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NZN designed the research study and curated, analyzed and validated the data. BMOS and BBDO performed the research and analyzed the data, NZN, BMOS and BBDO wrote the manuscript. JJEN conceptualized and supervised the study. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the manuscript.

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Effect of Combined Microbial Starter Fermentation and Packaging Type on Shelf Life, Quality, and Sensory Acceptability of *Bobolo*: a Traditional Cameroonian Fermented Cassava Product

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Abstract:

Bobolo, or Cassava stick, is a fermented product widely consumed in Cameroon. This cassava sub-product faces preservation challenges that limits its shelf life because of its high moisture content and the traditional use of Marantaceae leaves for packaging. The main objective of this study was to evaluate the combined effect of fermentation and packaging on the shelf life, quality, and sensory acceptability of Bobolo. To achieve this, the fermentation with and without a starter were compared with respect to detoxification and the acceleration of cassava roots softening. The cassava doughs from these two fermentation methods were wrapped in baking paper and cooked to produce Bobolo; the physicochemical, microbiological, and sensory qualities of the products were analyzed over a 9-day storage period and compared with Bobolo wrapped in Marantaceae leaves. The results show that the use of a microbial starter significantly accelerated retting of cassava roots and consequent softening, reducing the duration from 75 hours to only 26 hours. Additionally, the starter achieved a slightly higher level of detoxification (93.46%) compared to spontaneous fermentation (91.27%). Starter-fermented Bobolo packaged in baking paper showed the highest moisture loss of 12.73%, while maintaining good control of microbial growth during storage. Regarding sensory acceptability, spontaneous fermentation combined with traditional Marantaceae leaf packaging yielded the highest level of overall acceptability (7.84 ± 1.02). Conversely, products fermented with the starter and wrapped in baking paper were the least preferred (6.84 ± 1.33).

Keywords: Bobolo, cassava, detoxification, fermentation, packaging, preservation.



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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of principal source of calories in sub-Saharan Africa countries like Cameroon, where fermented products such as Bobolo are widely consumed (Eyenga *et al.*, 2024). Bobolo, also called *Baton de manioc* or cassava stick, is a steamed dough wrapped mainly in Marantaceae leaves and generally prepared from cassava roots through the retting process (spontaneous submerged fermentation) (Nardis *et al.*, 2021). Bobolo is appreciated for its soft texture, sour flavor, and cultural significance, but it faces severe post-production spoilage challenges due to its high moisture content (40-45%) and permeable traditional packaging (Tanyitiku, 2024). In fact, the Marantaceae leaves (e.g., *Megaphrynium macrostachyum* or *Sarcophrynium brachystachy*) used for wrapping, offer poor water vapor barriers (Onzo *et al.*, 2014; Kiki-Mvouaka *et al.*, 2023). This have consequence of promoting microbial growth and limiting shelf life to 2-3 days under ambient tropical conditions and exacerbating food waste and health risks from pathogens like *Bacillus cereus*, *Salmonella* spp. (Ekeledo *et al.* 2024).

Spontaneous fermentation of cassava depends on indigenous microbial flora and the hydrolytic enzymes they produced to soften roots, detoxify cyanogenic glucosides and provide the characteristic aromas of fermented products (Djoulde *et al.*, 2003; Padonou *et al.*, 2010; de Sousa Cavalcante *et al.*, 2025). However, this unit operation is inconsistent, taking 72-120 hours for retting and yielding variable detoxification (80-92%) influenced by environmental factors, microbial succession and cassava variety (Didier-Olivier *et al.*, 2025). Traditional Microbial starters such as Sta_96, have shown promise in reducing retting time by up to 50%, enhancing hydrolysis of hydrocyanic acid to achieve up to 98% detoxification, and improving the uniformity of African cassava products such as Bobolo or Chikwangue (Nardis *et al.*, 2021). This starter is composed of previously fermented cassava flour and a

defined consortium of Lactic acid bacteria, yeasts, molds and cyanide-degrading bacteria (e.g., *Bacillus subtilis*).

