

Study on the gelatinization properties and amylose content of rice varieties from Nigeria and Cameroun

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Abstract: Thirteen varieties (improved and local varieties) of non-parboiled milled rice (*Oryza sativa Linn*) grown in Nigeria and Cameroun were screened for gelatinization and amylose profile. Differential Scanning Calorimeter (DSC) was used in determining the gelatinization enthalpy (ΔH), onset (T_o), peak (T_p) and conclusion (T_c) temperatures. Results from DSC curves presented a single endothermic transition and a flow of maximum heating at peak temperatures from 67.66 and 81.27 °C. The enthalpy levels varied from 0.33 J/g for Panter, to 2.90 J/g for Jamila. Amylose content varied from 8.59 % for FARO 57, to 23.61 % for TOX 3145. Comparing samples of local varieties with those of improved varieties showed higher values for onset and peak gelatinization temperatures among local varieties. A significant and positive correlation was observed in onset temperature, peak and conclusion temperatures while amylose was negatively and weakly related to all gelatinization parameters.

Keywords: Non-Parboiled Rice, Improved Rice, Local Rice, Gelatinization, Amylose, DSC

1. Introduction

Hybridization between the two domesticated/cultivated rice species; *Oryza sativa Linn* and *Oryza glaberrima Steud* for desirable agronomic characteristics led to the production of New Rice for Africa known as NERICA [1,2]. Presently in Africa, there are several newly improved breeding lines, which are grown along with the previously cultivated varieties [3].

These numerous rice varieties in Africa have wide genetic variation [4].

The gene trait inherited from parental specie has been associated to amylose content in rice varieties. Kishine *et al.* [5] reported that NERICA varieties with gene inheritance from *O. glaberrima* had high amylose content while the varieties with gene from inheritance from the *O. sativa lines* had lower amylose content. Rice classification based on amylose content was described by Lawal *et al.* [6] as follows; waxy (0-2 % amylose),

very low amylose (2 – 12 %), low amylose (12 – 20 %), intermediate amylose (20-25 %) and high amylose (25-33 %).

Amylose and amylopectin are two glucose polymers in starch granules [6]. Amylose is essentially linear, consist of α -(1,4)-linked D-glucopyranosyl units while amylopectin is highly branched and made up of α -(1,4)-linked D-glucopyranosyl units joined through α -(1,6) linkages [7].

Approximately, 90% of milled rice (dry matter) comprised of starch and the eating as well as cooking quality of rice is influenced by the starch characteristics, which include amylose content, gelatinization temperatures [8].

Quantifying gelatinization characteristics of food is very relevant in food processing because it allows simulation of the cooking process for improved functional properties [8,9].

Amylose tends to act as a restraint to gelatinization because it diffuses out of the granules during swelling, making up the continuous phase (network) outside the

granules [10]. It has been reported that waxy starches usually swell to a greater extent than non-waxy starches [11, 12].

Varavinit *et al.* [11] reported a positive correlation of amylose to onset, peak and conclusion temperatures (0.84, 0.88, and 0.85, respectively). Their study suggested the prediction of amylose content of rice with gelatinization properties. A similar result by Park *et al.* [13] stated that gelatinization temperatures increase with higher amylose content in rice starches. Park *et al.* [13] postulated that the crystalline regions of non-waxy (high amylose) rice starch restricted the hydration of amorphous regions whereas waxy (high amylopectin) rice starch consisted mostly of crystalline regions and thus could begin gelatinization at a lower temperature.

Differential Scanning Calorimeter (DSC) has been used extensively to describe the gelatinization properties of rice flour. Normand and Marshall [14] demonstrated only one endothermic transition during gelatinization milled rice flour whereas milled whole grain rice exhibited two endothermic transitions.

This study therefore, aimed at using DSC in screening the gelatinization properties of selected improved and local rice varieties among the popular non-parboiled, milled rice in Nigeria and Cameroun.

