm0,2)10^{7a}$	$(4,7\pm0,3)10^{7a}$	$(3,1\pm0,1)10^{7ab}$	$(2,9\pm0,2)10^{7a}$
S4fbvp	4h	$(1,9\pm0,3)10^{6bc}$	$(8,9\pm1,4)10^{5cd}$	$(1,5\pm0,0)10^{7a}$	$(1,4\pm0,0)10^{7a}$	$(1,0\pm0,1)10^{7a}$
	24h	$(3,5\pm0,6)10^{6b}$	$(1,9\pm0,2)10^{6e}$	$(1,4\pm0,1)10^{7b}$	$(3,3\pm0,2)10^{7a}$	$(3,8\pm0,4)10^{7a}$

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel; h: hour.

Table.4 Growth of yeast strains at different temperatures after 4 hours and 24 hours of incubation

Yeast strains	Time	Yeast load (CFU/mL)		
		30°C	37°C	42°C
S1fbr	4h	$(6,5\pm0,8)10^{6bc}$	$(3,7\pm0,00)10^{6f}$	$(3,8\pm0,9)10^{5c}$
	24h	$(4,2\pm0,4)10^{7abc}$	$(3,3\pm 0,3)10^{7b}$	$(5,2\pm0,6)10^{5cd}$
S3fbr	4h	$(6,8\pm0,3)10^{5bc}$	$(6,9\pm0,5)10^{6e}$	$(2,4\pm0,2)10^{5c}$
	24h	$(4,9\pm0,1)10^{7a}$	$(4,6\pm0,2)10^{7ab}$	$(3,6\pm0,00)10^{5d}$
S3fbs	4h	$(5,7\pm0,2)10^{6bc}$	$(1,5\pm0,2)10^{7bc}$	$(1,4\pm0,1)10^{6a}$
	24h	$(4,5\pm0,2)10^{7ab}$	$(4,4\pm0,7)10^{7ab}$	$(5,4\pm0,3)\ 10^{6a}$
S5fbs	4h	$(7,8\pm4,2)10^{6bc}$	$(1,0\pm0,6)10^{7cd}$	$(1,8\pm0,9)10^{6a}$
	24h	$(5,1\pm2,7)10^{7a}$	$(3,4\pm1,8)10^{7b}$	$(3,8\pm2,1)10^{5d}$
S4ff	4h	$(1,1\pm0,3)10^{7ab}$	$(3,5\pm0,6)10^{6f}$	$(2,2\pm0,3)10^{6a}$
	24h	$(5,0\pm0,4)10^{7a}$	$(5,2\pm0,4)10^{7a}$	$(1,9\pm0,3)10^{6b}$
S5ff	4h	$(4,9\pm0,6)10^{6c}$	$(8,2\pm0,4)10^{6de}$	$(6,8\pm0,9)10^{5b}$
	24h	$(3,3\ \pm0,1)10^{7c}$	$(3,9\pm0,4)10^{7ab}$	$(7,7\pm0,6)10^{5c}$
S1fbvp	4h	$(2,2\pm0,6)10^{7a}$	$(2,4\pm0,1)10^{7a}$	$(7,3\pm0,4)10^{5b}$
	24h	$(3,6\pm0,3)10^{7bc}$	$(3,5\pm0,2)10^{7ab}$	$(7,9\pm0,9)10^{5c}$
S4fbvp	4h	$(1,1\pm0,1)10^{7ab}$	$(1,8\pm0,2)10^{7ab}$	$(1,7\pm0,2)10^{6a}$
	24h	$(2,37\pm0,24)10^{7a}$	$(4,9\pm0,5)10^{7ab}$	$(6,7\pm0,8)10^{7c}$

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel; h: hour.