The improvement of Sta_96 by its combination with a *Streptomyces* STF16, in previous work (Didier-Olivier *et al.*, 2026) have shown promise in retting of cassava roots to about 24 hours. Despite these biotechnological advances, no study to date has evaluated the effect of this enhancement on the microbiological, sensory, and physicochemical quality of cassava by-products obtained through retting, such as Bobolo, during storage. Furthermore, few investigations have examined the combined influence of such starter improvements and packaging innovations on fermented cassava foods, particularly regarding sensory acceptability, which remains a key determinant of consumer adoption in traditional markets.

This study aimed to evaluate the combined effects of spontaneous vs. starter-assisted fermentation and traditional Marantaceae leaves vs. baking paper packaging on Bobolo's shelf life and sensory acceptability by Cameroonian panelists. With hypothesis that starters would accelerate retting and detoxification while modern packaging extends stability, though sensory profiles might deviate from traditional preferences. These findings aim to inform scalable biotechnological interventions for enhancing food safety and marketability of indigenous African products.

MATERIALS AND METHODS

Cassava roots and starter culture preparation

Fresh bitter cassava (*Manihot esculenta* Crantz) roots of the local variety "Six-mois" were harvested at maturity (8 months) from farm in Goufan II-Bafia locality, Centre Region, Cameroon (geographical coordinates: 4°42'00" N, 11°15'00" E). this variety is typically characterized by dark green foliage, petioles that are green to reddish in color, and tubers with a

beige to light brown skin and white flesh, although these traits may vary according to plant populations and growing conditions. It was selected for its prevalence in Cameroonian fermentation processes, highly cyanogenic glycoside content (superior to 100 mg HCN/kg fresh weight), and high starch content (70-74% dry matter basis). Roots with no visible defects and 8-10cm diameter were selected for uniformity, washed under running tap water, peeled, and cut into 5-cm cylinders of approximately 200 g each. Two fermentation treatments were applied with twenty-five kilograms of peeled cylinders: (i) spontaneous fermentation (control) and (ii) starter-assisted fermentation. The starter culture consisted of a mixed consortium of previously fermented cassava flour, *Sta_96*, inoculated at 10^6 CFU/g of fermented flour, with *Streptomyces* sp. isolated from this *Sta_96*. The mixed starter was inoculated at 1g/100g of roots (total 1% w/w). Each treatment was submerged in sterile plastic containers filled with tap water in ratio of 1:1 (w/v) at ambient temperature ($28 \pm 2^\circ\text{C}$).

Retting progression was monitored over 3 days. Every 3 h, six randomly selected cylinders from each treatment were tested for firmness using a penetrometer. The softening level was expressed as the mean penetration depth (six measurements per cylinder) (Nardis *et al.*, 2016). The penetration measurements were performed using the Penetrometer RPN10 Berlin model; the pressure was applied at a controlled rate of 10mm/s to insure reproducibility and accuracy of the measurements. Penetration data obtained from each time point were compiled in Microsoft Excel 2016 and fitted using DM-fit software. The resulting sigmoid kinetics were modeled according to the equation described by Baranyi and Roberts (1994), thereby, enabling calculation of the retting time as follows:

$$D_r = \text{Lag} + (L/\mu)$$

Where, D_r , Lag, L and μ are respectively the retting time (h), the time required to initiate fermentation (h), the critical penetrometry index (cm/h) and the softening speed (cm/h).

The percentage reduction of the retting time of each strain was calculated as follows:

$$\begin{aligned} \text{Percentage of retting time reduction (\%)} \\ = \left(1 - \frac{\text{Retting time of strain}}{\text{Retting time of control}} \right) \times 100 \end{aligned}$$

