

# ASSESSMENT OF BIOCHEMICAL, NUTRITIONAL, AND DYNAMICS OF MICROORGANISMS DURING THE PRODUCTION OF COCOBACA (A FERMENTED CORN-BASED FOOD) PRODUCED IN ABIDJAN (CÔTE D'IVOIRE)

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**Abstract**– Production of maize-based street foods is a major source for maize consumption in Africa. However, most processors operate in unhygienic conditions and without risk control. This is the case for the production of *cocobaca*, a local fermented maize-based porridge consumed in Côte d'Ivoire. The aim of this study was to gain knowledge of biochemical changes and the dynamics of microorganisms during *cocobaca* production, with a view to improving control in the future. To this end, this work focused on the physico-chemical, biochemical and microbiological aspects of producing this fermented maize-based product. The pH of the maize paste obtained after grinding, which was  $3.83 \pm 0.01$ , decreased to  $2.93 \pm 0.03$  in the fermented maize paste. The pH of the *cocobaca* produced was  $3.42 \pm 0.03$ . Whereas a lower pH has an advantage in terms of food safety, as most spoilage bacteria do not grow at a lower pH. Fermentation also improved the bioavailability of macronutrients such as proteins, carbohydrates and total sugars. The concentration of minerals such as phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, copper and iodine increased after fermentation. The concentration of minerals such as phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, copper and iodine increased after fermentation. Fermentation also leads to the release of certain vitamins such as vitamin B1, vitamin B6, vitamin B9 and vitamin C. At the same time, lipids, fibre, ash, vitamin B2, vitamin B3, phenolic compounds and anti-nutritional compounds were reduced. In terms of microbiological results, the predominant microflora in the samples analyzed was aerobic mesophilic strains, followed by lactic acid bacteria and streptococci. Finally, coliforms, *E. coli*, staphylococci and *Clostridium perfringens* were not detected in the fermented dough. We can conclude from this study that the changes in biochemical composition during *cocobaca* production can be linked to the effect of fermentation. As a result, the risk of food-borne illnesses can be minimised by eliminating pathogens during production, leading to a stable, safe product that is suitable for consumption.

## INTRODUCTION

The production of cereal-based street foods is a major source for maize consumption in Africa. Indeed, over the last two decades, Africa has seen an increase in the commercialization of street foods, due to increased urbanization (Mensah, 2013; Lanmafankpotin *et al*, 2016; Bariol-Mathais, 2020; OECD/UNITED NATIONS, 2022). Côte d'Ivoire is no exception, with poverty forcing low-income

individuals to make do with ready-to-eat food (FAO, 1997). Similarly, lack of time, workplaces and schools that are gradually moving further away from where people live are leading them to prefer foods that are convenient, quick, easily accessible, and inexpensive (Ekpa *et al.*, 2019). These foods are defined by the FAO (1997) as “foods and beverages ready to be consumed prepared and/or sold especially in streets and similar public places”. Among these street foods are maize based foods.

Maize can be consumed in many forms (fresh roasted or boiled maize, steamed products, porridges, beverages and dough) (Sahoré *et al.*, 2007; Brou *et al.*, 2008; Adiko *et al.*, 2021). The diversity of maize-based foods involves widely applied processing technologies such as cooking, germination, milling and fermentation (Wakil and Daodu, 2011). From these methods come fermented maize-based foods such as *doklu*, *bassi*, *tôh*, *anangobaca* and *cocobaca* (Assohoun *et al.*, 2013; Soro-Yao *et al.*, 2013; Soro-Yao *et al.*, 2014; Aka-Gbezo *et al.*, 2017). *Cocobaca* is a traditional fermented porridge produced from maize or millet and consumed in Côte d'Ivoire. This fermented food is highly prized for its taste, flavor, availability and affordability for all social classes. In most regions of Côte d'Ivoire, *cocobaca* is consumed by all ages in various consistencies for breakfast, lunch and snacks. The technological process involves sorting (the bad maize seeds, the grains of sands are eliminated), soaking, washing, draining, grinding with the addition of spices, sieving, filtering, fermenting and cooking.

Fermentation is a process of desirable biochemical modification of the food matrix, brought about by microorganisms and their associated enzymes. The changes that occur during fermentation can be deleterious (production of toxins). Fermentation must therefore be controlled in order to obtain a product of nutritional and sanitary quality, with a stable taste and better preservation for the consumer (Izah *et al.*, 2016). In addition to developing pleasant flavors and improving the texture, preservation and digestibility of foods, fermentation also improves the nutritional value of fermented products through the synthesis of metabolites of nutritional interest and helps reduce the levels of anti-nutritional factors (Blandino *et al.*, 2003; Singh *et al.*, 2015).

This processing technique is used to produce *cocobaca*. However, during the production of *cocobaca*, as with most traditional African products are produced in unhygienic conditions (Kouamé *et al.*, 2019), the fermentation that takes place during this production is not subject to quality control. Nor is the processing environment monitored. What's more, the fermentation period depends mainly on the producers and the locality. However, fermenting for too long can lead to the loss of certain nutrients (proteins, vitamins, minerals and sugars) from the maize used for fermentation. In addition, the microorganisms involved in fermentation come mainly

from the environment, the fermentation materials and the water used (Bouatenin, 2013). When undesirable micro-organisms dominate the fermentation medium, they can produce products with unappreciated organoleptic properties and can lead to products that are harmful to consumers. In fact, there may be antagonism between the fermenting and pathogenic microorganisms for the use of substrates during fermentation. This antagonism can cause a reduction in the production of essential metabolites such as vitamins, sugars and organic acids, preventing acidification of the medium. This antagonist can adversely affect the texture, viscosity, taste and colour of the finished product. What's more, the finished product will not be of satisfactory microbiological quality and will present a health hazard for the consumer. In fact, most traditional fermented foods in Africa are threatened by a short shelf life (1 to 7 days) due to post-cooking contamination (Ntso *et al.*, 2016; Adesokan *et al.*, 2010). The aim of this study was to gain knowledge of biochemical changes and the dynamics of microorganisms during *cocobaca* production, with a view to improving control in the future.

