

# Chalky and Translucent Rice Grains Differ in Starch Composition and Structure and Cooking Properties

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## ABSTRACT

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Chalk is an important quality characteristic in rice and occurs most commonly when high temperatures are experienced during grain development. The aims of this report are to determine whether chalk affects cooking quality and to attempt to explain the effects on the basis of starch and protein in chalky and translucent grains. Three cultivars of rice were grown in the glasshouse at either 38/21°C or 26/15°C (day/night temperatures). Rice grown at the higher temperature contained more chalky grains. Grains in the inferior position were more susceptible to forming chalk

than were those in the superior position. The presence or absence of chalk affected cooking quality but neither amylose content, amylopectin structure nor protein composition explained the differences in cooking quality. However, the shape, size, and packing of amyloplasts and cells in chalky grains differed from those in translucent grains and might offer an explanation for the differences in cooking quality. It seems likely that the processes involved in the initiation or packing of amyloplasts are susceptible to high temperatures.

Australia is one of the smallest rice producers in terms of the world rice trade, yielding much less annually than other temperate rice-growing areas. However, the Australian rice industry is, proportionally, one of the largest exporters of rice; approximately 85% of the grain produced is sold to overseas markets. This is not the case in most other rice-growing countries, where the domestic market consumes the majority of the grain produced. Because the Australian rice industry cannot compete with other temperate rice-growing areas in terms of quantity, its marketing strategy has been to supply high-quality grain specifically to niche markets. Therefore, Australian rice must meet narrow specifications for each parameter of quality and undergo a stringent quality testing procedure before being sold to various markets.

Chalk, an opaque area in the grain, affects the visual appearance of white rice. Chalk mainly occurs at the center of the grain and can occupy >50% of the area of the grain. It is an undesirable characteristic in all markets except the arborio market; most markets will not accept rice that contains >2% of chalky grains. Here, we investigate whether chalk affects cooking quality or whether it is simply a cosmetic problem, and then we proceed to define the differences in the components of chalky and translucent grains.

Studies (Tashiro and Ebata 1975, Tashiro and Wardlaw 1991) have shown that high temperatures during specific stages of grain development tend to increase the occurrence of chalk in rice grains. However, another effect of high temperatures during grain development is a decrease in amylose content. The subsequent effect of this on the cooking quality of the rice might mask any effect of chalk on cooking quality (Tashiro and Ebata 1975). Because high temperatures seem to be the main cause of chalkiness in rice, the effect of high temperature on cooking quality complicates any work aiming to understand the effect of chalk on cooking quality.

Here, we report that chalky grains differ, independently of high temperature, from translucent grains in cooking properties, cellular structure, and the morphology and packing of the starch granule.

## MATERIALS AND METHODS

### Field Experiment

*Plant growth.* *Oryza sativa* (cvs. Millin and Amaroo) were sown on October 17, 1997, at the research farm of Yanco Agricultural Institute on a Birginbigil clay loam soil. Millin is a short duration

crop and reaches anthesis ≈10 days before Amaroo. Because of this, Millin experienced a sustained period of high temperatures (day/night 39/25°C) during grain development, whereas during grain development in Amaroo, the temperatures were, on average, 32/15°C.

*Harvest.* Mature grain was harvested from the field trials with a Hege header. Paddy rice was dried on aerated drying racks in low humidity to 14% moisture and placed in a warm room (28°C) for two days, reaching a final moisture content of ≈12%. Paddy rice (150 g) was dehulled (THU35A 250V 50Hz Test Husker, Satake), and then milled (McGil No. 2 Mill) for 60 sec.

### Glasshouse Experiment

*Plant growth.* *Oryza sativa* (cvs. Millin, Illabong, and Amaroo) were chosen for varying tolerances to forming chalk. Millin is a medium-grain rice that historically is unlikely to form chalk in high temperatures. Amaroo is another medium-grain rice, but with different textural properties, and is moderately resistant to forming chalk. Illabong is an arborio-style rice that generally forms chalk in the climate of Australia's rice-growing region.

Seeds of Millin, Amaroo, and Illabong were sown in a clay soil, in pots (20 cm diameter). About two weeks after germination, seedlings were thinned to five plants per pot and then grown in a glasshouse until plants reached anthesis. At anthesis, plants were moved to one of two controlled temperature environments for the remainder of grain development. In one glasshouse, plants were grown in a temperature regime of day/night 38/21°C, and in the other, plants were grown at day/night 26/15°C.

