Physicochemical Changes in Cassava Starch and Flour Associated With Fermentation: Effect on Textural Properties†

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Physicochemical changes in cassava starch and flour associated with fermentation were investigated and related to textural properties of its flour pastes. Cyanide and pH decreased, while crude protein, acidity, and apparent amylose content increased in the fermented products. Average starch granule diameter, solubility, and swelling power were depressed, while gelatinization enthalpy increased. Amylography of starch showed increased peak viscosity temperature, reduced peak, breakdown, and setback viscosities, while Texture Profile Analysis showed a decrease in hardness, cohesiveness, elasticity, and gumminess of the fermented flour paste. The altered textural properties were attributed to greater starch granule stability due to short amylose-like fragments formed by enzymatic hydrolysis of amylopectin.

Physikalisch-chemische Veränderungen in Cassavastärke und -mehl in Verbindung mit der Fermentation: Einfluß auf die texturalen Eigenschaften. Die physikalisch-chemischen Veränderungen in Cassavastärke und -mehl, verbunden mit Fermentation, wurden untersucht und mit den texturalen Eigenschaften ihrer Mehlpaste in Verbindung gebracht. Cyanid und pH-Wert verringerte sie, während Rohprotein, Azidität und scheinbarer Amylosegehalt die fermentierten Produkte erhöhten. Der durchschnittliche Stärkekorn-Durchmesser, die Löslichkeit und das Quellvermögen wurden herabgesetzt, während die Verkleisterungsenthalpie erhöht wurde. Die Amylographie der Stärke zeigte erhöhte Peakviskositäts-Temperatur, einen reduzierten Peak, Zusammenbruch uns Setback-Viskositäten, während die Textur-Profil-Analyse eine Verringerung der Härte, Kohäsivität, Elastizität und Gummiartigkeit der fermentierten Mehlpaste zeigte. Die veränderten texturalen Eigenschaften wurden größerer Stärkekorn-Stabilität zugeschrieben infolge kurzer amyloseartiger Bruchstücke, gebildet durch enzymatische Hydrolyse von Amylopektin.

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1 Introduction

Cassava (*Manihot esculenta* Crantz) is consumed in several parts of West and Central Africa in the form of a hot water flour paste called fufu. The flour is made by allowing peeled tuberous roots of the crop to ferment naturally while steeped in water. The fermented pulp is dried and milled into a fine flour which can then be reconstituted in boiling water into the paste (fufu) before consumption [17]. In a restricted variation, the product is also made from the unfermented flour, in which case only roots of the 'sweet' (implying low or absence of cyanogenic glucosides) variety are used. One major reason for fermenting cassava is to reduce this component in the 'bitter' varieties to innocuous levels [7, 12]. Currently attempts are being made to improve the process through the use of starter cultures [22].

Fufu has the potential for wider acceptability and consumption outside its present traditional base. However, one of the major drawbacks to the realization of this potential is the undesirable cohesiveness of the product. It appears that the product would be more widely acceptable if the cohesiveness could be decreased [18, 21]. However, although some attempts have been made to study the process of cassava fermentation, and some properties of the product have been reported [9, 19, 20, 22], emphasis has been placed on its chemistry and microbiology, with little effort expended toward developing an understanding of its physicochemical and textural properties. Development of information of this type is essential if we are to control product cohesiveness.

Moreover, if the process is to be commercially feasible, utilization of starter cultures which predominate the fermentation is essential. A better understanding of the in changes that take place during the process and how such changes relate to this textural property could be usefully exploited to improve the product for wider acceptability. A wider acceptability of the product could increase consumption of the locally available cassava crop and reduce the dependence on imported foods by countries of the region.

The objective of the present work is to investigate the ways in which natural and mixed culture fermentation of cassava roots affect the physicochemical and thermal properties of their starch and flour, and how such changes relate to textural properties of their flour paste.

