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VARIETAL DIFFERENCES IN PROPERTIES OF RICE STARCH GRANULES

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ABSTRACT

Crystallinity of native starch granules is now considered to be due to amylopectin instead of amylose. The crystalline fraction (Lintner starch), resistant to corrosion with 2.2 N HCl at 35°C, correlates positively with amylose content and gelatinization temperature of rice starch. Residual protein increases linearly with amylose content and is mainly the 60 k-Dalton *waxy* gene product whereas starch lipids are highest in intermediate and high amylose. Cooperative differential scanning calorimetry (DSC) of rice starch thermal properties in water showed an irreversible melting behavior. Both annealing and recrystallization take place during heating in the DSC. A three-phase model incorporating two distinct types of amorphous material and the crystalline domains of the amylopectin short-chain clusters was proposed to account for the thermal properties of granular starch/water mixtures. Thermomechanical analysis (TMA) volume expansion curves showed a one-stage swelling for waxy starch and a two-stage swelling for nonwaxy rice. The first is associated with the onset of gelatinization pehnomena (glass transition and partial melting) and the second coincided with the melting of starch crystallites. Retrogradation during storage of starch gels is also due to amylopectin.

Introduction

Starch is the major constituent of milled rice at 90% of dry matter (Juliano, 1985). It occurs as compound polyhedral granules 3-10 μm in size. It consists of a branched fraction, amylopectin and a linear fraction, amylose. Starch properties – amylose content, gelatinization temperature (GT), and gel consistency – index the eating quality of cooked rice. Raw starch granule properties such as GT are related to raw rice and cooking properties whereas cooked starch granule properties such as amylose content and gel consistency are more related to eating quality

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or cooked rice texture. Apparent amylose content of milled rice is classified as waxy (0-2%), low amylose (10-20%), intermediate amylose (20-25%), and high amylose (>25%). Final GT is classified as low (<70°C), intermediate (70-74°C), and high (>74.5°C) and is indexed by alkali spreading value in the breeding program as low (6-7), intermediate (4-5), intermediate-high (3), and high (2). Gel consistency is either hard (27-40 mm), medium (41-60 mm), and soft (61-100 mm).

Crystallinity of starch granules has been traditionally attributed to amylose because it readily forms a crystalline complex with 1-butanol but amylopectin does not (Lugay and Juliano, 1965). Recent studies have proven that crystallinity of starch granules is due to amylopectin (French, 1984). The crystalline fraction is more resistant to αamylolysis and acid corrosion than the amorphous fraction. Waxy rice starch granules are more susceptible to hog pancreatic α-amylase action than nonwaxy granules (Evers and Juliano, 1976). Susceptibility to corrosion with 2.2 N HCl at 35°C as indexed by loss of weight on treatment for 15 days may be an indicator of the amorphous fraction of rice starch granules (Maningat and Juliano, 1979).

Differential scanning calorimetry has been used to measure the enthalpy of gelatinization of rice starch (Stevens and Elton, 1971; Elberstein *et al.*, 1980; Maurice *et al.*, 1985). It was applied on rice starches that differ in amylose content and GT and are prepared from milled rice by sodium dodecyl benzene sulfonate (DoBS) extraction of protein (Russel and Juliano, 1983). Since these DoBS-prepared starches showed reduced enthalpy of amylose-lipid complex melting due to partial defatting, the study was repeated using starches prepared from milled rice by alkaline protease treatment, in conjunction with thermal mechanical analysis (dilatometry mode) (Biliaderis *et al.*, 1986). Starch granule is considered a partially-crystalline glassy polymer which, during gelatinization, is converted from glassy to rubbery amorphous materials plus crystallites (Biliaderis *et al.*, 1986).

Retrogradation of gelatinization is also due to amylopectin (Slade and Levine, 1987) which can be accelerated by four hours at 4°C (nucleation), followed by heating at 42°C for four hours at 80% rh (propagation) (Slade and Levine, 1987). The degree of gelatinization and retrogradation of cooked rice and rice starch can be determined by the β-amylase-pullulanse method (Matsunaga and Kainuma, 1981). Thirty freeze-thaw cycles were also used to accelerate retrogradation of gelatinized granules (Matsunaga and Kainuma, 1986).

