STUDIES ON RICE STARCH

THE ACCUMULATION AND PROPERTIES OF STARCHES IN THE STEM, POLLEN AND GRAIN OF SOME RICE VARIETIES GROWN IN SOUTHERN NEW SOUTH WALES

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BY

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Dear Mr Blakeney

The Head of the Department of Agricultural Chemistry has confirmed that the necessary corrections have been made to your thesis. Accordingly, I am pleased to advise you that you have been awarded the degree of Master of Science in Agriculture.

The next ceremony at which your degree could be conferred in person will be held on Saturday 14 March 1981. If you would prefer that the degree be conferred in absentia which could be done in the near future, would you please let me know.

I have available for return to you one copy of your thesis. Would you please advise me of the address to which it could be sent.

Yours sincerely

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TABLE OF CONTENTS

Page

PREFACE

The work reported in this thesis was carried out at the New South Wales Department of Agriculture's Agricultural Research Centre, Yanco between March 1972 and December 1979. The work was supported by the New South Wales Rice Industry through the Irrigation Research and Extension Committee; Rice Investigations Sub-Committee who provided some equipment and the services of a technical officer.

I thank my colleagues in the Rice breeding and Agronomy sections of Yanco Research Centre, Dr. D.J. McDonald, Mr. E.B. Boerema, Mr. L.G. Lewin and Mr. R.A. Hartley for advice and assistance in growing the rice trials. I thank Miss Kathy Tucker, Miss Deborah Cantrill, Miss Lynsey Welsh and Mrs. Helen Bourke for their technical assistance. Without the aid of these people the large amount of sample collection and preparation necessary for this study could not have been completed.

The scanning electron micrographs were taken at the Electron Microscopy Unit, Sydney University and I thank Dr. C.E. Nockolds and Miss D. Hughes for their advice and assistance.

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The scanning electron microscopy results were the basis of a paper delivered at the 6th World Cereal and Bread Congress, Winnipeg, Canada in September 1978. (Abstract appeared in Cereal Food World 23 (8) p.489 1978); Plates 7 and 10 also appeared in a research news item in the Agricultural Gazette of New South Wales (89 (4) p.47 1978). All other results are unpublished.

[Redaction]

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SUMMARY

A group of rice varieties known to differ in grain cooking quality were grown together in southern New South Wales. The accumulation and properties of starch were followed from seedling emergence to harvest.

Stem starch increased steadily in all varieties until flowering and then decreased as the grains filled and ripened. Starches isolated from rice stems 8 and 16 weeks after seedling emergence had similar properties for all varieties. The two varieties that have grain with waxy endosperm starch had non-waxy stem starch. The high levels of starch present in rice stems may make a significant contribution to grain yield and requires further study.

Pollen was collected and analysed for starch, protein, lipid, ash and sugar content. Rice pollen was found to contain about 22% starch. Within a variety starch isolated from pollen had a similar amylose content to mature endosperm starch. Rice varieties with waxy endosperm starch had waxy pollen starch. The analysis of rice pollen for starch and amylose content may be of use in rice breeding programmes. New crossbred lines could be sorted into amylose classes before harvest.

The accumulation and properties of starch in developing grain was investigated. All varieties were found to increase in amylose content and decrease in gelatinisation temperature as

ripening progressed. The molecular size of the amylose also increased during ripening. Mature grain was harvested and the Brabender paste viscosity, cooked grain texture and starch properties studied. All varieties had properties within the previously reported range.

Chalky cored endosperm is observed as an opaque centre in otherwise translucent grain. It detracts from the visual appeal of a rice sample and consequently reduces its market value. Light and scanning electron microscopic investigations of chalky cored endosperm revealed that it is due to small air spaces present in the endosperm tissue. Chalky tissue is less densely packed than translucent tissue. The properties of starch isolated from chalky and translucent parts of grain were similar.

Two methods of sugar analysis originally developed for clinical chemistry were adopted and standardised for general use.

ABBREVIATIONS

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
IRRI	International Rice Research Institute

INTRODUCTION

1. RICE GROWING IN SOUTHERN N.S.W.

Rice is grown within the irrigation areas associated with the Murrumbidgee - Murray river system. Yanco Agricultural Research Centre, where this study was undertaken is located at the south-eastern edge of the Murrumbidgee Irrigation area (fig. 1).

The New South Wales rice crop is grown exclusively under flood irrigation and operations are entirely mechanised. Planting is carried out in September and October either by combine or sod-seeder into a dry seedbed or by aerial sowing of pre-germinated seed into shallow water. Water is maintained on the crop for approximately four months. Before harvest the water is drained and the soil allowed to dry; normal mechanical header harvesting is then carried out. The crop is essentially disease-free and is troubled by few insects; weeds, mainly barnyard grass, can be a problem (Swain 1973).

The physiology of rice growth in southern New South Wales has been discussed by Boerema (1973) and is illustrated in figure 2. Temperatures at both ends of the rice growing season are low. Seedling emergence is slow but further vegetative development takes place during a period of increasing temperature and radiation. Panicle differentiation and growth occurs during the period of highest temperature and radiation. Grain set and ripening occupy a period of falling temperature. The

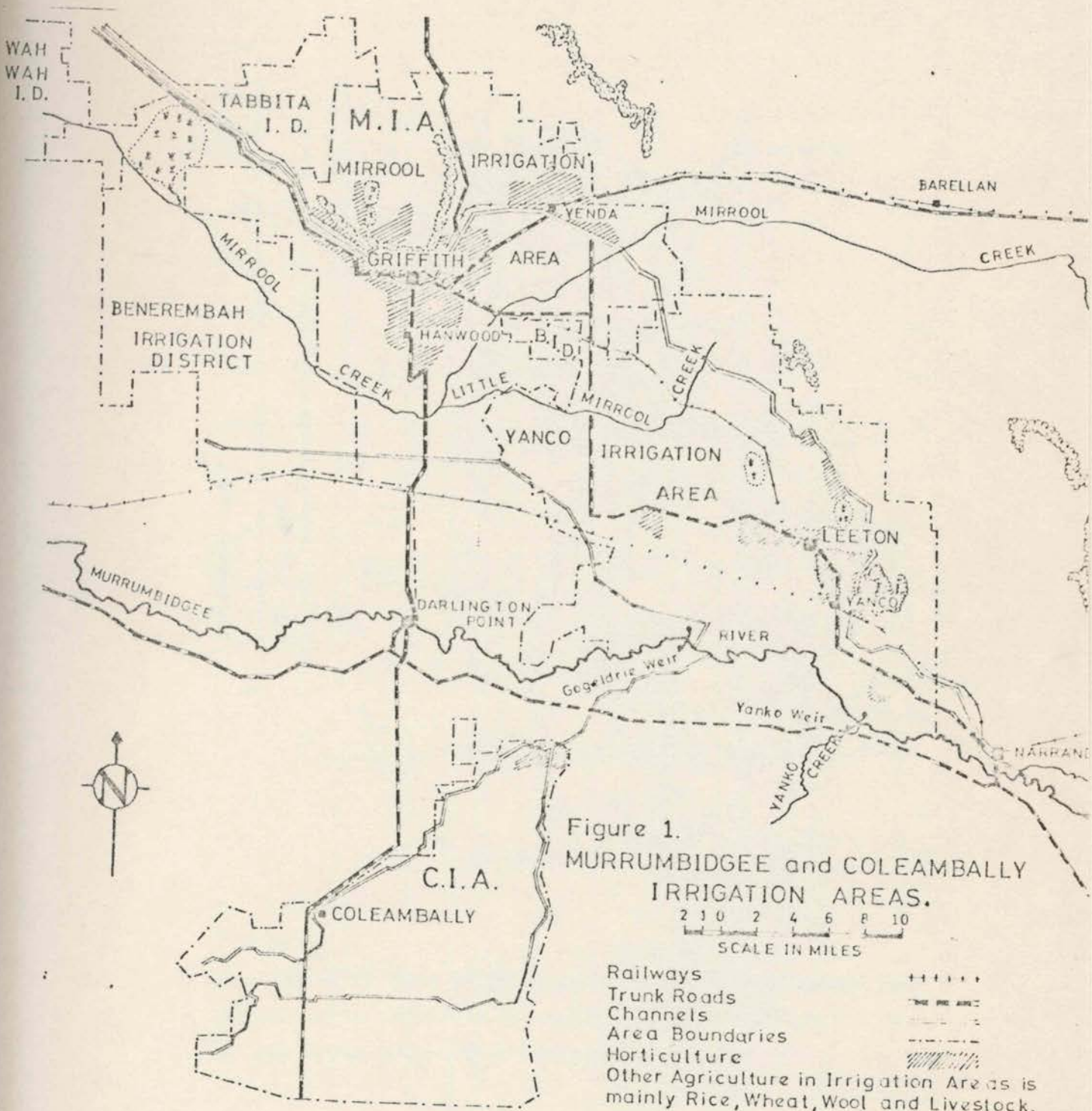


Figure 1.
MURRUMBIDGEE and COLEAMBALLY IRRIGATION AREAS.

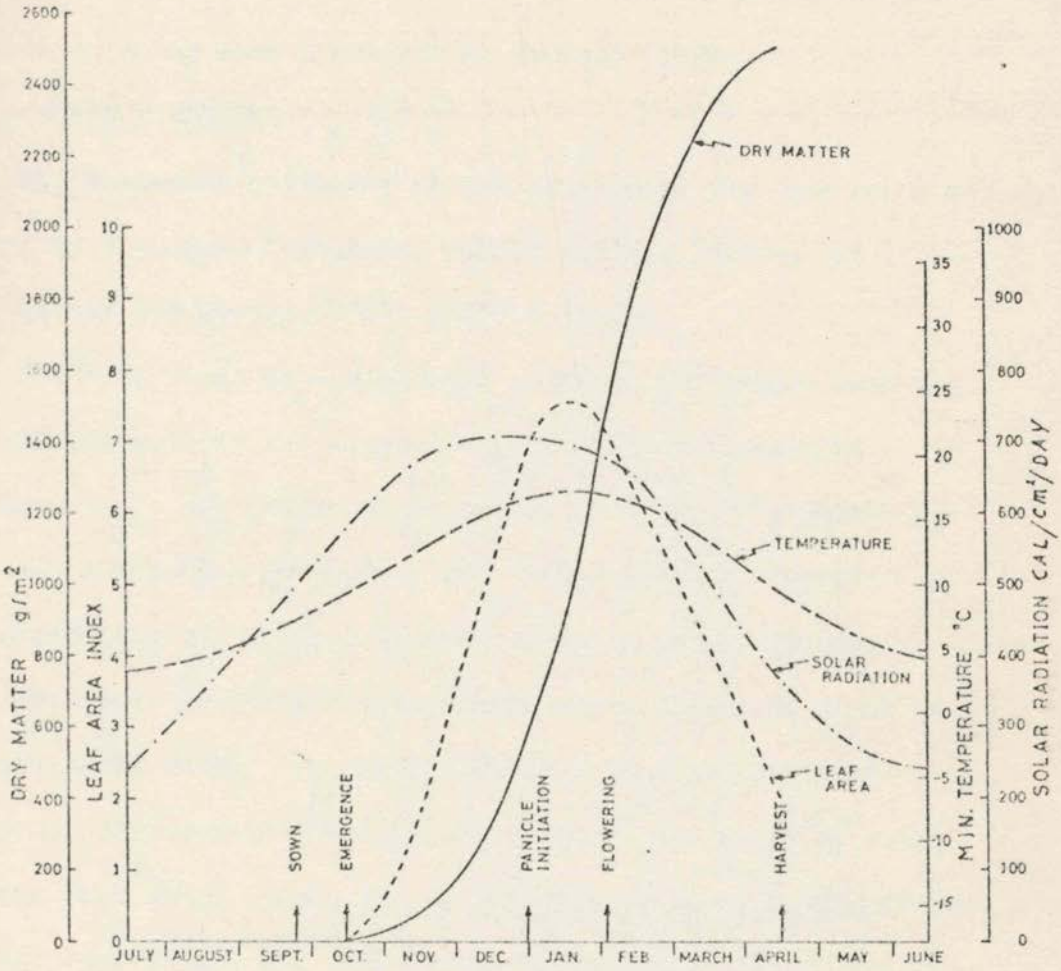


Figure 2.

The growth of rice in relation to minimum temperature and global solar radiation in the Murrumbidgee Irrigation Area of New South Wales (from Boerema 1973).

climatic effects on growth and yield of rice in the Murrumbidgee Irrigation area has been discussed by Boerema (1974).

2. RICE GRAIN STRUCTURE

The botanical structure of the rice grain has been described in detail by a number of workers, Santos (1933), Juliano and Aldama (1937), Little and Dawson (1960) and Cho (1967).

The rice fruit is a caryopsis in which the single seed is fused with the wall of the ripened ovary (pericarp), forming a seed-like grain. The caryopsis is called "brown rice" because it usually has a brownish pericarp. The surface of the caryopsis has ridges corresponding to those of the lemma and palea (hulls).

The rice caryopsis varies widely among cultivars in shape, size, length and width. The United Nations Food and Agriculture Organization classifies milled rice by length into sizes of extra long, more than 7mm.; long, 6.0 to 7.0 mm.; medium, 5.0 to 5.9 mm.; and short, less than 5 mm. The corresponding shape classifications for brown rice based on a length-to-width ratio are: slender, more than 3; medium, 2.4 to 3.0; bold, 2.0 to 2.39; and round, less than 2. (Chang and Bardenas, 1965). Traditional *Indica* varieties usually have long or medium sized grain. Traditional *Japonica* varieties have short grain.

3. RICE GRAIN COMPOSITION

Starch and protein together make up 98.5 percent of the constituents of dry milled rice (Juliano, *et al* 1964). Rice at 12

percent moisture has about 80 percent starch and 7 percent protein. Starch, a polymer of glucose, occurs in the endosperm as compound polyhedral granules, 4 to 8 µm in diameter. (Buttrose 1962, Schoch 1967). Protein is present as discrete particles, protein bodies, between the starch granules (Del Rosario *et al* 1968).

The protein content of milled rice ranges from 5 to 14 percent protein (at 12% moisture) (Juliano, 1966). Within the same variety, protein content can show a variation of 6 percent due to environment. Cagampang and co-workers (1966) found that at 12 percent moisture the protein content of various samples of the variety BP1-76 ranged from 8 to 14 percent. Starch content decreases with an increase in protein percentage. Rice grains also contain free sugars, principally sucrose; lipid, hemicelluloses and minerals.

The extensive world literature on rice grain composition has been collected (Juliano 1966) and reviewed (Juliano 1972).

4. RICE GRAIN DEVELOPMENT

Grain development in rice has been described by Hector (1936), Asada and Kasai (1963) and Saio (1964). Development follows a pattern common to other cereal grains.

At anthesis, the embryo sac contains an egg cell, a pair of synergid cells, and a number of antipodal cells, together with a large vacuolated central cell containing two polar nuclei.

Fertilization takes place when two male nuclei from a single pollen grain enter the embryo sac. One fuses with the egg nucleus to form the zygote and eventually the embryo. The other fuses with the two polar nuclei to form the primary triple fusion nucleus, which eventually develops into the endosperm. The triploid nature of this nucleus arises from the fact that it receives two sets of identical chromosomes from the maternal parent, and a single set from the male parent. (Simmonds 1978).