2 Materials and Methods

Fresh cassava roots of the 'Red Skin' variety and commercial cassava starch (used for comparison purposes) were purchased from a local market. *Bacillus subtillis* and *Candida krusei* were purchased from the American Type Culture Collection (Rockville, MD, USA), while *Lactobacillus plantarum* ATCC 33712 strain LA 102 No. 83 was supplied by the USDA-ARS Food Fermentation Laboratory (Raleigh, NC, USA). All other chemicals were laboratory-grade.

Flour and starch preparation

Native cassava flour: Fresh roots were peeled, washed, and chipped to about 0.50cm slices and dried at 40°C for 12h in a convection oven. The slices were ground in a laboratory mill and passed through a US No. 40 sieve to remove excess fibre.

Naturally fermented cassava flour (NF): The traditional method of preparing fermented flour was used. Fresh roots were peeled, washed, and cut into about 5cm discs and then immersed in tap water in a bucket, covered with cheesecloth, and allowed to ferment naturally. The temperature of the room varied between 23 and 25°C during the fermentation period. The end of fermentation occurred after 7 days when sample pulp crumbled between the fingers on slight pressing. Excess water was drained out and the solid matter was dried in a convection oven at 40°C for 12h. Dried pulp was ground into a flour with a laboratory mill, and excess fibre was removed by passing the ground material through a US No. 40 sieve.

Mixed culture fermented cassava flour (MCF): The procedure described by *Oyewole* [22] was followed, except that, for safety considerations, sterilization with mercuric chloride was omitted. It was assumed that, by adding 10^6 cells/mL, the added cultures would outgrow the indigenous ones. *B. subtillis, L. plantarum,* and *C. krusei,* each at 10^6 cells/mL, were used, and the apparatus was maintained in an incubation chamber at 30°C and 90% relative humidity. Roots became soft at the end of 4 days. Samples were withdrawn every 24h and analyzed for pH, acidity, and microbial count.

Native cassava starch: Fresh roots ('Red Skin') were peeled, chipped, and pulverized in a high speed, industrial Waring blender for 5min, suspended in 10 times its volume of distilled water, stirred for 5min, and filtered through double cheesecloth. The filtrate was allowed to stand for 1h for the starch to sediment, and the top liquor was decanted and discarded. The sediment was washed once with distilled water and dried at 40°C for 12h in a convection oven. The starch was hand-ground with mortar and pestle (to avoid granule disruption) and stored in sealed plastic jars.

Fermented cassava starch (NF and MCF): The extraction procedure was aimed at obtaining a starch with properties related to those of its parent flour. As such, roots were prepared, fermented, and the starch was extracted in each case as described above.

Proximate analysis

Proximate composition of starch and flour was determined by the *Standard Methods of AOAC* [2].

Amylose

Amylose contents of starch and flour were determined by the iodine binding method described by *McCready* and *Hassid* [15].

Average granule diameter

Average granule diameter of starch was measured microscopically. 100mg of starch were dispersed in 9.9mL of distilled water and held for 15min at 25, 60 or 85°C in a constant temperature water bath with constant stirring. Two drops of the suspension were placed on a slide, stained with two drops of 3% iodine solution, and observed under a microscope with a 40X objective and a micrometer eye piece. Twenty granules were selected randomly and their diameters measured. Each reading was multiplied by 2.75 (the constant for the 40X lens) to convert to microns. Three measurements were made per sample.

Solubility and swelling power

Solubility and swelling power of the starch at 85°C were measured by the method of *Schoch* [23].

Calorimetry

Onset and peak gelatinization temperatures and enthalpy were determined by Differential Scanning Calorimetry (DSC)

using a Perkin-Elmer Calorimeter (model DSC 4, Perkin-Elmer Corporation, Newalk, CT, USA). About 10mg of starch and 40mg of deionized water (weight fraction = 0.8) were weighed serially into DSC steel pans and sealed. A reference pan with an equivalent amount of water was also prepared. After the instrument was calibrated using Indium, the sample and reference pans were placed in the instrument and heated at a programmed rate of 10°C/min from 25-120°C. Data were recorded and analyzed by an attached Data Analysis Station.

