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Biochemical and Microbiological Changes of Cassava Dough Fermenting in Different Temperature Conditions

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Abstract: Cassava fermentation helps to preserve foods, provides a wide variety of textures and flavours and significantly improves the nutritional properties of the raw material. The production of attiéké from cassava in Côte d'Ivoire, typically occurs on a household or small industrial scale and consequently suffers from inconsistent product quality and may not always be safe for consumption. Therefore, this research was carried out to assess the biochemical and microbiological changes of cassava fermented dough during fermentation under different temperature conditions. Cassava doughs were inoculated with 10% of traditional starter and respectively incubated at 25, 30, 35, 45°C and ambient temperature. And then, pH, total titrable acidity, total sugar consumption, gas from fermentation and microorganism growth were assessed with time. There were different evolutions of studied parameters with time and in function of temperature of incubation. There were significant difference between effect of temperatures and 35°C seemed to be the best temperature for microorganism growth, acid production, sugar consumption and gas produced from fermentation. The results of the present research indicate that cassava dough fermentation, initiated by the inoculation of the spontaneous traditional starter was widely influenced by the temperature conditions and was optimum at 35°C for 12 h.

Key words: Fermentation, cassava dough, traditional starter, attiéké, temperature

INTRODUCTION

Cassava, the enlarged root of Manihot esculenta Crantz, is a staple food for over 500 million people in the developing world (Cock, 1985), of which 200 million live in subsaharian Africa (Madeley, 1993). It ranks fourth in the list of major food crops in developing country after rice, wheat and maize (Mlingi *et al*., 1992). Cassava has important agronomic advantages, but it has two important deficiencies. Firstly, the bitter varieties contain the toxic cyanogenic glucosides linamarin and (to a lesser extent) lotaustralin, which have fatal consequences when consumed in unprocessed foods (Kostinek *et al*., 2005). Secondly, it is very poor in protein, containing only about 1% (Sanni *et al*., 2002). So that, it is traditionally processed into a wide variety of products with different local names (Amoa-Awua *et al*., 1996). The processing methods cover a combination of procedures such as peeling, boiling, steaming, pounding, slicing, grating, roasting, soaking, pressing and fermentation depending on varieties. The most popular processing method, however, especially for the varieties high in cyanogenic glucosides, is by fermentation. And in Côte d'Ivoire, the most popular food derived from fermented cassava is attiéké. Attiéké was originally prepared and consumed exclusively in a restricted ethnocultural setting among the ethnic groups (Adjoukrou, Ebrié, Alladjan, Avikam, Ahizi) living in the laguna area in the south of the country. In recent years, the product has become popular beyond the boundaries of this area. It is now prepared and consumed everywhere in Côte d'Ivoire and even in the neighbouring countries. The cassava fermentation, during attiéké production requires the used of a traditional starter which production is linked to the ethnic groups. This starter constitutes the main source of microorganisms active in the dough fermentation. Microorganisms of such traditional starters, by virtue of their metabolic activities, contribute to the development of characteristic properties cassava of fermented foods (Holzapfel, 1997) the outcome and quality of which may not always be predictable or controllable. This is a major problem typically associated

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with traditionally fermented foods in Africa. Their preparation generally relies on chance inoculation and the result is often a product of inconsistent quality, poor hygiene, poor nutritional value and short shelflife. Moreover, cassava fermentation into attiéké is not yet controlled and most of the time, achieved in bad environment conditions and is under influence of several factors such as the quality and the rate of traditional starter added to the mash for fermentation, air condition and variation of temperature.

This study is carried out to assess the biochemical and microbiological changes of cassava dough fermenting in different temperature conditions.

MATERIALS AND METHODS

Traditional starter: Samples of ready to use traditional starter were obtained at a small-scale (women's enterprise) attiéké production in Abidjan.

Cassava roots: Twelve months-old freshly harvested cassava roots of the bitter variety were obtained from a farm of the University of Abobo-Adjamé (Abidjan, Côte d'Ivoire).

