Exothermic Transitions in Whole Grain Milled Rice and Milled Rice Flour Studied by Differential Scanning Calorimetry

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ABSTRACT

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Cooling curves for long, medium, and short grain varieties and very low amylose varieties of whole grain milled rice and their milled rice flours were generated by differential scanning calorimetry at a constant cooling rate of 1.0°C/min. Two successive heating and cooling cycles were run, but data were reported only for the two cooling cycles. Exothermic transitions were produced from all varieties, at least during the first cooling cycle. Exotherms were attributed to the crystallization of amylose-lipid complexes within the starch granule and were quantified by determining enthalpies (ΔH) for the crystallization process. Exotherm formation during the first cooling cycle was influenced by the amount of rice lipid removed from the kernel or flour, the presence of intact kernel structure, and the amylose content of the rice. In most cases, exotherms were produced during the second cooling cycle and were smaller in magnitude than those originating from the first cooling. The degree of amylose-lipid complex formation during the second cooling was independent of rice lipid, kernel structure, and amylose content. These exotherms appeared to depend only on the particular rice variety examined.

Exothermic transitions, which are produced by programmed heating or cooling of samples in a differential scanning calorimeter (DSC), result from the evolution of heat during a conformational change of a specific molecule over a given temperature range. For a macromolecule such as starch, exotherms are usually observed when starch changes from a random (amorphous) to a structured (semicrystalline) state. In the case of native rice starch, programmed heating in a DSC produced melting and crystallization of starch-lipid complexes within the starch granule (Biliaderis et al 1986a). An exotherm was recorded between 110 and 120°C, which Biliaderis et al (1986a) suggested was the result of crystallization of starch-lipid complexes. Another exotherm was postulated to occur at a lower temperature upon heating but could not be directly observed due to the masking effect of the more prominent starch gelatinization endotherm (Biliaderis et al 1986b). Observable exotherms also have been reported during programmed cooling of amylose-monoglyceride complexes (Eliasson and Krog 1985, Biliaderis et al 1985) and are thought to be due to the organization of helical, amylose-lipid inclusion complexes into a supramolecular, semicrystalline structure (Biliaderis et al 1985).

As noted above, exothermic transitions were seen at temperatures greater than 110°C for rice starch. In a practical sense, thermal events occurring above 110°C would have little application to rice cooking since during the cooking process (including pressure cooking) rice is usually not exposed to such elevated temperatures. However, as also noted above, exothermic events were observed during cooling of model systems using amylose and purified monoglycerides. Since cooked rice is always cooled before eating and during storage, could exotherms occur in more complex systems such as rice kernels and rice flour after simulated cooking in a DSC? Priestley (1977) stated that the presence of amylose-lipid complexes in parboiled rice was responsible for the insolubility of amylose in rice recooked in boiling water. The prevalence of amylose-lipid complexes in rice retarded the leaching of amylose into the cookwater during cooking. The formation of amylose-lipid complexes in whole kernel rice could influence its cooking qualities during and after cooking (Morrison and Azudin 1987) and could significantly influence the retrogradation of starch in stored cooked rice (Hibi et al 1990). Therefore, the presence of amylose-lipid complexes in whole grain milled rice or rice flour after cooking may have practical significance.

Two previous articles (Normand and Marshall 1989, Marshall et al 1990) report on the examination by DSC of thermal properties of whole grain milled rice and milled rice flour of representative long, medium, and short grain varieties and very low amylose varieties. These studies were concerned mainly with the gelatinization of starch, and no exotherms were observed. Attention was focused only on the first and second heating cycles; thermal transitions occurring during the first and second cooling cycles were not recorded. Our objective in the present study was to examine the cooling curves after simulated cooking of several varieties of whole grain milled rice and milled rice flour in the DSC and to report on conditions that affected the observed exotherms.

MATERIALS AND METHODS

Materials

Whole grain milled rice and milled rice flour were prepared as described by Normand and Marshall (1989) from varieties Lemont (long grain), Mars (medium grain), S-201 (short grain), and Calmochi (very low amylose). Solvents (hexane and chloroform-methanol [C-M]) used for lipid extraction of milled rice and milled rice flour were reagent grade.