Table.5 Growth of yeast strains at different NaCl concentrations after 4 hours and 24 hours of incubation

Yeast strains	Time	Yeast load (CFU/mL)		
		0%	1%	2%
S1fbr	4h	$(2,8\pm0,0)10^{6C}$	$(1,1\pm0,0)10^{6c}$	$(2,3\pm0,3)10^{5c}$
	24h	$(3,2\pm0,4)10^{7a}$	$(1,4\pm0,2)10^{6c}$	$(1,67\pm 0,09)10^{7d}$
S3fbr	4h	$(8,1\pm1,2)10^{5e}$	$(1,2\pm0,1)10^{6ab}$	$(3,2\pm0;3)10^{5c}$
	24h	$(4,1\pm0,1)10^{7a}$	$(4,4\pm0,2)10^{7a}$	$(4,9\pm0,0)10^{6ab}$
S3fbs	4h	$(4,1\pm0,3)10^{6b}$	$(3,3\pm0,4)10^{5abc}$	$(3,1\pm0,6)10^{5bc}$
	24h	$(3,6\pm0,0)\ 10^{7a}$	$(2,3\pm0,1)10^{7a}$	$(3,8\pm0,2)10^{7c}$
S5fbs	4h	$(4,2\pm2,2)10^{6b}$	$(1,2\pm1,0)10^{6abc}$	$(5,9\pm3,2)10^{5ab}$
	24h	$(4,3\pm2,3)10^{7a}$	$(3,4\pm1,8)10^{7a}$	$(2,3\pm1,2)10^{7bc}$
S4ff	4h	$(4,0\pm0,4)10^{6b}$	$(1,2\pm0,1)10^{5bc}$	$(2,2\pm0,2)10^{5c}$
	24h	$(4,2\pm0,3)10^{7a}$	$(1,92\pm0,06)10^{7b}$	$(4,4\pm0,3)10^{7abc}$
S5ff	4h	$(1,7\pm0,2)10^{6d}$	$(5,2\pm5,7)10^{5abc}$	$(6,3\pm0,6)10^{5ab}$
	24h	$(1,1\pm0,2)10^{7b}$	$(2,2\pm0,3)10^{6b}$	$(4,3\pm0,3)10^{5abc}$
S1fbvp	4h	$(1,6\pm0,0)10^{7a}$	$(1,9\pm0,2)10^{6a}$	$(2,5\pm0,6)10^{5c}$
	24h	$(4,7\pm0,3)10^{7a}$	$(3,9\pm0,1)10^{7a}$	$(4,2\pm0,1)10^{7bc}$
S4fbvp	4h	$(1,5\pm0,0)10^{7a}$	$(9,7\pm0,6)10^{5ab}$	$(6,6\pm0,2)10^{5a}$
	24h	$(1,4\pm0,1)10^{7b}$	$(4,7\pm0,4)10^{7a}$	$(5,3\pm0,4)10^{7a}$

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel; h: hour.

Table.6 Growth of yeast strains at different of bile salts concentrations after 4 hours and 24 hours of incubation

Yeast strains	Time	Yeast load (CFU/mL)		
		0,3%	0,6%	0,9%
S1fbr	4h	$(5,4\pm0,2)10^{6bc}$	$(1,1\pm0,0)10^{6d}$	$(2,6\pm0,3)10^{6c}$
	24h	$(1,5\pm0,1)10^{7b}$	$(2,2\pm0,0\ )10^{7bc}$	$(2,8\pm0,0)10^{7bc}$
S3fbr	4h	$(4,6\pm0,4)10^{6c}$	$(7,8\pm0,8)10^{6abc}$	$(5,4\pm0,3)10^{6b}$
	24h	$(3,3\pm0,3)10^{7a}$	$(1,5\pm0,1)10^{7c}$	$(4,1\pm0,1)10^{7ab}$
S3fbs	4h	$(6,7\pm0,9)10^{6bc}$	$(5,7\pm0,7)10^{6bc}$	$(4,6\pm0,6)10^{6b}$
	24h	$(4,7\pm0,3)10^{7a}$	$(3,1\pm0,3)10^{7ab}$	$(4,2\pm0,1)10^{7ab}$
S5fbs	4h	$(6,4\pm3,5)10^{6bc}$	$(8,5\pm4,6)10^{6ab}$	$(5,6\pm3,0)10^{6b}$
	24h	$(4,0\pm2,1)10^{7a}$	$(4,2\pm2,3)10^{7a}$	$(3,3\pm1,8)10^{7ab}$
S4ff	4h	$(7,4\pm0,6)10^{6b}$	$(7,8\pm1,9)10^{6abc}$	$(2,8\pm0,0)10^{6c}$
	24h	$(3,37\pm0,23)10^{7a}$	$(4,3\pm0,0)10^{6a}$	$(1,9\pm0,2)10^{7c}$
S5ff	4h	$(6,7\pm0,4)10^{6bc}$	$(5,0\pm0,3)10^{6c}$	$(1,4\pm0,0)10^{6d}$
	24h	$(1,9\ \pm0,3)10^{7b}$	$(1,9\pm0,7)10^{7bc}$	$(1,5\pm3,9)10^{6d}$
S1fbvp	4h	$(1,7\pm0,4)10^{7a}$	$(1,2\pm0,2)10^{7a}$	$(2,1\pm0,1)10^{7a}$
	24h	$(4,17\pm0,44)10^{7a}$	$(1,7\pm0,3)10^{7c}$	$(4,4\pm0,2)10^{7a}$
S4fbvp	4h	$(7,25\pm0,35)10^{6b}$	$(7,9\pm0,3)10^{6abc}$	$(6,4\pm0,9)10^{6b}$
	24h	$(5,0\pm0,7)10^{7a}$	$(4,3\pm0,3)10^{7a}$	$(5,0\pm0,3)10^{7a}$

