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New Source of Rice with a Low Amylose Content and Slow In Vitro Digestion for Improved Health Benefits

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Abstract: Rice provides 70% of dietary carbohydrates and other essential nutrients. Breeding for consumer preferences and health benefits are the main considerations. Rice with a low amylose content offers a good cooking quality with its soft and sticky texture but fast starch digestibility with a high sugar release. Therefore, to provide health benefits, it is important to identify rice varieties with slow starch digestibility and a low amylose content. A total of 167 indigenous upland rice germplasms were analysed for amylose content (AC) and in vitro starch digestibility. The results showed that 167 upland rice genotypes were mostly low in AC, which was related to a soft and sticky texture during cooking. Based on the glutinous and non-glutinous types, thirteen and nine indigenous upland rice germplasms were selected with a lower AC than RD6 and KDML105 (check varieties). The in vitro starch digestibility and the hydrolysis were different at each time point and different in each variety. In the glutinous group, ULR155, ULR138, ULR308, and ULR241 released less sugar and had slower starch digestibility than RD6. In the non-glutinous group, ULR219 and ULR264 showed lower AC, slower digestion, and lower sugar release than KDML105. The results suggest that six indigenous upland rice varieties will provide a rice source to accommodate consumer preferences while also offering health benefits.

Keywords: grain quality; indigenous upland rice; quality diversity; physicochemicals; rice starch

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1. Introduction

Rice (*Oryza sativa* L.) is an important crop for Thailand economy. Among the high-quality rice exports, KDML105 and RD6 provide good cooking and eating quality. Cooking and eating quality are important characteristics of rice, contributing to its economic value and its ability to accommodate the preferences of individual consumers [1]. Some consumers prefer their cooked rice to be hard and non-sticky, while others prefer it to be soft and sticky [2]. Rice-eating qualities such as taste, stickiness, and hardness are dependent on the rice grain composition and physicochemical properties. Rice is classified into two types based on starch content: glutinous and non-glutinous rice. Up to 90% of the dry matter in rice seed is starch [3], and the starch type affects the amylose content (AC). The amylose content (AC) is perhaps the most important component in terms of its impact on starch digestibility [4,5]. The low-amylose genotypes tend to offer a more rapid starch hydrolysis than that with a high AC [6–8]. Moreover, rice with a low AC is stickier and softer than the high-amylose genotypes and is classified as having good cooking quality, such as RD6 and KDML105 (Thailand's commercial varieties). However, most genotypes with good cooking quality have fewer health benefits.

Noncommunicable diseases (NCDs) are a critical concern for people around the world since they tend to be of a long duration and are caused by a combination of genetic, physiological, environmental, and behavioural factors. Healthy eating is the one factor with the ability to reduce the risk of diabetes and obesity. The glycemic index (GI) is used to indicate the levels of blood glucose in food. Carbohydrate digestion is the main factor affecting the absorption rates. The GI has a ranking (0–100) to show how quickly each carbohydrate-based food and drink raises blood glucose levels after eating. Diets involving a low GI index, means slows released of sugar after meal, which associated with reduced glycemic impact and more consistent postprandial insulin release, thereby avoiding the occurrence of hypoglycemia [9]. The glycemic index (GI) in rice grain is dependent on multiple factors, such as AC, dietary fibre content, physical size and form, post-harvest treatment, and cooking. Rice with a lower AC (high GI) is more likely to raise blood glucose than rice with a higher AC [10].

Rice variety with a low AC, a low GI, and good cooking quality while providing health benefits presents a challenge for rice breeders. Therefore, the identification of AC, GT, and starch digestion in rice holds significance for assessing the health benefits. A low AC in rice and slow sugar assimilation provide health benefits while offering good eating quality. Thus, the objective of this study is to characterise indigenous upland rice germplasms based on AC and GT as eating quality criteria and starch digestibility as a health benefit. The information in this study can be utilised as a genetic database and for developing new rice varieties to provide consumers with a better choice of product.

2. Materials and Methods

2.1. Genetic Plant Materials

In this study, 167 upland rice varieties were used, selected on the basis of grain yield as proposed by [11], with KDML105 and RD6 used as standard check varieties for non-glutinous and glutinous rice, respectively. The upland rice germplasm was collected from rice-growing areas in Thailand through the Rice Germplasm Collection Project of Khon Kaen University, Thailand.

2.2. Field Planting

The rice was planted under field conditions, using the direct seeding method and sprinkler irrigation until the maturity stage. Planting date was on the 2018 wet season (June–October) at the Agronomy Field Crop Station, Khon Kaen University, Khon Kaen, Thailand. The direct seeding method was applied with rows and plant spacings of 40×25 cm, respectively, and a plot size of 1.2×1.25 m, each plot having three rows. Fourteen days after seeding, the plants were thinned to maintain three plants per hill. Fertilizer was split-applied at a rate of 156.25 kg/ha N-P_2O_5-K_2O at 30 and 60 days after planting (DAP). Mature seeds at the complete panicle maturity stage were collected from the middle row of each plot and air-dried; the seeds were kept at a temperature of −4 °C in sealed polyethylene bags until analysis.

