Food Chemistry 69 (2000) 229–236

Food Chemistry

www.elsevier.com/locate/foodchem

Rapid communication

On the roles of protein and starch in the aging of non-waxy rice flour

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Received 11 May 1999; received in revised form 16 September 1999; accepted 18 September 1999

Abstract

Changes in pasting and thermal properties of non-waxy rice flour and its isolated starch during storage at 25, 35 and 45°C were compared. Aging had no apparent effect on the pasting behaviour of the isolated starch, but markedly affected that of the flour. Peak viscosity (V_p) of rice flour pastes generally increased with both temperature and time of storage, but reached a plateau within 4 weeks of storage at 45°C. Fresh flour paste exhibited lower V_p, a slower rise in apparent viscosity, and much better stability than an isolated starch paste. The addition of isolated oryzenin to isolated rice starch resulted in pasting behaviour which more closely approximated that of an extensively aged flour. DSC scans of fresh flour at ~13% moisture revealed a weak heat-irreversible endothermic event over the temperature range from 47 to 66°C which was attributed to the denaturation of oryzenin. This transition was shifted to higher temperatures with increasing storage temperature and time. It also became increasingly skewed and broadened until it was no longer detectable after the flour had been stored at 45°C for 8 weeks. Starch gelatinization characteristics of both flour and isolated starch, as determined by DSC, were apparently unaffected by aging. However, rice flour exhibited significantly higher onset temperature of gelatinization, but lower gelatinization enthalpy, than isolated rice starch. Similarly, pulsed NMR studies showed no apparent effect of aging on retrogradation behaviour of rice flour or starch gels. These results suggest that modification of the protein component, rather than starch, was primarily responsible for rheological changes associated with aging of rice flour.

Keywords: Viscosity; Thermal properties; Differential scanning calorimetry; Gelatinization; Retrogradation; Oryzenin

1. Introduction

It is common knowledge that the physicochemical properties of freshly harvested non-waxy rice can change quite dramatically during the first 3–4 months of storage (Barber, 1972; Chrastil, 1990a,b; Juliano, 1985; Villareal, Ressurreccion, Suzuki & Juliano, 1976). The most notable changes that occur during this natural aging process involve rheological properties that, in turn, have a significant effect on the cooking and eating qualities of rice (Perez & Juliano, 1981). Non-waxy rice flour, derived from inadequately aged grains, has also been observed by local manufacturers to give unsatisfactory performance in the preparation of several indigenous food products. Inconsistent quality brought about by variations in the aging process is also a problem. Thus, freshly milled rice flour usually has to be stored or aged under proper conditions of temperature, time and relative humidity to achieve a more consistent quality before usage. Studies of aging have, however, generally been focused on whole-grain rice rather than the flour.

The exact mechanism of postharvest aging of rice is not completely understood and differences in opinion do exist. Juliano (1985) and Zhou and Guo (1995) have discussed several possible mechanisms of aging. These include (1) changes in cell wall and proteins, (2) variations in non-soluble amylose, (3) effects of debranching enzymes on amylopectin, (4) interactions between proteins and breakdown products of lipid oxidation, and (5) starch–protein interactions. Experimental data obtained by Shibuya, Iwasaki and Chikubu (1977a,b) suggested that changes in the lipid and starch fractions had little influence on changes in rheological properties of rice during postharvest storage. There is, however, increasing evidence for the role of proteins (Barber,

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0308-8146/00/$ - see front matter
PII: S0308-8146(99)00271-X

1972; Chrastil, 1990b, 1994; Qiu, Jin & Zhou, 1998; Villareal et al., 1976). The aging process has been reported to be hastened by, among other factors, exposure to light and high temperatures, milling, and a high amylose content (Indudhara Swamy, Sowbhagya & Bhattacharya, 1978).

