European Journal of Nutrition & Food Safety
3(4): 134-145, 2013

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In vitro Starch Digestibility and Nutritional Composition of Improved Rice Varieties from Cameroun

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Author's contribution

This work was carried out in collaboration between all authors. Authors AMO and MN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first and final draft of the manuscript. Authors CAN, SAN and NW contributed to the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

Received 1st June 2013
Accepted 2nd October 2013
Published 6th November 2013

ABSTRACT

Aims: Resistant starch (RS), kinetics of starch digestion, predicted glycemic index (pGI) and nutritional composition were determined in two improved rice varieties from Cameroun.
Place and Duration of Study: Department of Bioresource Engineering, McGill University, Canada between December 2012 and March 2013.
Methodology: Non-parboiled and parboiled samples of TOX 3145 and NERICA-3 varieties were involved in this study. An *in vitro* enzymatic starch digestion method was applied to measure starch digestibility parameters. Standardized methods were adopted for proximate and mineral contents evaluation.
Results: The parboiled samples had significantly higher ($P<0.05$) resistant starch (8.35 - 11.07%) than the non-parboiled samples (3.81 - 4.84%). The values for pGI among

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samples ranged from 57.57 to 67.78%. Significantly higher values for protein, phosphorus and potassium were found among the parboiled samples ($P<0.05$). Nutritional composition was positively related to RS while pGI had inverse relationship with protein, ash, fat, phosphorus, potassium and RS.
Conclusion: Starch digestibility of these rice varieties was associated to their nutritional composition.

Keywords: Starch digestibility; milled rice; parboiling; resistant starch, improved rice variety; nutritional composition.

1. INTRODUCTION

Rice has become a staple food and a major source of energy in Cameroun [1]. The increased consumption rate of rice has made the screening of its glycemic index a relevant area of research interest. Glycemic index (GI) is a property of starchy food, which describes the rate of blood glucose absorption after consumption [2].

Milled rice is predominantly, starchy endosperm with a higher glycemic index compared to brown rice that contains the outer bran and germ portions [3]. In the process of milling, removal of only husk from paddy results to production of brown rice while removal of bran and most of the germ layer leads to production of milled rice (white rice).

Nutrients are more concentrated in the bran than the endosperm. It is a known fact that milling reduces the nutritional composition of rice grain, but on the other hand, the milling processes remove a large proportion of the anti-nutrient such as phytate, which might adversely affect the utilization of some of the nutrients [4]. The milled rice could be parboiled (parboiled milled grain) or non-parboiled (raw milled grain). Parboiling process involves three operations namely; soaking, steaming and drying of paddy.

The techniques in parboiling vary from traditional method to more sophisticated procedures [5]. These various parboiling operation differ in degree and intensity of temperature as well as soaking and steaming duration. The grain of parboiled rice is modified by the processes of soaking in hot water, steaming and drying it undergoes and this parboiled grain is not classed as cooked rice.

Thus, parboiled milled rice are grains of paddy which are milled after the process of parboiling whereas, non-parboiled milled rice are grains from milled raw paddy not subjected to parboiling process.

Doesthale et al. [6] studied the effect of milling on nutrient loss in parboiled and non-parboiled rice grain. Their study reported significantly lower milling losses for nutrients in parboiled than non-parboiled rice. Higher concentration of nutrient found in parboiled rice was attributed to nutrient solubilization and migration to the centre of the grain during starch gelatinization which occurs during the parboiling process [7,8].

However, Heinemann et al. [7] pointed the lack of uniformity in commercial parboiling processes in different countries as a hindrance to the conclusion of superior nutritional benefits of parboiled rice.

The benefits of parboiled rice grain over non-parboiled apart from firmer, stronger, less sticky grains, increase of milling recovery and decrease of cooking losses include increase in resistance starch fraction and lower glycemic index [9-12].

Resistant starch (RS) escapes enzymatic hydrolysis in the upper gastrointestinal tract which renders this fraction of starch unavailable to digestion with consequent reduction of post-prandial response. The relationship between RS and glycemic index of food had been described [13,12].

Modern research efforts of both national and international organizations have contributed to the improvement and development of newly improved rice varieties. The New Rice for Africa (NERICA) developed by AfricaRice (formerly West Africa Rice Development Association) are products of hybridization between the cultivated rice species of *Oryza glaberrima* and *Oryza sativa* [14]. In addition to the desirable agronomic characteristics (high yield, early maturity and resistant to drought, pests, diseases) of the improved varieties, the protein content is high [15,16].