In wheat it has been shown that gene products from these chromosomes are expressed in a quantitative manner in the offspring depending upon whether they are derived from the male or female parent (Kasarda *et al* 1976). Wheat grain development has been extensively studied in the last ten years using histochemical and electron microscope techniques, (Evers (1970), Simmonds (1972), Bennett *et al* (1973), Mares *et al* (1975, 1977), Morrison *et al* (1975), and Morrison and O'Brien (1976)). The pattern of development is complex. Immediately following fertilization, a period of free nuclear division takes place resulting, within 24 hours, in the formation of a multinucleate layer of cytoplasm lining the inner periphery of the embryo sac wall. Cell wall formation starts about 2-3 days after anthesis and is completed throughout the endosperm within the next 48 hours. By 4-5 days post anthesis, five to six layers of

large, vacuolated cells may be observed between the dorsal and ventral surfaces of the endosperm; those in the centre have already begun to accumulate starch. Nuclear division followed by cell wall formation (cytokinesis) occurs subsequently in the peripheral zone of the endosperm. Divisions may proceed in both radial and tangential directions, to build up large, irregular columns of endosperm cells. Until some 10-12 days post anthesis, in a total grain ripening time of 45-50 days, no distinction is possible between those peripheral cells lining the embryo sac which are destined to become endosperm cells, and those which will become aleurone cells. Metabolism appropriate to both cell types appears to occur, since small starch granules may be recognized in the outer layer of cells, small aleurone bodies are visible in the next inner layer and lipid droplets are widely distributed in both. However, between 12-14 days post anthesis a clear differentiation occurs, and the atypical organelles in the two cell types are reabsorbed. (Simmonds 1978). The last endosperm cells to be formed, the subaleurone cells, have a high protein content and only a small amount of starch. (Cobb 1904, Kent 1975).

5. PROPERTIES AND STRUCTURE OF STARCH

Starch is the major food reserve material of many organisms including most plants. Starch is deposited inside chloroplasts or

amyloplasts as granules. The size and shape of these granules varies widely.

Starch is composed of two polymers of α-D-glucopyranose, amylose and amylopectin. Amylose has an essentially linear structure; the glucose residues being linked (1→4) by α-glucosidic bonds. It is the minor component of most starches and usually has a molecular weight of the order of 10^5 - 10^6, (Williams 1968).

Amylopectin has a branched structure and involves α-(1→4) linked chains of D-glucopyranose units joined by α-(1→6) glucosidic branch points. The outer chains are those α-(1→4) chains from the non-reducing end groups to the outermost α-(1→6) branch points, and the inner chains are those α-(1→4) chains bounded at both ends by α-(1→6) branch points. Amylopectins have about 24 α-(1→4) bonds per every α-(1→6) bond. They have a molecular weight of the order of 10^7 or 10^8 making them one of the largest natural polymers (Erlander and French 1958). Starches may contain some material intermediate in structure between amylose and amylopectin (Banks and Greenwood 1975).

Until about 1940 most experiments were performed with whole starch and there was uncertainty about the heterogeneity of starch (Williams 1968). Meyer and co-workers (1940 a, b) established the heterogeneous nature of starch by examining the amylose that leached from incompletely gelatinized starch granules and showing that it was essentially a linear polymer. The residual material (amylopectin)

had a branched structure. Schoch (1945) later quantitatively separated starch into its two components. The method he developed used a selective precipitation dependent on the insoluble complex formed by the linear fraction with various polar organic compounds with relatively low water solubility, such as *n*-butanol, *n*-pentanol, thymol or nitromethane. Pure amylose and amylopectin can be prepared using thymol as the first precipitant, followed by recrystallisation using *m*-butanol (Greenwood, 1956). Greenwood and Robertson (1954) suggested that the mildest possible conditions be used for dispersion and fractionation. During fractionation, oxygen can degrade and chemically modify starch so that it is necessary to carry out dispersion and fractionation under nitrogen (Cowie and Greenwood 1957; Banks and Greenwood 1975). Adkins and Greenwood (1966) found that dimethyl sulphoxide could be used instead of alkali solutions to disperse starch granules. Muetgeert (1961) reviewed the fractionation of starch. The successful fractionation of starch was followed by the development of methods of structural analysis. Early studies concentrating on methylation and hydrolysis to provide information about linkages have been reviewed by Radley (1968).

Amylose gives a complex with iodine, in the presence of iodine ions, which has an intense blue colour. This complex involves the amylose helix enclosing a linear chain of iodine atoms. The

intensity and wavelength of absorption depends upon the chain length of the amylose (Baldwin *et al* 1944; Bailey and Whelan 1961). Under standardised conditions, amylose may be determined spectrophotometrically (McCready and Hassid 1943). A polymer containing a minimum of 18 glucose units is required for colour, and the maximum absorption is reached when the polymer exceeds 400 glucose units (Bailey and Whelan 1961). Amylopectin gives a much less intense, red-purple, colour (Bailey and Whelan 1961). Due to their comparative simplicity, colorimetric methods for measuring amylose have continued to be used in rice breeding programmes (Juliano 1971). A method of determination of amylose in whole starch by potentiometric titration of bound and free iodine present during titration, and which gives more accurate values than colorimetric methods, was developed by Bates and co-workers (1943) and has since been adapted by several workers (Banks and Greenwood 1975). Juliano (1979) has recently reviewed amylose analysis in rice and presented data comparing potentiometric, amperometric and colorimetric methods.

The determination of the chain length of amylopectin by titration of formic acid produced from periodate oxidation was introduced by Brown and co-workers (1945). Anderson and co-workers (1955), studied the periodate oxidation of 18 different starches by potassium metaperiodate and found that at constant temperature

the time taken for the uptake of periodate ion varied from starch to starch. Methods for the spectrophotometric determination of formic acid produced on periodate oxidation of carbohydrates have been reviewed by Kennedy (1972). Periodate oxidation can be used for the determination of amylopectin chain length in whole starch as the small amount of formic acid produced from amylose can be neglected. Amylopectin chain length may also be determined enzymically. In the presence of β-amylase and pullulanase (a bacterial enzyme that hydrolyses α-(1→6) linkages) amylopectin is quantitatively degraded to maltose and glucose. Glucose arises from those chains that contain an odd number of glucose units. By determining glucose and assuming a random distribution of odd chains, an estimate of chain length can be made (Lee and Whelan 1966; Adkins *et al* 1966; Banks and Greenwood 1970). The amylopectins of starches vary in chain length from 18 glucose units in sago to 30 glucose units in acorns (Williams, 1968). Much of what is known of the chemical structure of starch has been discovered using enzymic methods. Whelan (1971) reviewed the early enzymic exploration of starch structure. In early investigations β-amylase was used. It removes maltose units starting at the non-reducing end of an α-(1→4) D-glucopyranose polymer until two or three D-glucopyranose units away from a 1→6 branch point. α-amylase contaminants in early β-amylase preparations however gave misleading results. If amylose

had a completely unbranched structure it would be completely degraded by β-amylase, and this was found with early preparations of the enzyme. The concept of amylose as a simple, unbranched molecule persisted until 1949, when it was shown that with preparations free of α-amylase, amylose was incompletely degraded (Peat *et al* 1949, 1952 a, b). Later workers established that the incomplete degradation was not due to either barriers introduced by oxygen (Cowie and Greenwood 1957 b, c) or to the phosphate ester groups (Peat *et al* 1952 c; Banks and Greenwood 1961). α-(1→6) links have since been detected in pure amylose using an enzymic method based on yeast isoamylase which specifically hydrolyses α-(1→6) linkages (Kjolberg and Manners 1963). The use of pullulanase confirmed the presence of some branch points in amylose (Banks and Greenwood 1966). As amylose is an essentially linear polymer the degree of polymerisation can be estimated from solution viscosity (Cowie and Greenwood 1957a). The degree of polymerisation varies with the plant source and the stage of growth of the tissue.

Confirmation of the branched, tree-like structure of the amylopectin molecule proposed by Meyer and co-workers (1940 c) came from studies of the oligosaccharides produced by R-enzyme (α-(1→6) glucosidase from beans) on amylopectin β-limit dextrin, (Whelan 1971). More recently Gunja-Smith and co-workers (1970) have proposed a revised randomly-branched structure for amylopectin

based on work with *Cytophaga* isoamylase. However, Banks and Greenwood (1975) have discussed some inconsistencies in Gunja-Smith's data and regard the new structure with caution. Marshall (1974) has reviewed the applications of enzymic methods to the structural analysis of polysaccharides.

Most starches contain about 20% amylose. However, starches with widely differing amylose contents are known. Some plants produce starches with no amylose at all e.g. waxy cereals (Hixon and Brimhall 1968). Others produce starches with high levels of amylose. Maize containing the 'amylose extender' gene has starches containing up to 70% amylose and wrinkle-seeded pea starch contains 66% amylose (Hilbert and McMasters 1946; Potter *et al* 1953). Maize having the 'sugary' gene produces a branched glucose polymer called phytoglycogen that is water soluble and has a structure with more (1→6) branch points than amylopectin. (Banks and Greenwood 1975). Reviews on the amylose content of different varieties and parts of plants have been given by Williams (1968) and by Juliano (1979). The chemistry of starch has been extensively reviewed (Greenwood, 1956; Whelan, 1958; Whistler and Paschall, 1965; Radley, 1968; Banks and Greenwood, 1975).

6. STEM STARCH

In this thesis the term rice stem is used to describe the combined culm and leaf sheath (Chang and Bardenas 1965).

Carbohydrates, including free sugars and starch, are stored

in the stem of rice plants and may later be translocated to contribute to grain yield. The contribution of stored carbohydrates translocated from other parts of the plant to rice grain yield has been estimated to range between 0 and 40%. The subject has been reviewed by Yoshida (1972). Other cereals do not usually store starch in the stem and the contribution of reserve carbohydrates to yield is less important (Evans and Wardlaw 1976). Wheat, for instance, accumulates no starch in it s stems (Evans *et al* 1975). Wood (1960) stated that sucrose is the dominant accumulated carbohydrate in the wheat plant until approximately four weeks before harvest.

Most studies of rice stem starch have been made by Japanese workers. Nagai (1959) has reviewed the earlier Japanese literature; he states, that Togari and co-workers in 1954 found that immediately after transplanting, much starch accumulated in the leaf sheath and culm; as ripening progressed this starch decreased. He further states that Arashi and Eguchi in 1954 found that the starch content of individual leaf sheaths varied. The higher leaf sheaths contained the most starch. Nagai (1959) also reviewed the histochemical study of Baba and Kitsutaka, who in 1953 traced the accumulation of starch grains in the parenchymatous tissue at the base of the culm. They found, that after transplanting, a marked decrease in starch content occurred. Starch content then increased during tillering and then decreased again at stem elongation.

Accumulation then resumed with translocation taking place from the leaf sheath to the culm. A few days after flowering the culm and leaf sheath reached their maximum starch content. A higher stem starch content was observed in late maturing varieties and in low nitrogen (low growth) plots. Nagai (1959) finally reports that Sato in 1955 found that the size of the starch grains in rice stems varies in nearly direct proportion to the amount of starch accumulated. Matsubayashi (1967) reviewed the work of Yamada and co-workers from 1956. These researchers followed starch and sugar accumulation in the stems of the Japanese variety Norin No. 17 through early, ordinary and late season cultures. Stem starch increased steadily until just after flowering in all crops. However, transplanting caused a decline in stem starch accumulated during seedling bed growth. The maximum starch content reported was 34% (dry weight basis) just after flowering in early season culture.

In waxy cereals the waxy starch type is confined to the endosperm, pollen and embryo sac. The embryo, seed coat and other parts of the plant contain normal starch (Hixon and Brimhall 1968). Starch from the stems of waxy rice varieties stains blue with iodine/potassium iodide solution.

7. POLLEN STARCH

Grass pollens contain high levels of starch. Rice was the first plant found with a pollen containing waxy starch. Parnell

(1921) noted that the pollen from rice plants with waxy endosperms stained red with iodine; pollen from plants with normal endosperm stained blue with iodine and pollen grains from the F1 generation of a cross between waxy and normal endospermed rice exhibited dimorphism to iodine staining. Similar findings were later reported for maize (Demerec 1924) and barley (Kasiwaya 1930). Varieties with a waxy endosperm have non-waxy stem starches.

If the amylose content of rice pollen starch reflects the amylose content of endosperm starch, analysis of pollen starch amylose could be used as an early season selection tool in rice breeding programmes. It could also be used for selecting particular pollinators for use in complex crossing programmes where the initial crosses have not been fixed. Zuber and co-workers (1960) studied maize pollen starch with these aims in mind. They examined non-waxy maize pollens from selfed lines having endosperm amylose contents in the range 27.1 to 67%. They found that the pollen starches had amylose contents of only 11.4 to 15.5% and concluded that there was no apparent relation between the percentage of amylose in non-waxy endosperm and non-waxy pollen starch.

Banks and co-workers (1971a) expressed surprise at the results of Zuber and co-workers (1960) and on examining the pollen from two high amylose maize lines, found it to contain high amylose starch.

Rice pollen, like other grass pollens, is shed in the trinucleate stage (Kihara and Hirayoshi 1942; Matsushima 1970). Grass pollens usually contain over a third of their dry weight as

storage carbohydrate and have a short viability. Pollens that are shed in the binucleate stage have a low carbohydrate content and are relatively stable when stored for long periods (Brewbaker 1959). Amylase activity has been widely reported in pollen (Green 1891; Stanley and Linskins 1965) and any study of pollen starch needs to use material that has been rapidly processed to reduce degradative enzyme action.

8. STARCH SYNTHESIS AND PROPERTIES OF RICE GRAIN STARCH

Rice endosperm starch exists in compound granules, unlike other cereals except oats. Del Rosario and co-workers (1968) first noticed compound starch granules in 4 day-old grain. The components of a compound granule are called granula. The compound granules originate from single amyloplasts. Many small spherical granula are initiated in the proplastid stroma and by assuming a polyhedral shape in growth, soon fill the plastid. Fusions between granula often occur so that each granula may not be completely surrounded by a stroma layer (Buttrose 1960, 1962).

Initially, cereal starch granules occupy only a small part of the plastid volume. However, both plastid and starch granules grow rapidly until the latter occupies the whole volume. At maturity the rice starch granule has the structure of a pentagonal dodecahedron (Watabe and Okamoto 1960). The polyhedral shape is attributed to compression of the starch granules against one another as grain develops (Hoshikawa 1968). During grain growth starch granule size increases fastest in the cells in the centre of the endosperm.

Individual starch granules increase in size during grain development to have a diameter of between 3.3 and 10.6μm and a mean size of 6.3μm (Briones *et al* 1968). Little is known of the structure of rice starch granules. Their small size probably discourages histological studies. However, an electron micrograph of a rice starch granule corroded with hydrochloric acid revealed a layered structure that is characteristic of acid corroded cereal starches (IRRI 1970). Badenhuizen (1965, 1969) has reviewed the development of starches in higher plants. Briones and co-workers (1968) studied the changes in the physicochemical properties of starch in developing IR8 rice grains. They found that granule size and amylose content of the starch increased and gelatinization temperature decreased slightly during grain development. Iodine binding capacity increased from 4.68% in starch from 4 day-old grain to 5.40% in starch from ripe 39 day-old grain.

The development of starch in the grain of other cereals has been extensively studied. Barley, wheat and maize all show a steady increase in the proportion of amylose present in the starch (Matheson 1971; Boyer *et al* 1976). The increase found by Briones and co-workers (1968) for rice is comparatively small and they speculated that this may be due to the short ripening period of rice.

The amylose content of mature rice grain starches varies widely. Starch isolated from non-waxy rice varieties contains

between 8 and 37% amylose and may vary as much as 6% within a variety (Juliano 1970). Waxy rice starch has an apparent amylose content of 0.8 to 1.3%, the amylose is thought to be located at the centre of the granules (Juliano 1972). Phillips and Williams (1961) studied the varietal differences in the iodine binding capacities of crystalline rice amylose. All varieties were found to bind 18.9 mg iodine per 100 mg amylose. Estimated molecular weights ranged from 100,000 to 325,000. The properties of mature rice starch fractions have been reviewed by Juliano (1972).

9. RICE GRAIN APPEARANCE AND MILLING QUALITY

Rice milling removes the hull, germ and bran from grain with a minimum breakage of endosperm. The milling quality of rice is dependent on the yield of whole grain obtained. Because of the way rice is usually eaten, broken grain detracts from its appearance, quality and value. Grain breakage is a serious problem wherever rice is grown and processed.