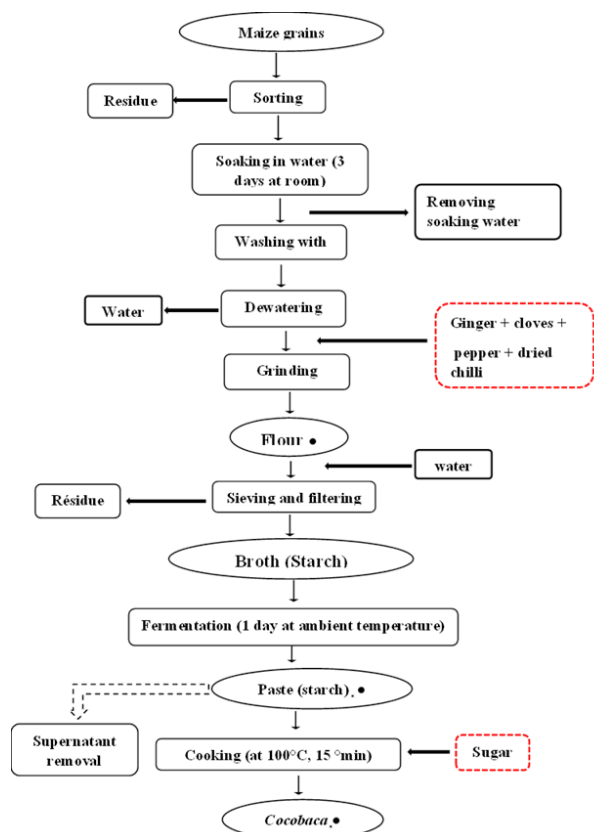
## MATERIALS AND METHODS

### Study material

Study material consisted of maize-based *cocobaca* and the intermediate products obtained during the production process, i.e. the paste obtained after grinding and the fermented paste.

### *Cocobaca* production process

To produce *cocobaca*, the corn kernels were soaked in water for 3 days at room temperature. After soaking, the maize was washed and placed in a colander to wring out the water for a few minutes. Next, the maize kernels were ground after adding the ingredients (ginger, cloves, pepper and dried chili pepper) to the corn kernels. The flour obtained at the end of grinding was sieved and filtered after adding water. The resulting paste was fermented for 24 hours at room temperature without removing the supernatant. Once fermentation was complete, cooking was carried out at 100 °C for around 15 minutes. Sugar was added and homogenized around 2 min from the end of cooking (Figure 1). The product can be consumed with or without sugar, depending on the consumer's preference.



**Fig. 1.** Traditional process for producing *cocoba* from maize  
 %: stages at which the samples were taken  
 •: broken red squares in the diagram indicate optional ingredients

### Sampling

Samples were taken before and after the fermentation stage, as well as from the finished product. They consisted of the paste obtained after grinding, the fermented paste and the *cocoba*. Three municipalities in Abidjan were chosen: Yopougon, Abobo and Port-Bouët. These communes were chosen because of their very high production and consumption of *cocoba*. The producers in these three communes are renowned for the quality of their product, according to consumers. In each commune, five (5) producers were chosen. These producers were chosen for the quality of their product and their willingness to participate in this study. A sample of each product (ground paste, fermented paste and *cocoba*) was taken from each producer, and three samples were taken. A total of 135 samples were taken for physico-chemical, biochemical and microbiological analysis.

### Determination of physico-chemical parameters

Titrate acidity and pH were determined using the method of Kimaryo *et al.* (2000). The quantity of soluble sugar was determined by the AOAC method (1995). A drop of the suspension prepared for titrate acidity was placed on the glass of the pocket refractometer (Model ATAGO POCKET REFRACTOMETER) to evaluate the quantity of soluble sugars. The quantity of soluble sugars was measured in the light, at the level of the instrument's eyepiece. Dry matter (DM) and water content were determined using the AOAC method (2000). This method consists in evaporating the water contained in the sample by drying in an oven at 105°C until constant mass is obtained.

### Determination of biochemical compound content

Proteins and lipids in the paste obtained after grinding, the fermented paste and the *cocoba* (finished product) were determined using the AOAC method (2000).

Protein content was determined based on total nitrogen using Kjeldahl (1976) technique. The mineral nitrogen content was obtained by assay after mineralization. The total protein content was then calculated by multiplying the amount of nitrogen by a conversion factor (6.25), i.e. 16% nitrogen in protein. Total lipid content was extracted with hexane (organic solvent) from samples using the SOXHLET extractor. After extraction, the solvent (n-hexane) was recovered using a rotary evaporator (HEIDOLPH, Germany) and the lipid content determined. Fiber content was also determined using the AOAC method (2000). Total carbohydrates were determined according to the calculation method recommended by FAO (2002). Similarly, the energy value was determined according to the method described by FAO (2002). Ethanol soluble sugars were extracted using the technique described by Martinez-Herrera *et al.* (2006). Total sugars were determined according to the method described by Dubois *et al.* (1956) using phenol and concentrated sulfuric acid. Reducing sugars, on the other hand, were determined according to the technique of Bernfeld (1955) using 3,5 dinitrosalicylic acid (DNS).

The ash content of the sample was determined using the AOAC method (1995). The principle of the method consists in incinerating 5 g of powder (contained in pre-dried porcelain crucibles) in a muffle furnace at 550°C to obtain the ash content. Mineral determination was carried out according to

the method described by Daji *et al.* (2023) using argon plasma ionizing source mass spectroscopy (ICP-MS). Five grams (5 g) of ash was homogenized in 10 mL of a mixture of hydrochloric acid (50%) and nitric acid (50%). The resulting mixture was filtered. The filtrate was made up to 100 mL with distilled water. Qualitative and quantitative determination was performed by spectrometry (ICP-MS) using a mineral standard solution.

### Detection and quantification of vitamins

Determination of the concentration of vitamins B1, B2, B3, B6, B9 and C was carried out by reversed-phase HPLC using the method described by Jin *et al.* (2012). Extraction of the water-soluble vitamins (B1, B3, B6 and C) from each sample involved dissolving 5 g of sample in 10 mL of a solution containing 0.05% phosphoric acid (v/v) and 0.3% sodium thiosulfate (w/v). Whereas for the preparation of vitamins B2 (riboflavin) and B9 (folic acid), 5g of sample was dissolved in 10 mL of 0.5% (v/v) ammonium hydroxide solvent. The mixtures were centrifuged at 4000 rpm for 20 min, then the collected supernatant was filtered through a 0.45 µm Millipore filter using a vacuum filter device. Then a 20 µL volume of each sample was injected into the Nucleodur HTEC C18 (250 mm×4.6 mm di, 5 µm, 100 Å, Alltech Associates Inc., Deerfield, IL, USA) high-performance liquid chromatography (HPLC) column (Agilent technologies, 1200 series, UK). The specific quantification wavelength was 275 nm for vitamins C, B1, B3 and B6, and 282 nm for vitamins B2 and B9.

