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ORIGINAL ARTICLE

# Effect of the Digestibility of Cassava Flour (*Manihot esculenta* Crantz) by Enzymes Extracted from Corn Malt

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## Abstract

The aim of this study is to determine the effect of the digestibility of cassava starch by the enzymes extracted from corn malt, which will constitute one of the answers to the problem of integrating local products into the process in a modern brewery. Cassava starch solutions of different concentrations (E0: 0 g/L; E1: 1 g/L; E2: 1.1 g/L; E3: 1.2 g/L; E4: 1.3 g/L; E5: 1.4 g/L and E6: 1.5 g/L) were prepared and subjected to two treatments (gelatinized and non-gelatinized) and 5 mL of each were placed in a test tube. Three millilitres (3 mL) of the solution containing amylases extracted from malt corn was then added to each of the test tubes containing the cassava flour solutions. All the treatments were subjected to three temperature stages (50 °C for 15 min, 90 °C for 20 min, and 100 °C for 75 min). Twenty-eight (28) objects (two duplicates) were experimented in a complete factorial design (2 treatments × 2 temperature levels). The results obtained showed that gelatinization had no effect, which could be due to the high optimum temperatures of corn enzyme activity. The concentrations also did not have significant differences which shows that these concentrations can well be used on an industrial scale to digest cassava starch by corn malt enzymes.

**Keywords** Hydrolysis · Digestibility · Enzymes · Starch · Malt · Cassava flour

## Introduction

Beer is a final product obtained after alcoholic fermentation, whose main raw materials are malt, hops and yeast (*Saccharomyces cerevisiae*) [1]. Since its discovery, beer has continued to develop, improve and move from continent to continent [1, 2], with a

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considerable increase in its consumption around the world [3]. This trend has increased since the 1960s [4, 5].

Today, beer has become a lucrative source of taxation and a commodity on a global scale. For many experts, the future of the global beer industry now lies in Africa [6]. Seeing this great demand for beer and also the costs linked to the importation of traditional raw materials (barley, malt and hops), technologists as well as brewing companies are forced to strengthen their local supply of raw materials and to raise the mismatches on the one hand between these raw materials and the brewing equipment for the production of classic beer and on the other hand between the raw material and the process of brewing classic beer [3–7]. There is therefore a more efficient use of brewing raw materials, or even the integration of certain local raw materials for obtaining the different types of beer at the industry level.

Cassava is an important potential source of starch which is the most commonly used polysaccharide as an ingredient in human food [8]. In effect, cassava (*Manihot esculenta* Crantz.) tuber is one of the most important root crops in the world. It is an important staple food for many Africans [9, 10] and also a good quality raw material for a number of industries [11]. Cassava contributes significantly to the economies of most tropical countries through its transformation into various products [11–13].

Rich in starch, cassava is often considered a source of carbohydrates, riboflavin, thiamin and nicotinic acid [10, 12], and its carbohydrate yield per hectare is about 40% higher than rice and 25% higher than corn, making cassava the cheapest source of calories for humans and animals [13], or even for industries. It is therefore a mixture of amylose, a polymer mainly composed of glucose linked by α-1,4 bonds, and amylopectin, a polymer of glucose linked by α-1,4 bonds and branched by α-1,6 bonds. Therefore, it is a major ingredient of plant food and an important raw material for industry.

Natural starch is a valuable ingredient for the food industry, widely used as a thickening, gelling, bulking, stabilizing, texturizing, moisturizing and anti-scratch agent [14]. However, the industrial uses of starch are limited by its physico-chemical and functional properties. Plant origin, environmental conditions and cultivar differences are known to influence starch function [15].

The hydrolysis of cassava starch into simple sugars can only be achieved by the application of exogenous enzymes, e.g. bacterial α-amylase and fungal amyloglucosidase, to convert the stored starch into monosaccharides, after which it is fermented with yeast to produce ethanol [16].

In this study, we chose to use enzymes from corn malt because the latter is already used as a source of hydrolytic enzymes in modern brewing. Malting of corn grains could also be a major source of hydrolytic enzymes needed to convert cassava starch into simple sugars [17] for the production of beer and other beverages [18, 19].