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel; h: hour.

Table.7 Viability rate of yeast strains in the presence of the digestive enzymes pepsin and pancreatin

	Viability rates of yeast (%)			
Yeast strains	Pepsin	Control pepsin	Control pancreatin	Pancreatin
S1fbr	(92,15±1,95)^{ab}	(94,93±0,85)^{a}	(89,56±1,09)^{a}	(83,68±2,70)^{b}
	(93,04±1,19)^{ab}	(97,39±2,22)^{a}	(96,34±1,59)^{a}	(98,30±2,07)^{a}
S3fbr	(95,54±2,50)^{a}	(90,83±3,73)^{a}	(92,34±2,94)^{a}	(85,04±1,51)^{b}
	(92,36±6,71)^{ab}	(93,32±0,99)^{a}	(91,43±2,33)^{a}	(88,71±4,80)^{ab}
S3fbs	(95,93±0,40)^{a}	(91,96±1,59)^{a}	(90,94±5,74)^{a}	(83,40±4,49)^{b}
	(89,29±4,94)^{ab}	(95,09±2,16)^{a}	(94,71±0,47)^{a}	(85,85±2,90)^{b}
S5fbs	(80,61±1,95)^{b}	(94,44±4,19)^{a}	(96,22±1,60)^{a}	(92,12±0,91)^{ab}
	(92,03±1,08)^{ab}	(96,43±3,21)^{a}	(92,13±1,87)^{a}	(83,03±0,33)^{b}
S4ff	(92,15±1,95)^{ab}	(94,93±0,85)^{a}	(89,56±1,09)^{a}	(83,68±2,70)^{b}
	(93,04±1,19)^{ab}	(97,39±2,22)^{a}	(96,34±1,59)^{a}	(98,30±2,07)^{a}
S5ff	(95,54±2,50)^{a}	(90,83±3,73)^{a}	(92,34±2,94)^{a}	(85,04±1,51)^{b}
	(92,36±6,71)^{ab}	(93,32±0,99)^{a}	(91,43±2,33)^{a}	(88,71±4,80)^{ab}
S1fbvp	(95,93±0,40)^{a}	(91,96±1,59)^{a}	(90,94±5,74)^{a}	(83,40±4,49)^{b}
	(89,29±4,94)^{ab}	(95,09±2,16)^{a}	(94,71±0,47)^{a}	(85,85±2,90)^{b}
S4fbvp	(80,61±1,95)^{b}	(94,44±4,19)^{a}	(96,22±1,60)^{a}	(92,12±0,91)^{ab}
	(92,03±1,08)^{ab}	(96,43±3,21)^{a}	(92,13±1,87)^{a}	(83,03±0,33)^{b}

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel; h: hour.