Methods

The preparation of samples for thermal analysis and a description of the calorimeter were described previously (Normand and Marshall 1989). To obtain data for this study, the DSC was programmed to complete two consecutive heating and cooling cycles. Samples with a moisture content of 70% were routinely heated from 20 to 110°C, held at 110°C for 5 min, then cooled to 20°C, and held for 5 min before repeating the second cycle under the same conditions. Heating and cooling rates were 1.0°C/min, with thermal data acquired at 10-sec intervals. Cooling curves were generated from each cooling cycle by comparing the sample to a water reference. The cooling curves were defined by their peak transition temperatures (T_p) and their exothermic enthalpies (ΔH). Enthalpies were calculated as the area under the transition curves defined by drawing a straight line between initial and final baselines (i.e., the points at which the initial and final heat capacities of the samples were constant).

Surface lipids were removed from whole kernel milled rice by the method of Hogan and Deobald (1961), except that hexane or C-M (2:1 v/v) were used as lipid solvents in place of petroleum ether. The extraction of lipid from rice flour was accomplished using AACC method 30-25 (AACC 1983), with hexane or C-M as the solvent. The percent of extractable lipid from whole grain milled rice was based on the amount of lipid removed from the same quantity of rice flour by C-M treatment. Both hexane and C-M were selected as lipid solvents to compare the effect on the rice cooling curves of two levels of lipid removal: low (hexane) and high (C-M).

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Amylose contents were determined by the method of Juliano (1971). Moisture values were obtained by oven-drying the samples at 105°C for 24 hr and were found to be 10–12% for all varieties.

RESULTS

Cooling Curves from Whole Grain Milled Rice and Milled Rice Flour

Thermal curves obtained during cooling of whole grain milled rice and milled rice flour samples revealed exothermic transitions that were present during the first and second cooling cycles (Figs. 1 and 2). Typical cooling curves are shown for S-201, Lemont, and Calmochi. These varieties were selected because they best illustrate the presence of the largest (S-201, Lemont) and smallest (Calmochi) exotherms of the four selected varieties. In every cooling curve examined, the single exotherm was the only transition observed between the temperature limits of 110 and 20°C. In addition, exotherms formed from the first cooling were always larger than those generated from the second cooling within every variety (Figs. 1 and 2).

Peak transition temperatures for kernels and flour samples are given in Table I. T_p values varied from 75 to 86°C depending on variety. The peak transition temperatures were about 10°C higher than those reported by Eliasson and Krog (1985) for amylose-monoglyceride complexes. Transition temperatures varied slightly with variety as Calmochi exhibited the lowest T_p. Exotherms generated during the first cooling always showed a higher T_p than during the second cooling.

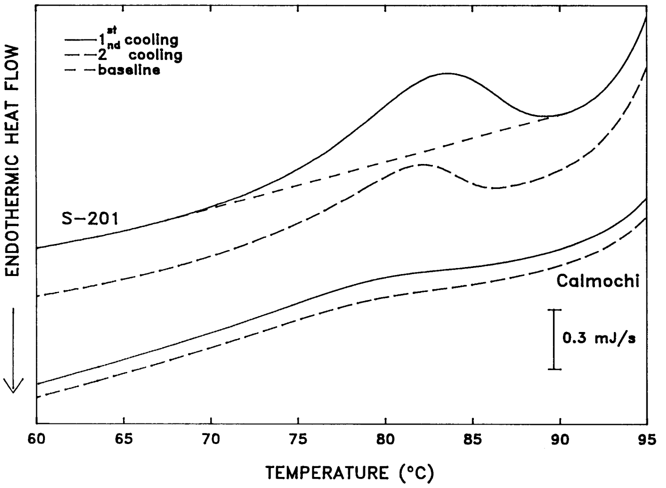

Fig. 1. Thermal curves of two different whole grain milled rice varieties (S-201 and Calmochi) obtained during the first and second cooling cycles in the differential scanning calorimeter. Scans were initiated at 110°C, and kernels were cooled to 20°C at 1.0°C/min. Water content of all samples was 70% (w/w).

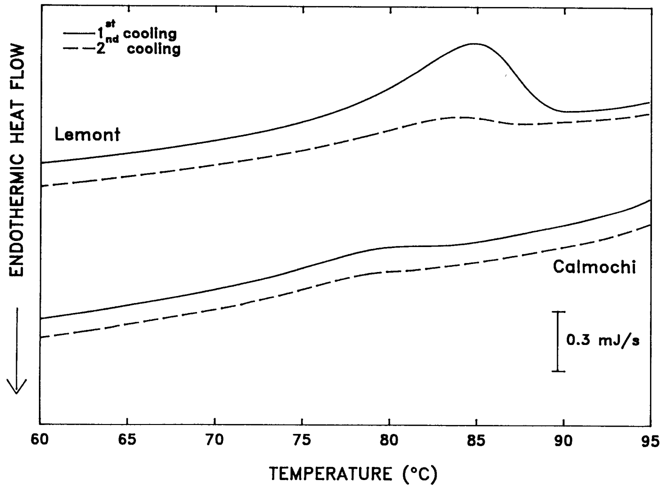

Fig. 2. Thermal curves of two different milled rice flours (Lemont and Calmochi) obtained during the first and second cooling cycles in the differential scanning calorimeter. Scans were initiated at 110°C, and flours were cooled to 20°C at 1.0°C/min. Water content of all samples was 70% (w/w).