The influence of protein content [17,18], moisture content [19], phosphorus contents [20,21,22] and resistant starch [23,24] on starch digestibility have been demonstrated. With the progressive release of newly improved rice varieties, it is relevant to evaluate the nutritional potential and glycemic index of each variety in order to provide optimal nutrition benefits to consumers.

There is paucity of literature data on nutritional composition and starch digestibility of newly improved rice varieties in different processed forms. This study aimed to screen the resistant starch fractions, predicted glycemic index, starch hydrolysis kinetics, proximate and mineral composition of two improved rice varieties grown in Cameroun.

2. MATERIALS AND METHODS

2.1 Samples

TOX 3145 and NERICA-3 rice varieties are improved lines of *Oryza sativa L* originated from AfricaRice. Samples of freshly harvested paddy of these two varieties were collected from a rice farmer in Ndop, Northwest Region of Cameroun. The varieties were harvested by panicle picking and separated into three portions. A portion from each rice variety was not parboiled (NP), dried to 12% moisture content before milling (NERICA-NP and TOX-NP samples). The other two portions from each variety were parboiled by two different methods, and then dried to moisture content of 14%.

Parboiled TOX 3145 variety was treated by Traditional (TOX-Trad samples) and IRAD-direct (TOX-IRAD) parboiling techniques.

Traditional parboiling was carried out by a farmer's group well known for parboiling rice at a semi-industrial scale in Cameroun. This traditional procedure involved no pre-cleaning of paddy, longer soaking and steaming time (18-20 hrs), soaking temperature of 80 ºC, non-uniform distribution of steam during steaming with traditional cooking equipment (barrel drums) and a three stone fire place.

The IRAD-direct parboiling was also carried out by the same farmer's group but the procedure involved an improved direct parboiling technology which has been code named a Uniform-Steam Parboiling system in IRAD (Unpublished report). The duration for soaking was 12 hrs at 80 ºC. This technique involved use of an improved steaming equipment (Vessel, stand, steam basket) and improved parboiling stove to ensure uniform distribution of steam.

On the other hand, NERICA-3 variety was parboiled by both subsets of IRAD parboiling process IRAD-direct and IRAD-indirect parboiling techniques.

Direct parboiling technique involves putting the rough rice (paddy) in water, placed on the fire before heated to 80 ºC. At 80 ºC temperature, the paddy was removed from the fire and left to stand in the water for 12 hr.

For indirect parboiling, water was heated to boiling point (98 ºC) and poured on paddy while stirring. The paddy was then left to stand in the water for 12 hr.

Both parboiled and non-parboiled samples were dehusked with Satake rice husker (THU 35A, Satake, Engineering Co. Ltd., Tokyo) and milled by a commercial roll milling facility. The milled rice grains were then brought to McGill University, Canada for analysis.

2.1.1 Sample preparation for analysis

2.1.1.1 Raw samples

Grains of the rice samples were individually ground using a coffee grinder (SUMEET Multi Grind, India) and passed through a 60 mesh (0.25 mm) sieve (CE Tyler, Ontario, Canada).

2.1.1.2 Cooked samples

Weight of 50±0.05 mg sample was weighed into capped tube and boiled in tap water (5 mL) for 30 min. Cooked samples were homogenized in cooking water for 1 min using Tissue-Tearor homogenizer (Biospec Products. Inc.) with controlled speed (level 2). The *in vitro* starch digestibility analysis commenced immediately after homogenization [25].

2.2 Nutritional Composition

Nutrient composition was determined by standard methods of the Association of the Official Analytical Chemists [26]. Moisture was estimated by drying in an oven at 105 °C until constant weight; ash contents by combustion of sample at temperature of 550 °C in muffle furnace; protein by nitrogen determination using Leco Nitrogen Analyzer (N x 6.25) while fat determination was by Soxhlet extraction using petroleum ether as solvent. The total carbohydrate content was determined by difference using the following formula: 100 - (weight in grams [protein + fat + water + ash] in 100 g sample).

Concentrations of phosphorus, potassium, iron and magnesium were determined by digestion of ground sample at 340 ºC using a digestion mixture of sulfuric acid, peroxide (30%), lithium and selenium. After digestion, phosphorus was estimated colorimetrically at the wavelength of 880 nm on a flow injector analyzer instrument (QuickChem series 8000, Lachat Instruments, CO, USA). Potassium, magnesium and iron were measured with a flame atomic absorption spectrophotometer (Perkin-Elmer 2380, Norwalk, Connecticut, USA).

