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Morphological, functional and pasting properties of starches separated from rice cultivars grown in Nigeria

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Abstract: This study was carried out to determine the composition, morphology, functional and pasting properties of rice starches isolated from different rice cultivars (IGR, EAR, ILR and N2R). The starches were isolated from their flours by using a modified deproteination method in 0.1% NaOH. The highest starch yield of 65.00% was obtained from EAR with a residual protein of 0.41% and the lowest starch yield of 45.70% from IGR with a residual protein of 0.42%. The apparent amylose (AAM) content of rice starches ranged from 21.88 to 26.04%. Rice starches contain 10.40-12.77%, 0.10-0.70% and 0.20-0.24% moisture, fat and ash contents, respectively. The sizes of the starch granules obtained from SEM were between 3-8 µm. Some of the granules were individual (single) while others were fused (compound granules). The rice starch granules were polygonal and angular-shaped. When heated from 55 to 95°C at 10°C intervals, starches with higher amylopectin content had higher swelling power (SP). Both SP and water solubility index increased with increased temperature. The bulk density, dispersibility and pH of the rice starches ranged from 0.41-0.56 g/ml, 75.10-82.12% and 5.3-6.9, respectively. Pasting parameters were evaluated using RVA. Significant differences were observed in individual pasting parameters of the rice starches especially in peak viscosity, trough viscosity, final viscosity and setback viscosity. The results revealed that cultivar difference has an effect on composition and pasting properties of rice starch.

Keywords: Rice starch, morphology, dispersibility, functional properties, pasting

Introduction

Traditionally, there have been basic attributes associated with rice starch that have given it merit over other cereal and non-cereal starches. These properties include hypoallergenicity, digestibility, bland flavor, small granule (3-10 µm), white colour, greater acid resistance, greater freeze –thaw stability of pastes and a wide range of amylose/amylopectin ratios. These novel and unique characteristics manifest itself in the different applications of rice starches.

There are two main polymers in rice starch granules, i.e. amylose (AM) and amylopectin (AP). Associated with these are the minor constituents like the proteins (0.25%) (Baldwin, 2001), the lipids (0.1-0.3%) (Morrison and Azudin, 1987), and the compounds of phosphorus. The rice grains contain four types of proteins (glutelin, prolamin, globulin and albumin) present in the endosperm. They adhere to the surface of the starch and are relatively difficulty to remove. The residual protein of rice starch depends on the method of isolation (Singh *et al*., 2000).

The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant (Bodenhuizen, 1969). The discrepancy in the size and shape of starch granules is attributed to biological origin (Delcour and Hoseney, 2010). Rice starch granules are very small, ranging from 3 to 10 µm (Ellis *et al*., 1998) with a unimodal distribution (Dang and Copeland ,2004). They are polygonal and angular-shaped (Singh *et al*., 2003).

According to Tester and Morrison (1990), starch swelling is a property of AP, whereas, AM has been known to restrict it (Park *et al*., 2007; Patindol *et al*., 2007). For this reason, the difference in swelling and pasting properties among starches should be attributed to variation in AP unit-chain length distribution. Other factors that affect the swelling power and solubility of starch granules are presence of lipids (Galliard and Bowler, 1987) and differences in morphological structures (Singh *et al*., 2003).

Pasting encompasses the changes that occur after gelatinization upon further heating and these include further swelling of granules, leaching of molecular components from the granules and eventual disruption of granules especially with the application of shear forces (Tester and Morrison, 1990). The effect of AM and AP on the pasting properties of rice starch has been widely reported(Park *et al*., 2007; Li *et al*., 2008). The viscosity parameters during pasting are cooperatively controlled by the properties of the swollen granules and the soluble materials leached out from the granules (Sandhu *et al*., 2004; Sandhu and Singh, 2007).

