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Classification of rice cultivars by using chemical, physicochemical, thermal, hydration properties, and cooking quality

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Abstract

The objective of this study was to classify the rice starch categorized by similarity on the processability of the cultivar by its food applicable properties for appropriate management of rice starch quality. Eight rice cultivars were tested to identify changes in chemical properties (amylose, protein and lipid content), physicochemical properties (grain hardness, peak viscosity, final viscosity, and setback and breakdown values), thermal (gelatinization temperature), and hydration (water absorbance) properties, during a 6 month storage period. The effect of over time on cooking quality was also analyzed. The eight cultivars included four cultivars classified as low amylose content (KDML 105, RD15 and PTT1), one medium amylose content (KTH17) and four high amylose content (PL2, RD31, SP1 and CN1). The results showed that during storage, the amylose, grain hardness, peak viscosity and final viscosity increased, while break down values decreased. Protein and lipid content and setback properties were unchanged. The gelatinization temperature positively correlated with amylose content. The highest onset, peak and conclusion temperature showed in RD31 (72.67 ºC, 77.08 ºC and 84.03 ºC, respectively). For hydration properties, the high amylose content rice showed the highest capacity to absorb water. The optimum cooking time positively correlated with amylose content. Classification of the rice cultivars using PCA resulted in two distinct groups, with the high amylose cultivars being well separated from the low and medium amylose cultivars. Applying our results to rice flour, incorporation of chemical and physicochemical properties in the analysis of rice flour allows better discrimination of the rice differently from using amylose content solely.

Keywords: Rice classification, Rice flour, Amylose, Pasting property, Principal component analysis

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1. Introduction

Rice is the staple commodity of people in most Asian countries, especially in the Southeast Asia. In general, rice samples are normally classified based on their amylose content, which is also used as the index of eating quality to predict the texture of the rice after cooking. Amylose content as a quality index helps the rice starch industry to produce a uniform rice product. However, it has been reported that rice classification by amylose content alone can misclassify its sensory qualities (Suwannaporn *et al*., 2007). Therefore, other parameters including physical qualities (grain size, color) and physicochemical qualities, or a combination of these factors, are recommended for use for more precise classification.

Rice grains exhibit distinct physicochemical properties, depending upon the cultivar, and the quality of the starch especially influences its cooking properties (Juliano, 1985). The amylose content of the rice starch is one of the important characteristics determining eating and cooking quality. Amylose content has long been used as a parameter to predict the texture of cooked rice. The suggested classification of amylose content in as waxy (0-5%), very low (5−12%), low (12−20%), intermediate (20−25%), and high (25−33%). However, as suggested in Champagne *et al*.(2001) reported that rice with similar amylose and protein content still manifests different textures. Factors affecting the textural quality of cooked rice include the rice cultivar, the amylose content, pasting properties, protein content, postharvest practice, moisture content, temperature, and storage period (Ramesh *et al*., 1999; Champagne, 1996; Pearce *et al*., 2001).

Additionally, other important parameters representing starch characteristics are water hydration properties including water absorption index (WAI), water solubility (WS), and swelling power (SP). The hydration properties of starch can be correlated with its pasting properties and physicochemical properties which are important for the use of starch in industry. Therefore, from the industrial standpoint, a simple and accurate approach to simplifying rice cultivars categorization is needed. Thus, it is plausible to classify the rice starch categorized by similarity on the processability of the cultivar by its food applicable properties for appropriate management of rice starch quality. Especially, in the case when significant differences in physicochemical properties occur even among the starch obtained from rice cultivars with similar amylose content (Ha *et al*., 2007; Yoon *et al*., 2009).

The physicochemical properties of freshly harvested rice change during aging of the rice (Villareal *et al*., 1976; Chrastil, 1990a; Chrastil, 1990b). The most important change that occurs during the aging process involves rheological properties, which contribute significantly to cooking and eating properties (Tan and Corke, 2002). The changes in rice properties during aging depend upon cultivar, storage conditions, and amylose content. To improve rice eating quality, the optimum aging period must be known. The chemical and physicochemical changes that occur during aging include the pasting properties and the thermal properties. Recently, rice pasting properties as determined by a Rapid Visco Analyzer (RVA) which reflects rice quality has received great attention and extensively used to predict the textural properties of cooked rice and for pasting comparison studies in starch applications. The advantage of using RVA is that it is simple, rapid, and applicable in rice industries. Therefore, the purpose of our study was to employ RVA profiling as an alternative quality classification method instead of using the amylose content as the sole quality index of rice. Changes in chemical and physicochemical properties during six months storage of eight rice cultivars were observed. Principal component analysis (PCA) was performed to differentiate rice characteristics as well as to observe the correlation among rice cultivars.

2. Materials and Methods

2.1 Chemicals

Chemicals and solvent used in this study were analytical grade from RCI Labscan Co., Ltd., Bangkok, Thailand. Potassium sulfate, Copper sulfate and boric acid were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Amylose from potato was purchased from Sigma–Aldrich Chemical. Co., (St. Louis, MO), USA.

2.2 Rice samples

Paddy of eight rice cultivars including high, medium, and low amylose content were obtained from the Northern part of Thailand. Low amylose rice samples were Khao Dawk Mali 105 (HM105), Pathum Thani 1 (PTT1) and RD 15, medium amylose rice sample was Khao Tah Haeng 17 (KTH17) and high amylose rice samples were Phitsanulok 2 (PL2), RD 31, Suphan Buri 1 (SP1) and Chai Nat 1 (CN1).

2.3 Storage conditions and preparation of rice flour

For storage conditions, paddy rice was stored at room temperature for 6 months. After each month, rice grains were de–husked by a milling machine (NW 1000 TURBO, Natrawee Technology Co., Ltd., Chachoengsao, Thailand) to obtain the polished rice. For rice flour preparation, the paddy samples were de-husked and polished with rice polishing machine. Polished rice grains were ground into flour using a blender and passed through a 100 mesh sieve screen. The obtained rice flour was kept in a zip-lock plastic bag and stored in freezer at -20 °C before analysis.

