Innovative Romanian Food Biotechnology *Vol. 10, Issue of March, 2012*

Received October 22, 2012 / Accepted January 21, 2012

RESEARCH ARTICLE

EFFECT OF CASSAVA VARIETY AND FERMENTATION TIME ON BIOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF RAW ARTISANAL STARTER FOR *ATTIÉKÉ* PRODUCTION

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Abstract
The objective of this study was to characterize an artisanal inoculum called "*Mangnan"*. It was used in fermenting cassava for the production of "*attiéké"* and it may be prepared from different cassava varieties and by different processing methods. Thus, the effects of varietal difference and fermentation time on biochemical and microbiological characteristics of this inoculum prepared from raw cassava roots, was investigated. Two cassava varieties; IAC and BONOUA were investigated at fermentation time of 0; 24; 48; 72 and 96 hours in ambient temperature (28-32°C) for 4 days. Quality parameters such as pH, total titratable acidity, total and reducing sugar consumption and microorganisms' growth were assessed with time.
All the analyzed parameters were significantly ($p<0.05$) affected by both cassava varietal differences and fermentation time. The changes in biochemical (decrease in pH, sugar consumption, and increase in titratable acidity) and microbiological characteristics were observed during cassava fermentation. The "IAC" variety of cassava and 72 hours of fermentation, gave the best results for processing of *Mangnan*, if its aim is to provide the maximum fermentative microorganisms (mainly lactic acid bacteria) in the process of *attiéké* production. These results serve as a useful guide in selecting cassava varieties and fermentation time for processing *Mangnan* and constitute a significant step in optimizing the production of *attiéké.*

Keywords: cassava varieties, fermentation, artisanal starter, quality parameters

Introduction

Cassava is grown widely in several parts of the world especially in the tropical regions and constitutes a significant proportion of the diet of the population. In Africa, it provides over 50% of the average daily caloric intake in some countries (Oyewole and Odunfa, 1992). There are many cassava varieties and in recent times, a number of regional programs have been initiated to breed improved varieties of cassava to increase yield and resistance to diseases. Various studies have shown that the physicochemical, functional and other quality characteristics of fufu, gari, cassava pellets and composite flours from cassava are significantly affected by varietal chemical composition differences (Safo-Kantanka and Owusu-Nipah, 1992). *Attiéké* is one of the starchy traditional fermented cassava products, the production of which involves the use of an artisanal starter cultures called `*mangnan*. The main purpose of using this inoculum is to improve the texture, colour and flavour of *attiéké* (Dziedzoave *et al*., 2000).

Assanvo (2002) found that the inoculum is the main source of microorganisms active in dough fermentation and responsible for organoleptic quality of fermented cassava products. Surveys by Assanvo *et al.,* (2006) from *attiéké* artisan producers, revealed that cassava variety used, had no effect on the preparation of the traditional starter, however the cultivar IAC (bitter variety) was preferentially used. The preparation method of

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this starter varies according to ethnic groups and production area. In Côte d'Ivoire, the most popular processing method (Adjoukrou method) is made by cooking and fermenting whole peeled cassava roots for 72 h. In other side, a traditional starter preparation method according to Abouré population, made by raw and fermenting whole unpeeled cassava roots, remains unknown and less used for *attiéké* production. Presently, attention is gradually being shifted to the improved varieties of cassava. There is thus the need to investigate the effect of these varieties and other starter preparation method, have on product quality in order to effectively optimize *attiéké* production.

The objective of this study was to standardize the manufacturing process of raw artisanal starter. Thus, it has been investigate how differences in cassava variety and fermentation time affect biochemical and microbiological characteristics of product.

Materials and Methods

Materials

The cassava roots used in this study were that of twelve months-old freshly harvested and obtained from a farm in N'dotré village, near of Anyama in Côte d'Ivoire. The cassava varieties selected comprised a local variety with low cyanide content, namely Bonoua and a bitter variety with high cyanide content, IAC (Improved African Cassava).

