7. Discussion

Amylose content is one of the most important factors affecting the cooking and sensory properties of rice. However, rice quality focuses mostly on amylose content and has not addressed amylose structure. It is well known that the cooking properties of different rices that have the same amylose content, can differ enormously[1-4.] An example of this in the Australian program are the three varieties Amaroo, Koshihikari and Millin. These have exactly the same amylose content but very noticeable differences in cooking properties, especially to Japanese palates. Amylose structure has been cited as the cause of differences between varieties of different sensory properties but identical amylose content[5-10], but no definitive answer is available. Here, we attempt to develop a method to investigate amylose structure using those three varieties of rice, Amaroo, Millin and Koshihikari and then to use that information to investigate the relationship between amylose structure and cooking properties. The cooking property chosen was resistant starch. Resistant starch is thought to be related to amylose content much more strongly than to amylopectin[11] because of the rapid way that amylose chains aggregate and resist attack by digestive enzymes. Further, as the world becomes familiar with nutritional requirements other than minerals and vitamins, and the requirement for starch in our diets for satiety and health[12-15], research on resistant starch is timely. The ultimate aim of this project is to deliver the outcomes in a form that that will benefit the rice improvement program.

Objective 1: Searching for allelic variation in the GBSS gene

The first objective towards finding differences in amylose structure was to seek differences in the sequence of the gene considered responsible for amylose synthesis, namely granule bound starch synthase (GBSS1). Sequence variation was sought by determining the sequence of the 8 exons of GBSS1. No differences were found during this project. However, simultaneously, a group in the US published two genetic differences in GBSS that explained more than 80% of the variation in amylose content of rice[16.] The first of these was a single nucleotide polymorphism at the splice site of intron 1. This SNP had been reported previously[17], but the functional significance and relationship with amylose content was reported by Ayres et al. (1997). The particular base determined whether the exon spliced correctly, which determined how much GBSS protein was translated. Many workers have concluded that this SNP defines the Wx^a and Wx^b allele[18-22]. The Wx^a allele is found in the indica and tropical japonica varieties and the Wx^b allele is found in temperate varieties. Amaroo, Koshihikari and Millin are all varieties with the temperate japonica class, so there would be no sequence difference at the splice site of intron 1 between these varieties. The second genetic variation was found in the flanking region of the gene. It is a simple sequence repeat (SSR) of alternating C and T [16, 23]. The number of dinucleotide repeats related well with amylose content. In the work reported here, the flanking region was not sequenced, thus the SSR was not detected. However, collaboration was generated with the American group, and the SSR was determined for a sub-set of the Australian collection. This is shown in Figure 4. Interestingly, the three varieties chosen all carry different CTn. Koshihikari is CT17, Millin is CT18 and Amaroo is CT 19. This was the only sequence variation found within the GBSS gene for these 3 varieties.

Amylose molecules are lightly branched[24, 25], thus a branching process is required to obtain that structure. Sequence variation was sought in Branching Enzyme 1 since several studies implicate BE1 in the synthesis of amylose. The earliest studies linking BE1 and GBSS are in vitro studies showing that BE I is much more likely to branch long chains like amylose rather than short chains like amylopectin [25, 26]. More recent studies using mutants show that the absence of BE1 does not affect the chain length distribution of amylopectin in rice, maize or wheat[27-29], suggesting either that BE1 is not involved in amylopectin synthesis, or that the other isoforms of BE can carry out the function of BE1 in its absence. BE1 has been linked genetically to GBSS[30] by the discovery of some interaction between the transcription of BE1 and GBSS, leading to the proposal that the expression of the BE1 and GBSS is coordinated[30]. These studies all suggest that BE1 does not play a crucial role in amylopectin synthesis, but that its kinetics and genetics could link it with the synthesis of amylose. However, no variation was found among the exons of that gene for the three varieties.

Thus, the outcome of the first objective was finding the variation in the SSR for the three varieties, as well as the rest of the Australian germplasm, in collaboration with Drs Bill Park and Nicola Ayres at Texas A & M University, College Station, Texas. The microsatellite is now being used successfully in the Australian Rice Improvement program.

