Variations in *in Vitro* Starch Digestion of Glutinous Rice Flour

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Although there have been extensive studies on nonglutinous cultivars of rice (*Oryza sativa* L.), relatively few studies have focused on glutinous rice. The present study was carried out to examine the *in vitro* digestion characteristics of the glutinous rice endosperm starches in relation to their physical traits. Twelve glutinous cultivars were classified into two physically different groups (groups I and II) on the basis of their X-ray diffraction patterns. Starches of rice flour from these cultivars were digested *in vitro* by α-amylase and thereafter by glucoamylase. The digestion percentages of starches by glucoamylase were significantly higher in the cultivars in group II than in those in group I at every sampling time, except for those at 24 h, and were also significantly higher at and after 6 h in the case of α-amylase digestion. These results suggest that the physical traits of glutinous rice starches in groups I and II may be closely associated with their biochemical traits. Kjeldahl N concentrations were not significantly different between groups I and II, suggesting that it is not related to the physical and chemical traits of glutinous rice.

Keywords: *Rice; Oryza sativa; starch; amylase; glucoamylase; nitrogen*

INTRODUCTION

Glutinous cultivars of rice (*Oryza sativa* L.) comprise one of the main crops in Thailand and Laos and are also minor crops in several other countries throughout the world. Generally, these starches consist entirely of amylopectin with a complicated structure (Kainuma, 1980). However, most studies of the physicochemical traits of white rice grains have focused mainly on the properties of nonglutinous rice. These studies reported mainly on the relationship between eating quality and amylose and/or Kjeldahl N content (Chikubu et al., 1983; Juliano et al., 1964; Williams et al., 1958), differences in elasticity and stickiness of cooked rice (Ebata and Hirasawa, 1982; Ebata et al., 1989; Okazaki and Miyazaki, 1966), and the interior disintegration of starch granules and/or storage cells with cooking (Okazaki and Miyazaki, 1966; Sasahara et al., 1980). In contrast, only two studies (Juliano et al., 1964; Nagato and Kishi, 1966) focused on the physicochemical traits of glutinous rice.

Many structural models concerning starches of rice and other crops have been proposed (Kainuma, 1980; Kainuma and French, 1971; Manners, 1985). However, it appears that none of these models has been successful for all starches, suggesting the complexity of starch structures. Furthermore, only two studies (Fukui et al., 1964; Fuwa, 1977) have examined the relationship between the structural traits of glutinous rice starches and their physical and biochemical characteristics. These facts suggest that studies on the relationship between the physical and biochemical traits of glutinous starches may provide a new means for the assessment of new cultivars of both glutinous and nonglutinous rice.

The present experiments were carried out to examine differences in the degree of *in vitro* starch digestion and Kjeldahl N concentrations between the white rice flours of glutinous rice cultivars having different physical traits.

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Table 1. Cultivars Used and Their N Concentrations

cultivar[a]	discriminant score[b]	N concn[c] (mg kg^{-1} DW)
group I		
Miyatamamochi	1.35	10.29 ± 0.16
Surugamochi	1.36	9.03 ± 0.20
Himenomochi	1.38	9.61 ± 0.36
Tanchomochi	1.45	8.66 ± 0.10
Kaguramochi	2.04	10.62 ± 0.39
Mangetsumochi	3.98	9.84 ± 0.33
group mean		9.67 ± 0.30
group II		
Jugoyamochi	−2.00	9.74 ± 0.25
Shigahabutaemochi	−1.42	10.21 ± 0.22
Kurenaimochi	−1.23	9.64 ± 0.43
Koganemochi	−0.36	11.18 ± 0.43
Himenomochi	−0.33	9.06 ± 0.29
Mochiminori	−0.17	7.88 ± 0.12
group mean		9.62 ± 0.45

[a] These glutinous rice cultivars belong to an eco(sub)species, *japonica*, that is one of the three eco(sub)species, *japonica*, *indica*, and *javanica*, of *Oryza sativa* L. [b] These values were used for classifying the cultivars on the basis of X-ray diffraction patterns (Zhang et al., 1993). [c] Data are the mean and standard error of three measurements.

MATERIALS AND METHODS

Samples. Twelve glutinous rice cultivars of the *japonica* eco(sub)species were used in this study (Table 1). The cultivars were classified into two groups (groups I and II; six in each group) on the basis of the X-ray diffraction patterns of their flours (Zhang et al., 1993) (Table 1). Seeds were planted in nursery paper pots, and the seedlings were grown in a greenhouse at Yamagata University for 30 days.

Thereafter, seedlings with three to four leaves were transplanted to a paddy field of the University farm in the Shonai plain of Yamagata prefecture and grown under ordinary cultural conditions. Seeds were harvested at ripened stages of each cultivar.

Sample Preparation. After drying at room temperature, the seeds were stored in vinyl bags with silica gel for 3 weeks until their water content reached ca. 140 g kg^{-1} of dry weight. The rate of milling was 10%. Starch flour was prepared

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according to the method of Yamamoto et al. (1973) as follows: Milled rice grains were ground below a 48-mesh flour by a mortar and pestle and passed through a 297-μm sieve. This flour (ca. 1 g) was suspended in 5 mL of 0.2% (w/v) NaOH and stirred at 5 °C for 3 h. A precipitate was allowed to settle and the supernatant removed. The residues were washed with distilled water until they showed no phenolphthalein color reaction and passed through a 100-mesh (150 μm) sieve. Finally, the starch flour was dehydrated by suspending it in alcohol and drying the solution at 30 °C for 15 h.