This paper summarizes our recent studies on varietal differences in properties of raw and cooked rice starch granules as affected by amylose content and GT and presents model systems to explain varietal differences in cooking and eating qualities of milled rice.

Materials and Methods

Rough rice samples were obtained mainly from the IRRI farm and dehulled in a THU-type Satake dehuller and milled either in a McGill Miller No. 3 or a Satake

TM-05 pearler. Starch was prepared from brown or milled rice either by DoBS extraction of protein or by alkaline protease digestion at 0.5% of the protein content, and sieving. To prepare it in retrograded form, starch was soaked for 10 minutes, cooked for 10 min. with 2.6 times by weight of water at 90°C, cooled for four hours or overnight at 40°C, and heated for four hours at 42°C at 80% rh (Slade and Levine, 1987).

Lintnerization of starch granules was done at 35 ± 1°C in 2.2 N HCl at a ratio of 1.5 g/100 ml for 15 days with daily hand shaking to resuspend the starch (Maningat and Juliano, 1979). Nonstarch lipids were first extracted with five volumes of $CHCl_3$-CH_3OH (2:1) for eight hours each, followed by two extractions with water-saturated butanol (WSB) for 30 minutes each including 10 min. centrifugation at 2000 g. The starch samples were air-dried and starch lipids were then extracted three times with five volumes of WSB for a total of 24 hours under N_2 atmosphere at 25-27°C (Maningat and Juliano, 1980). Residual starch was then extracted three times with five volumes of WSB (63:37 BuOH:H_2O) at 92°C for eight hours each. Extracted lipids were weighed after solvent removal.

Residual protein was measured by micro Kjeldahl analysis using the factor 5.95. SDS-polyacrylamide disc gel electrophoresis was performed on 10% gels, using standard proteins for molecular weight standards. Protein was extracted from rice starch granules by boiling for five minutes in 2.3% SDS, 5% β-mercaptoethanol, and 10% glycerol in 0.055 M Tris pH 6.8 buffer (Villareal and Juliano, 1986).

DSC studies were carried out on a Du Pont 1090 thermal analyzer equipped with a pressure DSC with 7-12 mg starch A Du Pont 943 thermomechanical analyzer equipped with a volume dilatometer probe was used on 250 mg starch to examine the volume expansion changes in 50% starch/water mixtures as a function of temperature (Biliaderis *et al., 1986).*

Results and Discussion

Degree of crystallinity

The degree of crystallinity, as indexed by 15-d Lintner starch granules, was lowest for waxy rice and highest for intermediate and high amylose granules (Figure 1). Lintner starch recovery correlated positively with amylose content (r = 0.44**) and final GT (r = 0.69**), despite the fact that amylopectin is the crystalline fraction of starch granule (French, 1984). An exception was IR48 starch which gave low Lintner starch recover (1.4%) similar to that of waxy rice. Another IR48 sample had 10% recovery. Highest Lintner starch recoveries (18-20%) were obtained for high GT intermediate amylose starches Ganagala and Yilan-chutze. Corrosion of the amorphous fraction was confirmed to be completed within seven to eight days and was shorter for low GT than for intermediate GT samples (Maningat and Juliano, 1979).

High-amylose rices differing in gel consistency – IR42 (hard), IR36 (medium), and IR32 (soft) – had similar X-ray diffractograms suggestive of similar degrees of crytallinity (S. Hizukuri, 1987 pers. comm.).