2.4 Amylose content analysis

Amylose content was determined using the Starch-iodine blue method as a modified assay by Juliano *et al*. (1981). Hundred milligram of milled rice flour was weighed in 100 mL volumetric flasks. One milliliter of 95% ethanol and 9 mL of 1 N NaOH was then added and heated subsequently in a boiling water bath for 10 min. The samples were diluted to 100 mL with distilled water and ready for analysis. Five milliliter of sample was withdrawn and made up volume to 100 mL with a solution mixture of 1 mL of 1 M acetic acid, 2 mL of iodine solution (0.2 g iodine and 2.0 g Potassium iodide in 100 mL distilled water) and distilled water. The test mixture was left for 20 min. Then the intensity of color developed was measured using spectrophotometer (DR 4000U, UV Visible Spectrophotometer, Hach, Loveland, Colorado, USA) at 620 nm against the reagent blank. Amylose content (%) was calculated from an absorbance of amylose standard curve.

2.5 Protein and fat content analysis

Protein content of rice flour was analyzed using Kjedahl method (AOAC, 1990) Lipid in the rice flour was extracted using the soxhlet extraction method (AOAC, 1990).

2.6 Pasting properties analysis

Pasting characteristics of the rice flour were analyzed using a Rapid Visco Analyzer or Rapid Visco Amylograph (RVA) (RVA-Super 3, Newport Scientific, Australia). The viscosity was measured during the heating and cooling of flour solution (Zhou *et al*., 2002). The rice powder that passed through the 120 mesh sieve was weighed to 3.000 ± 0.005 g. Then put the rice power into an aluminum cup filled with 25.000 ± 0.005 g and mixed with a plastic mixing blade. Then, the cup with mixture placed in the Rapid Visco Analyzer. The mixing speed was 160 rpm. The temperature schedule was started at 50°C for (0–1 min), then the temperature was increased (12.2 °C/min) to 95 °C within 2.5 min. Then, the temperature was decreased to 50 °C (12.2 °C/min) and maintained at 50 °C for 2.1 min.

2.7 Thermal properties

Thermal properties of rice flour were determined by using differential scanning calorimetry (DSC) (DSC 1, METTLER TOLEDO, Columbus, USA). Rice flour samples (10 mg) and distilled water were added to the DSC pan in a 1:2 ratio (w/w). The pan was hermetically sealed and allowed to equilibrate at 25 °C for 1 h. The samples were then heated from 10°C to 110 °C at 10 °C/min. An empty DSC pan was used as a reference. Onset, peak, conclusion temperatures and gelatinization enthalpy were determined (Purna *et al*., 2011).

2.8 Hardness

Hardness of the cooked rice was performed using a texture analyzer (QTS 25, Brookfield viscometers Ltd., USA). The rice samples were prepared by the modified method of Juliano *et al.* (1984). Rice samples 10 g cooked in 100 ml distilled water at 95 ± 1°C until white core disappeared. The cooked rice was drained of water completely using a strainer and surface moisture of the samples was blotted out. A 250 mm diameter probe was used to compress sample, with pre-test and post−test speeds of 1 mm/s and test speed of 0.5 mm/s. Parameters recorded from the test curves was hardness

2.9 Water absorption index (WAI)

WAI was measured according to the method of Medcalf and Gilles (1965). Starch suspension was heated at 80 °C. The result was calculated using the following equation:

Water absorption index = (wet sediment weigh) / (sample weight)

2.10 Cooking quality

Optimum cooking time was determined by the modified method reported by Juliano (1985). In a 250 mL beaker, about 100 ml distilled water was boiled (95 ± 1 °C) and 5 g of head rice samples dropped into it. Measurement of cooking duration was started immediately. After 10 min and every min thereafter, 10 grains of rice were removed and pressed between two clean glass plates. Cooking time was recorded when at least 90% of the grains no longer had an opaque core or uncooked center. The rice was then allowed to simmer for an additional 2 min to ensure that the core of all grains had been gelatinized. Optimum cooking time was taken as the established cooking time plus the 2 additional min.

2.11 Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) and Duncan's multiple range test at 5% probability level using SPSS version 17.0 software (IBM SPSS Statistics, USA). Principal Component Analysis (PCA) was employed to reduce the complexity of the data and performed using XLSTAT for Excel V. 19.4 (Addinsoft 2017, France).

3. Results and Discussion

3.1 Changes of chemical properties of rice flour during 6 months storage

Changes of chemical properties of rice flour during 6 months storage are shown in Fig 1 A-D. In Fig 1A, the results showed that the PL2 rice had the highest amylose content (36.77%) and the PTTT1 had the lowest amylose content (18.42%). The amylose content for the low, medium, and high amylose groups was in the range of 18.42−20.80%, 20.58−21.96%, and 29.92−36.77%, respectively. Generally, it was found that amylose content does not vary with storage time. During storage, the amylose content of the low and medium amylose samples were consistent but the amylose content of the high amylose rice increased after 4 months of storage. Villareal *et al*. (1976) and Chrastil (1990a) reported a slight increase of amylose content during storage. However, in our study, the amylose content did not change significantly, which is similar to the results reported by Kanlayakrit and Maweang (2013).

The hardness of cooked rice is shown in Fig 1B. The results show that the hardness value of high amylose rice is higher than that of low and medium amylose rice. This suggests that the hardness of cooked rice is influenced by the amylose content. This is due to the fact that amylose contains more crystalline regions, which in turn, show more compact starch granules and exhibit harder gel trends.

Storage time influences the texture of cooked rice. High amylose rice is more affected than low and medium amylose rice. This finding is similar to the results of Zhou *et al.* (2002) and Wiset *et al*. (2011) where milled rice and paddy rice were stored for 6 months at 30 °C resulting in an increase of the hardness of the aged rice. Juliano (1985), Meullenet *et al*. (1999), and Charstil (1994) also found that rice used for cooking had increased hardness over storage time. The hardness of aged rice when cooked was higher than that of freshly harvested rice when cooked because the amylose and amylopectin reaction contributes to the increase in the hardness of the cooked rice. The long amylopectin chains crystallize with an amylose molecule, which extends through several adjacent 'clusters', thereby contributing to double helices in several crystallites (Ong and Blanshard, 1995).

