
***In vitro* digestibility of dockounou, a traditional plantain derivate dish of Côte d'Ivoire**

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Abstract: The aim of this study was to identify sugar products from dockounou digestion. Tests of digestibility on various dockounou using a digestive juice of young snails namely *Archachatina ventricosa* and experimental acid hydrolysis were used. The results had showed that boiled dockounou were more hydrolysis than the backed one. Specifically, the rice boiled dockounou was more hydrolyzed than the maize boiled dockounou. Both rice boiled and baked dockounou are more hydrolyzed than the maize dockounou did. The sugars liberated for rice backed dockounou range from 0 to 0.80 μmol thereby it's 0 to 2.40 μmol for maize one. These results are significantly different ($p \leq 0.05$). Enzymatic or acid hydrolysis products of boiled and backed dockounou are only the glucose. The intensity of this reducing sugar liberation depends on the time. This information shows, in the whole, that dockounou is an energetic dish for children neither for diabetics.

Keywords: Ripe Plantain, Traditional Dish, Maize Flour, Rice Flour, Hydrolysis

1. Introduction

Musa (plantain and bananas) is the first fruit crop and produced throughout the world. It constitutes an important staple food in many developing countries, especially in Africa [1]. The world production of bananas and plantains (and the other types of this fruit) is estimated at 88 million tons per year [2]. Of the numerous edible varieties, plantain account for 19% and in 2010, its production was estimated at 8.462.530 tons in West Africa [3]. Côte d'Ivoire produces an average of 1 510 778 tons of plantain a year [4]. This production depends on a season marked by a period of abundance which goes from September to April, followed by a period of shortage which extends from May to August [5, 6]. However, a major problem of plantain is that the fruits are highly perishable. At ambient tropical temperatures, plantain and banana have an average market life of 1 to 10 days [7]. Indeed, plantain accounts 35 to 100% of post-harvest losses [8], generally resulting from diseases (crown rot, anthracnose, cigar-end rot, finger rot etc.), predator attacks (insects, birds, etc), physiological disorders (finger drop, peel splitting and chilling injury), mechanical damages induced by handling and transportation (impact, pressure, vibration, etc.) and the natural maturation process [9, 10, 11]. The difficulties of plantains preservation make the

fruits, while ripening at the ambient temperature, undergo depreciation and also qualitative and quantitative degradations along the distribution chain [12].

Nevertheless, several solutions were found to reduce the post-harvest losses; but fruit transformation into less perishable products (flours, chips, etc) is at far the most concrete of all, mainly as far as trading is concerned [8, 9, 11]. People have also invented several dishes (fried, boiled and roasted plantain, porridge, pounded plantain) using unripe and ripe plantain fruits [13, 14, 15, 16, 17]. One of these dishes, named "dockounou" in Côte d'Ivoire, involves senescent plantain pulp and can be considered as an interesting solution in reducing post-harvest losses [18]. "Dockounou" is a traditional pancake prepared by cooking or baking a mixture of pounded ripe plantains and a cereal flour [19]. It's considered as a ready to eat food because it can be immediately consumed on the point of sale [20]. The consumption of dockounou concerned all the Ivorian sectors of the society [21]. Therefore, the aim of this study is to identify mainly sugar products from dockounou digestion. It is hoped that the findings would be of benefit to diabetics specifically and consumers in generally.

2. Materials and Methods

2.1. Samples Collection and Preparation

Per seller, three (03) different samples of maize and rice boiled “dockounou” (Figure 1) and three (03) others of baked “dockounou” (Figure 2) were purchased from retail markets in the city of Abidjan in March 2013 after exhaustive inventory of traditional producers and sellers. All samples were collected in sterile conditions, and were transported aseptically in an icebox (4 - 6°C) to the laboratory for further analysis. Each type of collected samples were mixed together in a sterile mortar to constitute one unit sample of maize boiled “dockounou”, rice boiled “dockounou”, maize baked “dockounou” and one unit sample of rice baked “dockounou”. They were put in freezer at -18°C for preservation.



