Full Length Research Paper

Functional properties of amylopectin and amylose fractions isolated from bambarra groundnut (*Voandzeia subterranean*) starch

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Bambarra groundnut starch was fractionated into amylose and amylopectin fractions, and chemical modifications, through oxidation and acetylation, was applied to the amylose fraction. Percentage yield of amylose and amylopectin were 75% and 11% respectively. Proximate analysis revealed that percentage protein, ash, crude fibre and crude fat were below 1%. Swelling capacity and solubility of all the samples increased with increasing temperature. Water and oil absorption capacity revealed that hydrophobic tendency was greater than hydrophilic potentials. Gel forming capacity increased with increase in concentration of the samples and least gelation concentration was minimal in amylopectin fraction. Initial pasting temperature of native amylose reduced from $70^{o}C$ to $60^{o}C$ and $65^{o}C$ following oxidation and acetylation, respectively. Among the samples, highest pasting temperature was recorded in native amylopectin and values for peak viscosity during heating (Pv), hot paste viscosity at 95oC (Hv), viscosity after 30 min holding at 95oC (Hv30), cold paste viscosity (Cv), set back (SB) and breakdown (BD) were maximal in native amylose.

Key words: Bambarra groundnut, amylose, amylopectin, modifications.

INTRODUCTION

Using biopolymers in their native form often confront end users in various industries with difficulties. Chemical modifications such as oxidation and acetylation are applied to biopolymers to circumvent some of these undesirable properties in such polymers. In addition, such modifications have been used to impact certain properties in the polymer, and these depend on the end use of the polymer in various industries. In previous studies, several of such modifications have been applied to cellulose and starch (Kuakpetoon and Wang, 2001; Atichokudomchai et al., 2001, Forssel et al., 1995).

Oxidation of starch entails introduction of carbonyl and carboxyl groups on glucose units within the matrix of the polymer. Previous studies have shown that such modification brings about improvement in whiteness of the starch, and restricted retrogradation or "setting up" on standing (Kuakpetoon and Wang, 2001., Adebowale et al., 2002). Acetylation of biopolymers is obtained by esterification of native starch with acetic anhydride and the modified starches generally show better paste clarity, stability, increased resistance to retrogradation, and increased feeze-thaw stability (Adebowale and Lawal 2003b)

Previously, we have emphasised on the need to harness the potentials of underutilised legumes as invaluable sources of starch and protein concentrates (Adebowale et al., 2002, Adebowale and Lawal, 2003a).

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Producing starch and starch derivatives from conventional plants like cassava, maize, rice and potato, places too much demand on them, particularly as they have to meet the needs of both domestic and industrial uses. In addition, the significance of various modifications, both physical and chemical on the functional and physicochemical parameters of some underutilized legume starches have been previously investigated (Adebowale and Lawal, 2002., Adebowale and Lawal, 2003b,c). Here we present further progress in the fractionation of starch isolated from Bambarra groundnut (Voandzeia subterranean), an underutilized legume, into amylopectin and amylose.

Native starch is composed of almost linear amylose (an α-1,4 polymer) and amylopectin (a branched polymer) consisting of short linear α-1, 4 polymer chains linked to each other by α-1, 6 linkages. These two components form a semicrystalline structure in the starch granules, which consist of crystalline lamellae (ordered, tightly packed of parallel glucan chains) and amorphous lamellae (less – ordered regions) (Oates, 1997). Starches of different origins have different degrees of crystallinity (range about 15-45 %) (Zobel, 1988).

As in all chemical reactions, these modifications depend on prevalent environmental factors within the reaction system, such as the pH, reaction time, presence of catalyst and concentration (Whistler and Daniel, 1990).

This study was designed to investigate the physicochemical properties of native and chemically modified starch fractions of bambarra groundnut, with a view to providing information towards their effective utilisation, particularly in food industries.

MATERIALS AND METHODS

Materials

Bambarra groundnut seeds were obtained from Bodija market, Ibadan. The seeds were screened to eliminate the defected ones. Water was added to the samples and left overnight. The seeds were manually dehulled, air died at $30\pm2^{O}C$ then dry-milled to a fine powder. The flour was stored in polythene bags prior to use. All chemicals used in experiments were of analytical grade.