2. Materials and Methods

2.1. Samples

Rice varieties are *Japonica* subspecies, crossed between two *Oryza sativa* parents. The improved varieties are improved lines of *Oryza sativa L* originated from AfricaRice Center, Benin. Samples from Nigeria were five improved varieties (*FARO 44*, *FARO 57*, *FARO 60*, *FARO 61*) obtained from the Breeding Unit of Rice Research Program, National Cereal Research Institute (NCRI) Badeggi and four local varieties (*Kwandala*, *Yardass*, *Jeep*, *Jamila*) obtained from crop improvement unit of Kano State Agriculture and Rural Development Agency (KNARDA). From Cameroun, two improved varieties (TOX 3145, NERICA-3) and two local varieties (*Panter*, *Tianan*) were collected from a rice farmer in Ndop, Northwest Region of Cameroun.

Panicle picking was used in harvesting the samples and afterward samples were dried to 12 % moisture content before manually threshed and cleaned. The samples were dehulled using a rice dehuller (THU 35A, Satake, Engineering Co. Ltd., Tokyo) before milling (McGill mill, Model No. 2, Brookshire, Texas) for 30 seconds.

2.2. Sample Preparation for Analysis

Grains of the rice samples were individually ground using a coffee grinder (SUMEET Multi Grind, India) and passed through a 0.5 mm sieve (CETyler, Ontario, Canada).

2.2.1. Isolation of Starch

Starch was extracted from rice flour samples by alkaline deproteination method according to Lim *et al.* [15] with slight modification. About 100g of flour was mixed with 300 ml of 0.5 % NaOH. The mixture was constantly stirred for 4 h and left to stand for 24 h at 10 °C. The supernatant was decanted and the solid phase washed several times with distilled water until the pH of the filtrate was between 6.0 and 6.5. The isolated starch was oven-dried at 40 °C for 48 h and later ground in a mortar to pass through a 0.55 mm mesh sieve, then used for amylose determination.

2.3. Gelatinization

The gelatinization properties were examined by a Differential Scanning Calorimeter (DSC Q100, TA instruments, Wilmington, DE, USA). Heat flow and temperature calibrations of the DSC were performed using pure indium with 28.41 J/g heat of fusion and a melting temperature of 156.66 °C. The experimental values were within ± 3 % of the literature value.

A mass of 3 ± 0.01 mg was carefully placed at the centre of a pre weighed aluminium pan (40 μ l). Considering the moisture content of each sample, appropriate volume of distilled water was added to the pan by a micropipette to achieve a flour/ water ratio of 1: 2. This ratio of 1:2 accounts for more than 60 % moisture, which is the moisture content required for rice gelatinization and to obtain a single endotherm during DSC experiment [16]. The pans were hermetically sealed with TA sample encapsulating press. The sealed pans were stored at room temperature to stabilize for one hour before analysis. The sample pans were placed in the sample cells while an empty pan was placed in the reference cell of the DSC. Samples were heated from 35 to 100 °C at a rate of 10 °C/min. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures of gelatinization were determined. Gelatinization enthalpy (ΔH) was measured in J/g of dried sample weight.

2.4. Amylose Determination

The analytical procedure of Hoover and Ratnayake [17] was adopted in determination of amylose content. Isolated starch (20 mg) derived from procedure in 2.2.1 was vortexed with 8 ml of 90% dimethylsulfoxide (DMSO) then incubated in a shaking water bath at 85 °C for 15 min. Solution was subsequently cooled at room temperature for 45 min then diluted with distilled and deionized water (DDW) to 25 ml. Aliquot of 1ml from the diluted solution was added to 40 ml of DDW then mixed vigorously with 5 ml of iodine solution (0.0025 M I₂/0.0065 M KI mixture). Afterward volume was adjusted to 50 ml with DDW, mixed vigorously and allow to develop colour for 15 min. Absorbance was measured at 600 nm against a reagent blank. The percentage of amylose was calculated from an

equation obtained from the standard curve.