Cassava's raw artisanal starter preparation

About 1 kg of unpeeled cassava roots of both varieties were cut into slices of 3-5 cm^3, then divided into 5 parts of 200 g each. Four of the 5 parts of the cassava were wrapped in old jute sack (which have already been used for previous fermentation), placed in a basket and the whole was covered with a polyethylene bag to create anaerobic conditions. This was then allowed to ferment at ambient temperature (27-32°C) for 4 days. Fermentation was followed with time and samples of fermenting slices were aseptically taken for different analysis at the beginning and after 24, 48, 72 and 96 hours of fermentation.

pH determination

A sample of 10 g of fermenting cassava slices samples were blended with 20 ml of sterile distilled water and filtered through a Whatman's filter paper No. 1. The pH was then measured by using a pH meter (P107, CONSORT, bioblock Scientific, France).

Total titratable acidity

Ten grams of fermenting cassava slices samples were made into slurry using 100 ml distilled (CO_2-free) water in a flask. The slurry was then filtered using Whatman's No. 1 filter paper. Ten milliliters of the filtrate was titrated against 0.1 N NaOH using phenolphthalein as indicator; the total titratable acidity was calculated as a percentage of lactic acid.

Determination of total 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 slice, while the reducing sugars were quantified with dinitrosalicylic acid method described by Bernfeld (1955) and expressed in mg/100 g of fresh matter.

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 Laboratories, Combourg France). Tenfold serial dilution was prepared and spread-plated for microorganisms' count.

Aerobic mesophiles were counted by cultivation on Plate Count Agar (PCA Oxoid LTD, Basingstore, Hamsphire, England) after incubation at 30°C for 5 days.

Lactic acid bacteria were counted by cultivation on DeMan, Rogosa and Sharpe Agar (MRS, Merck 10660, Merck, Darmstadt, Germany) incubated

anaerobically in an anaerobic jar at 30°C, for 3 days.

The enumeration of coliforms was obtained by cultivation for 24 hours on Crystal-violet neutral-red bile lactose agar (VRBL, AFNOR NF ISO in July 4832, 1991) at 30°C, for the total coliforms and at 44°C, for the thermotolerant coliforms.

Enterococci were counted by cultivation on Bile esculin azide agar (BEA, ISO 7899/1) at 37°C, for 24-48 hours.

Enumeration of yeasts and moulds was carried out using Sabouraud chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich Chemie GmbH, India) after incubation at 25°C, for 5 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 at the significance defined at P≤ 0,05, using SPSS 11.5 software.

Results and discussion

The high counts of microorganisms found in cassava artisanal starter during fermentation indicated that this fermenting medium is suitable for microbial activities. Furthermore, the ambient temperature is always high enough to provide good growth conditions. Their initial counts at the beginning of the fermentation were not different ($p \leq 0.05$) and increased proportionally with the increase of fermentation time. Total aerobic mesophiles corresponded approximately to the sum of lactic acid bacteria, yeast and enterobacteria. Their counts were $2.4 \cdot 10^5$ and $3.2 \cdot 10^5$ CFU/g, respectively in the bitter and the sweet varieties (Figure 1). These counts increased with time, reaching highest values after 72 hours for IAC ($1 \cdot 10^{10}$ CFU/g) and BONOUA ($1 \cdot 10^{11}$ CFU/g) before decreasing slightly respectively to $1.3 \cdot 10^9$ CFU/g and $1.2 \cdot 10^{10}$ CFU/g at the end of fermentation (Figure 1). Examination of the enumerated aerobic mesophiles colonies indicated that substantial part of these microorganisms were similar to lactic acid bacteria isolated from fermenting cassava tubers. Indeed, lactic acid bacteria are often microaerophilic and able to grow on PCA (Sefa-Dedeh *at al*., 2004). During fermentation, they became dominant and contributed the most to the acidification of the product. At the start of fermentation, the load, not different at $p<0.05$, was low; $1.7.10^4$ CFU/g in bitter and $2.8.10^4$ CFU/g in the sweet variety. But, their number increased during fermentation for both varieties and reached the highest loads only after 72 hours before decreasing until the end of fermentation. Significant difference was observed in lactic acid bacteria loads between the two varieties from the 3rd day (72 h) to the end of fermentation (Figure 2). Yeast and mould initial load was very low; $3.3.10^3$ and 3.10^3 CFU/g respectively for bitter and sweet varieties. During fermentation, an increase was observed for all varieties, reaching highest values for the bitter ($2.5.10^7$ CFU/g) and the sweet ($1.6.10^8$ CFU/g) varieties after 72 hours (Figure 3).