2.3. Starch Type, Amylose Content, Gelatinisation Temperature, and Starch Purification and In Vitro Starch Digestibility Evaluation

The first experiment evaluated the starch type, amylose content, and gelatinisation temperature using the alkali spreading score (ASS) of 167 upland rice varieties. In the second experiment, 13 glutinous and 9 non-glutinous rice varieties were selected based on low amylose content to evaluate the in vitro starch digestibility.

2.3.1. Starch Type

In a preliminary study to establish the appropriate duration for staining flour rice, 0.1 g samples were stained with iodine solution (0.5 g of iodine and 2.5 g of potassium iodide in 1 L of deionised water). This solution was carefully applied to the rice flour samples for colour testing, inducing the formation of a stable blue helical inclusion complex between amylose and iodine. Visual colour differences were observed in the iodine-stained flour rice, with glutinous rice turning yellow after iodine staining and non-glutinous turning blue-black.

2.3.2. Amylose Content (AC)

The 167 varieties were analysed for AC. The milled rice was ground into flour at 0.1 g, weighed, and then dispersed in 1 mL of 95.0% ethanol and 9 mL of 1.0 N NaOH into a 100 mL volumetric flask. The solution was mixed with a magnetic stirrer for 10 min. Next, the samples were diluted to 100 mL with distilled water and 5 mL of gelatinised starch solution and placed in a volumetric flask, followed by the addition of 2 mL of acetic acid (57.75 mL in 1 L of water) and 2 mL of iodine solution (0.2% iodine + 2% potassium iodide). The mixture was adjusted, and the volume was increased to 100 mL with distilled water. All the contents were thoroughly vortex-mixed and allowed to stand for 10 min at room temperature. The absorbance was measured at 620 nm using a spectrophotometer (UV-6300PC spectrophotometer, VWR international, Shanghai, China) following [12]. As a control, NaOH solution was used. The AC of different varieties was calculated for comparison with a standard graph [13]. Amylose content in rice can be classified as 0–2.9% for waxy rice, 3–9.9% for very-low-amylose rice, 10–19.9% for low-amylose rice, 20–24.9% for medium-amylose rice, and more than 25% for high-amylose rice [14].

2.3.3. Gelatinisation Temperature (GT)

The alkali spreading value (ASV) of rice is a widely measured quality parameter and an accepted indicator of the GT class, estimated based on the alkali spreading score (ASS) of rice grain [15]. The standard evaluation system [16] was applied to score the ASS. Briefly, the sets of six whole milled kernels of each entry were placed in Petri dishes containing 10 mL of 1.7% (*w*/*v*) potassium hydroxide (KOH) solution. The kernels were arranged in such a way as to provide space between them for spreading. The plates were covered and incubated at room temperature for 23 h at 30 °C. The appearance and disintegration of kernels were visually rated based on the point numerical spreading scale (Figure 1). According to the scores, the GT of the rice grains was then classified in accordance [15] into four groups: high (1–2), high-intermediate (3), intermediate (4–5), and low (6–7) (Table 1).

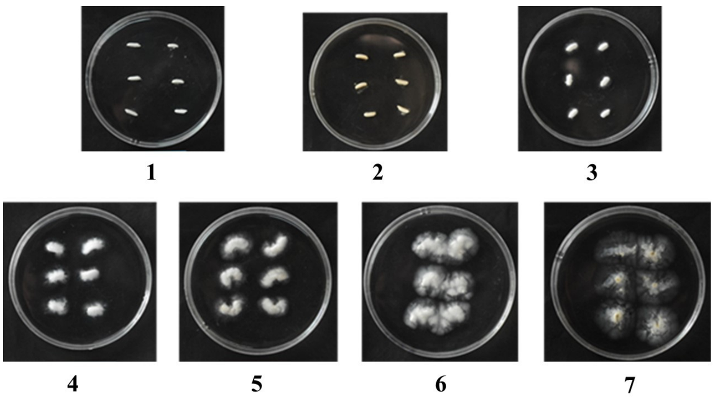

Figure 1. The seed alkali spreading score in 1.7% KOH.

Table 1. Degree of alkali digestion in the rice genotypes under study.

Alkali Spreading Score (ASS)	Alkali Spreading Value (ASV)	Gelatinisation Temperature (GT)	Cooking Time (min)
1–2	Low	High 75–79 °C	>24 min
3	Intermediate	High-intermediate 70–74 °C	20–24 min
4–5	High-intermediate	Intermediate 66–69 °C	17–24 min
6–7	High	Low 55–65 °C	12–17 min

2.3.4. Starch Purification and In Vitro Starch Digestibility

Thirteen glutinous upland rice and nine non-glutinous upland rice varieties were selected based on their low AC for in vitro starch digestibility. The amylose selection was based on RD6 and KDML105 as standard checks for glutinous and non-glutinous rice, respectively. The glutinous rice varieties with an AC of less than 6% were selected, while the selected non-glutinous varieties contained less than 16% amylose.

Starch Purification and Analyses

Rice starch was purified by alkaline extraction [17]. Filtered rice flour of 20 g was added to 50 mL 0.2% (*w*/*v*) NaOH and stirred using an incubator shaker (KS4000i-control-IKA, Life Science lab, Bangkok, Thailand) at 180 rpm and 25 °C for 3 h. The solution was filtered through four layers of straining cloth and centrifuged (BOECO centrifuge HC-240, Hamburg, Germany) at 5000 rpm for 30 min. The starch pellets were then redissolved in distilled water, adjusted to neutral pH, and washed with distilled water. Then, 3 mL of toluene was added to the starch pellets, which were washed five times with distilled water. The starch pellets were then dried at 40 °C for 48 h.