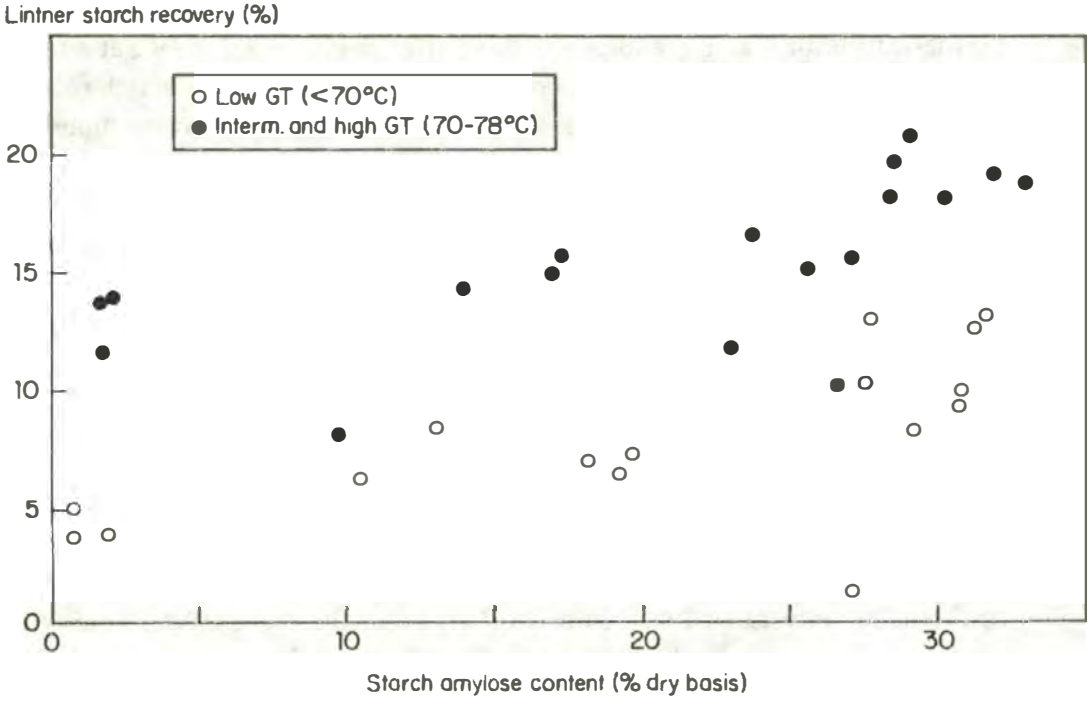

Figure 1. Scattergram of granule crystallinity indexed by Lintner starch recovery of rice starch granules differing in amylose content and final gelatinization temperature (Maningat and Juliano, 1979; IRRI, 1988, unpublished data).

Residual protein

The residual protein of indica rice starch is related positively to amylose content (Juliano, 1985). SDS-polyacrylamide disc gel electrophoresis confirmed that the major protein in nonwaxy starch (85-100%, mean 97%) was the *waxy* gene product with subunit molecular weight of about 60 k Daltons (Villareal and Juliano, 1986). The *waxy* gene product was absent in waxy starch granules. Japonica, javanica (bulu), and upland rice starch tended to have less *waxy* gene factor than indica rice starch at similar amylose content, thus verifying the observations of Sano *et al.* (1986).

Differential scanning calorimetry

Nonwaxy samples had lower gelatinization (melting) enthalpies than waxy starch of similar GT (Figure 2a). This observation can be explained by considering

that crystallization (exothermic) of amylose-lipid complexes occurs simultaneously and immediately after the onset of the thermal events. Intermediate and high GT starches had higher gelatinization enthalpies than low GT starches of similar amylose content, GT, but not amylose, correlated significantly with transition temperature, glass transition temperature (T_g), peak transition or gelatinization temperature (T_p), completion or melting temperature (T_c or T_m), (r = 0.95**-0.98**), and gelatinization enthalpy (r = 0.84**). Gelatinization enthalpy was higher for 50% rice starch pastes in water than for 20% starch pastes. Combined correlation coefficient of gelatinization enthalpy in Figure 2a (n = 38) was -0.58** for amylose and 0.78** for GT.