*Harvest.* The date of anthesis was recorded for each panicle. Panicles were harvested in duplicate from plants in both temperature conditions at either 7, 10, 12, 15, 20, and 25 days post anthesis (dpa) or at maturity. For each stage of development, the panicles from each harvest were dissected by separating grains in the superior position from grains in the inferior position (Fig. 1). The developing grains were carefully peeled to isolate the endosperm. The endosperm was immediately frozen in liquid nitrogen (LN<sub>2</sub>) and 10 were pooled. Grains harvested at maturity were dried and dehulled as described above and then milled for 1 min in a homemade small-sample miller.

### Measuring Chalkiness

The amount of chalk in the milled mature rice was measured using image analysis (Reece and Blakeney 1993). Briefly, after accounting for the background, chalk is expressed as the proportion of opaque relative to translucent areas in a single layer of white rice grains (130 cm<sup>2</sup>, ≈300 grains). In the field experiment, chalkiness was measured on all grains. In the glasshouse experiment, chalkiness was measured on grains that were either from the superior position or from the inferior position on the panicle (Fig. 1).

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## Grinding

For analysis with a Rapid Visco Analyser (RVA), mature polished rice was ground to pass through a 0.5-mm screen (Cyclotec 1093 sample mill, Tecator, Hoganas, Sweden). For starch and protein composition, grains were dried in an oven at 80°C overnight, and kept desiccated until they could be ground to a fine powder using a dental amalgamator (Ultramat 2, Southern Dental Industries, Melbourne, Australia). The flour was then brought to ambient moisture ( $\approx 13\%$ ) before use.

## Viscosity

In the field experiment, viscosity was measured on samples of chalky and translucent grains of Amaro and Millin. In the glasshouse experiment, viscosity was measured on grains from the inferior and the superior positions for each cultivar from both temperature treatments.

Ground sample (3 g) was weighed into an aluminum cup and distilled water (25 g) was added. The mixture was stirred to remove lumps. Viscosity was measured using a RVA (Newport Scientific model 3D) using Approved Method 61-02 (AACC 2000). To ensure that differences observed were not due to  $\alpha$ -amylase activity,  $\alpha$ -amylase was denatured by replacing 0.5 g of distilled water with  $\text{AgNO}_3$  solution (0.5 g, 10%, w/v) (Batey et al 1997). Viscosity was measured in duplicate samples as above.

## Sucrose Content

Grains from each stage of development were ground in  $\text{LN}_2$  using a precooled mortar and pestle. Tricine-NaOH buffer (Umemoto et al 1994) (pH 8, 100 mM, 0.5 mL) containing  $\text{MgCl}_2$  (8 mM), EDTA (2 mM), 2-mercaptoethanol (50 mM), and glycerol (12.5%, v/v) was added to the ground sample. The mixture was vortexed

on ice for 10 min and centrifuged ( $12,000 \times g$ ) for 10 min at 4°C. The supernatant was collected, and the pellet was resuspended in Tricine-NaOH buffer (0.5 mL), vortexed another 10 min on ice, and then centrifuged ( $12,000 \times g$ ) for 5 min. The two supernatants were pooled. Buffer was evaporated from duplicate aliquots (0.1 mL) using a vacuum concentrator (RC 10.09, Jouan). When dry, the sample was resuspended in benzoic acid (0.025M, 1 mL). The sucrose content of triplicate samples was determined by UV-visible spectrophotometry (UV-Vis 918, GBS Scientific Equipment, Australia) at 415 nm (Blakeney and Mutton 1980).

## Scanning Electron Microscopy

Milled rice was desiccated over silica gel for seven days, then broken along natural fracture planes and the pieces mounted on stubs. The specimens were platinum coated to a thickness of  $\approx 3$  nm. The specimens were examined using a field emission scanning electron microscope (Philips FESEM XL30FEG, 1kV accelerating voltage).