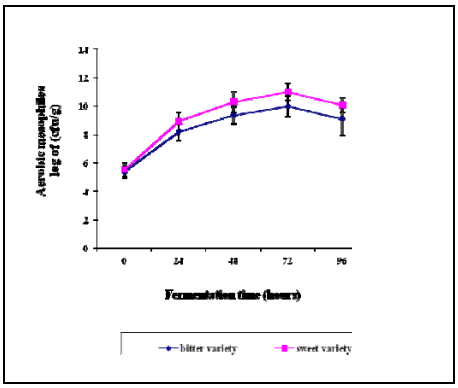

Figure 1. *Aerobic mesophiles bacteria dynamics during cassava fermentation*

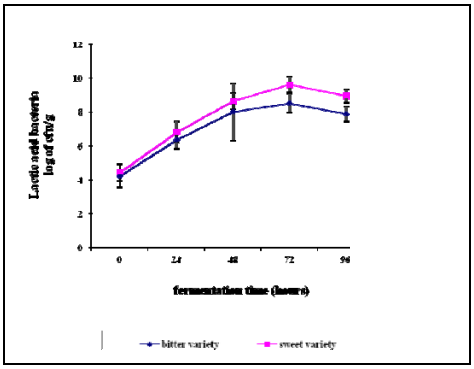

Figure 2. *Lactic acid bacteria evolution in cassava fermented as substrate*

These results are generally consistent with reports on other cassava fermentation (Amoa-Awua *et al*., 1996; Assanvo *et al*., 2006; Coulin *et al*., 2006). The predominance of lactic acid bacteria could be explained by the acid medium which is due to gradual decrease in pH during fermentation, through the production of a variety of organic acids such as lactic, acetic and formic acids, those contents depending on microbial growth. The lactic acid bacteria load was low in bitter variety (IAC) after 72 hours should be not only due to their low moisture content (Assanvo *et al*., 2006), but also to their high cyanogenic glycosides content which could have an inhibitory effect on microbial growth (Raimbault, 1995).

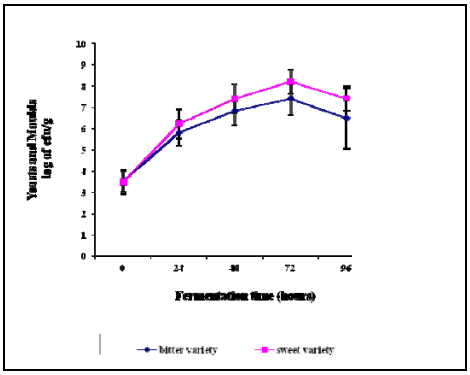

Figure 3. *Change in yeasts and moulds loads with fermentation time of cassava as fermentative substrate*

In other respect, the dynamics of growth, survival and biochemical activity 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 and the ability to colonize the food matrix (Giraffa, 2004). The rapid increase in microorganism load the first 72 hours of fermentation was due to 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 cells mortalities. The microbial changes in fermenting tubers are concomitant with important physico-chemical changes which vary according to fermentation time.

Among microorganisms counted from both raw traditional *attiéké* starters, the presence of coliform bacteria has been mentioned, but at reduced rate (Figure 4 and Figure 5). Their presence is evidence of possible faecal contamination of the starter, through the water or materials used or from the environment. However the presence of this microorganism is not alarming as its load decrease after 48 hours of fermentation when the pH becomes more acid (Desmazeaud, 1996). Moreover the antimicrobial property of lactic acid bacteria (bacteriocins productions, hydrogen peroxide, and other antimicrobial products) could have inhibitory effect on coliforms growth (Klaenhammer, 1993).