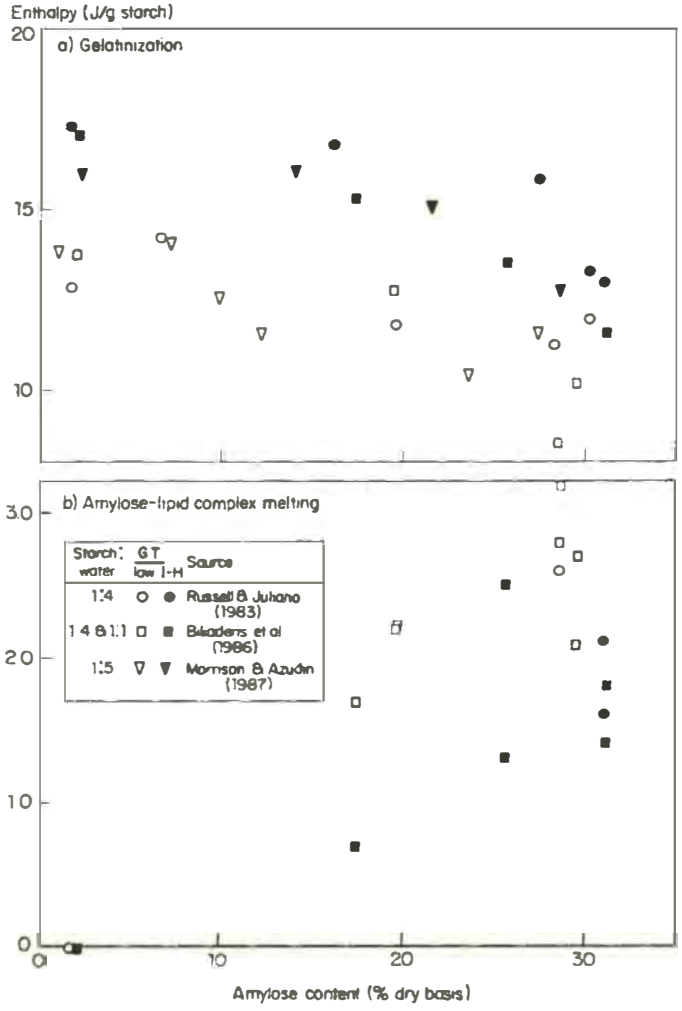

Figure 2. Effect of amylose content and gelatinization temperature of starch granules on enthalpy of (a) gelatinization and of (b) amylose-lipid complex melting prepared by dodecyl benzene sulfonate (Russel and Juliano, 1983) and by alkaline protease method (Biliaderis *et al.*, 1986; Morrison and Azuden, 1987). Data of Eberstein *et al.* (1980) are included.

Enthalpy of reversible amylose-lipid complex melting was present only in nonwaxy starch and tended to be higher for low GT starches ($r = -0.94^{**}$) in both 20% and 50% starch/water mixtures (Figure 2b). Combined correlation coefficient of enthalpy of amylose-lipid complex melting ($n = 17$) in Fig. 2b was 0.73** with amylose and 0.58* with GT. Intermediate- and high- amylose starches have similar enthalpies of amylose-lipid complex melting with IR480-5-9 having the highest enthalpy. IR480-5-9 also had the highest starch lipids (0.94%), as also reported by Morrison and Azudin (1987). Starch lipids tended to be highest for intermediate- and high-amylose rices but the reverse is true for nonstarch lipids of milled rice (IRRI, 1986) Figure 3). The leveling off of starch lipids content may be attributed to the poorer lipid binding of long chain amylopectin as compared to amylose: the higher colorimetric amylose value of high-amyose rice over low-amylose rice was shown to be due to high-iodine binding amylopectin (Takeda *et al.*, 1987). Starch lipids are mainly free fatty acids and lysophospholipids (Choudhury and Juliano, 1980; Maningat and Juliano, 1980). Phosphorus content of starch granules is mainly 6-phosphoglucose ester in wax rice, but is mainly lysophospholipids P in nonwaxy rice starch (Hizukuri *et al.*, 1983).