Starch isolation

The method of Sathe et al. (1981) as modified by Adebowale et al. (2002), was employed for the starch isolation. Occasional stirring was provided during all extractions.

Fractionation of amylose and amylopectin

Fractionation of amylose and amylopectin was carried out by following the general procedure of (Song and Jane, 2000). This consists of heating and stirring starch dispersion (0.8%, w/v in water) in water bath at $100^{O}C$ until starch is gelatinized. Starch solutions were filtered to remove insoluble residues, and the pH

was adjusted to 6.3 with a phosphate buffer. The solution was stirred in a boiling water bath for 2 h to disperse the starch molecules. Thereafter, n-Butyl alcohol was added (20%, v/v), and the solution was stirred at $100^{o}C$ for 1 h, followed by cooling to room temperature over a period of 24-36 h. Amylose butyl alcohol complex crystals was formed and precipitated during cooling, and was separated by filtration. The amylopectin remaining in the supernatant was recovered by adding excess methyl alcohol.

Amylose acetylation

Amylose acetylation was performed using the method of Wurzburg (1964). Native amylose (100 g) was dispersed in 500 ml of distilled water and stirred magnetically for 30 min. The pH was adjusted to 8.0 using 0.5 M NaOH. 10.2 g of acetic anhydride was added slowly to the mixture while maintaining a pH range of 8.0 – 8.5. The pH was finally adjusted to 4.5 with 0.5 M HCl. The amylose was filtered, and the residue obtained was washed four times with distilled water and air dried for 48 h at $30\pm2^{0}C$.

Amylose oxidation

Amylose oxidation was performed according to the method of Sathe and Salunkhe (1981). Native amylose (100 g) was mixed with 500 ml of distilled water and the pH of the mixture was brought to 9.5 with 0.3 M NaOH. 10 g of NaOCl were added dropwisely over a period of 2 h with constant stirring while a pH of 9.0-9.5 was maintained. The reaction vessel was cooled in a mixture of crushed ice and NaCl. The reaction was allowed to continue for 5 h after all the NaOCl had been added. The pH was brought to 7.0 with 0.3 M HCl and the slurry filtered through Whatman filter paper No 4. The amylose obtained was washed four times with distilled water and dried at $30\pm2°C$ for 48 h.

Extent of modification

The degree of substitution (DS) of the acetylated starch which is the moles of acetyl substituent per mole of D-glucopyranose unit was determined according to the method described by Würzburg, (1964).

The method of Parovuori et al. (1995) was used for the determination of carboxyl contents. 5 g of oxidized starch sample were slurried in 25 ml of 0.1 M HCl, and stirred for 40 min. The slurry was filtered through a medium porosity fritted glass crucible and the residue was washed with distilled water until it was free of chloride, (determined by silver nitrate test). The chloride free sample was dispersed in 300 ml of distilled water. The dispersion was heated in a steam bath and stirred continuously until the starch gelatinised. The hot sample was titrated with 0.1 M NaOH to a phenolphthalein end point. To quantify acidity due to other sources (mainly fatty acids complexed with amylase), 5 g of unoxidised starch were titrated to provide for a blank value.

$$Percent\ carboxyl = \frac{(sample\ titre\text{-}blank\ titre)\ ml \times alkali\ molarity \times 0.045 \times 100}{sample\ weight\ (g)}$$

The hydroxylamine method described by smith (1967) was used for the determination of carbonyl content. 2 g of oxidised starch were dispersed in 100 ml of distilled water and the suspension was gelatinised by heating in a boiling water bath and then cooled to $40^{o}C$. The pH was adjusted to 3.2, and 15 ml of hydroxylamine reagent was added (the hydroxylamine reagent was prepared by dissolving 25 g of reagent grade hydroxylamine hydrochloride in

water and adding 100 ml of 0.5 M NaOH. The solution was made to 500 ml with distilled water). The sample was covered with aluminium foil and placed in a water bath at 40°C. After 4h, the excess hydroxylamine was determined by rapid titration of the reaction mixture to pH 3.2 with 0.1 M hydrochloric acid.

$$\text{Percent carbonyl (C=O)} = \frac{\text{titre-sample titre) ml X acid molarity X 0.028 X 100}}{\text{dry sample weight (g)}}$$

Values presented as percentages are numbers of carboxyl (COOH) and carbonyl (CHO) groups per 100 anhydroglucose unit, AGU.

