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Kinetics of acidification and production of α-amylase, β-glucosidase and pectinase of three types of freeze-dried ferments for the production of quality attieke in Côte d'Ivoire

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ABSTRACT
The long-term availability of cassava ferment for the production of cassava of consistent, healthy quality is a problem in Côte d'Ivoire. To this end, three types of traditional ferment, namely braised, raw and boiled ferment, were freeze-dried. The aim of this work was to determine the ideal inoculation dose for each freeze-dried ferment for the production of attieke. For the same type of lyophilized ferment, doses of 5%, 10%, 15% and 20% were used to inoculate 4 batches of cassava dough. The fermentations were carried out for 24 hours at 35°C. During fermentation, samples were taken every 6 hours to assess the four main technological activities of a good Attieke ferment, i.e. rapid acidification, production of α-amylase, β-glucosidase and pectinases, corresponding to the respective attributes of acidity, flavour synthesis, detoxification and softening. Irrespective of the type of ferment considered, the results showed that the fermentation properties of the dough inoculated with 10% traditional ferment were similar to those of the dough inoculated with 10% freeze-dried ferment. The dough inoculated with 10% freeze-dried raw ferment had the highest acidity level of 0.97±0.1%, compared with 0.64±0.3% for the dough inoculated with 10% freeze-dried braised ferment and 0.63±0.2% for the dough inoculated with 10% freeze-dried boiled ferment after 24 hours of fermentation. Similarly, the production kinetics of α-amylolytic, β-glucosidase and pectinolytic activities are generally characterised by inactivation or repression after 18 h of fermentation, after which production is low or progressively reduced. At this time, the high amylolytic activity of 75.78±1.9 U/mL was recorded in the dough inoculated with 10% of the raw ferment. On the other hand, the high kinetics of β-glucosidase production (68.34±0.9 U/mL) was obtained in the cassava dough inoculated with 10% freeze-dried braised ferment. The highest yield of pectinolytic activity (24.5±0.09 U/mL) was obtained in cassava dough inoculated with 10% boiled freeze-dried ferment. The metabolic activity of the lyophilised ferment was maximal at an inoculation dose of 10%, after 18 hours of fermentation at 35°C.

KEYWORDS: Freeze-dried, Attieke ferments, Acidification, α-amylase, β-glucosidase, Pectinase

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INTRODUCTION

Attieke is a typical Ivorian food made from a traditional cassava ferment. The traditional inoculum is prepared by peeling roots and cooking them for 10 min. After cooling to 30°C, the cooked roots are placed in jute bags used for previous inoculum preparations. The roots are then left to ferment for three days at room temperature to facilitate the development of the microorganisms. The microorganisms thus cultivated are used to ferment the cassava paste used to make attieke. According to women producers, the traditional inoculum is important for the final quality of attieke (Coulin, 2004). However, there are several types of ferment, depending on the cooking method and the ethnic group. Boiled ferment is peeled, washed, lightly boiled in water, drained, cooled in the open air and then wrapped in jute bags. For braised ferment, the unpeeled tuber is lightly braised, cooled, peeled and then wrapped in a jute bag. Also for raw ferment, the unpeeled tuber is cut, washed lightly cooked in water, then wrapped in a jute bag. Each type of ferment is left to ferment in a warm (32°C to 40°C) area of the kitchen for 2 to 3 days (Bouatenin, 2013; Kakou et al., 2017). The use of such ferments to initiate cassava dough fermentation was intended to produce four (4) activities namely cassava dough softening, cassava dough souring, cyanogenetic glucoside reduction and volatile aromatic compound synthesis (Djeni, 2009). However, the technique for making attieke ferment differs between populations and production zones (Djeni 2009 ; Bouatenin 2013). The functional properties of these attieke ferments could thus vary not only according to the preparation technique, but also according to the species of microorganisms that ensure spontaneous fermentation. What's more, the rate of inoculation of the dough at the time of grinding varies from one producer to another and from one production zone to another. Similarly, after three days (3), these attiéké ferments lose their technological properties. For all these reasons, and because fermentation is not controlled, the quality of the finished product cannot be predicted (Kimaryo et al., 2000). However, the metabolic activity of these ferments is highest at 35°C coupled with a 10% inoculation dose, after only 12 hours of fermentation (Djeni et al., 2011). It is therefore necessary to find a ferment with empirical knowledge for the production of attieke with stable and/or improved quality through stabilization and preservation. According to Bouatenin et al., 2021, freeze-dried ferment is an alternative to traditional ferments that can ensure their shelf life and availability over a long period. For Karim et al., 2012, freeze-drying consists in removing water from the food, enabling stability by lowering water activity, thus extending product shelf lifeIn addition, this freeze-dried ferment must have the characteristics of a good cassava ferment, based on the attributes souring, aroma synthesis, detoxification and softening due to the four main respective technological activities, namely rapid acidification, production of α-amylase, β-glucosidase and pectinases that it produces (Djéni, 2009). In other hand, no study has been carried out not only on the technological properties of a freeze-dried cassava ferment, but also on the ideal dose of freeze-dried ferment to inoculate cassava dough for the production of quality attieke. The aim of this work is therefore to study the influence of the dose of freeze-dried ferment on the kinetics of production of technological properties during the fermentation of cassava dough.

MATERIALS AND METHODS

MATERIALS

The study material consisted of various ferments of braised, boiled and raw cassava and fresh roots of the IAC (Improved African Cassava) variety (Fig.1).

Braised ferment

Raw ferment

Boiled ferment

Cassava roots

Figure 1: Photograph of different types of attieke ferments and cassava roots

METHODS

Sampling of attieke ferments

Ready-to-use ferments were collected from 3 attieke producers in the villages of Anono (Abidjan) for the boiled type, Akrou (Jacqueville) for the braised type and Bonoua for the raw type. These producers were chosen based on the type of ferment used for the production of attiéké. At each production site, 6 samples of ferments were collected 3 times over a period of 3 years. A visit to the producers was carried out every year. These samples thus collected were packaged in batches of 1000 grams in sterile "stomacher" bags, placed in a cooler containing ice and transported to the biochemistry and microbiology laboratory of the NANGUI ABROGOUA University. A total of fifty-four (54) samples were collected under these conditions. These samples were immediately analyzed at the biochemistry and microbiology laboratory of NANGUI ABROGOUA University.