Among the eight rice starches examined, distinct evidence for both first- and second-order transition phenomena was shown in the thermal curves of IR2071-137-5. An incremental change in heat capacity (Cp) before the main melting peak was observed for the various starch/water mixtures examined, typical of an amor-

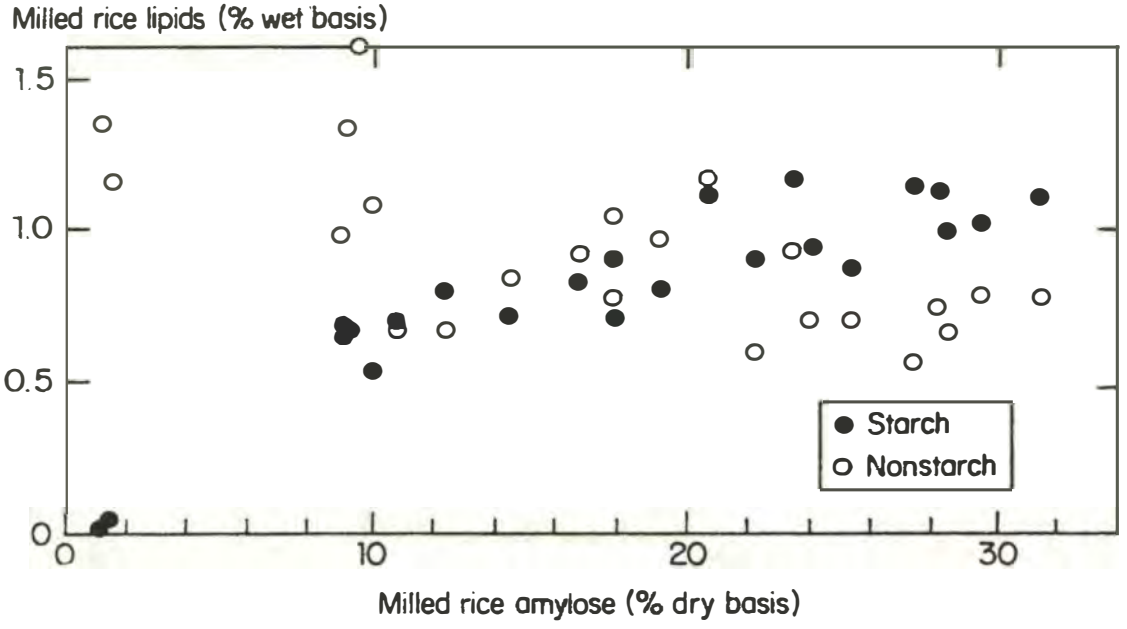

Figure 3. Scattergram of nonstarch and starch lipids of milled rices differing in amylose content (IRRI, 1986).

phous phase passing through the glass transition. All transition temperatures, T_g, T_p, and T_c (T_m), increased with decrease in moisture and converged to a high temperature region of 240-250°C for the dry sample. In particular, the T_g showed a monotonic increase at water contents <30%. Above this level, T_g remained constant at 68°C. There was a very good correlation between the volume fraction of water (v_1), and the melting temperature (T_m) at intermediate- or low-water contents (v_1 0.1-0.7). Thus, the minimum requirement for water to fully exert its plasticizing effect on IR2071-137-5 granules was 30%. Above this level, water forms a separate pure solvent phase outside the granules. T_m was depressed by water and was always above the T_g, indicating that regardless of the amount of water present, melting is T_g-dependent in that a previous softening (relaxation) of the amorphous parts is required before crystallite melting can commence.

Thermal behavior of granular starch can best be described by a three-phase model on the basis of its morphological and molecular features – a fully ordered cyrstalline phase (amylopectin) and two amorphous phases: (a) bulk amorphous regions (amylose) that are responsible for the Cp at T_g; and (b) nonordered intercrystalline materials within spherulites that do not contribute to this heat capacity change. The latter is under strain. The plasticization behavior of the two amorphous phases will depend on the true plasticizer (water) content as well as the nature of these regions.