2.3 Starch Fractions and Predicted Glycemic Index (pGI)

An *in vitro* method based on the procedure of Goni *et al.* [25] was adopted for determination of predicted glycemic index (pGI). The resistant starch (RS), digestible starch (DS) and total starch (TS) were measured according to AOAC 2002.02 using the Megazyme RS kit (Megazyme, Bray, Ireland).

2.4 Statistical Analysis

The experimental design consisted of two rice varieties (TOX 3145 and NERICA-3) subjected to three treatments (non-parboiled, two differently parboiled techniques) totalling 6 samples.

Data were expressed as mean±standard deviation of four replicate measurements. Variation in levels of starch fractions among samples were tested by a one way analysis of variance (ANOVA). The difference between means was determined by Scheffe's multiple comparison procedure test (P<0.05). Statistical software used was SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The kinetic parameters were calculated by a nonlinear regression in MATLAB (Version 7.6.0.324 R2008a, The Mathworks, Inc., Natick, MA, USA).

3. RESULTS

Proximate composition showed that protein and ash contents among samples ranged between 8.97 -14.98% and 0.52-1.19% dry matter, respectively (Table 1). The fat content among samples was statistically similar (P>0.05).

TOX-NP sample had the lowest values for fat (0.39%), protein (8.97%) and highest carbohydrate content (90.08%). The lowest ash content was observed in NERICA-NP (0.52%). However, NERICA-Direct had a significant highest protein content (14.98%) and lowest carbohydrate content (83.66%).

Generally, the parboiled samples had higher content of ash, protein and lower carbohydrate compared to their non-parboiled counterparts.

Table 1. Proximate composition of TOX 3145 and NERICA-3 varieties (% dry matter)

Samples	Fat %	Ash %	Protein %	Carbohydrate %
TOX- NP	$0.39{\pm}0.06^{a}$	$0.57{\pm}0.04^{b}$	$8.97{\pm}0.08^{d}$	$90.08{\pm}0.01^{a}$
TOX- IRAD	$0.44{\pm}0.03^{a}$	$0.88{\pm}0.01^{ab}$	$10.83{\pm}0.11^{c}$	$87.85{\pm}0.06^{b}$
TOX-Trad	$0.58{\pm}0.01^{a}$	$1\ .19{\pm}0.26^{a}$	$12.52{\pm}0.14^{b}$	$85.71{\pm}0.11^{c}$
NERICA-NP	$0.51{\pm}0.15^{a}$	$0.52{\pm}0.00^{b}$	$10.35{\pm}0.71^{cd}$	$88.63{\pm}0.86^{ab}$
NERICA-Indirect	$0.51{\pm}0.01^{a}$	$0.83{\pm}0.01^{ab}$	$11.31{\pm}0.18^{bc}$	$87.36{\pm}0.19^{bc}$
NERICA-Direct	$0.60{\pm}0.10^{a}$	$0.76{\pm}0.01^{ab}$	$14.98{\pm}0.12^{a}$	$83.66{\pm}0.00^{d}$

Mean±SD values with the same superscript letters in a column are not significantly different (P>0.05)

Table 2 presented varied concentration of phosphorus, potassium, magnesium and iron contents ranging from 0.176 to 0.289 g/100 g; 0.266 to 0.369 g/100 g; 0.173 to 0.281 g/100 g and 0.0 to 0.017 g/100 g, respectively.

Phosphorus and potassium composition in parboiled samples were significantly higher (P<0.05) compared to their non-parboiled counterparts.

Table 2. Mineral composition of TOX 3145 and NERICA-3 varieties (g/100 g dry matter)

Samples	Phosphorus	Potassium	Magnesium	Iron
TOX- NP	0.186 ± 0.002^{d}	0.273 ± 0.006^{b}	0.185 ± 0.003^{ab}	0.007 ± 0.000^{ab}
TOX- IRAD	$0.237\pm0.0.001^{c}$	0.369 ± 0.002^{a}	0.173 ± 0.005^{b}	0.004 ± 0.600^{b}
TOX-Trad	0.274 ± 0.007^{b}	0.341 ± 0.020^{a}	0.281 ± 0.050^{a}	0.017 ± 0.006^{a}
NERICA-NP	0.246 ± 0.005^{c}	0.266 ± 0.006^{b}	0.246 ± 0.001^{ab}	0.001 ± 0.000^{b}
NERICA-Indirect	0.289 ± 0.001^{a}	0.359 ± 0.020^{a}	0.248 ± 0.001^{ab}	0.000 ± 0.000^{b}
NERICA-Direct	0.236 ± 0.003^{c}	0.280 ± 0.007^{b}	0.214 ± 0.013^{ab}	0.000 ± 0.000^{b}

Mean±SD values with the same superscript in a column are not significantly different (P>0.05)

The starch fractions of TOX 3145 and NERICA-3 rice samples are presented in Table 3. The variation in TS among samples (82.19 - 89.44%) was not statistically different (P>0.05). The most predominant fraction of the TS was the DS fraction which varied between 72.22 to 85.63%.