Amylography

The Brabender Amylograph (model VAV 3113/67, C.W. Brabender, South Hackensack, NJ, USA) was used to study the effect of fermentation on the pasting properties of the starches and flours during heating, cooling, and shearing. 30g of starch or flour were weighed and stirred in 400g of distilled water, and then poured into the bowl of the amylograph. 20g of water were used to wash the remaining starch from the beaker to give a total slurry of 450g. The instrument was programmed to heat at 1.5°C per min for 45min to 92.5°C, held at that temperature for 15min, and cooled for 45min. Speed was maintained at 75rpm, and a 700cmg sensitivity cartridge was used. Curves of the amylograms were analyzed for peak, breakdown, and setback viscosities, and gelation and peak viscosity temperatures following the method described by *Dengate* [8].

Texture profile analysis (TPA)

An Instron Universal Testing Machine (UTM, model 1122, Instron Corporation, Canton, MA, USA) was used to study the texture profile of 30% flour gels following the method of *Bourne* [6]. Because cassava flour paste is an amorphous mixture that does not provide the necessary consistency for collecting useful information by TPA, a method of preparing the gel was developed. A 30% flour dispersion in water was made in a 10-mL, clear plastic syringe whose tip had previously been heat-sealed. The dispersion was thoroughly mixed, immersed in boiling water, and allowed to cook for 15min.
The piston was introduced into the syringe and pushed down until the gel assumed a volume of 10mL. The gel was allowed to equilibrate at 10°C for 6h. The first 1cm of the head of the syringe (and gel) was cut off with a sharp edge, and the remainder of the gel was pushed out with the piston. The middle 16mm (14mm diameter) portion of the gel was cut out and compressed twice (75% compression) with a force of 5kg in an Instron UTM fitted with a 50-kg load cell which was attached with a plunger having a 5.7cm diameter compression anvil. The measurements were performed on the Instron UTM with the crosshead speed set at 500mm per min and the chart speed was 1,000mm/min. Only gels made from the flours were used because the starch gels were too cohesive to be fractured under compression (a condition necessary for reliable TPA). Curves traced on the chart were analyzed for gel hardness, cohesiveness, elasticity, and gumminess.

3 Experimental design

Each procedure described above, with the exception of amylography, was conducted twice. The amylography procedure was conducted once. For each procedure, each determination was run in triplicate. The mean and standard deviation were calculated for each parameter measured.

4 Results and Discussion

Proximate composition of the native and fermented cassava starches and flours are shown in Tables 1 and 2. Fermenting the roots resulted in a decrease in soluble sugars, cyanide content, and pH of both starch and flour. This is in agreement with earlier studies [9, 12]. For the MCF product, the percent decrease in total soluble sugars was 94.7%, as compared to 78.9% for the NF product, reflecting higher microbial growth (microbial growth data not shown). The rate of pH decreased to about 4.5, and microbial counts were higher in the inoculated roots than in those fermenting naturally. The higher microbial population would also account for the higher consumption of soluble sugars and faster drop in pH.
There was an apparent increase in amylose content for both the starches and the flours prepared by natural and mixed culture fermentations. This unusual but persistent observation could be explained by the likely formation of amylose-like materials resulting from enzyme/acid hydrolysis of amylopectin at the amorphous regions of the starch granule.
Biliaderis et al. [5] showed that when starches were treated with amylases there was an initial attack on the amorphous regions of the starch granule, cassava starch being the most susceptible. *B. subtillis* is known to produce amylases [14]. *French* [10] observed that the intercrystalline amorphous areas in the starch granule are relatively susceptible to hydrolytic agents such as various enzymes and acids.
Adkins and *Greenwood* [1] reported an unusual iodine-binding behavior of amylomaize and attributed it to the presence of degraded amylose-like polysaccharides. *Banks* et al. [3] reported that, under standard conditions, a polysaccharide of 18 glucose units was the minimum necessary for the formation of an iodine complex, and that chains of 50 to 150g glucose units, resulting from both phosphorylase synthesis or enzyme degradation, gave blue color with iodine similar to those of amylose.

Table 1.
Proximate Composition of the Commercial, Native and Fermented Cassava Starches.

