Original Research Article

PhysiochemicalSelection of Potential Starters ofsome*Bacillus* Species Involved in the Fermentation of *Néré* Seeds (*Parkia biglobosa*) in Côte d'Ivoire

ABSTRACT

Aims:~~Research of~~Study of some*Bacillus sp*. starter strains to control ~~the~~ fermentation of ~~the seeds of~~*Parkia biglobosa* (*néré*)seeds.
Study design: Original research.
Place and Duration of Study: Between February and August 2023, this work was performed at Laboratory of Biochemistry, Microbiology, and Valorization of Agroresources, of the Agropastoral Management Institute, which is part of Peleforo Gon Coulibaly University (Korhogo, Côte d'ivoire).
Methodology: Fermented *néré* seeds were collected from *Soumbara* producers in the Korhogo region in northern Côte d'Ivoire. *Néré* seeds were subjected to a physicochemical analysis to determine the pH, titrable acidity, moisture, dry matter, and ash content. Subsequently, these ferment~~ing~~ (-ed) seeds were used for the isolation and enumeration of *Bacillus*~~genus~~ bacteria. Production of extracellular enzymes and the influence of stress conditions were carried out on these strains to select potential starters.
Results: The study of the physicochemical analysis of fermented *néré* seeds showed that these seeds have a pH of 6.59±0.04, close to neutrality, with a very low titrable acidity of 0.06±0.00%. The moisture content of the *néré* seeds is 38.76±0.13% with ~~(a)~~ high dry matter content of 61.24±0.22% coupled with a low ash content of 3.18±0.05%. Thirty-three (33) Gram-positive bacilli were isolated from *néré* seeds with a microbial load of 7.1 $\log_{10}$ cfu/g. The production of extracellular enzymes and the influence of stress conditions led to the selection of five *Bacillus* strains (BS 08, BS 14, BS 19, BS 22 and BS 31) as potential starters.
Conclusion: The control of the fermentation of *néré* seeds involves selecting strains with the best potential. Thus, five *Bacillus sp*. Isolates (BS 08, BS 14, BS 19, BS 22, and BS 31) were selected as potential starters for obtaining a *Soumbara* of exceptional nutritional and organoleptic quality.

Keywords: Soumbara, physico-chemical, Bacillus, technological properties, starters.

1. INTRODUCTION

Parkia biglobosa, commonly known as *néré*, is a species of tree in the *Fabaceae* family. This particular species of tree is indigenous to East Asia, Madagascar, America, Africa's Guinean and Sudanese regions [1]. In addition to being consumed for their sweet pulp, the pod fruits of *néré* (*Parkia biglobosa*) are also traditionally fermented and used as condiments in West Africa [2,3].This seasoning, also known as *Soumbara* in Côte d'Ivoire, is used in several

recipes as an emulsion to enhance the flavor of particular dishes[4].Due to the presence of glutamic acid, *Soumbara* is a highly popular condiment with a taste akin to cheese. Its strong smell and odor are ascribed to the molecules formated during the fermentation of the seeds, which also include ammonia, pyrazines, esters, acids, and ketones[5,6].This widely used culinary ingredient has intriguing nutritional benefits [6]. It is rich in minerals such as calcium, iron, and phosphorus as well as vitamins, especially vitamins B (thiamine, riboflavin and niacin). Furthermore, fermented *Parkia biglobosa* seeds are believed to have certain medical benefits, including a reduction in blood pressure, to prevent and/or to fight malnutrition and cardiovascular diseases [7,8].