### Determination of total polyphenol content and anti-nutritional factors

Polyphenols were determined according to the method described by Singleton *et al.* (1999) using the Folin Ciocalteu reagent. The amount of total soluble polyphenols in the extracts was then determined spectrophotometrically. Total flavonoid content was measured by colorimetric assay using the method described by Meda *et al.* (2005) using aluminum chloride. Tannins were determined by the method described by Bainbridge *et al.* (1996) using vanillin reagent. Phytates were determined by the method described by Latta and Eskin (1980) using Wade's reagent. The method used for oxalate determination was that described by Day and Underwood (1986).

### Microbiological analysis

Stock solution and decimal dilutions were carried

out in accordance with ISO 6887-2 (2017). Aerobic mesophilic germs (AMG) assimilated to total flora were enumerated in the mass on Plate Count Agar (PCA) after 72 hours incubation at 30°C in accordance with international standard ISO 4833 (2003). Neutral red crystal violet bile agar (VRBL agar) was used for coliform enumeration after 24 hours of culture at 30°C for total coliforms (NF V 08-050, 2009) and 44°C for fecal coliforms (NF V08-060, 2009). In addition, in accordance with NF ISO 7251 (2005), *E. coli* was detected by spreading 0.1 ml of the mother suspension or decimal dilutions on RAPID'E. Coli2 agar, followed by incubation at 37°C for 24 hours. In addition, Staphylococci were enumerated on Baird-Parker agar enriched with egg yolk and potassium tellurite for 24 to 48 hours at 37°C using the NM ISO 6888-1 method (2021). *Bacillus* enumeration was carried out on Mossel agar supplemented with egg yolk after 48 to 72 hours incubation at 30°C, using ISO 7932 (2004 /Amd 1: 2020). NM ISO 7937 (2009) was used for the detection of sulfite-reducing anaerobes after heat shocking the stock solution and dilutions (80°C for 15 min and immediately cooled). Salmonella testing was carried out using NF EN ISO 6579-1 (2017) in three stages.

Yeasts were counted on Sabouraud Chloramphenicol agar after 5 days incubation at 25°C in accordance with ISO 21527-1 (2008). Lactic acid bacteria were counted in accordance with NF ISO 15214 (1998) using MRS (Man Rogosa Sharpe) agar, which was incubated at 37°C for 24 to 48 hours in a candle jar. Tarzaghi and Sandine agar (M 17) was used to count streptococci after 48 hours incubation at 37°C using the Bio Rad 356-4174 method (2003).

### Statistical analysis

Assays were performed in triplicate, and the data presented were the means and standard deviation of these three determinations calculated with Excel 2016. The means obtained were compared by analysis of variance (ANOVA) using Duncan's multiple comparison test at a significance level of 5%. All statistical analyses were performed using XLSAT software version 2016.02.27444.

## RESULTS

### Physico-chemical parameters

The results of the physico-chemical analyses of the samples revealed variability in the physico-chemical

parameters of the various samples taken during *cocobaca* production.

The pH of the maize dough obtained after grinding, which was  $3.83 \pm 0.01$ , decreased to  $2.93 \pm 0.03$  in the fermented corn dough. The pH of the *cocobaca* produced (finished product) was  $3.42 \pm 0.03$ . The pH values of the samples were statistically different ( $P < 5\%$ ). The titratable acidity of the different samples correlated negatively with the pH value.

The soluble sugar extract of the samples analysed during *cocobaca* production decreased from  $0.93 \pm 0.06$  °Brix in the maize paste obtained after grinding to  $0$  °Brix in the fermented maize paste. The amount of sugars in the *cocobaca* was  $0.97 \pm 0.06$  °Brix. The dry matter content of samples analysed during *cocobaca* production decreased from  $42.01 \pm 0.82$  g/100g FM (maize paste obtained after grinding) to  $14.58 \pm 0.33$  g/100g FM (*cocobaca*). This value is not statistically different from that of unfermented corn dough at the 5% threshold (Table 1).

#### Macronutrient characteristics

Macronutrient results for samples analyzed during *cocobaca* production are reported in Table 2. The energy value obtained during *cocobaca* production increased from  $390.80 \pm 0.32$  kcal/100 g DM in the corn dough to  $406.54 \pm 0.25$  kcal/100 g DM in the finished product (*cocobaca*). These results are significantly different ( $P < 5\%$ ).

The protein content of the samples during

*cocobaca* production increased from  $9.82 \pm 0.03$  g/100 g DM in the unfermented maize dough to  $10.52 \pm 0.02$  g/100 g DM in the fermented corn dough. This content decreased to  $5.94 \pm 0.03$  g/100 g DM in the finished product (*cocobaca*). These results are statistically different at the 5% level. The lipid content of the samples decreased from  $3.61 \pm 0.03$  g/100 g DM (maize paste obtained after grinding) to  $1.44 \pm 0.01$  g/100 g DM (fermented maize dough). The differences observed in the samples taken during *cocobaca* production are statistically different at the 5% threshold. The carbohydrate content of the samples increased from  $79.75 \pm 0.03$  g/100 g DM (the maize dough obtained after grinding) to  $91.98 \pm 0.04$  g/100 g DM in the *cocobaca*. These contents showed a significant difference ( $P < 5\%$ ) (Table 2).

#### Composition of ethanol soluble sugars, fiber, and ash content of *cocobaca* and intermediate products

The total sugar content of samples taken during *cocobaca* production increased from  $10.18 \pm 0.15$  mg/100g DM (ground maize dough) to  $26.82 \pm 0.15$  mg/100g DM (fermented corn dough). However, this content decreased in the finished product ( $2.84 \pm 0.16$  mg/100g DM). The same applies to reducing sugars, which fell from  $4.54 \pm 0.13$  mg/100 g DM (unfermented maize dough) to  $1.44 \pm 0.28$  mg/100 g DM (finished product). The variations in sugar content are significantly different at the 5% threshold.