Objective 2: A method to measure amylose structure

In order to try to relate the variation in SSR with amylose structure, and in order to obtain some way to obtain information on the structure of amylose, a method to measure amylose structure was developed by using tools of chromatography. Size exclusion chromatography (SEC) is commonly used to analyse two properties of polymers. These properties are the hydrodynamic volume of the molecules in particular eluants and the molecular weight distributions of linear chains using standards of the same chemical structure as the polymer, or by using the principle of universal calibration to calibrate the column by using Mark Houwink parameters to relate the elution profiles of standards of a similar composition to the polymer of interest to the elution profile of the polymer of interest[31].

Many groups use SEC with pullulan standards to measure the 'MWD' of amylose. However, pullulan standards are not the same composition of starch, so their configuration in the eluant differs from that of a linear chain of starch, and consequently, the elution profile of a pullulan standard significantly under-estimates the MW of the starch molecule at the same elution volume31. This principle is not widely recognised in the field of starch chemistry, but it was observed in this work.

The first step towards measuring amylose structure is to separate the amylose from the amylopectin. It is well known that amylose exists as a non-crystalline polymer within the starch granule, and upon heating in water, it leaches, possibly though channels[32], from the granule to join the liquid phase of the cooking pot[33-35]. This is the first study where the amylose was leached directly from flour, rather than after purifying the starch. Purification of rice starch is not trivial, and the most commonly used method is the alkaline precipitation method. However, other studies in the laboratory have found that the alkaline precipitation method damages the long chains of amylose[36]. Moreover, more recent work in our laboratory shows that purification of starch by any method increases the solubility of amylopectin (Willoughy unpublished), thus separating amylose from flour should minimise the contribution from amylopectin. Figure 5

shows that amylose begins to leach above 90ºC, which is well above the gelatinisation temperature, thus other studies, relying on gelatinisation temperature, are not likely to collect all the amylose into the hot water soluble fraction. Further, the absorbance of the leached components of the intermediate amylose variety was higher than of the three low amylose varieties. The standard technique of Rapid Visco Analysis easily heats to that temperature, so the RVA was used to give a consistent set of heating/cooking conditions in order to investigate reproducibility of leaching of amylose without shear. The method was very reproducible, both within days and for different days. However, Figure 5 also shows that some leaching occurs from a waxy variety. This material could either be fragile outer layers of the starch granule that begin to dissolve[37], or it could be less crystalline molecules of amylopectin that dissolve more readily than semi-crystalline lamellae. The material in the supernatant, the hot water soluble (HWS) fraction, was analysed to determine the proportion of amylopectin molecules in it.

Two SEC columns were tested for measuring amylose and amylopectin molecules and the chains that comprise the molecules. Pullulan standards underestimate the MW of amylose (Ward et al. submitted), but they provide a broad guide to the separation capacity of the columns. Through the use of pullulan standards (Figures 6 and 7), the UH 250 was chosen for separating the chains of amylose from the chains of amylopectin, though this column required that the starch was debranched to linear chains[38], and the UH 500 was chosen for separating molecules in the HWS fraction of the starch (obtained from the modified RVA method). Thus the contribution of amylopectin to the HWS fraction could be determined.

Figure 8b shows that a waxy variety (amylopectin only) contains no chains in the HWS fraction that elute before 18 min, thus any chains eluting before 17.5 min on the UH500 could be attributed to amylose. Figure 8a shows the chains in the supernatant and the pellet of a non-waxy variety and shows that all the long chains (eluting before 18 min) are in the supernatant, and none are retained in the pellet. In other studies where a supernatant was obtained by leaching[33-35], some of the amylose always remained in the pellet.

In this study, all the amylose (long chains) was recovered in the HWS fraction, however, Figures 8b and 9 show that amylopectin contributes to the HWS fraction. Figure 8b, shows this conclusively since the figure shows the starch (debranched) in the HWS fraction of a waxy rice. Figure 9 shows the distribution of the whole molecules within the HWS fraction and of the chains (after debranching). The trace of the whole molecules shows a large peak in the exclusion zone (before 11 min), indicating elution of very large molecules. The separated linear chains do not show the large peak in the exclusion zone, and show a large peak beyond 18 min instead. This indicates that the process of debranching effectively deconstructs the large molecules in the exclusion zone to their constituent small branches, which elute beyond 18 minutes. Such large molecules made of small branches are highly likely to be amylopectin molecules, thus it must be concluded that amylopectin contributes to the HWS fraction. Furthermore, many amylopectin molecules elute in the separating phase of the column, along with the amylose molecules. These amylopectin molecules must be much smaller than those that elute in the exclusion zone. Different speeds and times of centrifugation did not cause the amylopectin molecules to precipitate (Figure 10), thus it was not possible to exclude amylopectin from the fraction without altering the fraction. However, the reproducibility of the method indicates that the contribution of amylopectin to the HWS fraction is not random, but depends on the variety. In terms of processing, cooking and sensory properties, the solubility of starch is likely to be a key

parameter, thus a contribution from amylopectin to the HWS fraction was accepted for the remainder of the work, and the amount was accounted for in any interpretation.

