Research Article

Production of Attieke by the Technique of Drying of Cassava Ferment

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The long-term availability of cassava ferment and the production of attieke of healthy and consistent quality are becoming serious problems in Côte d'Ivoire. The overall objective of this work was to assess the effect of several drying techniques on the performance of the traditional cassava ferment with a view to establishing a stabilized ferment for the production of attieke in Côte d'Ivoire. To do this, three drying techniques were used, namely, oven drying, sun drying, and freeze-drying. The end of the drying process is marked by the stabilization of the humidity rate of the ferment. The results obtained during the drying process indicate that the sun-dried ferment contains more GAM $(1.2 \pm 0.2) \times 10^8$ CFU/g than the other dried ferments. The freeze-dried ferment has the highest load of lactic acid bacteria $(3 \pm 0.2) \times 10^5$ CFU/g, while *Bacillus* was observed in large numbers in the ferment dried at 45°C $(7.1 \pm 0.6) \times 10^5$ CFU/g. The 37°C dried and freeze-dried cassava ferments recorded high yeast loads of $(7.6 \pm 0.5) \times 10^4$ CFU/g and $(7 \pm 0.4) \times 10^4$ CFU/g, respectively. Moulds $((5 \pm 0.2) \times 10^2$ CFU/g) were only detected in the 37°C dried culture. Moreover, during the fermentation of the cassava dough with the different ferments obtained after drying, a significant acidification occurs in the dough inoculated with the freeze-dried ferment $(2.9 \pm 0.07\%)$. However, the attieke produced with the freeze-dried ferment and the ferment dried at 37°C was the most appreciated by the panelists. Thus, freeze-drying and oven drying at 37°C are simple alternatives to the use of traditional ferments that can ensure their stability and the conservation of the cassava ferment over a long period of time.

1. Introduction

In Côte d'Ivoire, attieke is the most food consumed fermented cassava-based food [1]. It is dehydrated, steam-cooked cassava semolina with a slightly acidic taste and a whitish or light yellow color [2]. It is assimilated to couscous because of its granulation [3]. It is a traditional Ivorian dish that was once prepared and consumed in the family setting by lagoon peoples. Today, growing urbanization and increased demand have favored its popularity. Attieke has thus moved from the family setting to commercial production and is found throughout the country and even in countries of the West African subregion [4]. Attieke is also present in Europe, America, and Asia through the black African diaspora [1]. The preparation of attieke has become an income-generating

activity for women, and, as a result, it occupies an important place in the Ivorian economy [5]. However, despite its great popularity, the production of attieke still remains empirical. It covers a combination of steps including peeling, grinding, fermentation, pressing, granulation, drying, and steaming. Moreover, the development of the attieke industry is hampered by the irregularity of the product quality due to several factors, the most important of which is the fermentation which is carried out in an uncontrolled manner. Its preparation is generally linked to the “chance” of inoculation, and it often results in products of inconsistent quality, poor hygienic quality, low nutritional value, and short shelf life [6]. In addition, the laborious and time-consuming production of ferments is characterized by empirical control based on local traditions. As a result, the technological performance of these

traditional attieke ferments has been the subject of numerous studies for more than a decade, on the one hand for the control of fermentation and on the other hand for the selection of high-performance natural strains capable of directing fermentation [7]. However, the starters developed by research and development have many limitations as the work rarely takes into account the multiplicity of microbial activities required for a good fermentation of cassava. Also, their final shaping for use in the field is still not evident, coupled with fears about their use by traditional producers, with probably the additional costs of their acquisition that would limit their development. Thus, in the framework of this study, it appeared necessary to preserve the empirical assets by making them available for a sustainable intensification of attieke production with a stable and/or improved quality through the stabilization and conservation of the traditional ferment by drying. Therefore, the general objective of this work is to evaluate the effect of several drying techniques on the performance of the traditional attieke ferment in order to establish a stabilized ferment for attieke production in Côte d'Ivoire.

2. Materials and Methods

The biological material used to carry out this work consists of braised cassava ferment (Figure 1).