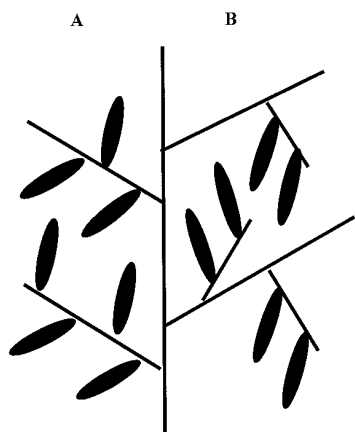
## Amylose Content

Amylose content was measured on mature grains of Millin and Amaro from the field experiment, and of Millin, Amaro, and Illabong from the glasshouse experiment. Chalky grains were separated from translucent grains. Chalky areas were predominantly in the centers of the chalky grains. Chalky centers were excised from  $\approx 30$  chalky grains, and translucent centers were excised from  $\approx 30$  translucent grains of either Amaro or Millin. The excised areas were amalgamated as described above and amylose content was measured on the flour (Juliano 1971, Blakeney et al 1994). Juliano (1971) reports no difference in amylose content between whole flour and defatted flour. Briefly, a subsample of flour (100 mg) was weighed into a 100-mL volumetric flask, wetted with ethanol (95%, 1 mL), then a solution of NaOH (1M, 9 mL) was added. The flasks were

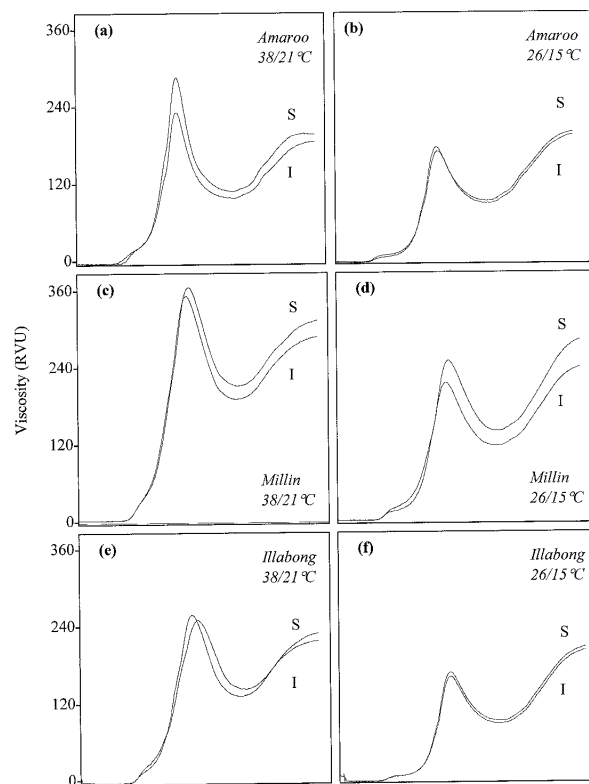
**TABLE I**  
Amount of Chalk (%) in Superior (S) and Inferior (I) Grains from Different Temperature Treatments in Two Experiments<sup>a</sup>

Cultivar	Field Experiment		Glasshouse Experiment			
	39/25°C	32/15°C	38/21°C		26/15°C	
			S	I	S	I
Amaroo		5.2	<1.0	2.0	<1.0	<1.0
Millin	12.5		5.9	10.7	2.6	5.2
Illabong			18.5	23.2	2.8	5.5

<sup>a</sup> Field experiment values for chalk in Millin were significantly higher than chalk in Amaro ( $P < 0.001$ ). Glasshouse experiment values in superior and inferior grains of Millin and Illabong for chalk is significantly higher when grown in high temperatures ( $P < 0.001$ ). In inferior grains of Amaro,  $0.06 < P < 0.1$ , and in superior grains of Amaro,  $0.1 < P < 0.2$ .



**Fig. 1.** Rice grain positions on the panicle: **A**, grains in superior position, branching from a main stem; **B**, grains in inferior position, attached to a superior branch.



**Fig. 2.** Viscosity curves of grains from superior (S) or inferior (I) position on the panicle from the glasshouse experiment: Amaro grown at day/night 38/21°C and 26/15°C (**a, b**); Millin grown at 38/21°C; and 26/15°C (**c, d**); Illabong grown at 38/21°C and 26/15°C (**e, f**).

placed in a sandbath (180°C) and refluxed for at least 5 min, allowing the ethanol to evaporate and the starch to gelatinize completely. The volume was then made to 100 mL with distilled water. An aliquot of each was pipetted into two test tubes. To each was added citric acid (0.1N, 2 mL), iodine solution (1 mL) containing I<sub>2</sub> (3.2 mM) and KI (48.2 mM), and distilled water (16 mL). Solutions were vortexed for 5 sec, left to stand for 20 min, then vortexed for another 5 sec. Absorbance was measured using a UV-visible spectrophotometer at 620 nm. Amylose in the starch solution was calculated from a standard curve of potato amylose.

