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# Effect of amylose content on structure, texture and $\alpha$ -amylase reactivity of cooked rice

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#### ABSTRACT

The susceptibility of cooked rice to  $\alpha$ -amylolysis were studied in four Taiwanese rice cultivars differing in amylose contents, i.e. Taichung Native 1(TCN1, *indica*), Taigung 9 (TG9, *japonica*), Taichung Sen Waxy 1 (TCSW1, *indica* waxy), and Taichung Waxy 70 (TCW70, *japonica* waxy). In addition, the correlation between  $\alpha$ -amylolysis and the microstructure and textural properties of the four rice cultivars was investigated. The hardness, gumminess and chewiness of cooked rice followed the order of TCN1>TG9>TCSW1>TCW70. However the waxy rice cultivars showed a higher extent and rate of  $\alpha$ -amylolysis. Using scanning electron microscopy, the microstructure revealed that the low-amylose rice and waxy rice cultivars contained hollow in the central endosperm. The internal hollow disappeared after  $\alpha$ -amylolysis, indicating that  $\alpha$ -amylase had penetrated the cooked grain, resulting in the inside-out hydrolysis. These results indicated that the textural properties of cooked rice are influenced mainly by its amylose/amylopectin ratio, followed by influencing the pattern of  $\alpha$ -amylolysis.

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#### 1. Introduction

Rice is the one of world's most important cereal crops for human consumption, providing staple food for over half the world's population. Rice variety, drying and storage conditions, rough rice moisture content, amylose content, starch type, degree of milling, water to rice ratio, cooking methods, pre-cooking and post-cooking processing are the deciding factors in the cooking and textural characteristics of cooked rice (Champagne et al., 1998, 1999; Meullenet, Marks, Griffin, & Daniels, 1999; Meullenet, Marks, Hankins, Griffin, & Daniels, 2000; Perez, Bourne, & Juliano, 1996; Perez, Juliano, Bourne, & Anzadua-Morales, 1993). In particular, a change in amylose content has a greater impact on the texture of the cooked rice than do physical attributes, such as granule morphology, crystallinity and size distribution (Ong & Blanshard, 1995).

The rate and extent of starch digestibility are affected by many factors, including: starch granule structure, crystal type, granule size, amylose/amylopectin ratio, molecular structure, and the

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interaction between starch and other components (Benmoussa, Moldenhauer, & Hamaker, 2007; Sasaki et al., 2009). Amylose content is known to have an obvious impact on starch hydrolysis, and high-amylose rice starch is expected to be resistant to digestion (Hu, Zhao, Duan, Linlin, & Wu, 2004).

There have been few attempts to clarify the dependence of the textural properties of cooked rice on the  $\alpha$ -amylolysis. Accordingly, this study attempts to elucidate the correlation between textural properties and microstructure and the phenomenon of  $\alpha$ -amylolysis of cooked rice. Four Taiwanese rice cultivars differing in amylose contents: Taichung Native 1(TCN1, *indica*), Taigung 9 (TG9, *japonica*), Taichung Sen Waxy 1 (TCSW1, *indica* waxy), and Taichung Waxy 70 (TCW70, *japonica* waxy), were selected for sample.

#### 2. Experimental

#### 2.1. Rice material

Milled rice of Taichung Native (TCN1, *indica* variety), Taigung (TG9, *japonica* variety), Taichung Sen Waxy 1 (TCSW1, *indica* waxy variety) and Taichung Waxy 70 (TCW70, *japonica* waxy variety) of first crop of 98' were obtained from the Taichung District Agriculture Improvement Station. The content of apparent starch was







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measured by using amyloglucosidase/ $\alpha$ -amylase method (Total starch assay procedure, Megazyme International Ireland Ltd., Sydney, Australia), and the quantities were 79.4, 77.13, 84.98 and 87.98 g/100 g dry weight for TCN1, TG9, TCSW1 and TCW70, respectively.

#### 2.2. Amylose analysis

Amylose content was measured by the modified method (Juliano et al., 1981). Milled rice grains were ground into flours with a cyclone sample mill (Cyclotec 1093, Tecator, Höganäs, Sweden) and passed through a 100-mesh (0.149 mm) sieve. Rice flour was defatted immediately using hexane with the Soxhlet apparatus. Then 100 mg of rice flour put in a conical flask, to which 1 mL of 95 mL/100 mL ethanol and 9 mL of 1 mol/L NaOH were added. The suspension was kept at ambient temperature for 16–24 h. and then distilled water was added to make 100 mL solution. A 5 mL aliquot of the solution was transferred to a 100 mL volumetric flask, and to adjust pH, 1 mL of 1 mol/L acetic acid was added. Then 2 mL of 0.2 g/ L iodine solution (I<sub>2</sub>: 2 g/KI: 20 g/L) and distilled water were added to make exactly 100 mL. Spectrophotometer measurements were made at 620 nm after the above starch-iodine solution was incubated for 20 min at ambient temperature. Standard curves was generated using the mixture of potato amylose (A0512, Sigma Chem. Co., St. Louis, MO, U.S.A.) and defatted TCW70 rice flour.