Fermentation of cassava dough: About 4 kg of freshly harvested cassava roots of bitter variety were peeled, washed and grated with a traditional grater. The cassava mash obtained was divided into 5 parts of 500 g each, then inoculated with 10% (w/w) of traditional starter and incubated, respectively at 25, 30, 35, 45°C and room temperature (27-28°C). The fermentation was followed with time and samples of fermenting dough were aseptically taken for the different analysis at the beginning and after 6, 12 and 18 h of fermentation.

Determination of ph and Total Titrable Acidity (TTA): Thirty gram of fermenting cassava dough sample were blended with 70 mL of sterile distilled water and filtered through a Whatman filter paper. The pH of 30 mL of the filtered solution was determined using a pH-meter (pH-meter P107, CONSORT, Bioblock scientific, France). Total Titrable Acidity (TTA) was determined using the standard method described by Amoa-Awua *et al.* (1996). Ten milliliter of filtered solution were titrated with NaOH 0.1 N, using 1% phenolphthalein as indicator. The volume of aliquot used was recorded to determine the amount of acid in the sample. The titrable acidity was calculated as percentage of lactic acid. The determinations were done in triplicates and the mean value recorded.

Determination of total sugars and reducing sugars: Water-soluble carbohydrates were determined by the phenol sulphuric acid method according to Dubois *et al.* (1956) and the values were expressed in g/100 g of fresh dough, while the reducing sugars were quantified as previously described by Bernfeld (1955) and expressed in mg/100 g of fresh matter.

Gas released from fermenting dough: The volume of gas produced during fermentation was measured on 300 g of cassava dough using an experimental device described Pol (1996).

Enumeration of microorganisms: Preparation of stock solutions, inoculation of agar plates, cultivation and quantification of microorganisms were carried out according to Coulin *et al.* (2006). For all determinations, 10 g of the samples were 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 for determination of microorganism counts. Enumeration of lactic acid bacteria was carried out using plates of DeMan, Rogosa and Sharp agar (MRS, Merck 10660, Merck, Darmstadt, Germany) which were incubated anaerobically in an anaerobic jar at 30°C for 3 days. Yeasts were enumerated on plates of Sabouraud chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich Chemie GmbH, Inda) incubated at 30°C for 4 days. Aerobic mesophiles were enumerated on plates of Plate Count Agar (PCA Oxoïd LTD, Basingstore, Hamsphire, England) and incubated at 30°C for 2 days.

Statistical analysis: Experimental results were subjected to Analysis of Variance (ANOVA) and differences between means were assessed by Duncan's new multiple range test using appropriate computer software

RESULTS AND DISCUSSION

Enumeration of microorganisms: The high counts of microorganisms found in cassava doughs during fermentation indicated that this fermenting medium is well-adapted for microbial activities. Moreover, the fermentation process was initiated by the inoculation of the spontaneous traditional starter containing a broad microflora. Growth of total aerobic mesophile, lactic acid bacteria and yeast in fermenting doughs under various temperature conditions are illustrated in Fig. 1-3. Total aerobic mesophiles corresponded approximatively to the sum of lactic acid bacteria and yeasts. The initial count at the beginning of the fermentation was 3.3×10^{7} cfu g^{-1} of dough. This count increased differently with the temperatures of incubation, reaching its highest values after 12 h for 35°C (8.1×10^{9} cfu g^{-1}) and 45°C

(Fig. 1). Examination of their colonies, cell morphologies and count of other microorganisms indicated that substantial part of aerobic mesophiles were similar to lactic acid bacteria enumerated in the fermenting doughs. Indeed, Lactic acid bacteria are often microaerophilic and able to grow on PCA. During the dough fermentation they became dominant and contributed the most to the acidification of the product. Their initial load was 4.1×10^{6} cfu g^{-1} of dough. Their number increased during fermentation for all temperatures but the highest loads were obtain after 12 h at 35°C (significant different with $p<0.05$) (Fig. 2). Cassava dough contained 2.3×10^{5} yeasts g^{-1} of dough at the beginning of the fermentation. Their growth was well enhanced at 35 and 30°C where their number reached, respectively 3.0×10^{7} and 10^{6} cfu g^{-1} after 12 h before decreasing (Fig. 3). At 25°C, their growth was low and at 45°C their load decreased from the beginning to the end of the fermentation. The dynamic of growth, survival and biochemical activitiy of microorganisms in foods are the result of stress reaction in response to the changing of the physical and chemical conditions into the food micro-environment (e.g., the gradient of pH, oxygen, water activity salt and temperature) and the ability to colonise the food matrix and grow with spatial heterogeneity (Giraffa, 2004).