In Vitro Starch Digestibility

The 20 mg of starch powder was added to 2.5 mL of distilled water, and the slurry was gelatinised by autoclave (HVS-BL, SUS304, Jiangsu, China) at 121 °C for 45 min. The gelatinised starch was dispersed by vortexing, and a 5 mL enzyme cocktail containing 14U porcine pancreatic α-amylase and 0.33U amyloglucosidase was added (Sigma-Aldrich, Inc., St. Louis, MO, USA); it was incubated with a continuous shaking water bath at 37 °C. Digested samples of 300 µL were collected at 0.5, 1, 2, 4, 6, and 24 h, respectively [18,19]. The released glucose was analysed using the DNS method [20].

2.3.5. Gel Consistency (GC)

The polished rice of 13 glutinous and 9 non-glutinous selected varieties and the check varieties were powdered using a pestle and mortar, followed by sieving with a 1 mm sieve. For the estimation of a gel consistency in the test tube, 100 mg of rice powder was taken, followed by the addition of 0.2 mL of ethanol containing 0.25% thymol blue and 2 mL 0.2 N Sodium hydroxide; they were then mixed well. All the samples were kept in a hot water bath for 8 min and then cooled for 5 min. Then, all the samples were vortexed and kept in an ice water box for 20 min. Then, all the tubes were taken out and laid horizontally on laminated graph paper for one hour to take the measurements. The varieties were grouped based on the length of the gel as hard (length of gel: less than 35 mm), medium hard (length of gel: 36–40 mm), and medium (length of gel: 41–60 mm) and soft (length of gel: 61–100 mm) [21].

2.4. Statistical Analysis

The measured data were subjected to analysis of variance using Statistix 10 software (Version 10.0 Copyright © 1985–2013, Analytical Software) following a completely randomized design (CRD), and the means with the least significant difference (LSD) were compared [22]. The correlation coefficient was analysed using Statistix 10 software according to Ward's method [22].

3. Results

3.1. Starch Type

Among the 167 indigenous upland rice varieties considered in this study, 107 were classified as glutinous and as 60 non-glutinous. The rice varieties were classified based on AC, with 57 waxy, 49 very low, 33 low, 16 intermediate, and 12 high. Moreover, the alkali spreading value (ASV) based on the alkali spreading score (ASS) was classified into four groups: 0 low, 4 intermediate, 98 high-intermediate, and 65 high; the same was performed for the gelatinisation temperature (GT) (Table S1). This section is divided into subheadings. It provides a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.2. Amylose Content

In this study, the 107 upland rice varieties in the glutinous group exhibited an AC ranging from 0.64 to 17.05% (Table S1) and can be divided into three groups: waxy, very low, and low amylose types, consisting of 57, 45, and 5 varieties, respectively (Figure 2a). At the same time, 60 upland rice varieties in the non-glutinous group exhibited an AC ranging from 11.16 to 37.56% (Figure 2b, Table S1). This group was divided into four categories: very low, low, intermediate, and high amylose types and consisted of 4, 28, 16, and 12 varieties, respectively (Figure 2). The check varieties, RD6 and KDML105, were classified as having a very low (7.33%) and low (19.94%) AC, respectively. Several of the indigenous upland rice varieties revealed a lower AC than the check varieties RD6 and KDML105.

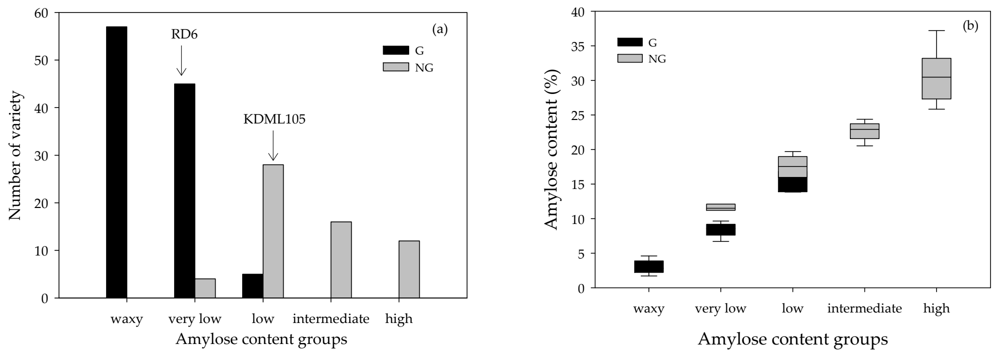

Figure 2. The 167 indigenous upland rice varieties: (**a**) Number of genotypes grouped based on amylose content; (**b**) Mean of amylose content in each group. The amylose content groups modified by Julino [14] as waxy = 0–5%, very low = 6–12%, low = 13–20%, intermediate = 21–25%, and high = 26–33%. G = glutinous rice and NG = non-glutinous rice, classified by the iodine test.