2.1. Sampling. The ready-to-use (braised) ferment was purchased at the large market in the commune of Abobo in the autonomous district of Abidjan in Côte d'Ivoire. In this commune, only one producer was chosen because of her willingness to produce the ferment. Three samples were taken from the producer, packaged in 500 g batches in "stomacher" bags and placed in a Camping Cooler (48QT) containing ice cream and then transported to the Microbiology and Food Biotechnology laboratory within 30 minutes. A total of three runs were carried out, i.e., nine samples were taken under the same conditions and analyzed immediately at the Laboratory of Biotechnology and Microbiology and Food (LMBA) of Nagui Abrogoua University (UNA).

2.2. Sample Preparation. The braised ferment collected from the producer was crushed using a Blender Binatone BLG-555-1.5L-450 blender. The resulting paste was divided into four parts at a weight of one kg. The first part is spread out on a tray covered with aluminium foil and put in the oven (SI4 854 C IX) at 37°C. Under the same working conditions, the second part is placed in the oven (SI4 854 C IX) at 45°C and the third part is exposed to the sun. The last part (remaining fermented cassava dough) is transferred to sterilized bottles, frozen for 24 hours at -80°C, and freeze-dried by a vacuum freeze dryer FD-80-A.

2.3. Determination of Humidity Rate. The method used for the determination of humidity rate and dry matter is that described by [8], which is based on dehydration by oven (Mettler, Germany) drying of samples to a constant

FIGURE 1: Braised ferment.

weight. Thus, 5 g of each sample is weighed into a glass capsule of known mass (m_0). The capsule containing the sample (m_1) is placed in the oven (Mettler, Germany) set at 105°C. The capsule, which is removed after 24 h, is placed in the desiccator. The whole (sample + capsule) is weighed (m_2) after cooling. Samples are taken every 24 hours, and the operation is repeated until a constant mass is obtained. The humidity rate is determined according to the following formula:

$$\text{humidity rate} = \frac{m_1 - m_2}{m_1 - m_0} \times 100. \quad (1)$$

m_0 is the mass of the empty crucible, m_1 is the mass of empty crucible + sample, and m_2 is the mass of the empty crucible + sample after drying.

2.4. Microbiological Analysis Carried Out during the Drying Process. In this part, a count was carried out not only to determine the level of contamination of the ferment but also to assess the viability of the fermenting germs during the drying of the ferment. Thus, microbial analyses were carried out to determine the microbiological loads of inoculum samples. Preparation of stock solutions, inoculation of agar plates, cultivation, and quantification of microorganisms were carried out according to [9]. For all determinations, 10 g of samples was homogenized in a stomacher with 90 ml of sterile peptoned buffered water (AES Laboratoire, COMBOURG France). Tenfold serial dilutions of stomacher fluid were prepared and spread plated to determine microorganism counts. Aerobic mesophilic was counted on PCA (plate count Agar) agar (Oxoid LTD, Basingstoke, Hampshire, England) after two days of incubation at 30°C according to AFNOR Standard NF V08-051,1999. Yeasts and moulds were enumerated on plates of Sabouraud chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich Chemie GmbH, Inda) incubated at 30°C for 4 days. Bacilli species were enumerated on plates Mossel agar (AES Laboratoire, COMBOURG France) after incubation at 30°C for 2 days. Enumeration of LAB was carried out using plates of de Man, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany) which were incubated under anaerobic conditions (Anaerocult A, Merck) at 37°C for 72 h.

2.5. Performance Tests of Dried Ferments and Determination of the Acidification Rate of the Fermenting Cassava Dough.

To test the performance of dried ferments (37°C, 45°C, sun-dried and freeze-dried ferment) for the production of attieke, the ferments are used to ferment different cassava doughs. For this purpose, a series of six erlenmeyers containing 250 g of cassava dough were used for each type of dried ferment. Four of these Erlenmeyers were inoculated with 10% of each dried ferment. The fifth Erlenmeyer flask containing 250 g of cassava dough was inoculated with 10% of traditional cassava ferment (positive control). The sixth Erlenmeyer flask contains only 250 g of cassava dough (negative control). During fermentation, samples are taken every six hours to determine the acidification rate of the medium. Thus, forty grams of inoculum samples were ground in 300 ml of distilled water in a porcelain mortar and then centrifuged at 4000 tours/min for 30 min. The pH was determined on 50 ml of the supernatant using a pH-meter (P107 Consort). Total titratable acidity (TTA) was determined by titrating 30 ml of supernatant used for pH determination against 0.1 M NaOH using phenolphthalein as indicator. TTA was calculated as percentage of lactic acid.