The fraction of RS ranged from 3.81 to 11.07 %. Highest value for RS was observed in TOX-IRAD (11.07%) while the two non-parboiled samples (TOX-NP and NERICA-NP) showed significantly lowest RS values (P<0.05). TOX-NP had the highest DS (85.63%) while NERICA-Direct had lowest DS (72.22%).

The comparison of samples between different parboiling techniques showed that TOX-IRAD had a higher RS (11.07%) with lower DS (76.08%) than its counterpart, TOX-TP (8.82 and 78.67%, respectively). Similar pattern was observed in samples of NERICA-3 variety. NERICA-Direct had higher RS (10.36%) with lower DS (72.22%) compared to NERICA-Indirect (8.35 and 75.95%, respectively).

Table 3. Starch fractions of TOX 3145 and NERICA-3 varieties (% dry matter)

Samples	RS	DS	TS
TOX- NP	3.81 ± 0.97^{b}	85.63 ± 6.88^{a}	89.44 ± 6.45^{a}
TOX- IRAD	11.07 ± 3.09^{a}	76.08 ± 5.40^{ab}	87.15 ± 2.39^{a}
TOX-Trad	8.82 ± 2.66^{a}	78.67 ± 8.13^{ab}	87.48 ± 5.57^{a}
NERICA-NP	4.84 ± 0.13^{b}	77.35 ± 2.10^{ab}	82.19 ± 2.24^{a}
NERICA-Indirect	8.35 ± 0.86^{a}	75.95 ± 2.83^{ab}	84.30 ± 3.63^{a}
NERICA-Direct	10.36 ± 0.24^{a}	72.22 ± 3.36^{b}	82.57 ± 3.58^{a}

Mean±SD values with the same superscript in a column are not significantly different (P>0.05)
TS= total starch; DS= digestible starch; RS= resistant starch.

The starch hydrolysis curves from 0 to 120 min for samples are presented in Fig. 1. All the samples portrayed rapid starch hydrolysis at the first 30 min of digestion. The curves for parboiled samples were lower than the non-parboiled samples. TOX-IRAD and NERICA-Direct did not achieve plateau before 90 min hydrolysis.

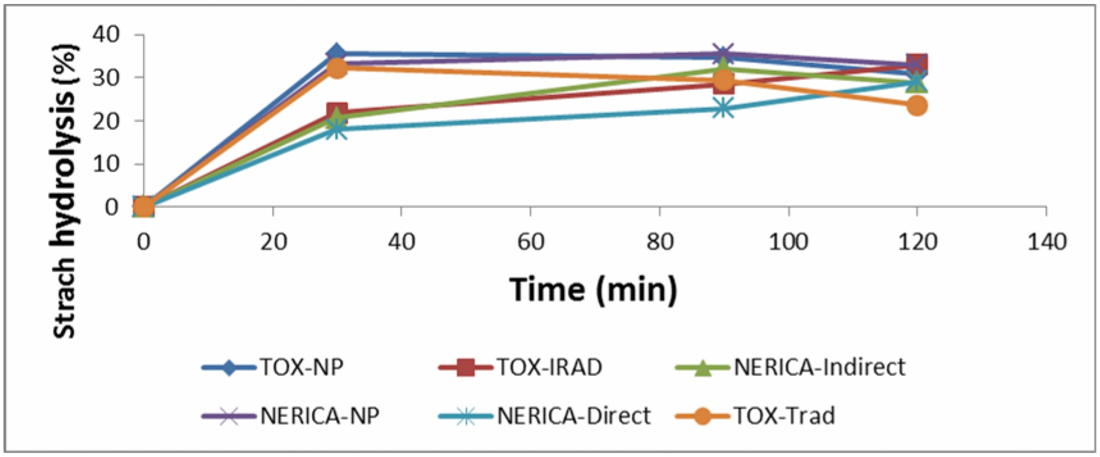

Fig. 1. Starch hydrolysis curves for rice samples (0-120 min).