3.3. Gelatinisation Temperature

According to the alkali spreading value (ASV), the rice was grouped by GT classification following [15] as high 75–79 °C, high-intermediate 70–74 °C, intermediate 66–69 °C, and low 55–65 °C. In this study, the 107 glutinous upland rice varieties can be divided into three groups: low, intermediate, and high-intermediate types consisting of 41, 65, and 1 varieties, respectively (Figure 3a). The 60 non-glutinous upland rice varieties were also divided into three groups: low, intermediate, and high-intermediate types consisting of 24, 33, and 3 varieties, respectively (Figure 3a). In addition, the check varieties, RD6 and KDML105, were classified as having intermediate GT. The alkali spreading score was negatively related to GT; for example, the high alkali spreading value (ASV) was low GT. The ASVs were grouped into three groups as intermediate, high-intermediate, and high on the average as 2.97, 4.88, and 6.27, respectively (Figure 3b). Several of the indigenous upland rice varieties revealed lower GTs than the check varieties (KDML105 and RD6). The AC was negatively correlated with the alkali spreading score (ASS) for both glutinous ($r = -0.1954$) and non-glutinous rice ($r = -0.338$) (Figure 4), indicating that a low ASS and high gelatinisation temperature (GT) are related to a high amylose content. Based on the AC and ASS, for the 13 glutinous and 9 non-glutinous upland rice varieties, the values of AC and GT were lower than for RD6 and KDML105 varieties and, therefore, selected for the in vitro starch digestibility test.

3.4. In Vitro Starch Digestion, Gelatinisation Temperature, and Gel Consistency of Selected Varieties

The in vitro enzymatic digestibility of 13 glutinous genotypes (Gs) and 9 non-glutinous genotypes (NGs) were selected based on their low AC and high grain yield (Table S1). The results revealed that a variety did not have a low GT even though it was selected based on a low AC, especially in non-glutinous rice. Moreover, gel consistency (GC) revealed that all varieties of glutinous rice were soft (more than 60 mm length) (Table 2). On the other hand, the non-glutinous rice of seven varieties was classified as hard (less than 35 mm length), ULR126 as medium hard, and ULR019 as medium (Table 3). The results of the enzyme hydrolysis percentage of glutinous and non-glutinous starch and the hydrolysis rate were different at each time point and variety (Tables 2 and 3). In addition, the enzyme hydrolysis percentage of glutinous and non-glutinous starch increased over time in all varieties (Tables 2 and 3). In this study, four of the 13 selected glutinous upland rice varieties, namely ULR241, ULR308, ULR138, and ULR155, released less sugar and slowly digestible starch than RD6 until six hours (Table 2). In addition, two of the nine varieties in the non-glutinous group, namely ULR219 and ULR264, contained more slowly digestible starch than KDML105 until four and two hours, respectively (Table 3). The results indicate that these varieties are sources of a low AC and slowly digestible starch than RD6 and KDML105.

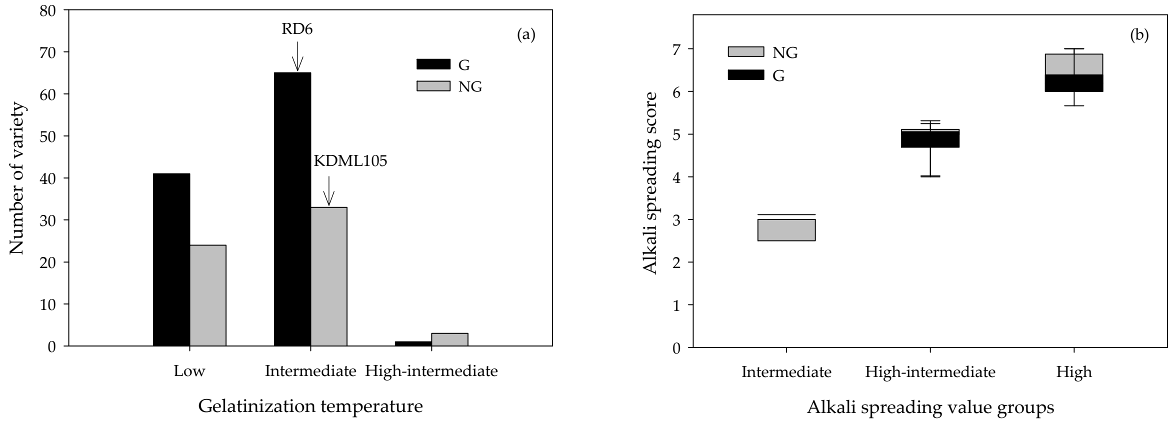

Figure 3. The 167 indigenous upland rice genotypes: (**a**) Number of genotypes grouped by gelatinisation temperature; (**b**) Mean of alkali spreading score in each group based on alkali spreading value. G = glutinous rice and NG = non-glutinous rice, classified by the iodine test.