2.5. Statistical Analysis

Data were subjected to one way analysis of variance (ANOVA) followed by Fisher's least significant-difference (LSD) test ($P < 0.05$) to determine the differences in amylose contents and gelatinization parameters among the thirteen samples. Results were expressed as mean \pm standard deviation of three replicates. Independent T-test was performed for differences in amylose contents and gelatinization parameters between local and improved rice varieties. The statistical software used was SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). A dendrogram was drawn using UnscramblerX version 10.3 (CAMO Software AS, Oslo, Norway) for cluster analysis.

3. Results and Discussion

Gelatinization properties of improved and local rice varieties obtained from DSC curves presented single endothermic transition (Fig. 1 and 2, respectively). The displayed endotherm curves confirmed an earlier report that milled rice flour exhibit only one endothermic transition during gelatinization [14].

Gelatinization parameters and amylose content showed significant variation among samples (Table 1). The gelatinization temperatures ranged from 63.42 to 78.34°C for onset temperature; 67.66 to 81.27°C for peak temperature; and from 72.03 to 90.18 °C for conclusion temperature. This result is similar to those reported by previous studies [4, 14, 18]. The study on 39 milled rice samples from West Africa reported ranges for onset temperature from 58 to 72 °C; peak from 66 to 77 °C and conclusion temperature from 70 to 83 °C [4].

Highest peak temperature (81.27 °C) was found in *FARO 57* which also had the lowest amylose (8.59 %). This implies high content of amylopectin in *FARO 57* sample in this study. This result agrees with the finding of highest peak temperature among waxy rice samples in an earlier

study by Zhu *et al.* [19]. Peak temperature represents half the conversion temperature of sample melting.

The lowest onset and peak temperatures with highest amylose content (23.61 %) was found in TOX 3145. This finding is contrary to the positive correlation of amylose to onset, peak and conclusion temperatures reported by Park *et al.* [11, 13]. This finding suggests that other factors such as starch structures, nutritional composition could have influenced the gelatinization of non-parboiled rice

The low enthalpy from 0.33 to 2.90 J/g found among samples in this present study is comparable to the enthalpy data (from 0.58 to 4.21 J/g) reported on rice varieties from West Africa [4]. Fan *et al.* [18] reported that non-starch components in rice flour such as protein, ash, fiber and lipids reduce enthalpy for gelatinization. Saif *et al.* [20] stated that starch concentration and varied level of water / starch ratio have significant influence on gelatinization properties of rice flour.

Low enthalpy values were also attributed to low molecular weight and chain length distribution of amylopectin [21]. This indicates the need for further study on starch structure of these non-parboiled rice samples in our study.

According to food classification based on amylose content [6], only *FARO 57* and TOX 3145 had very low amylose (8.59 %) and intermediate amylose (23.61 %), respectively. The other samples showed low amylose content (12 to <20 %). The observed levels of amylose are consistent with data in previous studies on milled rice varieties [22, 23]. Almost all samples studied by Bocevskaa *et al.* [22] had low amylose content (12–19.4 %) with exception of one variety that had intermediate amylose content (23.3 %). Similarly, amylose values between 2.3 % and 15.4 % were found among milled rice varieties involved in a study by Singh *et al.* [23].

The variation in amylose classification among samples between very low to intermediate agrees with literature that amylose content in rice is influenced by variety [24, 25].

Table 1. Gelatinization properties and amylose content of non-parboiled rice varieties