Thermomechanical analysis

Volume expansion at 90 and 95°C and total area of TMA curve were negatively correlated with amylose content ($r = -0.94^{**}$ to -0.97^{**}, and $r = -0.85^{**}$). Only a single stage gelatinization was noted for waxy rice as against a two-stage gelatinization for nonwaxy rice (Figure 4). It is interesting to note that despite the different heating rates employed (10°C/min. for DSC and 2°C/min. for TMA), the onset of both endothermic and dilatometric events occurred in the same temperature region. This is indicative of the changes in specific heat and expansion coefficient when passing through the glass transition. The sharp volume changes seen at the conclusion of the TMA curves correspond with the melting endotherm of the starch crystallites. The extended plateau in the TMA curve, following the glass transition region, can be attributed to the presence of: (a) new crystallites involving bundles of amylose-lipid helices; and/or (b) aggregated uncomplexed amylose chains or segments. The first stage is associated with the onset of the gelatinization phenomenon (glass transition and partial melting), and the second, with the melting of starch crystallites. Waxy rice starch, on other hand, after the glass transition region, mainly showed the second phase of volume expansion. This behavior clearly demonstrated the role of amylose in stablizing granule swelling during hydrothermal treatment.

Four different regions of volume expansion changes are noted: (1) form room temperature up to T_g, the sample undergoes reversible thermal expansion; (2) at T_g, the granules show an irreversible swelling due to the relaxation of the amorphous

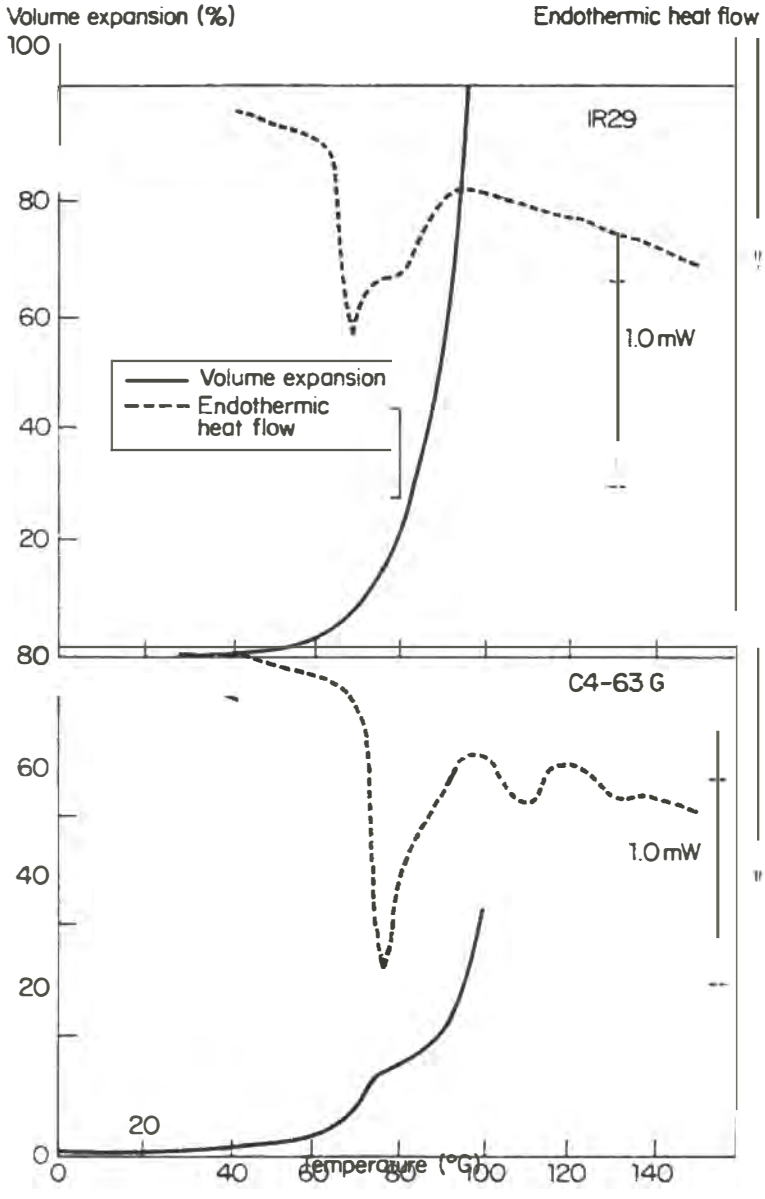

Figure 4. Differential scanning calorimetry and thermomechemical analysis thermal curves of IR29 (waxy) and C4-63G (nonwaxy) rice starches (Biliaderis *et al.*, 1986). Heating rate or DSC is 10°/min. and for TAM, 2°C/min.