Measured variable[1)]	Type of cassava starch			
	Commercial	Native	Fermented naturally	Fermented with culture
Total carbohydrate (%)	99.44 ± 0.1	99.03 ± 0.1	99.32 ± 0.1	99.31 ± 0.3
Apparent amylose (%)	19.76 ± 2.3	16.71 ± 1.0	20.35 ± 2.0	18.23 ± 2.2
Total sugars (μg/mL)	37.63 ± 1.9	35.23 ± 2.3	11.43 ± 1.4	7.33 ± 1.3
Crude fibre (%)	0.15 ± 0.04	0.23 ± 0.06	0.13 ± 0.12	0.11 ± 0.06
Ash (%)	0.19 ± 0.03	0.22 ± 0.04	0.19 ± 0.00	0.11 ± 0.08
Lipids (%)	0.13 ± 0.02	0.20 ± 0.03	0.14 ± 0.03	0.08 ± 0.01
Crude protein (%)	0.09 ± 0.01	0.31 ± 0.01	0.38 ± 0.01	0.40 ± 0.01
Cyanide (mg/kg)	1.41 ± 0.19	1.94 ± 0.00	ND	ND
Acidity (g/dL) as acetic acid	0.43 ± 0.06	0.37 ± 0.06	0.73 ± 0.12	0.68 ± 0.01

[1)] Values are expressed on a dry weight basis and each represents an average and a standard deviation of two runs, with each analyzed in triplicate. ND = not detectable.

Table 2.
Proximate Composition of Native and Fermented Cassava Flours.

Measured variable[1)]	Type of cassava flour		
	Native	Fermented naturally	Fermented with culture
Total carbohydrate (%)	93.17 ± 0.3	95.97 ± 0.3	96.40 ± 0.2
Apparent amylose (%)	14.57 ± 0.7	16.45 ± 1.0	19.16 ± 0.1
Total sugars (μg/mL)	88.50 ± 0.9	18.67 ± 0.9	4.67 ± 0.07
Crude fibre (%)	1.33 ± 0.08	1.03 ± 0.12	1.11 ± 0.05
Ash (%)	2.68 ± 0.22	1.16 ± 0.05	1.16 ± 0.03
Lipids (%)	0.87 ± 0.07	0.52 ± 0.05	0.31 ± 0.01
Crude protein (%)	1.95 ± 0.05	1.81 ± 0.05	2.02 ± 0.11
Cyanide (mg/kg)	8.14 ± 0.54	2.21 ± 0.09	1.90 ± 0.12
Acidity (g/dL) as acetic acid	0.43 ± 0.06	0.73 ± 0.06	0.88 ± 0.11

[1)] Values are expressed on a dry weight basis with each representing an average and a standard deviation of two runs, with each analyzed in triplicate.

The recent work of *Thorn* and *Mohazzed* [25] has established, that, after side chains are split off, there are saccharide chains of more than 40 glucoside residues in the basic structure of potato amylopectin. Such linear residues would likely participate in iodine binding and contribute to blue color formation. Thus, it is probable that the apparent increase in amylose content in the fermented cassava starches is due to intensification of the blue color by linear fractions, resulting from enzyme/acid hydrolysis of amylopectin at the amorphous regions of the starch granule during the fermentation.

Table 3 shows the effects of fermentation on some physicochemical properties of the starch. No significant differences were observed on the average granule diameter at 25°C between the native and fermented products. The unchanged diameter at 25°C suggests that, during fermentation, granule surface corrosion, if any, was minimal. *Leach* and *Schoch* [14] observed that the surface areas of potato and cassava starch granules were not very susceptible to enzyme attack. A decrease in average granule diameter at 60°C (below the gelatinization range) and at 85°C (above the gelatinization range) of the fermented cassava starches was, however, observed. Average granule diameter, solubility, and swelling power were reduced by about 21.2%, 26.5%, and 12.1%, respectively, in the NF starch, while in the MCF product the reductions were 19.4%, 37.8%, and 15.5%, respectively. According to *Leach* et al. [13], the major factor that controls the swelling behavior of a starch is the strength and character of the micellar network within the granule. Solubilization also reflects the extent of intermolecular cross-bonding within the granule [23].