Rice varieties	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Amylose (%)
Improved					
FARO 44	63.50 \pm 2.06f	69.28 \pm 1.48e	79.66 \pm 0.78f	2.51 \pm 0.32ab	16.54 \pm 1.47bc
FARO 52	63.74 \pm 0.55ef	68.79 \pm 0.28e	77.70 \pm 1.10f	1.54 \pm 0.74cde	15.86 \pm 1.16bc
FARO 57	76.47 \pm 0.51b	81.27 \pm 0.44a	89.55 \pm 0.47ab	2.13 \pm 0.06bc	8.59 \pm 1.24d
FARO 60	76.97 \pm 0.17ab	80.31 \pm 0.12ab	90.18 \pm 2.74a	1.17 \pm 0.01ef	18.49 \pm 2.01ab
FARO 61	78.34 \pm 0.37a	81.22 \pm 0.35a	87.86 \pm 0.91abc	1.28 \pm 0.23def	12.96 \pm 2.32bcd
TOX 3145	63.42 \pm 0.04f	67.66 \pm 0.16f	74.02 \pm 1.14g	0.66 \pm 0.16fg	23.61 \pm 2.32a
NERICA- 3	73.76 \pm 0.36c	76.90 \pm 0.43c	83.56 \pm 0.47e	0.62 \pm 0.12fg	12.46 \pm 0.17cd
Local					
Kwandala	75.88 \pm 0.06b	79.31 \pm 0.02b	87.87 \pm 0.49abc	2.00 \pm 0.26bcd	18.57 \pm 4.34ab
Yardass	76.89 \pm 0.73ab	80.81 \pm 0.30a	87.80 \pm 0.28bc	2.34 \pm 0.43ab	16.26 \pm 3.77bc

Jeep	77.46±0.21ab	80.75±0.16a	86.87±0.51cd	1.38±0.13def	13.39±3.05bcd
Jamila	65.72±0.47d	70.34±0.85d	79.66±0.23f	2.90±0.78a	16.71±3.38bc
Panter	76.84±0.37ab	80.00±0.28ab	85.47±0.64ed	0.33±0.08g	11.74±1.07cd
Tianan	65.21±0.30ed	69.86±0.30ed	72.03±0.39g	1.21±0.09ef	13.68±0.97bcd

Mean± SD values within same column followed by same letters are not significantly different ($P>0.05$)

Table 2. Comparison of gelatinization parameters and amylose content between improved and local varieties

Parameters	Local	Improved	T-value	P-value
To (°C)	73.00±5.60	70.88±6.75	-0.86	0.09
Tp (°C)	76.84±5.02	75.06±6.03	-0.81	0.14
Tc (°C)	83.28±6.02	83.21±6.13	-0.03	0.81
ΔH (J/g)	1.69±0.92	1.42±0.72	-0.86	0.53
Amylose (%)	15.06±3.31	15.50±4.79	0.27	0.30

$P>0.05$ is not significant

The comparison between improved and local varieties in gelatinization properties and amylose content is presented in Table 2. Local varieties showed higher values for onset temperature (73.00 vs. 70.88 °C), peak temperatures (76.48 vs. 74.06 °C), conclusion temperature (83.23 vs. 83.21 °C) and enthalpy (1.69 vs. 1.42 J/g) than the improved varieties. The mean value for amylose content among improved varieties (15.50 %) was higher compared to local varieties (15.06 %). The P-values presented in Table 2 indicated statistical similarity in parameters between the two groups of rice varieties. However, observation of variation in values for thermal properties and amylose content between improved and local varieties was very weak and insignificant ($P>0.05$). This finding indicated similarity among these rice varieties in thermal properties and in amylose content.

Table 3. Correlation Coefficient (r) between gelatinization parameters and amylose content of samples.

Parameter	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Amylose (%)
To (°C)	1				
Tp (°C)	0.994*	1			
Tc (°C)	0.902*	0.923*	1		
ΔH (J/g)	-0.183	-0.114	-0.097	1	
Amylose (%)	-0.215	-0.226	-0.206	-0.080	1

** Correlation is significant at 0.01 level.

The relationship between gelatinization parameters and amylose content is shown in Table 3. The onset, peak and conclusion temperatures were highly and positively correlated ($P<0.01$). The correlation between peak temperature with onset and conclusion temperatures were $r=0.994$ and 0.923 , respectively. The enthalpy and amylose content had inverse and weak correlation with

gelatinization temperatures ($P>0.05$). However, positive relationship of amylose with gelatinization properties had been described previously [10, 11, 13, 26].