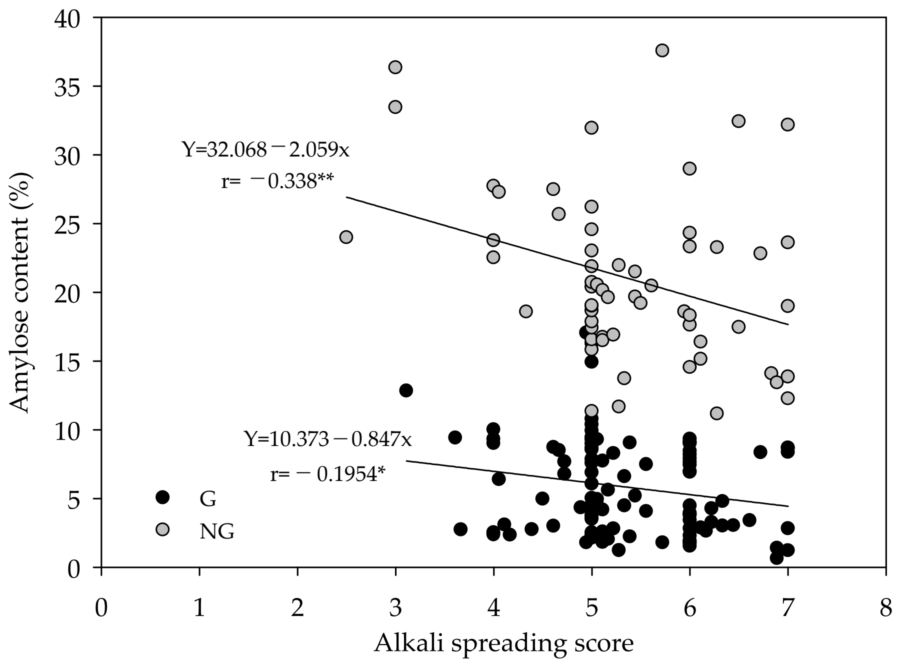

Figure 4. The relationship between amylose content (AC) and alkali spreading score (ASS) of 167 upland rice varieties. * and ** significant at $p < 0.05$ and $p < 0.01$, respectively.

Table 2. Gelatinisation temperature (GT), gel consistency (GC), and starch digestibility of 13 glutinous upland rice varieties and the RD6 standard checks.

Var	GT	GC (mm)	Percentage of Enzyme Hydrolysis of Gelatinised Starch (Time: Hours)					
			0.5 h	1 h	2 h	4 h	6 h	24 h
ULR003	L	67	50.0 ^{ab} ± 0.07	55.9 ^{ab} ± 0.83	59.1 ^{ab} ± 0.32	62.2 ^{a} ± 0.46	61.4 ^{a} ± 0.67	61.2 ^{ab} ± 0.49
ULR008	I	69	43.4 ^{bc} ± 2.18	47.4 ^{c} ± 1.47	56.1 ^{bc} ± 3.12	61.0 ^{a} ± 3.55	59.2 ^{a} ± 3.51	61.2 ^{ab} ± 0.63
ULR017	I	61	50.2 ^{ab} ± 1.43	49.5 ^{abc} ± 0.45	59.3 ^{ab} ± 0.99	60.0 ^{a} ± 1.91	59.6 ^{a} ± 0.42	61.5 ^{ab} ± 0.55
ULR020	L	72	30.1 ^{de} ± 0.59	52.5 ^{abc} ± 1.50	59.9 ^{ab} ± 0.71	60.1 ^{a} ± 1.00	61.6 ^{a} ± 1.75	59.8 ^{ab} ± 0.60
ULR056	I	77	50.7 ^{ab} ± 3.70	52.0 ^{abc} ± 3.97	57.6 ^{abc} ± 0.08	61.1 ^{a} ± 0.36	65.1 ^{a} ± 0.34	58.8 ^{ab} ± 0.64
ULR138	L	66	8.6 ^{f} ± 0.46	12.3 ^{f} ± 0.69	15.4 ^{f} ± 1.21	20.9 ^{e} ± 0.03	28.6 ^{d} ± 0.18	47.8 ^{d} ± 0.87
ULR155	I	73	7.9 ^{f} ± 0.12	10.7 ^{f} ± 0.32	14.0 ^{f} ± 0.96	27.6 ^{cd} ± 3.10	50.6 ^{b} ± 0.87	56.6 ^{bc} ± 4.03
ULR241	L	83	13.8 ^{f} ± 0.58	21.6 ^{e} ± 2.56	25.7 ^{e} ± 0.59	31.1 ^{c} ± 0.79	39.1 ^{c} ± 0.32	58.8 ^{ab} ± 1.37
ULR243	H-I	96	54.7 ^{a} ± 0.89	55.3 ^{abc} ± 1.33	61.9 ^{a} ± 0.46	62.2 ^{a} ± 0.94	61.2 ^{a} ± 0.90	60.8 ^{ab} ± 0.38
ULR251	I	91	25.5 ^{e} ± 0.97	31.3 ^{d} ± 3.80	36.7 ^{d} ± 1.28	55.8 ^{ab} ± 2.83	59.3 ^{a} ± 0.24	61.5 ^{ab} ± 1.86
ULR308	L	72	13.9 ^{f} ± 3.43	15.9 ^{ef} ± 4.08	17.9 ^{f} ± 3.76	23.0 ^{de} ± 3.13	32.0 ^{cd} ± 4.60	51.9 ^{cd} ± 3.36
ULR364	L	80	55.3 ^{a} ± 0.90	56.5 ^{a} ± 0.69	60.1 ^{ab} ± 0.31	60.2 ^{a} ± 1.66	61.8 ^{a} ± 0.39	62.9 ^{a} ± 0.67
ULR394	L	76	37.6 ^{cd} ± 7.94	47.9 ^{bc} ± 2.92	53.1 ^{c} ± 1.30	58.9 ^{a} ± 1.61	62.2 ^{a} ± 3.00	58.3 ^{ab} ± 0.77
RD6	I	79	24.1 ^{e} ± 0.25	38.0 ^{d} ± 1.20	40.9 ^{d} ± 0.49	51.5 ^{b} ± 0.52	61.0 ^{a} ± 0.99	63.9 ^{a} ± 0.04
F-test			**	**	**	**	**	**
CV (%)			13.78	10.03	5.29	6.10	6.67	4.58