Table.8 Self-aggregation rates (%) capacity of yeasts strains after 4 hours and 24 hours of incubation

Yeast strains	**Self-aggregation rates (%)**	
	4 hours	24 hours
S1fbr	(75,70±1,0)^{a}	(79,68±0,5)^{a}
S3fbr	(51,13±2,64)^{c}	(85,38±0,88)^{a}
S3fbs	(84,96±0,1)^{b}	(91,24±1,1)^{a}
S5fbs	(63,73±1,79)^{d}	(94,49±0,73)^{a}
S4ff	(53,88±0,17)^{d}	(88,98±1,44)^{a}
S5ff	(50,61±1,96)^{d}	(88,44±0,79)^{a}
S1fbvp	(73,06±2,73)^{d}	(81,57±2,01)^{a}
S4fbvp	(80,15±2,62)^{d}	(89,75±0,35)^{a}

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel; h: hour.

Table.9 Adhesion rate of yeasts strains to hydrocarbons after 4 hours and 24 hours of incubation

Yeast strains	Adhesion rates of yeast (%)	
	Xylene	Chloroform
S1fbr	(17.6 ±3.4)^{c}	(32.10±1.3)^{d}
S3fbr	(33.57±0.61)^{b}	(85.06±2.74)^{a}
S3fbs	(31.70±1.8)^{b}	(70.30±1.0)^{b}
S5fbs	(52.94±1.49)^{a}	(62.63±1.94)^{c}
S4ff	(31.43±0.81)^{b}	(70.95±2.89)^{b}
S5ff	(14.81±1.67)^{c}	(33.92±1.52)^{d}
S1fbvp	(46.87±0.18)^{a}	(34.85±1.62)^{d}
S4fbvp	(40.25±1.04)^{d}	(57.38±1.99)^{e}

In the same column and for the same time, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S: yeast strain; ff: braised cassava ferment; fbr: braised cassava ferment; fbs: boiled cassava ferment without the peel; fbvp: boiled cassava ferment with the peel; h: hour.

Table.10 Degradation of phytates by yeast strains in corn and rice flours

Food	Fermentation	Yeast strains	Phytates concentrations (µg/mL)	Degradation rate (%)
Local rice flour	Before fermentation		164.21±9.66^{a}	-
	Before	Control	157.71±10.37^{a}	3.98±0.66^{i}
	fermentation	S3fbs	43.33±0.77^{b}	73.55±2.03^{cdef}
		S4fbvp	40.74±2.13^{b}	75.11±2.76^{cde}
Industrial rice flour	Before fermentation		75.41±3.86^{a}	-
	Before	Control	73.91±1.74^{a}	1.92±2.71^{i}
	fermentation	S3fbs	33.09±1.74^{b}	56.01±4.56^{gh}
		S4fbvp	39.92±2.13^{b}	47.07±0.11^{h}
White corn flour	Before fermentation		82.24±1.93^{a}	-
	Before	Control	79.74±2.64^{a}	3.05±0.93^{i}
	fermentation	S3fbs	17.10±1.16^{b}	79.18±1.90^{bcd}
		S4fbvp	5.08±0.39^{c}	93.82±0.32^{a}

In the same column, charges bearing the same letters are not significantly different at the 5% threshold according to Tukey's test. S3fbs: strain 3 of boiled cassava ferment without the peel; S4fbvp: strain 4 of boiled cassava ferment with the peel; Control: sample without yeast strains.

Figure.1 Photographs of traditional cassava ferments (Figure.1-A, 1-B, 1-C and 1-D) and of hulled corn grains (Figure.1-E) and rice (Figure.1-F and 1-G).