### HPLC

Starch was debranched by the alkali procedure (Batey and Curtin 1996) with some modifications. Briefly, rice flour (50 mg) was wetted with ethanol (95%, 0.5 mL), then NaOH (0.25M, 1 mL) and distilled water (3.5 mL) was added to the powder. The vials were refluxed and the starch gelatinized as described above. After cooling to 50°C, sodium acetate buffer warmed to 50°C (0.2M, pH 4, 1 mL) and glacial acetic acid (32 µL) was added. An aliquot (1 mL) was mixed with isoamylase (7 µL) and incubated at 50°C for 2 hr. The process was then continued according to the alkali method described by Batey and Curtin (1996).

HPLC was performed on debranched starches using a Waters 2690 Alliance and a Waters 2410 Refractive Index detector. The program Millennium was used to control the pump and to acquire and process data. Chromatography was performed on a Waters Ultrahydrogel 250 gel permeation column at 60°C using a mobile phase of ammonium acetate (0.05M) at a flow rate of 0.5 mL/min. Run time was 50 min.

### Gel Electrophoresis

Rice grains (chalky and translucent) of Millin, Illabong, and Amaro were ground using an amalgamator as described above. Proteins were extracted from 50 mg of rice flour in Tris-HCl buffer (pH 6.8, 125 mM, 1 mL) containing SDS (4%), urea (4M), glycerol (20%, v/v), and 2-mercaptoethanol (5%) (Mizuno et al 1993). The mixture was vortexed for 1 min and allowed to stand at room temperature overnight. The mixture was centrifuged (5 min, 10,000 × g), and proteins in the supernatant solution were separated using SDS-PAGE with a Hoefer 10- × 8-cm Mighty Small, using a 10% gel and all solutions as described in the operator manual. Gels were dried (Hoefer Easy Breeze) after staining with Coomassie Blue R-250 as described in the operator manual.

## RESULTS

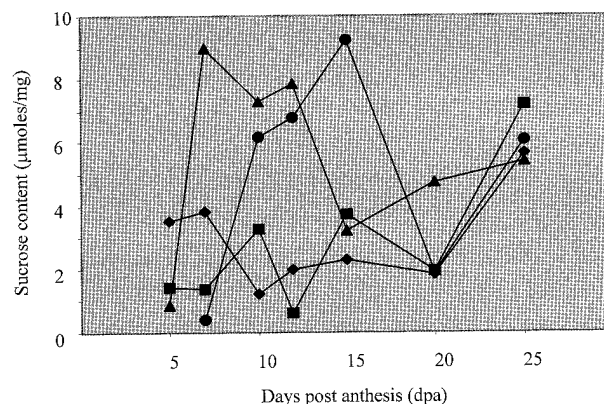
Table I shows that grains from the field experiment of Amaro contained significantly less chalk than those of Millin. In the glasshouse experiment, the amounts of chalk differed significantly between the 38/21°C and 26/15°C treatment groups for Millin and Illabong ( $P < 0.001$ ). In all cultivars in the glasshouse experiment, more chalk was present in inferior grains than in superior grains.

Figure 2 shows the viscosity curves of grains from either the superior or inferior position of Amaro, Millin, and Illabong grown

at either 38/21°C or 26/15°C during grain development. The characteristics of the viscosity curves differed between the two temperature treatments. In grains of all three cultivars grown at 38/21°C, pasting temperatures and peak viscosities were higher than in grains grown at 26/15°C. Furthermore, setback was negative for grains grown at 38/21°C and positive for grains grown at 26/15°C. These trends were observed in grains from both the superior and the inferior position on the panicle. The addition of AgNO<sub>3</sub> indicated that α-amylase did not influence the viscosity curves (data not shown). Also, grains in a superior position on the panicle always had a higher peak viscosity than those in an inferior position.

Figure 3 shows the sucrose content of developing endosperms of Millin grown at 38/21°C and 26/15°C. The pool of sucrose in the endosperm was larger in grains from plants grown at 38/21°C and reached a maximum size at ≈12–15 dpa, decreasing after this point. However, in plants grown at 26/15°C, the pool of sucrose in the endosperms fluctuated little from anthesis until 20 dpa, but then it increased between 20 and 25 dpa. Changes in the sucrose content of endosperms of Millin and Illabong were similar (data not shown).