Fig 1C shows the water absorption index (WAI) of different rice cultivars during six months storage. At the beginning of storage, all rice samples exhibited different WAI characteristics. The WAI values of rice starches from similar amylose groups, especially CN1, was relatively higher than other cultivars in spite of containing a high amylose content.

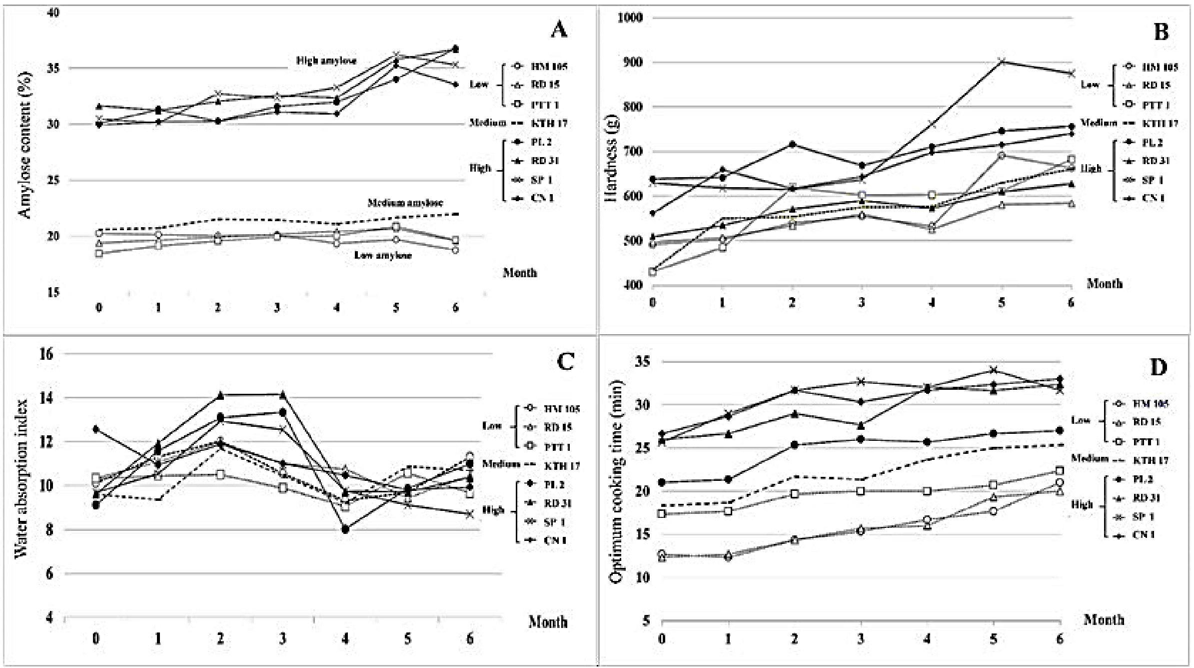

Figure 1 Amylose content (A), Hardness (B), Water absorption index (WAI) (C) and Optimum cooking time (D) of different rice flour during 6 months storage

Over the first three months of storage, the WAI increased and then started to decline, the phenomenon likely associated with the lower hydration process of starch granules. The WAI of high amylose rice was higher than that of low and medium amylose rice. The highest and lowest WAI was found in RD31 (14.16) and PL2 (8.02), respectively. Lee *et al*. (2012) have also reported that the WAI of high amylose rice is higher than that of low and medium amylose rice. However, a different finding was reported in (Juliano and Perez, 1984) which was that low amylose content rice can absorb a higher volume of water than high amylose rice. This could be explained by the amylose containing more crystalline regions, resulting in the difficulty of water molecules to migrate inside the grains. These various findings indicate that the amylose content does not accurately represent the processability properties, including the WAI.

The cooking time of rice grains of different rice cultivars is shown in Fig 1D. The cooking time of all the rice cultivars ranged from 12.33 min (KDML105) to 33.0 min (CN1). The results show that high amylose rice exhibit longer cooking times than low and medium amylose rice. It was also found that high amylose rice requires high temperature and energy and consequently require more cooking time. The results for all the samples indicate that the cooking time increased with increased storage time. This finding is in agreement with the results of Villareal *et al*. (1976), who reported that storage time effects gelatinization of rice flour, and are positively correlated. This increase was due to the greater mobility and subsequent restructuring of starch and changes in the protein bodies present in the grain. Moritaka and Yasumatsu (1972) proposed a mechanism of aging involving lipid, protein, and amylose. Lipids form free fatty acids, which can link with amylose, carbonyl compounds, and hydroperoxides to form a complex amylose or carbonyl structure which can accelerate protein oxidation and condensation and create an accumulation of volatile carbonyl compounds. Protein oxidation (formation of di-sulphide linkages from sulphydryl groups), together with an increase in the strength of micelle binding of starch, inhibits swelling of the starch granules and affects the texture of the cooked rice.

The protein and fat content of the rice flour of each cultivar was measured, and showed a slight change during the storage time (Table 1). The rice flour from the PTT 1 cultivar had the highest protein content at all times during the storage period, compared to other samples. The protein content of all the rice flour samples changed only slightly during the storage period. This finding agrees with Kanlayakrit and Maweang (2013) who reported that the protein content of paddy and milled rice after ten months in storage did not significantly change. In addition, it was noticed that the protein content of the rice flour samples did not correlate with the amylose content.