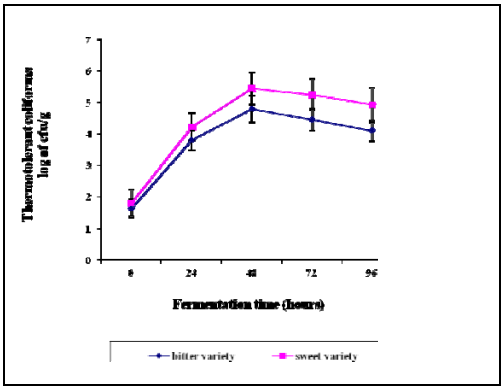

Figure 4. *Change in thermotolerant coliforms loads with fermentation time of cassava during artisanal starter obtaining*

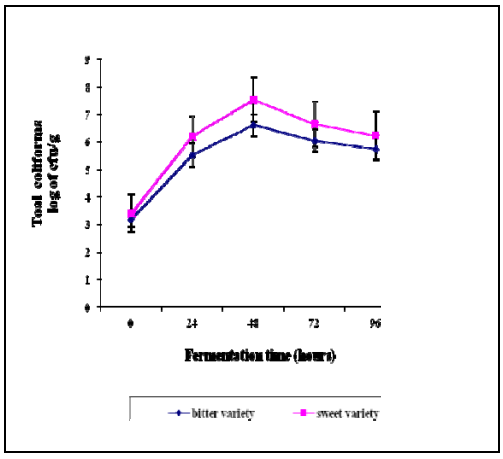

Figure 5. *Change in total coliforms loads with fermentation time of cassava during artisanal starter obtaining*

Changes in pH and titratable 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 was ranged between 6.34±0.3 and 6.58±0.25. During the first 48 hours of fermentation, there was a gradual but a quick fall of pH values to reach 4.76±0.31 and 4.21±0.36 respectively in the bitter and sweet varieties. But after this time, a very small decrease of pH was observed (Figure 6). Similar results were found by Coulin *et al. (*2006).

The trend in total titratable acidity was directly opposite that observed for pH. There was a gradual increase in total acidity during the 96 hours of cassava fermentation ranging from 0.02 to 0.09% and 0.02 to 0.08% respectively in the sweet and bitter varieties (Figures 7).

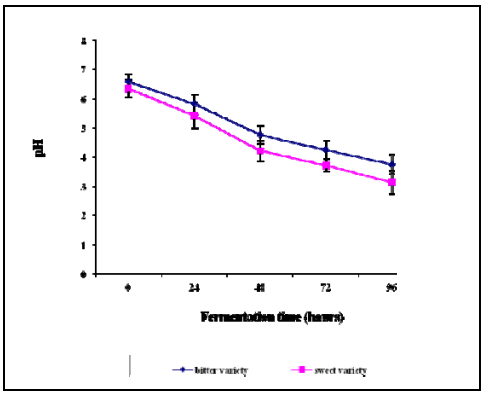

Figure 6. *Change in pH during cassava varieties fermentation for artisanal starter obtaining*

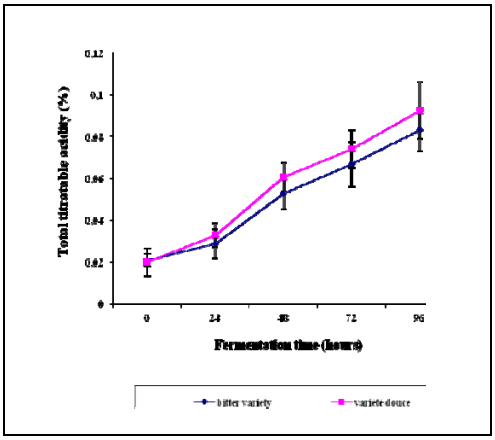

Figure 7. *Change in total titratable acidity with fermentation time of cassava during artisanal starter obtaining*