Harsh threshing and transport can cause some paddy grain to break, however most breakage occurs during milling. Rice millers attempt to minimise breakage by the optimal setting of their mills.

Under good milling and handling conditions yields of whole grain vary with variety and harvest environment. The two major causes of breakage during milling are suncracking, also called

internal fissuring or checking and chalky core, also called opaque endosperm. Suncracking results when harvesting is delayed, when too rapid drying of the crop takes place or when rain falls on a ripe crop. The cause is mechanical; fluctuations in temperature and moisture content cause the outer portion of the grain to expand faster than the centre resulting in the formation of stress cracks (Rind 1962).

Some suncracking can be found in nearly all rice samples and is common in all cereal grains (e.g. Chung and Converse 1971). Rice grains can contain many visible cracks without breaking and the relationship between visually observed checking and whole grain mill yield is usually poor. Most checking cracks only weaken the grain rather than break it. The critical factor is the speed of moisture transfer (McDonald 1967).

Checking was a very serious problem in the Australian rice industry. It was largely overcome by McDonald and co-workers in the late 1950's by encouraging all rice farmers to harvest at high moisture, between 18 and 22%, followed by cool air drying. All the New South Wales rice crop is now handled in this way in co-operatively owned Marketing Board sheds (Anon 1970; McDonald 1967).

Australian average commercial whole grain yields of medium grained rice are now over 60%, paddy basis, and among the highest in the world.

Chalky core, the other major cause of grain breakage is also undesirable because it detracts from the visual appearance of the sample. Chalky core is observed as a small, white, opaque region that occurs in otherwise clear translucent grain.

Grain appearance is of paramount importance to rice quality, as most rice is bought and sold on sample appearance. The New South Wales rice industry currently exports over 90% of its production and is extremely jealous of its reputation for selling high quality translucent grain. To ensure translucency of premium samples some grain shipments are passed through electronic sorting machines (Sortex) that remove grain with a chalky core. Machines of this type are also used for sorting parboiled rice (Garibaldi 1974; Anon 1976). Ikehashi and Khush (1979) in reviewing the methodology of assessing appearance of rice grain state that chalkiness and whiteness of miled rice are two of the most conspicuous factors determining its commercial value.

The chalky portions of rice grains have been shown by del Rosario and co-workers (1968) at I.R.R.I. to be endosperm cells with a loose packing of starch granules. The appearance of chalk is seasonal. In some seasons a variety will have a majority of clear translucent grain while in others it will be badly chalked.

Some varieties never develop chalk. It is a major aim of the N.S.W. rice breeding programme to develop rice varieties that do not produce chalky core. Nagato (1962) demonstrated that the chalky core portion at the centre of a rice grain had a low specific gravity and was soft compared with the translucent endosperm surrounding it. Ando and Ichikawa (1974) have commented upon the random, irregular packing of chalky endosperm cells. Using electron microscopy, they found that single starch granules were massed loosely together, so that large irregular openings could be seen.

The opaque appearance of chalky endosperm is due to the inclusion of minute air spaces. These air spaces probably also contribute to the softness of chalky endosperm as they reduce the area available for starch-protein adhesion. The adhesion between starch and protein has been proposed as one of the major factors in wheat grain hardness. (Simmonds *et al* 1973). Air spaces in soft wheat endosperm tissue are a well-known phenomenon and are considered to contribute to its softness (Percival 1921) (Stenvert and Kingswood 1977).

The opaque nature of chalky cored grains should not be confused with the opaqueness characteristic of waxy rices. Watabe and Ohamoto (1960) showed that waxy rice starch granules have a distinctive rough surface compared with granules isolated from non-waxy varieties. The opaque nature of waxy rice grain is

attributed to light defraction in the air spaces associated with this rough surface.

10. RICE GRAIN COOKING AND EATING QUALITY

Rice consuming people of the world have definite ideas concerning the texture, flavour and other cooking characteristics of rice. The desired properties may change from one ethnic group or geographical region to another (Juliano et al 1964a). In evaluating cooking quality an attempt is made to relate chemical and physical properties of the grain to the desired subjective characteristics of the cooked rice.

Starch makes up approximately 90% of the dry weight of milled rice. The physical and chemical properties of this starch have a predominant effect on grain cooking and eating quality.

Rice varieties differ greatly in cooking and processing qualities. Among varieties, grain size and shape are generally associated with cooking characteristics. Usually short and medium grain varieties become somewhat sticky on cooking. Although this type is preferred in some northern Asian countries, there is a distinct preference in Australia and most western countries for the drier, flaky quality usually found in long grain varieties. The short grain varieties are used for making puffed rice and are also used successfully for pre-cooked canned rice and in dry quick-cooking rice products in the United States of America (Kester 1959). Both types are used in the manufacture of dry breakfast cereals

and both are used in the parboiling process. Length of grain is not completely reliable as an indicator of the cooking quality of rice, however, as a number of varieties give cooked products different from the type expected on the basis of grain length classification (Halick and Kelly 1959).

Gelatinization temperature The cooking of rice consists mainly of gelatinizing the starch. Gelatinization temperature is related to cooking time, granula size, molecular size of the starch sub-fraction polymers and their relative amounts (IRRI 1965; Beachell and Halick 1957; Onate *et al* 1964).

Viscograph studies are undertaken to simulate cooking and to study changes occurring during pasting, cooking and cooling of aqueous starch systems. The temperature of initial increases in viscograph viscosity of a 10% rice powder suspension provides a measure of gelatinization temperature. Halick and co-workers (1960) found a highly significant correlation, 0.939, between gelatinization temperature obtained with the viscograph and the birefringence-end-point method (BEPT). Thus, results obtained from viscograph studies are reportedly accurate indicators of gelatinization temperature.

Gelatinization temperature is influenced by environment. Beachell and Stansel (1963) noted that cooler temperatures, especially during ripening, produced starch with a lower gelatinization temperature. Kihara and Kajikawa (1960) noted that high temperatures during ripening gave a slower rate of alkali digestion of the

endosperm which indicated a high gelatinization temperature range. Suzuki (1979) recently reviewed the use of the viscograph (amylograph) for determining rice cooking quality. Varieties have been classified on their gelatinization temperatures (Beachell and Stansel 1963). Those with gelatinization temperatures of less than 69°C are considered to be low gelatinizing; 70-74°C, intermediate; and greater than 75°C are classed as high gelatinizing types (Beachell and Stansel 1960). Several methods for estimating gelatinization temperature are used extensively in rice breeding programmes. Little and co-workers (1958) applied the alkali test originally developed by Jones (1938) as a rapid screening method. Water absorption of milled rice when cooked in excess water at 77°C and at 82°C also provides a relative means of estimating gelatinization temperature. Halick and Kelly (1959) found a highly significant negative correlation of water absorption with gelatinization temperature. Short grains exhibiting high water uptake generally have low gelatinization temperature. Other tests similar to water absorption and related to gelatinization characteristics are available, (Rao *et al* 1952).

Conflicting reports on the relation between gelatinization temperature and amylose content are given in the literature. Halick and co-workers (1960), studying American rice varieties concluded that amylose content of rice had little, if any, relation to gelatinization temperature. However Juliano and

co-workers (1964b) found that amylose content was positively, but not significantly, correlated with gelatinization temperature. When two (Century Patna-231 and Taichung (Native) 1) of the 16 varieties they studied were excluded they found a highly significant positive correlation. Century Patna-231 has a high gelatinization temperature and a low amylose content while Taichung (Native) 1 has a low gelatinization temperature and high amylose content. They concluded that generally, the greater the amylose content, the higher the gelatinization temperature.

Reyes and co-workers (1967) attributed the slow rate of cooking of varieties with a high gelatinization temperature to differences in the internal structure of starch granules and to a higher degree of starch crystallinity.

Within a variety gelatinization temperature usually increases with protein content. Juliano and co-workers (1965) observed this trend to be inconsistent and concluded that gelatinization temperature tends to be sensitive to protein level. In the endosperm, protein forms a matrix around the starch granules. Possibly more heat energy is required to cross or break the protein matrix and to gelatinize the starch in high protein grains. Bhattacharya (1979) has recently reviewed the literature on rice starch gelatinization temperature and its determination.

Amylose content and cooked grain texture Texture awareness is related to flavour intensity and, in bland foods like rice, as flavour becomes less important, texture assumes greater prominence (Szczesniak and Kleyn 1963; Szczesniak 1971).

The texture of cooked rice has been demonstrated to be correlated with both protein and amylose content. Amylose content has been shown by numerous workers to be very important to the cooking and processing qualities of rice. Cooked rice with high amylose content is flaky and dry, while rice with low amylose is sticky and moist when cooked (Williams *et al* 1958). Juliano and co-workers (1965) found that cooked high-amylose rice is flaky and dry and low-amylose rice is sticky and moist. A highly significant partial correlation between assessed tenderness and amylose content was obtained ($r = -0.881^{**}$ with amylose content independent of protein content). These workers also found that protein content is an important index of tenderness and cohesiveness of cooked rice when its interaction with amylose is minimized. Cooked rice tenderness, cohesiveness, and starch composition are also related (Juliano *et al* 1964c; Williams *et al* 1958). Cohesiveness is probably a product of the amylopectin fraction, since waxy rice whose starch is essentially pure amylopectin is very sticky. The hardness of cooked rice may be caused by the retrogradation of amylose after cooling.

Rice varieties are now commonly grouped on the basis of their amylose contents into waxy (0-2% amylose), very low amylose

(2-9%), low amylose (9-20%), intermediate amylose (20-25%) and high amylose (25% and greater) (IRRI 1972). From 14,261 rice cultivars in the IRRI world collection, Gomez (1979) found the intermediate-amylose group to be the largest, with 5,633 cultivars, followed by the high-amylose group with 4,340 cultivars, and the low-amylose group with 2,218 cultivars.

Due to its relationship with cooked grain texture, amylose content is widely used as an index of cooking quality in rice breeding programmes. Its use greatly increases the effectiveness of selection. However, the levels of amylose which denote good cooking quality in southern New South Wales appear to be different to those widely used in tropical areas. Until the present time, selections have been made on the basis of the standards used in the tropics. Unfortunately, genotypes containing 23-24% amylose, which might be expected to be dry-cooking without being excessively hard, have proved to be generally soft-cooking and slightly sticky. It is important that the appropriate levels of amylose be determined, so that varieties can be produced in New South Wales which better satisfy the cooking quality requirements of export markets in Asia and elsewhere.

Water absorption and volume expansion during cooking are directly affected by amylose content. Waxy rice expands the least during cooking, and its cooked grain has the heaviest bulk density. Cooked-grain resistance to disintegration is also related to amylose

content, with high-amylose rice being the most resistant and waxy rice the least resistant. The tendency to disintegrate may be amplified by soaking cooked grain overnight in water (Juliano 1979a).

Amylose content alone is not an adequate index of quality. However, its use in breeding programmes greatly reduces the quantity of material which must be more thoroughly evaluated in more time consuming tests. The amylose test has the added advantage that it can be performed on very small samples of grain.

It is not safe to assume that the choice of a higher level of amylose for rice grown in temperate areas will automatically indicate better cooking quality. Unduly high amylose contents are almost invariably associated with very hard texture and are just as unsatisfactory as low levels of amylose. Cagampang and co-workers (1973) found that many varieties did not have the expected gel consistency values predicted from amylose content.

Although it is widely believed that the growing environment affects amylose content and the relationship between amylose and cooking quality, there is no substantial data to support this. The amylose content of a rice variety can vary by as much as 6 percentage points (Juliano 1972) and the same rice varieties grown under different environments can be classed into different groups. When 34 rice varieties were tested in five countries, nine were classified as intermediate at IRRI in the Philippines, 23 in Taiwan, 12 in Peru, 4 in Brazil and 16 in Sri Lanka (Paule *et al* 1979). Temperature during grain ripening has been

shown to affect amylose content (Suzuki and Murayama 1967; Nikuni *et al* 1969; Ebata and Nagato 1967; Resurreccion *et al* 1977). Amylose content generally decreased as the mean temperature increased. However, response to temperature may differ, depending on whether the variety is *japonica* or *indica* (Resurreccion *et al* 1977). Other factors observed to influence rice texture include the age of the sample and the molecular properties of its starch. Freshly harvested rice cooks soft and starchy as compared with aged samples of the same variety (Desikachar and Subrahmanyan 1960; Barber 1972).

Efforts are now being made to objectively evaluate cooked rice texture; the various methods used have recently been reviewed (Suzuki 1979; Laignelet and Feillet 1979; Blakeney 1979).

Juliano has reviewed the literature on rice grain quality on several occasions (1972) (1973) (1977) (1979b). Hampel (1968) has reviewed rice grain quality tests used in world trade and Webb and co-workers (1972, 1979) have described the rice grain quality evaluation methods used in the United States.

AIM OF THE INVESTIGATION

The aim of the present work was to study the development and properties of starch in rice plants grown under temperate conditions in southern New South Wales.

MATERIALS AND METHODS

1. RICES

The rices used in this work were all grown in the field at Yanco Agricultural Research Centre, Yanco, N.S.W. Australian methods of rice culture have been described by McDonald (1963 a, b). All plots were 30 m x 1.5 m, were grown by the combine sowing method (Boerema 1969) and treated with 78 Kg/ha of ammonium sulphate as a single basal dressing. A standard post-emergence commercial treatment (4 Kg/ha) of Molinate was used to control barnyard grass (Swain 1973). Trials were grown in the 1972/73 rice season for the initial pollen starch grain development and grain starch trials, in 1973/74 for the main pollen starch trial and in 1974/75 for the stem starch trial. The varieties used and their origins are given in table 1.

2. PLANT AND GRAIN SAMPLES

Plants were cut for stem starch samples just above ground level at fortnightly intervals. Developing grain samples were hand harvested from the mid portion of panicles every four days beginning 4 days after mid flowering. All samples were immediately taken to the laboratory for analysis. The ripe remainder of the plots was harvested using a mechanical small plot harvester. Brown rice was prepared using a Satake laboratory sheller and then polished to remove about 10% of the bran by

Table 1.

Rice Varieties used in the Experiments

Caloro	Early Caloro is an Australian selection from Caloro introduced from California in 1922. Caloro was selected from the Japanese variety Early Wateribune. Caloro and its selections were the basis of the southern New South Wales rice industry between 1930 and 1956. Early Caloro is a soft cooking short grain.
Calrose	Calrose was introduced from California in 1956. It was selected from the cross (Caloro x Calady) x Caloro. It is currently the most widely grown variety in southern New South Wales. Calrose is a soft cooking medium grain.
Kulu	Kulu was selected at Yanco Agricultural Research Centre from the cross Bluebonnet 50 x Calrose and released in 1967. Kulu is a soft cooking long grain.
Inga	Inga was selected at Yanco Agricultural Research Centre from the cross Century Patna x Calrose and released in 1973. Inga is a long grain that is firmer cooking than Kulu but still classified as soft. Inga is the principle long grain variety grown in southern New South Wales.
YR57	YR57 is an unreleased crossbred selected from the cross CI 9434 x Calrose made at Yanco Agricultural Research Centre in 1964. YR57 is a firm cooking long grain.
Ali Combo	Ali Combo was introduced from Madagascar in 1963. It is a very firm cooking long grain.
Madinika	Madinika 1191 was introduced from Madagascar in 1965. It is a large, long grained, firm cooking variety.
Tarra 140	Tarra 140 was selected at Yanco Agricultural Research Centre from the cross Caloro II x ((Delitus x Caloro) x Caloro II) made in 1965. It is a waxy, medium grained variety.
Kuro Mochi	Kuro Mochi 22 was introduced from Japan in 1965. It is a waxy, short grained variety.

weight in a Satake MC250 one-pass rice pearler. Grain samples from pollen trials were hand harvested and then test tube milled (Scott *et al* 1964).