Preparation of freeze-dried ferment

Once in the laboratory, each type of attieke ferment collected was ground using a Binatone BLG555-1.5L-450 Blender mixer. The ground material was transferred into sterilized bottles, frozen for 24 hours at -80 ° C and freeze-dried using a FD-80-A vacuum freeze-dryer.

Preparation of cassava Dough

The fresh cassava roots of the IAC variety purchased at the large market in the Abobo commune are first peeled and washed with plenty of water by rubbing them with a sponge, then disinfected for 15 minutes with 1% mercury chloride in 70% ethanol and rinsed in sterile distilled water according to the Bouatenin method (2013). The cossettes (cassava pulp cut into small pieces) are then heated for 5 minutes to inactivate endogenous linamarase. The sterilized pulps are crushed using a traditional grater previously disinfected with 70% alcohol under aseptic conditions.

Fermentation process

The fermentation process was carried out as described by Djeni (2009). A set of five (5) Erlenmeyer flasks containing 250 g of cassava dough was used for each type of ferment. The doses of ferments used were 5%, 10%, 15% and 20% (based on dry matter). A batch of dough inoculated with 10% traditional ferment was used as a control. The fermentations were carried out in duplicate and the Erlenmeyer flasks were incubated for 24 hours at 35°C in a thermostatic water bath (GFL, Hanover-Vinnhorst, Germany). During the fermentation, samples were taken every 6 hours to evaluate the technological properties. All experiments were repeated three times and the average was retained

Acidification during cassava dough fermentation

The pH and titratable acidity (expressed as a percentage) of the different cassava doughs undergoing fermentation were determined using the AOAC (1995) method. 10 g of each cassava fermenting dough was homogenized in 20 mL of distilled water. The pH was obtained by inserting the electrode of a pH meter (P604, Consort, France) into the mixture. After reading the pH, a volume of 10 mL of the supernatant is removed and 3 drops of 1% phenolphthalein are added before titrating the acidity with a standard sodium hydroxide solution (0.1N). The end of the titration is indicated by a noticeable pink colour.

Evaluation of enzymatic activities during cassava dough fermentation

α-Amylase activity : The determination of α-amylase activity is carried out according to the method described by Vazquez et al. (2004). To detect α-amylase activity, 100 mL of distilled water is added to 10 g of fermenting cassava dough and centrifuged at 600 rpm for 40 minutes. The supernatant obtained is dialysed at a suitable concentration at 4°C. The dialysate is the crude enzyme preparation. The enzyme-substrate reaction mixture consists of 100 µL of starch (1 %) dissolved in 125 µL of sodium phosphate buffer (0.2 M) at pH 6.9. To this mixture 75 µL of enzyme extract is added. This reaction medium is incubated in a water bath at 37°C for 30 minutes. The enzymatic reaction is stopped by adding 300 µL of DNS solution. The medium is then homogenised and heated in a boiling water bath for 5 min, then cooled to room temperature for 10 min. The absorbance is measured at 540 nm using a spectrophotometer against the control (containing all products except the enzyme extract) after the addition of 2 mL of distilled water. One unit (U) of amylase was defined as the amount capable of releasing one micromole (µmol) of glucose/min into the reaction medium under the above conditions.

β-Glucosidase activity : β-Glucosidase activity was determined according to the method of Gallo (2004) using 4-nitrophenol-β-D-glucopyranoside as substrate. To 10 g of fermenting cassava dough, 100 mL of homogenised distilled water is added and centrifuged at 6000 rpm for 40 minutes. The supernatant obtained is diluted to a suitable concentration at 4°C. The reaction medium used consists of 125 µL of 100 mM acetate buffer pH 5.0, 50 µL of appropriately diluted enzyme solution and 75 µL of 5 mM pNP-glycoside. The reaction mixture was incubated at 37°C for 10 minutes. The reaction was stopped by heating the mixture to 95°C for 5 min. Enzymatic activity is manifested by the appearance of a characteristic yellow colouration of paranitrophenol (pNP), which is released in alkaline medium by hydrolysis of pNP-

glycoside. The amount of pNP released is determined using a spectrophotometer (Spectronic 20 D+) at a wavelength of 410 nm against a control containing no enzyme solution. These are the standard assay conditions used in this study. Enzyme activity is defined as the amount of enzyme capable of releasing one micromol (µmol) of product into the reaction medium under the conditions described above.

Pectinolytic activity : The pectinolytic activity assay was performed according to the method of Macedo et al. (2000). To measure pectinolytic activity, 10 g of fermenting cassava paste were dissolved in 200 mL of distilled water. The mixture was homogenized and then 10 mL of the mixture were taken and centrifuged at 6,000 rpm/40 min. The supernatant obtained was dialyzed at 4 °C against distilled water for 4 hours after adding 80% ammonium sulfate to precipitate all proteins. The dialysate constitutes the crude enzyme preparation. The enzyme-substrate reaction mixture is composed of 4 mL of 0.2% polygalacturonate dissolved in a 0.1 M acetate buffer of pH 5. One (1) mL of enzyme solution is added and the whole is incubated for 10 min at 40 °C. The reaction is stopped by adding 1 mL of DNS (3,5-dinitrosalycilic acid), at 100 °C for 15 min in a water bath. After cooling the tubes, 1 mL of distilled water is added. The optical density of each tube with its control is read at 540 nm on a spectrophotometer. The pectinolytic activity is expressed in International Units per milliliter of reaction medium. One unit of pectinolytic activity represents the quantity of enzyme that releases one micromole of galacturonic acid per minute.