In addition, the fiber content of the samples decreased progressively from  $1.04 \pm 0.03$  g/100 g DM

**Table 1.** Physico-chemical characteristics of *cocobaca* and intermediate products

Food	Samples	pH	Titratable acidity (%)	SG (°Brix)	Dry matter (g/100 g FM)
<i>Cocobaca</i>	CPB	$3.83 \pm 0.01^a$	$0.09 \pm 0.01^c$	$0.93 \pm 0.06^a$	$42.01 \pm 0.82a$
	CPF1	$2.93 \pm 0.03^c$	$0.13 \pm 0.03^a$	$0^b$	$38.74 \pm 0.06b$
	CPF	$3.42 \pm 0.03^b$	$0.11 \pm 0.02^b$	$0.97 \pm 0.06^a$	$14.58 \pm 0.33c$

Values in the table are means  $\pm$  standard deviation of three trials for each parameter. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product), SG: soluble sugar, FM: fresh matter.

**Table 2.** Energy value and macronutrient content of *cocobaca* and intermediate products

Food	Samples	Energetic value (kcal/100g DM)	Proteins (g/100 g DM)	Lipids (g/100 g DM)	Carbohydrates (g/100 g DM)
<i>Cocobaca</i>	CPB	$390.80 \pm 0.32^c$	$9.82 \pm 0.03^b$	$3.61 \pm 0.03^a$	$79.75 \pm 0.03^c$
	CPF1	$403.63 \pm 0.10^b$	$10.52 \pm 0.02^a$	$1.44 \pm 0.01^c$	$87.16 \pm 0.02^b$
	CPF	$406.54 \pm 0.25^a$	$5.94 \pm 0.03^c$	$1.65 \pm 0.03^b$	$91.98 \pm 0.04^a$

Values in the table are means  $\pm$  standard deviation of three trials for each parameter. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product), MD: dry matter.

(maize dough obtained after grinding) to  $0.31 \pm 0.01$  g/100 g DM (finished product). The dry matter content of samples analyzed during *cocobaca* production decreased from  $42.01 \pm 0.82$  g/100g MF (unfermented maize dough) to  $14.58 \pm 0.33$  g/100g MF (*cocobaca*). The ash content of samples taken during *cocobaca* production also decreased from  $5.77 \pm 0.02$  g/100 g DM (maize dough obtained after grinding) to  $0.06 \pm 0.00$  g/100 g DM (fermented maize dough). These results are significantly different at the 5% threshold (Table 3).

### Mineral content of *cocobaca* and intermediate products

Minerals detected during *cocobaca* production included phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, copper, zinc and iodine at varying levels. The potassium content of the samples increased from  $2.10 \pm 0.00$  mg/100g (maize dough after grinding) to  $3.10 \pm 0.14$  mg/100g in the fermented corn dough. Calcium content increased from  $0.81 \pm 0.03$  mg/100g (maize dough after grinding) to  $1.48 \pm 0.01$  mg/100g (fermented dough). This content decreased to  $0.78 \pm 0.04$  mg/100g in the finished product. In addition, the magnesium content of the samples analyzed increased from  $1.30 \pm 0.00$  mg/100g (paste obtained after grinding) to  $1.91 \pm 0.00$  mg/100g (finished product).

Iron content increased from  $1.11 \pm 0.01$  mg/100g (maize dough after grinding) to  $1.62 \pm 0.01$  mg/100g (fermented dough). This content decreased in *cocobaca* ( $1.02 \pm 0.02$  mg/100g). Iodine content increased from  $0.61 \pm 0.01$  µg/100g (paste obtained after grinding) to  $0.81 \pm 0.01$  µg/100g (finished product). These results are statistically different at the 5% threshold (Table 4).

### Variation in vitamin content during *cocobaca* production

Table 5 shows the concentrations of vitamins B1, B2, B3, B6, B9 and C obtained from the various samples

analyzed. The concentration of vitamin B1 increased non-significantly from  $3.76 \pm 0.98$  mg/100 g to  $3.89 \pm 0.12$  mg/100 g from maize paste after grinding to fermented maize paste. This concentration dropped to  $1.13 \pm 0.01$  mg/100 g in *cocobaca*. Vitamin B6 concentration during *cocobaca* production increased from  $0.54 \pm 0.03$  mg/100 g FM (maize dough after grinding) to  $0.82 \pm 0.01$  mg/100 g FM (fermented maize dough). The vitamin B9 concentration of the samples increased from  $242.76 \pm 5.23$  µg/100 g FM (maize dough after grinding) to  $246.72 \pm 8.80$  µg/100 g DM in the fermented corn dough. This concentration dropped to  $238.97 \pm 2.56$  µg/100 g DM in *cocobaca*. In addition, the vitamin C concentration of the samples increased from  $0.24 \pm 0.03$  mg/100 g FM (maize paste after grinding) to  $3.77 \pm 0.00$  mg/100 g DM in fermented maize paste. This content subsequently fell in the finished product ( $0.04 \pm 0.01$  mg/100 g FM). These variations are statically different at the 5% threshold.

### Variations in phenolic compounds during *cocobaca* production

Statistical analysis revealed a significant difference ( $P < 5\%$ ) in total polyphenol content between the different samples taken during *cocobaca* production.

Total polyphenol content during *cocobaca* production decreased from  $401.79 \pm 9.46$  mg/100g DM (unfermented maize dough) to  $205.69 \pm 7.18$  mg/100 g DM (*cocobaca*). In addition, a reduction in total flavonoid content from  $54.67 \pm 0.81$  mg/100g DM (unfermented maize dough) to  $5.56 \pm 0.52$  mg/100g DM (*cocobaca*) was observed. This reduction was significant ( $P < 5\%$ ) (Table 6).

### Variation in anti-nutritional compounds during *cocobacca* production

In general, the phytate content at the end of fermentation of all the products studied was at trace levels and below the WHO limit for phytates in foodstuffs, which is 22.10 mg/100 g DM. Oxalate levels obtained after fermentation during *cocobacca*

**Table 3.** Composition of ethanosoluble sugars, fiber, and ash content of *cocobaca* and intermediate products

Food	Samples	Total sugars (mg/100 g DM)	Reducing sugars (mg/100 g DM)	Fibers (g/100 g DM)	Ash (g/100 g DM)
<i>Cocobaca</i>	CPB	$10.18 \pm 0.15^b$	$4.54 \pm 0.13^a$	$1.04 \pm 0.03^a$	$5.77 \pm 0.02^a$
	CPF1	$26.82 \pm 0.15^a$	$2.42 \pm 0.44^b$	$0.82 \pm 0.02^b$	$0.06 \pm 0.00^c$
	CPF	$28.41 \pm 0.16^a$	$2.54 \pm 0.28^b$	$0.31 \pm 0.01^c$	$0.12 \pm 0.02^b$

Values in the table are means  $\pm$  standard deviation of three trials for each parameter. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product), MD: dry matter.

