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# Effect of Accelerated Retting Process on Physiochemical and Pasting Properties of Cassava (Manihot esculenta Crantz) Flours

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#### Authors' contributions

This work was carried out in collaboration among all authors. Author NNZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RN and JJEN managed the analyses and the literature searches of the study. All authors read and approved the final manuscript.

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# **ABSTRACT**

**Aims:** To compare the physicochemical (water, total titratable acid, amylose and amylopectin contents), functional (swelling and solubility powers) and pasting characteristics of cassava flour retted in two different ways.

**Study Design:** Flours were produced from two cassava cultivars subjected to natural and accelerated fermentations using a multi-strain mixed starter.

**Place and Duration of Study:** Department of Microbiology of the University of Yaounde 1 and Department of Food Science and Nutrition at the University of Ngaoundere, both in Cameroon, between November 2019 and February 2020.

**Methodology:** The cassava roots of each cultivars were divided into two groups and fermented by submerging peeled roots in tap water. One group of each cassava cultivar was inoculated with 1% (w/w) of the starter, while the other was subjected to a spontaneous fermentation. Retting stopped after the softening of the roots; that is after twenty-six hours for the inoculated groups, and after seventy-two hour for the control groups. The fermented roots were then squeezed, dried and

grounded in a blender. Flours obtained were subjected to physicochemical, functional and pasting analysis.

**Results:** Flours produced in accelerated fermentation (AF) were characterized by their low amylopectin contents (46.59% and 43.57%), swelling power (6.71% and 7.56%), solubility (6.03% and 7.96%), and peak viscosity (6182 cP and 5676 cP) for Six-mois and Mintol-meko flours respectively. Whereas setback viscosity (1032 cP and 1068 cP), stability (0.27 and 0.24) and final viscosity (3565 cP and 3566 cP) of flours from natural fermentation (NF) were lower than those from AF.

**Conclusion:** The accelerated fermentation reduces the tendency of paste to downgrade, that is responsible for staling in baked products and could therefore produce flours that are less fluid.

Keywords: Cassava; retting process accelerated fermentation; pasting properties; multi-strain starter; Cameroon.

### 1. INTRODUCTION

Cassava (Manihot esculenta Crantz) is a staple food for about 800 million individuals in the world [1]. Its roots contain about 70% of starch, making it the main source of carbohydrate for the sub-Saharan populations [2]. However, cassava tubers present two major drawbacks: their short shelf life [3] and the presence of cyanogen compounds whose hydrolysis leads to the production of hydrogen cyanide, toxic to humans and animals [4].

In order to reduce the amount of cyanogen compounds and postharvest losses of cassava roots [5], and to improve the organoleptic quality of cassava by-products [6], many populations in tropical areas use retting or liquid fermentation despite the fact that the process takes many days to accomplish (3-7days) [7]. Among the products obtained from transformation, fermented cassava flour is ranked first as the most consumed cassava byproducts [8]. It is cooked by mixing in boiling water to form a somehow acidic dough called fufu, which is consumed either with a sauce or with vegetables. The gelling capacity of a flour being one of the most important criteria in the production of starchy foods, texture related properties of the flour are consequently crucial criteria for the rating of a starchy food.

Some research studies show that the physicochemical and functional properties of cassava flours are related to the type of cassava cultivar [9,10]; and the fermentation process can modify the pasting properties of some tubers like Taro (*Colocossia esculenta*) [11]. Moreover, reducing the retting time and cyanogen using a starter made by previously fermented-cassava chips have been shown [12]. But, the impact of the use ofpreviously fermented-cassava chipsas

starter on pasting properties of the cassava byproducts that depends on retting process has not been studied.

This work aimed to compare the physicochemical, functional and pasting characteristics of cassava flour retted in two different ways.

### 2. MATERIALS AND METHODS

#### 2.1 Sampling

Ten months old cassava of two bitter local cultivars were harvested in *Bafia* and *Eseka*, two localities characterized by their high activities in cassava processing in the Central region of Cameroon. These cultivars were selected because they are the most processed species in these two localities. Local names were identified and morphological characterization of each cultivar was described (Table 1). These characteristics were mainly the color of the leaves, stems, petioles and skin roots [13]. After harvest, tubers were transported in polyethylene bags to the laboratory. The starter used in this work were produced in the laboratory as described by Nkoudou et al. [12].