Influence of Lipid Removal on Exothermic Transitions

Biliaderis et al (1985) and Eliasson and Krog (1985) determined that both lipid and starch (amylose) were necessary for the appearance of exotherms during cooling of previously heated amylose-monoglyceride complexes. In the intact rice kernel, both starch-associated lipid (starch lipid) and amylose can normally be found in the starch granule (Azudin and Morrison 1986, Morrison 1988), while sources of nonstarch lipid are usually outside of and separate from the granule (Morrison 1988).

Exposure of whole kernels to the apolar solvent hexane resulted in the removal of only a small percentage of the lipid, as follows: Lemont, 30%; Mars, 33%; S-201, 26%; and Calmochi, 46%. Apolar solvents are not effective at extracting starch lipids (Morrison 1988). Therefore, hexane probably extracted only nonstarch lipid from the kernels. C-M, a more polar lipid solvent than hexane, removed more lipid than hexane did, as follows: Lemont, 78%; Mars, 86%; S-201, 74%; and Calmochi, 87%. C-M may have removed nonstarch and some starch lipid from the kernels because Soxhlet extraction of starch granules with methanol is an effective method of removing starch lipids (Morrison 1988). Since C-M removed more lipid than hexane did, we can evaluate the amount of lipid removed as it influenced ΔH. The data in Table II show that treatment with lipid solvents actually increased ΔH during the first cooling in every variety except Calmochi when extracted and unextracted kernels were compared. When hexane and C-M treatments were compared, C-M extraction caused a decrease in ΔH during the first cooling (Table II).

Enthalpies were lower during the second cooling than the first cooling in both treated and nontreated samples. In some cases, particularly for Mars and Calmochi, no transitions were observed during the second cooling (Table II).

Enthalpies for milled rice flour and flour extracted with lipid solvents are presented in Table III. Exposure of flour to hexane resulted in removal of 39, 49, 41, and 62% of the nonstarch lipid from Lemont, Mars, S-201, and Calmochi, respectively. In contrast, treatment of rice flour with C-M removed essentially all of the lipid in all varieties. Treatment of flour with these lipid solvents decreased ΔH in all varieties when exotherms generated during the first cooling were compared. In addition, flours exposed to C-M showed consistently lower ΔH values than nontreated or hexane-treated samples.

As with the kernels, flour enthalpies were lower after the second cooling and demonstrated no consistent pattern.

When enthalpies generated during the first cooling for unextracted kernel and flour samples were compared within each variety (Tables II and III), values for kernels were consistently lower.

Influence of Amylose Content on Exothermic Transitions

Thus far we had determined the effect of lipid removal on the extent of exotherm formation. Since amylose may also be

TABLE I
Transition Temperatures (°C) for Cooling Curves of Unextracted Whole Grain Milled Rice and Milled Rice Flour[a]

Rice Variety	Whole Kernel First Cooling	Whole Kernel Second Cooling	Flour First Cooling	Flour Second Cooling
Lemont	82.5 ± 0.4	82.0 ± 0.4	84.7 ± 0.3	81.3 ± 0.4
Mars	81.6 ± 0.1	NT[b]	84.1 ± 0.4	75.4 ± 1.3
S-201	83.2 ± 0.8	81.7 ± 0.4	85.4 ± 0.1	83.9 ± 0.6
Calmochi	79.3 ± 0.2	78.4 ± 0.2	79.3 ± 0.1	78.6 ± 0.4

[a] Values are means ± SEM of triplicate determinations; moisture content of samples was 70%.

[b] No transition was observed.