The primary objective of this study was to probe further into the roles of the starch and protein fractions in the aging of rice flour. Changes in pasting and thermal properties of flour from freshly harvested non-waxy rice grains and the corresponding isolated rice starch during short-term storage at different temperatures were compared. Oryzenin, the major protein in rice, was also extracted from fresh rice flour and added back to isolated starch to observe its effects on pasting properties.

2. Materials and methods

2.1. Non-waxy rice flour

Freshly harvested paddy (Makmur 77 variety) was immediately dried to a moisture content of ~13% (db) in a tray drier at 45°C. The dried paddy was then dehulled and milled using Minghetti rice processing machines (P. Minghetti, Vercelli, Italy). The milled rice was ground into flour using a ball mill and passed through a 0.125 mm sieve.

2.2. Starch isolation

Starch was isolated using the method described by Juliano (1984) involving repeated steeping of rice flour in NaOH solution. The isolated starch was dried in an oven at 40°C for 48 h to give a moisture content close to that of the rice flour. The dried starch was ground into powder and passed through a 0.125 mm sieve.

2.3. Extraction of oryzenin

Oryzenin was extracted from fresh rice flour using the method described by Chrastil (1990b). The extract was dried in vacuo at room temperature.

2.4. Storage trials

Storage trials were conducted using a completely randomized design. Rice flour and isolated starch were placed in air-tight glass bottles and stored in the dark at 25, 35 and 45°C in thermostatically controlled incubators. Triplicate samples were withdrawn for analysis after an initial period of two weeks (zero time) and at appropriate time intervals thereafter. Statistical comparison of means was conducted using the Student *t* test or analysis of variance (ANOVA) followed, if necessary, by Duncan's multiple range test.

2.5. Chemical analysis

Moisture content was determined by drying samples in an air-oven at 105°C to constant weight. Amylose content of the isolated starch was determined using the method of Williams, Kuzima and Hlynka (1970). Total protein ($N \times 5.95$) and fat contents were analyzed using approved methods of the American Association of Cereal Chemists (1984). The results were expressed on dry weight basis.

2.6. Pasting characteristics

A Rheomat 115 viscometer (Contraves, Germany) was used to study the pasting behaviour of non-waxy rice flour and isolated starch slurries. Measurements were carried out using the MS-DIN 125 measuring system at a shear rate of 4.4 s^{-1}. A homogenized slurry sample (17.5 ml) was transferred into a measuring tube that was then immediately introduced into the heating chamber of the instrument maintained at 96°C by a circulating water bath. Changes in viscosity were monitored over a period of 20 min. In all cases, the concentration of starch in the slurry was adjusted to ~12% (w/v) by taking into consideration the protein content of the sample.

2.7. Differential scanning calorimetry (DSC)

To obtain the starch gelatinization thermal profiles, rice flour and isolated starch slurries containing one part of dry matter to two parts of distilled water were prepared by gently stirring the dry powders with the required amounts of water in beakers. Samples of ~10 mg (accurately weighed to 0.01 mg) were filled into aluminium pans that were then hermetically sealed. The prepared samples were left to stand for 1 h at room temperature (~25°C) before DSC measurements were carried out using a DuPont 2910 differential scanning calorimeter fitted with a standard DSC cell. Heat-flow and temperature calibrations were performed using pure indium. An empty aluminium pan was used as a reference to balance the heat capacity of the sample pan. All samples were heated from 10 to 120°C at 10°C min^{-1}. Transition temperatures (onset, T_o; peak, T_p; conclusion, T_c) and overall gelatinization enthalpy (ΔH_G, expressed as J g^{-1} dry matter) were determined as described by Seow and Teo (1993).

To study the thermal properties of the "dry" rice flour and starch (containing ~13% moisture), samples of ~5 mg (accurately weighed to 0.01 mg) were sealed in hermetic aluminium sample pans and heated from 20 to 150°C at a rate of 10°C min^{-1}.