3. POLLEN COLLECTION

Pollen was collected from the plots at the mid flowering stage. Collections were made on sunny days in the early afternoon between one and two o'clock when pollen shed is heavy. Pollen was collected using an ordinary domestic vacuum cleaner powered by a portable generator and fitted with a long extension of 15mm diameter plastic tubing. Immediately on collection the samples were brushed through a 9XX flour silk (aperture width 0.15mm) to remove insects, anthers and other non pollen contaminants. Half the sample was then immersed in cold ($4^{o}C$) 0.01M $HgCl_2$ solution, the other half was sealed in a plastic vial and frozen at $-20^{o}C$. Where the amount of pollen collected from a variety was less than 5g all the pollen was immersed in cold $HgCl_2$ solution and no compositional analysis made.

4. CHEMICALS

Glucose and sucrose standards were BDH Aristar (B.D.H. Chemicals Ltd., Poole, England, U.K.). Fructose was Pfanstiehl C.P. Special (Pfanstiehl Laboratories Inc., Waukegan, Illinois, U.S.A.) and Maltotriose was Calbiochem 93%, Glucose-free (Calbiochem (Aust.) Carlingford, N.S.W.). All other chemicals were analytical reagent grade. The enzymes used have been specified in individual methods.

5. PREPARATION OF PARAHYDROXYBENZOIC ACID HYDRAZIDE

Parahydroxybenzoic acid hydrazide, PAHBAH, is simple and cheap to produce in the laboratory. Methyl-4-hydroxybenzoate (p-hydroxybenzoic acid methyl ester, Methyl paraben) is converted to the hydrazide simply by adding hydrazinium hydrate. 400g Methyl-4-hydroxybenzoate (Ajax Unilab Cat. 327) was suspended in 1 l. of 5% w/v sodium bicarbonate solution and mixed well; some frothing occurred. The suspension was filtered through miracloth (Calbiochem (Aust.), Carlingford) and the paste placed in a 2 l. polypropylene beaker. In a fume hood 500 ml of hydrazinium hydrate (Ajax Unilab Cat. 1093) was added, the beaker placed in a boiling water bath and heated until a clear solution was obtained. On cooling PAHBAH precipitated; the yield was improved by neutralising the excess hydrazine with hydrochloric acid to pH 7.5. The precipitate was collected on miracloth, washed with cold distilled water and dried at 50^{o}C in an oven.

Miracloth was preferred for collecting the final precipitate as it is very fast filtering and unaffected by residual hydrazine. Filter paper tended to disintegrate and contaminate the product.

6. PREPARATION OF SOYBEAN BETA AMYLASE

Soybean β-amylase free from α amylase was prepared basically by the method of Peat and co-workers (1952a). Soybeans (Clarke 63 c.v.)

were obtained from a variety trial grown at Leeton Agricultural Research Station. The beans were roller milled on a Brabender Quadrumat Junior flour mill the hulls passing over the 78 grit gauze sieve into the bran tray. The flour was defatted with cold acetone. Two hundred grams of flour in a litre of acetate buffer pH 5.4 containing 0.1M tri sodium EDTA with a few drops of octan-2-ol was mechanically shaken for two hours, centrifuged at 2000G for 10 minutes and the precipitate discarded. Normal sulphuric acid was added dropwise to the supernatant to reduce the pH to 4.8, the mixture was centrifuged and the precipitate discarded. The supernatant was held between 60 and 61°C in a heated water bath for 30 minutes, allowed to cool to room temperature, centrifuged and the precipitate discarded. Ammonium sulphate, 41.8g per 100ml supernatant, was added with stirring. The mixture was centrifuged and the precipitate collected, dissolved in 50ml of water and dialysed against distilled water at 4°C for 48 hours. The enzyme solution was stored under toluene at 4°C.

The absence of α-amylase in the soybean β-amylase preparations was checked using the very sensitive sodium starch glycollate - glucoamylase method of Marshall and co-workers (1977, Marshall 1978). Sodium starch glycollate was purchased from B.D.H. Ltd. and a highly purified, α-amylase free preparation of *Aspergillus niger* glucoamylase (amyloglucosidase item 171581) from Calbiochem (Aust.) Ltd., Carlingford.

No glucose was detectable after incubating 1 ml of the soybean β amylase preparation and 1 ml of the substrate preparation described by Marshall and co-workers (1977) together at 40°C for 30 minutes. It is concluded that the soybean β amylase preparations were α amylase free.

7. PREPARATION OF STEM AND GRAIN STARCHES

Starch was prepared from stems, developing grains and mature grains by modifications of the method of Adkins and Greenwood (1966). This method has the advantage that it produces no obvious modifications to the starch granules. Rice stems were cut into short lengths soon after collection and then blended in a Sorvall omnimix (Ivan Sorvall Inc., Newtown, Connecticut, U.S.A.) with acetate buffer, pH 6.5 containing 0.01M mercuric chloride. The resulting slurry was screened through a No. 9 nylon flour bolting cloth (aperture width 0.14 mm) fixed in a wooden frame. Additional buffer was used to wash the residue on the screen. Screen residues were re-extracted a total of five times.

Screenings were then re-blended in a Waring blendor and passed over a No. 15 nylon flour bolting cloth (aperture width 0.08 mm). The starch suspension was allowed to settle overnight and most of the supernatant removed by gentle suction.

Protein and lipid material was removed, without degrading the starch, by shaking an aqueous suspension of the starch overnight with one-eighth its volume of toluene; the proteins are denatured

at the water toluene interface. The samples were centrifuged at 2000G for 10 minutes to separate the starch granules. Five to seven extractions with fresh toluene were required to produce a clear toluene layer and starch with a protein (N x 5.95) content of less than 0.25%. The final extraction was allowed to settle under gravity overnight. The starches were washed successively with ethanol, acetone and diethyl ether and dried in a vacuum desiccator.

Developing rice grains were pulled from the panicles and blended in a Waring blendor with the acetate, mercuric chloride buffer before screening and protein extraction. Care was taken to always sample grains from the middle region of the panicles. Mature rice grain and dissected endosperm fragments were initially soaked at $4^{o}C$ for 52 hours in the buffer before blending in a Waring blendor, screening and protein extraction.

8. PREPARATION OF POLLEN STARCH

Pollen starch was prepared after the method of Banks and co-workers (1971a). Rice pollen in an excess of 0.01M $HgCl_2$ solution was homogenised at maximum speed in a Sorvall omnimixer and the resultant slurry screened over a 15XX flour silk (aperture width 0.08mm). The residue screen overs were re-extracted a total of five (in some cases six) times until a sample of the residue gave a negative test for starch when stained with iodine/potassium iodide solution. The combined filtrates were mixed and allowed

to sediment in tall cylinders for 36 hours at 4°C. The supernatant was removed by gentle suction. The sedimented material was suspended in sodium chloride solution (0.01M) and shaken with one-eighth volume of toluene overnight on a reciprocating shaker. The starch was allowed to sediment, the toluene layer removed, added to ten volumes of water and nitrogen bubbled vigorously through the mixture to release starch granules held in the toluene. The mixture was allowed to stand for 3 hours, the toluene and clear aqueous layers removed from the sediment and rejected. The starch sediments were combined, suspended in sodium chloride solution (0.01M) and the extraction with toluene repeated. A total of 10 to 12 extraction cycles were necessary to achieve a clear toluene layer, signifying the removal of all contaminating protein and lipid material. Differential sedimentation in water in 2 l. measuring cylinders at room temperature was used to remove contaminating macerated pollen tissue. The starch was collected on glassfibre paper (Whatman GF/C) on a Buchner funnel, washed with ethanol, acetone and diethyl ether and dried in a vacuum desiccator.

The starches from all rice varieties had a pure white colour and contained little contamination when examined with iodine/potassium iodide stain under a microscope.

9. MEASUREMENT OF DEVELOPING STARCH GRANULE SIZES

Starch granule sizes were measured using the method of Matheson and Wheatley (1962). Starch samples were stained with an 0.2% w/v iodine in 2% w/v potassium iodine aqueous solution and photomicrographed. A section of a stage micrometer was photographed at the same magnification with each batch. The high contrast negatives were projected onto a screen using a roll film projector so that 100 mm represented 10μ. Sizes were measured on the screen to the nearest millimeter.

10. LIGHT AND SCANNING ELECTRON MICROGRAPHS

Light microscopy was carried out on a Leitz Orthoplan microscope fitted with an Orthomat W automatic microscope camera (Ernst Leitz GMBH, Wetzlar, West Germany).

Black and white photomicrographs were made using Kodak Technical Pan film SO 115 and were developed to a contract index of 2.9 using Kodak D 19 developer. Colour photomicrographs were made using Kodak Ektachrome 160 colour slide film.

Scanning electron microscopy was carried out at the Electron Microscopy Unit, Sydney University. Specimens were attached to stubs using double sided adhesive tape and coated with a thin layer of gold in a vacuum splutter coater. They were examined using a JSM-U3 scanning electron microscope fitted with a T.V. scan and relevant fields were recorded on Kodak Panatomic 120 film.

11. ANALYSIS OF RICE POLLEN

Frozen pollen samples were analysed for nitrogen using a micro Kjeldahl method, AACC method 46-13, lipid using AACC method 30-10, and ash using AOAC method 08-01. Moisture was determined using a vacuum oven method, AACC 44-40.

Starch was determined using the semi-micro method for cereal grains proposed by Banks and co-workers (1970). This method involves solubilizing the starch with hot aqueous calcium chloride, subjecting the extract to the concurrent action of α amylase and amyloglucosidase and specifically determining the resulting glucose with glucose oxidase.

Free reducing sugars and sucrose were determined on homogenised 80% ethanol extracts.

12. IODINE SOLUTION FOR STAINING STARCH IN POLLEN GRAINS

Initially pollen was stained for starch using chloral hydrate solution containing iodine/iodide as suggested by Parnell (1921). Several variations of concentration were tried but all gave uneven staining. A solution of iodine/iodide in methoxyethanol gave good uniform staining. The solution contained iodine (0.2g) potassium iodide (20g) and distilled water to 1 litre. This solution was diluted 1:1 with 2-methoxyethanol as required. Anthers or pollen are mounted directly in the stain; if staining is too intense the material is washed with 50% (v/v) aqueous 2-methoxyethanol.

13. ESTIMATION OF STARCH IN STEMS AND GRAINS

The method of Hassid and Neufeld (1964) was used. This method is basically that developed by Pucher and co-workers (1948) and involves the extraction of starch with perchloric acid, precipitation as the starch-iodine complex followed by hydrolysis with dilute acid to glucose. The glucose produced was determined by the PAHBAH reducing sugar method.

14. ESTIMATION OF PROTEIN

Nitrogen content of rice grains and starch was determined by the Macro Kjeldahl method (AACC method 46-12). Protein content was calculated by multiplying Kjeldahl nitrogen by 5.95. This factor is based on the nitrogen content of the major rice protein, glutelin, of 16.8% (Juliano 1972).

15. MOISTURE CONTENT

Plant samples were dried to constant weight at 65^{o}C in a forced air dehydrater. The moisture content of starch and grain samples was determined using AACC method 44-16.

16. ESTIMATION OF STARCH AMYLOSE CONTENT

The iodine binding capacity of starches was determined by the method of Banks and co-workers (1971b) (Banks and Greenwood 1975). This method is a development of the differential potentiometric technique originally proposed by Gilbert and Marriott (1948) and later adopted by Anderson and Greenwood (1955). The amylose content of starches was calculated by assuming that

pure amylose binds 19.5 g iodine per 100 g (Banks and Greenwood 1967).

17. DISSOLUTION OF POLLEN STARCHES

Starches were pretreated with DMSO by the method of Banks and co-workers (1971). The starches (200 mg.) were dissolved in dimethylsulphoxide with gentle mechanical stirring to give 2.5% (w/v) solutions. Ethanol (2 vols.) was added to precipitate the starch which was washed five times with ethanol and then dried overnight in a vacuum oven at 65°C. The non-granular starches were dissolved in dimethylsulphoxide (8 ml) and aliquots of 0.5 ml taken for measuring iodine binding capacity.

18. PREPARATION OF AMYLOSE AND AMYLOPECTIN FRACTIONS FROM STARCH

The method of Banks and Greenwood (1967) was used for the mature grain starches. Wet starches were used as isolated. Dry starches were wet by soaking in distilled water for 36 hours at 4°C under toluene. Wet starch (30 g) was dissolved in 500 ml of dimethylsulphoxide (Merck, Darmstadt, West Germany zur synthese grade Art.802912 99% by gas chromatography) with gentle mechanical shaking for 60 hours at 35°C under dry oxygen free nitrogen. No precipitate was detected on centrifuging this solution for thirty minutes at 2000g. The solution was then poured into two volumes of butan-1-ol and the resulting non-granular precipitate washed repeatedly with butan-1-ol in order to remove residual dimethylsulphoxide. This butan-1-ol-moist precipitate is added to boiling oxygen free water under nitrogen and the boiling continued for one hour.

The dispersion was then cooled to 60°C and powdered thymol (1 g/l) added. After standing for three days at room temperature, the amylose-thymol complex was collected and dispersed in boiling oxygen free water. Boiling was continued for 45 minutes. The solution was cooled and butan-1-ol added. After standing overnight, the amylose-butan-1-ol complex was removed by centrifugation and stored.

Amylopectin was obtained from the supernatant remaining after removal of the amylose-thymol complex. Excess thymol was removed by extraction with peroxide free diethylether and the polysaccharide precipitated under nitrogen with 2 volumes of ethanol.

In the other trials only small quantities of starch were available. Dry granular starches were first rendered amorphous by a method similar to that of Matheson (1971). Starch (2 g) was suspended in 100 ml of dimethylsulphoxide in a 250 ml conical flask. Each flask was fitted with a rubber bung containing a Pasteur pipette to act as a gas bubbler and a short piece of glass tubing plugged with glass wool to allow gas to escape. The bulb ends of the Pasteur pipettes were attached via flexible plastic tubes to a dry nitrogen supply. The flasks were placed in an orbital shaking water bath at 35°C and shaken gently for 60 hours. The solution was then poured, with stirring, into ethanol, centrifuged and the precipitate washed successively by centrifuging with ethanol, acetone and peroxide free diethylether. The amorphous

starch was then suspended in a little peroxide free diethylether and 20 ml of oxygen free 0.05M NaCl solution added and the starch mixed to a paste. A further 180 ml of NaCl solution was added and oxygen free nitrogen bubbled through the mixture which was then heated at 100°C for 30 minutes. This solution was poured into two volumes of butan-1-ol and fractionation continued as in the grain starches method. Matheson (1971) has stressed the importance of using peroxide free ether in the washing and dissolution of starches to avoid depolymerisation.

The wet amylose-butan-1-ol complex excludes weighing for determining the concentration of the solution. The advantage of using the butan-1-ol complex is that it is easily and completely soluble in cold water, whereas the dried amylose is difficult to dissolve in boiling water and requires either aqueous alkali or dimethylsulphoxide as a solvent. A rapid approximate determination of amylose content was made using the colorimetric reaction with iodine (McCready and Hassid 1943). Accurate estimations of amylose content were made by hydrolysing the complex with acid to form glucose followed by a reducing sugar determination (Adkins *et al* 1969).

19. LIMITING VISCOSITY NUMBER

The molecular weights of amylose and amylopectin were estimated by the limiting viscosity number method as described by Greenwood (1964).

20. DETERMINATION OF AMYLOPECTIN CHAIN LENGTH OF STARCH SAMPLES

(a) Chemical method using periodate oxidation.