For the freshly harvested rice, the highest lipid content was found in the HM105 cultivar (2.51%) and the lowest in the RD15 cultivar (0.65%) at 2 months storage time. Both the HM105 cultivar and the RD15 cultivar are in the low amylose group, so these results indicate that lipid content and amylose content are not related. Also, no obvious changes in protein and lipid levels were seen over the 2 months storage period, which shows that protein and lipid content is not influenced by storage time. However, suggested that there are chemical interactions that occur during rice storage, including interaction between proteins, a breakdown products of lipid oxidation and starch–protein interactions (Sodhi *et al*., 2003). In addition, it has also been reported that lipids formed free fatty acids and complexes with amylase, carbonyl compounds, and hydroperoxides, which can quicken protein oxidation and condensation and increase the rate of accumulation of volatile carbonyl compounds. Protein oxidation is the formation of di–sulfide linkages from sulfhydryl groups and together with an increase in the strength of Mitchell binding of starch (Sodhi *et al*., 2003).

Table 1 Protein and lipid content of rice flours during 6 months storage

Rice cultivar		Storage time (months)						
		0	1	2	3	4	5	6
Protein (%)								
Low amylose	KDML 105	8.58±0.33bc,A	8.74±0.55ab,A	8.68±0.46bc,A	8.31±0.28b,A	8.53±0.30bc,A	8.82±0.28bc,A	7.80±0.51c,B
	RD 15ns	8.42±0.15c	8.30±0.21b	8.60±0.54bc	8.52±0.38b	8.99±0.18ab	8.42±0.29bc	8.11±0.18a
	PTT 1ns	9.46±0.18a	9.01±0.18a	9.39±0.27a	9.32a±0.13b	9.49±0.47a	9.35±0.31a	9.21±0.48ab
Medium amylose	KTH 17	9.06±0.17ab,A	8.29±0.23b,B	8.62±0.16bc,B	8.65±0.29ab,B	8.86±0.20ab,B	9.28±0.24a,A	7.33±0.33c,C
High amylose	PL 2	8.34±0.09c,B	8.42±0.45ab,AB	8.44±0.18b,AB	8.42±0.14b,AB	8.13±0.16c,C	8.84±0.41b,AB	9.05±0.24a,A
	RD 31	9.47±0.19a,A	8.99±0.47a,ABC	8.42±0.16b,BC	9.34±0.18a,A	9.25±0.30a,AB	7.22±0.33e,D	6.56±0.34ab,C
	SP 1	8.32±0.15c,C	8.580.33ab,BC	9.07±0.66ab,AB	9.28±0.25a,A	8.99±0.21ab,AB	8.28±0.39cd,C	8.17±0.23bc,C
	CN 1	6.95±0.35d,C	7.02±0.18c,BC	7.37±0.28c,BC	7.24±0.21c,BC	7.22±0.15d,BC	7.98±0.47d,B	8.80±0.35ab,A
Lipid (%)								
Low amylose	KDML 105	1.94±0.06a,D	1.72±0.04a,F	2.51±0.06a,A	2.27±0.07a,B	2.09±0.06a,C	1.82±0.04a,E	1.680.04±c,G
	RD 15	1.20±0.07d,C	1.37±0.10c,B	1.57±0.10d,A	0.73±0.04f,F	0.96±0.03e,E	1.08±0.01f,D	1.58±0.04d,A
	PTT 1	1.47±0.11b,C	1.06±0.07f,E	0.65±0.08h,G	1.29±0.06d,D	0.88±0.01f,F	1.63±0.04d,B	1.86±0.04a,A
Medium amylose	KTH 17	1.15±0.06e,F	1.26±0.06d,E	1.36±0.10f,D	1.47±0.03b,B	1.66±0.04c,A	1.07±0.06f,G	1.42±0.01f,C
High amylose	PL 2	1.06±0.07f,F	1.18±0.10e,E	1.42±0.04e,D	1.16±0.03e,E	1.65±0.07c,C	1.70±0.05c,B	1.80±0.06b,A
	RD 31	1.00±0.03g,D	0.94±0.03g,E	0.90±0.03g,F	1.14±0.01e,C	1.38±0.04d,B	1.36±0.03e,B	1.54±0.02e,A
	SP 1	1.27±0.10c,E	1.53±0.06b,C	1.79±0.06b,AB	1.38±0.06c,D	1.78±0.01b,B	1.81±0.02a,AB	1.82±0.04b,A
	CN 1	0.85±0.06h,F	1.55±0.07b,D	1.70±0.01c,B	1.15±0.06e,E	1.64±0.04c,C	1.74±0.04b,A	1.65±0.05c,C

Note: Means in the same column followed by the same lowercase superscript letters are not different by Duncan's multiple range test at *p>0.05*

Means in the same row followed by the same uppercase superscript letters are not different by Duncan's multiple range test at *p>0.05*

ns No significant difference at *p>0.05*

3.2 Changes of pasting properties of rice flour during 6 month storage

The pasting properties of the rice cultivars under different storage times are shown in Table 2. The highest peak viscosity (PV) was found in RD15 (5759 cP) and the lowest in PL2 (868 cP). Comparing the actions of amylose and amylopectin, the latter plays a major role in swelling of the starch while the amylose acts as a diluent (Swamy *et al*., 1978). It was reported by Tester and Morrison (1990) that amylose content is negatively correlated with starch granule swelling. This agrees with results of our study in which the starch granules with a high amylose content swelled at a slower rate than those with high amylopectin, resulting in lower peak viscosity values.

Breakdown (BD) values of the 8 rice cultivars ranged between 550−4193 cP. Breakdown values indicate the ease with which the starch granules are broken when heated after reaching maximum swelling at peak viscosity. Similar to peak viscosity, the low amylose provides high breakdown, while the medium and high amylose rice starches provide low breakdown, indicating a negative correlation. Samples in the RD15 sample, which is in the low amylose group, exhibited the highest breakdown value of 4193 cP, while the PTT1 cultivar showed the low breakdown value of 1296 cP. This could be explained by the effect of the high protein level in the PTT1 cultivar. The protein−starch interaction in rice grains can hinder the rate of granule swelling, resulting in lower stability of the starch granules. Zhou *et al*. (2002) also reported that protein oxidation and increases in the strength of the micelle binding of starch could inhibit swelling of the starch granules.