Figure.2 Production diagram of corn and rice flour

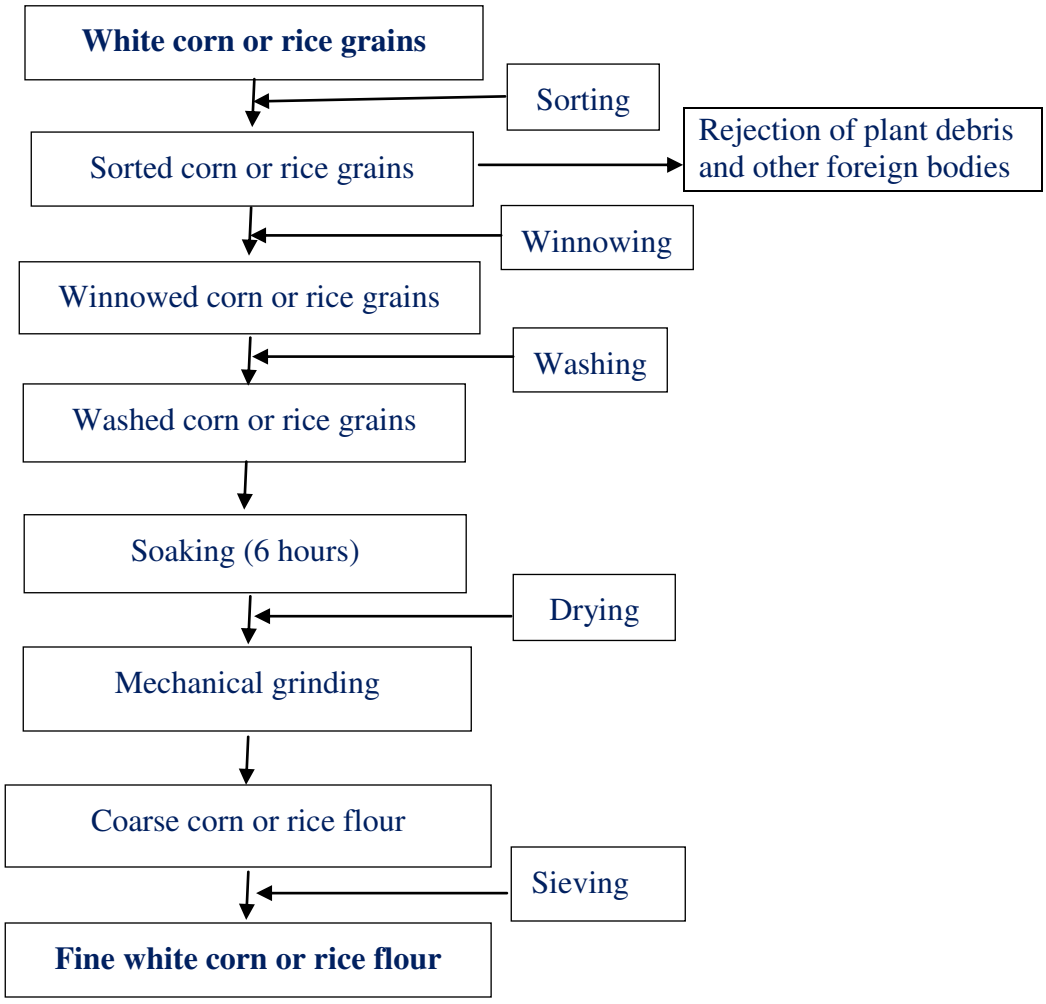
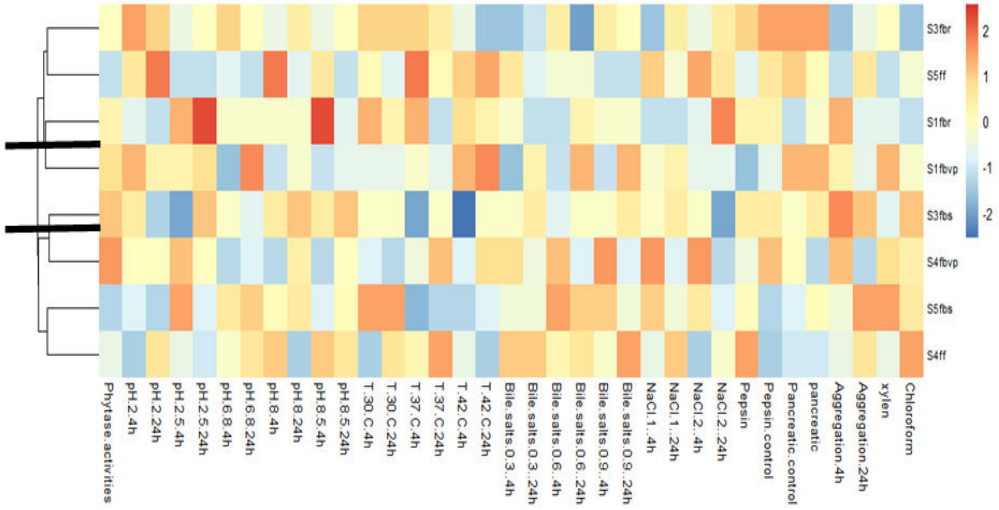