RESULTS AND DISCUSSION

To produce Attieke, women producers use a ferment to inoculate the cassava dough. This inoculum has enormous technological properties, namely the softening of cassava dough, the souring of cassava dough, the reduction of cyanogenetic glucosides and the synthesis of volatile aromatic compounds. The production of these activities would be due to the production of enzymes such as α-amylase, β-glucosidase and pectinase. (Bouatenin, 2013; Djeni 2009). However, it is highly vulnerable due to its high water content. Indeed, after three days, it loses its technological properties, which could lead to extreme physico-chemical and microbiological changes in the cassava dough. In order to ensure the long-term availability of the ferment and improve the stability of finished products, the introduction of a rapid, less costly preservation technique such as freeze-drying would be of paramount importance. Work carried out by Bouatenin et al (2021) on the effect of several drying techniques on traditional cassava ferment for attieke production showed that attieke made from freeze-dried ferment was the most appreciated. The use of freeze-dried ferments at different concentrations (5%, 10%, 15%, 20%) to ferment cassava dough will enable us to determine the ideal inoculation dose. In the course of the various fermentation tests carried out, there was significant acidification, reflected in a rapid drop in pH and an increase in acidity, whatever the inoculation rate and type of ferment. However, this variation is more pronounced in doughs inoculated with traditional ferments and cassava doughs inoculated with 10% freeze-dried ferments after 24 hours of fermentation. Under the same fermentation conditions, the pH and acidity of doughs inoculated with the different freeze-dried ferments are not significantly different at the 5% threshold ($P > 0.05$) when the inoculum used is 10%. At this stage, the dough inoculated with 10% lyophilized raw ferment records the highest acidity of 0.97±0.1% and that of the dough inoculated with the traditional raw ferment was 0.93±0.4%. Dough inoculated with traditional braised ferment recorded an acidity level of 0.62±0.09%, while that of dough inoculated with 10% freeze-dried braised ferment was 0.64±0.3%. While, the dough inoculated with the traditional boiled ferment recorded an acidity rate of 0.52±0.07percentage and that of the dough inoculated with 10% of the freeze-dried boiled ferment was 0.63±0.2 percentage (Table 1).

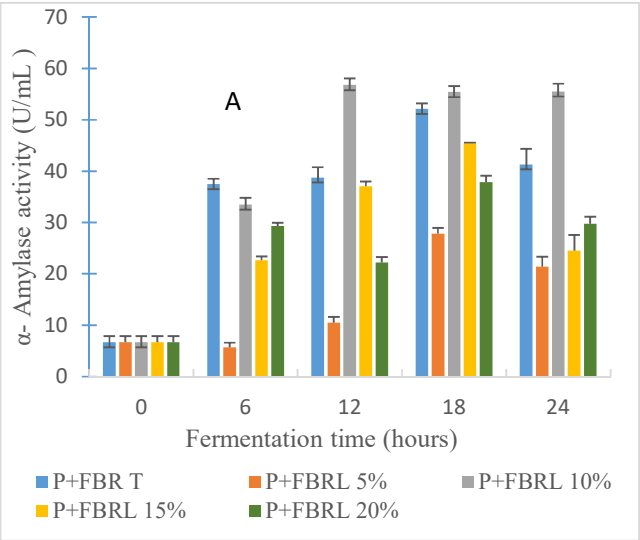
Table 1: Effect of ferment dose during acidification of cassava dough