Figure 1. Boiled dockounou



Figure 2. Baked dockounou

2.2. Preparation of Substrate Solutions

Fifty (50) grams of each type of dockounou sample were deleted in 200 mL of distilled water. After 30 minutes of decantation, the supernatant which content sugar reducing were eliminated. This operation is repeated several times to eliminate all reducing sugars. To verify that there is no reducing sugar, a test of [22] method was made on the last supernatant. In the same way, a thin-layer chromatography is made with the deposit according to [23] method.

The spot obtained was dried at 50°C for 24 hours in an etuve. The dry spot was reduced in powder in a mortar and for each sample, the substrate was prepared in 100 mM (pH 5.6) acetate buffer for enzymatic hydrolysis and in distilled water for acid hydrolysis.

2.3. Enzymatic Extract Preparation

Several young giant snails *Archachatina ventricosa* with a live weight of 30 – 40 g were gathered from the botanic garden

of the Félix Houphouët-Boigny university. They have been abandoned without feed during three days. Each snail was killed according to [23] method and his mucous collected according to [24]. The mucous was filtered with gaze (a medical sterile compress). The filtrate obtained was separated in several aliquots of 1 mL and preserved in freezer at -18°C. Many dilutions of each aliquot for digestibility tests were made to find the adequate initial rate conditions for the experimental. So, the 1/150 dilution was retained for the various enzymatic tests.

2.4. Proteins of Brut Enzymatic Extract Test

Proteins content in the brut enzymatic extract was determined according to [25] method using Folin-Ciocalteu reagent.

2.5. In Vitro Enzymatic Hydrolysis

In vitro enzymatic digestibility was performed during 24 hours with 200 µL of reactional medium which content respectively: 100 µL of acetate buffer (100 mM, pH 5.6), 80 µL of the substrate and 20 µL of enzymatic extract diluted solution. The reactional medium was incubated at 37°C for 24 hours. At each intermediate time (1h, 2h, 4h, 8h, 12h, 16h, 20h and 24h), an aliquot of 100 µL was taken to reducing sugar quantity test and 5 others µL were used in thin layer chromatography according to [23] method to identify the sugars which were appeared.

2.6. Acid Hydrolysis

It was realized with the chloridric acid 2.2 N during seven weeks. One (01) % of substrate solution was diluted in chloridric acid solution 4.4 N initial concentration to obtained final concentration 2.2 N in 200 µL of solution. This reactional medium was incubated at ambient temperature (25°C) during 49 days. Every week, an aliquot of 100 µL was test to quantify reducing sugar content and 5 others µL were taken to identify the sugar types appeared on thin layer chromatography according to [23] method.

2.7. Statistical Analysis

Each sample was analyzed in triplicate and data were reported as means. Differences between means were performed by analysis of variance (one way ANOVA) using StatPlus 2008 (Analystsoft Inc) software. Statistical significance was stated at $p \leq 0.05$.

3. Results

3.1. Enzymatic Hydrolysis

“Fig.3” presents the evolution of reducing sugars quantity liberated in time during the enzymatic hydrolysis. The kinetic of this hydrolysis shows, in the whole that, the boiled dockounou were more hydrolysis than the baked one. Specifically, the rice boiled dockounou was more hydrolyzed than the maize boiled dockounou. Values obtained for the both

dockounou are significantly different ($p \leq 0.05$). Indeed, total sugars liberated range from 0 to 5.10 μmol in 12 hours for rice boiled dockounou then it ranged from 0 to 4.74 μmol for maize one. After the 14th hour, the inverse situation was observed. Maize boiled dockounou liberate more sugars (5.14 to 5.70 μmol) than the rice boiled dockounou (5.16 to 5.56 μmol).

Both boiled and baked dockounou are more hydrolyzed than the maize dockounou did. The sugars liberated for rice baked dockounou range from 0 to 0.80 μmol however it's 0 to 2.40 μmol for maize one. These results are significantly different ($p \leq 0.05$). This hydrolysis is very important for the rice baked dockounou up to the 12th hour, while it's linearly for maize one's up to the 24th hour.