The rapid increase in microorganism loads the first 12 h of fermentation was due to the abundance of nutrient useful for their growth and also to absence of inhibitory substances. After this period, growth in the fermenting medium became hard because of the decrease of nutrients, competition between microorganisms, high acidity explaining the cell mortalities. According to Thomas *et al.* (2001), bacterial growth and metabolism affect yeast growth and yeast cells lost viability at a rapid rate and to a greater extent, if bacteria had a chance to grow in the medium prior the yeast inoculation. In the same way, Thomas *et al.* (2001) showed that at lower pH values, acetic acid is a potent inhibitor of yeast growth. Our results are generally consistent with reports on other cassava fermentations (Amoa-Awua *et al.*, 1996; Obilie *et al.*, 2004). Microbial growth during attiéké fermentation, after inoculation of cassava dough with a traditional starter showed that the fermentation could be optimal at 35°C for 12 h.

The microbial changes in fermenting doughs are concomitant with important physico-chemical changes which vary according to the incubation temperature.

Changes in pH and total titrable acidity: pH is a critical factor in developing flavour and aroma of foods (Montet *et al.*, 2006; Panda *et al.*, 2007). In present study, the pH of the initial dough was 5.89. There

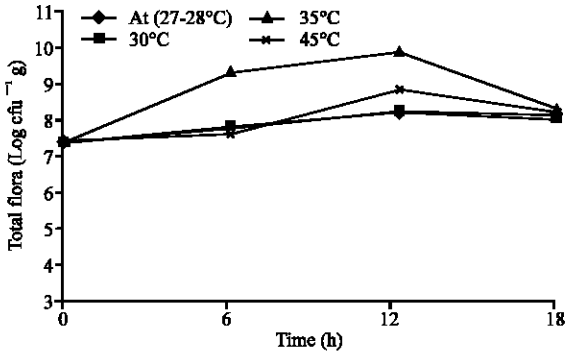

Fig. 1: Effect of temperature on aerobic mesophiles growth during cassava doughs fermented with 10% of traditional starter

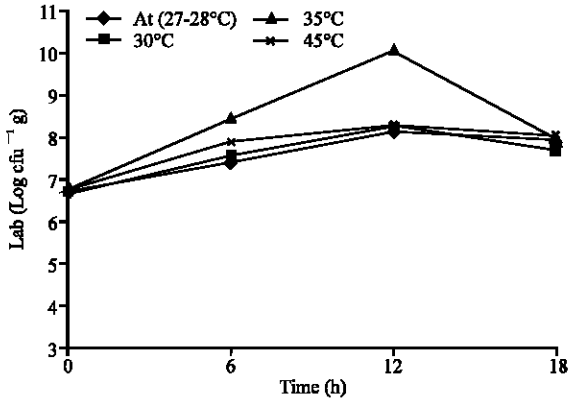

Fig. 2: Effect of temperature on lactic acid bacteria growth during cassava doughs fermented with 10% of traditional

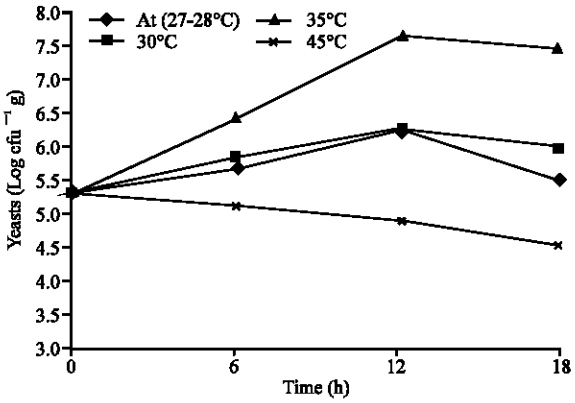

Fig. 3: Effect of temperature on yeasts growth during cassava doughs fermented with 10% of traditional starter