Detoxification assessment

Cyanide detoxification was quantified by measuring residual hydrogen cyanide (HCN) equivalents in retted cassava roots each 3 hours using the method of Bradbury *et al.* (1999). Briefly, 0.1 g in triplicate samples of fermented cassava root was placed into screw-cap vials containing 1 mL of phosphate buffer (pH 6.0) and a strip of picrate paper. Vials were sealed and incubated at 30°C for 18 h to allow cyanide interaction with the picrate paper. Thereafter, the picrate papers were transferred into test tubes containing 5 mL distilled water and boiled for 5 min. Following boiling, the strips were removed and the solution was allowed to cool to room temperature ($28 \pm 2^\circ\text{C}$) before further analysis. The absorbance of the cooled solution was measured at 510 nm using a UV-Vis spectrophotometer Jenway 7305 model. The blank control consisted of the boiled solution from a picrate paper strip that had not been exposed to cyanide to ensure accurate baseline correction. Total cyanide content (ppm) was calculated by multiplying the absorbance by 396, as described by Bradbury *et al.* (1999) and expressed as mg HCN/kg fresh weight. Detoxification efficiency was calculated as:

$$\% \text{Detoxification efficiency} = \left(1 - \frac{[\text{HCN}]_{\text{retted}}}{[\text{HCN}]_{\text{raw}}} \right) \times 100$$

Bobolo production and packaging

Bobolo production was processed according to the protocol of Eyenga *et al.* (2024). Fully retted roots were dewatered (moisture ~50%), by pressing through muslin cloth in order to allow expressed liquid to drain. Then, grated mechanically, the water content of the resulting paste was adjusted to approximately 55% and portioned into 200-g units. Four treatments were

prepared (n=12 per treatment, 3 replicates × 4 storage times):

- T1: Spontaneous fermentation + Marantaceae leaves (traditional).
- T2: Spontaneous fermentation + baking paper.
- T3: Starter fermentation + Marantaceae leaves.
- T4: Starter fermentation + baking paper.

Marantaceae leaves (*Megaphrynium macrotachyum*) were collected in a local market and washed with tap water to remove dust. Baking paper (food-grade, silicone-coated, 45 g/m²) was cut to 60×20 cm sheets. Dough portions of the two type of fermentation type were wrapped in double layer in leaves and paper, traditionally tied using thread derived from dried banana leaves, steamed in a traditional aluminum pot at 100°C, for 45 min until internal temperature reached 90°C. after cooking, Bobolo were cooled to 25°C, and stored at ambient conditions of the room temperature of 28 ± 2°C and 80 ± 5% relative humidity for 0, 3, 6, and 9 days.

Physico-chemical analyses

Physico-chemical analyses consisting in determination of moisture content and texture of Bobolo samples during 9 days storage were performed in triplicate per treatment/time point. Moisture content was determined using loss-on-drying thermogravimetric method in electronic moisture analyzer MCA110-1Infitek model (Infitek Co., Ltd. Jinan, Shandong Province, China). Briefly, small sample (typically 2-5g) were placed on aluminum pan inside the analyzer, and weighed automatically to obtain the initial mass (M_{initial}). The mass sample obtained was then heated by an integrated halogen lamp at 105°C until the mass loss became constant (M_{dry}). The value of moisture content of sample was given automatically by analyzer after calculation as:

$$\text{Moisture content (\%)} = \frac{M_{\text{initial}} - M_{\text{dry}}}{M_{\text{initial}}} \times 100$$

The texture (firmness) of Bobolo was evaluated using a GY-M15 penetrometer (Shenzhen Di Era Electronic technology Co., Ltd., Shenzhen, Guangdong, China), operating on the principle that firmness is defined as the force required to push a flat-tip probe into the sample to a fixed depth of 10 mm. Briefly, cylindrical Bobolo slices (3 cm in diameter × 3 cm in length) were placed horizontally, and the flat-tip probe of the penetrometer was pressed vertically into the center of the upper surface under a constant applied force of 15 Newtons. Three replicate measurements were performed per sample, and the mean penetration force (expressed in kg/cm²) was calculated and reported as an index of hardness.