2.8. Retrogradation of flour or starch gels

The pulsed nuclear magnetic resonance (NMR) method, described by Teo and Seow (1992), was used to study retrogradation of flour or starch gels. A Minispec PC 100 pulsed NMR spectrometer (Bruker, Germany) operating at 20 MHz was employed. Rice flour or isolated starch gels at a dry matter-to-water ratio of 1:2 were prepared and retrogradation studies were conducted at 5, 15 and 25°C.

3. Results and discussion

3.1. Chemical composition

Table 1 shows the protein and fat contents of the non-waxy rice flour and isolated starch studied, as well as the amylose content of the latter, before and after storage at 25, 35 and 45°C for 14 weeks. As expected, statistical analysis showed that these parameters remained unchanged over this period of storage, in agreement with the findings of other researchers (Barber, 1972; Rajendra Kumar & Zakiuddin Ali, 1991). No significant effects of storage on total starch, amylose or amylopectin contents of rice were noted by Qiu et al. (1998). Noomhorm, Kongseree & Apintanapong, 1997) also reported that the total amylose and protein contents of waxy rice did not change during four months of storage at 28–30°C. However, Chrastil (1990b) did detect a small but significant increase in amylose content during storage of milled non-waxy rice grains at 40°C over an extended period of 12 months, although total protein and starch contents remained unchanged.

3.2. Pasting behaviour

Typical viscograms of pastes, prepared from fresh rice flour and flour aged at 35°C, are shown in Fig. 1. Similar patterns were observed regardless of aging temperature. The most distinctive change on aging of the material is the increase in peak viscosity (V_p) of the paste, an effect that is generally well-known (Barber, 1972; Dhaliwal, Sekhon & Nagi, 1991; Indudhara Swamy, Sowbhagya & Bhattacharya, 1978; Perdon, Marks, Siebenmorgen & Reid, 1997; Shibuya, Iwasaki & Chikubu, 1977b; Rajendra Kumar & Zakiuddin Ali, 1991). The magnitude of this increase is obviously dependent on storage temperature. Fig. 2 shows, in greater detail, the effect of storage temperature on V_p of rice flour. When stored at the relatively low temperature of 25°C, there was a slow but gradual increase in V_p that occurred only after a long induction period of 6–7 weeks. Conversely, when stored at the relatively high temperature of 45°C, an increase in V_p occurred immediately, reached a maximum value within 4 weeks, and levelled off on further storage. Rice flour, aged at the intermediate temperature of 35°C, exhibited V_p values between that aged at 25 and 45°C. Peak viscosity increased gradually to a level approaching the maximum value attained for samples aged at 45°C for 14 weeks.

In contrast, V_p of isolated starch pastes did not exhibit any significant ($P < 0.05$) change even after 14 weeks, regardless of the temperature of storage (Table 1). This lack of effect of aging on pasting behaviour of isolated starches is in agreement with previous observations based on Brabender amylography (Shibuya et al., 1977b; Rajendra Kumar & Zakiuddin Ali, 1991). As shown in Fig. 3, there were also other distinct differences in pasting behaviour between rice flour and isolated rice starch that may possibly be accounted for by factors other than starch. For example, isolated starch exhibited a faster rise in paste viscosity than flour when heated under the same conditions. Rajendra Kumar and Zakiuddin Ali (1991), who made similar observations, attributed this effect to the absence of other components (such as proteins and cell wall constituents) in isolated starch. Fresh rice flour paste also exhibited considerably lower V_p than the isolated starch paste but much improved stability, as indicated by the gentler decline in viscosity after the peak. These observations, which are in agreement with those obtained by Shibuya et al. (1977b) and Hamaker and Griffin (1990), suggest that interactions between starch and other flour constituents could be responsible for the aging of flour. Aging could affect or modify such interactions through its effects, not so much on starch, but on those other constituents. It is interesting to note that Chrastil (1990b) reported

Table 1
Contents of selected chemical components and peak viscosity of fresh and aged (14 weeks at 25, 35 and 45°C) rice flour and isolated starch[a]