Mean ± SED in the columns with superscript letter are significantly different at $p < 0.05$ by LSD. GT = gelatinisation temperature, L = low, I = intermediate, H-I = high-intermediate, ** significant at $p < 0.01$.

Table 3. Gelatinisation temperature (GT), gel consistency (GC), and starch digestibility of 9 selected non-glutinous upland rice varieties and the KDML105 as a standard check.

Var	GT	GC (mm)	Percentage of Enzyme Hydrolysis of Gelatinised Starch (Time: Hours)					
			0.5 h	1 h	2 h	4 h	6 h	24 h
ULR 040	L	32	54.6 ^{a} ± 0.08	56.0 ^{a} ± 0.28	57.9 ^{ab} ± 0.11	59.8 ^{ab} ± 1.72	54.7 ± 1.53	51.3 ^{d} ± 0.24
ULR 126	I	39	49.6 ^{a} ± 1.87	55.4 ^{a} ± 0.74	53.0 ^{b} ± 0.58	58.3 ^{abc} ± 0.14	60.5 ± 4.17	66.6 ^{a} ± 0.18
ULR 128	L	14	43.6 ^{abc} ± 1.99	53.2 ^{a} ± 0.04	55.3 ^{ab} ± 0.63	56.5 ^{abc} ± 1.93	61.3 ± 0.69	64.3 ^{abc} ± 0.18
ULR 129	I	26	44.7 ^{ab} ± 6.39	49.8 ^{a} ± 2.00	53.1 ^{ab} ± 2.82	56.3 ^{bc} ± 2.35	64.9 ± 0.75	61.9 ^{c} ± 0.56
ULR 132	L	9	37.2 ^{bc} ± 1.59	48.7 ^{a} ± 1.70	51.4 ^{b} ± 0.68	57.0 ^{abc} ± 0.73	62.5 ± 0.94	66.0 ^{ab} ± 1.70
ULR 219	I	24	8.4 ^{d} ± 2.69	13.3 ^{c} ± 1.33	22.4 ^{d} ± 0.39	35.5 ^{d} ± 0.57	49.7 ± 2.12	66.0 ^{ab} ± 0.15
ULR 264	I	25	12.9 ^{d} ± 3.60	18.6 ^{c} ± 5.14	26.3 ^{d} ± 4.53	51.4 ^{c} ± 2.51	60.5 ± 5.05	65.4 ^{abc} ± 1.64
ULR 414	I	30	51.9 ^{a} ± 1.47	56.3 ^{a} ± 0.28	59.5 ^{ab} ± 1.26	63.5 ^{a} ± 0.26	63.5 ± 0.69	62.4 ^{bc} ± 0.89
ULR 019	I	41	54.3 ^{a} ± 0.88	57.2 ^{a} ± 0.69	61.8 ^{a} ± 0.43	62.5 ^{ab} ± 0.19	61.8 ± 0.88	61.8 ^{c} ± 0.53
KDML105	L	61	31.7 ^{c} ± 1.64	37.5 ^{b} ± 0.81	42.6 ^{c} ± 0.54	52.0 ^{c} ± 0.77	59.9 ± 0.21	65.2 ^{abc} ± 0.38
F-test			**	**	**	**	ns	**
CV (%)			13.93	9.63	8.06	5.78	8.91	2.72

Mean ± SED in the columns with superscript letter are significantly different at $p < 0.05$ by LSD. GT = gelatinisation temperature, L = low, I = intermediate, H-I = high-intermediate, ** significant at $p < 0.01$.

The calculation of enzyme hydrolysis rates in the glutinous and non-glutinous rice varieties reveals a rapidly diminishing rate after 1 h in all cultivars (Figure 5), indicating that these gelatinised starches were rapidly digested during the earlier period. Moreover, four selected glutinous upland rice varieties, namely ULR241, ULR308, ULR138, and ULR155, and two in the non-glutinous group, namely ULR219 and ULR264, showed lower enzyme hydrolysis rates than the check varieties RD6 and KDML105, respectively (Figure 5). The low rates of enzyme hydrolysis indicate the presence of a slowly digestible starch in the rice in comparison to other varieties (Tables 2 and 3).

Interestingly, the results show that the same starch and waxy types in the glutinous and non-glutinous rice had different digestion rates. Moreover, the results indicate that the selection criteria for grain quality are not only based on AC or GT but also on in vitro starch digestibility. The rice varieties with a slow digestion, low AC, low GT, and soft gel consistency, namely ULR241, ULR308, ULR138, and ULR155, in glutinous rice and ULR219 and ULR264 in non-glutinous rice, with a low AC, low GT, and hard gel consistency, demonstrate high cooking quality and offer a healthier carbohydrate choice.