Microbiological analyses

Twenty-five grams of each category samples were homogenized in 225 mL peptone water (0.1%) using a Stomacher bac. Decimal dilutions were realized from 10⁻¹ to 10⁻⁹, and plated on: Plate Count Agar at 37°C for 48 h for total mesophilic count (ISO4833-1); Mannitol Salt Agar (MSA) at 37°C, for 48 h incubation, for *Staphylococcus* spp. Count (ISO 6888-1); Oxytetracycline Yeast Extract Agar at 25°C, for 5 days; for yeasts and molds count (ISO 21527-1); Mac Conkey Agar at 37°C, for 48 h, for enterobacteria count (ISO 21528); Thioglycolate Agar supplemented with copper sulfate (2g/l), for Hydrogen sulfite (H₂S) gas complexation, at 37°C for 48 h, for Anaerobic sulfite reducing bacteria count (ISO 15213). After incubation time the plates containing between 30 and 300 colonies were selected and the counts for each microbial family were used to calculate colony-forming units per gram as follows:

$$\log_{10} N = \log_{10} \left[\frac{\sum C}{(n1 + 0.1n2)d} \times \frac{10}{\text{weight of sample taken}} \right]$$

where:

- N : number of counts,

- $\sum C$: sum of colonies counted on all plates,
- n_1 : number of plates counted at the first (lowest) dilution ,
- n_2 : number of plates counted at the next higher dilution,
- d : dilution factor ratio between the two consecutive dilutions (usually 10).

Sensory evaluation

Sensory acceptability was assessed on day 0 by 120 untrained Cameroonian panelists aged between 20 and 50 years, and regular Bobolo consumers. For each sample, panelists were first asked to score the overall liking using a 9-point hedonic scale anchored at 1= Extremely dislike, 9= Extremely like. Next, they were asked to precise how they perceived six sensory characteristics: whitish color; fermented odor; sweet taste; sour taste; sticky and elasticity, by using a 3-point JAR scale : 1 = not enough, 2= just about right and 3 = too much. Products were coded with three-digit random numbers, served at 40°C in randomized order with water/rusk for palate cleansing in odorless plastic containers (Plaehn and Home, 2008). Testing occurred in a sensory booth under white light.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$) to compare treatments and times. Fermentation times and detoxification were compared by t-test. Results are means \pm SD ($n=3$). Sensory analysis and statistical analyses were performed using XLSTAT 2020 (Addinsoft, Paris France).

RESULTS

Fermentation kinetics and detoxification efficiency

Starter-assisted fermentation significantly accelerated cassava root retting compared to spontaneous fermentation. The time required for complete softening decreased from 75.00 ± 3.00 h (spontaneous) to 26.00 ± 2.00 h (starter) (Table 1). Detoxification efficiency reached $93.70 \pm 0.70\%$ in starter-fermented roots versus $91.60 \pm 2.50\%$ in spontaneously fermented roots (Table 1). Raw cassava exhibited baseline HCN levels of 113.8 ± 4.1 mg/kg fresh weight.

Table 1. Detoxification efficiency and retting time of bitter cassava roots under spontaneous and starter-assisted fermentation ($n=6$).

Parameter	Spontaneous	Starter	p-value
Retting time (h)	75.00 ± 3.00^b	26.00 ± 2.20^a	<0.001
HCN raw (mg/kg fresh weight)	113.80 ± 4.10	113.80 ± 4.10	-
HCN retted (mg/kg fresh weight)	9.50 ± 2.50^a	7.10 ± 0.80^a	<0.05
Detoxification (%)	91.60 ± 2.50^a	93.70 ± 0.70^a	<0.05

The results are presented as the means \pm standard deviations. In the same line, the values followed by different lowercase letters (a, b, c, d...) are significantly different via Duncan's multiple range test.

Physico-chemical changes during storage

Water content remained stable across treatments initially (day 0: 49.3-52.8%) but declined more rapidly in Paper-wrapped Bobolo (Figure 1A). Spontaneous-fermented Bobolo packaged in Marantaceae leaves showed the

slowest moisture loss of 2.55% (from 52.90% day 0 to 47.92% day 9) compared to spontaneous + leaves (7.52%). The hardness across treatments increases from 11 kg/cm^2 at day 0 to more than 14 kg/cm^2 at day 9, with the highest value in starter-fermented Bobolo (Figure 1B).