Final viscosity (FV) for the low amylose group was in the range of 2035−2615, for the medium amylose group, 1935−2449, and the high amylose group was 1300−3669 cP. This indicates that the final viscosity positively correlated with the amylose content. Setback (SB) occurred when the cooling starch molecules began to re−associate in an ordered structure (Atwell *et al*., 1988). SB values indicate the hardness of the cooled gel paste. Low SB values signify a low rate of starch retro gradation. Similar to the trend of FV, a positive correlation between SB and amylose content was observe. It is well documented that rice pasting behavior is affected by starch−lipid complex formation in the rice molecules. The higher endogenous lipid content in the high amylose group contributed to more complex formation between amylose and lipid, and thereby restricted granular swelling (Chrastil, 1990b)

Table 2 Pasting properties of rice flours during 6 months storage

Rice cultivar	Viscosity (cP)	Storage time (months) 0	1	2	3	4	5	6
Low amylose								
KDML105	PV	5586±57.51^{a}	4887±47.96^{ab}	4188±38.42^{b}	3668±21.92^{c}	2438±26.31^{c}	2269±2.83^{c}	2194±80.61^{c}
	BD	3964±82.50^{a}	3325±67.65^{ab}	2686±52.80^{b}	2191±43.84^{c}	1136±27.74^{cd}	913±14.14^{e}	902±41.72^{e}
	FV	2615±1.41^{a}	2556±4.95^{ab}	2496±8.49^{b}	2482±31.11^{c}	2290±16.82^{d}	2363±38.18^{c}	2257±20.51^{d}
	SB^{ns}	993±23.57	994±14.74	995±5.90	1006±9.19	988±19.92	1007±26.87	965±18.38
RD 15	PV	5759±35.45^{a}	5032±37.94^{b}	4302±20.51^{c}	3737±18.38^{d}	2499±27.02^{e}	2278±5.66^{e}	2344±83.44^{e}
	BD	4193±105.13^{a}	3507±3.54^{b}	2820±71.18^{c}	2269±4.16^{d}	1169±25.46^{e}	896±50.31^{e}	883±8.49^{e}
	FV	2704±59.40^{a}	2636±51.62a^{b}	2586±31.82^{abc}	2546±28.99^{c}	2351±34.08^{c}	2410±13.44^{c}	2446±114.55^{c}
	SB	930±4.24^{c}	1117±1.41^{b}	1251±4.24^{a}	1145±23.33^{ab}	1172±35.84^{ab}	1213±28.99^{ab}	1218±123.04^{ab}
PTT1	PV	2401±25.46^{b}	2073±31.82^{b}	4302±25.92^{a}	2253±9.54^{b}	2379±24.04^{b}	2318±21.92^{b}	2247±3.54^{b}
	BD	1296±73.54^{ab}	763±88.16^{b}	1337±15.56^{a}	1042±70.71^{ab}	873±14.74^{b}	824±36.77^{b}	908±74.95^{b}
	FV	2035±8.49^{c}	2427±1.41^{b}	2668±43.84^{a}	2356±10.15^{b}	2678±4.95^{a}	2741±12.16^{a}	2654±0.71^{a}
	SB	1137±39.60^{a}	1112±1.41^{ab}	1087±1.41^{ab}	1078±10.69^{bc}	1021±3.54^{d}	1028±18.03^{cd}	1082±11.31^{b}
Medium amylose								
KTH17	**PV**	3333±18.03^{a}	2573±31.82^{ab}	3024±32.53^{b}	2531±53.16^{b}	2413±59.69^{b}	2222±69.30^{b}	2124±2.83^{b}
	BD	2068±38.00^{a}	1474±5.66^{ab}	1671±24.04^{c}	1348±44.64^{bc}	1142±56.90^{bc}	850±38.18b^{c}	762±12.73^{bc}
	FV	2133±11.59^{ab}	1935±53.74^{b}	2197±8.49^{ab}	2185±24.25^{ab}	2309±41.40^{ab}	2405±14.14^{a}	2449±2.83^{a}
	SB	869±21.20^{d}	837±16.26^{d}	1222±9.23^{a}	1003±15.31^{c}	1037±22.27^{c}	1033±16.97^{c}	1087±12.73^{b}

Note: PV: Peak Viscosity, BD: Breakdown Value, FV: Final Viscosity, SB: Setback

Means in the same row followed by the same superscripts are not different by Duncan's multiple range test at $p>0.05$

Table 2 Pasting properties of rice flours during 6 months storage (continue)