TABLE II
Enthalpy (ΔH) Data from Cooling Curves of Whole Grain Milled Rice Extracted with Hexane or Chloroform-Methanol[a]

Rice Variety	Unextracted First Cooling	Unextracted Second Cooling	Hexane First Cooling	Hexane Second Cooling	Chloroform-Methanol First Cooling	Chloroform-Methanol Second Cooling
Lemont	0.77 ± 0.11	0.44 ± 0.10	1.33 ± 0.18	0.80 ± 0.05	0.99 ± 0.15	0.86 ± 0.14
Mars	0.32 ± 0.08	NT[b]	0.75 ± 0.04	NT	0.70 ± 0.12	0.50 ± 0.04
S-201	0.83 ± 0.15	0.40 ± 0.06	1.19 ± 0.07	0.33 ± 0.08	1.04 ± 0.08	0.61 ± 0.12
Calmochi	0.24 ± 0.09	0.20 ± 0.08	0.22 ± 0.06	0.13 ± 0.03	0.15 ± 0.02	NT

[a] Values are given as J/g of sample as means ± SEM of triplicate determinations; moisture content of samples was 70%.
[b] No transition was observed.

TABLE III
Enthalpy (ΔH) Data from Cooling Curves of Milled Rice Flour Extracted with Hexane or Chloroform-Methanol[a]

Rice Variety	Unextracted First Cooling	Unextracted Second Cooling	Hexane First Cooling	Hexane Second Cooling	Chloroform-Methanol First Cooling	Chloroform-Methanol Second Cooling
Lemont	1.72 ± 0.21	0.15 ± 0.02	1.48 ± 0.08	0.50 ± 0.05	0.90 ± 0.06	0.82 ± 0.13
Mars	1.31 ± 0.23	0.16 ± 0.02	1.11 ± 0.04	NT[b]	0.72 ± 0.03	0.44 ± 0.19
S-201	1.60 ± 0.02	0.15 ± 0.07	1.18 ± 0.17	0.15 ± 0.02	0.70 ± 0.06	0.48 ± 0.15
Calmochi	0.27 ± 0.07	0.12 ± 0.04	0.22 ± 0.01	0.16 ± 0.02	NT	NT

[a] Values are given as J/g of sample as means ± SEM of triplicate determinations; moisture content of samples was 70%.
[b] No transition was observed.

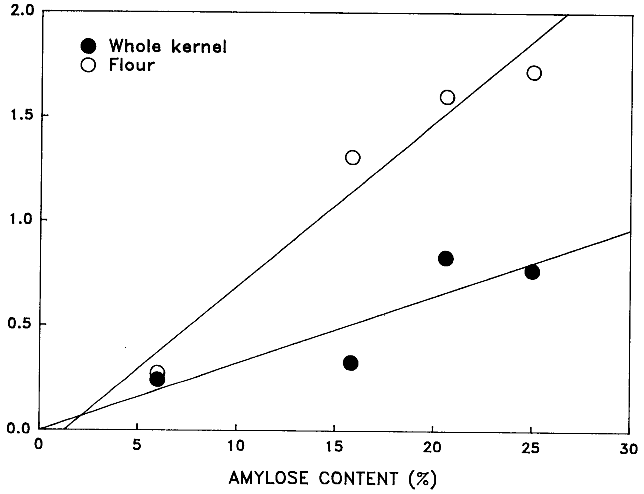

Fig. 3. Relationships between the amylose content of four different rice varieties (Lemont, 25.0%; Mars, 15.8%; S-201, 20.6%; and Calmochi, 6.6%) and enthalpies (ΔH) associated with the crystallization of amylose-lipid complexes. For whole kernel (●) samples, $R = 0.87$ and $P < 0.20$; for flour samples (○), $R = 0.97$ and $P < 0.05$. Enthalpies were obtained from exotherms generated during the first cooling cycle.

involved in the production of exothermic transitions during cooling, we wanted to determine the relationship between amylose content of the rice varieties and the degree of exotherm formation (ΔH). Figure 3 shows this relationship for data obtained during the first cooling. For intact kernels, only a weak linear relationship ($P < 0.20$) was seen between amylose content and ΔH. However, flour samples showed a strong linear relationship, significant at the $P < 0.05$ level. Data from the second cooling showed no direct relationship between variables for either kernel or flour, and the relationships are not shown. At least for the first cooling, amylose content of the rice appeared to be important in determining the extent of exotherm formation.

DISCUSSION

We have shown the presence of exothermic transitions during cooling of whole kernel milled rice and milled rice flour when these samples were subjected to simulated cooking in a DSC. The involvement of lipid (Tables II and III) and amylose (Fig. 3) in exotherm formation in our studies and the previous observations of exotherm formation during cooling of amylose-lipid complexes (Eliasson and Krog 1985, Biliaderis et al 1985) suggest that the exotherms we observed were due to the formation of amylose-lipid complexes.