Amylopectin apparent chain length was estimated from the formic acid produced after periodate oxidation (Anderson *et al* 1955). Amylopectins or starch granules (150 mg) were suspended in 30 ml, 0.56M potassium chloride solution and 10 ml, 0.2M sodium metaperiodate in a glass stoppered 50 ml conical flask. The mixture was gently shaken on an orbital shaker, in the dark at 4°C for 20 days. At the completion of incubation, 10 ml samples were withdrawn and added to 0.5 ml ethylene glycol in another 50 ml conical flask. After shaking in the dark at room temperature for 20 minutes, carbon dioxide free nitrogen gas was bubbled through the samples for 10 minutes. The solutions were titrated under nitrogen with standard 0.01M sodium hydroxide solution using a pH meter and autotitrator linked directly to an XY recorder. The amount of alkali used at pH 6.25 was determined from the graph.

When amylopectins were not isolated, the external chain length of the amylopectin fraction was calculated from the percentage of amylopectin in the granules. The small contribution of formic acid from amylose was neglected.

(b) Enzymic method using β-amylase and pullulanase.

Some estimations of amylopectin average chain length were made using the enzymic method of Adkins and co-workers (1966). The determinations were made using enzymes purchased from Boehringer

Mannheim (Aust.), pullulanase from *Aerobacter aerogenes* (Cat. 108944 20 units/mg) and crystalline sweet potato β-amylase (Cat. 102822 500 units/mg).

21. GLUCOSE DETERMINATION USING GLUCOSE OXIDASE

Glucose was determined using a modification of the glucose oxidase-peroxidase method proposed by Trinder (1969a, b, c).

The modified reagent contained di-sodium hydrogen orthophosphate (24.8 g), sodium di-hydrogen orthophosphate (12.4 g), benzoic acid (4.0 g), 4-amino-phenazone (Eastman, sold as 4-amino-antipyrine Cat.6902) (0.2 g) and *p*-hydroxybenzoic acid (3.0 g). All these chemicals were dissolved in approximately 1800 ml of water in a 2 l. volumetric flask and the enzymes glucose oxidase (Boehringer 105139 200 units/mg) (40 mg) and peroxidase (Boehringer 108073 250 units/mg) (10 mg) added. The solution was then diluted to 2 l., covered with aluminium foil to protect it from the light and stored at 4^{o}C. This solution is stable and gives similar standard curves for at least 4 months.

Samples were diluted to contain between 0.2 and 1.5 g/l of glucose. Diluted sample (0.1 ml) was placed in a test tube and 5.0 ml of reagent added. The tube mixed and incubated in a 40^{o}C water bath for 15 minutes mixed and then read at 510 nm against a reagent blank. Glucose standards were included in all sequences.

22. DETERMINATION OF β-AMYLOLYSIS LIMITS OF STARCH

β-amylolysis limits were measured by a method similar to that of Peat and co-workers (1952c). Starch (25 mg), wetted

with benzene, was dissolved in 10 ml, 0.25M sodium hydroxide solution by heating for three minutes in a boiling water bath. The solution was cooled and neutralised with N-sulphuric acid. Acetate buffer (0.2 M; pH 4.8; 10 ml) was added and the solution made up to nearly 50 ml with distilled water and placed in a water bath to equilibrate at 40°C. β-amylase solution (1 ml) was added, the volume made up to 50 ml, the flask well mixed and a layer of toluene added to cover the digest. The flasks were incubated at 40°C for one hour. Aliquots (1 ml) were taken and the maltose produced estimated by the PAHBAH method.

23. CLARIFICATION FOR SUGAR ANALYSIS

The plant extract or β-amylase digest was mixed well and a 10 ml aliquot placed in a 100 ml wide mouthed volumetric flask. Twenty ml of barium hydroxide octahydrate solution (57 g/l) was added with mixing followed by 20 ml of zinc sulphate heptahydrate solution (50 g/l). The flask was then filled to volume with benzoic acid solution (3 g/l), mixed well and after 5 minutes filtered through Whatman No. 40 filter paper. A clear extract was obtained.

24. ESTIMATION OF REDUCING SUGARS

Reducing sugars were determined by a modification of the *p*-hydroxybenzoic acid hydrazide (PAHBAH) method proposed by Lever (Lever 1972, 1977; Lever *et al* 1973) for blood sugar analysis.

Samples of clarified, diluted extracts or digests (0.5 ml or 0.1 ml with 0.4 ml water) were added to test tubes using Gilson

microlitre samplers. PAHBAH working solution (5 ml) was added and the tube mixed and then heated in a boiling water bath for 4 minutes. The tubes were removed, 10 ml of distilled water added and the contents mixed on an electric vibrating tube mixer. The colour was read against water at 415 nm. Glucose and maltose standards (0.2 g/l and 0.4 g/l for 0.5 ml samples) were included in each batch.

PAHBAH working solution was prepared by dissolving solid parahydroxybenzoic acid hydrazide in an alkaline solution at the rate of 5 g per litre. The alkaline diluent is 0.5M sodium chloride containing 0.05M tri sodium citrate and 0.01M calcium chloride. It is made by dissolving 14.704g of trisodium citrate dihydrate and 1.47g calcium chloride dihydrate in 500 ml of water in a 1 l. volumetric flask. Twenty grams of sodium hydroxide dissolved in 250 ml water is added with mixing and after cooling, the flask was diluted to volume.

PAHBAH working solution was made up each day as required. The solution is stable overnight in the dark at $4^{o}C$; solutions that developed any yellow colour were discarded. The reagent may be stabilised by the addition of sodium sulphite (5g/100ml) (Lever *et al* 1973). The colour formed with reducing sugars is stable for at least one hour.

25. ESTIMATION OF SUCROSE

Sucrose was estimated by determining reducing sugars before and after invertase digestion.

Two 0.1 ml samples of clarified diluted extract were placed in test tubes. Invertase solution (0.4ml) was added to one (PAHBAH 1) and water (0.4ml) was added to the other (PAHBAH 2). The tubes were incubated at room temperature for 30 minutes, 5ml of PAHBAH working solution added and a reducing sugar determination carried out on each tube as described. Sucrose was determined by deducting the absorbance obtained without invertase from the absorbance obtained with invertase and comparing with a standard curve (Table 3).

Sucrose = PAHBAH 1 - PAHBAH 2

Sucrose standards (0.5 g/1 and 1.0 g/1) and a glucose standard 1.0 g/1) were included in each batch as a check on both parts of the method.

Invertase solution was prepared by dissolving 50mg of purified invertase from *Candida Utilis* (Sigma grade X Cat I 4753 α-galactosidase free, 400-800 units per mg) in 200ml of maleic acid buffer pH 5.5 (Maleic Acid 5.8g, Sodium Hydroxide 2.1g per litre adjusted to pH 5.5).

26. DETERMINATION OF GELATINIZATION TEMPERATURE

A method similar to that of Hoseney and co-workers (1971) was used. A slurry of 0.4g of starch in 10ml of water was heated in a water bath held at 50 ± 0.5°C by a Braun Model 1480 thermomix (B. Braun Melsungen AG. West Germany). The starch slurries were allowed to equilibrate for twenty minutes. The temperature was then increased in 2°C steps with 15 min. equilibration after each temperature increase. After each equilibration, samples were taken for microscopic

examination. The bath temperature was increased until all the starch had completely gelatinized. Each sample was examined under a polarizing microscope and the proportion of starch granules that retained birefringence determined. The percentage of gelatinized starch granules was plotted against temperature and the temperature required to gelatinize 50% of the granules interpolated from the graph.

27. DETERMINATION OF THE VISCOSITY OF STARCH AND RICE FLOUR PASTES

The viscosity of rice starch and flour pastes was determined using a Brabender Viscograph (Brabender OHG Duisburg, West Germany) (Halick and Kelly 1959). Milled rices were ground to flour in a Brabender Quadrumat Junior laboratory roller mill and passed through a 9xx flour silk screen; overs were reground until they passed the screen.

(i) Rice flour Viscographs Four hundred and fifty ml of distilled water was added to 50g of rice flour in a commercial aluminium milk shake container. The suspension was mixed on a milk shake machine (Woodson Model LAI; A.M. Wood & Sons, Sydney Australia) for 30 seconds and then rapidly poured into the bowl of the Brabender Viscograph. The amylograph was started at 50°C, 75 r.p.m. and fitted with a 700 cmg sensitivity cartridge. Once temperature had stabilised the graph was started and temperature programmed to rise at 1½°C/min to 95°C. The temperature was then held at 95°C for 20 minutes before a cooling cycle

at 1½°C/min back to 50°C. Rice flour gelatinization temperatures were determined in the Brabender viscograph using 100g flour in 400 ml of water and a 350 cmg sensitivity cartridge. Constant rate heating at 1½°C/min was started at 40°C and the graph terminated when the pen went off scale. Gelatinization temperature was calculated from the graph by extrapolating the line to zero viscosity and calculating the temperature at this point from the set rate of temperature increase (Beachell and Stansel 1963).

(ii) Rice Starch Viscographs were done on the dry starches as isolated in a similar manner to the flour viscographs except that 40g of starch in 460 ml of water was used.

28. COOKED RICE TEXTURE

Cooked rice texture was determined using a Kramer shear cell on an Instron model 1140 universal testing machine by the method described by Blakeney (1979).

RESULTS AND DISCUSSION

A. ANALYTICAL PROCEDURES

In the course of this research, it was necessary to investigate some of the analytical procedures used. Two methods of sugar analysis originally developed for clinical chemistry were extensively modified.

1. Enzymic determination of amylopectin chain length.

In method 20b, amylopectin is degraded by the concurrent action of β-amylase and pullulanase. Pullulanase is known to specifically hydrolyse the α-(1→6) linkages in amylopectin and consequently the entire polysaccharide molecule is rendered susceptible to β-amylase action. At high concentrations of β-amylase, chains having an even number of glucose residues are converted to maltose. Chains having an odd number of glucose residues are converted to maltose and one glucose molecule, which can be specifically estimated using a glucose oxidase method. The pullulanase and β-amylase used must be maltase (α-glucosidase) free. Amylopectin average unit chain length is then calculated on the assumption of a random distribution of even and odd length chains.

Initially several attempts were made to isolate pullulanase using cultures of *Aerobacter aerogenes* isolated by a colleague and the method of Ueda and Ohba (1972) (Price 1968). All attempts failed to produce a culture filtrate that would degrade Pullulan (Sigma P4516). It was concluded that only specific strains of *Aerobacter aerogenes* contained pullulanase.

Pure pullulanase and β-amylase were later purchased commercially.

The enzymic method is claimed to be more reproducible than periodate oxidation (Adkins *et al* 1966).

2. Glucose determination using glucose oxidase.

Trinder (1969b) uses the relatively non toxic dye 4-amino-phenazone in place of the more commonly used *o*-tolidine and *o*-dianisidine that are considered carcinogenic (Trinder 1969a).

Trinder's (1969b) reagent uses phenol to couple with peroxide and 4-amino-phenazone in the presence of peroxidase for colour formation. The presence of phenol necessitates a 2 part reagent and in an effort to overcome this disadvantage a number of substituted phenols were tried. The hydroxybenzoic acids were all found to give some colour in the system. *m*-hydroxybenzoic acid gave the best colour yield followed by *p*-hydroxybenzoic acid. *o*-hydroxybenzoic acid gave a relatively poor colour yield (Fig. 3). Method 21 was developed. The use of *p*-hydroxybenzoic acid in place of phenol resulted in the preparation of a stable single solution reagent of equivalent sensitivity to Trinder's original reagent. Meiattini and co-workers (1978) have recently reported on a cholesterol oxidase method where they use *p*-hydroxybenzoic acid and 4-amino-phenazone in one reagent for colour formation. The incubation time of this method is critical as the colour was found to develop and then fade along a continuous curve (Fig. 4). All determinations using the method were done sequentially so that accurate incubation timing of each tube was possible.

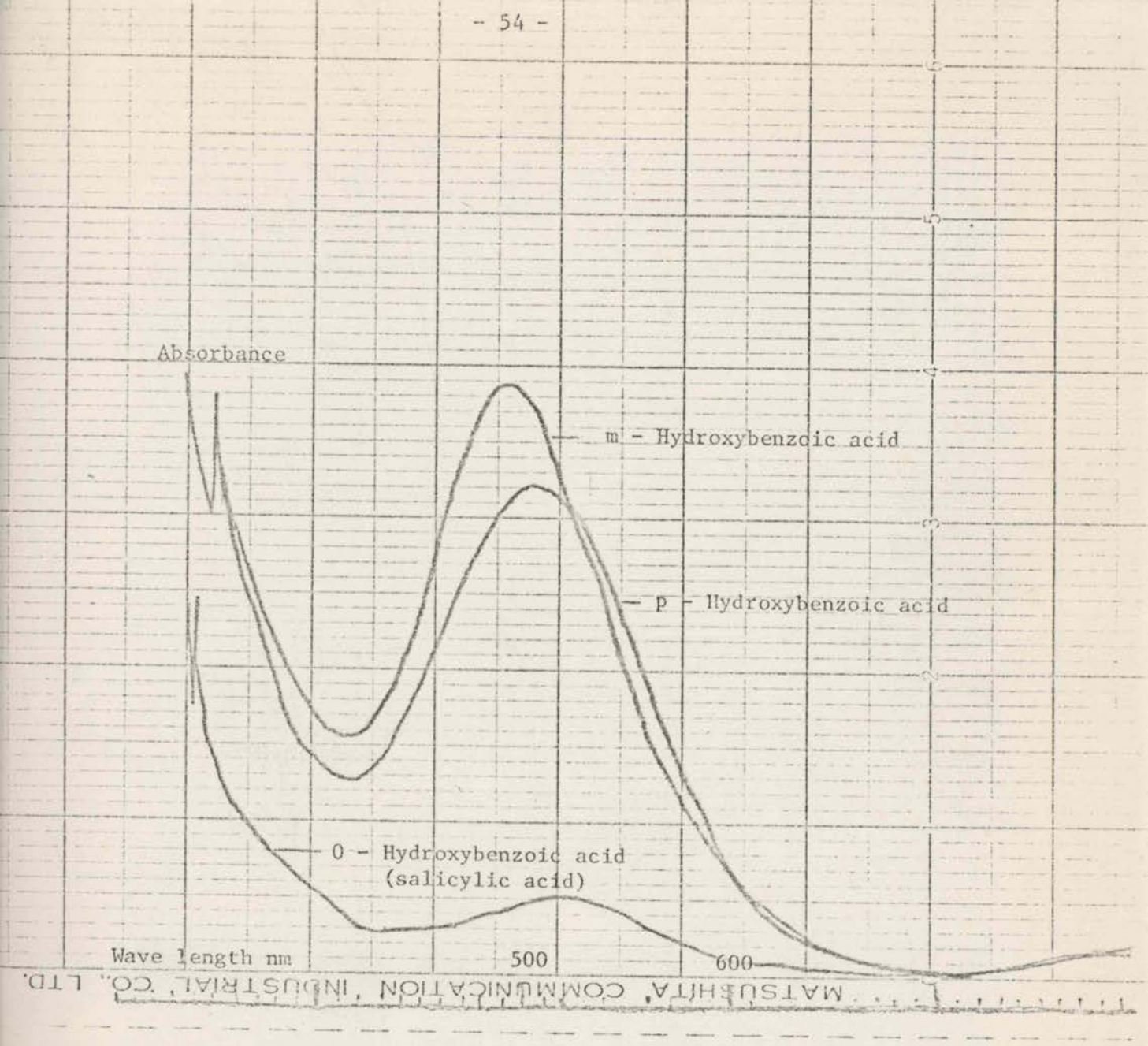


Figure 3.

Absorption spectra of the three hydroxybenzoic acid; 4-aminophenazone derivatives in modified Trinder glucose oxidase reagents. (hydroxybenzoic acids 1.5 g/l, Glucose standard 0.4 g/l).