A deeper understanding of amylose structure can be gained by knowing the branching frequency, the length of the backbones and branches, and the number of amylose molecules. Snippets of this information can be obtained by labelling of the amylose molecule and obtaining the number average and the weight average degree of polymerisation, and then obtaining the same information for the amylose chains. Two conditions must be met in order to draw valid conclusions from such a study, (i) each amylose molecule or chain must be labelled and visible to the detector, and (ii) each chain must carry only one label.

Reductive amination of the reducing end of starch molecules has been used previously to introduce a label onto a starch chain[8, 39-42]. The chemistry of this method is well established and involves attaching a primary amine, carrying a fluorophore, onto the reducing end of the starch molecule. There is only one reducing end on each starch molecule, thus only one label is introduced per molecule and the first condition is met. Efficiency of labelling across a range of chain lengths has been tested up to DP 135 for one of the fluorophores (APTS)[41], and in that study, efficiency decreased slightly, although reproducibly, as the chain length increased. That fluorophore has been optimised for fluorophore assisted capillary electrophoresis, but the method is useful only for amylopectin chains. In another study, starch was chemically purified, and amylose collected from that and labelled with 2 AP[24]. Labelling efficiency was tested and was reasonable. However, all our attempts to use that technique on amylose were unsuccessful. Attempts to use other fluorophores were made, but the cost, time and irreproducibility rendered the technique impractical. The major difference between the work here and the other study[24], is that we used amylose that had not been collected by passage through a number of chemical reactions. The methods used in that study to purify starch have been found to alter the structure and the solubility of amylose[36]; this could explain why we were unsuccessful with the technique.

Late in the term of this project, labelling of amylose structure was revisited. A new label was discovered - a chromophore with simpler labelling, better chemistry and lower cost relative to the fluorescent options. Since the chemistry of the new label – ANDS – is essentially based on the same principles as the fluorescent labels described earlier, in that only the reducing end is labelled, we can be sure that each chain is labelled only once. In order to measure labelling efficiency, we can take advantage of pullulan standards, of known molecular weight, and the simple mathematical relationship between the response of the UV detector and the response of the Refractive Index (RI) detector[31]. The UV detector is a number sensitive detector, and the signal represents $P(M)$, ie the proportion (P) of chains of a particular molecular weight (M), but the RI detector is mass-sensitive, and for most polymers, the signal represents $M^2 P(M)$[31]. The two signals can therefore be related by mathematically transforming the signal from one detector to the other so long as the molecular weight of the polymer is known. Figure 11 shows the pullulan standards after labelling with ANDS, and shows the RI and UV signals. The UV signal certainly decreases as the molecular weight increases (high molecular weight elutes earlier that low) (Figure 11). The same mass of each standard was labelled, thus for the higher molecular weight pullulans, the number of molecules is fewer than for lower molecular weight pullulans. If labelling efficiency is independent of molecular weight, we expect that the transformed UV signal will be equivalent to the RI signal. If labelling efficiency decreases as molecular weight increases, we expect the transformed UV signal to be lower than the actual RI signal. Figure 12 shows the UV trace expressed as a signal from the RI, and shows that the signals are essentially

equivalent. Thus we can be confident that labelling efficiency is maintained up to a molecular weight of about 800 000.