Rice is grown in all ecological and dietary zones of Nigeria, with different species possessing adaptation traits for each ecology. The two commonly

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grown species in Nigeria are *Oryza sativa* and *Oryza glabberima* (Abulude, 2004). Although rice has been the subject of much investigation in the area of basic production in Nigeria, it has not benefited from the kind of value added research required for economic competitiveness on an international scale. Hence, rice producers in the country are small farmers who are left entirely on their own to keep the sub-sector afloat against so many odds. To encourage industrialist to engage in large scale rice cultivation in Nigeria, there is a need for a systematic study that would reveal that functional ingredients can be developed from Nigeria local rice cultivars and that these ingredients would stand the world market competitiveness. One of such functional ingredient from rice is starch. Starch is the major component of rice constituting about 90% of its dry matter (Patindol *et al*., 2009). Literature review reveals scanty or virtually no information on the composition and the properties of starch derived from local rice cultivars in Nigeria. The existing researches in the literature have been on rice gain itself, e.g. effect of parboiling treatments on physicochemical qualities of local rice cultivars in Nigeria (Otegbayo *et al*., 2001) and chemical composition of the rice grain (Oko and Ugwu, 2011). In view of this, there is the need to isolate starch from these cultivars of rice grown in Nigeria and study their properties. The purpose of this investigation was to isolate starch from local cultivars of Nigerian rice and study their composition, morphology, some functional and pasting properties. Apart from engaging the interest of large scale industrialist, the result of this study may also motivate government to support farmers to increase their output, thereby creating wealth and employment in the country.

Materials and Methods

Materials

Dried rough rice samples given local names of Igbemo rice (IGR), Efon Alaye rice (EAR) and Ilaje rice (ILR) were purchased from farmers. Igbemo and Efon Alaye are located in Ekiti state and Ilaje is in the riverine area of Ondo state, all in Nigeria. Lastly, Nerica II rice (N2R) was generously donated by I.I.T.A.(Internatonal Institute of Tropical Agricultural) Ibadan, Oyo state in Nigeria. All the rice are upland rices, with the exception of Ilaje rice, which is lowland rice. All chemicals used in experiments were of analytical grade as results expressed as mean ± standard deviation (SD) of n = 3.

Isolation of rice starch

Rice starch was isolated from rice flour by using the alkaline deproteination method of Lim *et al*. (1999) with some modifications. Rice grain was first dehulled and ground to powder using a laboratory grinder. Rice flour (200 g pass through 1 mm sieve screen) was mixed with 500 ml of 0.1% NaOH. The mixture was stirred on a magnetic stirrer for 3h, and stored at 4°C overnight. The supernatant was decanted, and fresh volume of sodium hydroxide was added to the solid phase and stirred for another 3h at ambient temperature. The procedure was repeated twice after which the solid phase was washed with 0.1% NaOH, blended and filtered. Distilled water was added to the filtrate and allowed to stand for 3 h.The supernatant was decanted and distilled water was added again. The procedure was repeated several times until the pH of the filtrate was between 6.0 and 6.5. The starch residue was collected and dried in a vacuum oven (CN505F, YOGOII, Genlab Widnes, England) at 40°C for 48 h.

Proximate compositions of isolated rice starches

Apparent amylose content (%) was determined by colorimetric iodine assay index method, according to Juliano (1985). The moisture, protein, lipid, and ash content in rice starch were determined using procedure of AACC method (2000).

Morphology of rice starch granules

The morphology of rice starch granules was evaluated by scanning electron microscope (SEM) (QUANTA FEG 250 ESEM).Starch samples were suspended in 95% ethanol and mounted on circular aluminium stubs with double-sided sticky tape. The starch granules were evenly distributed on the surface of the tape, and the ethanol was allowed to evaporate. The samples were then coated with 12 nm gold, examined and photographed at an accelerating voltage of 5 kv with a magnification of x5000 and x10000.

Functional properties

Swelling power and solubility

Swelling power and water solubility index (WSI) determinations were carried out in the temperature range 55-95°C at 10°C intervals using the method of Leach *et al*. (1959) and Holm *et al*. (1985), respectively.