Proximate analysis

Moisture content, Ash, crude fibre, crude protein, and fat were determined by AOAC methods (1990)

Effect of temperature on solubility and swelling

1.0 g of sample was accurately weighed and quantitatively transferred into a clear dried test tube and weighed (w_1). The starch was then dispersed in 50 cm^3 of distilled water using a blender.
The resultant slurry was heated at temperatures of 60°C, 70°C, 80°C and 90°C respectively for 30min in a water bath (using temperature regulated water bath). The mixture was cooled to room temperature and centrifuged (500 revolutions per min, for 15 min).
Aliquots (5 ml) of the supernatant were dried to a constant weight at 110°C. The residue obtained after drying the supernatant represented the amount of starch solubilised in water. Solubility was calculated as g per 100 g of sample on dry weight basis.
The residue obtained from the above experiment (after centrifugation) with the water it retained was quantitatively transferred to the clean dried test tube used earlier and weighed and recorded as W2.

$$\text{Swelling of starch} = (W_2 - W_1) \text{ weight of sample}$$

Oil and water absorption capacities

Oil and water absorption capacities were determined by the method of Beuchat (1977). 10 ml of distilled water or oil (Executive Chef Oil, Lever Brothers (Nigeria) Plc, Lagos, Nigeria) was added to 1 g of sample, and mixed thoroughly with a Variwhirl mixer (Model A901, salver chem. Chicago, IL, USA) for 30 s and allowed to stand for 30 min. Then the volume of the supernatant was recorded. The mass of oil or water absorbed was expressed as gg^{-1} starch on a dry weight basis.

Gelation Properties

Gelation studies were investigated, employing the method of Coffman and Garcia (1977). Samples of starch (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g) were combined with 5 ml portions of distilled water in test tubes and blended by a Variwhirl mixer (Model A901, salver chem. Chicago, IL, USA) for 5 min. The test tubes were then heated for 30 min at 80°C in a water bath, followed by rapid cooling under running cold tap water. The test tubes were further cooled and held at 4°C for 2 h. The lowest gelation concentration was determined as that concentration when the sample did not fall down or slip from the inverted test tube.

Pasting properties

Brabender viscographic studies of the starch samples were investigated using 80 gl^{-1} dispersion of the starch. A Brabender viscoamylograph (Type VA-V, Brabender GmbH, Duisburg, Germany) equipped with a 700 μg sensitivity cartridge was used. The slurry was heated from 30°C to 95°C, and kept at this temperature for 30min, before cooling down to 50°C. A constant rotational velocity of 75 revolutions per min was maintained and the heating or cooling rate was 1.5°C min^{-1} throughout the process.

Statistical analysis

Analyses were done in triplicate. Analysis of variance was performed to calculate significant differences in treatment means, and LSD ($P< 0.05$) was used to separate means (SAS, 1988).

RESULTS AND DISCUSSION

The results of proximate analysis of amylopectin, native and chemically modified amylase fractions of Bambarra groundnut starch is presented in Table 1. Percentage yield of amylose, calculated on dry native starch basis was 75%, while that of amylopectin was 11%. Starch is composed of two fractions, amylose fraction, which constitutes the bulk of the amorphous region and amylopectin fraction, which constitute the crystalline fraction. In this sense, the results obtained here suggest that Bambarra groundnut starch is composed of largely amorphous fractions, and this ultimately affects the physico chemical parameters. The values agree with previous studies on chemical composition of some legume starches (Hoover and Manuel, 1996a). The yield of acetylated amylose and oxidized amylose were 84% and 74% respectively, based on native amylose. Lower yield in oxidised derivative might be attributed to degradative oxidation of glycosidic bonds in amylose, a development that probably results in loss of mass. In all the samples, values obtained for percentage protein, ash, crude fibre and crude fat were below 1%. This result establishes high level of purity of starch fractions. It is also noteworthy that some values were even low beyond detectable readings, this lends credence to the fact that the amylopectin, amylose and the derivatised amylose were pure. No marked changes were observed in moisture content of the samples. Also oxidation and acetylation did not change the moisture content of native amylose, probably because they were stored under the same condition or the level of modification was not high enough to cause significant changes in moisture content of the samples compared with native amylose.