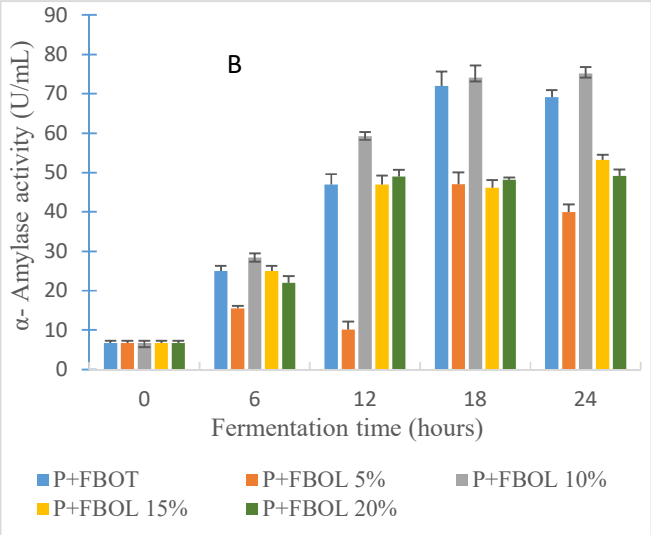
				Fermentation time (hours)				
		Inoculum rate (%)		0	6	12	18	24
Braised ferment	pH	Control	10	7.22±0.1^{a}	5.89±0.07^{a}	4.8±0.9^{a}	3.15±0.02^{a}	3.03±0.2^{a}
		After freeze-drying	5	7.22±0.1^{a}	4.35±0.5^{b}	4.6±0.1^{a}	4.46±0.1^{b}	4.11±0.2^{b}
			10	7.22±0.1^{a}	4.15±0.07^{b}	3.24±0.07^{b}	3.12±0.5^{a}	3.01±0.09^{a}
			15	7.22±0.1^{a}	4.2±0.3^{b}	4.18±0.07^{a}	4.23±0.1^{b}	4.14±0.2^{b}
			20	7.22±0.1^{a}	4.2±0.4^{b}	4.24±0.2^{a}	4.23±0.4^{b}	4.12±0.7^{b}
	Titratable acidity (%)	Control	10	0.09±0.07^{a}	0.17±0.09^{a}	0.29±0.02^{a}	0.5±0.09^{a}	0.62±0.09^{a}
		After freeze-drying	5	0.09±0.07^{a}	0.06±0.01^{b}	0.02±0.01^{b}	0.02±0.01^{b}	0.03±0.01^{b}
			10	0.09±0.07^{a}	0.19±0.05^{a}	0.33±0.07^{a}	0.54±0.02^{a}	0.64±0.3^{a}
			15	0.09±0.07^{a}	0.13±0.02^{a}	0.19±0.09^{c}	0.39±0.09^{c}	0.4±0.05^{a}
			20	0.09±0.07^{a}	0.13±0.1^{a}	0.29±0.1^{a}	0.37±0.07^{c}	0.3±0.2^{c}
Boiled Ferment	pH	Control	10	7.22±0.1^{a}	5.39±0.09^{a}	4.42±0.5^{a}	3.18±0.07^{a}	3,13±0.4^{a}
		After freeze-drying	5	7.22±0.1^{a}	5.94±0.07^{a}	4.61±0.4^{a}	4.56±0.9^{b}	4.42±0.07^{b}
			10	7.22±0.1^{a}	5.32±0.5^{a}	4.38±0.01^{a}	3.13±0.4^{a}	3.01±0.2^{a}
			15	7.22±0.1^{a}	4.98±0.2^{b}	4.46±0.9^{a}	4.28±0.2^{b}	3.78±0.1^{c}
			20	7.22±0.1^{a}	4.98±0.07^{b}	4.44±0.07^{a}	4.33±0.4^{b}	3.71±0.09^{c}
	Titratable acidity (%)	Control	10	0.09±0.04^{a}	0.17±0.01^{a}	0.29±0.07^{a}	0.5±0.07^{a}	0.52±0.07^{a}
		After freeze-drying	5	0.09±0.04^{a}	0.09±0.02^{b}	0.16±0,07^{c}	0.2±0.2^{b}	0.31±0.05^{b}
			10	0.09±0.04^{a}	0.1±0.5^{a}	0.29±0.07^{a}	0.59±0.06^{a}	0.63±0.2^{c}
			15	0.09±0.04^{a}	0.16±0.1^{a}	0.22±0.07^{a}	0.26±0.04^{b}	0.45±0.04^{b}
			20	0.09±0.04^{a}	0.21±0.1^{a}	0.18±0.1^{c}	0.53±0.1^{a}	0.5±0.1^{a}
Raw ferment	pH	Control	10	7.22±0.09^{a}	5.23±0.7^{a}	4.42±0.2^{a}	3.42±0.2^{a}	3.4±0.2^{a}
		After freeze-drying	5	7.22±0.09^{a}	6.2±0.1^{b}	5.41±0,9^{b}	4.02±0.01^{b}	3.94±0.7^{b}
			10	7,22±0.09^{a}	5.14±0.01^{a}	4.17±0.09^{a}	3.39±0.9^{a}	3.02±0.1^{a}
			15	7.22±0.09^{a}	5.45±0.09^{a}	4.7±0.07^{a}	3.41±0.1^{a}	3.18±0.3^{a}
			20	7.22±0.09^{a}	5.14±0.07^{a}	4.65±0.3^{a}	4.42±0.07^{b}	3.29±0.09^{ab}
	Titratable acidity (%)	Control	10	0.09±0.07^{a}	0.27±0.1^{a}	0.45±0.07^{a}	0.89±0.09^{a}	0.93±0.4a^{a}
		After freeze-drying	5	0.09±0.07^{a}	0.25±0.01^{a}	0.35±0.3^{c}	0.21±0.02^{c}	0.29±0.02^{c}
			10	0.09±0.07^{a}	0.27±0.2^{a}	0.45±0.1^{b}	0.95±0.01^{a}	0.97±0.1^{a}
			15	0.09±0.07^{a}	0.22±0.05^{a}	0.45±0.07^{a}	0.5±0.09^{b}	0.4±0.07^{b}
			20	0.09±0.07^{a}	0.18±0.03^{b}	0.41±0.09^{a}	0.29±0.07^{c}	0.7±0.09^{d}

Considering each type of ferment, for each parameter, in the same column, the mean values followed by different alphabetical letters are statistically different ($P \leq 0.05$) (DUNCAN multiple t-test).

Indeed, acidification is one of the important technological properties sought in attieke ferments. This acidification contributes to the improvement of the taste, texture and shelf life of the final product According to Montet et al. (2006) and Panda et al. (2007). It could be said that the organoleptic qualities of the finished products that will be obtained from 10% of freeze-dried ferment will be more improved than those of each traditional ferment especially. However, the high activity of production of titratable acidity is related to the diversity of fermentative microorganisms. This acidification of fermenting doughs is a probable effect of the activity of lactic acid bacteria and yeasts, according to the work of (Djoulde et al., 2015; Oduah et al., 2015). These authors have shown, during their work on fermented products, that lactic acid bacteria and yeasts, which make up the majority of the microflora, transform sugars into organic acids. This leads to a decrease in the pH of the medium, making it more acidic. The high activity of acidity production is related to the diversity of microorganisms with significant

enzymatic activities (α amylase, β-glucosidase and pectinase). It should be noted that regardless of the type of ferment incriminated, the production of these enzymes is greater in cassava doughs inoculated with traditional ferments and doughs inoculated with 10% of each freeze-dried ferment than other cassava doughs inoculated with 5, 15 and 20% of freeze-dried attiéké sourdough. Thus, the mechanism of production of α amylase in doughs inoculated with 10% of boiled and braised ferment is by repression characterized by an increase in enzymatic activity in the culture medium up to 18 hours of fermentation, beyond which the increase is slowed down or stopped. The enzymatic activity therefore remains constant or increases slightly when the fermentation is prolonged beyond this optimal duration. At this time, the optimum α amylase value in dough inoculated with 10% boiled ferment was 74.12±3.1 U/mL, and that in dough inoculated with 10% freeze-dried braised ferment was 55.41±2.1 U/mL. The kinetics of α-amylase production in dough inoculated with 10% of the raw ferment is by inactivation. Inactivation occurs after 18 hours of fermentation, with enzyme production of 75.78±1.9 U/mL (Fig. 2).