Soumbara production method is a family-based, empirical and traditional process involving three crucial steps: double cooking the seeds, alkaline fermenting the cotyledons and drying the fermented product [9]. According to Adjoumani et al. [10], fermentation remains the most important step in producing high-quality *soumbara*. It is a metabolic process involving a variety of microorganisms, including yeasts, bacteria and fungi with a predominance of *Bacillus* bacteria [11]. In Côte d'Ivoire, process of fermenting *néré* seeds to produce *soumbara* is still entirely traditional, involving a wide variety of methods and uncontrolled microbial flora. Moreover, the production and commercialization of *soumbara* occur under unfavorable conditions and do not always respect to good hygiene practices, making them vulnerable to various microbiological contaminations caused by pathogenic bacteria that are generally linked to food-borne diseases [12,13]. It would be wise for this widely consumed food ingredient to be as safe as possible, i.e. exempt from all pathogenic micro-organisms, to avoid the risk of intoxication which could lead to more serious health problems. The most appropriate alternative for controlling this ingredient is the selection and inoculation of *Bacillus sp*. with beneficial effects, also known as starters in Côte d'Ivoire [10]. Several studies in the sub-region have reported that *Bacillus sp*. are main microorganisms responsible in this alkaline fermentation and produce several metabolites that are involved in the development of the flavour, taste and texture of *soumbara*. In addition, antimicrobial compounds produced by *Bacillus sp*. have been identified as affecting the preservation and health safety of *soumbara* [14,15,16,17]. However, in Côte d'Ivoire, very few studies on the fermentative properties and inoculation of *Bacillus sp*. for the production of *soumbara* have been conducted. Only studies conducted are those by Kouakou et al. [9], which examined the technological properties of *Bacillus sp*. and carried out fermentation trials with potential starters (unpublished results). These studies alone could not elucidate the full potential of *Bacillussp*. bacteria isolated in Côte d'Ivoire. Thus, the objective of this work is to study the technological properties of *Bacillus sp*. isolated from *soumbara* in Côte d'Ivoire in order to select potential starters.

2. MATERIAL AND METHODS

2.1. Materials

Fermented *néré* seeds, known as *soumbara*, constitute the biological material (Figure 1). These seeds were collected from three production units in the region of Korhogo (9° 27′ 41″ North, 5° 38′ 19″ West) under aseptic conditions after 72 hours of fermentation at a rate of 100 g per production unit. These samples, transported in a cool box, were acheminated to the Laboratory of biochemistry, microbiology and valorization of agroresources of the Agropastoral Management Institute at the Peleforo Gon Coulibaly University of Korhogo for physico-chemical and microbiological studies.

A **B**

Fig 1: Fermentation of *néré* seeds (A)and *soumbara* (B)

2.2. Methods

2.2.1.Physicochemical analyses of *soumbara* seeds

2.2.1.1. pH and titrable acidity of soumbara seeds

pH of *soumbara* seeds was determined according to the protocol described by N'goran-Aw et al.[18]. Twenty (20) g of *soumbara* were added to 100 mL of distilled water. The obtained solution was filtered and the electrode of the previously calibrated pHmeter (HANNA, Germany) was immersed in the solution under agitation. The pH value was read directly on the pH meter's screen. Regarding the titrable acidity, three (3) drops of 1% phenolphthalein solution were added to 10 mL of the previously obtained filtrate. The obtained solution was titrated against a NaOH solution (0.1 N) until a pale pink endpoint was reached[19]. Titrable acidity was evaluated according to the following formula:

$$\text{Titrable acidity (\%)} = \frac{\text{V(NaOH)} \times \text{N(NaOH)} \times 0.09 \times 100}{\text{V(Test sample)}}$$

2.2.1.2. Determination of moisture and dry matter content of soumbara seeds

The procedure for determining the moisture content was described by AOAC[20]. To dehydrate the samples, they were oven dried to a constant weight. Thus, five (5) milligrams (m0) of *soumbara* seeds were placed within a glass capsule. The whole (sample and capsule) (m_1) was placed in a 105°C oven for 24 hours, and then cooled in a desiccator and weighted (m_2) once more. The moisture content (H) as a percentage of the mass was computed using the equation below:

$$H(\%) = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100$$

Dry matter (DM) content of the sample was estimated using the following formula and was given as a percentage of its raw mass:

$$DM(\%) = 100 - H(\%)$$

2.2.1.3. Determination of ash content of soumbara seeds

Ash content (A) of *soumbara* samples was determined according to the method proposed by AOAC [20]. Five (5) g (P0) of *soumbara* were mineralized at 550°C for 6 hours in a muffle furnace (SX-4-10 Series, Tianjin, China) until the complete destruction of organic matter. After cooling in a desiccator, the ash was weighed (P1) and the ash content (percentage) was determined using the following formula:

$$A(\%) = \frac{(P_0 - P_1) \times 100}{P_0}$$

2.2.2. Microbiological analysis of *soumbara* seeds

Isolation and enumeration of bacteria of the genus *Bacillus* involved first preparing the stock solution by weighing 25 g of *soumbara* seeds, which are homogenized in 225 mL of buffered peptone water. Next, decimal dilutions were performed ranging from 10^{-2} to 10^{-6}. Finally, these decimal dilutions (100 µL) were used to inoculate nutrient agar plates supplemented with 50 µg/L of nystatin to inhibit the growth of fungal strains. The agar plates inoculated by spreading were incubated at 30°C for 48 hours. After incubation, Petri dishes containing characteristic Bacillus sp. colonies ranging from 15 to 150 were selected for enumeration. The number of microorganisms expressed as cfu/g was determined in accordance with standard NF ISO 7218 [21] using the formula:

$$N = \frac{\sum C}{(n1 + 0.1n2) \times d \times V}$$

Characteristic *Bacillus sp.* colonies were identified using the following biochemical tests: Gram coloration, catalase and sporulation test. Colonies identified such as *Bacillus sp*. were stored in nutrient broth supplemented with 20% glycerol at -20°C for any further tests.

2.2.2. Study of the technological properties of *Bacillussp.* involved in *soumbara* fermentation

2.2.2.1. Production of extracellular enzymes in solid medium

Néré seeds are composed of pulp that is rich in pectin, proteins (30–47%) and carbohydrates (13–17%) [22]. *Bacillus sp*. isolates capable of degrading these substrates will lead to interesting chemical and biochemical modifications associated with the final products of *soumbara*. Method used for the production of extracellular enzymes (pectinase, amylase and protease) is that described by Ouattara et al.[23]. Screening was carried out in Petri dishes with the following composition: 0.28% $(NH_4)_2SO_4$, 0.6% KH_2PO_4, 0.2% KH_2PO_4,

0.02% yeast extract, 0.01% $MgSO_4H_2O$, 1.5% agar and 1% substrate (pectin or starch or skim milk) at pH 6.0. Seven (7) µL of bacterial suspension with an optical density of 1 at 600 nm prepared in tryptone salt were used to inoculate wells made in the agar. The inoculated culture media were incubated at 30°C for 48 hours. After incubation, agar plates were flooded with a solution of iodine and potassium iodide (5 g potassium iodide + 1 g iodine + 330 ml distilled water) to reveal the clear zones around the wells, indicating the presence of activity enzymatic.

2.2.2.2.Temperatureand pH effects on the growth of isolates that produce extracellular enzymes

Influence of temperature and pH on*Bacillus sp*. growth that produce extracellular enzymes were evaluated as described by Yao et al.[24] in broth medium. Two hundred (200) µL of bacterial suspension with an optical density of 1 at 600 nm was used to inoculate 5 mL of nutrient broth. Concerning the influence of temperature, the cultures were incubated at temperatures ranging from 30 to 50°C. For the influence of pH, nutrient broth was prepared at different pH values (4 to 8) and incubated at 30°C. After 48 hours of incubation, the absorbance was measured at 600 nm against sterile nutrient broth with a spectrophotometer (Pioway Medical Lab-UV752, Singapore).

2.3. Data Analysis

Data were presented as mean ± standard deviation after the samples were analyzed in triplicate. Duncan's test at the 5% level was utilized to identify significant differences between means, and analysis of variance (ANOVA) was carried out using SPSS Statistics 20.0 software.

3. RESULTS AND DISCUSSION

3.1. Assessing the Physico-Chemical Characteristics of *Soumbara* Seeds

Physico-chemical analyses of *soumbara*seeds are summarized in Table 1. The results show that pH of *soumbara* is near neutral with a value of 6.59 correlated with a low titrable acidity (0.06%). Our results are in agreement with those of Camara et al.[25] and Kambiré et al.[26]who, working on *soumbara* samples in Côte d'Ivoire, obtained pH values ranging from 6.60 to 7.45 and from 6.03 to 6.8 with titrable acidity between 0.07 and 0.3% respectively. Studies by Niamké et al.[27] showed that unfermented *néré* seeds flours has an acid pH of between 5.24 and 5.32. The pH and acidity obtained in our research are directly linked to the alkaline fermentative activity of *Bacillus sp*. strains, which are the dominant microorganisms in microflora. According to Atere et al.[28], the hydrolysis of proteins during fermentation of *néré* seeds leads to the production of amino acids and the release of ammonia, which is alkaline in nature, increases the pH, and decreases the titrable acidity of fermented *Parkia biglobosa* seeds. Fermented *néré* seeds have a high water content of 38.76% and a dry matter content of 61.24%. Studies by Makanjuola and Ajayi[29] indicated that *néré* seeds have a water content of 12.76% before cooking and fermentation. T, these authors obtained a water content at the end of fermentation (3 days) for *Parkia biglobosa* seeds of 42.80%. During the process of transforming *néré* seeds into *soumbara*, certain stages require the addition of water: soaking and cooking. These steps will encourage the seeds to take on water, thereby increasing the water content even before fermentation begins. Several authors reported similar assertions[12,29]. This high water content could be a problem to the conservation of *soumbara*, which is not the case because after fermentation, drying is carried out to reduce the high water content.