Table 4. Mineral content of *cocobaca* and intermediate products

Food Samples	P ( $\mu\text{g}/100\text{g}$ )	K ( $\text{mg}/100\text{g}$ )	Ca ( $\text{mg}/100\text{g}$ )	Mg ( $\text{mg}/100\text{g}$ )	Na ( $\text{mg}/100\text{g}$ )	Mn ( $\mu\text{g}/100\text{g}$ )	Fe ( $\text{mg}/100\text{g}$ )	Cu ( $\mu\text{g}/100\text{g}$ )	Zn ( $\mu\text{g}/100\text{g}$ )	I ( $\mu\text{g}/100\text{g}$ )
<i>Cocobaca</i> CPB	197.4 $\pm$ 2.40 <sup>b</sup>	2.10 $\pm$ 0.00 <sup>b</sup>	0.81 $\pm$ 0.03 <sup>b</sup>	1.30 $\pm$ 0.00 <sup>c</sup>	0.42 $\pm$ 0.00 <sup>c</sup>	20.12 $\pm$ 0.13 <sup>b</sup>	1.11 $\pm$ 0.01 <sup>b</sup>	28.94 $\pm$ 0.57 <sup>b</sup>	34.54 $\pm$ 0.71 <sup>a</sup>	0.61 $\pm$ 0.01 <sup>b</sup>
CPF1	281.51 $\pm$ 12.62 <sup>a</sup>	3.10 $\pm$ 0.14 <sup>a</sup>	1.48 $\pm$ 0.01 <sup>a</sup>	1.51 $\pm$ 0.01 <sup>b</sup>	0.81 $\pm$ 0.00 <sup>b</sup>	30.12 $\pm$ 1.26 <sup>a</sup>	1.62 $\pm$ 0.01 <sup>a</sup>	43.97 $\pm$ 1.13 <sup>a</sup>	14.04 $\pm$ 1.36 <sup>b</sup>	0.81 $\pm$ 0.01 <sup>a</sup>
CPF	311.76 $\pm$ 0.91 <sup>a</sup>	2.21 $\pm$ 0.00 <sup>b</sup>	0.78 $\pm$ 0.04 <sup>c</sup>	1.91 $\pm$ 0.00 <sup>a</sup>	1.21 $\pm$ 0.00 <sup>a</sup>	14.18 $\pm$ 0.06 <sup>c</sup>	1.02 $\pm$ 0.02 <sup>c</sup>	23.27 $\pm$ 0.14 <sup>c</sup>	34.74 $\pm$ 0.71 <sup>a</sup>	0.81 $\pm$ 0.01 <sup>a</sup>

Values in the table are means  $\pm$  standard deviation of three trials for each parameter. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product).

production were also below the WHO standard threshold of 105 mg/100 g DM. The oxalate content of samples collected during *cocobaca* production dropped from 110.00  $\pm$  5.50 mg/100g DM (unfermented maize dough) to 25.67  $\pm$  3.18 mg/100g DM in the finished product. Results for samples analyzed during *cocobaca* production also showed a significant decrease ( $P < 5\%$ ) in tannin content, from 60.93  $\pm$  0.23 mg/100g MS (unfermented maize dough) to 19.19  $\pm$  0.49 mg/100g MS (*cocobaca*) (Table 7).

#### Evolution of the load of quality-altering microorganisms during *cocobaca* production

The results of the microbiological study of samples collected during the *cocobaca* production process are shown in Figure 2. The highest average loads were obtained with aerobic mesophilic germs (AMG), which decreased from 7.34 log (CFU/g) in the paste maize obtained after grinding to 3.56 log (CFU/g) in the finished product. This variation is significant at the 5% threshold. However, loads of total coliforms, thermotolerant coliforms, *E. coli* and *Staphylococcus* were only detected in the paste obtained after grinding, with respective loads of

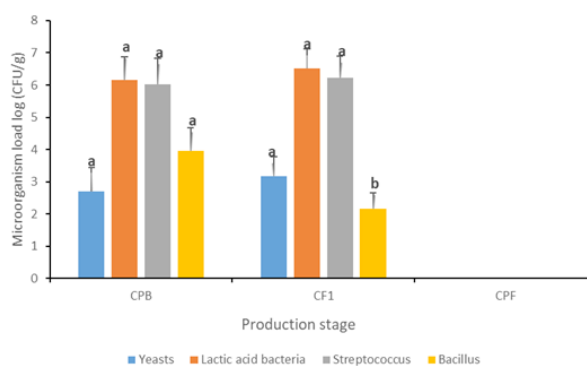


Fig. 2. Evolution of microbial germ load during *cocobaca* production. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product).

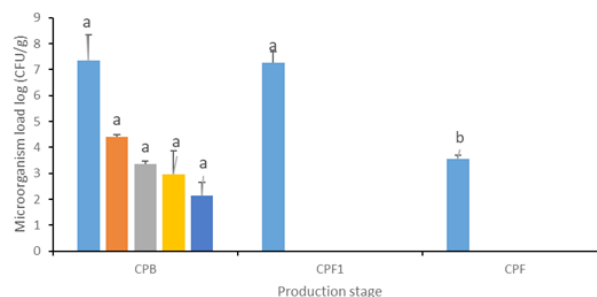


Fig. 3. Evolution of fermentative microbial load during *cocobaca* production. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product).

4.40 log (CFU/g), 3.36 log (CFU/g), 2.96 log (CFU/g) and 2.15 log (CFU/g).