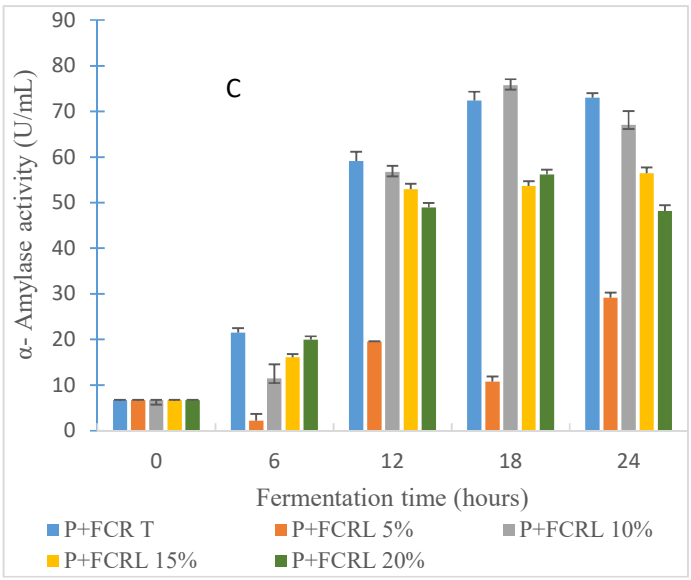
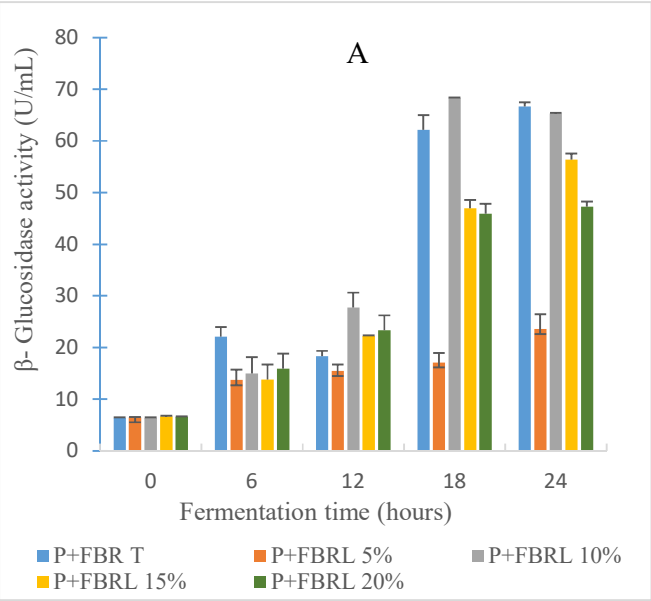
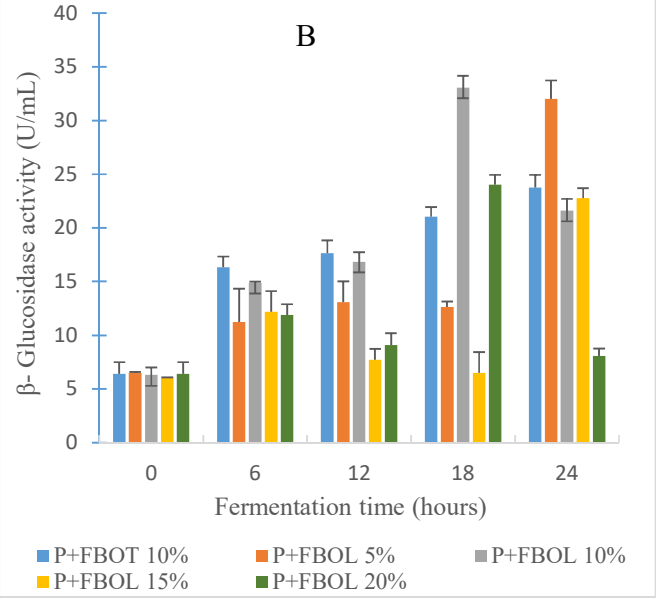


Figure 2 : Production kinetics of α-amylase of different freeze-dried ferments inoculated into cassava dough during fermentation. (**A:** Cassava dough controlled by braised ferments, **B:** Cassava dough controlled by boiled ferments; **C:** Cassava dough controlled by raw ferments). **P+FBRT:** Dough inoculated with traditional braised ferment; **P+FBRL 5%:** Dough inoculated with 5% freeze-dried braised ferment; **P+FBRL 10%:** Dough inoculated with 10% freeze-dried braised ferment; **P+FBRL 1 5%:** Dough inoculated with 15% freeze-dried braised ferment; **P+FBRL 20%:** Dough inoculated with 20% freeze-dried braised ferment ; **P+FBOT:** Dough inoculated with traditional boiled ferment; **P+FBOL 5%:** Dough inoculated with 5% freeze-dried boiled ferment; **P+FBOL 10% :** Dough inoculated with 10% freeze-dried boiled ferment; **P+FBOL 15% :** Dough inoculated with 15% freeze-dried boiled ferment; **P+FBOL** 20% : Dough inoculated with 20% freeze-dried boiled ferment; **P+FCRT :** Dough inoculated with traditional raw ferment; **P+FCRL 5%:** Dough inoculated with 5% freeze-dried raw ferment ; **P+FCRL 10%:** Dough inoculated with 10% freeze-dried raw ferment; **P+FCRL 15%:** Dough inoculated with 15% freeze-dried raw ferment; **P+FCRL 20%:** Dough inoculated with 20% freeze-dried raw ferment.

The production of this enzyme could be linked to the presence of fermentative microorganisms, notably yeasts and lactic acid bacteria (Kostinek et al., 2007), and starch, which is a potential source of energy for these fermentative organisms (Kostinek et al., 2007) during fermentation. Indeed, in both yeast and bacteria, α-amylase transcription is induced indirectly by polysaccharides such as starch, and the synthesis-secretion system is regulated by glucose and disaccharides such as maltose or trehalose. Maltose and glucose are the products of starch hydrolysis by α-amylase. Maltose is a catabolic inducer and glucose is the catabolic intermediate of the repressor (Yamana 1980 ; Henkin et al., 1991 ; Bouatenin et al., 2016). The various kinetics obtained have shown that the adaptation time for induction and active secretion of α amylase varies according to the ferments. The fermentation time required to obtain the maximum amount of α amylase in a cassava dough inoculated with 10% traditional or freeze-dried ferment is estimated at 18 hours. The reduction or cessation of α amylase at the end of fermentation can be explained by instability of the enzyme or inhibition of α amylase by glucose, which is a hydrolysis product of the enzyme (Nazir et al., 2009). However, of all the fermenting cassava doughs, the best yield was obtained in the dough inoculated with 10% raw ferment after 18 hours of fermentation. Furthermore, the kinetics of β-glucosidase production as a function of fermentation duration showed inactivation after 18 hours of fermentation. This kinetics is observed in cassava doughs inoculated with 10% braised, boiled and raw freeze-dried ferment. At this time, the production of β-glucosidase activity in these fermented doughs were 68.34±0.9 U/mL,

33.08±1.1 U/mL and 29.87±3.1 U/mL respectively. Under the same fermentation conditions, the high β-glucosidase activities of cassava doughs inoculated with traditional braised, raw and boiled ferments were 62.15±1.5 U/mL, 25.76±1.9 U/mL, 21.06±0.9 U/mL, respectively (Fig. 3).