Material	Aging temperature (°C)	Moisture (% db)	Protein (% db)	Fat (% db)	Amylose (% dry starch)	Peak viscosity (Pa s)
Flour	Fresh	12.6	8.0	1.4	20.7	0.52
	25	12.7	8.1	1.3	20.4	0.58
	35	12.4	7.9	1.3	20.9	0.62
	45	12.6	8.0	1.4	20.6	0.64
Starch	Fresh	12.4	0.5	1.0	20.7	0.66
	25	12.4	0.5	0.9	20.4	0.67
	35	12.3	0.4	1.1	20.6	0.68
	45	12.3	0.4	0.9	20.9	0.67

[a] The results are means of triplicate determinations. Variation from the mean was less than ±3%.

that the number of disulphide (-SS-) bonds and the average molecular weight of oryzenin increased significantly during storage of rice grains. Associated with such changes were decreases in protein solubility and reversible binding of oryzenin to starch molecules. In a related study, Chrastil and Zarins (1992) found that low-molecular weight peptide subunits of oryzenin decreased and high-molecular weight subunits increased during storage. Qiu et al. (1998) also recently reported that oryzenin in rice stored at 38°C for 6 months had a significantly lower content of free thiol groups and a higher molecular weight than the protein in newly harvested rice. We suspect that as starch-oryzenin interaction decreases during aging, its influence on paste viscosity also considerably lessens, thereby allowing the predominant starch fraction to more fully exert its effects on rheological properties. Thus, with aging, the pasting behaviour of rice flour comes ever closer to that of the isolated starch, particularly in terms of peak viscosity, although breakdown of the swollen starch granules may continue to be retarded by the presence of protein and/or other constituents. Cleavage of disulfide linkages of proteins (on addition of dithiothreitol to rice flour) or protein hydrolysis through the action of proteinases have been shown to lead to a decrease in Brabender amylographic paste viscosity (Hamaker & Griffin, 1990). These findings lend further support to the belief that protein plays a more prominent role than starch in the rice aging process.

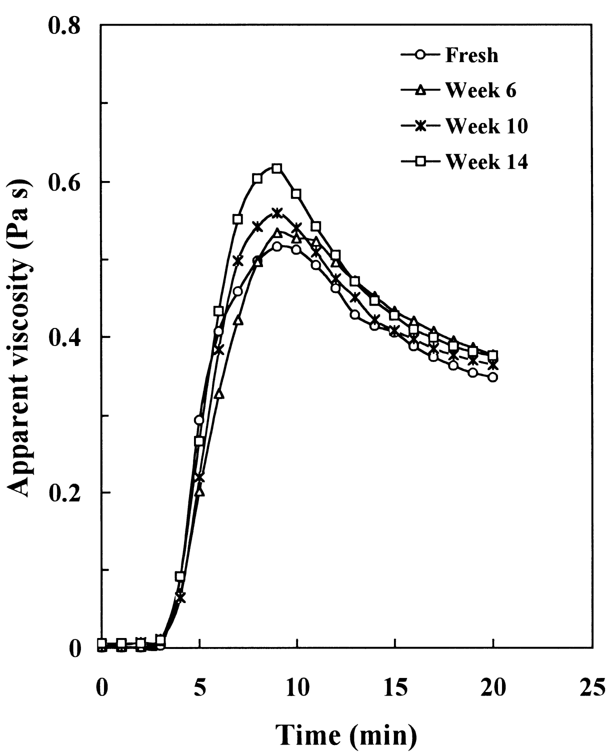

Fig. 1. Effects of aging at 35°C on pasting characteristics (96°C, shear rate 4.4 s^{-1}) of rice flour slurries containing ~12% starch.