Cereals contain both α- and β-amylases, although α-amylases account for approximately 30% of the total protein synthesized during germination [20]. The two amylases can be distinguished from each other. α-Amylases are inactivated at a pH between 4.8 and 5.0. However, in this pH range, β-amylases are stable. β-amylases are inactivated at pH between 6.0 and 7.0, whereas at this range, α-amylases are stable [20]. α-Amylase is a key enzyme that catalyzes the endo cleavage of α-1,4-glycosidic bonds in storage starch and releases short oligosaccharides and α-limit dextrins [21].

## Material and Methods

The corn and cassava used in this study were obtained from a farm in the town of Kenge in the province of Kwango in the Democratic Republic of Congo. The homogeneity of raw materials was obtained after a visual inspection and any material gnawed, attacked by insects, and so forth was eliminated from the lot.

As for the cassava tubers, they had been soaked in water for 3 days and then left to dry for 4 days in the sun at temperatures varying between 29 and 35 °C. The product obtained was ground and sieved (diameter 1 cm).

### Corn Grain Malting and Enzyme Extraction

Corn grains were steeped for 48 h and then germinated in the dark for 72 h. The green malts obtained were dried at 40 °C in an oven for 48 h and then ground and sieved (diameter 0.5 mm). Corn malt amylases were extracted using a 50 mM acetate buffer (pH 5) as described by Amisi et al. [22]. One kilogram of corn malt flour was mixed with 20 L of buffer at ambient temperatures; the mixture was left to stand for 20 min and then centrifuged at 1600 rpm for 5 min. After the malting process, the resulting malt had 285 U/g of α-amylase, 60 U/g of β-amylase, 12 U/g of β-glucanase, 44% of soluble nitrogen and 34% of soluble nitrogen after 2 h boiling.

### Digestibility of Cassava Flour

The choice of starch hydrolysis conditions is also one of the key factors in the success of the hydrolysis of the starch during the brewing process. It is evident that corn amylases can maintain their hydrolytic activity at relatively high temperatures. The results of Biazus et *al*. [23] showed that the optimal temperatures of corn α- and β-amylases are 90 and 50 °C, respectively, while some other studies have shown that the optimum of corn β-amylase would be around 55 °C while that of α-amylase would be around 70 °C [24, 25].

In this study, the choice of temperatures was dictated both in accordance with the results of these studies [23–25] and with the constraints of the technical parameters of brewing (gelatinization of cassava starch at high temperature).

The digestibility of cassava flour (gelatinized and non-gelatinized) by amylases extracted from corn malt (α- and β-amylases) was evaluated sequentially at 90 °C and 65 °C, respectively, for α- and β-amylases. Cassava flour solutions of different concentrations (E0: 0 g/L; E1: 1 g/L; E2: 1.1 g/L; E3: 1.2 g/L; E4: 1.3 g/L; E5: 1.4 g/L and E6: 1.5 g/L) were prepared and subjected to two treatments (gelatinized and non-gelatinized) and 5 mL of each were placed in a test tube. Three millilitres (3 mL) of the solution containing the amylases extracted from malt corn were then added to each of the test tubes. All the treatments were subjected to three temperature stages (65 °C for 15 min, 90 °C for 20 min and 100 °C for 75 min). The digested samples obtained were then centrifuged at 5000 rpm for 5 min using Sigma 3-16KL IVD Refrigerated Centrifuge. After each centrifugation, the supernatant and settled material were collected separately.

In short, twenty-eight objects (two duplicates) were experimented with in a complete factorial design (2 treatments × 2 temperature levels).

Gelatinization was carried out as described by Li et al. [26], and Ubwa et al. [27, 28], with some slight modifications. To do this, 1 L of distilled water was poured into Erlenmeyer flasks (reaction vessels) and placed in a water bath. When the temperature in the

reaction vessel reached 45 °C, different quantities of cassava flour were added (1 g, 1.1 g, 1.2 g, 1.3 g, 1.4 g and 1.5 g). The water bath was set to increase the temperature by 1 °C per minute until the gelatinization temperature was obtained (78 °C).

### Reducing Sugar Measurements

Reducing sugars in supernatants was determined using the method described by Miller [29] and Nirmala and Muralikrishna [30], slightly amended by Amisi et al. [25, 34, 35]and Bwanganga et al. [31]. This method tests the presence of free carbonyl groups of sugars by an oxidation–reduction reaction between the carbonyls free from oxidizing sugars and the carboxylic acid function of dinitrosalicylic acid.