The observed changes in pH and total titratable acidity during the cassava roots fermentation were probably due to the accumulation of organic acids mainly lactic and acetic acids produced by lactic acid bacteria which constituted the dominant specific microbiota (Guiraud *et al*., 1995; 1998; Kimaryo *et al*., 2000; Djoulde, 2003; Obilie et Okomas, 2004; Kobawila *et al.,* 2005; Coulin *et al*., 2006; Panda *et al*., 2007). Even though total acidity values were higher in the sweet variety, statistical analysis did not show any significant difference ($p<0.05$) between these two cassava varieties used.

Total and reducing sugars

Total sugars content, initially ranged between 21 and 22 mg/g of fresh matter, decreased proportionally with the increase of the duration of fermentation for all varieties. It was clear that due to the amylolytic activity of the microbiota of the traditional artisanal starter, a part of starch in cassava tubers was converted to sugar and consequently to lactic acids, during fermentation (Giraud *et al.,* 1993; Zhang and Chen, 2000). The decrease was more accentuated after only 48 hours of fermentation in sweet variety (13±1.34) than bitter variety (16.24±1.07) (Figure 8).

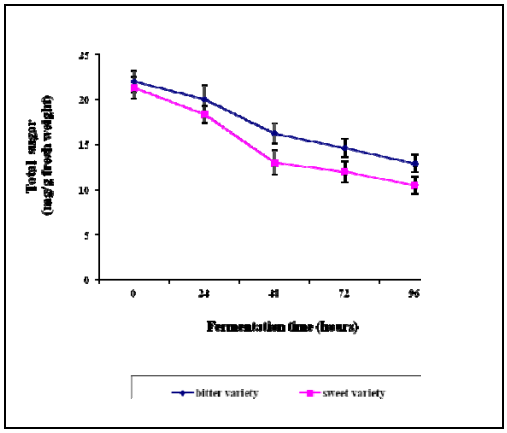

Figure 8: *Change in total sugar rate with fermentation time of cassava during artisanal starter obtaining*

But the differences observed were not statistically significant ($p<0.05$). This might be explained by the low cyanogenic glycosides level and the high moisture content in BONOUA cultivar (sweet variety). Indeed, according to Spier *et al.* (2006),

ambient temperature (30 °C) combined with high moisture content (90 %) lead to highest α-amylase produced by Lactic acid bacteria for cassava starch hydrolysis.

A similar trend was observed in reducing sugar content, but at a reduced rate (Figure 9).

However, all the fermentable sugars generated after starch hydrolysis did not be converted to lactic acids. A substantial portion was probably utilized by microorganisms for growth and multiplication.

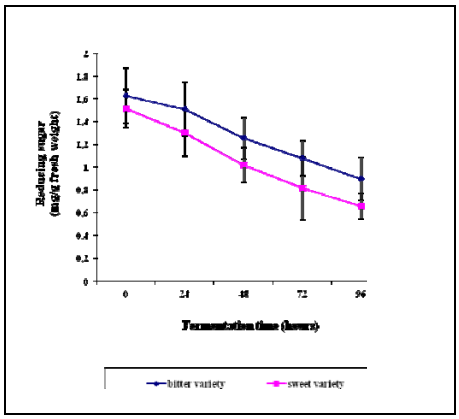

Figure 9. *Change in reducing sugar rate with fermentation time of cassava during artisanal starter obtaining*

Conclusion

Consequently to our investigations, it could be noticed that cassava fermentation for raw artisanal starter preparation is widely influenced by cassava variety and fermentation time. The highest microbial (lactic acid bacteria load) and physico-chemical (pH, total acidity) activities were obtained with sweet variety (BONOUA) and the optimal time of fermentation was determined at 72 hours.

Thus, the root of sweet cassava variety, that present the highest fermentative microorganism (*Lactobacillus*, *Enterococcus*, and fungi) is the best one that could be used in the production of this artisanal stater.

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