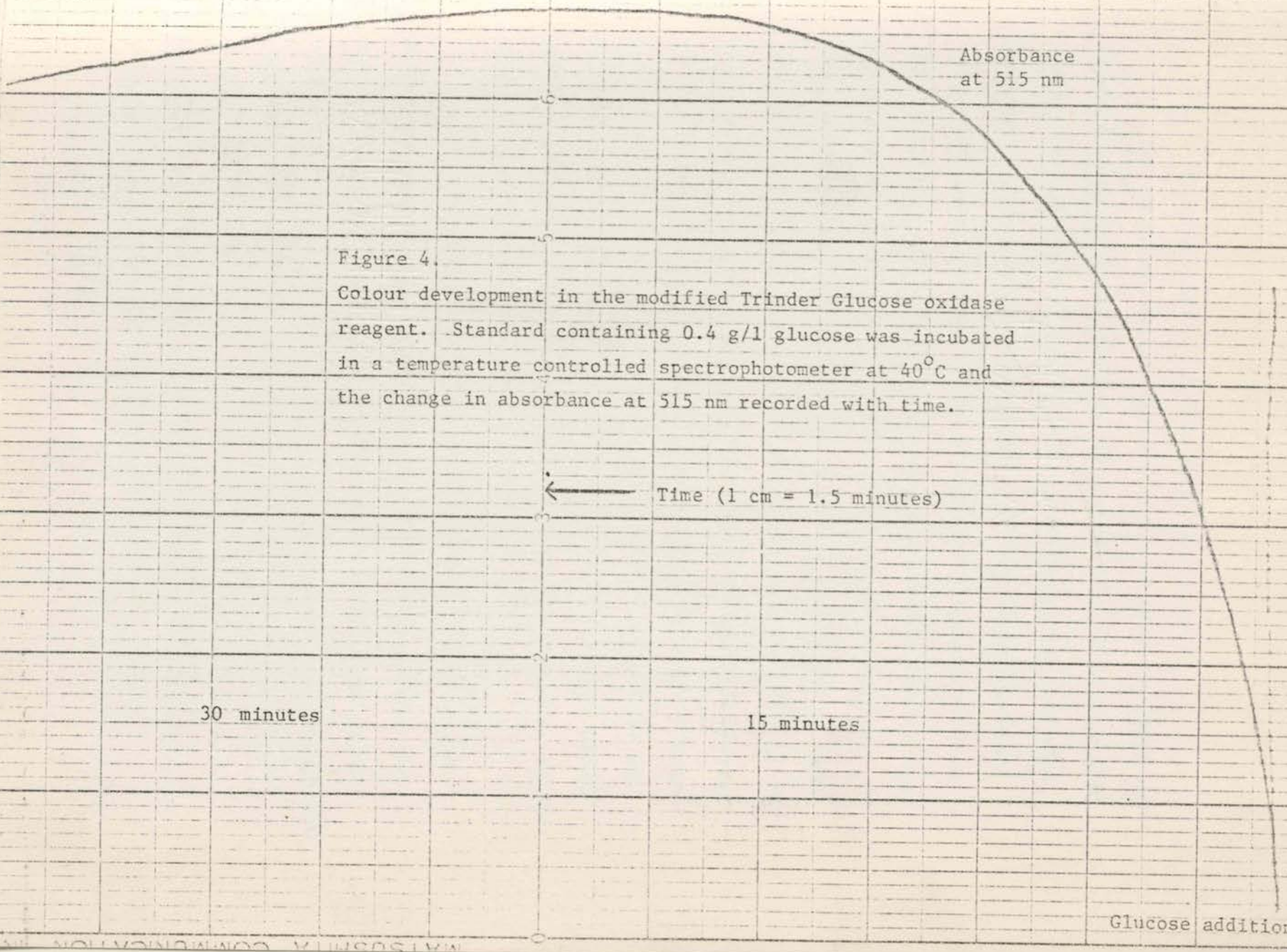


Figure 4.
Colour development in the modified Trinder Glucose oxidase reagent. Standard containing 0.4 g/l glucose was incubated in a temperature controlled spectrophotometer at 40°C and the change in absorbance at 515 nm recorded with time.

← Time (1 cm = 1.5 minutes)

3. Clarification of extracts for sugar analysis.

The clarification and deproteination of grain extracts and enzyme digests for sugar analysis has always been difficult. The traditional use of lead acetate introduces heavy metal ions that are incompatible with many subsequent analytical methods, while sodium tungstate fails to inactivate all enzyme activities (Rose 1968). Extraction with alcohol necessitates an extra heating step and losses due to evaporation can make calculation of extract concentrations difficult. Methods for the clarification of sugar extracts from foods have recently been reviewed by Lee (1978). The zinc ferrocyanide method gives effective clarification but leaves a residue of potassium acetate in the sample. The sodium benzoate-hydrochloric acid method proposed by Rose (1968) is difficult to filter due to the fine benzoic acid precipitate produced. Barnes (1978) overcame this problem by filtering through glass fibre filter paper.

The zinc sulphate barium hydroxide double precipitation method has been widely used for blood samples (Fales 1963). The amount of clarifying solutions was determined by trial and error using a series of β-amylase digests. The same amount has subsequently been found adequate for clarifying flour and potato digests and several fruit juices. The concentration of barium hydroxide was increased from that recommended for blood to give a slightly alkaline filtrate which is desirable when using the PAHBAH reaction.

To check that the clarification and deproteination method was not removing any sugar from the samples, a large digest of amylopectin and soybean β-amylase was divided into 2 samples and spiked with C^{14} (u) glucose and C^{14} (u) maltose respectively. Carbon 14 labelled sugars were purchased from the Radiochemical Centre (Aust.). To subsamples of each were added various amounts of the clarifying solutions of barium hydroxide and zinc sulphate. All subsamples were then made to volume and filtered in the usual manner except for one set of untreated samples that were diluted to volume but not filtered. Aliquots of the filtrates and the two unfiltered controls were placed in scintillation vials, Instagel (Packard Instruments) added and the C^{14} content counted on a Liquid Scintillation Spectrometer (Packard Model 3255). The counts obtained were adjusted for quench using an internal C^{14} toluene standard. The means of ten counting sequences were compared using Duncan's new multiple-range test (Steel and Torrie 1960). None of the treatments were significantly different and it is concluded that the method does not remove any sugar from the sample. The filtrates contained no detectable protein by the Coomassie brilliant blue test (Bradford 1976).

4. Determination of reducing sugars with parahydroxybenzoic acid hydrazide

A new colorimetric reaction for reducing sugars was discovered by Lever (1972). When glucose or other reducing sugars react with parahydroxybenzoic acid hydrazide (PAHBAH) in aqueous alkali,

4-hydroxybenzoylhydrazones of glyoxal and methylglyoxal are produced; these form yellow chelates with calcium.

Colour yields for various carbohydrates and standard tables for the PAHBAH method (Method 24) are presented in tables 2 and 3. All the sugars tested had peak absorbances in the region of 410nm. PAHBAH working solution water blanks have zero absorbance between 410 and 420nm. (Fig. 5).

The PAHBAH method has several advantages over the older reducing sugar methods that are based on copper, ferricyanide or 3, 5 dinitrosalicylic acid reduction. These methods are all less specific than PAHBAH reacting with non sugar reducing substances (Southgate 1976; Lever *et al* 1973). They require more care with heating and cooling procedures. The *o*-toluidine method is a comparable type of reaction but uses reagents that are more toxic and corrosive than PAHBAH (Ferraro *et al* 1976). Very few substances have been found that interfere with PAHBAH (Lever 1972). High protein levels can cause an increase and calcium complexing agents a decrease in colour yield. The latter problem is easily overcome by the addition of excess calcium.

5. Determination of sucrose.

In Method 25, the invertase digestion proceeds rapidly at room temperature ($20^{\circ}C$) and for up to 20g/l of sucrose is complete in 20 minutes. Digestions were run with added glucose without affecting the rate of digestion or the relative amount of sucrose

Table 2. Comparative specificity of the PAHBAH method for carbohydrates

Carbohydrates	Response relative to glucose PAHBAH[a]
Glucose	100
Mannose	100
Galactose	68
Fructose	100
Ribose	68
Xylose	68
2-keto Gluconic acid	86
Galacturonic acid	70
Maltose	115
Sucrose	0
Maltotriose	130
Raffinose	0
Stachyose	0
Ascorbic Acid	13

[a]manual PAHBAH with 1 mM carbohydrate solutions

Table 3.

Absorbances obtained with some sugar standards using the PAHBAH method

Sugar Concentration in grams per litre		Water	Invertase	Sugar Concentration in grams per litre		Water	Invertase
Glucose	0	0	0	Fructose	0	0	0
B.D.H. Aristar	0.2	.13	.15	Pfanstiehl	0.2	.15	.16
	0.4	.23	.25	Levulose C.P.	0.4	.26	.27
	0.6	.34	.36	Special	0.6	.36	.38
	0.8	.45	.46		0.8	.45	.47
	1.0	.57	.58		1.0	.58	.60
	1.5	.87	.87		1.5	.88	.87
Sucrose	0	0	0	Maltose	0	0	0
B.D.H. Aristar	0.2	0	.17	Monohydrate	0.2	.09	.11
	0.4	0	.28	Calbiochem	0.4	.16	.19
	0.6	0	.39	A Grade	0.6	.21	.23
	0.8	0	.53		0.8	.27	.31
	1.0	0	.62		1.0	.33	.36
	1.5	0	.92		1.5	.51	.55
Raffinose	0	0	0	Melibiose	0	0	0
pentahydrate	0.5	0	.30	monohydrate	0.5	.27	.28
Sigma	1.0	0	.52	Sigma	1.0	.53	.55
	1.5	0	.84		1.5	.80	.81
Maltotriose	0	0	0	Stachyose	0	0	0
>93%	0.5	.14	.19	$4.5H_2O$	0.5	0	.27
Calbiochem	1.0	.27	.31	per Mole	1.5	0	.49
B Grade	1.5	.40	.43	Sigma			

All samples were 0.1 ml with 0.4 ml of water or invertase added. Absorbances were read on a Varian 635 spectrophotometer at 415 nm in 1 cm silica cells against water.

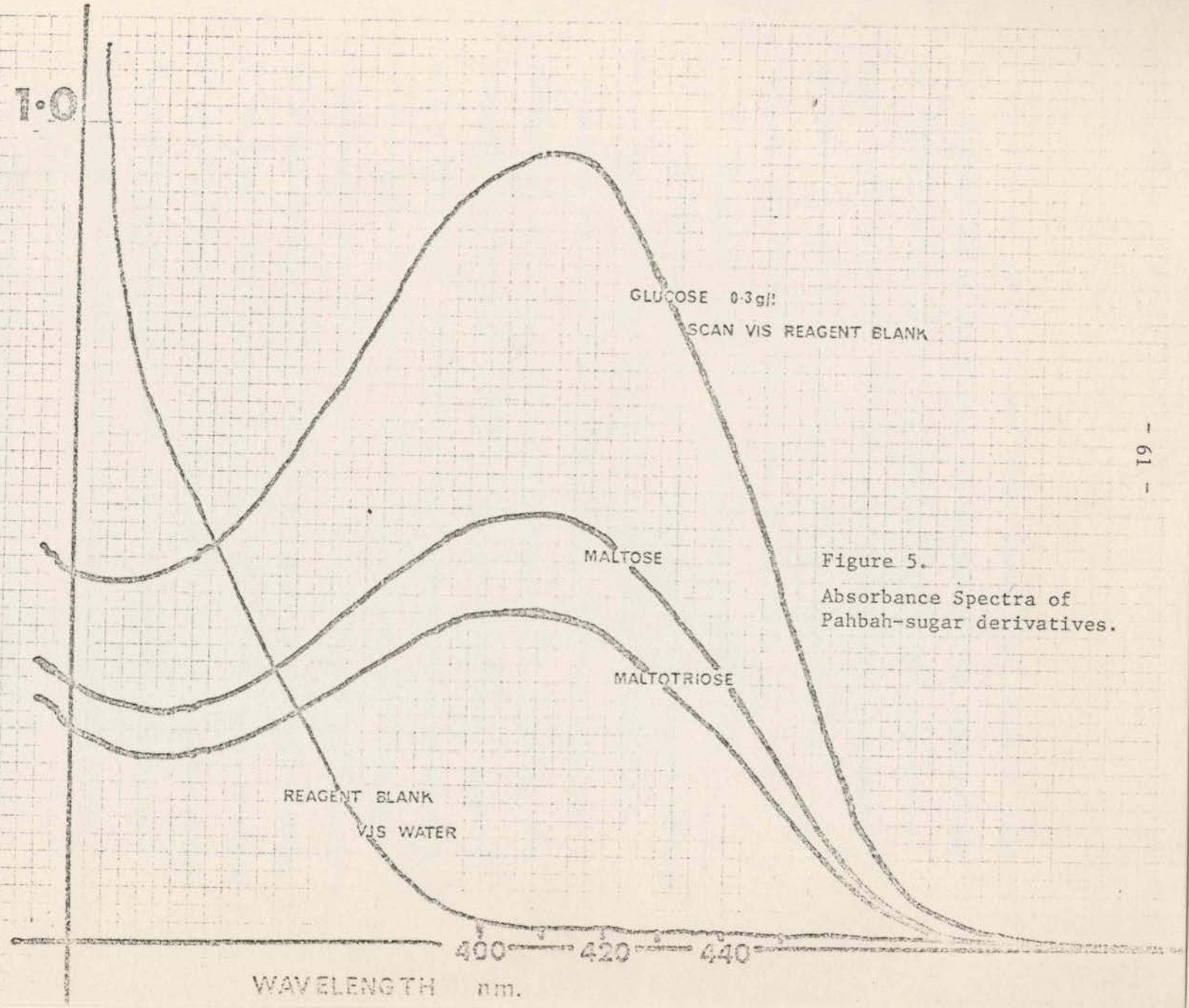


Figure 5.

Absorbance Spectra of Pahbah-sugar derivatives.

found from the "with invertase" PAHBAH assay. The method is specific for sucrose only in the absence of raffinose and the raffinose series of oligosaccharides which also release PAHBAH active sugars when treated with invertase. Representative samples of pollen were analysed for raffinose using the α-galactosidase method of Schieweck and Busching (1969); none was detected.

6. Viscosity of starch and rice flour pastes.

In Method 27 the manner of flour preparation is very important. Flours produced on different mills and sieved in different ways can produce totally different viscogrammes. Figure 6 shows two viscogrammes for a sample of the Yanco crossbred YR 13-89-11. The viscogramme was produced from flour milled in a Brabender Quadrumat mill as described. Flour for the lower viscogramme was produced in an Allis Chalmers laboratory roller mill to pass a 12xx silk screen. It is thought that the level of starch damage produced during milling influences the rate of hydration of the flour and the final viscosity of the paste. Flour samples prepared in attrition mills as recommended by Halick and Kelly (1959) have been found to give variable viscogrammes. Flour moisture content and sample size are also extremely important. Peak viscosity was found to vary expotentially with sample size.

B. STARCH PROPERTIES

The development and properties of rice starches were investigated throughout plant growth. The rice varieties used were chosen because they were known to differ in cooking quality.