### 2.2 Production of Fermented Flours

The fermented flours were produced according to the protocol described in our previous work [12] with slight modifications: The cassava roots of each cultivar were peeled and cut into cylindrical blocks of 4 cm long and 5 cm in diameter; then washed and separated in two groups of three batches of 500 g each. A total of 12 batches (2 cassava varieties x 2 groups per cultivar x 3 batches for each group) was obtained. Fermentation was carried out by submerging peeled roots of each batch in 500 ml of

Table 1. Cultivar morphological description

Cassava cultivars names	Morphological characterization		
	Stems	Petioles	Skin roots
Six-mois	chestnut	Green-red	White
Mintol-Meko	White/green	White	White

Sterilized tap water. This was performed in plastic containers covered with muslin to prevent the entry of flies. One group of each cassava cultivar was inoculated with 1% (w/w) of the multi-strains starter (previously fermentedcassava flour), while the other was subjected to a spontaneous fermentation and called the control or non-inoculated group. Retting was conducted, at room temperature (25±3°C) and stopped after the softening of the roots. This was observed after 26 hrs for the inoculated groups, and after 72 hours for the control groups. The fermented roots were then squeezed, dried in an oven at 40°C for 24 hours and grounded in a blender. The flours obtained were sieved, preserved in airtight plastic bags and labeled NF and AF respectively for the control batch (Natural Fermentation) and the batches fermented with the starters (Accelerated Fermentation). Thus, all samples were prepared and preserved at 4°C for further analyses.

# 2.3 Chemical Analysis

#### 2.3.1 Determination of water content

The water content of the flour samples obtained from fermented cassava was determined using the method described by Nuwamanya et al. [10] with a slight modification. Briefly, 10 g of the sample to be analyzed was dried in an oven at 105°C for 5 hours. The dried sample was transferred into a desiccator to cool down at room temperature and then weighed. This procedure was repeated several times until the weight was constant. The water content of the sample was then determined using the formula:

Water Content (%) = 
$$\frac{\left(W_i - W_f\right)}{W_f} \times 100$$
 (1)

Where:  $W_i$ : the initial weight of the flour sample and  $W_f$ : the weight of the sample after drying and cooling.

#### 2.3.2 Determination of total titratable acid

The amount of acid present in flour samples was determined according to AOAC (Association of Official Analytical Chemists. Official Methods of

Analysis, 13th ed. Washington DC, 1980). For this, 3 drops of phenolphthalein were added to 10 ml obtained from a mixture of 10 g of a flour and 90 ml of distilled water. Each mixture is titrated with a solution of NaOH (0.1 N) contained in a graduated burette until the appearance of a pink color indicating the equivalence. Each ml of the volume of NaOH dispensed is equivalent to 9mg of lactic acid.

# 2.3.3 Determination of the amylose and amylopectin contents

The percentages of amylose and amylopectin of the fermented flour samples were determined by colorimetric method at two different wavelengths according to Jarvis and Walker [14] and cited in [15]. The absorbance at 580 nm was used for the detection of starch and amylopectin, while amylose was detected at 720 nm. This was made possible by the use of standardization curves obtained from a pure Irish potato starch containing amylose at 20% and amylopectin at 80%. The percentages of amylose and amylopectin were determined according to the formulae below:

Amylose (%) = 
$$\frac{OD_{580nm} \times 10}{a} \times 100$$
 (2)

Amylopectin (%) = 
$$\frac{OD_{720nm} \times 10}{a'} \times 100$$
 (3)

Where: a, is the slope of the calibration curve of amylose and a' the slope of the calibration curve of amylopectin.

# 2.4 Determination of the Swelling and Solubility Powers

The swelling and solubility powers of the fermented flour samples were determined by the method described by Farasara et al. [16], with slight modifications. A suspension [8.3% (w/v)] of each flour sample was heated at 85°C for 30 min. The mixture was homogenized every 5 min in order to avoid the agglutination of flour at the bottom of the heating container. Thereafter, the sample was cooled for 10 min and centrifuged at 4000 rpm for 15 min. The supernatant obtained was poured into a glass petri dish, dried at 110°C

for 5 hours and then weighted. The solubility and swelling power were calculated using the following formulae.