2.6. Classification by Rank of Attieke Products. After all analyses, each ferment is used for the preparation of attieke. On the basis of the average scores, the panelists drew up a ranking of the different attieke samples submitted for their assessment. Ranking tests are carried out according to the ISO 8587-2006 standard. A tasting, followed by an evaluation of the attieke samples, is carried out by subjects, 70 in number (made up of UNA students and staff of the Food Biotechnology and Microbiology laboratory). The evaluation of the attieke samples was done by assigning them marks on a structured 5-level rating scale (from very unpleasant = 1 to very pleasant = 5), expressing the general impression of their preference. Finally, the samples are ranked according to their preference (from most liked to least liked) by the different subjects (Supplementary File: Tasting Sheet).

2.7. Statistical Analysis. The software R.3-01, ANOVA method with Duncan's post-hoc test, and 5% significance level was used to calculate the means and standard deviations of the physicochemical and microbiological parameters studied. It was also used to compare the means of the physicochemical and microbiological parameters of the samples and to determine whether the differences observed in the means of the physicochemical and microbiological parameters were significant at the 5% significance level.

3. Results and Discussion

Traditional cassava processors use an inoculum to ferment the cassava paste during the production of attieke. This inoculum has the capacity to reduce the fermentation time and improve the texture and flavour of the final product [10]. Beyond 3 days of fermentation, the quality of the ferment remains undesirable and could lead to extreme physicochemical and microbiological changes in the cassava dough. In order to facilitate

access and availability of the ferment, the implementation of a quick and less expensive preservation technique such as drying would be a necessity. The tests carried out during this study highlighted the effect of several drying techniques on the performance of the ferment and the quality of the attieke. The end of drying during the different tests was marked by the stabilization of the dry matter. Indeed, the drying time of the ferments varies according to the temperature. That of the sun-exposed ferment was 8 days, while that of the dried ferments under study (37°C and 45°C) was 3 days and that of the freeze-dried ferment was 2 days (Figure 2).

The long drying time of the ferment exposed to the sun would be due to the air temperature. These results are confirmed by those of [11]. This author has shown, during the drying of mint leaves, that the air temperature has a considerable influence on the drying speed. At the end of the drying process, the microbial load was reduced in the analyzed ferments. This could be due to the effect of temperature on the growing germs. For [12], the temperature has a considerable influence on microbial growth. However, the high AMG load of $(1.2 \pm 0.2) \times 10^8$ CFU/g was detected in the sun-dried ferment (Table 1). This load is significantly different ($P < 0.05$) from that obtained in other ferments. This can be explained by the fact that, during sun drying, the material is exposed to an invasion of insects such as flies, which would bring additional germs to the material. In addition, exposure to sunlight in the ambient air promotes contamination by microorganisms [13]. Also, at the end of drying, a diversity of microorganisms that participate in fermentation such as yeasts, moulds, *Bacillus*, and lactic acid bacteria (LAB) was enumerated in the analyzed ferments. LAB is the most dominant. These results are in agreement with those of [6, 9, 14] which indicate a dominance of lactic acid bacteria in traditional cassava-based fermented products or starters. The lyophilized ferment $((3 \pm 0.2) \times 10^5$ CFU/g) contains the most and the sun-dried ferment the least LAB $((5 \pm 0.1) \times 10^2$ CFU/g). The difference in LAB load in these ferments was significantly different ($P < 0.05$) (Table 1). This indicates that LABs are capable of withstanding low freeze-drying temperatures [15]. They had indicated in their work on the study of cold adaptation that lactic acid bacteria are able to withstand freezing temperatures, but this cold resistance was species dependent. In fact, lactic acid bacteria synthesize in the presence of cold, proteins called cold-induced proteins (CIPs) that enable them to improve the efficiency of their metabolic processes [16]. In addition, lactic acid bacteria, thanks to the organic acids they produce, guarantee food safety and also give it very special characteristics in terms of aroma and texture. These LAB also participate in the reduction of cyanide rate in cassava and are responsible for the sour taste in fermented products due to the production of lactic and acetic acids [17]. It could be said that the product derived from lyophilized ferment (LF) would be of good organoleptic quality. The LABs enumerated in ferments dried at 37°C and 45°C and sun-dried would be mesophilic LABs capable of growing at temperatures between 37 and 45°C [15]. It should also be noted the massive presence of yeasts and *Bacillus* in the ferments analyzed. Yeasts had previously been identified as the second predominant germs involved in cassava