Table 4 shows varied kinetics of *in vitro* starch digestibility among samples. Extent of starch hydrolysis as indicated by equilibrium concentration (C_∞) was highest in the NERICA-NP (34.26%) followed by TOX-NP (33.68%). NERICA-Direct had the lowest C_∞ (27.26%).

The lowest value for kinetic constant (k) parameter was observed in NERICA-Direct (0.03 m^{-1}) followed by NERICA-Indirect and TOX-IRAD (0.04 m^{-1}). However, NERICA-NP, TOX-NP and TOX-Trad had values of (0.2, 0.11, 0.2 m^{-1}, respectively).

The values for pGI varied from 57.57 to 67.78% among samples. The highest pGI was found in NERICA-NP (67.78%) followed by TOX-NP (66.99%) whereas NERICA-Direct had a significant lowest pGI (57.57%).

Table 4. Kinetics parameters and predicted glycemic index rice samples

Samples	(C_∞)*(%)	(k)* (min^{-1})	H_{90exp} (%)	pGI	R^2
TOX- NP	33.68	0.20	34.60 ± 1.12^{a}	66.99 ± 0.89^{a}	0.98
TOX- IRAD	31.69	0.04	28.59 ± 2.73^{ab}	62.17 ± 2.19^{ab}	0.98
TOX-Trad	28.47	0.20	29.49 ± 2.06^{ab}	62.89 ± 1.65^{ab}	0.93
NERICA-NP	34.26	0.11	35.58 ± 0.13^{a}	67.78 ± 0.10^{a}	0.99
NERICA-Indirect	30.97	0.04	32.06 ± 1.58^{a}	64.96 ± 1.27^{a}	0.98
NERICA-Direct	27.26	0.03	22.86 ± 0.70^{b}	57.57 ± 0.56^{b}	0.96

*Mean±SD values within same column followed by same letters are not significantly different (P>0.05). *Parameters of model equation $C= C_\infty (1-e^{-kt})$; C_∞ = equilibrium concentration; k = kinetic constant; $H_{90exp;}$ experimental values for total starch hydrolysis at 90 min; pGI = predicted glycemic index.*

Table 5 presented the relationship of pGI and starch fractions with nutrient composition. RS had significantly positive relationship with protein (r= 0.630; P<0.05), ash (r=0.788; P<0.01), fat (r=0.634; P<0.05), phosphorus (r= 0.659; P<0.05) and magnesium (r= 0.728; P<0.01). On the other hand, RS was inversely related to carbohydrate (r= -0.57; P>0.05), DS (r= -0.747; P<0.01) and pGI (r= -0.143; P>0.05). An inverse but weak correlation was observed between pGI and RS (-0.143; P>0.05).

The pGI showed negative correlation with protein, ash, fat, phosphorus, potassium and RS, although the correlation was not significant (P>0.05).

Table 5. Pearson correlation coefficient (r) between some parameters of starch digestibility and nutrient composition

Parameters	Ash	CHO	Protein	Fat	P	Mg	K	RS
Ash	1							
CHO	-0.459^{NS}	1						
Protein	0.428^{NS}	-0.951**	1					
Fat	0.383^{NS}	-0.659*	0.769*	1				
P	0.523^{NS}	-0.768**	0.895**	0.777**	1			
Mg	0.264^{NS}	-0.370^{NS}	0.56^{NS}	0.336^{NS}	0.655*	1		
K	0.643*	-0.704*	0.648*	0.351^{NS}	0.605*	0.065^{NS}	1	
RS	0.788**	-0.568^{NS}	0.630*	0.634*	0.659*	0.728**	0.395^{NS}	1
DS	-0.529^{NS}	0.178^{NS}	-0.305^{NS}	-0.320^{NS}	-0.467^{NS}	-0.671*	0.035^{NS}	-0.747**
pGI	-0.293^{NS}	0.400^{NS}	-0.299^{NS}	-0.113^{NS}	-0.212^{NS}	0.110^{NS}	-0.227^{NS}	-0.143^{NS}

*Levels of significance: **P< 0.01, * P< 0.05; NS: Not significant (P>0.05)*
CHO=carbohydrate; P=Phosphorus; Mg=Magnesium; K=Potassium

4. DISCUSSION

The proximate composition of samples in this study is consistent with a previous report on improved rice varieties [16]. Adu-Kwarteng et al. [16] asessed the nutritional composition of 10 newly improved rice varieties in Ghana. Their study reported protein values between 5.95 to 9.16% and ash content of 0.45-0.59%.