Eliasson and Krog (1985) and Biliaderis et al (1985) found endothermic transitions during heating of amylose-lipid complexes, which were due to melting or dissociation of these complexes, prior to the observation of exothermic transitions. After completion of the first and second heating cycles of the samples found in Table II, Marshall et al (1990) found no observable endotherms in untreated or treated samples that could be attributed to melting of amylose-lipid complexes in whole kernel milled rice. They observed a starch gelatinization endotherm but no second, higher temperature endotherm. Since exotherms attributed to crystallization of amylose-lipid complexes were observed in the present study (Figs. 1 and 2), we believe that melting of these complexes did occur, but the endothermic transitions due to the melting were obscured by much larger starch gelatinization endotherms. Heating curves of untreated (Normand and Marshall 1989) and treated (Marshall, Normand and Goynes, *unpublished observations*) flour samples in Table III showed small endothermic transitions, with transition temperatures of 95–100°C, except for Calmochi exposed to C-M treatment, where no endotherm was found. If these small endotherms represent melting of amylose-lipid complexes, then the absence of an exotherm in Calmochi treated with C-M (Table III) is to be expected.

The appearance of exotherms from the formation of semicrystalline, amylose-lipid complexes provides an explanation for several of our results. In flour samples exposed to the first cooling cycle, enthalpies were lowest when the greatest amount of lipid was removed (Table III). Therefore, removal of lipid reduced the degree of amylose-lipid crystallization, and the more lipid removed, the less crystallization achieved. In untreated flour, starch surface lipids may penetrate the starch granule during gelatinization (cooking) and form inclusion complexes with the amylose in the granule (Morrison 1988). Removal of starch surface lipids with hexane or C-M prior to heating in the DSC would reduce the contribution of starch surface lipid involved in crystallization and decrease the enthalpy. With C-M treatment of flour samples, less than 1% of the total rice lipid may remain, but exotherms were still observed (Table III), except for Calmochi. The origin of the residual lipid after C-M treatment was not determined and could consist of both starch and nonstarch components. The absence of an exotherm in Calmochi treated with C-M (Table III) suggests that the amount of lipid available to complex with amylose is very small.

The classes of lipids comprising the starch and nonstarch fractions and an identification of the free fatty acids in these lipids have been determined for rice (Azudin and Morrison 1986, Morrison 1988). The nonstarch lipids are predominantly triglycerides, but the starch lipids are primarily lysophospholipids (LPL) and free fatty acids (FFA) for nonwaxy rice, since starch granules from waxy varieties have little if any lipid (Azudin and Morrison 1986). LPL and FFA are remarkable similar in their composition, consisting of palmitic, linoleic, and oleic acids, with minor amounts of other fatty acids (Morrison 1988). Both LPL and FFA (also as monoglycerides) could complex with starch during rice cooking, since Biliaderis et al (1986a) and Ko and Park (1989) have shown that rice starch can interact with monoglycerides and lysolecithin, respectively, and affect the thermal properties of the starch.

We also observed that exothermic enthalpies for kernels were lower than comparable enthalpies for flour samples (compare unextracted data in Tables II and III). A major difference between kernel and flour was the absence of structural integrity in the flour. Therefore, kernel structure may influence formation of semicrystalline, amylose-lipid complexes. In the kernel, most starch granules are densely packed and highly ordered in structures within cells that make up the starchy endosperm. Little if any surface lipid may be present between granules. In contrast, granules in flour form clumps of different sizes, and some are even found as individual granules. Very likely they have adsorbed lipid on the granular surface during grinding of the kernels. For amylose in kernels, the major source of lipid would be readily accessible inside the granule. When kernel integrity is destroyed, not only starch lipid but adsorbed lipid originally from outside the granule may now complex with the amylose. Thus, in flour, more total lipid is available, and more interaction occurs between amylose and lipid during heating in the DSC. This eventually leads, upon cooling, to an increase in enthalpy of crystallization for the amylose-lipid complex. The presence of intact kernel structure also increased gelatinization enthalpies and starch gelatinization temperatures compared with flour samples of the same rice variety. (Normand and Marshall 1989).