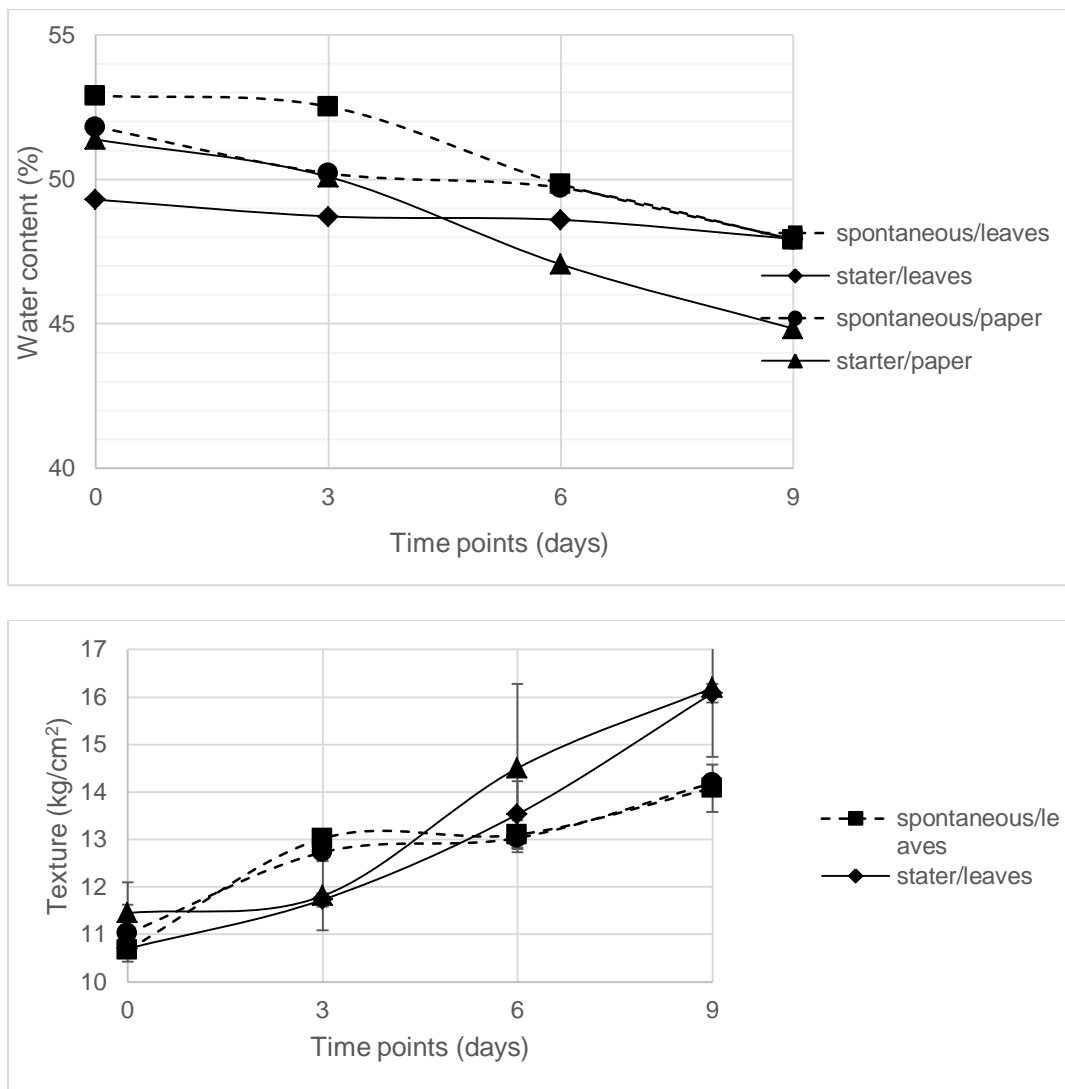


Fig. 1. Evolution of **(A)** Water content and **(B)** texture in Bobolo during 9-day storage under four treatments: spontaneous/leaves (■), spontaneous/paper (●), starter/leaves (◆), starter/paper (▲). Values are means \pm SD (n=3).