Solubility (%) = 
$$\frac{\text{weight of dried supernatant}}{\text{weight of dried flour matter}} \times 100$$
 (4)

Swelling Power (%) =

$$\frac{\text{weight of sediments}}{\text{weight of dried flour matter}} \times (100 - \%\text{Solubity})$$
 (5)

## 2.5 Pasting Properties of the Flours

The pasting properties of different flours were determined with a Rapid Visco-Analyser (RVA-4, New Port Scientific, and PETERN Instrument-Australia). Flour suspension 12.5% (w/v) (3.5 g dry basis suspended in distilled water and the total weight adjusted to 28 g) was equilibrated for 1 min at 50°C, heated to 95°C for 5 min. at the rate of 9°C /min. The samples were thereafter held at 95°C for 2 min. cooled to 50°C for 5 min. at 9°C/min and held again at 50°C for 1 min. The stirring speed was 160 rpm. From the resulting curve, the Pasting temperature (temperature at the onset in viscosity rise) was determined. The viscosity; the Breakdown viscosity (difference between the peak viscosity and the minimum viscosity during the holding phase at 95°C) and the Setback viscosity (difference between the minimum viscosity at 95°C and the end viscosity) were recorded.

### 2.6 Statistical Analysis

All measurements were performed in triplicate according to experimental plan. The comparison of the mean values obtained was done by ANOVA on SPSS 16.0 (SPSS International Chicago, USA) at 95% confident level. The significant differences between the mean values were performed using the LSD procedure.

#### 3. RESULTS AND DISCUSSION

# 3.1 Chemical Composition of the Fermented Flours

Analysis of the chemical composition (water, amylose and amylopectin contents) of different cassava flours was obtained using two varieties of cassava which were fermented differently as shown in Table 2.

#### 3.1.1 Moisture content

The water content of the different samples varies from 4.92% to 9.8%. These values are below the authorized limit of the FAO, which prevents the

proliferation of the microorganisms responsible for the deterioration of the flours. No significant difference was observed between samples retted for 72 hours and those obtained from accelerated fermentations, regardless of the cassava cultivar.

# 3.1.2 Total titratable acidity (TTA)

Total titratable acid of flours, varies between 0.24 and 0.32 (mg equivalents of lactic acid). Flours with higher contents are those obtained from natural fermentation. The TTA of flours of AF was Non-significant difference was observed with flours obtained from AF. Then the use of starter is characterized by the diminishment and standardization of the quantity of acid produced during the fermentation.

# 3.1.3 Amylose and amylopectin contents

The standard curves of amylose and amylopectin of respective flours are represented in Fig. 1. The regression equations obtained are y = 24.55x for amylose and y = 11.42x for amylopectin with the R square of 0.994. The apparent amylose content (AAM) varies from 11.89±1.09% to 14.75±0.29%. The amylose values found are within a range of varieties low in amylose content as described by Nkoudou et al. [12] and are more suitable as thickener. The AAM of the flours samples resulting from the natural fermentation (NF) of the two cultivars were lower than those of accelerated fermentation (AF). For Six-mois cultivar, AAM of NF flour (13.36 ± 0.58%) differs significantly from the AAM of AF flour (14.75  $\pm$  0.29%); showing the extensive degradation of amylose durina fermentation. Contrary to amylose contents, flours samples obtained from the cassava roots subjected to NF, were the ones that showed the highest amylopectin (AP) contents. Values obtained respectively for Six-mois and Mintolmeko cultivars were47.98  $\pm$  2.48% and 49.13  $\pm$ 0.25% for NF, and 46.59±3.72% 43.56±3.034% for AF. The use of starter seems to increase the ratio AAM/AP hence leading to the production of flour with an increased retrogradation potential, that is responsible for staling in bakery [17,18].