Regarding the dry matter, the fermented *néré* seeds have a content of 61.24%. Work of Makanjuola and Ajayi[29] reported that the dry matter content of locust bean seeds was 47.20%, close to our obtained results. However, the studiesrealized by Kambiré et al.[26]andTankoano et al.[30] have shown that the dry matter content of *soumbara* is above 90%. Dry matter content accounts for the macromolecule composition of *soumbara*. Indeed, this fermented condiment is rich in proteins (41-43%), lipids (20-43%) and carbohydrates (13-17%)[22,31,30] which makes it a good food source to meet nutritional needs and growth deficiencies. In addition, *soumbara* is believed to have certain medical benefits, including a reduction in blood pressure, to prevent and/or to fight malnutrition and cardiovascular diseases[30].

Ash content of *soumbara* is low at 3.18%. The ash content reflects the mineral composition of the *soumbara*. Our values are in agreement with those of Omafuvbe et al. [12] who obtained an ash content of 3.55% at the end of *soumbara* fermentation in Nigeria. In fact, these authors showed that the ash content of the seeds decreases when they are transformed into *soumbara*. Untreated *soumbara* seeds had an ash content of 5.40%. After cooking and fermenting these *néré* seeds, the ash content fell to 4.90 and 3.55% respectively. According to Makanjuola and Ajayi[29], some minerals can be leached out during soaking and cooking, leading to a decrease in ash content. In addition, Oladunmoye[32]reported that during fermentation, microbes also use some of the minerals up as nutrients source for growth.

Comment [D1]: It needs evidence, what is the mineral source for microorganism, if it wasn't the fermented substrate it self?
Add reference shows this theory.

Table 1.Physico-chemical parameters of *soumbara* seeds

Parameters	Unit	Means ± SD	Methods
pH	-	6.59 ± 004	Glass electrode pHmeter [18]
Titrable Acidity	(%)	0.06±0.00	Titrimetry dosage method[20]
Moisture	(%)	38.76±0.13	Dehydration method[20]
Dry Matter	(%)	61.24±0.22	Dehydration method[20]
Ash	(%)	3.18±0.05	Mineralisation method[20]

3.2. *Bacillus sp*. isolated, characterized and enumerated from *soumbara* seeds

Enumeration of *Bacillus sp*. after 3 days of fermentation of the *néré* seeds yielded a load of 7.1 $\log_{10}$ cfu/g. During fermentation, the increase in this bacterial population could be explained by their ability to break down more complex compounds into simpler ones[33]. A total of thirty-three (33) large, plate colonies with smooth or irregular contour and white or beige colour, gram-positive bacilli, catalase-positive and sporulating, were isolated from the fermentation of *néré* seeds. *Bacillus sp*. have been isolated from *soumbara* in West Africa and form part of the dominant fermentation flora[34,12,35,19,36,37,10]. *Bacillus sp*. present in our study would come from utensils, the environment and the raw material used for *soumbara* production[10]. Study by Kouamé et al.[38] have reported that the spore-forming capacity of *Bacillus sp*. is an advantage over other microorganisms when it comes to developing in *soumbara*. In addition, the near-neutral pH favours the growth of bacteria of the *Bacillus* genus [24]. Until confirmation of the species by molecular characterization, these presumptive *Bacillus sp*. named BS 01 to BS 33 were kept for screening on technological properties.