Figure 4 shows SEM images of the surface of a transverse section of the center of a translucent grain and a chalky grain. Figure 4a shows that cells were packed tightly together, and cell contents (amyloplasts) were packed tightly within the cells. This contrasts with the image in Fig. 4b, which shows a structural difference in the middle of the area, with airspaces and irregular cells. Figures 4c and d show cells that have broken open, revealing amyloplasts and starch granules. The amyloplasts in the translucent grain (Fig. 4c) were ≈12 µm in diameter, compound, and tightly packed with starch granules. Conversely, in chalky grain (Fig. 4d), not all cells were tightly packed with amyloplasts, and in the less ordered cells, there were a few amyloplasts of 12 µm, and the rest of the starch granules were not packed into compound amyloplasts. Figures 4e and 4f show the amyloplasts and individual starch granules of translucent



**Fig. 3.** Change in sucrose content over time (µmol/mg) of developing grains of Millin, Amaro, and Illabong rice grown at 38/21 and 26/15°C. ▲ = 38/21°C superior, ● = 38/21°C inferior, ◆ = 26/15°C superior, ■ = 26/15°C inferior.

**TABLE II**  
Amount of Amylose (%) in Chalky (C) and Translucent (T) Grains from Field and Glasshouse Experiments<sup>a</sup>

Cultivar	n	Field Experiment				Glasshouse Experiment			
		39/25°C		32/15°C		38/21°C		26/15°C	
		C	T	C	T	C	T	C	T
Amaroo	2	— <sup>b</sup>	—	17.1	17.4	··· <sup>c</sup>	14.7	···	19.9
Millin	4	16.3	17.1	—	—	14.4	14.0	18.8	16.9
Illabong	4	—	—	—	—	16.6	14.8	19.5	19.3

<sup>a</sup> Field experiment values were highly significant (Amaroo 0.02 <  $P < 0.03$ , Millin  $P < 0.001$ ). Glasshouse experiment values were significant (Millin grown at 26/15°C 0.005 <  $P < 0.01$ , Illabong grown at 38/21°C  $P < 0.001$ ); all others not significant ( $P > 0.10$ ).

<sup>b</sup> No sample.

<sup>c</sup> Insufficient sample.

and chalky grains. The amyloplasts of translucent grains (Fig. 4e) are regular, compound and tightly packed. In chalky areas (Fig. 4f), most starch granules are single, but some show evidence of compound organization.

Table II shows the amylose content (%) of mature grains from the field and glasshouse experiments. The amylose content of chalky grains was significantly lower than that of translucent grains for both Millin ( $P < 0.001$ ) and Amaroo ( $0.02 < P < 0.03$ ) in the field experiment. In the glasshouse experiment, the amylose content of both chalky and translucent grains from plants grown at 38/21°C was significantly lower than that of both chalky and translucent grains from plants grown at 26/15°C for all three cultivars ( $P < 0.001$ ). Furthermore, at each temperature treatment, the amylose content was always slightly higher in chalky grains than it was in translucent grains (Millin 26/15°C,  $0.005 < P < 0.01$ ; Illabong 38/21°C,  $P < 0.001$ ; all others not significant).