(7.4×10^{8} cfu g^{-1}) before decreasing , respectively to 1.8×10^{8} and 1.6×10^{8} cfu g^{-1} at the end of the fermentation

was a gradual but quick fall of pH during the first 12 h. of fermentation for all temperature of incubation. But, after this time, a very small decrease of pH was observed (Table 1). Similar results were found by Coulin *et al.* (2006). The decrease of pH during the cassava dough fermentation was probably due to the accumulation of organic acids mainly lactic and acetic acids produced by lactic acid bacteria which constituted the dominant microflora (Giraud *et al.*, 1998; Kimaryo *et al.*, 2000; Kobawila *et al.*, 2005; Coulin *et al.*, 2006; Panda *et al.*, 2007).

The initial total titrable acidity value for the non-fermented dough was somewhat low (0.09%). The results showed that cassava underwent acidification during fermentation and this is responsible of the sour taste of the final product. The total titrable acidity increased from 0.09-0.72%, 0.80, 0.98, 1.06 and 0.82% after 18 h of fermentation, respectively for 25, 30, 35, 45°C and ambient temperature (Table 1). Analyse of variance showed that temperature significantly changed the percentage of total titrable acidity of fermenting dough, with respect to 35 and 45°C. The rapid increase of titrable acidity is associated with the production of organic acids during fermentation.

Total sugars: Total sugars content was found to decrease proportionally with the increase in the duration of fermentation, with respect to the doughs incubated at 30, 35 and 45°C, where the initial value fall from 2.9 g/100 g, respectively to 0.58, 0.36 and 0.45 g/100g only after 6 h of fermentation (Table 1). But, the maximum decrease was observed at 35°C. It was clear that, due to the amylolytic activity of the microflora of the traditional starter, a part of starch in cassava dough was converted to sugar and consequently to lactic acids, during organic acid metabolism (Giraud *et al.*, 1993; Zhang and Chen, 2000). Indeed, according to Spier *et al.* (2006) temperature of 30°C combined with 90% of moisture lead to highest α-amylase production for cassava starch hydrolysis. Moreover, Spier *et al.* (2006) achieved great yields of α-amylase in temperature between 30 and 37°C. However, all the fermentable sugars generated after starch hydrolysis did not be converted to lactic acids. A substantial portion was probably utilized by microorganisms present in the fermentation medium for their normal metabolism.

Table 1: Changes in pH, total titrable acidity, total sugars and reducing sugars during cassava fermenting at various temperature conditions