#### 2.3. Cooking

Rice sample of 5 grains were mixed with water (in a ratio of 2:2.33 g/g) in a beaker and was sealed with a sheet of thin aluminum foil. Thirty min later, the samples were put in an automatic rice cooker (TAC-10H Tatung Co., New Taipei City, Taiwan) and cooked for 15 min. The rice grains were kept covered for additional 30 min. After cooking, the rice grains were allowed to sit for 30 min at ambient temperature before preparation for further analysis.

#### 2.4. Texture analysis

The textural properties of cooked rice were determined by using a texture analyzer (Stable Micro System, TA-XT2i, Surrey, UK). After cooking, one kernel of cooked rice was immediately placed inside the test cylindrical probe of 100 mm diameter and compressed the kernel to 0.5 deformation at a pre-test speed of 2.0 mm/s, test and post-test speed of 5.0 mm/s. A force—time curve was obtained from the test and the measurements of hardness, cohesiveness, gumminess, chewiness and resilience were computed using the Texture Expert software supplied with the instrument.

#### 2.5. Enzyme hydrolysis

Rice sample of 5 grains were mixed with water (in a ratio of 2:2.33 g/g) in a screw cap tubes. After the cooking procedure mentioned in section 2.3, 20 mmol/L phosphate buffer (pH 6.9,

with 0.02 g/100 mL Na N<sub>3</sub>) was added to make 5 mL solution. A  $\alpha$ amylase solution (0.5 mL, 600U, EC3.2.1.1 *Bacillus* sp., Sigma Chemicals Co., St. Louise, MO, U.S.A.) was added to the cooked rice suspension. The sealed tube was then placed in a shaking water bath 37 °C for 10, 20, 30, 40, 50, 60, 180, 360, 540, 720, 1080, 1440 min. Next,  $\alpha$ -amylase was inactivated immediately by placing the tubes containing the aliquots in boiling water for 5 min. The amount of hydrolyzable carbohydrate and reducing sugar were then quantified according the phenol-H<sub>2</sub>SO<sub>4</sub> method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and Somogyi-Nelson method (Somogyi, 1945), respectively. All measurements were performed in triplicate.

#### 2.6. Scanning electron microscopy (SEM)

The morphological changes of cooked rice grains were observed using a scanning electron microscopy (JSM-5400, JEOL, Tokyo, Japan) at 10 kV. The cooked rice grains were freeze-dried by liquid nitrogen. After cutting using scalpel, the cross section of cooked grains was observed. Samples were attached to an SEM stub using a double-backed cellophane tape. The stub and sample were coated with gold—palladium by an SPI-Module Sputter Coater and then examined and photographed.

#### 2.7. Statistical analysis

Analysis of variance and the significance of differences among samples were analysed with the ANOVA procedure and Duncan's multiple range test of SAS for Windows R 8.0 (SAS Institute Inc., Cary, NC), respectively.

#### 3. Results and discussion

#### 3.1. Amylose content and textural properties

The cultivar TCN1 had high amylose content (31.9195 g/100 g dry weight), and the cultivar TG9 had an intermediate amylose content (17.78 g/100 g dry weight). The remaining two cultivars, TCSW1 and TCW70, were waxy, with starch consisting exclusively of amylopectin (Table 1).

Table 1 shows some of the textural properties of the cooked rice grains. TCN1, with the highest amylose content, had the highest hardness, whereas *japonica* waxy, TCW70, was the least one. This clearly indicates that the higher the amylose content, the higher hardness will be. TCN1 also had the higher values for cohesiveness, gumminess, chewiness and resilience: 0.688, 362, 360 and 0.859, respectively. All of the textural parameters demonstrated a strong positive correlation with amylose content (Table 1), indicating that amylose content is the most important influence on each of these parameters. Previous finding concerning the influence of amylose content on rice texture were confirmed: cooked rice with low amylose content is soft and sticky, while rice with high amylose is firm and fluffy (Perdon, Siebenmorgen, Buescher, & Gbur, 1999).

#### Table 1

Amylose contents and textural parameters of freshly cooked rice by textural profile analysis (TPA).

Variety	Amylose content (g/100 g)	Hardness (g)	Cohesiveness (dimensionless)	Gumminess (g)	Chewiness (g)	Resilience (dimensionless)
TCN1 TG9 TCSW1 TCW70	$\begin{array}{l} 31.91 \pm 0.18a,^a \\ 17.78 \pm 0.22b \\ 0.92 \pm 0.09c \\ 1.00 \pm 0.09c \end{array}$	$526 \pm 17a$ $347 \pm 11b$ $175 \pm 4c$ $114 \pm 13d$	$\begin{array}{l} 0.688 \pm 0.003a \\ 0.574 \pm 0.020b \\ 0.436 \pm 0.025c \\ 0.480 \pm 0.023c \end{array}$	$362 \pm 13a \\ 199 {\pm} 9b \\ 76 {\pm} 5c \\ 55 {\pm} 4d$	$360 \pm 14a \\ 197 \pm 7b \\ 73 \pm 5c \\ 53 \pm 4c$	$\begin{array}{l} 0.859 \pm 0.034a \\ 0.672 \pm 0.030b \\ 0.503 \pm 0.054c \\ 0.588 \pm 0.052c \end{array}$

<sup>a</sup> Means  $\pm$  standard deviations, n = 3 and 5 for amylose content and TPA analysis, respectively; means within the same column with different letters are different significantly at p < 0.05.

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