#### Evolution of fermentative microorganisms during *cocobaca* production

The results of the microbiological analysis of the samples collected during the *cocobaca* production process are shown in Figure 3. These results showed a clear increase in the average yeast load from 2.70 log (CFU/g) in the pulp obtained after grinding to 3.17 log (CFU/g) in the fermented pulp. In addition, the lactic acid bacterial load increased from 6.15 to 6.51 log (CFU/g), from the post-grinding dough to the fermented dough. This load decreased to 2.10 log (CFU/g) in the finished product. In addition, the *streptococcus* load of the paste obtained after grinding to fermented paste had increased from 6.02 to 6.22 log (CFU/g). The *Bacillus* load was reduced

**Table 5.** Vitamin content of *cocobaca* and intermediate products

Food	Samples	Vitamin B1 (Thiamin) mg/100 g FM	Vitamin B2 (Riboflavin) µg/100 g FM	Vitamin B3 (Niacin) µg /100 g FM	Vitamin B6 (Pyridoxine) mg/100 g FM	Vitamin B9 (Folic acid) µg/100 g FM	Vitamin C (ascorbic acid) mg/100 g
Cocobaca	CPB	3.76 ± 0.98 <sup>a</sup>	17.16 ± 0.63 <sup>a</sup>	20.81 ± 1.20 <sup>a</sup>	0.54 ± 0.03 <sup>b</sup>	242.76 ± 5.23 <sup>a</sup>	0.24 ± 0.03 <sup>b</sup>
	CPF1	3.89 ± 0.12 <sup>a</sup>	11.41 ± 0.94 <sup>b</sup>	0 <sup>b</sup>	0.82 ± 0.01 <sup>a</sup>	246.72 ± 8.80 <sup>a</sup>	3.77 ± 0.00 <sup>a</sup>
	CPF	1.13 ± 0.01 <sup>b</sup>	0 <sup>c</sup>	0 <sup>b</sup>	0.04 ± 0.00 <sup>c</sup>	238.97 ± 2.56 <sup>a</sup>	0.04 ± 0.01 <sup>c</sup>

Values in the table are means ± standard deviation of three trials for each parameter. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product), FM: fresh matter.

**Table 6.** Phenolic compound content of *cocobaca* and intermediates

Food	Samples	Total polyphenols (mg/100g DM)	Total flavonoids (mg/100g DM)
<i>Cocobaca</i>	CPB	401.79 ± 9.46 <sup>a</sup>	54.67 ± 0.81 <sup>a</sup>
	CPF1	243.39 ± 3.19 <sup>b</sup>	41.67 ± 0.23 <sup>b</sup>
	CPF	205.69 ± 7.18 <sup>c</sup>	5.56 ± 0.52 <sup>c</sup>

Values in the table are means ± standard deviation of three trials for each parameter. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product), MD: dry matter.

**Table 7.** Variation in anti-nutritional compounds during production of unfermented maize *cocobaca*

Food	Samples	Phytates (mg/100 g DM)	Oxalates (mg/100 g DM)	Tannins (mg/100 g DM)
<i>Cocobaca</i>	CPB	70.42 ± 0.17 <sup>a</sup>	110.00 ± 5.50 <sup>a</sup>	60.93 ± 0.23 <sup>a</sup>
	CPF1	16.61 ± 0.51 <sup>b</sup>	29.33 ± 3.18 <sup>b</sup>	27.17 ± 0.68 <sup>b</sup>
	CPF	0.85 ± 0.17 <sup>c</sup>	25.67 ± 3.18 <sup>b</sup>	19.19 ± 0.49 <sup>c</sup>
Standard (WHO)	-	22.10	105	

Values in the table are means ± standard deviation of three trials for each parameter. Values with identical superscript letters are not statistically different at  $P > 0.05$ . CPB: *Cocobaca* paste after grinding, CPF1: *Cocobaca* paste fermented one day, CPF: *Cocobaca* (finished product), MD: dry matter.

from 3.97 log (CFU/g) in the paste obtained after grinding to 2.17 log (CFU/g) in the fermented paste. The variation in the load of all the fermentative microorganisms from ground paste to fermented paste, with the exception of *Bacillus*, was not significant at the 5% threshold. However, this variation from the fermented paste to the finished *cocobacca* product is significant at the 5% threshold.

## DISCUSSION

The aim of this study was to gain knowledge of biochemical changes and the dynamics of microorganisms during *cocobaca* production, with a view to improving control in the future. pH is an important factor during fermentation and microbial growth. A low pH level increases food safety. In this study, pH decreased with a simultaneous increase in

acidity during the *cocobaca* paste fermentation phase as observed in millet sourdough studies (Bender *et al.*, 2018). The drop in pH of fermented samples was promoted by the production of organic acids, mainly lactic acid and other acids (acetic acid, propionic acid....) by microbial activity from carbohydrate pathway metabolism (Ruiz-Rodríguez *et al.*, 2019; Degrain *et al.*, 2020; Banwo *et al.*, 2021). The reduction in the refractometric dry extract value observed in fermented *cocobaca* maize pulp was probably due to the bioconversion of fermentable sugars during fermentation. These results are in line with those of Gbohaida *et al.* (2016), who showed a rapid decrease in refractometric dry extract after 48 hours of cashew juice must fermentation. This variation was also a function of fermentation strains.

In addition, the gradual increase in energy value from unfermented corn pulp samples to the finished

product could be explained by the different treatments and the digestion of starch. The high-water content of unfermented corn dough samples in the finished product was due to the addition of water during soaking and cooking. Decreased lipid content was detected in both fermented corn dough and finished product. This could be associated with leaching or high microbial activities during fermentation (Kumoro *et al.*, 2020; Ambarsari *et al.*, 2022). Some previous work indicated that *Lactobacillus spp.* produced higher lipolytic enzymes, leading to greater lipid degradation during fermentation (Gong *et al.*, 2020). On the other hand, the protein content increased significantly after fermentation. This increase could be explained by the enzymatic hydrolysis (protease activity) of complex proteins into peptides and amino acids caused by the fermenting micro-organisms. A similar result concerning protein after microbial fermentation was also reported in the case of a study on fermented sorghum flour (Istianah *et al.*, 2018). The clear shift in carbohydrate content from unfermented corn dough samples to the finished product would indicate that the degree of fermentation was insufficient to induce complete hydrolysis in the amorphous starch region (Ambarsari *et al.*, 2022). In addition, a study on millet flour showed an improvement in carbohydrates and energy value following fermentation and malting (Adebisi *et al.*, 2017). Also, the high total sugar content of the fermentation phase could be explained by the enzymatic hydrolysis of starch by microflora. According to various studies, at the start of the fermentation process, sugar levels appear to be higher, but as fermentation progresses, sugar levels show a downward trend (Budhwar, 2020). In addition, a prolonged period of fermentation can lead to the use of sugar by the fermentative microflora. This has led to a reduction in reducing sugars in the fermented dough. These results are in line with the work of some researchers who have shown that fermented mixtures contain reducing or soluble sugars that are used by the microflora as a carbon source, and the fermented food product may eventually contain a lower amount of sugars (Budhwar *et al.*, 2020).