Microbiological quality during storage

Total mesophilic counts appeared to increase across all treatments (Table 2), with highest growth in spontaneous + leaves (from 3.91 day 0 to 7.03 day 9). In starter-fermented samples, yeast and mold counts were generally lower (2.82 log CFU/g and 3.69 log CFU/g), corresponding to physical observations on day 6 for baking paper and day 3 for Marantaceae leaves, respectively (Figure 2). Indicator bacteria-enterobacteria and *Staphylococcus*

spp.-are quasi-absent at day 0 to day 3, and appeared in day 6, particularly in sample from spontaneous fermentation wrapped in baking paper. Anaerobes sulfite reducing bacteria were mainly observed in spontaneous fermentation with continuous increasing from 1.00 log at day 3 to 4.02 log CFU/g at day 9. Assisted starter-fermentation seems to permit a good regulation of microbes with reduced total density of aerobes mesophilic flora, and significant limitation of contaminant indicator microorganisms.

Table 2. Changes in total mesophilic aerobes, enterobacteria, *Staphylococcus* spp., fungi and Anaerobe sulfite reducing bacteria (log CFU/g) during storage.

Packaging type	Fermentation type	Time point (days)	Microbial flora log CFU/g				
			TMA	ENT	STA	FON	AnSR
Baking paper	Spontaneous	0	3.32±0.29 ^{bc}	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
		3	4.43±0.61 ^{bcd}	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
		6	4.60±0.12 ^{cd}	0.88±1.25 ^{ab}	2.37±0.46 ^c	3.26±0.21 ^{cd}	0.88±1.25 ^{ab}
		9	6.34±0.06 ^{ef}	3.12±0.07 ^d	2.16±0.37 ^{bc}	5.24±0.57 ^f	3.38±0.12 ^{cd}
	Starter	0	3.00±0.00 ^b	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
		3	3.39±0.10 ^{bc}	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
		6	3.52±0.35 ^{bc}	0.75±0.04 ^{ab}	0.00±0.00 ^a	2.53±0.22 ^b	2.65±0.91 ^c
		9	3.55±0.32 ^{bc}	0.84±1.20 ^{ab}	2.08±0.12 ^{bc}	2.82±0.02 ^{bc}	2.34±0.36 ^c
Marantaceae leaves	Spontaneous	0	3.91±0.28 ^{bcd}	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
		3	4.47±0.10 ^{cd}	0.92±1.30 ^{ab}	0.65±0.91 ^a	0.00±0.00 ^a	1.00±1.41 ^{ab}
		6	5.18±0.16 ^{de}	1.00±1.41 ^{ab}	0.80±1.13 ^a	4.07±0.53 ^e	3.48±0.50 ^{cd}
		9	7.03±0.46 ^f	2.23±0.33 ^{bc}	3.18±0.36 ^c	5.06±0.41 ^f	4.02±1.17 ^d
	Starter	0	1.52±2.15 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
		3	3.39±0.10 ^{bc}	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
		6	3.69±0.42 ^{bc}	0.73±1.04 ^{ab}	1.00±1.41 ^{ab}	2.75±0.39 ^{bc}	2.15±0.21 ^{bc}
		9	4.36±0.06 ^{bcd}	0.84±1.20 ^{ab}	2.63±0.57 ^c	3.69±0.06 ^{de}	4.15±0.82 ^d

The results are presented as the means ± standard deviations. In the same line, the values followed by different lowercase letters (a, b, c, d...) are significantly different ($p < 0.05$) via Duncan's multiple range test. TMA: total mesophilic aerobes; ENT: enterobacteria; STA: *Staphylococcus* spp.; FON: Fungi (Yeast and molds); AnSR: Anaerobe sulfite-reducing bacteria