Although *Leach* et al. [13] showed that the presence of substances such as lipids or phosphate groups affect swelling, cassava starch and flour contain low levels of lipids and phosphate groups. Their influence on swelling power would, therefore, be minimal.

Hwang and *Kokini* [11] have recently observed that side branches function to prevent intermolecular association of carbohydrate polymers. As such, water molecules can more readily penetrate the intermolecular spaces, resulting in enhanced solubility. For the same reason, the presence of side branches promotes swelling and subsequent gelatinization. During the swelling of native starch, some of the water taken up hydrogen bonds with the free (OH) groups in the amorphous areas of the granule. When some of them are hydrolyzed, as occurs during fermentation, there is possible intermolecular hydrogen-bonding of the fragments, resulting in a reduction of the free (OH) groups where the water molecules would normally hydrogen-bond. This would lead to less water uptake and less swelling during heat treatment. The reduction in average granule diameter, solubility, and swelling power of the fermented starches at 60 and 85°C are, therefore, likely related to alterations in the internal granule structure following enzyme/acid action.

Table 3 also shows the effects of fermentation on the thermal properties of the cassava starch. Onset peak gelatinization temperatures remained unchanged, while gelatinization enthalpies were increased by 13.1 and 13.4% in the NF and MCF starches, respectively. Higher enthalpy and temperature of gelatinization reflect a greater internal granule stability. *Biliaderis* [4] has noted that onset and peak gelatinization temperatures, as well as gelatinization enthalpies as measured by the DSC, reflect a change of order within the starch granule.

Zobel [26] showed an increased trend of gelatinization enthalpies with increasing amylose content of the B type crystalline starches and explained that high heat of gelatinization of high

Table 3.
Some Physical Properties of Commercial, Native and Fermented Cassava Starches.

Measured variable[1)]	Type of cassava starch			
	Commercial	Native	Fermented naturally	Fermented with culture
pH	6.58 ± 0.16	6.9 ± 0.0	4.41 ± 0.02	4.51 ± 0.02
AGD[2)] (μm) 25°C	16.00 ± 1.0	17.24 ± 1.0	15.79 ± 1.0	16.10 ± 1.0
... 60°C	36.49 ± 3.7	41.17 ± 3.3	36.82 ± 3.1	34.72 ± 4.0
... 85°C	57.90 ± 3.2	68.49 ± 4.1	54.06 ± 6.8	58.45 ± 3.5
Swelling power (g/g)	27.32 ± 0.9	28.70 ± 1.5	25.22 ± 0.3	24.25 ± 0.7
Solubility (%)	32.29 ± 2.0	29.71 ± 1.3	21.83 ± 2.3	18.48 ± 1.0
Gelatinization onset temperature (°C)	68.40 ± 0.5	67.67 ± 0.5	66.77 ± 0.2	69.08 ± 0.9
Gelatinization peak temperature (°C)	74.69 ± 0.7	73.04 ± 0.5	72.60 ± 0.3	73.99 ± 0.4
Gelatinization enthalpy (mJ/mg)	12.75 ± 0.2	12.75 ± 0.6	14.42 ± 0.8	14.46 ± 0.7

[1)] Each value represents an average and a standard deviation of two sample determinations each analyzed in triplicate.
[2)] The standard deviations of Average Granuale Diameter (AGD) at 60 and 85°C were adjusted to those at 25°C to account for natural granule size variation.

amylose starches could be attributed to the extensive association between the amylose chains. *Zobel* [27] also found that, in crystalline type B starches, the amylose content appears to have no effect on gelatinization temperature, although *Takeda* and *Hizukuri* [24] reported that gelatinization temperature was increased by an increase in amylose content of various starches. The formation of new hydrogen bonds, as postulated above, explains the observed increase in gelatinization enthalpies of the fermented starches.