In the case of mineral composition, Adu-Kwarteng et al. [16] reported lower values for phosphorus (0.121-0.199 g/100 g) and potassium (0.071-0.102 g/100 g) compared to values in this present study. The variation could be attributed to geographical location and varietal differences [16].

The significantly lower iron content among the NERICA-3 samples in comparison with samples of TOX variety supports varietal differences as a factor influencing nutritional composition.

Observation of generally poor iron content among samples is not surprising since milled rice is characterized by very low iron content [27].

The finding of higher levels of protein and ash among the parboiled samples as compared to their non-parboiled counterparts is in agreement with literature report [28]. Damir [28] attributed the increased ash content in parboiled rice to penetration of water soluble mineral salt during soaking and steaming process.

This study therefore, supports nutrient solubilisation and consequent higher concentration of nutrient in the endosperm during parboiling [7,8]. This explains the higher losses of nutrients during milling in non-parboiled than parboiled rice [6].

Similarly, the higher phosphorus and potassium among parboiled samples can be explained by their significantly higher protein and ash contents.

This finding however, portrays the relevance of parboiling process on enhanced nutritional value of rice grain particularly on starch digestibility.

Various nutrients including protein, moisture, and phosphorus in food have been associated to starch digestibility [17,19-22]. High protein content in food as well as protein-starch complex were related to low glycemic response [17,18]. Previous studies have demonstrated the influence of high phosphorus content in lowering starch digestibility rate [20,21].

The RS content among studied samples are comparable with literature data on milled rice [29, 30, 25]. The finding of higher RS with a lower DS fraction in parboiled rice than non-parboiled rice is consistent with literature documentation [31-34].

Walter et al. [29] reported a lower range (0.6-5.0%) of RS in non-parboiled samples compared to 2.3-5.8% found in parboiled rice samples. The DS fractions in their study had mean values of 82.4 for non-parboiled rice and 73.9% for parboiled samples.

The tendency of gelatinized starch during parboiling to undergo retrogradation upon cooling leads to resistant starch formation in parboiled rice grain [35].

The variation found in RS fraction between the different parboiled samples could be attributed to application of different degree of heat treatment during the different parboiling process. The protocol of traditional parboiling involved non-uniform distribution of steam during parboiling which could have influenced the sampled grain.

RS fraction has demonstrated a significant influence on glycemic index of rice [30,24,36,25].

The pGI values among samples are within the moderate glycemic index of food classification [37]. This result is consistent with previous reports on milled non-parboiled rice. Hu et al. [30] reported 60.1-106.3% glycemic index in milled rice varieties. Miller et al. [38] showed glycemic index values between 64 and 93%.

This finding of higher pGI among the non-parboiled samples (NERICA-NP and TOX-NP) is in agreement with literature reports that parboiling elicits lower glycemic response compared to the non-parboiled rice [9-12].

Moreover, the lower pGI among parboiled samples supports earlier reports that higher protein and phosphorus content in food reduced rate of starch digestibility [17,20- 22]. The highly significant and positive correlation (r=0.895; P<0.01) between phosphorus and protein content in our study confirms the influence of protein and phosphorus on starch digestibility.

Deepa et al. [23] reported similar correlation of DS and RS with proximate nutrients in brown rice varieties.

Our study therefore, supports the literature fact that nutritional composition influences starch digestibility. The formation of complex with starch by protein, fat and phosphorus hinders starch susceptibility to amylolytic enzymes action [17,19-23].

The starch kinetic was described by a non-linear model [25] which indicates higher resistance to enzymatic hydrolysis with a lower rate of starch digestion in NERICA-Direct and TOX-IRAD compared to other samples in this study.

5. CONCLUSION

The nutritional and starch digestibility profiles of parboiled and non-parboiled rice samples were highlighted. The parboiled samples had higher RS, lower pGI, and kinetic constant compared to non-parboiled samples. RS had a significant positive relationship with nutritional composition, while pGI was inversely correlated to protein, ash, fat, phosphorus, potassium and RS.

This study portrayed the relationship of nutritional composition with starch digestibility in rice. There is need to extend this study on more rice varieties that are processed with similar parboiling techniques.

ACKNOWLEDGEMENTS

This study was supported by the Canadian International development Agency (CIDA) project on enhancing food security in Africa through the improvement of rice post-harvest handling, marketing and the development of new rice-based products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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