The crude fiber content of both the fermented dough and the finished product decreased during *cocobaca* production. Fermenting and cooking food leads to the solubilisation of fibre, which indirectly results in a reduction in crude fibre content (Adebisi

*et al.*, 2017; Arora *et al.*, 2010; Luithui *et al.*, 2018). In addition, the considerable reduction in the ash content of fermented maize dough samples can be attributed to losses due to leaching (extraction of soluble compounds from the ash on contact with water) of soluble inorganic salts during fermentation (Akinola *et al.*, 2017).

While a significant increase in minerals such as phosphorus, potassium, calcium, magnesium, sodium, manganese, iron, copper and iodine were observed during *cocobaca* production, zinc (Zn) content declined. However, some studies have demonstrated their benefits for the human organism. This high bioavailability in fermented maize dough is thought to be due to the activity of enzymes produced by the fermenting germs during the fermentation step. The significant increases in all minerals are in line with the report by Hejazi *et al.* (2016) who described that fermentation by lactic acid bacteria and yeast improves the quality of cereal foods. In addition, anti-nutritional factors (which could limit mineral availability by forming insoluble complexes within the food matrix) can be degraded by microbial enzymes, leading to a release of available minerals (Adebo *et al.*, 2022). In addition to degrading antinutrient content, fermentative germs can also improve mineral bioavailability by producing organic acids. These organic acids can form soluble and absorbable ligands with minerals, thus preventing the formation of insoluble complexes with phytates (Rousseau *et al.*, 2020).

Higher mineral availability is important for nutrition, as these micronutrients are essential for proper metabolic function, growth, and development of the human organism. Potassium and phosphorus are essential mineral components for growth, cell metabolism and tissue and nerve repair, stimulating the body's physiological activities (Babatundé and Adeola, 2021). While iron, sodium and zinc are important for the normal functioning of the body system and play a role in balancing oxidative stress for the prevention of mutagenesis (Banwo *et al.*, 2020; Yiannikourides *et al.*, 2019); Singh *et al.*, 2019). In addition, sodium is essential for physiological activities, including muscle contraction and fluid control in the body (Euh *et al.*, 2021). In addition, iodine is essential in the fight against goiter. Calcium and magnesium are useful for the proper development and formation of bones in infants and children (Banwo *et al.*, 2021; Nkhata *et al.*, 2018).

The non-significant increase in the concentration of vitamins B1 and B9, as well as the significant increase in vitamin B6 in fermented maize pulp, can be partly attributed to microbial activity during fermentation, where substances are broken down, making them less assimilable. Fermentation considerably improves the availability of water-soluble vitamins. Natural fermentation breaks down poly- and oligosaccharides that cannot be digested by the body, releasing certain amino acids and improving the availability of group B vitamins (Ochanda *et al.*, 2010). The increase in ascorbic acid (vitamin C) content during fermentation was in line with studies on malted millet, mung bean and chickpea by Obadina *et al.* (2017). This increase was certainly due to the germination/malting stage, which is stimulated by the enzymatic hydrolysis of starch by amylases and diastases, thus intensifying the availability of glucose for vitamin C biosynthesis (Ochanda *et al.*, 2010; Banwo *et al.*, 2021). The low or trace concentrations of water-soluble vitamins B2 and B3, may have been lost during the fermentation and cooking phase due to leaching. This is in agreement with the loss of thiamine during germination of brown rice due to leaching (Ochanda *et al.*, 2010).

In addition, the vitamins B1, B6, B9 and C obtained during *cocobaca* production are an advantage for the human organism. Vitamin B1 has real physiological effects by improving the utilization of sugars, amino acids and fatty acids for energy production; in the event of deficiency, we will see an increase in blood pyruvate, then lactate with metabolic acidosis (Baudin, 2019). In addition, vitamin B6, or pyridoxine, is the coenzyme of numerous enzymes, including transaminases, deaminases and decarboxylases, which produce neurotransmitters such as serotonin, dopamine, amphetamines and Gaba (gamma-aminobutyric acid). It is essential for amino acid and protein metabolism (cofactor of around 60 enzymes) (Baudin, 2019). Vitamin B9, on the other hand, is essential during pregnancy to meet maternal needs and ensure proper development of the fetus and placenta. In France, the recommended milk and preventive treatment for pregnant women is supplemented with vitamins D and B9 (Le Guyader and Garçon, 2019). Vitamin C acts as a cofactor for various enzymes and as an antioxidant in human biology. Vitamin C plays a key role in the synthesis of catecholamines, collagen, cortisol, neurotransmitters and peptide hormones, immune

cell functions, as well as iron and folic acid metabolism (Dresen *et al.*, 2023).

However, the observable reduction in total polyphenols and flavonoids during *cocobaca* production could be explained by the enzymatic effect of fermentative strains and heat treatment. In the same context, some studies have shown that fermentation and enzymatic treatment reduce polyphenols (11-22%) and flavonoids (40-51%) in millet bran (Luithui *et al.*, 2018; Coulibaly *et al.*, 2011). In addition, a reduction in polyphenols may also be due to the presence of phenolic oxidase during germination (Tajoddin *et al.*, 2014; Tian *et al.*, 2019).

In addition, phytates, oxalates and tannins are the main anti nutrients that limit the nutritional value of cereals. Their progressive reduction from fermented dough to finished product (*cocobaca*). The reduction of phytates, oxalates and tannins below the WHO threshold is due to the fermentation effect. The degradation of three anti nutrients is caused respectively by the activity of phytase, oxalate decarboxylase and tanniase triggered by fermentative microflora (Ojo and Enujiugha, 2018). Reducing the quantity of these antinutrients in a food is beneficial for the body because it increases the bioavailability of essential minerals and certain compounds (proteins and carbohydrates) for the consumer (Ojo and Enujiugha, 2018). This can reduce the digestive problems caused by anti nutrients. In addition, phytates and oxalates were below the WHO threshold. The result is like the findings of Ojo and Enujiugha (2018), who observed that fermented samples of *ogi* (fermented porridge produced in Nigeria) formulated from ground corn and bean mixtures had low levels of tannins, oxalates and phytate. Millet fermentation was also found to reduce certain anti nutrients, notably phytic acid and tannins (Coulibaly *et al.*, 2011). According to their results, enzymatic treatment significantly reduced the levels of anti nutritional factors in millet broth (Azza Alwohaibi *et al.*, 2022). In another study designed to examine the effect of fermentation on anti-nutritional activity, maize flour was subjected to fermentation with a consortium of lactic acid bacteria (LAB) at 12-hour intervals. Results showed that with increasing fermentation time, a considerable reduction in anti nutrients was observed in fermented maize flour (Chibuikwe Ogodo *et al.*, 2014). The microorganisms mainly used were *S. boulardii*, *L. plantarum* and *L. casei*. Both types of fermentation technique significantly reduced phytic

acid, trypsin inhibitor and tannic acid content (Budhwar *et al.*, 2020).