3.3. Technological properties of *Bacillus*sp. and selection of potential starters

Bacillus sp. strains isolated were screened for their ability to produce extracellular enzymes in a solid medium (Table 2). Of the thirty-three (33) *Bacillus sp*. ~~Strains~~isolates, eighteen (18) or 54.55% were able to degrade pectin by producing pectinase and use it as a carbon source. The activity diameters obtained following the expression of these enzymes vary between 1 and 3.2 cm depending on the strain tested. With regard to the production of amylolytic enzymes, only eleven (11) isolates were able to degrade starch, i.e. 33.33%, with halo diameters of between 1.3 and 2.7 cm. Finally, fourteen (14) isolates produced proteases in solid medium with production halos between 1.2 and 3.6 cm. Eight (8) isolates (24.24%) out of the 33 strains distinguished themselves by producing all three extracellular enzymes investigated, namely pectinases, amylases and proteases (Table 3). Studies by Kouakou et al. [9] and Dabiré et al.[17] on *Bacillus sp*. isolated from *soumbara* in Côte d'Ivoire and Burkina Faso showed that they produce several cellular enzymes such as cellulases, amylases, pectinases, proteases and lipases. Our results show that *Bacillus sp*. ~~Isolated~~isolateshave interesting technological properties. The ability of the isolates to secrete these extracellular enzymes is an advantage for *soumbara*. These enzymes produced by *Bacillussp*. are capable of breaking down macromolecules (pectin, proteins and carbohydrates) present in *soumbara* into simpler molecules. Proteins are hydrolyzed into peptides and amino acids, and polysaccharides and pectin into simple sugars. These metabolites are used in chemical reactions and play an important role in the organoleptic and nutritional quality of *soumbara*[22,9,17]. The eight (8) isolates that produce both these three types of extracellular enzymes were retained for the influence of temperature and pH on microbial growth in order to select potential starters.

Table 2. Production of extracellular enzymes by *Bacillus sp*. strains isolated

Extracellular enzymes	Halo diameter	Number of isolates	percentage
Pectinase	[1-3.2 cm]	18	54.55
Amylase	[1.3-2.7 cm]	11	33.33
Protéase	[1.2-3.6 cm]	14	42.42

Table 3. Enzymatic activity of eight *Bacillus sp.*isolated from fermenting *néré* seeds

Bacillus strains	Enzyme activity (diameter of clear zone in cm)		
	Pectinase	Amylase	Protéase
BS 08	2.90 ± 0.1^{b}	2.65 ± 0.05^{a}	3.30 ± 0.06^{a}
BS 11	2.67 ± 0.06^{c}	2.15 ± 0.05^{c}	2.67 ± 0.12^{b}
BS 14	2.73 ± 0.06^{c}	2.70 ± 0.10^{a}	2.37 ± 0.07^{c}
BS 16	3.07 ± 0.12^{a}	2.32 ± 0.03^{b}	3.17 ± 0.29^{a}
BS 19	2.40 ± 0.10^{d}	2.41 ± 0.08^{b}	2.74 ± 0.05^{b}
BS 22	2.17 ± 0.06^{e}	1.60 ± 0.04^{d}	2.12 ± 0.10^{d}
BS 24	2.60 ± 0.10^{c}	1.68 ± 0.06^{d}	3.20 ± 0.02^{a}
BS 31	3.20 ± 0.0^{a}	2.11 ± 0.15^{c}	2.76 ± 0.07^{b}

Data are represented as means±SEM (n=3). Mean with different letters in the same column are statistically different ($p<0.05$).

3.4. Growth under stress conditions

3.4.1. Influence of temperature

Temperature and pH are parameters that can influence the growth of *Bacillus sp*. during the fermentation of *néré* seeds. Table 3 shows the influence of temperature on the microbial growth of the eight *Bacillus* isolates. These results show that these isolates have good resistance to the different temperatures studied, with growth peaks at temperatures of 30, 35 and 40°C. Strains BS 11, BS 16 and BS 19 had a peak at 30°C with optical densities of 0.721±0.03, 0.944±0.01 and 0.848±0.02 respectively. As for isolates BS 14, BS 22 and BS

24, their growth peaks at 35°C with optical densities of 0.961±0.03, 1.194±0.04 and 0.860±0.04 respectively. The peak at 40°C was obtained with strains BS 08 and BS 31. These results confirm those of Yao et al.[24] and Ehon et al. [39] who showed that *Bacillus sp*. strains isolated respectively from cocoa and *attiéké* in Côte d'Ivoire are capable of growing up to 50°C with a maximum growth between 30 and 40°C. During fermentation of *néré* seeds, the temperature ranges between 25 and 40°C [22]. Therefore, *Bacillus sp*. isolates capable of growth will be able to degrade the various substrates (proteins, pectins, and carbohydrates) and produce metabolites for the development of the nutritional and organoleptic characteristics of *soumbara*.