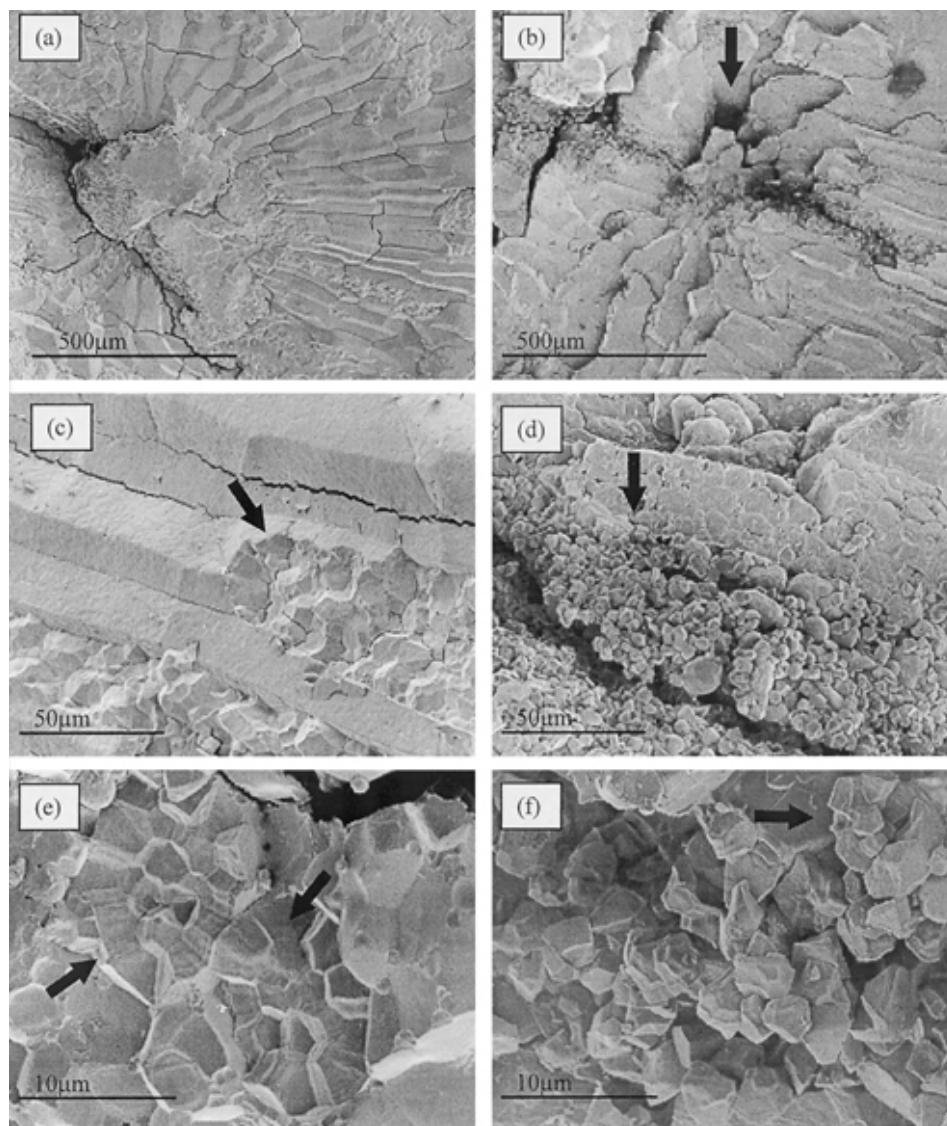
Figures 5a and b show viscosity curves of chalky and translucent grains of Amaroo and Millin from the field experiment. In both cases, translucent grains have higher peak and final viscosities than chalky grains.

Table III shows the amounts of each starch fraction from de-branched starch, separated by HPLC, of translucent and chalky grains of Amaroo and Millin. For both cultivars there were no significant differences between chalky and translucent grains in the amount of high or low molecular weight glucans in the amylopectin fraction. The amylose in chalky and translucent grains of Millin was not significantly different either, however there was a slightly significant difference between chalky and translucent grains of Amaroo ( $0.06 < P < 0.1$ ).

There was no discernible difference in the protein composition of chalky and translucent grains from any cultivar grown in the glasshouse when separated on polyacrylamide gels (results not shown).

## DISCUSSION

Rice grown in Australia undergoes stringent quality testing before being sold to various markets. Chalk is one quality parameter that is very closely monitored. Most markets will not accept rice that contains >2% of chalky grains because the rice is not visually pleasing. We attempted to determine whether chalk affects cooking



**Fig. 4.** Fractured plane of translucent (a,c,e) or chalky (b,d,f) grain: **a**, center of a translucent grain at low magnification **b**, center of a chalky grain where overall structure appears less ordered and less tightly packed (magnification showing regular cellular structure); **c**, at higher magnification, amyloplasts packed together inside a cell that has broken open; **d**, a cell of the chalky grain that has broken open, exposing loosely packed amyloplasts and single starch granules; **e**, at very high magnification, the regular size and shape of starch granules in a translucent grain packed into the amyloplasts (indicated by arrows); **f**, in the chalky grain, starch granules appear to very easily fall away from one another when an amyloplast (indicated by an arrow) is broken open.

quality and to explain the effects on the basis of the composition of chalky and translucent grains. Previous studies show that growing plants at high temperatures leads to higher numbers of chalky grains (Tashiro and Ebata 1975, Tashiro and Wardlaw 1991). Many other studies report a variety of responses to high temperatures, all of which can change different components of the grain and cooking quality. In using high temperatures to induce chalk, we must account for the effects of high temperatures on cooking quality separately from the effects of chalk on cooking quality.