Bulk density

This was determined by the method of Wang and Kinsella (1976) with slight modification. 10 ml capacity graduated cylinder was filled with the starch powdery sample. This was done by gently tapping the bottom of the cylinder on the laboratory bench several times until there is no further dimunition of the sample level after filling to the 10 ml mark.

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of samples (g)}}{\text{Volume of sample (ml)}}$$

Dispersibility

This was determined by the method described by Kulkarni *et al*. (1991) as recently modified by Akanbi *et al*. (2009).

pH

Rice starch samples (5 g) were weighed in triplicate into a beaker, mixed with 20 ml of distilled water. The resulting suspension stirred for 5min and left to settle for 10 min. The pH of the water phase was measured using a calibrated pH meter (Benesi, 2005).

Pasting properties of starches

The pasting properties of the rice starches were evaluated by using a Rapid Visco Analyzer (Newport Scientific, RVA Super 3, Switzerland). Starch suspensions (9%, w/w; dry starch basis, 28 g total weight) were equilibrated at 30°C for 1 min, heated at 95°C for 5.5min,at a rate of 6°C /min, held at 95°C for 5.5 min, cooled down to 50°C at a rate of 6°C/min and finally held at 50°C for 2mins. It was a programmed heating and cooling cycle. Parameters recorded were pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), or trough viscosity (TV), final viscosity (FV), and peak time (PTime). Breakdown viscosity (BV) was calculated as the difference between PV minus MV, while total setback viscosity (TSV) was determined as the FV minus MV. All determinations were performed in triplicate.

Statistical analysis

Experimental data were analysed using Microsoft Excel and SPSS V. 12 .0.

Results and Discussion

Isolation and composition of starches

Table 1 presents the data on yield, chemical composition and apparent amylose (AAM) content of rice starches obtained from the four cultivars of rice flour. The starch yield range from 45.70%-65.00% and this is in accordance with works previously carried out on long-grain rice starch from Houston, Texas, by other researchers (Wang and Wang, 2004). IGR has the lowest starch yield while EAR has the highest. Moisture content (10.40-12.77%), lipid content (0.10-0.70%) and protein content (0.40-0.43%) of isolated rice starches are similar to that previously obtained by Li *et al*. (2008). The slight variation in moisture content (10.40-12.77%) of the rice starches might be attributed to differences in cultivar (Chen *et al*., 2003). These values also agree with those reported in literature by Huaisan *et al*. (2009) and Li *et al*. (2008). The residual protein content of the four starches was slightly different averaging 0.41% (Table 1). These results are in accordance with previous literature reports of rice starch isolated using the traditional alkaline steeping method with residual protein in the range of 0.07-0.42% (Lumdubwong and Seib, 2000 ; Hagenimana *et al*., 2005). The residual protein of N2R starch was the lowest and that in ILR starch was the highest. Poor solubility of prolamin in NaOH and the high solubility of glutelin, globulin and albumin in NaOH had been previously reported in the literature (Cardoso *et al*., 2007a; Cardoso *et al*., 2007b). It is possible that ILR flour contain more prolamin when compared to the other rice flours. The rice starches were also similar in fat content and N2R cultivar had the highest (AAM) content and ash content (Table 1). The AAM content of rice starches ranged between 21.88% and 26.04%. The results for AAM content are consistent with those reported by Jane *et al*. (1999) and Lii *et al*. (1996). The difference in AAM content among starches from different rice cultivars may be due to different factors such as genotype, environmental conditions, and cultural practice (Kim and Wiesenborn, 1995) and is affected by the climatic conditions and soil type during growth (Asaoka *et al*., 1985; Morrison and Azudin, 1987). Based on the classification of rice starch by amylose content (Juliano, 1992); IGR, EAR and ILR starches are intermediate (20-25% AM) and N2R starch is high (>25% AM).