**Fig. 2.** Physical changes in Bobolo during storage period. Black arrows indicate the presence of molds.

Sensory acceptability

Significant ($p < 0.05$) differences in overall acceptability based on 9-point hedonic scale

were noted in sensory evaluation on day 0 (Table 3). The spontaneous fermentation with Marantaceae leaves were highest scored among the treatments (7.84 ± 1.02), not significantly

different from spontaneous fermentation packaged with paper sample (7.52 ± 1.38). Products from starter fermentation wrapped with baking paper received the lowest overall score (6.84 ± 1.33). More than 50% of consumers rated all sensory attributes as “Just about right” for products from spontaneous fermentation, independently of packaging type (Table 3). Products packaged the same way but produced by starter fermentation received “just about right” ratings from over 55% of panelists for most

attributes; these ratings did not differ significantly from those of the most preferred products. The only exception was color, which approximately 44% of panelists judged to be too dark. Bobolo from accelerated fermentation packaged in baking paper received the lowest overall acceptability score, as it was judged dark (38.24%), inelastic (39.71%), and under-sweet (45.59%) by panelists.

Table 3. Consumers response percentages and overall acceptability scores (means \pm SD, n=120 panelists) on sensory attributes of Bobolo treatments on day 0.

Sensory attributes	JAR levels	Baking paper		Marantaceae leave	
		Starter	Spontaneous	Starter	Spontaneous
Color	Too clear	19.12%	7.35%	8.82%	2.94%
	JAR	42.65%	80.88%	47.06%	70.53%
	Dark	38.24%	11.76%	44.12%	24.47%
Odor	under fermented	16.18%	27.94%	13.24%	16.18%
	JAR	54.41%	55.88%	63.24%	76.47%
	Too fermented	29.41%	16.18%	23.53%	7.35%
Texture	Inelastic	39.71%	36.76%	39.71%	20.59%
	JAR	36.76%	57.35%	47.06%	61.76%
	Elastic	23.53%	5.88%	13.24%	17.65%
Stickiness	Non-sticky	26.47%	26.47%	26.47%	14.71%
	JAR	52.94%	67.65%	61.76%	73.53%
	Too sticky	20.59%	5.88%	11.76%	11.76%
Sweet taste	Under-sweet	45.59%	32.35%	45.59%	39.71%
	JAR	48.53%	66.18%	50.00%	57.35%
	Too sweet	5.88%	1.47%	4.41%	2.94%
Acidity	Not sour enough	23.53%	32.35%	33.81%	23.53%
	JAR	63.24%	66.18%	60.29%	75.00%
	Too sour	13.24%	1.47%	5.88%	1.47%
Overall acceptability		6.84 ± 1.33^d	7.53 ± 1.38^a	7.43 ± 1.40^a	7.84 ± 1.02^a

The results of overall acceptability are presented as the means \pm standard deviations. In the same line, the values followed by different lowercase letters (a, b) are significantly different ($p < 0.05$) via Duncan's multiple range test. JAR= Just About Right.

DISCUSSION

The biotechnological efficacy of microbial starter was confirmed by the results of significant reduction in retting time, from 75 h under spontaneous fermentation to 26 h with the starter culture. This substantial decrease indicates that the selected consortium greatly accelerated cassava root softening and tissue maceration, most likely through the coordinated metabolic activities of its dominant members,

namely *Streptomyces* sp. and Lactic acid bacteria. These dominant microorganisms in the starter are known to secrete extracellular pectinases and cellulases that hydrolyze pectin and related polysaccharides in the cell wall, thereby, accelerating the rapid disintegration of cassava tissue (Alqahtani *et al.*, 2020; Alam *et al.*, 2022). Similar observations have been reported in African cassava fermentations, where inoculated or controlled fermentations

consistently shortened retting duration compared with traditional spontaneous processes (Hasan *et al.*, 2020 ; Ickofa *et al.*, 2020).

The improvement in detoxification, although modest, was statistically significant: starter fermentation achieved 93.70% detoxification compared with 91.60% for spontaneous fermentation. This difference reflects the more efficient *Streptomyces* sp.-driven linamarase activity, which hydrolyzes linamarin, than that occurring during spontaneous microbial succession. In contrast, spontaneous fermentation depends on a natural microbial succession that is less predictable and may not sustain consistent enzymatic activity throughout processing. This 2.1% gain, demonstrates a more consistent and controlled detoxification process. In practical terms, both treatments reached detoxification levels compatible with safety requirements, so the additional reduction may not translate into a major nutritional or toxicological advantage. Nevertheless, the starter-assisted process is valuable because it helps standardize fermentation performance and reduces variability linked to cassava genotype and environmental conditions that can influence microbial activity and detoxification efficiency.