Starch Digestion. The starch flour (50 mg) was suspended in 1.5 mL of a 0.033% (w/v) solution of α-amylase (Wako Pure Chemical Industries Ltd., Tokyo, Japan) according to the method of Fuwa et al. (1977). The flour−enzyme mixture was incubated at 37 °C, and then the reaction mixture (0.15 mL) was sampled at 1, 3, 6, 12, and 24 h after the start of incubation.

At the same time, mixtures of the flour and α-amylase (0.15 mL) were prepared and incubated for 24 h as in the above α-amylase digestion but without periodic sampling. The mixtures were then cooled to 0 °C to inactivate α-amylase activity and vacuum-dried. The dried reaction mixture was dissolved in 5 mL of a 0.2% (w/v) solution of glucoamylase (Wako Pure Chemical Industries, from *Rhizopus* sp.). Glucoamylase digestion (0.5 mL reaction mixture) was conducted under the same incubation conditions and sampled at the same time intervals as in the α-amylase digestion. After starch digestion by α-amylase and glucoamylase, the total water-soluble carbohydrate concentrations (TWSCC) were determined using 12.5 μL of the reaction mixture by the phenol−sulfuric acid method (Dubois et al., 1956). This was then used to calculate the percent digestion of the rice flour.

After the seeds were dried at 85 °C for 3 days, the Kjeldahl N concentration was determined using a Kjeltec 1026 system (analyzer) (Nippon General Co., Ltd., Tokyo, Japan).

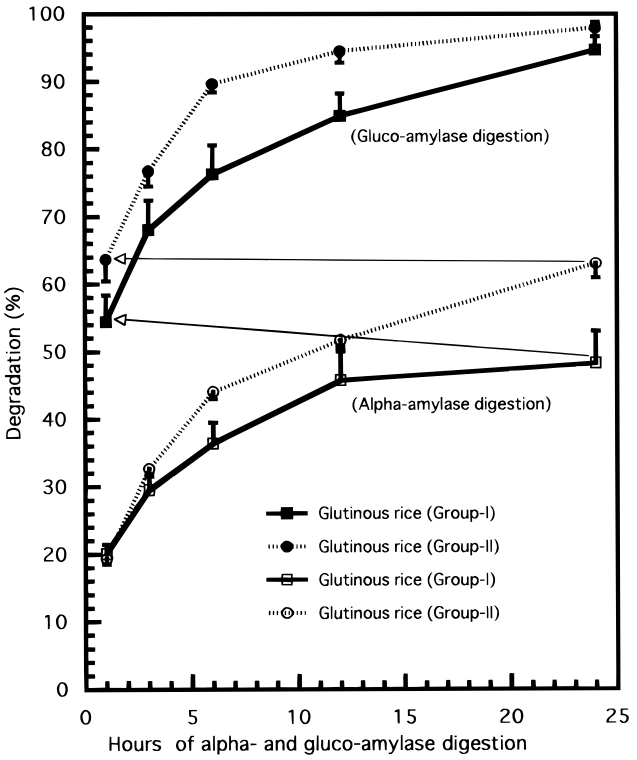

Figure 1. Percent digestion of starch by α-amylase and glucoamylase with time. Vertical bars indicate 0.5 standard error. Solid arrows indicate that the cultivar groups I and II were treated with glucoamylase after digestion by α-amylase for 24 h.

RESULTS AND DISCUSSION

The percent digestion of different rice flours by α-amylase tended to be significantly higher in group II than in group I of *japonica* glutinous rice by 6 h of incubation (Figure 1). The percent digestion of the same rice flours by glucoamylase was also higher in group II than in group I except at 24 h, at which time the difference was insignificant (Figure 1). The correspondence between the digestibility of the starches and their X-ray diffraction patterns (Zhang et al., 1993) suggests that the biochemical and physical traits of these types of rice are closely associated.

The results of the α-amylase and glucoamylase digestions appear to indicate that the endosperm starches of glutinous cultivars in group II have a looser structural arrangement at the molecular level than do those in group I. This would allow the enzymes to penetrate rapidly into the starch granules in group II.

The present results concerning glutinous rice may also prove to be useful in evaluating breeding programs that are designed to improve the quality of nonglutinous rice. Hitherto, attempts to improve the latter have mainly focused on decreasing the amylose content (Chikubu et al., 1983; Juliano et al., 1964; Williams et al., 1958). However, the amylose content of nonglutinous rice is now quite low (amylopectin accounts for >80% of the mass of milled nonglutinous rice grains). Thus, other approaches to improving quality, such as changing the structure of the amylopectin, may be more rewarding. The present biochemical method, which appears to reveal information about the structure of amylopectin, may thus be useful in this regard.

Kjeldahl N concentration of milled rice grains exhibited nonsignificant variations between group I and group II cultivars (Table 1). Variance analyses showed that cultivar variations in Kjeldahl N concentrations were significantly different ($p < 0.005$) within groups and throughout the 12 cultivars (data not shown). Thus, it appears that the Kjeldahl N concentration was not related to the X-ray diffraction patterns or digestibility of glutinous rice and, furthermore, may change greatly with environmental factors.

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