Morphological properties of rice starch granules

The starch granules of IGR, N2R, ILR and EAR are shown in Figure 1. They are all small in size and fall within the range (3-10 µm) reported in the literature (Lindeboom *et al*., 2004). The SEM micrographs of the four alkali-isolated starches show individual granules and cluster of granules (Figure 1). The differences in the pictures are the variations in the numbers of individual granules and compound granules. These compound granules were attributed to the presence of residual protein as indicated by Cardoso *et al*. (2006) or due to the drying conditions that produce slight gelatinization on the surface of granules and cause the granules to adhere together to form aggregates as proposed by Newman *et al*. (2007). It seems the contribution of residual protein is more than that of gelatinization in the formation

Table. 1. Yield, chemical composition and amylose content of starches from four rice cultivars grown in Nigeria

Rice Cultivars	Yield (%)	Moisture (%)	Protein (%)	Fats (%)	Ash (%)	Amylose (%)
IGR	45.70±0.1	10.90±0.01	0.42±0.01	0.10±0.01	0.20±0.01	21.88±0.01
EAR	65.00±0.26	10.40±0.02	0.41±0.01	0.70±0.1	0.22±0.01	22.90±0.01
ILR	48.40±0.12	12.77±0.03	0.43±0.01	0.50±0.1	0.23±0.01	22.64±0.01
N2R	60.80±0.1	11.74±0.01	0.40±0.01	0.40±0.1	0.24±0.01	26.04±0.01

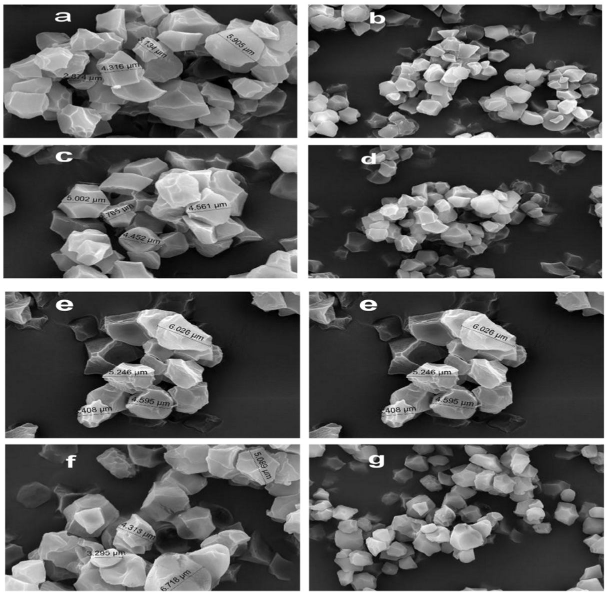

Figure 1. SEM micrographs of four alkali-isolated rice starches of magnification (Mag) x 10,000 and 5,000. a, b; c, d; f, g are SEM images of IGR, N2R and EAR starches of Mag. X 10,000 and x 5,000 respectively with the exception of e. Both are ILR starches of Mag. X 10,000

of compound granules. The alkali gelatinization of starches obviously depends on the concentration of the alkali (NaOH). Compared with thermal starch gelatinization, less is known about alkali gelatinization (Yamamoto *et al*., 2006). Since these starches were extracted at low alkali concentration (0.1% NaOH), the occurrence of the phenomenon of alkali gelatinization was completely rule out. This position is clearly in consonance with literature view of Cardoso *et al*. (2007a) which state that progressive loss of granular morphology due to alkaline gelatinization is likely to occur when treatment of the rice flour is done with NaOH concentration higher than 0.24% (W/V).

Functional properties of rice starches

The values for bulk density, dispersibility and pH are summarized in Table 2.The bulk densities of the rice starches ranged from 0.41 to 0.56 g/mls, the highest bulk density of 0.56 g/mls for ILR starch and the lowest value of 0.41 g/mls for N2R starch. Dispersibility is a measure of reconstitution of starch flour in water, the higher the dispersibility the better the flour reconstitutes in water (Kulkarni *et al*., 1991). The dispersibility of the isolated rice starches ranged from 75.10 to 82.125%, the highest value was obtained for EAR starch and the lowest value for ILR starch. Since the higher the dispersibility the better the starch flour reconstitutes, the values obtained for these rice starches (75.10 - 82.12%) are better than the 40.667% obtained by Akanbi *et al*. (2009) for breadfruit starch. pH is an important property in starch industrial applications, being used generally to indicate the acidic or alkaline properties of liquid media. pH values for the rice starches ranged from 5.3 to 6.9, this shows that the rice starches have low acid content.