Table 4 shows the effect of fermentation on the pasting properties of cassava starch. Although natural fermentation had no effect on the pasting temperature, that of MCF starch was found to be higher than that of the native starch. Temperature at peak viscosity was increased in both fermentations, while on the other hand, peak, breakdown, and setback viscosities were reduced by the fermentations.

Table 4.
Pasting Properties of 6.7% Native or Fermented Cassava Starch-Water Dispersions and Effect of pH on the Properties of 6.7% Dispersion of the Native Starch Only.

Measured variable[1)]	Type of Cassava		
	Native	Fermented naturally	Fermented with culture
Pasting temperature (°C)	42	42	43.5
Peak viscosity temperature (°C)	63	69	72
Peak viscosity (BU)	1070	770	850
Breakdown viscosity (BU)	575	310	360
Setback viscosity (BU)	275	190	230
	pH (of native starch only)		
	6.9	5.5	4.5	3.5
Pasting temperature (°C)	42	42	42	42
Peak viscosity temperature (°C)	61.5	62	61.5	60
Peak viscosity (BU)	1070	1070	1070	990
Breakdown viscosity (BU)	560	570	590	650
Setback viscosity (BU)	280	280	225	190

[1)] Each value represents a mean of two sample determinations.

During the fermentation of the roots, the pH of the pulp was reduced from 6.9 to about 4.5. In order to understand the role of pH on the pasting properties of the starch, native starch was acidified and its pasting properties studied. Acidification of the native starch with citric acid (Table 4) did not affect its peak viscosity between pH 6.9 and 4.5. However, a reduction in peak viscosity was observed when the pH of the native starch was lowered to 3.5, suggesting that the observed differences between the native and fermented cassava starches were due mainly to the enzyme action. The rheological changes also reflect the greater internal stability of the fermented starch granule, resulting in reduced swelling and amylose leaching. *Miller* et al. [16] suggested that exudate network formation was a likely factor influencing starch paste viscosity. Table 5 shows the effect of fermentation on some textural properties at 10°C of 30% cassava flour gels. Hardness, gumminess, cohesiveness, and elasticity of flour gels at 10°C were reduced in the fermented products. Gel hardness and gumminess have been associated both to the degree of granule swelling and network formation by leached amylose [4].

Since cohesiveness is associated with the intermolecular forces within the food system, a reduction in this property also suggests a reduction of such forces. The failure of the starch granules to release sufficient amylose would account for the reduction in the cohesiveness of the fermented products. This

Table 5.
Effect of Fermentation on Some Textural Components at 10°C of 30% Cassava Flour Gels.

Parameter[1)]	Type of Cassava flour gel		
	Native	Fermented naturally	Fermented with culture
Hardness (kg)	2.25 ± 0.02	2.03 ± 0.03	2.13 ± 0.05
Cohesiveness	0.79 ± 0.02	0.75 ± 0.06	0.68 ± 0.04
Gumminess (kg)	1.79 ± 0.04	1.53 ± 0.14	1.46 ± 0.12
Adhesiveness (mm)	24.3 ± 2.01	15.33 ± 4.7	8.33 ± 2.1
Elasticity (mm)	11.33 ± 1.04	11.17 ± 0.76	10.67 ± 0.58

[1)] Each value represents a mean of two sample determinations each replicated three times.

is closely tied to the reduced solubility, swelling power, and restriction to granule size increase during heating, a consequence of greater internal granule stability of the fermented starches.

5 Conclusion

Fermented cassava starch and flour were found to have an apparent increase in amylose content as determined by the iodine binding method. Average granule diameter, solubility, and swelling power of the cassava starch at 60 and 85°C were found to be depressed by fermentation, while their gelatinization enthalpies increased. It is concluded that these changes are due to the formation of amylose-like fragments caused by enzymatic hydrolysis of amylopectin at the amorphous regions of the starch granule. Thus, the fragments could realign and form new hydrogen bonds, resulting in greater internal granule stability. A more stable granule would account for the observed reduced solubility and swelling power of the fermented starches. This, in turn, would account for the reduced cohesiveness and other textural components of the flour pastes. A further investigation of this stability of the fermented starch granules is necessary.

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