Physicochemical Changes in Cassava Starch and Flour Associated with Fermentation, Effect on Textural Properties *

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Abstract

Physicochemical changes in cassava starch and flour associated with fermentation were investigated and related to textural properties of the flour paste. 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. Texture Profile Analysis of the fermented flour paste showed a decrease in hardness, cohesiveness, elasticity, and gumminess. The altered textural properties were attributed to greater starch granule stability caused by realignment of short amylose-like fragments formed by enzymatic hydrolysis of amylopectin.

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 (Numfor and Lyonga, 1987; Okezie et al., 1989; Hahn, 1989). 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 (Cooke and Maduagwu, 1978; Ketiku et al., 1978). Currently attempts are being made to improve the process through the use of starter cultures (Oyewole, 1990).

Fufu has the potential for wider acceptability and consumption outside its present traditional base. However, one drawback to the realization of this potential is the undesirable cohesiveness of the product. It appears that the product would be more widely acceptable if its cohesiveness could be decreased (Odigboh and Mohsenin, 1975; Osunji, 1983). Although some attempts have been made to study the process of cassava fermentation, and some properties of the product have been reported (Collard and Levi, 1959; Akinrele et al., 1962; Longe,

1980; Ogunsua, 1980; Dougan et al., 1983; Phan and Mercier, 1984; Okafor et al., 1984; Ezeala, 1984; Oyewole, 1990), 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.

Also, if the process is to be commercially feasible, utilization of starter cultures which predominate the fermentation is essential. A better understanding of the changes that take place during the process and how such changes relate to the products cohesiveness 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 the flour paste.

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 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 0.50 cm slices and dried at 40°C for 12 hr 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 5 cm 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 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 12 hr. 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 (1990) 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. krusel,* 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 24 hr and anlyzed for pH, acidity and microbial count.

Native cassava starch: Fresh roots were peeled, chipped, and pulverized in a high speed, industrial Waring blender for 5 min, suspended in 10 times its volume of distilled water, stirred for 5 min and filtered through double cheesecloth. The filtrate was allowed to stand for 1 hr 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 12 hr 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 (AOAC, 1984).

Amylose
Amylose contents of starch and flour were determined by the Iodine binding method described by McCready and Hassid (1943).

Average granule diameter
Average granule diameter of starch was measured microscopically. 100 mg of starch were dispersed in 9.9 mL of distilled water and held for 15 min 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 (1964).

Calorimetry
Onset and peak gelatinization temperatures and enthalpy were determined by Differential Scanning Calorimetry using a Perkin-Elmer Calorimeter (model DSC 4, Perkin-Elmer Corporation, Newalk, CT, USA). About 10 mg of starch and 40 mg 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 was recorded and analyzed by an attached Data Analysis Station.

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 (1978). Because cassava flour paste is an amorphous mixture that does not provide the necessary consistency for collecting useful information by Texture Profile Analysis (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 15 min.

The piston was introduced into the syringe and pushed down until the gel assumed a volume of 10 mL. The gel was allowed to equilibrate at 10°C for 6 hr. The first 1 cm 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 16 mm (14 mm diameter) portion of the gel was cut out and compressed twice (75% compression) with a force of 5 kg in an Instron UTM fitted with a 50-kg load cell which was attached with a plunger having a 5.7 cm diameter compression anvil. The measurements were performed on the

Instron UTM with the crosshead speed set at 500 mm per min and the chart speed was 1,000 mm per 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.

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 significant decrease in soluble sugars, cyanide content, and pH of both starch and flour. This is in agreement with earlier studies (Ketiku et al., 1978; Longe, 1980; Ezeala, 1984). The decrease in total soluble sugars was much greater in the MCF product, reflecting higher microbial growth (not shown). The rate of pH decrease 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. (1980) 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 (Leach and Schoch, 1961). French (1984) observed that the intercrystallins amorphous areas in the starch granule are relatively susceptible to hydrolytic agents such as various enzymes and acids.

Adkins and Greenwood (1966) reported an unusual iodine-binding behavior of amylomaize and attributed it to the presence of degraded amylose-like polysaccharides. Swanson (1948), Bailey and Whelan (1961), and Banks et al. (1974) reported that, under standard conditions, a polysaccharide of 18 glucose units was the minimum necessary for formation of

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
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	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 sample determinations 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
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	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 sample determinations each analyzed in triplicate.

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 ±0.2	4.51 ±0.2
AGD[2]	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	18.45 ±1.0
Gelatinization peak 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	73.40 ±0.5	72.60 ±0.3	73.99 ±0.4
Gelatinization enthalpy (mJ/mg)	12.75 ±0.2	12.75 ±0.6	14.42 ±0.81	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 Granule Diameter (AGD) at 60 and 85°C were adjusted to those at 25°C to account for natural granule size variation.

Table 4. Effects of Fermentation on some Textural Components at 10°C of 30% Cassava Flour

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 ±1.7	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 thrice.

an iodine complex, and that chains of 50 to 150 glucose units, resulting from both phosphorylase synthesis or enzyme degradation, gave blue color with iodine similar to those of amylose. The recent work of Thorn and Mohazzed (1990) 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 observed 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 (1961) 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 (1965), 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 (Schoch, 1964).

Although Leach et al. (1959) showed that the presence of substances such as lipids or phosphate groups also affect swelling, cassava starch and flour contain low levels of lipids and phosphate groups. Their influence on swelling power would, therefore, be minimal.

What should account for the decreased swelling and solubility of the fermented starches?

Hwang and Kokini (1991) 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 these branches 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 (1992) 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 (1984) 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 amylose starches could be attributed to the extensive association between the amylose chains. Zobel (1988) also found that, in crystalline type B starches, the amylose content appears to have no effect on gelatinization temperature, although Tekeda and Hijukuri (1974), Madamba et al. (1975), and Seog et al.

(1978) 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 some textural properties at 10°C of 30% cassava flour gels. Hardness, gumminess, cohesiveness, and elasticity of flour gels at 10°C where 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 (Biliaderis et al., 1990).

Since cohesiveness is associated with the intermolecular forces within the food system, a reduction in the value of 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 is closely tied to the reduced solubility, swelling power, and restriction to granule size increase during heating, a consequences of greater internal granule stability of the fermented starches.

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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