Now that separation and measurement of amylose is validated and understood, we can investigate the structure of amylose in the three varieties, Amaroo, Millin and Koshihikari. Figure 13 shows the elution profile of the hot water soluble fraction of each. There is very little difference between the varieties, but as shown earlier, a significant amount of amylopectin co-elutes with the amylose, obscuring any differences between the varieties for the distribution of amylose. In SEC, the elution time of a molecule does not depend on its molecular weight, but on its hydrodynamic volume. This means that a long amylose molecule and a small amylopectin molecule could occupy the same volume in the eluent, and thus elute at the same time. In order to determine the amount of amylose and amylopectin in each fraction, a long SEC column was used to fractionate the samples. Figure 14 shows the colour of each fraction when mixed with iodine and shows which fractions contain amylose. Most fractions show blue staining, which is the expected colour of the amylose iodine complex (figure 14). Figure 15 shows the separation of each fraction on the UH 500, and shows that there are basically three groups. The earliest fractions have most starch eluting in the exclusion zone (before 10 min), and in the separating phase of the column, the starch in the earliest fraction elutes at about 12 min. The middle fractions also contain starch in the exclusion volume, but in the separating phase, the peak is at about 14 min, representing decrease in size of the molecules. The latest fractions also contain starch that elutes in the exclusion zone, and the molecules that elute in the separating phase are smaller again. Figure 15 shows the elution profiles of the fractions for each variety.

Figure 15 shows that the early fractions of Millin contain much more starch than the early fractions of the other two varieties (note the scale), and for Millin, most of it elutes in the void volume. Comparison with the elution profile of the debranched fractions (Figure 16), most of the starch in the exclusion zone is amylopectin. The starch that elutes in the separating phase of the column is long linear chains. Millin contains more long linear chains of amylose than do the other two varieties. For the intermediate fractions, Figure 16 shows that for Millin and Koshihikari, there are 3 populations: the exclusion zone (amylopectin), and two peaks in the separating phase. However, upon debranching there is a normal distribution of chains. The intermediate fractions contain chains of lower molecular weight than the early fractions, and again, Millin has the most. However, for the last few fractions, with shorter linear chains (Figure 17), Koshihikari seems to have the most. Recently, the UH 500 column was calibrated for determining the molecular weight distribution of linear chains of amylose (Ward et al.). Extrapolating from that work, for the chains in the early fraction (Figure 17) the peak maximum is about DP 5000, in the intermediate fractions the peak maximum is about DP 2000 and in the late fractions they are about DP 500.

The differences in distribution of amylose chains amongst the varieties suggest different functional properties of the rice. During cooking, the starch granule gelatinizes and the amylose leaches out of the granules[35, 43] into a continuous phase[44]. For a variety like Millin, which has twice as many long chains as Koshihikari (Figure 17), the concentration of the continuous phase would be much higher than it is for Koshihikari, and the proportion of long chains in that phase would be higher than for Koshihikari. Aqueous solutions of amylose (as would be found in cooked rice) are inherently unstable, and thus the amylose is prone to forming a viscoelastic paste or a gel[45]. Whether a paste forms or a gel forms relates to the concentration of the amylose in the solution and the chain length of the amylose[45]. The rate of aggregation of amylose, is strongly

related to chain length in that chains of DP 80 aggregate rapidly and chains of DP 2000 aggregate slowly, and the structure of the resultant aggregate differs[45]. In a study using synthetic amylose, it was found that for long chains of amylose, gelation was favoured over precipitation, and on standing, the gels exuded some water. For short chains (DP 500), some gelation occurred at higher concentrations, but precipitation predominated[45]. Thus, the higher concentration of long chains in the amylose of Millin would suggest that the amylose of Millin is more prone to forming a gel than the amylose of Koshihikari, and on cooling, the gel would harden for Millin. The amylose in Koshihikari would precipitate, leaving essentially a visco-elastic paste, that would retrograde very slowly[46]. Thus, investigating amylose structure can provide a significantly better insight into cooking properties than can the measure of amylose content. Figure 18 shows that the three rice varieties are similar on the outside, but clearly they differ on the inside.

Objective 3: Relationship between amylose and resistant starch

Resistant starch is that portion of the starch that is not digested in the gut and moves through the digestive tract to the large intestine[47]. It acts in a similar matter to dietary fibre and ferments in the colon. It is believed to contribute to good bowel health, acting as a substrate for colonic bacteria to produce butyrate. Butyrate and other short-chain fatty acids, such as propionate may reduce the risk of colorectal cancer and aid in management of inflammatory bowel conditions[48]. Several studies show that resistant starch can act as a prebiotic, an agent that can carry bacteria, such as lactobacillus, to the colon, enabling this beneficial bacteria to colonise the colon more easily (refs), and assist in the treatment of conditions, such as infectious diaorrhea[12, 15, 49-53].