Effect of temperature on swelling power and solubility

Effect of temperature on swelling power and solubility of amylopectin, native and chemically modified amylose

Table 1. Proximate analysis of amylopectin, native and chemically modified amylose fractions of Bambarra groundnut starch.

Sample	Parameters (%)					
	Yield	Protein	Ash	Crude fibre	Crude fat	Moisture
N. amyl	75*	0.4	0.4	0.9	0.6	10.2
A. amyl[a]	84**	0.1	0.1	0.3	ND	10.3
O.amyl [b]	74**	ND	0.1	ND	0.5	10.4
N. ampt	11*	ND	0.1	ND	1.4	10.3

N. amyl: Native amylose; A. amyl: Acetylated amylose; O. amyl: Oxidised amylose; N. ampt: Native amylopectin.

* Value was calculated on dry native starch basis
** Value was calculated on dry native amylose basis
[a]Degree of substitution (moles of acetyl substituent per mole of D-glucopyranose unit) = 0.33
[b]COOH = 0.27(%). CHO = 0.29(%) (Values are numbers of carboxyl and carbonyl groups per 100 anhydroglucose unit,
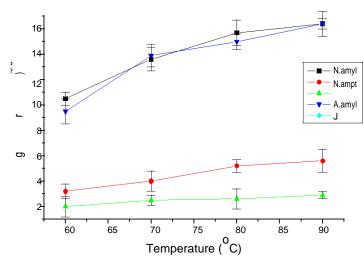AGU) ND Not detected.

Figure 1. Effect of temperature on swelling power of amylopectin, native and chemically modified amylose fractions of Bambarra groundnut starch. N. amyl: Native amylose; A. amyl: Acetylated amylose; O. amyl: Oxidised amylose; N. ampt: Native amylopectin.

Figure 2. Effect of temperature on solubility of amylopectin, native and chemically modified amylose fractions of Bambarra groundnut starch. N. amyl: Native amylose; A. amyl: Acetylated amylose; O. amyl: Oxidised amylose; N. ampt: Native amylopectin.
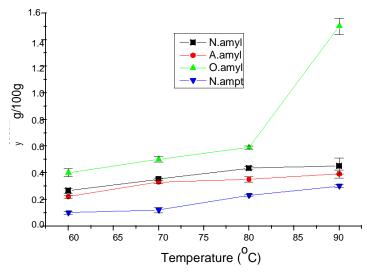
fractions of Bambarra groundnut starch are presented in Figures 1 and 2. The results indicate that swelling capacity and solubility of all the samples increased with increase in temperature. Maximal swelling capacity and solubility in all cases was observed at 90° C. This suggests that increase in temperature enhanced penetration of water into the granules of the samples. Thermodynamic mobility of particles increased as temperature increased, thereby facilitating penetration of water into the granules. In previous studies, (Adebowale and Lawal, 2002, 2003b) we have reported increase in swelling capacity with rise in temperature for various legume starches. It is also reasonable that increasing temperature weakened the intragranular binding forces of both native and modified amylose derivatives, thereby facilitating less restricted swelling and enhanced leaching of granular particles which led in increased solubility. However, restricted swelling in amylopectin fraction could be attributed to its crystalline nature. The crystalline arrangement prevented easy penetration of water thereby limiting both swelling capacity and solubility.

Water and oil absorption capacity

Water and oil absorption capacities of the samples are presented on Figure 3. The result indicates that hydrophobic tendency was greater than hydrophilic properties in all the samples. The result also indicates that water absorption capacity was minimal in native amylopectin, where least value of 5.5 ml/10 g sample was recorded. Maximal water and oil absorption capacities were observed in native amylose. Because of the amorphous nature of amylose, both oil and water were absorbed faster compared with crystalline amylopectin. Acetylated amylase has better oil and water absorption capacities than oxidized amylose. Earlier, Sathe and Salunkhe (1981) and Hoover and Vasanthan (1994) have reported increased water and oil absorption capacity following acetylation.

Table 2. Gelation properties of amylopectin, native and chemically modified amylose fractions of Bambarra groundnut starch.