The dinitrosalicylic (DNS) reagent was prepared by mixing 0.75 g of 3,5-dinitrosalicylic acid, 22.5 g of sodium potassium tartrate and 1.2 g of NaOH in that order in 75 mL of distilled water (this reagent has been stored at 4 °C and should not be used after 15 days). The calibration was prepared with glucose (0 – 0.5 – 1 – 1.5 and 2 g/L). The different musts obtained were diluted (dilution 1/100). Then, 0.5 mL of each sample (including calibration solutions) was added to 0.5 mL of DNS solution. The mixture was stirred and boiled for 5 min and cooled in an ice bath and then 5 mL of distilled water was added before reading the absorbance at 540 nm. The quantity of sugar released is expressed in g of glucose equivalent (Supplementary Tables 1 and 2). These two tables give the values of different concentrations of the calibration solution (glucose) read using a visible UV spectrophotometer ($\lambda = 540$ nm). Using the equation obtained from the calibration curve (supplementary Fig. 1), the quantity of reducing sugars expressed in equivalent grams of glucose had been calculated. The calibration curve had been used to understand the instrumental response to the analyte and predict the concentration in our samples during saccharification. A standard solution of glucose at various concentrations with a range including the unknown of interest and the instrumental response at each concentration is recorded. For more precision and to understand the error, the response at each concentration was repeated to obtain an error bar. The data was then fitted to a linear function so that unknown concentrations could be predicted.

## Results and Discussion

The digestibility of cassava flour (gelatinized and non-gelatinized) by amylases extracted from a corn malt (α- and β-amylases) was evaluated at 65 °C and 90 °C for β- and α-amylases, respectively. The results obtained show that flour concentration (from 1 to 1.5 g/L) and gelatinization or not (treatments) had no effect on digestibility as shown in Tables 1 and 2 of the analysis of variance and Figs. 1 and 2 related to comparisons of means.

From Tables 1 and 2, it can be seen that gelatinization has no significant effect at the 5% threshold on α and β-amylase activities even though there is a false significance of the effect of concentration due to the fact that the control alone (E0: 0 g cassava flour/L) was significantly different from the other treatments. This is confirmed by the Fisher pairwise comparison in Tables 3 and 4. The latter shows the simultaneous confidence level of the analysis carried out, which is = 80.64%.

The breakdown of starch into fermentable sugars is essential for several industrial processes, including the production of alcoholic beverages such as beer. The digestibility

**Table 1** Analysis of variance 2 factors (gelatinization: two levels — gelatinized and non-gelatinized flour) and flour concentration (7 levels: 0, 1, 1.1, 1.2, 1.3, 1.4, 1.5 g/L)

| Source | DF | Adj SS | Adj MS | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Treatments | 1 | 31.64 | 31.643 | 1.40 | 0.250 |
| Concentration of substrate (g/L) | 6 | 600.79 | 100.132 | 4.44 | 0.005 |
| Error | 20 | 450.98 | 22.549 | | |
| Lack-of-fit | 6 | 43.38 | 7.231 | 0.25 | 0.952 |
| Pure error | 14 | 407.60 | 29.114 | | |
| Total | 27 | 1083.42 | | | |

**Table 2** Analysis of variance 2 factors (gelatinization: two levels — gelatinized and non-gelatinized flour) and flour concentration (7 levels: 0, 1, 1.1, 1.2, 1.3, 1.4, 1.5 g/L)

| Source | DF | Adj SS | Adj MS | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Treatments | 1 | 13.44 | 13.44 | 0.48 | 0.497 |
| Concentration of substrate (g/L) | 6 | 743.28 | 123.88 | 4.41 | 0.005 |
| Error | 20 | 561.60 | 28.08 | | |
| Lack-of-fit | 6 | 154.01 | 25.67 | 0.8 | 0.533 |
| Pure error | 14 | 407.60 | 29.11 | | |
| Total | 27 | 1318.33 | | | |