There are four types of resistant starch[54]: Type 1 is physically unavailable for digestion, such as brown rice where the bran layer might restrict access for digestive enzymes; Type 2 is naturally-occurring resistant starch granules, for example in green bananas,; Type 3 is retrograded starch which becomes difficult to digest because of the crystals formed; and Type 4 is chemically modified.

Resistant starch (RS) is rather difficult to measure. However, in vitro methods to measure resistant starch have recently been developed[55]. The method developed by McCleary et al. (2002) mimics the processes of digestion in vivo. Figure 3 shows the method, and it was tested with a number of varieties of rice, ranging in amylose content, to ensure that all the starch is accounted for. Table 3 shows that all the starch was accounted for, when moisture content is factored in. Despite the fact that all the starch is accounted for, it must be stressed that this is an in vitro method, using a limited number of enzymes, in conditions that probably are not the same as those in the gut. In this work, we determine the structure of resistant starch from freshly cooked rice and from retrograded rice.

The propensity of amylose to form gels and aggregations has led to a general assumption that amylose relates strongly to resistant starch, and a previous measurement of RS from rice also suggest that the molecule is amylose[56]. Table 4 shows the varieties used, their origin, the amylose content, CTn and RS content. The lowest RS is 0.9% and the highest, excluding the amylose extender, is 3.7%. Figure 19 shows that RS does relate reasonably well to amylose content and reasonably well to the proportion of long chains (as measured by SEC). The hot water soluble fraction contains all the amylose and a small amount of the amylopectin, so potentially was an ideal sample for measuring the structure of RS, but Figure 20 shows that there is very little relationship between the amount of hot water soluble starch and the RS content. In order to

characterise the structure of the RS molecules, RS was collected during the process of measuring it at the stage just prior to the final hydrolysis. The RS molecules are much smaller than expected (Figure 22), eluting at the end of the separating phase of the UH 500 column, and necessitating the use of the UH 250 column (Figure 23). RS molecules elute after amylose (11 min) and before and with amylopectin. The signal obtained by SEC is consistent with the RS content obtained spectrophotometrically (Figure 23).

The UH 250 column has recently been calibrated for the molecular weight analysis of linear chains of starch[31]. Amylose molecules are essentially linear, and if the RS is actually portions of amylose chains, the molecular weight of the RS should agree with the predicted molecular weight given by the calibration for that particular elution time. Figure 25 shows the calibration and shows that the molecular weight of the RS is higher than it would be if it was linear chains. Thus, it is likely that at least some of the RS is not linear chains.

The RS from freshly cooked rice of some Australian and Asian rices was examined by SEC for whole molecules and the chains obtained after debranching. Figure 26 shows that the Asian rices are generally higher in RS than the Australian rices (except for Doongara), though they are also higher in amylose content and also are indica varieties. Comparing Figure 26b with Figure 24 shows that the amylopectin chains for full flour elute between 16 and 19 min, with most short chains eluting just before 18 min. However, the elution profile of the chains comprising the RS molecules differs from that of amylopectin chains in that it is not bimodal, but a normal distribution. Further, the longer chains of amylopectin elute between 15.5 and 17 min, and this is exactly the region that the chains of the RS molecules elute (Figure 26b). Figure 27 shows the SEC trace of amylose in the full flour and in the RS for a number of varieties, and shows that very little amylose remains. Either the amylose is easily digested by the enzymes, contributing to the non-resistant starch, or the amylose chains are partially digested and small chains remain. In order to examine the chain length distribution of the RS molecules more closely, capillary electrophoresis (CE) was used. CE gives a series of well separated peaks, each corresponding to a particular chain length of the starch[41]. It is useful in the range of DP 1 – 80. Figure 28 shows the CE trace of chains of amylopectin from full flour. The shortest chains are DP 6 and the longest detected are DP 87. Most chains are in the range of DP 10 – 15. Figure 28 shows the CE traces of six Australian rices and shows a large peak of DP 1 and 2 (glucose and maltose) and shows proportionally much fewer chains of DP 9 – 15, indicating that these were digested and formed part of the non-resistant starch. Further, the chain length distribution of the RS from freshly cooked rice is unique to each variety, suggesting that the tertiary architecture of the molecule affects the capacity of the digestive enzymes to access the amylopectin. Thus, we conclude that RS from freshly cooked rice is a mixture of shortened amylose chains and the remainder of digested amylopectin. The structure of the amylopectin determines how much of the molecule the enzymes can access.