Concentration (%,w/v)	N.amyl	O.amyl[b]	A.amyl[a]	N.ampt
2	- liquid	- liquid	- liquid	+ firm gel
4	- viscous	- liquid	- viscous	+ v. firm gel
6	- viscous	- viscous	+ gel	+ v. firm gel
8	+ gel	- viscous	+ gel	+ v. firm gel
10	+ gel	+ gel	+ firm gel	+ v. firm gel
12	+ firm gel	+ firm gel	+ v. firm gel	+ v. firm gel
14	+ v. firm gel	+ firm gel	+ v. firm gel	+ v. firm gel
LGC	8	10	6	2

N. amyl: Native amylose; A. amyl: Acetylated amylose; O. amyl: Oxidised amylose; N. ampt: Native amylopectin
[a]Degree of substitution (moles of acetyl substituent per mole of D-glucopyranose unit) = 0.33
[b]COOH = 0.27(%). CHO = 0.29(%) (Values are numbers of carboxyl and carbonyl groups per 100 anhydroglucose unit, AGU).
LGC: Least gelation concentration.

Table 3. Pasting characteristics of amylopectin, native and chemically modified amylose fractions of Bambarra groundnut starch.

Sample	Tp (^{o}C)	Pv (BU)	Hv (BU)	Hv$_{30}$ (BU)	Cv (BU)	SB (BU)	BD (BU)
			Paramete				
N.amyl	70	850	720	730	2050	1200	120
O.amyl[b]	60	755	655	670	1500	745	85
A.amyl[a]	65	760	700	710	1850	1090	50
N.ampt	85	610	550	580	750	140	30

Values are means of triplicate determinations. Tp : initial pasting temperature; Pv : peak viscosity during heating;
Hv: hot paste viscosity (at 95 ^{o}C); Hv$_{30}$ viscosity after 30min holding at 95 ^{o}C; Cv: cold paste viscosity (at 50 ^{o}C);
SB: setback value = Cv – Pv; BD: breakdown = Pv – Hv$_{30}$; BU: Brabender unit.
[a]Degree of substitution (moles of acetyl substituent per mole of D-glucopyranose unit) = 0.33
[b]COOH = 0.27(%). CHO = 0.29(%) (Values are numbers of carboxyl and carbonyl groups per 100 anhydroglucose unit, AGU).

Gelation properties

Table 2 presents the gelation properties of amylopectin, native and chemically modified amylose fractions of Bambarra groundnut starch. Using the least gelation concentration (LGC) as the index of gelation, the result obtained in this study indicates that lowest LGC was observed in amylopectin and the highest in oxidised amylose. It is also noteworthy that amylopectin formed a gel at a very low concentration of 2%. The gel strength of amylopectin is attributed the rigidity provided by its crystalline nature. These crystalline areas, both within the swollen granules and in the aqueous solution between the granules, improved the strength and rigidity of starch gel. In all the samples investigated, increasing concentration facilitated gelation properties. This suggests that enhanced interaction occurred among the binding forces as the concentration increased. Probably, introduction of carbonyl and carboxyl groups caused intermolecular repulsion that limited interaction of oxidized amylose molecules, which led to reduction in gelation properties.

Brabender amylographic studies

Amylographic characteristics of amylopectin, native and chemically modified amylose fractions of bambarra groundnut starch are presented on Table 3. Initial pasting temperature of native amylose reduced from 70°C to 60°C and 65°C following oxidation and acetylation respectively. Among the sample, the highest melting temperature was observed in native amylopectin. Values for peak viscosity during heating (Pv); hot paste viscosity (at 95°C) (Hv); viscosity after 30 min holding at 95°C (Hv30); Cv: cold paste viscosity (at 50°C), set back and breakdown were maximal in native amylose.

Cooking starch fraction slurry by suspending it in water and increasing the temperature gradually increases the viscosity to a maximum, known as peak viscosity. The viscosity drops as heating continues and finally increases again on cooling. Changes in granule dimensions and structure during the cooking process are accompanied by significant changes in the viscosity and other rheological properties. Information obtained from pasting characteristics is vital when considering them as a

component of a food product. Amylopectin exhibited higher pasting temperature because of its high crystalline nature which resisted gelatinization compared with amorphous amylose.

The reduction in cold pasting values of acetylated and oxidized amylose informs that new substituent groups have been introduced into the modified derivatives. The substituent groups restrict the tendency of the molecules to realign after cooling, thus facilitating a lower setback value for the modified derivatives.

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