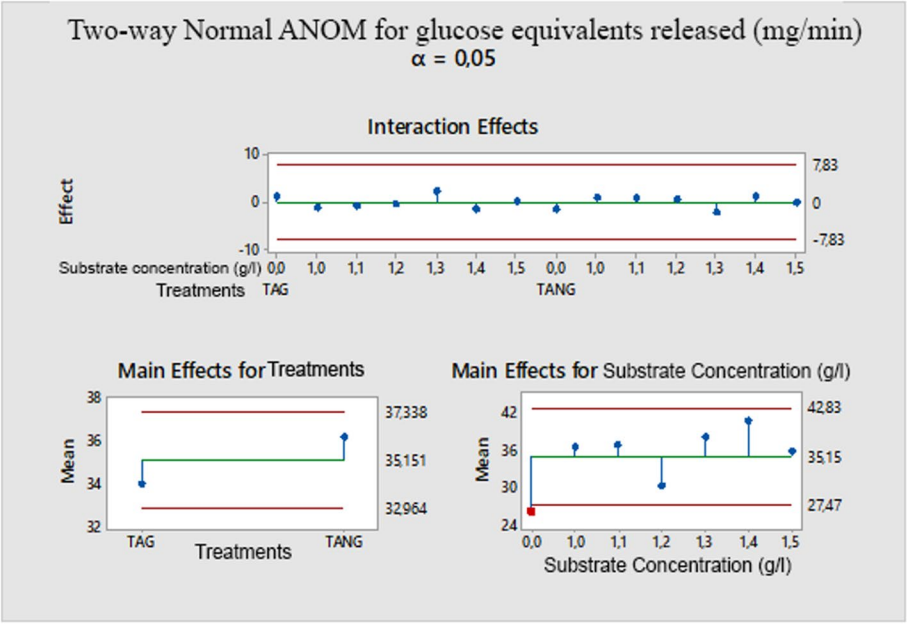


**Fig. 1** Comparison of means for Table 1: Analysis of variance 2 factors (gelatinization: two levels — gelatinized and non-gelatinized flour) and flour concentration (7 levels: 0, 1, 1.1, 1.2, 1.3, 1.4, 1.5 g/L) (response: glucose equivalents released in mg/L)

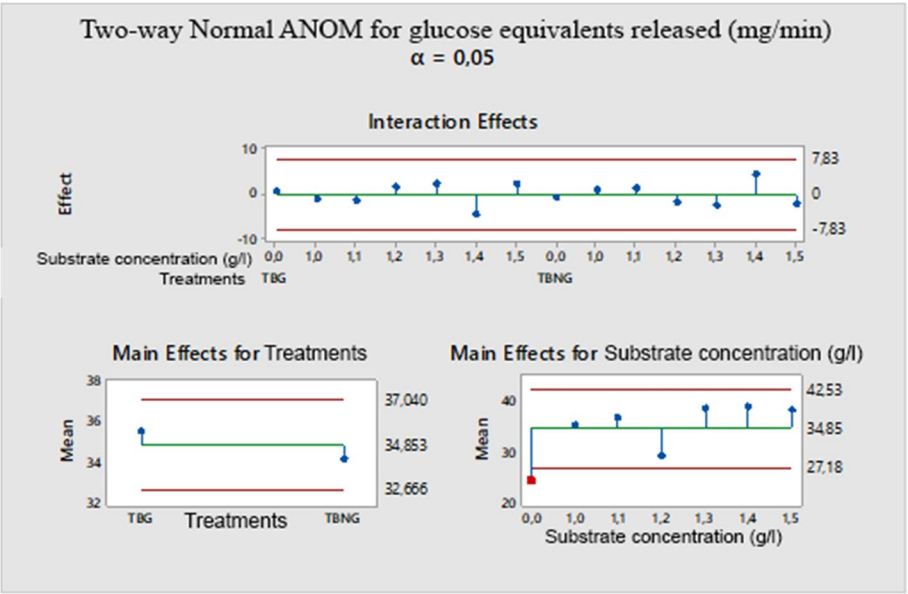


**Fig. 2** Comparison of means relative to Table 2: Analysis of variance 2 factors (gelatinization: two levels — gelatinized and non-gelatinized flour) and flour concentration (7 levels: 0, 1, 1.1, 1.2, 1.3, 1.4, 1.5 g/L) (response: glucose equivalents released in mg/L)

**Table 3** Grouping information using the Fisher LSD method and 95% confidence

| Factors | *N* | Mean | Grouping |
|---|---|---|---|
| TAN | 7 | 67.03 | A |
| TBG | 7 | 65.70 | A |
| TBN | 7 | 62.92 | A |
| TAG | 7 | 62.78 | A |