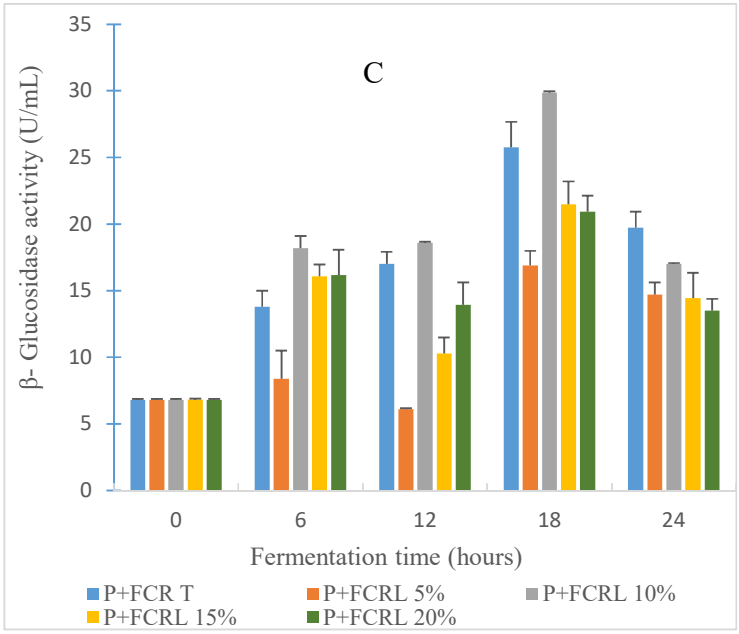
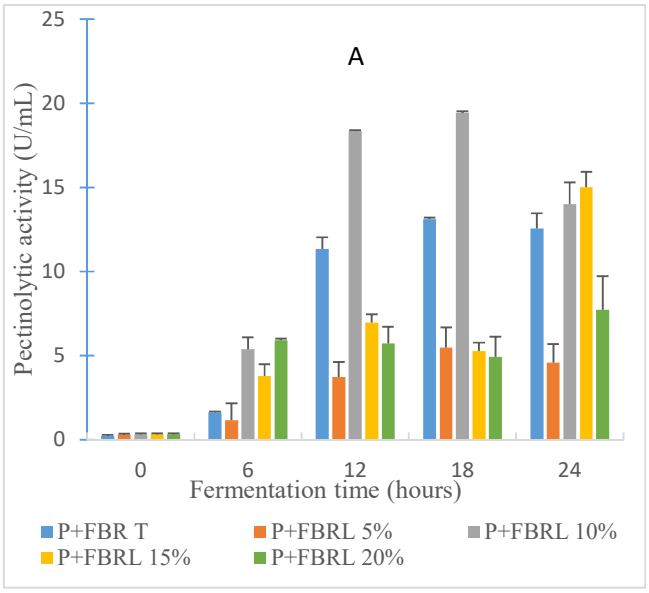
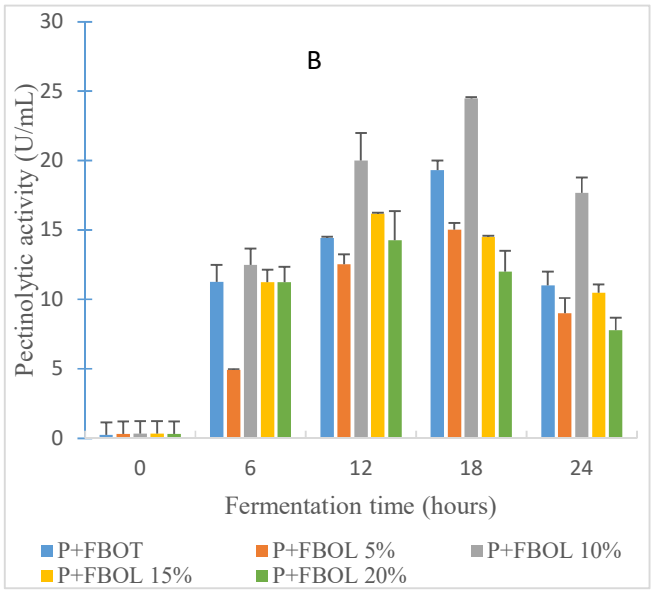


Figure 3: Production kinetics of β- glucosidase of different freeze-dried ferments inoculated into cassava dough during fermentation. (**A:** Cassava dough controlled by braised ferments, **B:** Cassava dough controlled by boiled ferments; **C:** Cassava dough controlled by raw ferments). **P+FBRT:** Dough inoculated with traditional braised ferment; **P+FBRL 5%:** Dough inoculated with 5% freeze-dried braised ferment; **P+FBRL 10%:** Dough inoculated with 10% freeze-dried braised ferment; **P+FBRL 1 5%:** Dough inoculated with 15% freeze-dried braised ferment; **P+FBRL 20%:** Dough inoculated with 20% freeze-dried braised ferment ; **P+FBOT:** Dough inoculated with traditional boiled ferment; **P+FBOL 5%:** Dough inoculated with 5% freeze-dried boiled ferment; **P+FBOL 10% :** Dough inoculated with 10% freeze-dried boiled ferment; **P+FBOL 15% :** Dough inoculated with 15% freeze-dried boiled ferment; **P+FBOL 20% :** Dough inoculated with 20% freeze-dried boiled ferment; **P+FCRT :** Dough inoculated with traditional raw ferment; **P+FCRL 5%:** Dough inoculated with 5% freeze-dried raw ferment ; **P+FCRL 10%:** Dough inoculated with 10% freeze-dried raw ferment; **P+FCRL 15%:** Dough inoculated with 15% freeze-dried raw ferment; **P+FCRL 20%:** Dough inoculated with 20% freeze-dried raw ferment.