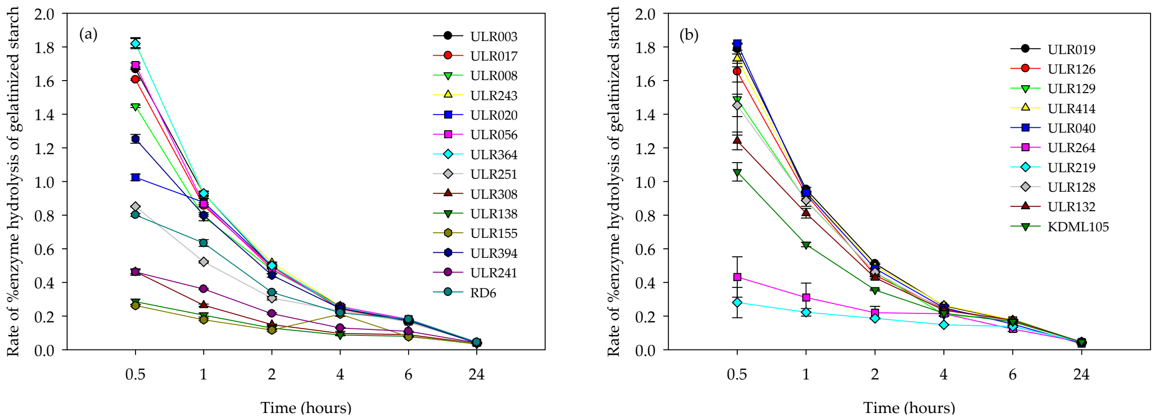

Figure 5. In vitro enzyme hydrolysis rates for gelatinised rice starches: (**a**) Thirteen glutinous varieties and check varieties (RD6); (**b**) Nine non-glutinous varieties and check varieties (KDML105).

4. Discussion

4.1. Amylose Content, Gelatinisation Temperature, and Gel Consistency

Cooking and eating quality are the most important traits in rice for meeting consumer behaviour/needs and are used in the selection criteria of a breeding scheme. Quality traits such as AC, GT, and starch content are considered by consumers when making health benefit decisions. In this study, 167 upland rice genotypes in Thailand exhibited high diversity in AC and GT. In general, most of the glutinous rice genotypes had a lower AC than the non-glutinous ones. The selection of a low AC in glutinous rice was due to the preference of consumers, especially those in the northern and northeastern regions of Thailand. The low amylose content was related to the stickiness and softness of cooked rice. Products containing intermediate to high amylose include rice noodles, rice flour, and traditional rice snacks. Consequently, there were fewer high-intermediate AC varieties in the germplasms than low amylose. The low AC was related to the softness and stickiness of the cooking quality. In this study, the intermediate to high AC was found only in the non-glutinous rice varieties. Interestingly, rice genotypes with a high AC showed a lower blood glucose and insulin response, making them suitable for diabetics [12]. The AC linear chains, bound by α-1,4 glycosides links and behaving essentially as a non-branched molecule, were related to the starch granules being packed into crystallites and becoming more exothermic, forming an amylose–lipid complex with greater stability [23]. This affects the water uptake, resulting in swelling, gelatinisation [24], and the release of sugar during digestion. However, rice genotypes with a high AC become dry, non-sticky, and hardened during cooking [25]; therefore, the high AC in both glutinous and non-glutinous rice genotypes affects their stickiness or texture, resulting in low cooking and eating quality. In addition, many factors affect the physicochemical properties of rice starch, such as the variety, composition, and structure of starch granules, processing method, storage conditions, and temperature during grain development [26,27]. Since the variety factor effect cannot be controlled by management, identifying a fixed variety with good cooking quality is the main goal of a breeding programme. In this study, AC was found to be negatively correlated with the ASS (Figure 4), indicating that rice needs more time and higher cooking in the low-ASS group. The ASS was related to the gelatinisation temperature (GT), where a low ASS had a high GT.