#### 3.2 Swelling Power and Solubility

The swelling power of a flour in excess water as well as its solubility differ significantly, with respect to the sample analyzed. Solubility and swelling are often used to evaluate the extent of the interactions between starch chains. For the Six-moiscultivar, the swelling power of the flour

obtained by NF is 8.17% while those obtained by AF is 6.71%. The results obtained with Mintolmeko are in the same direction with those of Sixmoiscultivar. The reduction noted on swelling power with the AF was also observed for the solubility percentage; showing that the reduction of amylopectin or increment of amylose all together contribute to reduce swelling and solubility capacities of the flour. Strong negative correlation between amylose and swelling was reported by Tomoko et al. [19] in wheat starches. These authors found that, reduced amylose content starch results to greater swelling. On the other hand, greater swelling reduced the quantity of free water and it is associated with higher pasting viscosity [20]. The highest solubility value (10.05%) was obtained with NF of Mintol-meko and the lowest solubility (6.03%) with AF of Sixmois. These results show that, irrespective of the cassava cultivar, higher swelling power and solubility percentages of flours are obtained with NF. Then NF flours could be hydrolyzed easily to produce sugars without using energy as compared to AF flours, which are presumed to have strong associative bonds [21].

# 3.3 Pasting Properties

Pasting properties of the different flours analyzed by RVA are summarized in Table 4. There were no significant differences (p>0.05) in pasting temperature of flours irrespective of the nature of cassava fermentation. The highest peak viscosity (6652cP) was obtained with NF of Six-mois cultivar while the lowest (5676cP) was obtained with AF of Mintol-meko. Irrespective of the cassava cultivar, flours from the NF have higher peak viscosities than those resulting from AF. These observations differ from those of Malumba et al. [18] who found that, cassava flours issued

Table 2. Moisture, amylopectin and amylose contents of fermented cassava flours

Physicochemical parameters	Flour samples	Cassava cultivars	
		Six-mois	Mintol-meko
Moisture (%)	NF	9.80±0.00 <sup>a</sup>	4.92±0.03 <sup>a</sup>
. ,	AF	6.47±0.17 <sup>b</sup>	5.18±0.19 <sup>a</sup>
TTA (mg eq. of Lactic Acid)	NF	$0.32 \pm 0.02^{b}$	$0.27 \pm 0.02^{a}$
,	AF	$0.24 \pm 0.02^a$	0.25 ±0.01 <sup>a</sup>
Apparent Amylose AAM (%)	NF	13.36±0.58 <sup>b</sup>	11.89±1.09 <sup>a</sup>
, , ,	AF	14.75±0.29 <sup>a</sup>	13.73±1.09 <sup>a</sup>
Amylopectin, AP (%)	NF	47.98±2.48 <sup>b</sup>	49.13±0.25 <sup>b</sup>
	AF	46.59±3.72 <sup>b</sup>	43.56±3.03 <sup>a</sup>
Ratio AAM/AP	NF	0.27	0.24
	AF	0.31	0.31

The results are presented in form: Mean ± Standard deviation. For each physicochemical property, means in the same row followed by different superscripts are significantly different (P = .05). NF: natural fermentation; AF: accelerated fermentation; TTA: Total Titratable Acid

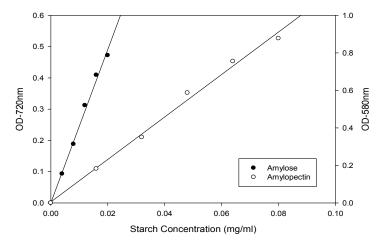


Fig. 1. Standards curves of amylose and amylopectin for cassava flour. OD: optic density

Table 3. Swelling and solubility powers of fermented cassava flours

Functional properties	Flour samples	Cassava varieties	
		Six-mois	Mintol-meko
Swelling power (%)	NF	8.17±0.01 <sup>a</sup>	7.68±0.01 <sup>b</sup>
	AF	6.71±0.00 <sup>d</sup>	7.56±0.01 <sup>c</sup>
Solubility (%)	NF	8.04±0.06 <sup>b</sup>	10.05±0.07 <sup>a</sup>
	ΔF	6 በ3+በ በ4 <sup>d</sup>	7 96+0 06 <sup>c</sup>