FIGURE 2: Variation in the humidity rate of traditional mashed cassava ferment during drying. FD at 37°C: ferment dried at 37°C; FD at 45°C: ferment dried at 45°C; FSD: ferment sun-dried; LF: lyophilized ferment.

TABLE 1: Microbial loads of the different ferments before and after drying.

Microbial load (CFU/g)						
<i>Before drying</i>		$(8 \pm 0.7) \times 10^{8a}$	$(3 \pm 0.3) \times 10^{6a}$	$(2.1 \pm 0.2) \times 10^{6a}$	$(6.1 \pm 0.3) \times 10^{5a}$	$(1 \pm 0.1) \times 10^{5a}$
<i>After drying</i>	FD at 37°C	$(3 \pm 0.4) \times 10^{6b}$	$(1 \pm 0.1) \times 10^{5b}$	$(1 \pm 0.8) \times 10^{5b}$	$(7.6 \pm 0.5) \times 10^{4b}$	$(5 \pm 0.2) \times 10^{2b}$
	FD at 45°C	$(2.5 \pm 0.7) \times 10^{6b}$	$(2 \pm 0.1) \times 10^{5b}$	$(7.1 \pm 0.6) \times 10^{5b}$	$(4.2 \pm 0.9) \times 10^{4b}$	nd
	FSD	$(1.2 \pm 0.2) \times 10^{8a}$	$(5 \pm 0.1) \times 10^{2c}$	$(9.2 \pm 0.5) \times 10^{2c}$	$(3.5 \pm 0.2) \times 10^{3c}$	nd
	LF	$(8.4 \pm 0.4) \times 10^{5b}$	$(3 \pm 0.2) \times 10^{5b}$	$(3.2 \pm 0.3) \times 10^{5b}$	$(7 \pm 0.4) \times 10^{4b}$	nd

For the same parameter and in the same column, the mean values followed by the same alphabetical letter are not statistically different ($P < 0.05$) (Duncan multiple t test), GAM: mesophilic aerobic germs; LAB: lactic acid bacteria; FD at 37°C: ferment dried at 37°C; FD at 45°C: ferment dried at 45°C; FSD: ferment sun-dried; LF: lyophilized ferment, nd: not detected.

fermentation after lactic acid bacteria that can contribute to the flavour development of fermented products [18]. High loads were detected in the ferment dried at 37°C ($(7.6 \pm 0.5) \times 10^4$ CFU/g) (Table 1). This load is significantly different ($P < 0.05$) from that obtained in other ferments. This is due to the fact that yeasts, like other microorganisms, can only function in an optimal temperature range, up to a critical temperature beyond which they cannot survive. Thus, the work of [19] showed that yeasts are able to resist in a medium with an acidic pH of 3 to 5 and temperatures ranging from 25°C to 37°C. Among the ferments analyzed, the ferment dried at 45°C has the highest *Bacillus* content ($(7.1 \pm 0.6) \times 10^5$ CFU/g) (Table 1). This load is significantly different ($P < 0.05$) from that obtained in other ferments. For [7], some *Bacillus* are capable of producing polygalacturonic activity at 45°C necessary to attack the plant cell walls of cassava roots composed of pectin and cellulose. The use of these enzymes is the basis of clarification for the softening of cassava pulp during fermentation. These microorganisms attack the skeletal structures of the cell partition (pectin) of cassava tubers and thus contribute to the softening of the cassava paste rapidly during fermentation. However, the monitoring of