	Temperature (°C)	Fermentataion period (h) 0	6	12	18
pH	25	5.89±0.14^{a}	5.2±0.51^{a}	4.50±0.08^{b}	4.30±0.02^{c}
	30	5.89±0.14^{a}	4.81±0.27^{a}	4.30±0.14^{a}	4.10±0.1^{b}
	35	5.89±0.14^{a}	4.6±0.26^{a}	4.20±0.02^{a}	4.00±0.01^{ab}
	45	5.89^{a}±0.14^{a}	4.4±0.05^{a}	4.10±0.05^{a}	4.00±0.03^{a}
	AT	5.89±0.14^{a}	4.8±0.34^{a}	4.30±0.03^{a}	4.20±0.08^{b}
Total titrable acidity (%)	25	0.09±0.01^{a}	0.30±0.2^{a}	0.60±0.02^{c}	0.72±0.04^{c}
	30	0.09±0.01^{a}	0.42±0.15^{a}	0.69±0.06^{b}	0.80±0.13^{bc}
	35	0.09±0.01^{a}	0.55±0.09^{a}	0.80±0.05^{ab}	0.98±0.6^{ab}
	45	0.09±0.01^{a}	0.56±0.07^{a}	0.88±0.01^{a}	1.06±0.05^{a}
	AT	0.09±0.01^{a}	0.49±0.06^{a}	0.74±0.05^{b}	0.82±0.08^{bc}
Total sugars†	25	2.90±0.09^{a}	1.25±0.18^{b}	0.45±0.10^{a}	0.33±0.07^{a}
	30	2.90±0.09^{a}	0.58±0.11^{ab}	0.33±0.04^{a}	0.26±0.03^{a}
	35	2.90±0.09^{a}	0.36±0.1^{a}	0.28±0.09^{a}	0.26±0.08^{a}
	45	2.90±0.09^{a}	0.45±0.11^{a}	0.35±0.15^{a}	0.33±0.14^{a}
	AT	2.90±0.09^{a}	0.87±0.05^{ab}	0.77±0.05^{a}	0.33±0. 07^{a}
Reducing sugars*	25	1.11±0.05^{a}	2.55±0.8^{a}	2.41±0.80^{a}	2.21±0.20^{a}
	30	1.11±0.05^{a}	2.80±0.6^{a}	2.52±0.20^{a}	1.88±0.13^{a}
	35	1.11±0.05^{a}	2.44±0.11^{a}	2.06±0.44^{a}	1.82±0.10^{a}
	45	1.11±0.05^{a}	3.17±0.26^{a}	2.91±0.78^{a}	2.49±0.81^{a}
	AT	1.11±0.05^{a}	4.82±0.72^{b}	3.61±0.53^{a}	2.41±0.85^{a}

Values are means of three determinations; AT = Ambient Temperature Fermentation, In a column, means values followed by different superscript are statistically different ($p \leq 0.05$), *: (mg/100g of fresh matter); †: (g/100g of fresh matter)

Gas released from the fermentation: Heterofermentative lactic acid fermentation occurs in most of the spontaneously fermented cassava products (Giraud *et al.*, 1998). In this kind of fermentation, microorganisms produce lactic acid and other compounds such as acetic acid, alcohols and CO_2 by the phosphocetolase way. The results showed different evolutions ($p<0.05$) in gas production for each temperature of incubation (Fig. 4). Gas released during fermentation could be associated to the dynamic of microorganisms. At 35°C, where growth of both lactic acid bacteria and yeasts was most enhanced, the maximal amount (108 mL) of gas was obtained and remained steady for 2 h during the fermentation. This could be explained by the fact that starch hydrolysis and fermentation of simple sugars were naturally most intensive at highest temperature due to the faster microflora development (Gotcheva *et al.*, 2001). Beyond this temperature, the gas released reached his highest

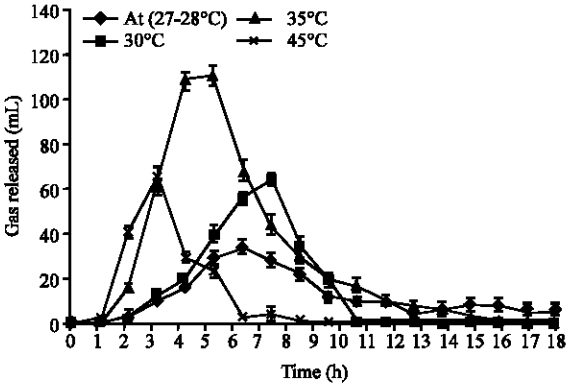

Fig. 4: Effect of temperature on gas released during cassava doughs fermented with 10% of traditional starter

value (62 mL) only after 3 h of fermentation and became nil at the 6th h. In the same time yeasts growth decreased considerably. In all cases, due to the decrease of heterofermentative bacteria and alcoholic yeasts, the amount of gas released became almost nil after 12 h of fermentation.

CONCLUSION

The results of the present research indicate that cassava dough fermentation, initiated by a spontaneous traditional starter is widely influenced by the temperature conditions. The highest microbial and physico-chemical activities were obtained at 35°C and the optimal time of fermentation was determined here at 12 h. However, identification of global conditions for a controlled fermentation may be considered for further development aims at improving attiéké production.

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