3.4.2. Influence of pH

Figure 1 illustratesthe microbial growth of the eight (8) *Bacillus sp*. strains producing extracellular enzymes at different pH levels. All the isolates were able to grow at the different pH levels studied with varying growth rates. pH 4 showed the lowest optical density values for all isolates in general. The growth peak of the strains is at pH 7, except for strain BS 22 which showed a peak at pH 6 with an optical density of 0.688±0.01. These results demonstrate that *Bacillus sp*. isolated from our study allow for adaptation and a high growth capacity in both acidic and basic environments. Several studies have revealed that the pH during the fermentation of locust beans generally varies between 6 and 8[22,10,26,28]. Thus, five (5) isolates that exhibit good microbial growth above OD 0.4 at 600 nm can be proposed as potential *Bacillus* starters for the control of *soumbara* fermentation. These are the isolates BS 08, BS 14, BS 19, BS 22 and BS 31.

Table 4.Impact of temperature on eight *Bacillus sp*. growth

***Bacillus*strains**	**Optical density at 600 nm**				
	30°C	**35°C**	**40°C**	**45°C**	**50°C**
BS 08	0.834±0.04^{c}	1.065±0.03^{b}	1.133±0.01^{a}	0.630±0.02^{d}	0.310±0.01^{e}
BS 11	0.721±0.03^{a}	0.620±0.02^{b}	0.565±0.05^{b}	0.353±0.02^{c}	0.054±0.02^{d}
BS 14	0.725±0.02^{b}	0.961±0.03^{a}	0.694±0.03^{b}	0.546±0.03^{c}	0.240±0.05^{d}
BS 16	0.944±0.01^{a}	0.746±0.02^{b}	0.466±0.01^{c}	0.362±0.01^{d}	0.162±0.01^{e}
BS 19	0.848±0.02^{a}	0.641±0.02^{b}	0.538±0.03^{c}	0.311±0.02^{d}	0.311±0.02^{e}
BS 22	0.941±0.03^{b}	1.194±0.04^{a}	0.719±0.03^{c}	0.595±0.02^{d}	0.271±0.02^{e}
BS 24	0.651±0.03^{b}	0.860±0.04^{a}	0.647±0.04^{b}	0.442±0.04^{c}	0.153±0.02^{d}
BS 31	0.552±0.04^{c}	0.728±0.02^{b}	0.923±0.02^{a}	0.391±0.02^{d}	0.122±0.02^{e}

Data are represented as means±SEM (n=3). Mean with different letters in the same line are statistically different ($p<0.05$) according to Duncan's test

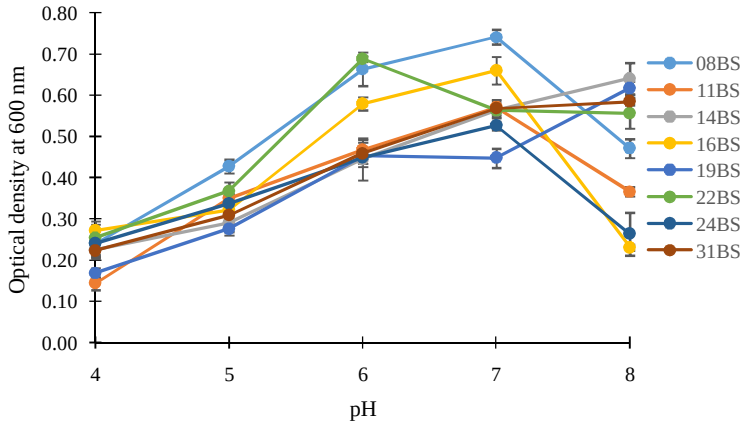

Fig. 2.Impact of pH on the microbial growth of eight extracellular enzyme-producing *Bacillus* isolates

4. CONCLUSION

Soumbara is a fermented condiment widely consumed in West Africa, particularly in Côte d'Ivoire. This condiment is still fermented empirically by the microorganisms that colonize the *néré* seeds. This study aimed to establish *Bacillus sp*. starters for controlling the fermentation of *néré* seeds. But, first of all, a physicochemical analysis of these fermenting grains was conducted. She revealed that these fermented seeds have a pH of 6.59, which is close to neutrality with a low titrable acidity (0.06%). These seeds have a dry matter content of 61.24%, which suggests they have a high content of complex macromolecules with a moisture content of 38.76% and an ash content of 3.18%. Thirty-three (33) *Bacillus sp*. bacteria were isolated from fermented *néré* seeds. The study of technological properties (extracellular enzyme production) and the influence of stress conditions (temperature and pH) led to the selection of five potential starters (BS 08, BS 14, BS 19, BS 22 and BS 31) for the control of *soumbara* fermentation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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