Bacillus in the different ferments according to temperature and drying time could be explained by the fact that temperatures applied to the ferments in a short time cannot be significantly destructive to either the vegetative flora or the *Bacillus* spores. Like *Bacillus*, mould plays an essential role in the degradation of cassava tissue, which is necessary for a good texture of the dough. The presence of mould was also observed at the beginning of drying in the different ferments analyzed and at the end of drying of the dried ferment at 37°C (Table 1). This is due to the fact that mould is present at room temperature and at different pH levels. The studies carried out by [20] show that the moulds have a growth temperature between 4 and 30°C; the optimum pH is between 4 and 6.5, but they have the possibility to colonize very acidic environments (pH 2) via acidophilic moulds and also basic moulds (up to pH 11). In addition, moulds can also grow on the surface of food without being desired [21]. The presence and multiplication of these microorganisms in these ferments lead to important biochemical modifications of the dough during fermentation for the production of attieke. In various fermentation tests, significant acidification occurs during the fermentation process, resulting in a rapid decrease in pH.

FIGURE 3: pH (a) and titratable acidity (b) of fermenting cassava pastes inoculated with the ferments obtained after drying. PwF: fermented paste without ferment; P + FD 37°C: paste inoculated with the ferment dried at 37°C; P + FD 45°C: paste inoculated with the ferment dried at 45°C; P + FD in the sun: paste inoculated with the ferment dried in the sun; P + LF: paste inoculated with Lyophilized ferment, P + TF: paste inoculated with traditional ferment.

After 24 hours of fermentation, the pH of the doughs inoculated with the different ferments drops from 0.8 to 1.48 units (Figure 3(a)). However, the titratable acidity of the different fermenting dough increases. This increase in acidity is more pronounced in the paste inoculated with the lyophilized ferment ($2.9 \pm 0.07\%$) than in other fermenting dough (Figure 3(b)). The difference is not significant at the 5% threshold ($P > 0.05$). This acidification is attributed to the LABs that colonize the dried ferments put in place. These results are in agreement with those of [22]. These authors attribute to lactic acid bacteria the main role of rapid

acidification of food products. Indeed, the high activity of production of titratable acidity is related to the diversity of fermentative microorganisms. A co-metabolism between yeast and other microorganisms, in particular lactic acid bacteria, could be suggested, whereby the bacteria produce acid in the medium for the growth of the yeast and then these yeasts provide vitamins and other growth factors for the bacteria. Therefore, one could say that the paste inoculated with the lyophilized ferment (P + LF) has a microflora suitable for the production of attieke. After all analyses, each ferment is used for the preparation of attieke. On the basis of the

TABLE 2: Test of ranking by rank of attieke from different ferments.

Samples	Average scores	Average ranks (according to the assessment)
Attieke without ferment	1.25 ± 0.3 ^a	4.32 ± 1.07 ^a
Attieke from lyophilized ferment	3.96 ± 0.8 ^b	2.07 ± 0.53 ^b
Attieke from the ferment dried at 37°C	3.93 ± 0.6 ^b	2.1 ± 0.9 ^b
Attieke from the ferment dried at 45°C	3.17 ± 0.5 ^c	3.36 ± 0.89 ^c
Attieke from the ferment sun-dried	2.21 ± 0.95 ^d	4.71 ± 1.32 ^a
Attieke from traditional ferment	3.28 ± 1.2 ^{bc}	3.35 ± 0.77 ^c

For the same parameter and in the same column, the mean values followed by the same alphabetical letter are not statistically different ($P < 0.05$) (Duncan multiple t test).

TABLE 3: Descriptive characteristics of attieke produced with the different dried ferments.