Moisture stability during 9 days storage showed packaging to be a more dominant factor than fermentation type. The sharper decrease in moisture observed in samples packed in baking paper can be attributed to the partial permeability of this material, which permits gradual water loss (Tanyitiku, 2024). As moisture content declines, the product becomes less hydrated and the matrix of the cassava-based paste or dough becomes denser and less pliable, resulting in a firmer texture (Bouniol *et al.*, 2021). In other words, moisture loss and textural hardening appear closely linked, since reduced water availability decreases softness and increases perceived stiffness. In contrast, samples wrapped in Marantaceae leaves retained moisture more effectively at the beginning of storage, which helped preserve a softer texture and delayed firming. This higher water retention likely maintained greater plasticity in the product structure, limiting the

compactness that develops during drying. Nevertheless, leaf-wrapped samples also appeared to favor faster product deterioration, probably because of water vapor condensation, that creates a favorable environment for microbial growth as suggested by Onzo *et al.* (2014).

For this, progressive increase of TMA flora was noted during storage with more increase in spontaneous fermented samples than those of starter fermentation, suggesting the better control of dominant flora and limitation of opportunistic microorganisms by the used of starter (Brauman *et al.*, 1994). Moreover, the late appearance of enterobacteria and staphylococci, especially from day 6 onward in spontaneous fermentations and in baking-paper packages, indicates secondary contamination linked to the surrounding environment, which weakens microbiological stability. Their low presence in starter-fermented samples reflects better sanitary control, probably due to the more effective action of the inoculated microflora (Djoulde *et al.*, 2015). Fungal flora became more abundant at the end of storage, particularly in spontaneously fermented products wrapped in Marantaceae leaves. This increase can be explained by the high moisture retained by the leaves, which favors mold growth. Sulfite-reducing anaerobes followed the same trend, with higher counts in spontaneous fermentation, indicating an increased contamination risk. Better control of microbial growth in starter-fermented samples may be attributable to activities associated with *Streptomyces* sp. in the starter. These include: the production of antimicrobial secondary metabolites, the competitive colonization and rapid growth on available substrates, the secretion of lytic enzymes (chitinases, glucanases, proteases) and volatiles compounds that inhibit pathogens (Barbuto *et al.*, 2021).

Sensory acceptability results indicate that both fermentation method and packaging material strongly influenced consumers' perception of Bobolo. The highest overall score was obtained for spontaneous fermentation combined with Marantaceae leaves, suggesting that this

traditional processing route produces sensory attributes that are more familiar and preferred by consumers. This preference likely reflects development of the characteristic sour, fermented aroma and flavor associated with conventional Bobolo, together with the specific contribution of Marantaceae leaves, which may help preserve desirable odor, texture, and visual appearance during processing and storage (Eyenga *et al.*, 2024). In contrast, starter-fermented Bobolo, particularly when packed in baking paper, received the lowest acceptability scores. This lower appreciation may indicate that the controlled fermentation produced a sensory profile perceived as less typical of the traditional product. Consumers often associate fermented cassava foods with a specific combination of acidity, aroma, softness, and packaging-related cues; when one of these elements changes, the product may be judged as less authentic or less appealing, even if technologically improved (Tomlins *et al.*, 2007; Diallo *et al.*, 2013). Baking paper may also have altered moisture retention and aroma development, resulting in a product that was less soft, less aromatic, or less consistent with the expected sensory identity of Bobolo.

CONCLUSION

This study showed that consumer preference for Bobolo is not determined by fermentation alone, but by the interaction between fermentation method, packaging, texture, and aroma. While starter fermentation may improve process control, safety, and standardization, the traditional combination of spontaneous fermentation with Marantaceae leaves appears to preserve the sensory qualities most valued by consumers. This suggests that any technological improvement intended for adoption should not only ensure microbiological or processing advantages, but also maintain the familiar organoleptic characteristics that define product identity and consumer acceptance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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