The results are presented in form: Mean ± S.E.M. For each functional property, means in the same row followed by different superscripts are significantly different (P = .05). NF: natural fermentation; AF: accelerated fermentation

Table 4. Pasting properties of cassava flour Six-mois and Mintol-meko retted differently

Pasting properties	Flour samples	Cassava varieties	
		Six-mois	Mintol-meko
Pasting temperature, Pt (°C)	NF	71.7±1.4 <sup>a</sup>	70.4±1.2 <sup>ab</sup>
	AF	72.2±0.1 <sup>a</sup>	71.2±0.0 <sup>b</sup>
Peak viscosity, Pv (cP)	NF	6652±7 <sup>a</sup>	6615±10 <sup>a</sup>
	AF	6182±336 <sup>b</sup>	5676±73 <sup>b</sup>
Breakdown viscosity, <i>Bv</i> (cP)	NF	2947±223 <sup>a</sup>	4186±6 <sup>a</sup>
	AF	3524±362 <sup>b</sup>	3281±120 <sup>b</sup>
Setback viscosity, Sv(cP)	NF	1032±115 <sup>a</sup>	1068±2 <sup>b</sup>
	AF	1162±2 <sup>c</sup>	1171±53 <sup>a</sup>
Final viscosity, Fv (cP)	NF	3565±22 <sup>a</sup>	3566±348 <sup>a</sup>
	AF	3454±21 <sup>a</sup>	3467±59 <sup>a</sup>
Stability ratio (Bv/Pv)	NF	0.44	0.63
	AF	0.57	0.58
Setback ratio (Fv/Sv)	NF	3.45	3.34
	AF	2.97	2.96

The results are presented in form Mean ± S.E.M. For each parameter, means in the same row followed by different letter are significantly different (P=.05). NF: natural fermentation; AF: accelerated fermentation

from a 4-days fermentation by microbial culture had a higher peak viscosity than those derived from a 7-days natural fermentation. However, it is known that, softening of cassava tubers is generally accompanied by the release of reducing sugars in the medium that are quickly consumed by the microorganisms present in starter to produce organic acids. This difference could be also explained by the level of organic acids produced during the two kinds of fermentation. Indeed [22] observed the decrease of viscosity of cassava starch with acid concentrations above 0.5% (v/v) and [23] found that, the 1 mM of organic acids (lactic, acetic, succinic, malic and citric acids) concentration reduced the peak viscosity of non-waxy rice flour. The results of peak viscosity agree with the amylopectin contents shown on Table 2. The high values of peak viscosity of the flours from natural fermentation are attributed to high amylopectin content and consequently to the swelling of the starch from the flour [24]. Contrarily to the peak viscosity, setback viscosity of flour obtained from NF was lower than those from AF. The lowest setback viscosity (1032cP)

was that of NF of Six-mois and the highest (1171cP) was that of AF of Mintol-meko. The low values of setback viscosities observed in NF, causing a higher setback ratio (Fv/Sv): this coefficient being 3.45 for NF and 2.97 for AF of Six-mois. The same trend was observed with the Mintol-meko cultivar. These data confirm that flour of NF have a higher retrogradation rate because of their low setback viscosity [25], due to the reassembling of the amylose molecules, which diffuse out of the starch granule during the heating phase [24].

# 4. CONCLUSION

The impact of reducing the retting time process using multi-strain mixed starters on physicochemical properties of cassava fermented flour was investigated in order to increase its productivity. It appears from this study that the acceleration of fermentation reduced the amylopectin content, the swelling power and solubility in cassava fermented flour. One of the characteristics of the flour obtained with the use of starter is their low peak viscosity

compared with flour obtained by the natural fermentation. Considering that the reduction of viscosity was related to the degradation rate and the softening degree of cassava roots, the acceleration of retting process would then reduce the thickening power of the flour obtained. Consequently, flour from the natural retting could be more suitable in baking. But accelerated retting process has the advantage to reduce the tendency to downgrade that is responsible for staling in baking, and consequently, could produce flour that is less fluid and more suitable in infant food. This study suggest the possibility of using the starter for the production of flours with specific characteristics that contribute to the identity of the product.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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