Multivariate analysis was applied by average linkage clustering to establish similarities in gelatinization properties among samples. Figure 3 presents the similarities among the 13 samples into four major clusters. The first and third clusters comprised only one variety (TOX 3145 and NERICA-3, respectively). The second group contained four varieties (Tianan, Jamila, FARO 52 and FARO 44) while fourth cluster comprised seven varieties (Yardass, Kwandala, FARO 60, FARO 57, Panter, Jeep, FARO 61). TOX 3145 and NERICAC-3 indicated diverse thermal characteristics from the other rice samples.

The distinct characteristic of TOX 3145 from the other samples could be explained by its low onset, peak, conclusion temperatures, enthalpy and highest amylose content.

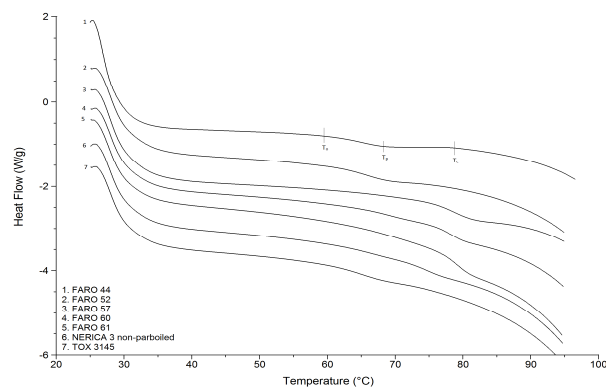


Figure 1. The gelatinization endotherm curves of seven improved varieties of non-parboiled rice as determined by differential scanning calorimetry (DSC).

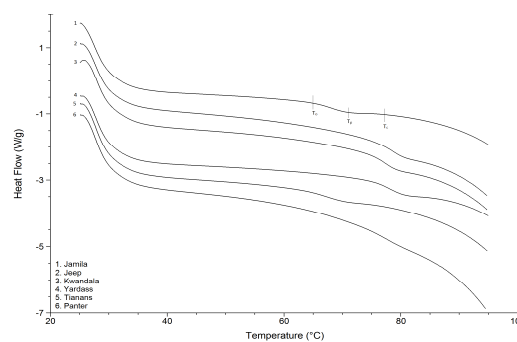


Figure 2. The gelatinization endotherm curves of six local varieties of non-parboiled rice as determined by differential scanning calorimetry (DSC).

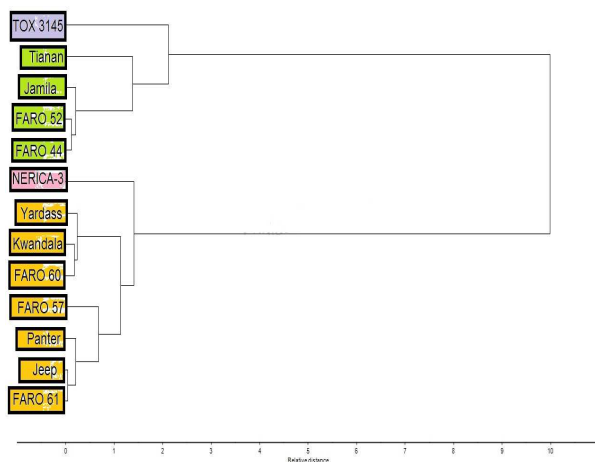


Figure 3. Dendrogram based on gelatinization characteristics and amylose content.

4. Conclusion

This study provides information on gelatinization properties and amylose contents of rice samples from Cameroun and Nigeria. The local varieties had higher onset and peak gelatinization temperatures than the improved varieties. The enthalpy levels were generally low among samples. A significant and positive correlation was observed among onset temperature, peak and conclusion temperatures while amylose was inversely related to all gelatinization parameters.

There is need for further study on influence of factors such as starch structures, nutritional composition on the gelatinization properties of these milled rice.

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