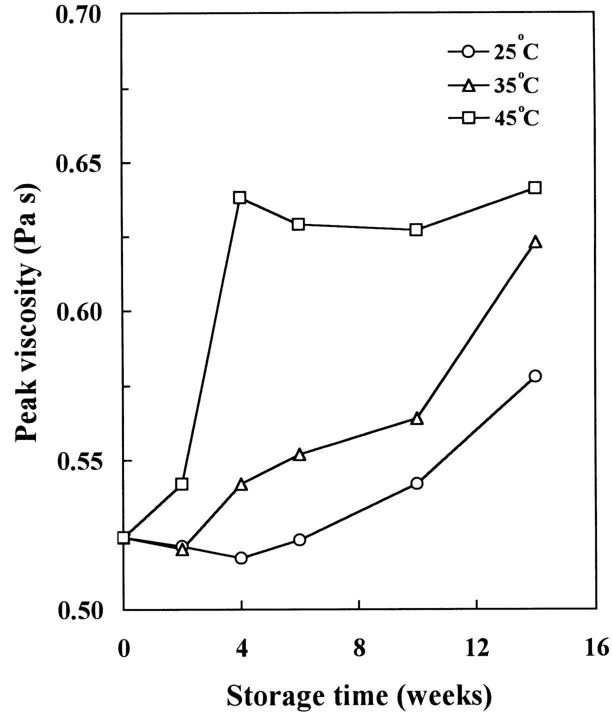

Fig. 2. Effects of aging temperature and time on peak viscosity (96°C, shear rate 4.4 s^{-1}) of rice flour pastes.

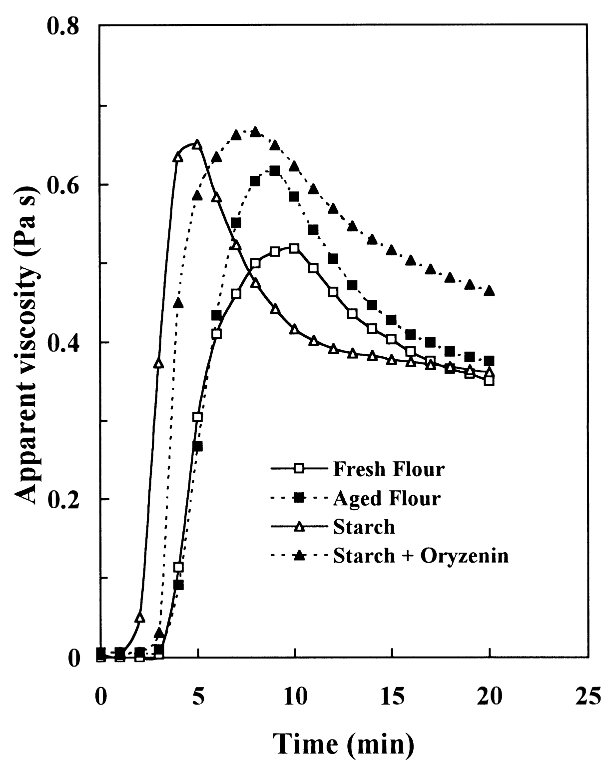

Fig. 3. A comparison of the pasting characteristics (96°C, shear rate 4.4 s^{-1}) of fresh flour, flour aged at 35°C for 14 weeks, isolated starch, and starch + oryzenin mixture.

To validate, to some extent, the above assertions, we carried out an experiment in which we added isolated oryzenin to fresh isolated starch to give a protein content close to that of the original flour, before determining the pasting behaviour of the mixture or "reconstituted flour" under the same conditions. A comparison of the viscograms obtained is shown in Fig. 3. It is clear that the initial rate of increase in apparent viscosity, peak viscosity, and breakdown of the paste on adding oryzenin to isolated starch became more closely similar to those of an extensively aged (14 weeks at 35°C) flour sample. It is reasonable to expect that external addition of isolated oryzenin would not lead to starch-protein interaction or binding that was as strong as that found naturally in the fresh flour. It is also possible that the conditions of isolation of both starch and protein may have altered, to some degree, their structure and properties.