### Effect of High Temperatures on Chalkiness

Illabong is an arborio rice; this class of rice is unique in that a chalky center is a normal and desirable quality. However, Table I shows that when Illabong did not experience temperatures >30°C during its growth, the incidence of chalk was very low. The same response, though not so obvious, was noted in Amaro and Millin. This study and others (Tashiro and Ebata 1975, Tashiro and Wardlaw 1991) indicate that rice forms more chalk in the grain when the plant experiences high temperatures during grain development. In all cultivars, and in both temperatures, grains in the inferior position on the panicle formed more chalk than did those in the superior position (Table I). This could be due to differences in the development of grains in superior and inferior positions on the panicle, which could be due to vascular supply or events in the endosperm. Presuming that the vascular system supplying a panicle of rice is similar to that supplying a head of wheat (O'Brien et al 1985), materials such as sucrose (the primary form in which carbon is transported to the grain) and amino acids traveling in the phloem would reach superior grains before inferior grains. However, Fig. 3 indicates that chalk is not due to insufficient vascular supply. In each temperature regime, inferior grains contain as much sucrose as superior grains. Therefore, it is more likely that chalk is related to metabolic events within the developing grain that utilize carbon in sucrose for starch deposition. In high temperatures, inferior grains, therefore, must be more susceptible than superior grains to those other metabolic events as the amount of chalk in inferior grains is higher than in superior grains.

### Effects of High Temperatures on Cooking Quality

Viscosity curves are routinely used to indicate the cooking quality of rice (Blakeney et al 1994, Okadome et al 1998). The difference between the final and peak viscosities (setback) correlates with the amylose content of the grain (Juliano et al 1964); a more positive setback relates to a higher amylose content. Also, final viscosity is related to softness of the gel, with a low final viscosity correlating to a softer gel. Figures 2a,c,e show that superior and inferior grains grown at 38/21°C have different peak and final viscosities and different setbacks from those grown at 26/15°C. The lower peak viscosity in the 26/15°C treatments (Figs. 2b,d,f) was not due to the action of  $\alpha$ -amylase. The negative setback for grains grown at 38/21°C (Figs. 2a,c,e) contrasted with the positive setback for grains grown at 26/15°C (Figs. 2b,d,f). Pasting temperature was also higher for the grains grown at 38/21°C. Taken together, the differences in functional properties and pasting temperatures of grains grown at 38/21°C and of grains grown at

26/15°C suggest different starch or protein compositions or different degrees of crystallinity within the starch granules (Tester et al 1999).

The shape of the viscosity curve for grains in the superior and inferior positions on the panicle does not vary significantly, but the height of the curves indicate that inferior grains are a little softer than superior grains (Fig. 2). The differences were present independently of the growing temperatures. This indicates that superior and inferior grains could always differ in quality. Umemoto et al (1994) report that inferior grains have less amylose than do superior grains (this was also the case in the glasshouse experiment in this study, Table II), which could explain this difference in texture between inferior and superior grains.

Table II shows that the amylose content of all grains grown in the higher temperatures was lower than of those grown at lower temperatures. According to Ayres et al (1997), variation in the amylose content of Californian rice cultivars can be explained by a single-nucleotide polymorphism in an allele of the waxy gene encoding the granule bound starch synthase (GBSS) enzyme. This polymorphism is also temperature sensitive (Larkin and Park 1999), with fewer GBSS transcripts accumulating in grains grown at high temperatures. Amaro, Millin, and Illabong are all descendants of Californian cultivars that carry the temperature-sensitive allele, and it has been determined that these cultivars also carry the temperature sensitive allele (unpublished data). Therefore, higher temperatures would lead to less amylose in the grains of all three cultivars.

### Effects of Chalkiness on Cooking Quality

Grains from the field experiment were separated into chalky and translucent grains. Figures 5a,b show that viscosity of chalky grains differs from that of translucent grains, indicating different cooking quality. Differences in cooking quality may be explained by different starch and protein compositions.