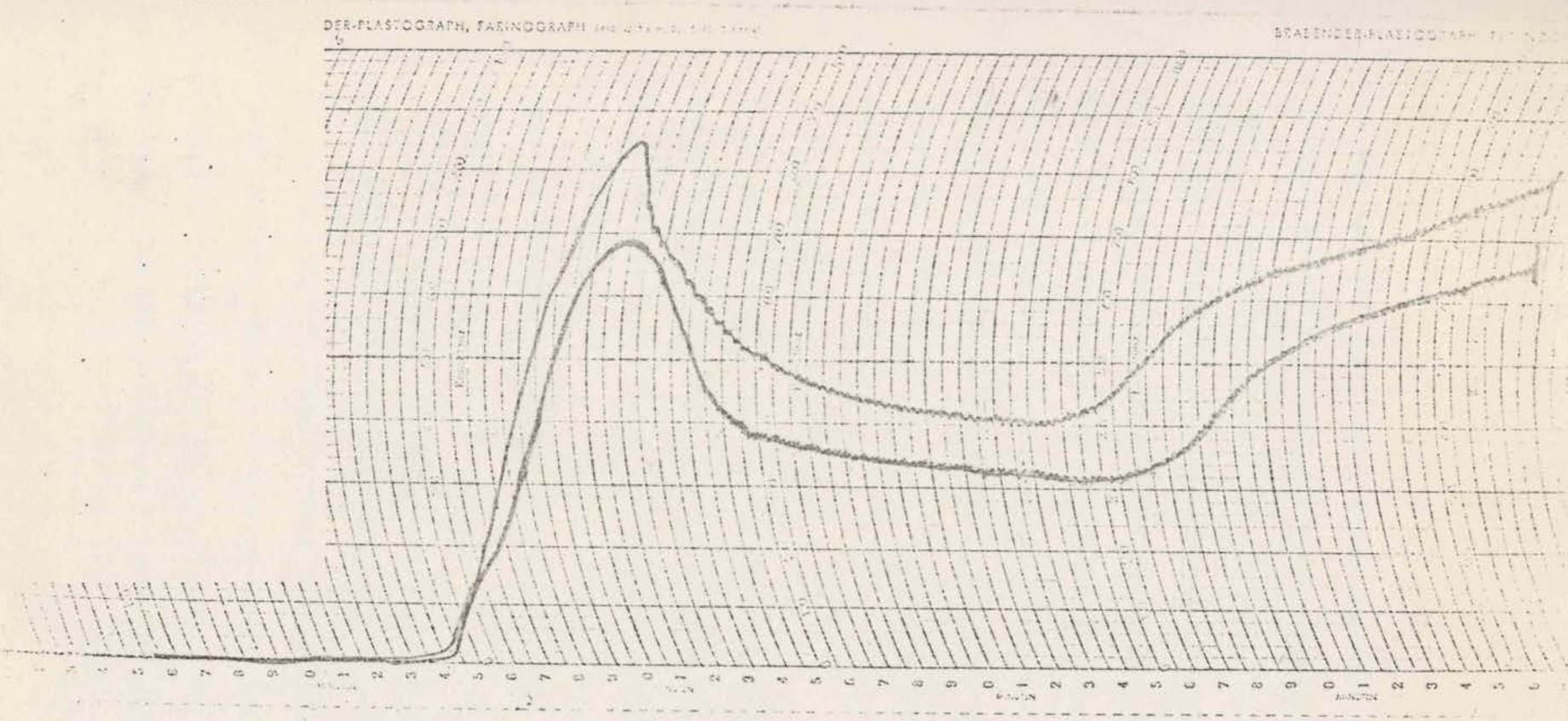


Figure 6.

Two viscogrammes showing the effect of different milling methods on the paste viscosity of a sample of YR 13-89-11 rice flour.

Starches isolated from stems and pollen were investigated to establish if within a variety, they are similar to the mature endosperm starch. Grain was collected from anthesis to maturity and the development and structure of endosperm starches investigated to see how the properties of these starches related to the cooking qualities of the grain. Due to the importance of appearance to rice market quality, the properties of starches isolated from the chalky and translucent parts of chalky cored grain were investigated to establish if any variance in starch type was associated with the chalky core character.

1. Rice stem starch.

In the series of rice varieties of diverse genetic background that were grown during the 1974/75 rice season, dry matter accumulation for all varieties (Table 4) followed the previously described patterns (Boerema 1973). The non-adapted, imported varieties Ali Combo and Madinika had generally slower growth curves and Ali Combo produced the least total dry matter.

All varieties accumulated stem starch (Table 5). Starch levels began to rise after emergence and accumulation continued until after flowering when starch content dropped rapidly. Some varieties, notably Calrose, started to accumulate stem starch again in the final weeks before grain harvest (Table 5). The rate of starch accumulation was similar for all varieties; the curve for Inga is illustrated in figure 7.

Table 4.

Rice Plant Above Ground

Total Dry Matter ('00 g/M^2)

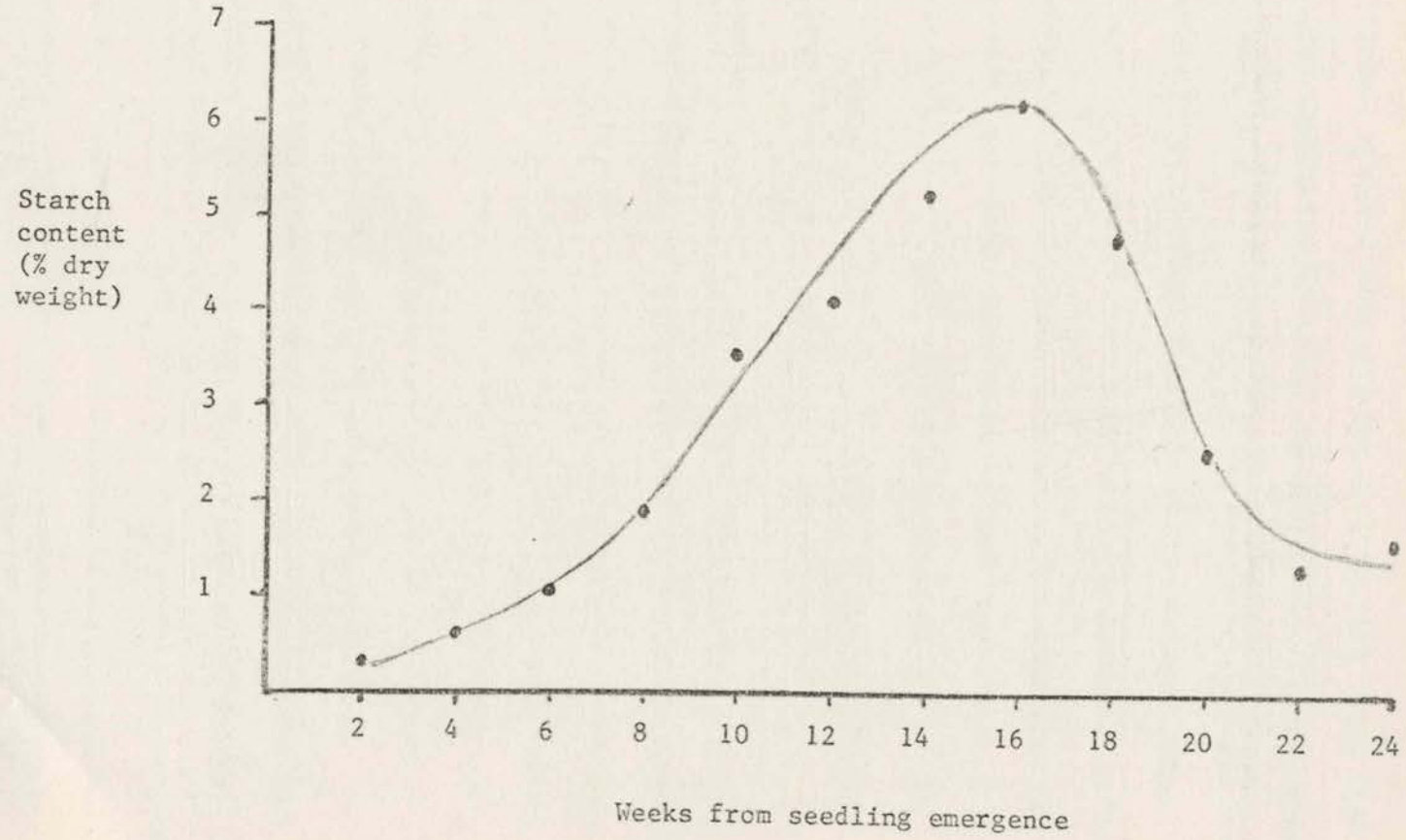
Weeks from emergence	Caloro	Calrose	Kulu	Inga	YR57	Ali Combo	Madinika	Tarra 140	Kuro Mochi
2	0.43	.42	0.31	0.38	0.29	0.17	0.29	0.34	0.26
4	0.86	.83	0.64	0.59	0.51	0.36	0.52	0.66	0.57
6	1.63	1.52	1.21	0.96	0.94	0.84	1.02	1.11	0.89
8	2.51	2.34	2.27	2.16	1.83	1.57	2.30	2.16	1.85
10	3.97	4.13	4.11	3.86	3.49	3.30	3.95	3.87	3.34
12	6.48	6.38	6.07	5.89	5.24	4.96	5.67	5.98	5.11
14	9.42	10.02	9.37	10.64	9.86	7.87	8.96	9.26	8.31
16	12.39	13.46	11.83	12.86	11.34	9.48	9.49	12.73	10.41
18	16.34	17.84	16.49	16.17	16.13	11.49	11.23	16.08	14.73
20	19.67	20.41	19.83	18.96	17.33	13.82	14.34	19.34	16.20
22	21.43	23.93	21.21	20.92	18.20	14.76	18.31	21.34	17.16
24	22.64	25.13	21.30	21.84	18.44	15.30	19.82	21.67	18.20

Table 5. Stem Starch as Percentage of Rice Stem Dry Wt.

Weeks from emergence	Caloro	Calrose	Kulu	Inga	YR57	Ali Combo	Madinika	Tarra 140	Kuro Mochi
2	0.52	0.42	0.23	0.31	0.33	0.23	0.18	0.24	0.19
4	1.13	1.02	0.89	0.63	0.42	0.84	1.09	0.86	0.84
6	2.16	2.14	1.67	1.04	1.21	1.55	2.33	1.59	1.32
8	3.23	3.06	2.84	1.93	1.88	1.96	3.04	2.73	2.46
10	3.80	3.53	3.10	3.66	4.24	3.03	3.92	3.21	3.54
12	4.93	4.13	4.07	4.28	4.29	4.23	4.61	4.11	4.94
14	6.02	5.89	5.66	5.36	5.40	4.89	4.96	5.57	6.22
16	6.14	6.23	5.92	6.31	5.68	5.14	6.27	5.83	6.17
18	3.66	3.74	4.06	4.84	6.11	5.23	6.18	4.21	3.83
20	2.23	1.67	2.55	2.51	4.31	3.66	3.37	2.44	2.66
22	1.45	1.61	1.02	1.46	1.86	2.04	1.64	1.62	1.68
24	1.43	2.48	1.33	1.63	1.44	1.50	0.84	2.33	1.97

Figure 7.

Accumulation of Starch in the Stems of Inga Rice



The starches isolated were all found to be relatively free from contamination when examined under the microscope. Starch granules isolated from stems in the first month after emergence were all round in shape. Average granule diameter at this stage was 4μm. With subsequent isolations, starches increased in size and, where confined by other granules, developed polyhedral shapes. The mean size of stem starch granules apparently varied with total starch content although some small granules could be found even in the 16 weeks after emergence preparations when starch content reached a maximum for most varieties. When starch content started to fall, many of the granules isolated were partially damaged and some stained readily with Chlorazol Violet R (C.I.32445). This stain is used to identify damaged starch in wheat flour preparations (Flint and Moss 1970). Plate 1 illustrates stem starch from the variety Inga at 16 weeks after emergence. At this stage the larger granules are 100μm in diameter. However, many smaller granules are present and some granules are beginning to show evidence of enzymic breakdown.

Stem starch levels reached over 6% of the total dry weight in many of the rice varieties tested. This is similar to levels reported by Japanese workers (Nagi 1959) and represents a considerable energy reserve for the plant. All rice varieties tested seem to mobilise this reserve during grain filling. However, to determine the amount that is translocated to grain, would require further studies using radio labelled stem starches. The large amounts of

starch involved can be appreciated when stem starch is expressed in terms of starch yield per square metre (Table 6).

Starch was isolated from all varieties at 8 and 16 weeks after emergence in sufficient quantities for the determination of amylose contents, β-amylolysis limits and amylopectin average chain lengths (Tables 7 and 8). Stem starches isolated from the two varieties with waxy endosperm starch, Tarra 140 and Kuro Mochi, were normal, not waxy starches. Stem starches isolated from all varieties at both sampling dates had similar amylose contents. There was no apparent relationship with the known amylose contents of grain endosperm starch (Tables 7 and 8). β-amylolysis limits were similar for all varieties and consistent with a 15% amylose content. Amylopectin average chain lengths were similar for most varieties. At 16 weeks after emergence, starch isolated from Kulu and the local waxy variety Tarra 140, had significantly higher amylopectin average chain lengths. The Japanese waxy variety, Kuro Mochi had a significantly low average chain length.

The properties of rice stem starch as determined in this experiment are very uniform between varieties. In a rice breeding programme, stem starch would be of no use in predicting the properties of grain endosperm starch. The amounts of stem starch accumulated however, are large and an investigation to determine what proportion of stem starch is translocated to grain, would be worthwhile. Rice

Plate 1. Rice stem starch isolated from the variety Inga 16 weeks after emergence. The microscope was focused to emphasise the layered structure of the grains (X 350).

Table 6.

Rice Stem Starch (g/M^2)

Weeks from emergence	Caloro	Calrose	Kulu	Inga	YR57	Ali Combo	Madinika	Tarra 140	Kuro Mochi
2	.224	0.18	0.07	0.12	0.10	0.04	0.05	0.08	0.05
4	.970	0.85	0.57	0.37	0.21	0.30	0.57	0.57	0.48
6	3.52	3.25	2.02	1.00	1.14	1.30	2.38	1.76	1.17
8	8.11	7.28	6.45	4.17	3.44	3.08	6.99	5.90	4.55
10	15.09	14.67	12.74	14.13	14.80	10.00	15.48	12.42	11.82
12	31.95	26.35	24.70	25.21	22.48	20.98	23.59	24.27	25.24
14	56.71	59.61	53.03	57.03	53.24	38.48	44.44	51.58	51.69
16	76.07	83.86	70.03	81.15	64.41	48.73	59.50	74.22	64.23
18	59.80	66.72	66.95	78.26	98.55	60.09	69.40	67.70	57.01
20	43.86	54.49	50.57	47.34	74.69	50.58	48.33	47.19	43.09
22	31.07	38.53	21.63	30.54	33.85	30.11	29.73	34.57	28.83
24	32.38	62.32	28.33	35.60	26.55	22.95	16.65	50.49	35.85

Table 7. Some Properties of Rice Stem Starches at 8 weeks after emergence

Rice Variety	Amylose content[a] (% Whole Starch)	β amylolysis limit (as % Maltose of Whole Starch)	Amylopectin average chain length[b] (anhydroglucose units)[c]
Caloro	15.4	64	26
Calrose	15.6	63	27
Kulu	16.2	64	29
Inga	14.8	64	26
YR57	16.4	66	24
Ali Combo	15.3	64	25
Madinika	16.8	65	24
Tarra 140	14.7	63	27
Kuro Mochi	16.4	65	24

[a] Calculated from iodine binding capacity assuming that pure rice stem amylose binds 19.5g iodine per 100g

[b] Assuming that the contribution of amylose to formic acid production is negligible.

[c] LSD (5%) 1.8 units.

Table 8. Some Properties of Rice Stem Starches at 16 weeks after emergence

Rice Variety	Amylose content[a] (% Whole Starch)	β amylolysis limit (as % Maltose of Whole Starch)	Amylopectin average chain length[b] (anhydroglucose units)[c]
Caloro	16.2	63	24
Calrose	15.4	63	26
Kulu	15.8	65	28
Inga	14.6	64	24
YR57	16.3	64	25
Ali Combo	16.1	63	25
Madinika	17.2	65	24
Tarra 140	15.3	64	28
Kuro Mochi	15.7	66	23

[a] Calculated from iodine binding capacity assuming that pure rice stem amylose binds 19.5g iodine per 100g

[b] Assuming that the contribution of amylose to formic acid production is negligible

[c] LSD (5%) 1.7 units.

varieties with the ability to accumulate stem starch efficiently and then also translocate it to the grain may have a potential yield advantage.

2. Rice Pollen Starches

In February 1973 pollen was collected from a headland of the variety Inga. Separation and analysis methods were developed using this sample. The following season, a number of rice varieties were grown specially for pollen collection and starch analysis. They included the local variety Baru (Boerema, McDonald and Lewin 1974) not otherwise used in these experiments. In the second season the variety Madinika flowered very poorly and insufficient pollen was collected to make starch.