Figure.3 Hierarchical heat map showing the classification of yeasts strains isolated from traditional cassava ferments based on probiotic property analysis parameters



In an approach to evaluate a relationship between yeast and surface cells in the gastrointestinal tract, several adhesion tests were performed. The majority of yeast isolates exhibited a strong capacity for self-aggregation and solvent adhesion. These results are consistent with those of Oliveira *et al.,* (2017) and Fernández-Pacheco *et al.,* (2018) who reported the self-aggregation capacities of yeast strains isolated during the evaluation of their probiotic potential. The strong self-aggregation capacity would prevent the invasion of various other microorganisms through the formation of a biofilm and then also lead to increased persistence in the gastrointestinal tract which would only be possible thanks to surface proteins (Collado *et al.,* 2008). The criterion of adhesion to epithelial cells is widely taken into account in the evaluation of the probiotic properties of a yeast strain. Thus, the adhesion of the strains studied to xylene and chloroform would mimic the conditions of epithelial cells. In this study, the studied strains showed solvent adhesion capacity, therefore indicating their abilities to adhere to the gastric mucosa with low kinetic elimination. Which implies that the yeast strains studied can adhere to the gastric mucosa thus offering the prospective function as a potentially probiotic supplement for human well-being as reported by Helmy *et al.,* (2019). These results are similar to those of Lara-Hidalgo *et al.,* (2019) who noted high rates of adhesion of yeast strains to hydrocarbons, notably chloroform and xylene. This behavior can also lead to a reduction in the adhesion of pathogens and their toxins to intestinal epithelial cells by attaching to the same receptor sites.

In the gastrointestinal tract, several enzymes are involved in the digestion of food consumed, notably pepsin (pH 2, stomach) and pancreatin (pH 8, small intestine). These enzymes are also considered selection criteria because they are capable of preventing the beneficial actions of certain probiotics. Indeed, after being ingested, the yeasts encounter drastic conditions in the stomach, since the food remains there for around 4 hours at a pH close to 2.

At pH 2, most yeasts show very good growth in depending on the exposure time and the conditions of their growth medium. Thus, certain strains tested during this work all showed resistance to pepsin and pancreatin. These results are similar to those obtained by Porru *et al.,* (2018) and Sen and Mansell (2020) who showed that yeast species could maintain viability in a simulated gastric environment. Also, the work of Palla *et al.,* (2019) showed that the beneficial actions of yeast strains are not affected by the presence of digestive enzymes, notably pepsin.

At the end of this work, it appears that the demonstration of phytase production made it possible to reveal significant phytase activities of yeast strains isolated from traditional cassava ferments. The yeasts studied were able to tolerate, grow and survive stimulated gastrointestinal conditions including different pH (acidic and basic), temperature, different concentrations of NaCl and bile salts. The results indicate that two yeast strains selected on the basis of the probiotic profiles studied and the phytase production capacity were mainly able to

degrade more than 50% of the phytates contained in corn and rice flour. Due to these interesting probiotic properties, these yeast strains could be considered as promising candidates for future biotechnological developments.

Data

Data used to support the findings of this study are included in the article.

Author Contributions

Zamblé Bi Irié Abel Boli: Designed the study, carried out the literature searches, drafted the experimental protocol and participated in writing the final manuscript. Ekoua Regina Krabi: Drafted the experimental protocol and participated in writing the final manuscript. Koky Marc Celaire N'sa: Conceived the experimental protocol and analysed the data. Koffi Maïzan Jean-Paul Bouatenin: Participated in writing and conceived the study. Wahauwouele Hermann Coulibaly: Corrected the experimental protocol and the final manuscript. Konan Rocard Konan: Participated in writing the final manuscript. Rose Koffi-Nevry: Participated of the designed the study, corrected the experimental protocol and the final manuscript. Marina Koussemon: Corrected the experimental protocol and the final manuscript.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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