3.3. Thermal properties

The DSC thermal profiles of rice flour (13% moisture) aged at three different temperatures up to 14 weeks are shown in Fig. 4. Fresh rice flour was found to exhibit a weak heat-irreversible endothermic transition over the temperature range from 47 to 66°C in the DSC thermogram. As shown in Fig. 4a, a small remnant of this transition was still detectable when a freshly prepared isolated starch sample, at about the same moisture content, was scanned. Both temperature and time of aging profoundly affected the shape, size and position of the endothermic event. Details of transition temperatures and enthalpy associated with this event are given in Table 2. The transition was shifted to higher temperatures and became increasingly skewed and broadened with increasing storage time, the effects being more pronounced at higher storage temperatures. Broadening of the endotherm and its shift to higher temperatures continued until it was no longer detectable after 8 weeks of storage at 45°C over the range of temperature scanned. Prior to its disappearance, the transition enthalpy (ΔH) did not appear to be affected by the conditions of aging applied in the present study, with mean ΔH values ranging from 0.8 to 1.5 J g^{-1} (dry flour basis).

The origin of the weak endothermic transition observed is of particular interest. DSC scans of low-moisture polysaccharide systems generally give rise to a characteristic "sub-Tg endotherm" centred at about 60°C (Appelqvist, Cook, Gidley & Lane, 1993; Cooke, Gidley & Hedges, 1996; Kalichevsky, Jaroszkiewicz, Albett, Blanshard & Lillford, 1992; Shogren, 1992; Thiewes & Steeneken, 1997). This "sub-T_g endotherm" has been hypothesized to arise from enthalpy relaxation and shown to increase in magnitude on physical aging at temperatures below the glass transition temperature (T_g) (Thiewes & Steeneken, 1997). Our results indicated

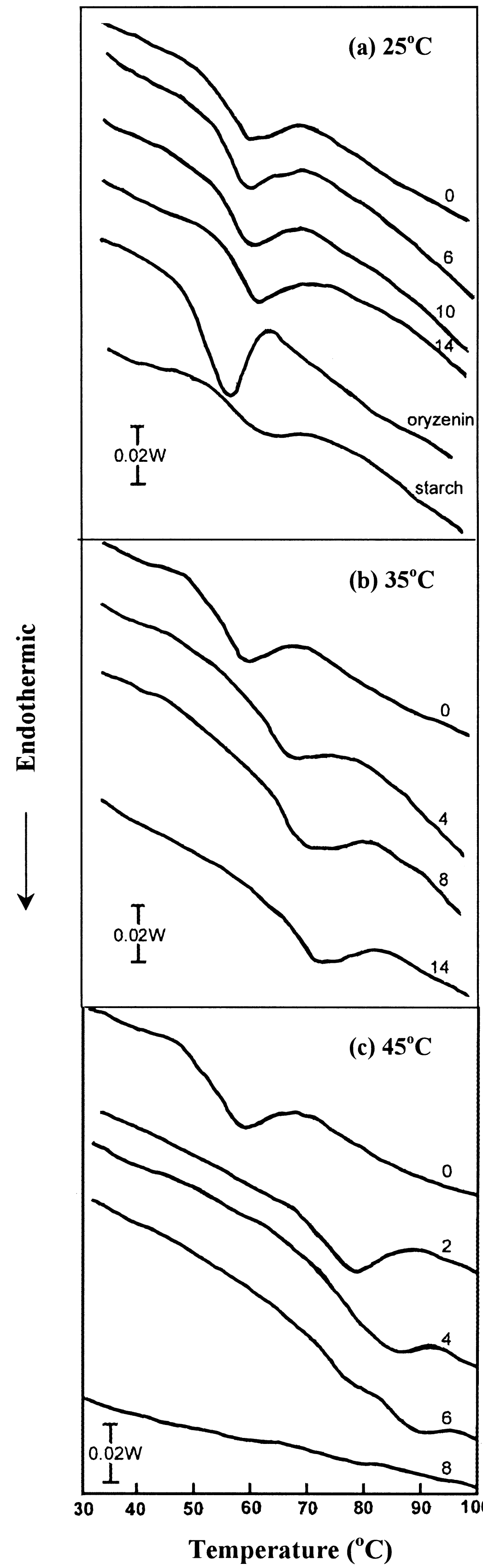

Fig. 4. Effects of aging temperature and time on DSC thermal profiles of rice flour at ~13% moisture (Numbers represent aging time in weeks).