Kernel integrity may also be important in explaining the observation that untreated rice kernels exhibited lower enthalpy values than did kernels treated with hexane or C-M, except for Calmochi (Table II). Marshall et al (1990) found lower starch gelatinization temperatures in kernels exposed to hexane or C-M and attributed these results to disruption of kernel structure by the lipid solvents. In the present situation, two opposing processes may be at work. Disruption of kernel structure by exposure to lipid solvents may increase lipid availability to the starch granule as proposed earlier, but extraction of lipid will conceivably reduce the total lipid available to be complexed with amylose. The results in Table II suggest the influence of disrupted kernel structure on controlling the degree of amylose-lipid melting and subsequent crystallization. The lack of an increase in enthalpy in treated Calmochi kernels may reflect limiting amounts of both lipid and amylose, since Calmochi is a very low amylose rice.

We noted in the results section that peak transition temperatures for the exotherms were higher in kernel and flour (Table I) than for literature values (Eliasson and Krog 1985) describing peak transition temperatures in amylose-monoglyceride complexes. Eliasson and Krog (1985) used higher moisture (85–95%) and a faster heating rate (10°C/min) for their DSC samples than we did. However, at least for starch-monoglyceride complexes, moisture content and heating rate has little effect on peak transition temperatures produced by melting of these complexes (Biliaderis et al 1986a). This comparison emphasizes the difference between simple, two-component model systems and the more complex kernel and flour systems. The comparison may further suggest that the complexes formed in kernel and flour may be quite different in lipid composition and amylose-lipid structure.

We conclude from our results that the exotherms observed during the first cooling cycle were influenced by the amount of lipid removed, the structural integrity of the kernel, and the amylose content of the rice.

Exotherms produced during the second cooling cycle were always smaller than those generated during the first cooling cycle. This observation indicated that disruption of the semicrystalline complex occurred during the second heating cycle, and only partial or incomplete formation of this complex was seen during the second cooling cycle. However, factors existing within a particular rice variety appear to influence the extent of partial crystallization.

Since amylose-lipid complexes may exist in whole grain milled rice and milled rice flour after cooking, their presence should be considered in describing the properties of cooked rice, especially retrogradation, and the properties of rice flour in various baking applications.

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LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 30-25, approved April 1961, revised October 1981. The Association: St. Paul, MN.

AZUDIN, M. N., and MORRISON, W. R. 1986. Non-starch lipids and starch lipids in milled rice. J. Cereal Sci. 4:23.

BILIADERIS, C. G., PAGE, C. M., SLADE, L., and SIRETT, R. R. 1985. Thermal behavior of amylose-lipid complexes. Carbohydr. Polym. 5:367.

BILIADERIS, C. G., PAGE, C. M., and MAURICE, T. J. 1986a. On the multiple melting transitions of starch/monoglyceride systems. Food Chem. 22:279.

BILIADERIS, C. G., PAGE, C. M., MAURICE, T. J., and JULIANO, B. O. 1986b. Thermal characterization of rice starches: A polymeric approach to phase transitions of granular starch. J. Agric. Food Chem. 34:6.

ELIASSON, A.-C., and KROG, N. 1985. Physical properties of amylose-monoglyceride complexes. J. Cereal Sci. 3:239.

HIBI, Y., KITAMURA, S., and KUGE, T. 1990. Effect of lipids on the retrogradation of cooked rice. Cereal Chem. 67:7.

HOGAN, J. T., and DEOBALD, H. J. 1961. Note on a method of determining the degree of milling of whole milled rice. Cereal Chem. 38:291.

JULIANO, B. O. 1971. A simplified assay for milled rice amylose. Cereal Sci. Today. 16:334.

KO, J.-H., and PARK, K.-H. 1989. Differential scanning calorimetric study of amylose-lipid complex and amylose content in rice starch. Korean J. Food Sci. Technol. 21:556.

MARSHALL, W. E., NORMAND, F. L., and GOYNES, W. R. 1990. Effects of lipid and protein removal on starch gelatinization in whole grain milled rice. Cereal Chem. 67:458.

MORRISON, W. R. 1988. Lipids in cereal starches: A review. J. Cereal Sci. 8:1.

MORRISON, W. R., and AZUDIN, M. N. 1987. Variation in the amylose and lipid contents and some physical properties of rice starches. J. Cereal Sci. 5:35.

NORMAND, F. L., and MARSHALL, W. E. 1989. Differential scanning calorimetry of whole grain milled rice and milled rice flour. Cereal Chem. 66:317.

PRIESTLEY, R. J. 1977. Studies on parboiled rice. Part 3: Characteristics of parboiled rice on recooking. Food Chem. 2:43.

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