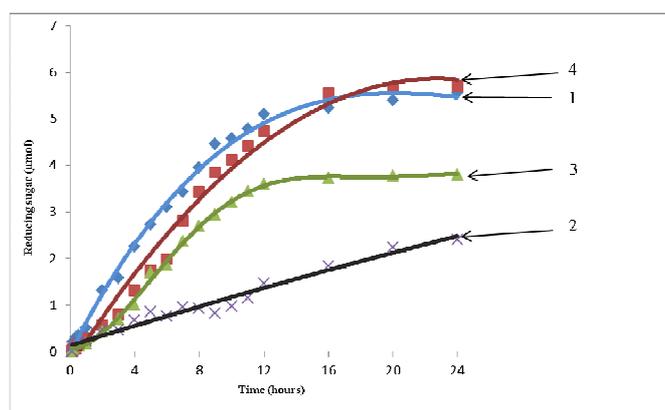


Figure 3. Kinetics hydrolysis curves of products stemming from enzymatic hydrolysis of various types of dockounou.

1: rice boiled dockounou ; 2: maize backed dockounou; 3: rice backed dockounou ; 4: maize boiled dockounou

The results had permitted to determinate the different initial rate values of the kinetic enzymatic hydrolysis. In table 1, were reported these values which varied according dockounou and are significantly different at $p \leq 0.05$ with the three others. Indeed, the initial rate of hydrolysis value of rice boiled dockounou ($9.97 \cdot 10^{-3}$ UI/mg of proteins) are the more highest than the three others dockounou's which were respectively $3.38 \cdot 10^{-3}$ UI/mg of proteins for maize boiled dockounou, $3.31 \cdot 10^{-3}$ UI/mg of proteins for rice baked dockounou and $2.8 \cdot 10^{-3}$ UI/mg of proteins for maize baked dockounou and are not significantly different ($p \geq 0.05$).

Enzymatic profile was shown by Figure 4. The results showed that it was appeared on the chromatogram only reducing sugars which had the same molecular weight as the glucose. The intensity of this appearing depends on the time. Indeed, it's more important as the time increase.

Table 1. Initial rate of enzymatic hydrolysis of various types of dockounou.

Types of dockounou	Initial rate (UI/mg of proteins)
Rice boiled dockounou	$9.97 \cdot 10^{-3} \pm 0.85^a$
Maize boiled dockounou	$3.31 \cdot 10^{-3} \pm 0.97^b$
Rice backed dockounou	$2.80 \cdot 10^{-3} \pm 0.45^b$
Maize backed dockounou	$3.38 \cdot 10^{-3} \pm 0.97^b$

Means followed by different letters (a or b) are significantly different at $p \leq 0.05$