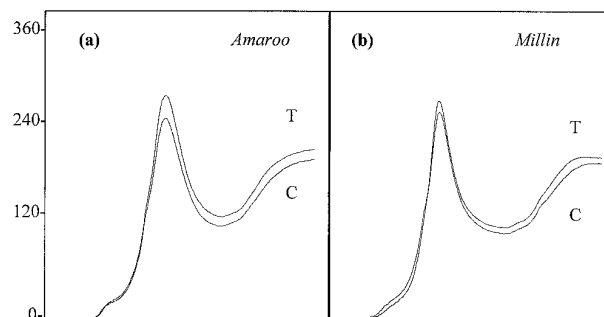
The amylose content of chalky and translucent grains from Amaro or Millin grown in the field was significantly different ( $P < 0.03$ ) (Table II). The amylose content of chalky and translucent grains was not significantly different in most cases in plants grown at either 38/21°C or 26/15°C in the glasshouse; Millin grown at 26/15°C and Illabong grown at 38/21°C were the exceptions ( $P < 0.01$ ) (Table II). Data in Table III shows that GPC-HPLC did not reveal differences in the structure of long-chain glucans between chalky and translucent grains. However, technology such as capillary electrophoresis (O'Shea et al 1998) could provide more detailed information on the fine structure of amylopectin. In wheat, high temperatures during grain development lead to differences in the types of proteins accumulated in the grain, which leads to different cooking properties (Blumenthal et al 1997). However, there were no differences in the protein compositions of chalky or translucent rice (data not shown). Therefore, it seems that the molecular structure of starch and proteins in chalky grains is not measurably different from that of translucent grains.

**TABLE III**  
Distribution (%) of Amylose and High Molecular Weight (HMW) and Low Molecular Weight (LMW) Glucans in Chalky (C) and Translucent (T) Grains of Amaro and Millin<sup>a</sup>

Cultivar	n	Amylose		HMW Glucans		LMW Glucans	
		C	T	C	T	C	T
Amaro	3	8.23 <sup>b</sup>	5.54 <sup>b</sup>	21.92	23.69	69.76	70.77
Millin	2	5.80	5.56	22.75	23.40	71.46	71.05

<sup>a</sup> From the field experiment. HMW and LMW glucans are shouldering peaks of the amylopectin fraction.

<sup>b</sup> Significant at  $0.06 < P < 0.10$ ; all others not significant.



**Fig. 5.** Viscosity curves of grains of Amaro (a) and Millin (b) from the field experiment. T = Translucent grains; C = chalky grains.

Chalk mostly occurs in the center of a grain and can occupy >50% of the area of the grain. Figure 4 shows that the ultrastructure of chalky areas differs greatly from that of translucent areas. In chalky areas, the air spaces, the presence of single granules rather than compound amyloplasts, and the disorganized cellular structure all offer the opportunity for increased water absorption during cooking, and thus a softer cooked grain. In chalky areas, the spaces and disorganized packing suggest that the concentration of starch is lower per square millimeter in chalky than in translucent centers. However, the error inherent in current methods of starch analysis could mask small differences in starch concentration. Also, it is most likely that individual granules swell differently from granules packed into compound amyloplasts, and that air spaces in chalky areas would allow greater swelling of cells and granules during cooking to occupy the spaces. Therefore, in chalky grains, greater swelling of less starch would dilute the concentration of starch in the gel and lead to a softer cooked rice.

Very little is known about the factors or processes that affect the packing of granules into amyloplasts or the initiation of amyloplasts, but these are the key differences observed in chalky and translucent areas. Juliano and Bechtel (1972) have shown that the starch granules in the rice grain increase in size first and fastest at the center of the endosperm and that starch synthesis begins in the center and moves outward. Therefore, at early stages of development, starch and starch granules are being synthesized in the middle of a milky sac. It would seem that, at this stage, high temperatures can disrupt this process, and perhaps chalk is a result of such a disruption.

## CONCLUSIONS

The presence of chalk in rice grains is not merely a cosmetic factor. High temperatures during grain development lead to an increased incidence of chalky grains. However, chalky grains differ from translucent grains in cooking properties, in cellular structure, and in the morphology and packing of the starch granule, independently of high temperature, indicating that the formation of chalk in a grain is not controlled solely by environmental factors. Even though different starch and protein compositions seem likely between chalky and translucent grains, more sensitive techniques must be used to ascertain whether or not this is the case. Future work should allow us to explain the variations in amylose content at different growth temperatures by screening for a temperature-sensitive polymorphism in an allele encoding for granule-bound starch synthase. More information about the initiation and packing of amyloplasts is needed to understand how high temperatures affect individual starch granules and amyloplasts, changing their morphology and packing so much as to cause air spaces and the appearance of chalk in grains.

## ACKNOWLEDGMENTS

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