Rice cultivar	Viscosity (cP)	Storage time (months)						
		0	1	2	3	4	5	6
High amylose								
PL2	PV	1343±19.66^{ab}	1270±4.95^{ab}	1688±4.95^{b}	1236±28.22^{a}	1054±34.43^{ab}	906±4.95^{b}	868±1.41^{b}
	BD^{ns}	356±9.29	380±14.85	522±2.12	241±15.28	318±1.53	183±22.63	192±26.87
	FV	2009±20.22^{ab}	1824±26.16^{ab}	1482±35.36^{b}	1836±26.73^{ab}	1419±39.93^{b}	1346±4.95^{b}	1301±21.92^{b}
	SB	1022±15.72^{b}	934±6.36^{c}	1309±38.18^{a}	841±23.12^{d}	683±13.32^{e}	623±22.63^{f}	625±6.36^{f}
RD31	PV	2319±23.71^{ab}	2128±2.83^{ab}	2358±20.51^{c}	2633±30.37^{a}	2227±60.51^{ab}	2049±33.94^{ab}	1932±31.82^{b}
	BD	1090±8.02^{b}	1121±6.36^{b}	1079±72.12^{b}	1300±36.66^{a}	987±24.58^{b}	795±32.53^{bc}	764±16.26^{c}
	FV	2760±10.12^{a}	2408±10.61^{b}	3299±185.97^{a}	3152±9.02^{a}	2675±43.68^{a}	2679±34.65^{a}	2509±31.82^{ab}
	SB	1531±10.69^{c}	1400±19.80^{d}	2020±134.35^{a}	1818±13.58^{b}	1435±9.45^{d}	1425±33.23^{d}	1341±16.26^{d}
SP1	PV	2320±26.99^{a}	2262±14.85^{a}	2507±26.16^{c}	2174±13.58^{a}	1859±53.70^{b}	1725±47.38^{b}	2210±50.20^{a}
	BD	1104±28.88^{a}	1111±12.73^{a}	1221±14.85^{a}	968±25.42^{b}	749±23.43^{c}	544±45.96^{d}	776±17.68^{c}
	FV	2694±16.04^{b}	2582±4.95^{b}	3280±21.21^{a}	2826±20.21^{a}	2473±31.77b^{c}	2315±11.31^{c}	3054±44.55^{a}
	SB	1478±17.35^{c}	1431±2.83^{d}	1994±19.80^{a}	1620±43.29^{b}	1363±2.08^{e}	1133±9.90^{f}	1620±12.02^{b}
CN1	PV	2759±12.42^{a}	2289±6.36^{ab}	2826±18.38^{ab}	2602±8.08^{ab}	2382±77.95^{ab}	2334±215.67^{ab}	1583±26.87^{b}
	BD^{ns}	1122±8.50	967±2.12	1180±45.96	1044±25.48	838±100.07	667±4.95	550±5.66
	FV	3324±33.20^{a}	2855±2.12^{a}	3669±31.11^{a}	3268±32.01^{a}	3152±283.82^{a}	3452±214.25^{a}	2160±18.38^{b}
	SB	1687±29.26^{bc}	1533±6.36^{d}	2024±58.69^{a}	1709±14.93^{b}	1607±113.61^{cd}	1785±3.54^{b}	1127±14.14^{e}

Note: PV: Peak Viscosity, BD: Breakdown Value, FV: Final Viscosity, SB: Setback

Means in the same row followed by the same superscripts are not different by Duncan's multiple range test at p>0.05

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During rice aging, a number of physiological and physicochemical changes occur, including the pasting properties, which can be attributed to characteristics of the starch granules. As storage time increases, a decrease of PV and BD can be observed but also an increase of FV and SB. A significant decrease in PV indicates that aged rice is more resistant to swelling than freshly harvested rice (Keawpeng and Venkatachalam, 2015). A longer storage time results in lower BD in all rice samples. These findings are in accordance with results of Tulyathan and Leeharatanuluk (2007) who found that PV and BD of KDML105 decreases with longer aging times. The decrease in BD as storage time increases indicates the reduction of capacity of starch granules to rupture after cooking (Zhou *et al*., 2003). The increase of FV and SB in aged rice samples has also been reported by several researchers (Kanlayakrit and Maweang, 2013; Zhou *et al*., 2003; Park *et al*., 2012; Katekhong and Charoenrein, 2012a; Katekhong and Charoenrein, 2012b; Tananuwong and Malila, 2011; Sowbhagya and Bhattacharya, 2001). Katekhong and Charoenrien (2012b) reported that the increase of FV and SB with longer rice storage duration was due to the granules being stronger after longer storage resulting in some of the starch granules not being disrupted by cooking. The FV and SB may occur by the rearrangement of leached amylose and also of the granules which have not been disrupted.

3.3 Changes of thermal properties of rice flour during 6 month storage

Results of the thermal properties are illustrated in Table 3. Several concepts have been proposed to explain the gelatinization process. Starch gelatinization is an endothermic process that corresponds to the loss of starch crystalline in the starch granules under particular heat and moisture conditions. Gelatinization describes the range of irreversible changes when starch is heated in excess water (Lee *et al*., 2012). The peak temperature is the initiation of position where the endothermic reaction occurs at the maximum. Conclusion temperature is defined as when all the starch granules are fully gelatinized, and the curve then remains stable (Tester and Morrison, 1990). Table 3 illustrates, for freshly harvested rice (zero month storage), the highest onset, peak and conclusion temperature found in the high amylose group. These highest values were found in RD31 sample (T_0 72.45 °C and T_p 76.01 °C) and CN1 sample (T_c 81.75 °C). Comparing the onset, peak and conclusion temperatures and ΔH_{gel} among rice cultivars with different amylose content, it was found that, in general, high amylose rice showed slightly higher values than those of low and medium amylose rice, which could be affected by starch structure properties. The starch with low amylose contains more amorphous and less crystalline regions, leading to a lower gelatinization temperature. The lower gelatinization temperatures for rice flours indicate that less energy is required for starch gelatinization.

Table 3 Thermal properties of different rice flour during 6 months storage

Thermal properties		Rice cultivar	Storage time (months)		
			0	3	6
Onset temperature (T_O, ºC)					
	Low amylose	KDML 105	63.22	63.42	63.93
		RD 15	64.37	64.17	64.31
		PTT1	63.32	63.23	63.68
	Medium amylose	KTH17	64.25	64.54	63.2
	High amylose	PL2	61.32	62.09	60.41
		RD31	72.45	72.67	72.24
		SP1	62.43	73.00	72.14
		CN1	71.43	72.18	71.96
Peak temperature (Tp, ºC)					
	Low amylose	KDML 105	70.52	70.87	70.86
		RD 15	70.70	70.87	70.86
		PTT1	68.71	69.02	69.52
	Medium amylose	KTH17	70.03	70.99	69.86
	High amylose	PL2	67.38	67.99	68.04
		RD31	76.01	77.08	76.84
		SP1	68.88	76.85	77.02
		CN1	76.20	76.71	77.01
Conclusion temperature (Tc, ºC)					
	Low amylose	KDML 105	78.00	78.29	77.72
		RD 15	77.52	78.01	77.5
		PTT1	75.75	75.41	75.95
	Medium amylose	KTH17	76.52	77.19	77.18
	High amylose	PL2	73.04	73.34	76.16
		RD31	80.58	84.03	82.57
		SP1	75.54	81.59	82.8
		CN1	81.75	82.61	82.49
Gelatinization Enthalpy (ΔH gel; J/g)					
	Low amylose	KDML 105	2.20	2.18	2.18
		RD 15	2.26	2.23	2.15
		PTT1	2.88	2.58	2.50
	Medium amylose	KTH17	2.58	2.29	2.04
	High amylose	PL2	1.66	1.34	1.40
		RD31	3.04	2.76	2.57
		SP1	2.23	3.09	2.14
		CN1	2.66	2.68	2.25