Table 2. Bulk density, dispersibility and pH of rice starches

Rice cultivar	Bulk density g/ml)	Dispersibility (%)	pH
IGR	0.48±0.0220	80.02±0.2664	6.9±0.0046
EAR	0.50±0.0210	82.12±0.2654	6.0±0.0045
ILR	0.56±0.0220	75.10±0.2655	6.5±0.0044
N2R	0.41±0.022	79.03±0.2655	5.3±0.0044

The values for swelling power (SP) (g/g) and water solubility index (WSI) (%) for the rice starch samples heated from 55 to 95°C are listed in Table 3. The SP started to rise drastically at 75°C for N2R and EAR starches ; at 65°C for ILR and IGR starches. Similar results were obtained by Lii *et al*. (1995) working on starches derived from indica and japonica rice. IGR starch, with the smallest apparent amylose (AAM) content, had the highest SP during heating. The data were identical to those reported by Yang *et al*. (1988), Lii *et al*. (1995) and Yeh and Li (1996). Both the SP and the WSI of starch increased as the temperature increased. The difference in SP among starches from different rice cultivars indicate variation in the strength of associative bonding forces within the granules (Leach *et al*., 1959). The highest SP shown by IGR starch might be indicative of weak bonding forces within its granules and the fact that it is less compact when compared to the other starch granules. The difference in AAM content and starch granular properties may also have affected the SP and solubility of starches (Singh and Singh, 2001). Starch granules become increasingly susceptible to shear disintegration as they swell and starches with lower AAM content (higher AP content) swell more than those with higher AAM content. This is corroborated by the work of Tester and Morrison (1990) which reported that AP contributes to swelling of starch granules and pasting, whereas AM and lipids inhibit swelling. It is not a coincidence that N2R starch with the highest AAM content has the lowest SP and IGR starch with the lowest AAM has the highest SP.

Table 3. Some properties of IGR, EAR, ILR, and N2R starches at different temperatures

Starch	Temp (°C)	Swelling power (g/g)	WSI (%)
IGR	55	3.68±0.11	0.91±0.12
	65	17.32±0.25	3.95±0.17
	75	19.36±0.13	8.53±0.24
	85	22.31±1.16	11.05±0.25
	95	32.45±1.181	11.59±1.12
EAR	55	2.41±0.11	0.80±0.17
	65	2.60±0.35	0.91±0.26
	75	7.36±0.12	3.37±0.24
	85	16.25±1.22	13.19±1.12
	95	27.54±1.32	16.97±1.14
ILR	55	2.93±0.12	0.42±0.13
	65	9.16±0.13	2.12±0.16
	75	10.18±0.25	2.78±0.23
	85	11.92±1.17	4.10±1.12
	95	29.24±1.26	18.11±1.22
N2R	55	2.11±0.11	0.70±0.12
	65	2.58±0.25	0.81±0.17
	75	7.32±0.13	3.27±0.24
	85	16.21±1.16	13.16±0.25
	95	24.53±1.181	16.76±1.12

Pasting properties of rice starches

Pasting properties of different rice starches are summarized in Table 4. Pasting temperature (PT) of different rice starches ranged from 60.70 to 62.10°C, the highest for IGR starch and the lowest for N2R starch. But PT range from 79.1°C to 79.5°C has been previously reported (Huaisan *et al*., 2009). Peak viscosity (PV) is the maximum viscosity attained by gelatinized starch during heating in water. It indicates the water binding capacity of the starch granule (Shimelis *et al*., 2006). PV was found to be lowest for EAR starch and highest for ILR starch. Trough viscosity (TV) was found to be the lowest for EAR starch (133.60RVU) and the highest for N2R starch (277.33RVU).Breakdown viscosity (BV) (measure of the vulnerability or susceptibility of the cooked starch to disintegration). BV ranged between 40.92 RVU to 52.50 RVU, the lowest for N2R starch and the highest for ILR starch. The values of BV of the starch samples vary significantly. The higher the breakdown in viscosity, the lower the ability of the starch sample to withstand heating and shear stress during cooking (Adebowale *et al*., 2005). Therefore, N2R (40.92 RVU) and EAR (42.67 RVU) starches might be able to withstand more heating and shear stress compared to starches from IGR (46.92 RVU) and ILR (52.50 RVU) because of their lower breakdown value.