The high production of β-glucosidase in fermenting doughs especially in cassava dough inoculated with 10% braised ferment could be related to the hydrolysis of various compounds containing β-glucosidase bonds such as cyanogenic glucosides (Djeni, 2009; Bouatenin, 2013). As cassava contains large quantities of these cyanogenetic glucosides, β-glucosidase-producing microorganisms would probably be able to hydrolyze linamarin into glucose and cyanohydrin acetone, and use the glucose for their own metabolism (Bouatenin, 2013). The production of β-glucosidase during the fermentation of cassava dough has also been demonstrated by (Djouldé et al., 2005). In their work, these authors set up a detoxification and protein enrichment ferment for cyanogenic cassava. They also demonstrated that β-glucosidase participates in cassava detoxification by degrading linamarin. One of the technological

properties of attieké ferments is the ability to soften cassava dough during fermentation through the production of pectinase (Djouldé et al., 2005). Thus, the kinetics of pectinolytic activity production in cassava dough inoculated with 10% boiled freeze-dried ferment (24.5±0.09 U/mL) and that inoculated with 10% braised freeze-dried ferment (19.42±0.09 U/mL) with the highest yields is marked by inactivation. This inactivation is characterized by an increase in enzyme production up to 18 hours of fermentation, after which production is progressively reduced. The kinetics of pectinolytic activity production in cassava dough inoculated with 10% raw freeze-dried ferment is constitutive, reaching a maximum value of 16.5±2.3 U/mL after 24 hours of fermentation (Fig. 4).





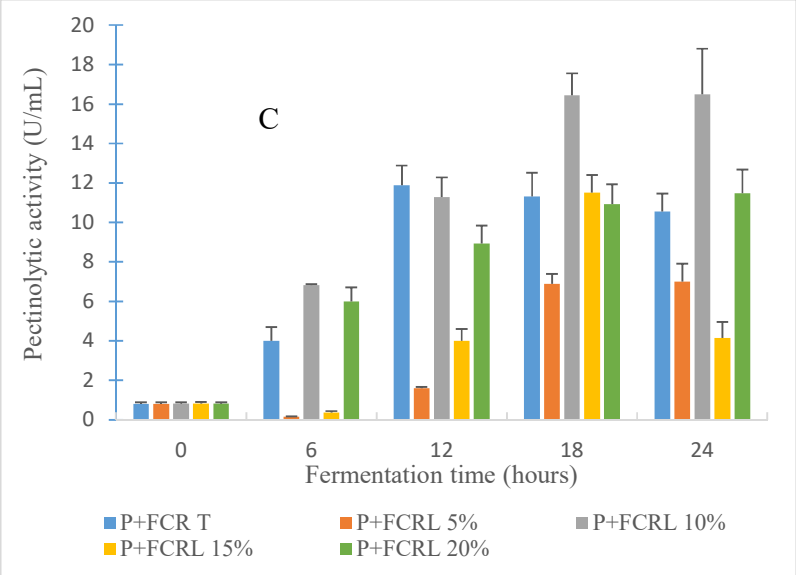


Figure 4 : Production kinetics of pectinolytic activity of different freeze-dried ferments inoculated into cassava dough during fermentation (**A:** Cassava dough controlled by braised ferments, **B:** Cassava dough controlled by boiled ferments; **C:** Cassava dough controlled by raw ferments). **P+FBRT:** Dough inoculated with traditional braised ferment; **P+FBRL 5%:** Dough inoculated with 5% freeze-dried braised ferment; **P+FBRL 10%:** Dough inoculated with 10% freeze-dried braised ferment; **P+FBRL 1 5%:** Dough inoculated with 15% freeze-dried braised ferment; **P+FBRL 20%:** Dough inoculated with 20% freeze-dried braised ferment ; **P+FBOT:** Dough inoculated with traditional boiled ferment; **P+FBOL 5%:** Dough inoculated with 5% freeze-dried boiled ferment; **P+FBOL 10% :** Dough inoculated with 10% freeze-dried boiled ferment; **P+FBOL 15% :** Dough inoculated with 15% freeze-dried boiled ferment; **P+FBOL 20% :** Dough inoculated with 20% freeze-dried boiled ferment; **P+FCRT :** Dough inoculated with traditional raw ferment; **P+FCRL 5%:** Dough inoculated with 5% freeze-dried raw ferment **; P+FCRL 10%:** Dough inoculated with 10% freeze-dried raw ferment; **P+FCRL 15%:** Dough inoculated with 15% freeze-dried raw ferment; **P+FCRL 20%:** Dough inoculated with 20% freeze-dried raw ferment.

The pectinase production observed in fermenting cassava doughs reflects the ability of the microorganisms in the lyophilized ferments used to excrete this enzyme during fermentation. Bouatenin et al. (Bouatenin 2013; Bouatenin et al., 2021) have attributed the role of pectinoytic activity production to a category of microorganisms present in cassava ferments, particularly Bacillus and moulds. These key microorganisms are involved in the degradation of cassava tissues through the production of enzymes such as polygalacturonases, pectin esterases and cellulases, which they release (Ouattara et al., 2008; Ehon et al., 2015), allowing the softening of cassava dough during fermentation. To facilitate data interpretation and select potential lyophilized enzymes whose inoculation doses are ideal for attieke production, a principal component analysis was performed using titratable acidity and enzyme activity data. Figure 5 shows the principal component analysis highlighting several groups of Attieke fermentation rates. The cassava doughs inoculated with 10%, 15% and 20% of the raw lyophilized ferment and the raw traditional ferment are distinguished from the other attieke

ferments. However, the cassava dough inoculated with 10% of the raw freeze-dried ferment is similar to that inoculated with 10% of the raw traditional ferment. As for the dough inoculated with 15%, 20% of the braised or boiled freeze-dried ferment, it is similar. The dough inoculated with 10% of the braised freeze-dried ferment, the dough inoculated with 10% of the boiled freeze-dried ferment, the dough inoculated with 10% of the braised traditional ferment, and the dough inoculated with 10% of the boiled traditional ferment produce the technological properties (acidification, α-amylase, β-glucosidase, pectinase) in a similar way (Fig. 5).