granular material (mainly amylose) not bound or loosely connected to crystallites; (3) the intermediate plateau region, above the T_g, where the swollen granular structure does not show pronounced dimensional changes after partial melting. Secondary phenomena such as annealing of starch crystallites and crystallization of amylose-lipid complexes may also occur in this region; and (4) the abrupt volume expansion changes due to melting; the molecules are pulling out of the crystallites as are the crystallites from each other (Biliaderis *et al.*, 1986).

The application of DSC and TMA on whole grain milled rice would result in more relevant data on rice cooking and cooked rice. Cooking of rice grain takes longer than cooking of starch granules because of the water absorption and thermal gradients from the surface to the core of the grain.

Retrograded starch

Surprisingly, all retrograded starches of high-amylose rices IR8, IR42, IR32, and IR36 showed optimum lintnerization in 3-5 days in 2.2 N HCl at 35°C of 60-67% (mean 64% regardless of gel consistency differences, corresponding to 33-40% lintner or retrograded starch. In the native granule, low GT rices such as IR8 and IR42 had lower Lintner starch yields (6-8%) than intermediate GT rices such as IR32 and IR36 (10-12%). Defatting of the starch with WSB at 92°C before gelatinization to minimize amylose-lipid complexing gave similar Lintner starches for the gelatinized starch granules. However, hydrolysis of cooked starch with β-amylase-pullulanase increased with butanol defatting.

Summary and Conclusions

Although crystallinity of rice starch granules is due to amylopectin, crystallinity indexed by Lintner starch yield correlated positively with amylose content and GT. Protein in starch granules was mainly the waxy gene product with 60 kDalton molecular weight. Lipids were mainly free fatty acids and lysophospholipids and were highest in intermediate and high amylose and low GT rices. Enthalpy of gelatinization correlated positively with GT but was highest in waxy rice due to amylose lipid complex crystallinization (exothermic) in nonwaxy starch. Thus at intermediate- to low-water contents, the DSC thermal curve is not representative of the initial crystallite profile, but reflects the composite thermal effect of several processes that occur simultaneously during heating in the DSC – melting, annealing, and crystallization. This implies that melting is irreversible (nonequilibrium). The role of water (at levels <30%) is essential in that, acting as a plasticizer, it decreases the glass transition temperature of the amorphous parts of the granules, which in turn facilities the melting or reorganization of the starch crystallites and amylose-lipid complexes to occur at lower temperatures, as evidenced in the DSC thermal profiles of IR2071-137-5.

On the basis of well-known morphological and molecular characteristics of the amylopectin molecule, as well as the organization of the starch granule, a three-phase model is proposed to account for the thermal behavior of granular starch: starch crystallites (amylopectin), a bulk amorphous phase (mainly amylose) that can undergo thermal activation at T_g i.e., contribute to incremental heat capacity change associate with second-order transitions) and an intercrystalline amorphous phase (amylopectin region of dense branching) that does not possess the typical properties that characterize the previous phase.

The TMA volume expansion curves (50% starch) of the nonwaxy samples exhibited a two-stage swelling pattern. The first stage is associated with the onset of the gelatinization phenomena (glass transition and partial melting), and the second, with the melting of starch crystallites. On the other hand, the waxy rice starches after the glass transition region mainly showed the second phase of volume expansion. Volume expansion at 90 and 95°C and total area under the TMA curve correlated negatively with amylose content but not with GT. This behavior clearly demonstrated the role of amylose in stabilizing granule swelling during hydrothermal treatment.

The retrograded fraction obtained by lintnerization was shown to be similar in soft and hard gel high amylose cooked rice starch granules.

Acknowldgment

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