Comparison of the gelatinization properties of milled rice flour made from freshly harvested and aged rice revealed that aged rice had higher values of thermal characteristics than those of freshly harvested rice. Similar findings were reported by Champagne (2001), Meullenet *et al*. (1999) and Katekhong and Charoenrien (2012b). The higher enthalpy of gelatinization and peak temperature of aged rice reflected the loss of double helical and crystalline structure. The increase in both the peak temperature and the conclusion temperature, as induced by the storage period, indicated that it took longer to disorder the structure of aged rice grain during the gelatinization process. These results indicated that storage of rice increases the rigidity of the granules at the end of the storage period, resulting in increased energy being required to disrupt the structure of the starch granules (Zhou *et al*., 2010).

Table 4 shows correlation coefficient (r) of factors used in the study during 6 months storage of 8 rice samples. The size of correlation coefficient was interpreted according to Rule of Thumb as reported by Schober, *et al*., 2018. The interpretation of correlation was described as follows: 0.00−0.010 is negligible correlation, 0.10−0.39 is weak correlation, 0.40-0.69 is moderate correlation, 0.70−0.89 is strong correlation, and 0.90−1.00 is very strong correlation. When considering the correlation coefficient greater than 0.5 (in the moderate range), it was found that parameters including amylose, hardness, cooking time, breakdown, setback, and final viscosity play important roles in indicating relationship, either positive or negative, to one another. This indicates that those mentioned variables are potential for being used as factors to differentiate differences in rice samples.

Table 4 Correlation coefficient (r) of factors used in the study during 6 months storage of 8 rice samples

Variables	**Amylose**	**Protein**	**Lipid**	**Hardness**	**PV**	**BD**	**SB**	**FV**	**WAI**	**Cooking time**
Amylose	1									
Protein	-0.295	1								
Lipid	0.022	-0.107	1							
Hardness	0.608	-0.210	0.280	1						
PV	-0.041	0.155	-0.071	-0.212	1					
BD	-0.524	0.066	0.106	-0.605	0.309	1				
SB	0.444	-0.272	-0.140	0.055	0.229	-0.091	1			
FV	0.096	-0.253	-0.077	-0.109	0.278	0.220	0.827	1		
WAI	0.117	-0.029	-0.174	-0.181	0.381	0.117	0.420	0.248	1	
Cooking time	0.867	-0.288	0.015	0.645	-0.109	-0.657	0.568	0.263	0.072	1