Naturally occurring RS in rice is quite low, but different ways of handling and cooking rice can augment it. For example, parboiling increase it[57] as does different baking methods[58]. The first type of RS resists digestion because it is physically inaccessible to the enzymes. A study with hamsters fed cooked white rice, cooked brown rice, and cooked white rice plus bran shows that classic responses linked with RS are much higher for the cooked brown rice[13]. Cooked white rice with added bran elicited the same responses as cooked white rice alone[13], suggesting that the bran layer protected the rice from digestion. In this study, we found that the RS content of brown rice was almost twice as high as from white rice, consistent with the findings of Kahlon et al. (2000),

and further establishing the health benefits of brown rice. Cooking rice by the rapid-boil method caused a decrease in RS, suggesting that the molecules that form the RS are leached. Fresh gels showed almost no RS, but after one week, staled gels showed a 5-fold increase in RS. Over a week, the amylose and amylopectin molecules retrograde[59], and the retrograded starch would be inaccessible to the enzymes.

Resistant starch was examined in cooked and retrograded rice. Tables 5 and 6 shows the values. Table 6 shows the values for RS in freshly cooked and in retrograded, and shows the difference for each variety upon retrogradation. Koshihikari grown in Japan, IR 64 and PSBRc12 did not retrograde at all, and these are varieties known, at least anecdotally, not to retrograde. Koshihikari grown in NSW did retrograde, indicating an environmental effect on propensity to retrograde. Varieties known to retrograde, anecdotally, contained twice as much RS in the retrograded state than in freshly cooked (highlighted Table 6). These varieties are Kyeema, PSBRc 10, 98 and 18. We therefore suggest that the increase in RS content from freshly cooked to retrograded provides a quantifiable measure of the susceptibility of rice to retrogradation, and importantly, it is a test conducted on cooked rice grains, not on flour. However, it is not a method for routine screening, but perhaps for pre-release of late generation lines.

Variety	RS fresh	RS retro	% difference
Amaroo	1.26	1.67	32.54
Basmati	2.67	3.7	38.58
Doongara	2.15	3.17	47.44
Koshihikari	1.30	1.73	33.08
Koshihikari-Japan	0.97	0.99	2.06
Kyeema	0.89	1.6	79.78
Langi	1.24	1.5	20.97
Opus	1.17	1.41	20.51
IR60	2.99	4.5	50.50
IR8	3.40	3.9	14.71
IR5	3.38	4.85	43.49
IR64	2.54	2.57	1.18
IR24	0.83	1.2	44.58
AE	6.24	11.97	91.83
PSB Rc10	1.88	4.2	123.40
PSB Rc98	3.09	5.9	90.94
PSB Rc12	2.47	2.6	5.26

Table 6: RS content of fresh and retrograded rice, and difference between the values (%)

Figure 30 shows that the RS content of retrograded rice relates quite well to amylose content and to the RS content of freshly cooked rice, but as shown in the Table above, the fact that it doesn't relate perfectly is an important finding for the quantification of retrogradation.

The structure of RS molecules from retrograded rice (Figure 31a) shows no detectable difference from that of freshly cooked rice. Notable is that Koshi from NSW showed more RS than Koshi from Japan, indicating an environmental effect on RS or retrogradation. Debranched RS from retrograded rice does show a difference in structure from fresh rice (Figure 32) in that the debranched RS from retrograded rice contains a population of chains of about DP 7-12 that is not

present in the RS from freshly cooked rice. Retrogradation of amylopectin would occur in the samples, which would involve recrystallisation of amylopectin chains 60, and in the crystallised state, they would be protected from enzymic digestion.

8. Conclusions

A method to measure the structure of amylose has been developed and will be useful in the future to understand the relationship between amylose structure and cooking properties. Interpreting the chain length distribution of Millin and Koshi in terms of polymer science indicates that amylose from Millin probably forms a gel, which is capable of hardening over time, whereas the amylose from Koshi is more likely to form a visco-elastic paste, which is not capable of hardening to the same degree as Millin. Amylose appears to be related to the amount of resistant starch that occurs both naturally and in retrograded rice, but the main remaining molecules in a sample of resistant starch are the dextrins of amylopectin. Retrogradation is not simple to measure, but a quantifiable measure of retrogradation of cooked rice appears to be the increase in RS content between freshly cooked and retrograded rice. However, the method is not optimal for routine screening, but could conceivably be used for late-generation screening.

9. References

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