Means that do not share a letter are significantly different

Where *TAN* temperature of α-amylase, non-gelatinized flour, *TAG* temperature of α-amylase, gelatinized flour, *TBN* temperature of β-amylase non-gelatinized flour, *TBG* temperature of β-amylase, gelatinized flour

of cassava flour by corn enzymes or simply the hydrolysis of its starch will depend on the choice of the right temperature and pH conditions. The choice of starch hydrolysis conditions is also one of the key factors in the success of this process (hydrolysis). Biazus et al. [23] report that α-amylase retains 80% activity in a pH range between 4.0 and 6.5. This is similar to literature reports, as the optimal pH of most α-amylases has been reported between pH 4.5 and 6.5 [27, 28, 37–39].

For this work, gelatinization which is a most remarkable transformation that starches undergo during their heat treatments in an aqueous medium [32], characterized by the irreversible modification of several parameters including granule size and crystallinity [33] had no effect, which could be due to the high optimal temperatures of corn enzyme

**Table 4** Fisher individual tests for differences of means

| Difference of levels | Difference of means | SE of difference | 95% CI | *T*-value | Adjusted *P*-value |
|---|---|---|---|---|---|
| TBN – TAN | −4.11 | 4.83 | (−14.08, 8.86) | −0.85 | 0.403 |
| TAG – TAN | −4.25 | 4.83 | (−14.22, 5.72) | −0.88 | 0.387 |
| TBG – TAN | −1.34 | 4.83 | (−11.31, 8.63) | −0.28 | 0.784 |
| TAG – TBN | −0.14 | 4.83 | (−10.11, 9.83) | −0.03 | 0.977 |
| TBG – TBN | 2.77 | 4.83 | (−7.20, 12.74) | 0.57 | 0.572 |
| TBG—TAG | 2.91 | 4.83 | (−7.06, 12.88) | 0.60 | 0.552 |

Simultaneous confidence level = 80.64%. *TBN* temperature of β-amylase, non-gelatinized flour, *TAN* temperature of α-amylase, non-gelatinized flour, *TAG* temperature of α-amylase, gelatinized flour, *TBG* temperature of β-amylase, gelatinized flour

activity [23, 34]. According to Malumba et al. [32], this phenomenon activates at 70 °C for barley malt amylase and above, which is well below the optimal temperature of α-amylase but close to that of β-amylase. Indeed, the likely optimal temperatures for β-amylase would be at 50 °C, and for α-amylase at 90 °C [22]. The concentrations did not have significant differences, which shows that these concentrations can also be used on an industrial scale to digest cassava starch by corn malt enzymes.

## Conclusion

This study reveals that the hydrolysis of cassava flour by enzymes extracted from corn malt is not dependent on the effect of temperature through gelatinization, and even less on the concentration of cassava starch.

The results obtained show that there are no significant differences at the 0.5% level between different treatments. Gelatinization did not affect the secondary structure of starch, showing that the treatment did not affect the reducing sugar measured too. The gelatinization can only affect the solubility of the sample before the enzyme reaction. Its effect can be different considering the strategy of hydrolysis performed. SSA (simultaneous solubilization and saccharification activities) strategies are only effective with thermally resistant α-amylases. This could be due to the high optimal temperatures of the corn amylase enzymes. The concentration also did not have a significant effect on the hydrolysis of cassava starch flour by corn enzymes. This thermal stability is a good factor to justify the use of this malt in industrial processes.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12010-024-05044-9.

**Author Contribution** All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were carried out by Arthur Kapepa Amisi and Erick A. Chimanuka, under the supervision of Jean-Claude T. Bwanganga, Laboratory Manager. Guelor Kasereka and Roger V. Kizungu participated in the analysis of the data collected. The first draft of the manuscript was written by Arthur Kapepa Amisi, and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

**Data Availability** The authors declare that additional data in this manuscript are available at the complementary information. They will also be available on request from the first author.

## Declarations

**Ethics Approval** The authors of this research did not involve human or animal subjects. So, no ethical approval is required.

**Consent to Participate** The authors declare that this study has not required a consent to participate because it does not involve human subjects.

**Consent for Publication** The authors declare that the manuscript does not contain any individual person's data in any form (including any individual details, images or videos). No consent to publish is required.

**Competing Interests** The authors declare no competing interests.

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