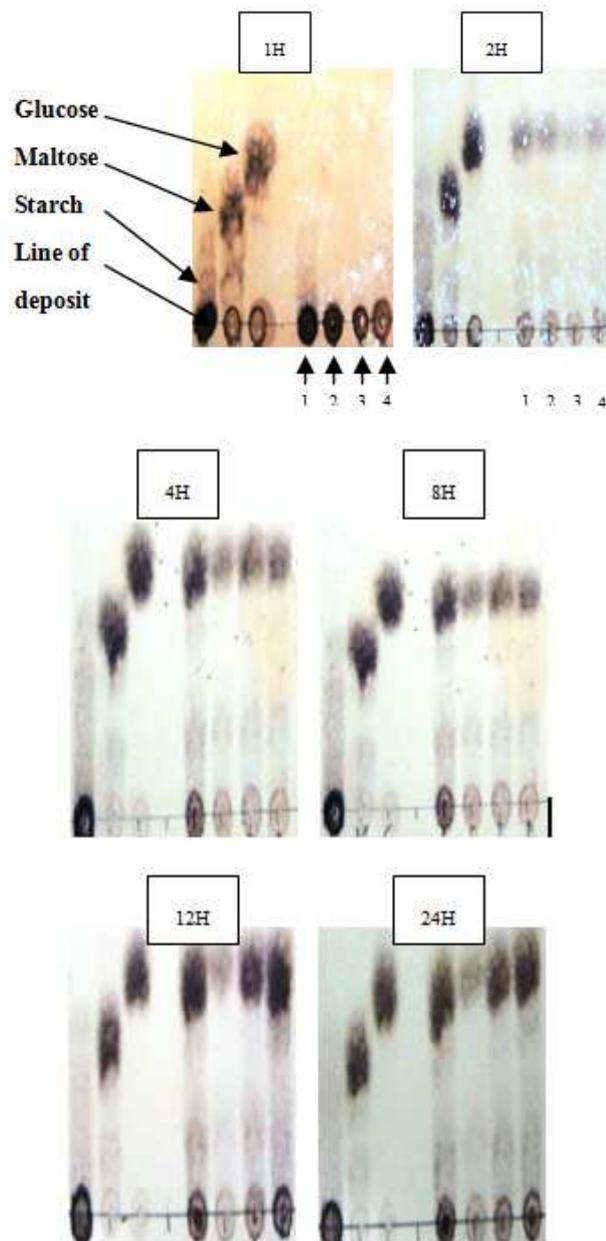


Figure 4. Enzymatic hydrolysis thin layer chromatographic profile of various types of dockounou.

1: rice boiled dockounou ; 2: maize backed dockounou; 3: rice backed dockounou ; 4: maize boiled dockounou

3.2. Acid Hydrolysis

During all the experimental, reducing sugars liberated by acid hydrolysis of rice boiled dockounou are more important than the others; whatever, those for rice baked dockounou are the lowest (Figure 5). Until the 21th day, it observed an important increase quantity of reducing sugars from rice and maize boiled dockounou (ranged from 0 to 0.85 and 0 to 0.59 μmol respectively and statistically different ($p \leq 0.05$)) and maize baked dockounou ranged from 0 to 0.55 μmol . After this day, the increase is relatively weak up to the 35th day (ranged from 0.85 to 0.88; 0.6 to 0.73 and 0.55 to 0.63 μmol respectively).

The initial rate values of the acid hydrolysis which were determined through the graphics were consigned in table 2. These values are significantly different at $p \leq 0.05$ and revealed that the boiled dockounou are the more highest initial rate values ($16.30 \cdot 10^{-6}$ and $8.90 \cdot 10^{-6}$ UI/mL of HCl for maize and rice dockounou respectively which are statistically different at $p \leq 0.05$) than the baked dockounou ($2.08 \cdot 10^{-6}$ and $3.71 \cdot 10^{-6}$ UI/mL of HCl for maize and rice dockounou respectively which are not significantly different at $p \geq 0.05$).

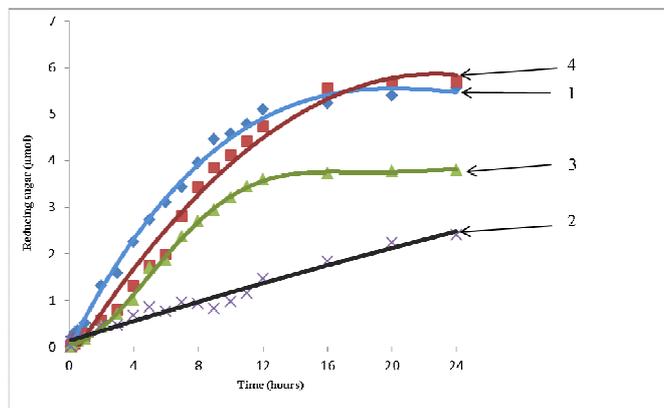


Figure 5. Kinetics hydrolysis curves of products stemming from acid hydrolysis of various types of dockounou.

1: rice boiled dockounou ; 2: maize backed dockounou; 3: rice backed dockounou ; 4: maize boiled dockounou

Table 2. Initial rate of acid hydrolysis of various types of dockounou.

Types of dockounou	Initial rate (UI/mL of HCl 2.2N)
Rice boiled dockounou	$8.90 \cdot 10^{-6} \pm 0.96^b$
Maize boiled dockounou	$16.30 \cdot 10^{-6} \pm 1.08^a$
Rice backed dockounou	$3.71 \cdot 10^{-6} \pm 0.70^c$
Maize backed dockounou	$2.08 \cdot 10^{-6} \pm 0.37^d$

Means followed by different letters (a, b, c, d) are significantly different at $p \leq 0.05$