Setback is a measure of recrystallization of gelatinized starch during cooling. There are significant differences in the values of setback viscosity (SV) for the isolated starches. N2R starch with the highest AAM content showed the highest SV; in contrast IGR starch with the lowest AAM content (Table 1) has the lowest SV (Table 4). This is in absolute agreement with works in the literature (Chang and Liu, 1991; Gudmundsson, 1994) that constantly link high amylose content with the tendencies of syneresis and retrogradation, especially in legume starches (Adebowale and Lawal, 2003). The difference in setback among different starches may be due to the amount and the molecular weight of AM leached from the granules and the ghost of the gelatinized starch (Loh, 1992). Final viscosity (FV) (indicates the ability of the starch to form a viscous paste) for different rice starches ranged from 189.67 RVU to 329.92 RVU, the lowest shown by EAR starch and the highest by N2R starch. It has been reported in previous work by Miles *et al*. (1985) that an increase in final viscosity might probably be due to the reassociation of amylose molecules. The lowest values of PV, TV and FV, for EAR starch compared to the other starches are to be noted; despite the slight differences in amylose content. According to Juliano *et al*. (1987), varietal differences in pasting characteristics of starch can be attributed to the differences in amylopectin molecular structure rather than amylose. It is also possible that the differences in the degree of randomly limited branching in AM content might have also contributed to varietal differences. Other reasons for differences may be inherent differences in the structure of starch or maybe due to different degree of interactions between starch and its associated compounds during pasting (Zhang and Hamaker, 2008).

Table. 4. Pasting properties of starches from different cultivars

Rice Cultivars	PV (RVU)	TV (RVU)	BV (RVU)	FV (RVU)	SV (RVU)	Peak Time (Min)	PT (°C)
IGR	220.50±0.1	173.15±0.1	46.92±0.1	223.67±0.1	50.08±0.1	6.68±0.1	62.10±0.1
EAR	176.33±0.2	133.6±0.2	42.67±0.2	189.67±0.2	56.00±0.2	6.36±0.2	61.85±0.2
ILR	279.69±0.2	227.1±0.2	52.50±0.2	301.17±0.2	74.00±0.2	6.78±0.2	61.20±0.2
N2R	268.25±0.1	227.33±0.1	40.92±0.1	329.92±0.1	102.5±0.1	6.42±0.1	60.70±0.1

PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SV, setback viscosity; PT, pasting temperature.

Conclusions

Variation in the yield, composition, functional and pasting properties of starches from different rice cultivars were observed. The highest starch yield was from EAR and the lowest from IGR. The composition of the starches varies significantly. N2R starch showed the highest apparent amylose (AAM) content and lowest residual protein content, in contrast the lowest AAM was associated with IGR starch. The highest bulk density and lowest dispersibility for ILR starch might probably be due to its possession of the highest residual protein content.

Both the swelling power and water solubility index of the rice starch increased with increased temperature. Differences in composition resulting from varietal differences resulted in variations in the properties of these starches, especially the pasting parameters. Cooked ILR starch had the highest peak viscosity and breakdown viscosity when compared to the other starches. In contrast, N2R showed the highest final viscosity and setback viscosity. Varietal differences in pasting properties were attributed to the differences in amylopectin molecular structure rather than amylose. The composition and pasting properties of these rice starches indicate that they can be used in the food industry and non-food applications such as in paper and textile industries.

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