Types of attieke						
Characteristics of attieke	Attieke from the ferment dried at 37°C	Attieke from the ferment dried at 45°C	Attieke from lyophilized ferment	Attieke from the ferment sun-dried	Attieke from traditional ferment	Attieke without ferment
Granulation	Heterogeneous, less sticky, and less rounded grains	Heterogeneous grains, less sticky, and less rounded	Heterogeneous, less sticky, and rounded grains	Heterogeneous grains, separated, and rounded	Heterogeneous grains, less sticky, and less rounded	Very sticky grains, not rounded
Color	Yellowish	Yellowish	Yellowish	Whitish	Whitish	Whitish
Flavor	Good aroma	Slight aroma	Very good aroma	No flavor	Good aroma	No flavor
Taste of attieke	Slightly sour, sour, and sweet	Slightly acidic	Very sweet, slightly sour, and acidic	Very sour	Slightly sour, acidic, and less sugary	Fade

average scores, the panelists drew up a ranking of the different attieke samples submitted for their assessment. Following this ranking, attieke from the freeze-dried and 37°C dried ferments were the most appreciated with mean rank values of 2.07 and 2.1, respectively (Table 2). The preference of these attieke over the other types can be justified by their granulometry (heterogeneous grains, less sticky, and rounded) and their organoleptic characteristics (good aroma, slightly sour, and tart taste). In addition, studies conducted by [23] have shown that attieke is composed of a mixture of medium and small grains with a sticky texture, often yellow or cream (shiny) in color, with a pleasant odour and a slightly acidic taste. Attieke from ferments dried at 37°C and freeze-dried are composed of heterogeneous, less sticky, and rounded grains. This attieke is similar to the traditional attieke which is yellowish in color with slightly sour, acidic sweet taste, and a pleasant aroma (Table 3). Indeed, according to [24], the preference of a food can be defined not only by its granulometry but also by its taste. It is clear, as established by [25] Oduro, that a good quality cassava-based fermented product should have a slightly sour taste that is not aggressive and without atypical odour, as is the case for attieke produced with lyophilized ferment and ferment dried at 37°C, hence their good appreciation by the panelists.

4. Conclusion

The effect of drying on the performance of the cassava ferment for attieke production allowed us to know that the drying time of the ferments varies according to the temperature. That of the sun-exposed ferment was 8 days while that of the dried ferments (37°C and 45°C) was 3 days and that of the freeze-dried ferment was 2 days. At the end of the drying process, the ferment germs, namely, lactic acid

bacteria (LAB), *Bacillus*, yeasts, and moulds, were detected in the ferments analyzed. The high LAB load was detected in lyophilized ferment (LF), while the high yeast loads were observed in the 37°C dried ferment and in the freeze-dried ferment. The ferment dried at 45°C was the most *Bacillus*-loaded of the ferments tested. The presence of mould was also observed at the beginning of the drying process in the different ferments analyzed and at the end of the 37°C dried ferment. When different fermentation tests were carried out, significant acidification occurred, resulting in a rapid decrease in pH and an increase in the soluble sugar content. The attieke from the freeze-dried ferment and ferment dried at 37°C are the most appreciated by the panelists. These two ferments are then retained for further work.

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.

Authors' Contributions

This work was carried out in collaboration between all authors. Koffi Marcellin DJE and N'dédé. Theodore DJENI were responsible for study design and supervision of work. Koffi Maïzan Jean-Paul BOUATENIN, Kohi. Alfred KOUAME and N'guessan Ghislain KOFFI were responsible for laboratory work, data analysis, and manuscript preparation.

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Supplementary Materials

A tasting, followed by an evaluation of the *attieke* samples, is carried out by the subjects, 70 in number (made up of UNA students and staff of the Food Biotechnology and Microbiology laboratory). The evaluation of the *attieke* samples was done by assigning them marks on a structured 5-level rating scale (from very unpleasant = 1 to very pleasant = 5), expressing the general impression of their preference. Finally, the samples are ranked according to their preference (from most liked to least liked) by the different subjects. (Supplementary Materials)

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