4. Discussion

The study had revealed that products of the enzymatic or acid hydrolysis of boiled and backed dockounou are only glucose as reducing sugar. The intensity of the spots of glucose obtained on the various chromatographic patches varied according to the duration of the hydrolysis. What confirms the speed of the curves of the kinetics of hydrolysis. The obtained sugar would result from insoluble fibers and the other glucidic constituents especially that free sugars were eliminated by the wash. Indeed, the content of ripe banana crude fiber range from 0.58 to 1.21% [26, 27, 28]; for baked products, the insoluble fiber ranged from 0.89 to 8.64 % [26]. Also, some authors [29, 30] pointed out that the dietary fiber yields in flours were not affected by the heating like wise stage of plantain maturation. These assertions showed effectively that the glucose which was released after hydrolyze is from insoluble fiber and other carbohydrates not soluble in water. The Life Sciences Research Office of the

Federation of American Societies for Experimental Biology defines fiber as “the endogenous components of plant materials in the diet which are resistant to digestion by enzymes produced by humans. Simply put, dietary fiber, including cellulose, hemicelluloses, pectin, and lignin, comes from the cell walls of plants plus other indigestible components of plants [31]. But, it is well known that fiber as cell wall content, can be digested by among animals like snail. Indeed, according to [32], snail digestive juice is known to be a complex mixture of enzymes and also, many reports indicated the presence of hydrolyzing enzymes in the digestive juice of snail [33]. Also, according to [34], other author products of the action of snail juice fractions on some Yeast cell-walls are glucose and mannose which can be detectable. These reports were confirmed by the acid hydrolysis. The same authors are reported that digestive juice of snail content abundant carbohydrases like exo-amylases and α -glucosidase activities [35, 36]. The snail digestive juice action had showed that the boiled dockounou were more digestible than those for backed. This fact is confirmed by the initial rates obtained, in the both hydrolysis, for all sample which are higher for boiled dockounou than the backed one. This can be explained by the hydro-thermic treatment used, which probably modify enzymes affinity for his substrates content in the food. Indeed, according to a report [37], there is respectively an earning and a loss of humidity in the processes of cooking with water and with the oven. In fact, during the boiled process, water and heat favors the gelatinization of carbohydrates contained in rice and corn. This hydro-thermic treatment pulls the disorganization of the structure of carbohydrates [38, 39]. According to another report [39] the water and the heat swells gradually entailing an increase of the surface of available attack of glucidic constituents (starch, amylose, amylopectin). All these structural modifications are going to favor the hydrolysis by the enzymatic raw extract.

Concerning baked dockounou, the dry heat treatment dehydrates glucidic constituents. This loss of water would strengthen the crystalline phases of the granules of carbohydrates. What would prevent the restructuring and the relaxation of constituents and thus their complete gelatinization which doesn't facilitates the hydrolysis action of enzymes.

The results are showed that whatever the mode of cooking, dockounou prepared with the ground rice are more easily hydrolyzed than dockounou prepared with the corn starches. This can be explaining by the size of grains of the starch, the presence of micro-pore on their surface, the degree of physical damage and the content in amylose grains of starch [40, 41, 42]. Moreover, some authors [43] are reported that the rice mush flour is more hydrolysis than the maize flour mush. They are showed that the digestion rate of rice is better than the maize one. Also, other authors [36] are reported that the variability of the starch grain size could influence the enzymatic attack considerably. This shows that glucidic chain in rice, after the digestion, would liberate rapidly more sugars than the maize did.

Besides the weak rate of hydrolysis of backed dockounou beyond 24 hours of enzymatic hydrolysis shows that carbohydrates stemming from these preparations are resistant in enzymes. These glucidic constituents could present beneficial effects at the level of the colon. Indeed, these slowly digestible carbohydrates could behave as fibers in the bowel. What would favor the increase of the production of saddles, the stimulation of the fermentation stomach pain, the decrease of the cholesterol level on an empty stomach and the decrease of the glycaemia.

5. Conclusion

The digestibility tests using enzymatic or acid hydrolysis are shown in the whole that the various dockounou studied liberate glucose as the most important reducing sugar from these hydrolysis. This sugar would result from insoluble fibers and the other insoluble glucidic constituents in the dockounou. These results permit to recommend the dockounou to African young boy or children who need more energetic resources to growth however it recommend it recommend to diabetic to pay more attention for these dishes.

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