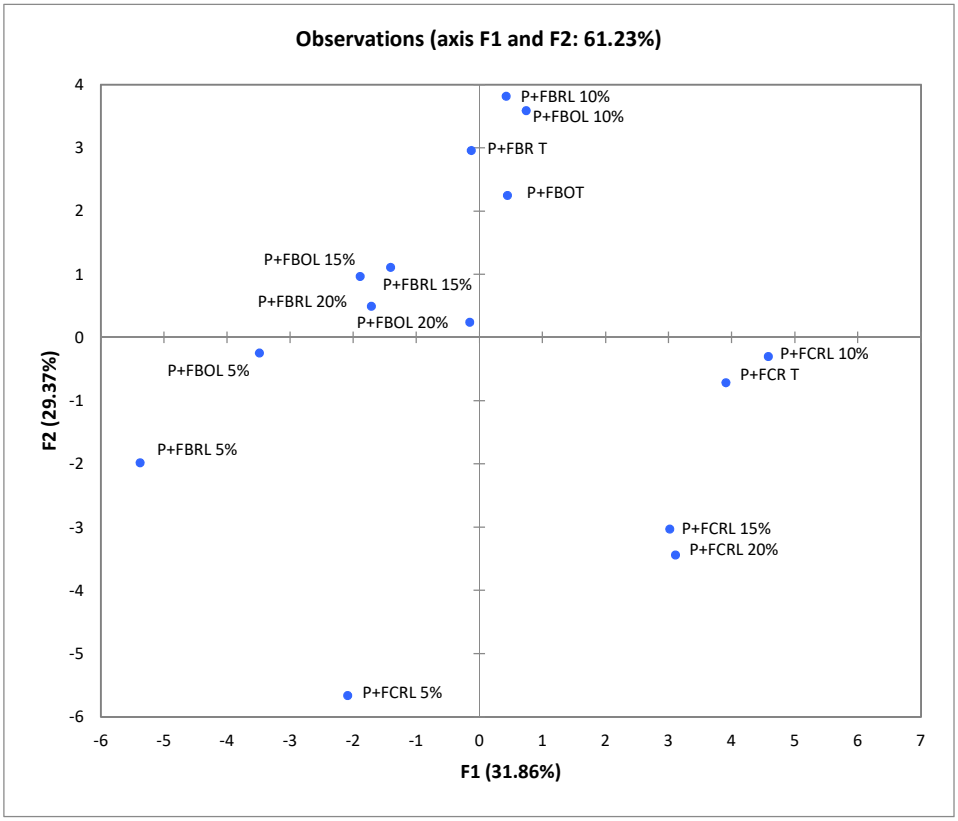


Figure 5: Principal component analysis (PCA) of fermenting cassava doughs.

P+FBRT: Dough inoculated with traditional braised ferment; **P+FBRL 5%:** Dough inoculated with 5% freeze-dried braised ferment; **P+FBRL 10%:** Dough inoculated with 10% freeze-dried braised ferment; **P+FBRL 1 5%:** Dough inoculated with 15% freeze-dried braised ferment; **P+FBRL 20%:** Dough inoculated with 20% freeze-dried braised ferment ; **P+FBOT:** Dough inoculated with traditional boiled ferment; **P+FBOL 5%:** Dough inoculated with 5% freeze-dried boiled ferment; **P+FBOL 10% :** Dough inoculated with 10% freeze-dried boiled ferment; **P+FBOL 15% :** Dough inoculated with 15% freeze-dried boiled ferment; **P+FBOL 20% :** Dough inoculated with 20% freeze-dried boiled ferment; **P+FCRT :** Dough inoculated with traditional raw ferment; **P+FCRL 5%:** Dough inoculated with 5% freeze-dried raw ferment ; **P+FCRL 10%:** Dough inoculated with 10% freeze-dried raw ferment; **P+FCRL 15%:** Dough inoculated with 15% freeze-dried raw ferment; **P+FCRL 20%:** Dough inoculated with 20% freeze-dried raw ferment.

On the other hand, the dough inoculated with 5% freeze-dried boiled ferment, the dough inoculated with 5% freeze-dried braised ferment and the dough inoculated with 5% freeze-dried raw ferment form a group in which the technological properties are different in each

dough fermented for 24 hours. It should be noted that, regardless of the type of ferment considered, the fermentation properties of a dough inoculated with 10% traditional ferment are similar to those of a dough inoculated with 10% freeze-dried ferment. In short, the metabolic activity of freeze-dried ferment is highest at a 10% inoculation dose, after 18 hours of fermentation at 35°C. Freeze-dried braised ferment, freeze-dried raw ferment and freeze-dried boiled ferment are therefore selected for the production of each type of attieke in Côte d'Ivoire.

CONCLUSION

The aim of this work was to study the influence of the dose (5, 10, 15 and 20%) of freeze-dried sourdough on the kinetics of the production of technological properties during the fermentation of cassava dough.During the different fermentations carried out, it was also clearly demonstrated that the production of titratable acidity and hydrolytic enzymes in cassava dough was influenced by the inoculation dose. The metabolic activity of freeze-dried enzymes was highest at a 10% inoculation dose, after 18 hours of fermentation at 35°C. Under these fermentation conditions, the technological properties of a dough inoculated with 10% of a traditional ferment and one inoculated with 10% of a freeze-dried ferment were similar, irrespective of the type of ferment used. Thus, freeze-dried braised ferment, freeze-dried raw ferment and freeze-dried boiled ferment will be an alternative for the formulation of attieke ferments that can ensure their preservation and long-term availability for the production of consistent quality attieké in Côte d'Ivoire.

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Data Availability
The data files associated with this study are available upon request to the corresponding author.

Conflicts of Interest
The authors declare that they have no conflicts of interest regarding the publication of paper.

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