Table 2
Effects of aging on transition temperatures and enthalpy of the endothermic event attributed to oryzenin denaturation[a]

Aging temperature (°C)	Aging time (weeks)	Transition temperature (°C)			ΔH (J g^{-1} dry flour)
		T_o	T_p	T_c	
25	0	47.2	56.5	65.7	0.9
	2	45.9	54.5	65.7	1.1
	4	46.9	55.2	67.6	1.2
	6	50.1	57.6	70.6	1.1
	8	48.6	56.0	70.1	1.1
	10	50.1	56.4	70.3	1.0
	14	52.3	60.6	71.8	1.3
35	0	47.2	56.5	65.7	0.9
	2	56.6	63.1	71.5	0.8
	4	56.9	64.9	79.7	1.1
	6	59.4	67.5	81.1	0.9
	8	61.1	67.9	81.6	1.0
	10	59.8	68.4	82.6	0.9
	14	60.1	69.6	86.9	1.5
45	0	47.2	56.5	65.7	0.9
	2	65.7	73.7	85.7	1.0
	4	68.7	81.3	90.5	1.1
	6	68.2	82.1	93.8	0.8
	8	– (nd)[b]	–	–	–
	10	–	–	–	–
	14	–	–	–	–

[a] The results are means of triplicate samples (standard deviation $< 1.2°C$ or < 0.3 J g^{-1}).
[b] nd = Transition was not detected.

no increase in transition enthalpy during storage. In fact, the transition disappeared after prolonged aging. It is, therefore, unlikely that the weak endothermic transition observed in the present study could be synonymous with this so-called "sub-T_g endotherm".

We suspected that this weak endotherm resulted from protein denaturation and supportive evidence was obtained when a similar DSC scan of a dried sample of isolated oryzenin revealed a similar endothermic transition, albeit bigger and occurring over a slightly lower temperature range from 45 to 62°C (Fig. 4a). The slight discrepancies in size, shape and position of the endotherms may be accounted for by (a) differences in moisture content, (b) differences in sample size (c) influence of other constituents present in rice flour as compared with isolated oryzenin, and/or (d) changes in properties of oryzenin resulting from the extraction procedure.

Native oryzenin thus appeared to be susceptible to conformational alterations during aging, even at relatively low moisture contents and storage temperatures. Progressive formation of intermolecular covalent disulphide cross-links (and possibly hydrogen bonds), as the flour aged, probably made the protein less soluble and less prone to bind with other flour constituents such as starches. Thus, the state of oryzenin appears to be a crucial factor influencing the functional properties of rice. DSC studies of the thermal behaviour of the isolated starch and oryzenin fractions during storage should provide additional evidence in support of this hypothesis. To the best of the authors' knowledge, the use of DSC to study the effects of aging on thermal transitions of rice flour at low moisture levels has hitherto not been explored.

Where starch gelatinization was concerned, typical DSC thermograms (not shown), similar to those reported by other researchers (e.g. Biliaderis, Page, Maurice & Juliano, 1986; Normand & Marshall, 1989), were obtained. At the moisture levels studied, starch gelatinization gave rise to a single large endothermic transition. Table 3 gives details of the calculated transition temperatures and enthalpy associated with starch gelatinization (ΔH_G) for both rice flour and isolated starch, before and after aging at various temperatures for 14 weeks. It is evident that starch gelatinization characteristics remained unchanged during aging of both rice flour and isolated starch. DSC studies by Qiu et al. (1998) have similarly revealed no change in peak temperature before and after storage of rice. However, the onset gelatinization temperature (T_o) of rice flour was consistently and significantly ($P < 0.5$) higher while gelatinization enthalpy was markedly lower (even after taking into consideration the weight of other flour constituents) than the corresponding values for isolated starch, although peak (T_p) and conclusion (T_c) temperatures were practically unaffected. These results reinforce the view that non-starch flour constituents (particularly protein) are more directly responsible for postharvest changes in rheological properties of rice than the starch component.