The microbiological study of samples collected during the *cocobaca* production process enabled us to assess the dynamics of quality-altering and fermentative flora. AMG, coliforms (total and thermotolerant), *E. coli* and *Bacillus* in the paste obtained after grinding were observed with a high AMG load. AMG and *Bacillus* were also detected in the fermented dough. Pathogenic microorganisms such as *Salmonella* and *Clostridium*, on the other hand, were not detected throughout the production process. The presence of AMG, coliforms (total and thermotolerant), *E. coli* and *Bacillus* in the ground dough could be attributed to cross-contamination from the working environment and utensils used during soaking and the water used for soaking, as well as a temperature (ambient) that is favorable to the development of microorganisms. Traditional production practices for *ogi* (fermented porridge produced in Nigeria), involving rudimentary and unhygienic utensils and carried out in an uncontrolled environment, are common. These practices can lead to unintentional contamination of the finished product with microbes (Fagunwa *et al.*, 2023). Moreover, traditional village fermentation has little or no control over microbial growth, which explains the high number of micro-organisms found in all products. The low pH of fermented products and heat treatment (cooking) would make them fit for consumption. However, poor hygienic conditions in which they are processed and handled can lead to the introduction of other micro-organisms, including pathogens (Mwizerwa *et al.*, 2018). The low pH of fermented products and heat treatment (cooking) would make them fit for consumption. However, the poor hygienic conditions under which they are processed and handled can lead to the introduction of other micro-organisms, including pathogens (Mwizerwa *et al.*, 2018). In addition, many toxins are also heat-stable, so they remain active and pose a danger to foodstuffs even if they are heat-treated. Similar results were obtained by Batool *et al.* (2012) who mentioned the high number of pathogenic bacteria found, is certainly due to their presence initially on the cereal grains from which the *ogi* was obtained. The presence of high numbers of pathogenic bacteria generally indicates information about food processors, raw materials, processing conditions, storage conditions and product handling. Studies carried out by Kouamé *et al.* (2019) on the

identification of hazards and critical control points during the production of *attieke*, a cassava-based fermented food produced in the Ivory Coast, revealed the presence of pathogenic bacteria at high levels. The observation of the *Bacillus* genus from the ground dough to the end of the fermentation stage could be explained by their adaptation to acidic environments and could undoubtedly have a fermentative role. In addition, *Bacillus* genus dominated throughout the processing phase of “*iru*”, a Nigerian fermented condiment made from *Parkia biglobosa* grains, in their phylogenetic studies. However, in other studies, specific species such as *B. subtilis*, *B. licheniformis* and *B. amyloliquefaciens* have been proposed as starter cultures (Aderibigbe *et al.*, 2011; Adewumi *et al.*, 2019). The absence of pathogens in the fermentation phase would be due to the effect of fermentation and the acidity of the medium created by the development of fermentative germs. Lactic acid bacteria and Streptococci were observed in the ground dough, and their load was even more pronounced in the fermented dough. Although yeasts are involved in fermentation, they had a low load during *cocobaca* production. Fermentative germs are also known for their tolerance of acidic environments and their ability to break down fermentable substrates derived from plant matter. This explains the high load of lactic acid bacteria, which are the main micro-organisms that play an essential role in *cocobaca* fermentation. Similarly, Ljabadeniyi (2007) reported that during fermentation (after wet milling of fermented maize) of *ogi*, lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus fermentum*) and yeast (*Saccharomyces cerevisiae*) were the predominant isolates. The majority of microorganisms present in soaked and fermented maize for *ogi* production have been reported to participate in the fermentation of other local foods. Oyewole and Isah (2012) also mentioned that *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Streptococcus*, *Penicillium* and *Saccharomyces* are important in the fermentation of local foods. These microorganisms have the ability to ferment certain carbohydrates and are responsible for fermenting maize to produce porridge (Odunfa, 2015; Amusa *et al.*, 2015; Ozoh and Kuyanbana, 2015; Okoli *et al.*, 2023). Further work on the correct characterisation of the fermentative microflora during *cocobaca* production, such as the isolation of the predominant fermentative strains, could help to understand the biochemical and microbiological changes during the production of this food. This

understanding of the process can be used to better control it in the future and ensure consistency of flavor, texture, safety and nutrition.

### CONCLUSION

The aim of this study was to gain knowledge of biochemical changes and the dynamics of microorganisms during *cocobaca* production, with a view to improving control in the future. The results of the sample analyses revealed changes in the physico-chemical and nutritional parameters essential for safety and nutritional value. The lowest pH and highest acidity were obtained in the fermented paste. The results also indicated that at the fermentation stage, there was an improvement in protein, carbohydrate and total sugar content. In addition, a significant increase in most minerals was observed in the fermented maize dough and some in the finished product. As far as vitamins are concerned, a slight increase in vitamins B1, B6, B9 and C was observed, with the exception of vitamins B2 and B3. In addition, a significant reduction in phenolic compounds and anti-nutritional factors (phytates, oxalates and tannins) was observed in the samples analysed after 24 hours of fermentation. This reduction is a factor that could have a positive impact on the nutritional value of *cocobaca*. As far as the microbiological results are concerned, the spoilage germs detected in the unfermented dough were eliminated in the fermented dough; with the exception of *Bacillus* and GAM, which were present in both the unfermented and fermented doughs. Fermentative germs were also observed in the unfermented dough. However, the highest loads were obtained in the fermented dough with lactic acid bacteria and Streptococci. Spontaneous fermentation contributed to the elimination of pathogens.

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**Conflict of Interest:** None.

### Declaration of competing interest

The authors declare that they have no known

competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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