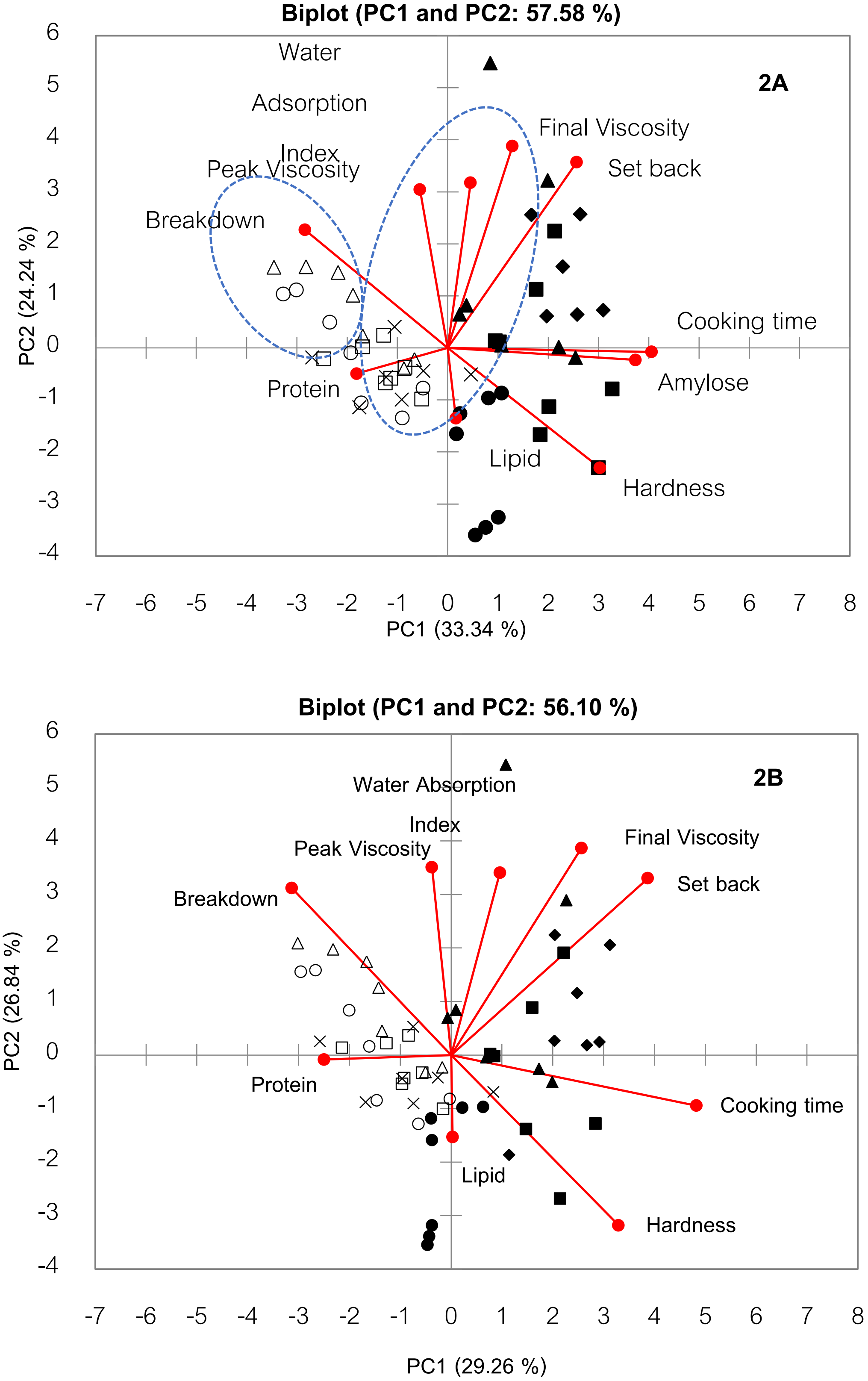

Figure 2 A; Biplot of Chemical properties (Amylose, Protein and Lipid content), Pasting properties, Hardness, Water adsorption index and cooking time. B; Biplot of Chemical properties (Protein and Lipid content, except amylose content), Pasting properties, Hardness, Water adsorption index and cooking quality

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Data obtained in our study were used to classify all rice cultivars, using Principal Component Analysis as shown in Fig 2 A−B. From fig, the 1st and 2nd components altogether contributed to 56−58% of total variance. Fig 2 A−B are results of employing chemical properties, pasting properties, hardness, and water adsorption index and cooking time, except amylose content was also included in Fig 2A. The results indicated that both figures showed similar classification patterns in which high amylose rice samples were clearly differentiated from low and medium amylose rice. The loading plot (Fig 2A) clearly shows that the amylose content exhibits a negative correlation with the breakdown, peak viscosity, and protein content, while being positively correlated with the setback, final viscosity hardness, and cooking time. Two distinct groups of rice were initially identified. First, the rice cultivars with high amylose cultivars (high in SB, FV, hardness, and cooking time) clearly showed similar characteristics**.** However, the low and medium amylose cultivars (high in protein, breakdown, and peak viscosity values) were more variable, and not able to easily characterised. When other parameters were subsequently considered, rice samples with similar amylose content could be separated into distinct different groups.

Fig 3 illustrated classification results using Hardness, Breakdown, Setback, Final Viscosity and cooking time as differentiating parameters. It can be seen that selection of these 5 factors with correlation coefficient greater than 0.5 (Table 4) can increase total variance contributed by 1st and 2nd PCs to 86%. From Figure, the final viscosity, set back, cooking time and hardness showed high correlation with high amylose rice sample; whereas breakdown parameter correlated well with low amylose rice sample.

Classification of rice amylose content by discriminant analysis of physicochemical properties of 9 rice cultivars was carried out by Suwannaporn *et al*. (2007). They reported that protein content, which plays a major role in cooked rice texture, did not correlate with amylose content. In addition, discriminant analysis results showed that only pasting attributes could discriminate the amylose groups but not texture. Another study for rice classification approach using hydration and pasting properties of their starches instead of amylose content was conducted by Lee *et al*. (2012) in which 12 different cultivars were classified without considering the effect of aging. It was also reported that samples were separated into different groups although they possessed similar amylose content. They suggested that classification using the concept of practical process ability is a more appropriate and useful system than the classical approach of considering the amylose content only.

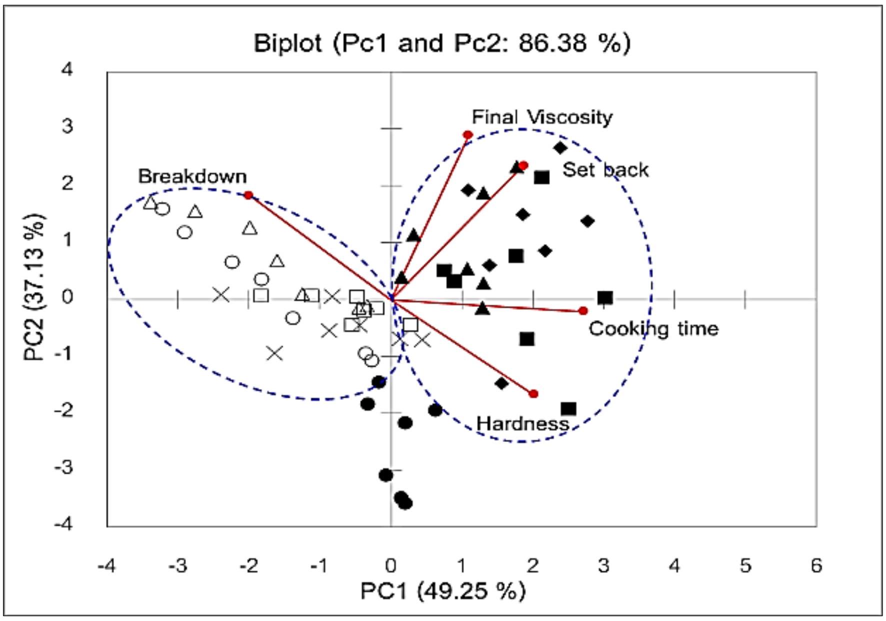

Figure 3 Classification results using hardness, breakdown, setback, final viscosity and cooking time as differentiating parameters

4. Conclusions

From this study, it was found that grouping of rice cultivars suitable to the rice industry do not solely depend on amylose content, but importantly depend on a combination of many factors, including the interaction between rice starch and other components such as protein, and fat, as well as thermal and pasting parameters, all of which play an important role in precise rice classification. The aging process also affects changes in many aspects of rice grains, contributing to the classification results. Our approach to the classification of rice, based on the combinations of chemical and physico-chemical properties of the rice cultivars, is more precise for rice classification and is therefore a valid alternative classification method of benefit to the industry. Therefore, classification of the rice starch categorized by similarity on the process ability of the cultivar by its food applicable properties was appropriate for management of rice starch quality.

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