Alkali digestion is one of the important indicators of the eating, cooking, and processing quality of rice grains [28]. The upland rice genotypes can be classified into four groups from low to high GT (Figure 3). The rice genotypes with low alkali digestion had a high gelatinisation temperature [29], meaning that they required more water and time to cook than those with intermediate and low GTs [27]. Typically, the gelatinisation characteristics of rice are determined using differential scanning calorimetry (DSC), which is a thermal method for assessment [30], or a rapid visco analyzer (RVA), as it is useful for achieving a better understanding of the development of an enzyme-resistant (RS) starch [31]. The DSC and RVA results were related to the degree of starch gelatinisation (DSG), which were found to be dependent on steaming times in the parboiling process of rice [31], the hydration and irreversible swelling of the granule, molecular order, melting of starch crystals, and starch solubilization [32]. In addition, the DSC and RVA techniques require specialized equipment and usefulness for a smaller number of samples or variety to clearly classify the variation of starch in each variety. So, in many varieties or in the first selection process, the quick and easiest technique was suitable. In addition, the gelatinisation onset (GT_O) had a positive correlation with the pasting onset (PT_O) temperatures [30] and DSG [32]. In this study, most upland rice genotypes (58.7%) had an intermediate GT, and 38.9% had a low GT (Figure 3). The rice genotypes with a low to intermediate GT exhibited good cooking qualities such as water absorption, moistness, volume expansion, and softness upon cooling [33]. Since the AC and ASS were negatively correlated, they can predict each other. Moreover, the texture was related to the amylose and amylopectin contents in the rice seeds. Most of the study results showed the correlation between AC and physicochemical traits, such as the rapid visco analyzer (RVA) result, setback viscosity (SBV), and consistency viscosity (COV) [34], indicating that starch granules influenced by the amylose amount were associated with cooking quality, such as starch gelation time, as measured using RVA, SBV, and COV. Therefore, it was important that the AC and GT be included in the selection criteria. Moreover, in some eating and cooking quality selections, we used gel consistency (GC) as a selection criterion; it is a suitable indicator to measure cooked rice flour or milled rice with the index of cooked rice texture, especially for high-amylose-contents rice [35]. The relationship between AC and GC was dependent on genotype and starch but GT was significantly negatively correlated with GC [36]; in this study, they were not related because the primary selection was based on AC. However, in the low-AC group of glutinous rice was had a soft-gel consistency classification (Table 2). The gene control of cooking quality trait was reported; for example, GC may be controlled by several minor genes aside from the *Wx* in chromosomes 1, 2, 6, and 7 [35,37–40]. Moreover, it has been reported that AC controlled by a major gene is tightly linked to waxy (*Wx*) locus on chromosome 6, which encodes the granule-bound starch synthase (GBSSI) [41,42], and chromosomes 2, 3, 4, 5, 6, 7, 8, 10, and 11 [35,43–46]. The *Wx* locus region was reported to also affect both GC and GT [47]. For GT, a major QTL was identified at the region corresponding to the alkali degeneration locus (*alk*), and some minor QTLs were also detected on chromosomes 3, 5, 8, and 9 [46].

4.2. Starch Digestion

Blood glucose levels after meals are influenced by the enzymatic digestion of starch, which is an important metabolic response. Therefore, the rate of starch digestibility in the human small intestine is extremely important for the provision of both health and nutritional benefits [48]. The starch digestion rate is an important consideration for rice consumers concerned about health, particularly those with diabetes. The in vitro methods of starch digestion in this study were easy to perform, they saved cost, and they were related to GI determination, for which in vitro protocols are often modified to fulfil the experiment requirements. Amylose content was the one parameter of interest when studying the GI of starch. The amylose content is also correlated with starch digestibility, which can be described as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [49]. So, high amylose content was correlated with a higher percentage of RS [50], which in consequence will result in a lower GI [51]. Moreover, total starch hydrolysis was related to sucrose release or enzyme hydrolysis [51]. In this study, the in vitro enzymatic digestibility of 13 selected glutinous genotypes (G) and 9 selected non-glutinous genotypes (NG) with check varieties RD6 and KDML105 showed that some varieties had a slower sugar release during digestion (Tables 2 and 3, Figure 5). Generally, waxy or low-amylose genotypes release more sugar and rapidly digestible starch than those with intermediate to high AC [7,8,52] due to the significant proportion of amylopectin in starch granules. The branched chains of amylopectin, especially the short lateral chains, allow for hydration via hydrogen bonds to form gels with the tendency to retrograde [53], relating to the softness in cooking quality. However, the quality of cooking depends on not only AC, GT, and digestion but also on the degree of crystallinity [54], protein [55], lipid [56], starch granule size [57], and amylopectin chain length [58]. To benefit consumer health, the ideotype of rice grain quality is low amylose and an intermediate GT with low digestion. In this study, we identified the glutinous upland rice varieties (ULR138, ULR155, ULR241, and ULR308) and non-glutinous upland rice varieties (ULR219 and ULR264) with a low AC and slowly digestible starch (Tables 2 and 3).

According to the results of this study, upland rice varieties ULR241, ULR308, ULR138, and ULR155 (glutinous rice) and ULR219 and ULR264 (non-glutinous rice) have high cooking quality and offer a healthier carbohydrate choice. The findings reveal that when varieties exhibit low AC with slow starch digestion, the selection criteria should not only be based on AC or GT but also on in vitro starch digestibility. Moreover, good-cooking-quality rice varieties with health benefits can be found in local rice germplasms, which may be useful for extending planting or breeding programmes in the future.

5. Conclusions

Indigenous upland rice germplasms in Thailand exhibited differences in AC, GT, and starch digestibility. Most upland rice germplasms can be classified as having waxy to a low AC and an intermediate GT. Glutinous rice varieties ULR155, ULR138, ULR308, and ULR241, as well as non-glutinous rice varieties ULR219 and ULR264, exhibited a low AC, along with low to moderate GTs, slow digestibility, and reduced sugar release compared to the check varieties. The rice varieties identified as having low AC and slow starch digestibility in this study can be utilised as genetic resources for rice production and parental lines in future rice breeding programmes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102622/s1, Table S1: Amylose content (AC), alkali spreading score (ASS), gelatinisation temperature (GT), and starch type of 167 indigenous rice varieties.

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