Table 3
Effects of aging at different temperatures for 14 weeks on gelatinization properties of rice flour and isolated starch (at dry matter/water ratio of 1:2)[a]

Material	Aging temperature (°C)	Transition temperature (°C)			ΔH_G (J g^{-1} dry flour or starch)
		T_o	T_p	T_c	
Flour	Fresh	69.9	77.7	89.9	7.5
	25	70.1	77.9	89.6	7.6
	35	70.0	77.7	90.2	7.8
	45	70.2	78.0	89.8	7.5
Starch	Fresh	65.8	76.7	88.9	12.2
	25	66.1	76.2	90.0	12.6
	35	66.6	76.9	90.2	12.5
	45	66.5	76.9	90.0	13.0

[a] The results are means of triplicate samples (standard deviation $< 1.2°C$ or < 1.1 J g^{-1}).

Table 4
Effects of aging (14 Weeks at 25, 35 and 45°C) on retrogradation rate constants (k) and Avrami exponents (n) of 1:2 rice flour-water and isolated starch-water gels stored at different temperatures

Material	Aging temperature (°C)	$k \times 10$ (day^{-1})			n		
		5°C	15°C	25°C	5°C	15°C	25°C
Flour	Fresh	9.3	6.3	4.1	0.7	0.6	0.5
	25	9.0	6.2	3.9	0.7	0.7	0.6
	35	9.0	6.2	3.6	0.7	0.7	0.6
	45	8.7	6.5	3.7	0.8	0.7	0.7
Starch	Fresh	12.2	8.6	5.0	0.7	0.7	0.8
	25	11.7	8.7	5.5	0.6	0.7	0.7
	35	11.2	8.9	5.3	0.7	0.7	0.8
	45	11.3	8.7	5.3	0.7	0.7	0.7

3.4. Retrogradation

The patterns of retrogradation of rice flour and starch gels were qualitatively similar to those reported for other non-waxy starch gels and starch-based products (Seow & Teo, 1996; Teo & Seow, 1992). The NMR data obtained, which reflected amylopectin retrogradation, were analyzed using the Avrami equation, as described by those authors, in order to determine the rate of retrogradation (k) and the Avrami exponent (n). The results are given in Table 4. As expected, rate of retrogradation increased as storage temperature of the gels was lowered from 25 to 5°C. The negative temperature coefficient exhibited by amylopectin recrystallization over this range of storage temperature is in agreement with previous experimental results (Slade & Levine, 1987; Teo & Seow, 1992). Aging clearly did not affect the retrogradation behaviour of rice flour or starch gels. However, the rate of retrogradation of flour gels was considerably lower than that of isolated starch gels at any given gel storage temperature. This may be due to the slight difference in actual starch concentration in the flour and isolated starch gels. However, a more likely reason is that other flour components may delay starch retrogradation. The Avrami exponent (n) remained more or less constant, suggesting that aging or the presence of non-starch constituents did not affect the recrystallization behaviour of amylopectin, at least in terms of crystal morphology. It can be surmised that aging had not affected the starch component in any way that could cause changes in its retrogradation behaviour.

4. Conclusion

The results of the present study support previous suggestions that oryzenin-starch interactions could be the major factor responsible for rheological changes associated with aging of rice. Such interactions, which could influence starch properties such as swelling and solubility, are probably altered as a consequence of changes in structure and properties of oryzenin, rather than starch, during storage.

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