The pollen grains of all the varieties studied were approximately 40µm in diameter and had a high starch content (Table 9). Pollen in fresh anthers readily stained for starch. However, day-old dry pollen stained weakly. The variety Ali Combo flowered very unevenly and the observed lower starch content of its pollen may be due to a greater proportion of old pollen in the sample.

The clarity of the 2-methoxyethanol based iodine stain is shown in plate 2 where pollen in the anther of a plant segregating for the waxy starch character is illustrated. The staining method has subsequently been used at Yanco Agricultural Research Centre for examining the purity of waxy plants selected for use in pure seed schemes. When iodine stained pollen grains were observed at

Table 9. Composition of Rice Pollens

Variety	Nitrogen[a]	Lipid[a]	Ash[a]	Starch[a]	Free Reducing Sugars[a] (as glucose)	Free Sucrose[a]
Caloro	3.24	2.73	2.46	23.4	4.7	5.3
Calrose	3.18	3.10	2.38	22.6	5.1	5.7
Kulu	2.94	2.89	2.33	23.4	5.1	5.4
Inga	2.96	2.84	2.47	23.7	4.6	5.8
Ali Combo	3.31	3.12	2.36	19.4	4.8	5.4
Tarra 140	2.97	2.77	2.38	21.6	6.2	5.9

[a] all figures are expressed on a percent dry weight basis

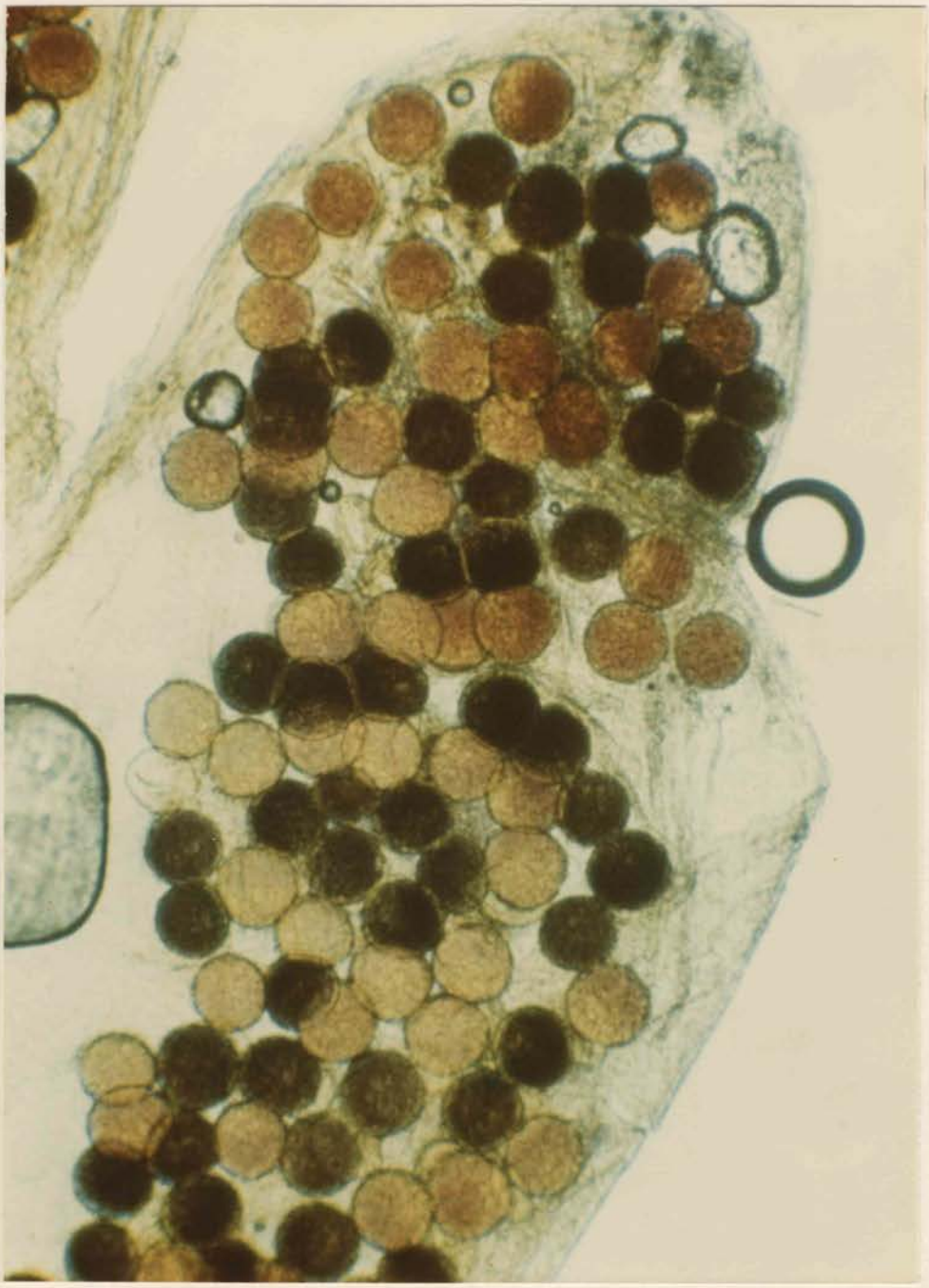


Plate 2. Pollen grains in the anther of a rice plant segregating for waxy and normal starch (X 250)

high magnification, some of the internal structure of the grains became visible. It was observed that the iodine stain was principally associated with small rod shaped granules (plate 3). On analysis the rice pollens all contained similar levels of nitrogen, lipid ash, starch and sugars (Table 9). These results, particularly the high starch contents, are similar to the figures quoted for maize pollen by Todd and Bretherick (1942).

The level of free sugars in the waxy variety Tarra 140 may be significant as waxy varieties usually also have a high free sugar level in their endosperms and are known in some parts of the world as "sweet rices". Unfortunately there was insufficient of the other waxy pollen Kuro Mochi for compositional analysis.

The isolated pollen starches were examined by microscopy. The starch grains from all varieties were similar. The grains were cylindrical - many with enlarged ends; others were ovoid. They ranged in size from 0.5 to 1.0μm in length and 0.3 to 0.7μm wide. Some of the cylindrical grains appeared to be two ovoid grains joined together or to be a single grain with two hills. The extremely small size of pollen starch made light microscopy difficult. Electron microscopy was not available at the time this part of the study was made. A photomicrograph of pollen starch isolated from the second season crop of Inga is presented in plate 4. All the pollen starches isolated were observed using an iodine/iodide stain; few non-starch contaminants were observable. Staining the

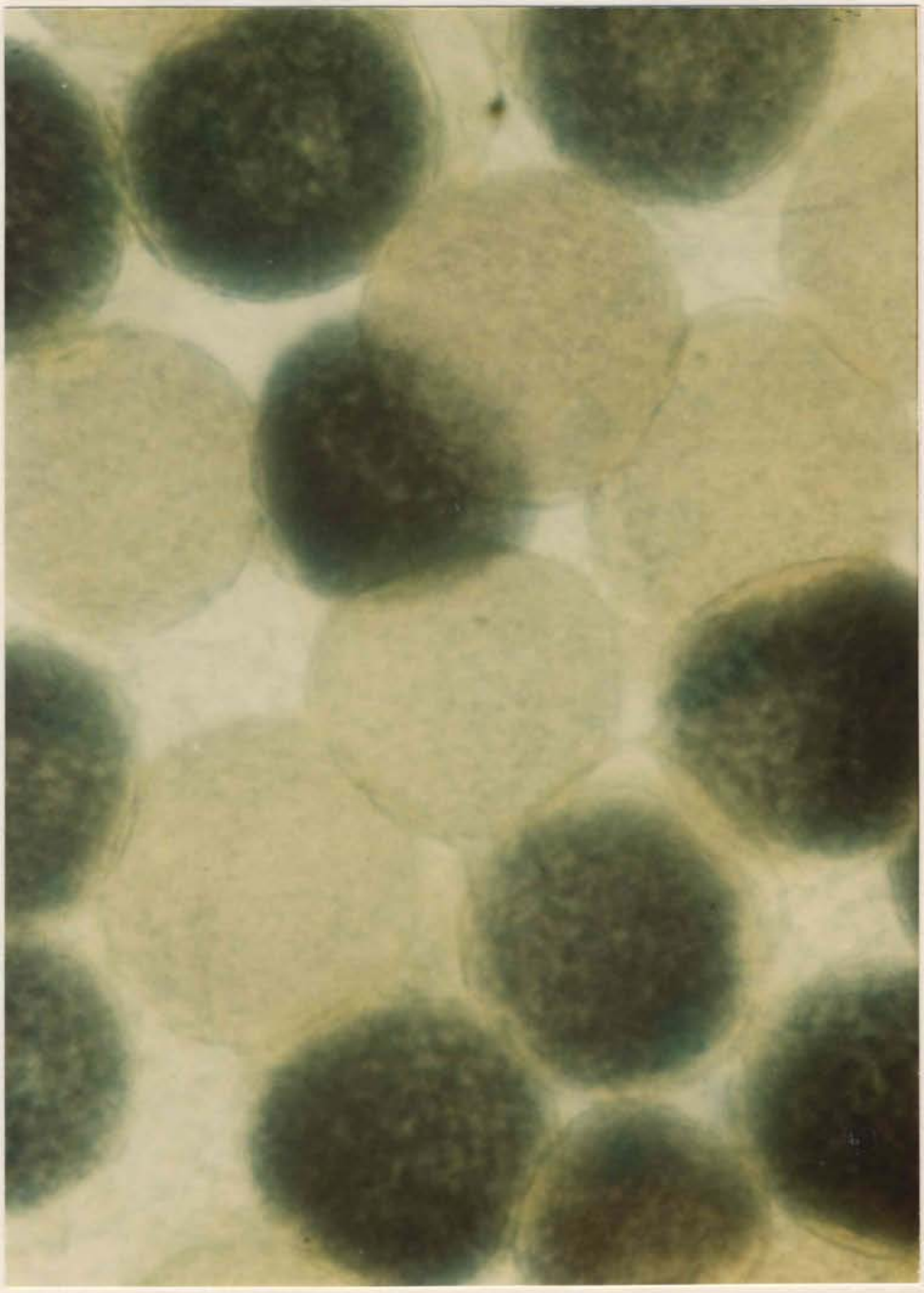


Plate 3. Segregating pollen grain at higher magnification. The stained starch granules can be seen within the pollen grains (X 1000)

Plate 4. Rice pollen starch granules isolated from the variety Inga (X 8000)

starch preparation with dilute solutions of Chlorazol Violet R (C.I. 32445) and Ponceau 2R (C.I.16150), (Flint and Moss 1970) failed to detect any damaged starch or any protein. Starch determinations were made on the isolated pollen starches using the α-amylase, amyloglucosidase digestion method (Banks *et al* 1970) followed by glucose oxidase determination of the resultant glucose. The determination all produced near quantitative recoveries of the added starches. It is concluded that the starches isolated were pure and uncontaminated by pollen cell debris.

The amylose contents of the pollen starches and the endosperm starches later isolated from the same plots were estimated from the iodine binding capacities of the starch (Table 10). The two varieties with waxy endosperms have waxy pollen starches. In the non waxy endosperm varieties, the pollen starch iodine binding capacities of the two varieties that normally have high amylose content, endosperm starches were also high. There is not complete agreement between the two columns of amylose content data (Table 10). Some of the potentiometric plots, particularly Kulu, had indefinite inflection points. Indefinite inflection points are attributed to starches containing amylose with a shorter chain length. The problem can be overcome by titrating at a lower temperature (Adkins and Greenwood 1966). This problem was also experienced by Banks and co-workers (1971a) with high amylose maize pollen starch.

Table 10. Properties of Pollen and Endosperm Starch

Variety	Starch[a]	IBC[b] mg %	Pollen Starch Amylose % [c]	Endosperm Starch Amylose %[c]
Caloro	23.4	4.2	21.5	20.4
Calrose	22.6	4.1	21.0	19.8
Baru	21.8	4.2	21.5	21.2
Kulu	23.4	4.2	21.5	21.7
Inga Season 1	23.7	4.4	22.6	21.8
Inga Season 2	22.8	4.3	22.1	23.8
Ali Combo	18.6	5.3	26.7	28.5
YR 57	22.4	5.2	27.2	25.8
Tarra 140	21.6	0.2	1.0	0
Kuro Mochi	20.5	0.2	1.0	0

[a] percent dry wt. of pollen

[b] Iodine binding capacity at 20.0°C by potentrametric titration

[c] $\frac{\text{IBC} \times 100}{19.5}$

The results obtained are in agreement with the findings of Banks and co-workers (1971a) for high amylose maize pollen starch but contrary to those of Zuber and co-workers (1960) for maize pollen starches. For use in a rice quality breeding programme, the results show promise in predicting grain starch amylose content from the pollen starch values. Low amylose lines could be eliminated before harvest. However, later analysis of the endosperm starch of intermediate amylose lines may still be necessary. To be of practical use a simple, micro method for determining pollen starch amylose content is needed. It is possible that a method based on initial dimethylsulphoxide extraction combined with colorimetric estimation of amylose content and an enzymic estimation of total starch could be used.

3. Developing Grain Starches

In the 1972/73 trial, developing grains were pulled from the mid region of panicles every four days after mid-flowering. Care was taken that grains from the same region of the panicles were always selected to ensure the same age of grain between samplings.

Total grain starch content developed rapidly. At 4 days after flowering, grains from all varieties contained a high starch level (Table 11). The initial high starch level is due to pericarp starch. Pericarp starch breaks down in the first 16 days after flowering. Endosperm starch is low at 4 days and increases throughout

Table 11. Starch Content of Developing Grain (mg/100mg of caryopsis)

Days after mid anthesis	Caloro	Calrose	Kulu	Inga	YR57	Ali Combo	Madinika	Tarra 140	Kuro Mochi
4	33.5	32.2	27.4	31.4	26.5	36.4	38.9	28.2	38.3
8	43.4	41.4	36.4	38.3	42.6	41.4	43.4	37.6	40.2
12	43.9	46.6	42.2	40.4	43.7	46.5	45.2	46.3	40.6
16	52.4	55.3	53.2	54.6	48.8	45.8	47.4	49.3	43.6
20	57.3	59.5	58.3	57.6	50.4	47.4	51.9	53.6	49.7
24	62.8	67.1	65.8	62.9	58.8	51.6	55.0	59.8	53.5
28	68.3	72.5	71.6	73.4	60.6	57.2	58.4	69.5	61.4
32	76.9	81.3	75.8	78.6	62.3	62.3	63.2	74.8	73.8
36	78.4	82.7	84.2	79.7	67.5	68.4	69.7	81.7	79.7
40	80.2	80.2	83.6	82.6	78.9	72.6	71.4	83.3	84.4
44	82.6	83.0	83.9	84.8	79.4	78.3	77.6	83.4	84.8
48	82.4	81.3	82.3	81.4	81.6	78.4	79.3	83.6	84.7
52	83.1	82.4	82.3	81.7	80.2	76.8	78.6	83.2	84.0

grain ripening, declining slightly during grain desiccation. High pericarp starch levels in young developing wheat grains has been reported by Jenkins and co-workers (1975). Paul and co-workers (1971) also noted an initially high level of starch in developing rice grains. In their study, starch accumulated at a maximum rate between 12 and 16 days after anthesis.

Starch grain properties were followed throughout grain development (Table 12). Least significant differences for all of table 12 are presented at the bottom of table 12g. All varieties showed an increase in amylose content (as determined by iodine binding capacity) and a decrease in gelatinization temperature as they ripened. Increases in the amylose content of rice starch during ripening have also been reported by Kester and co-workers (1963). Briones and co-workers (1968) noted an increase in the amylose content and a decrease in gelatinization temperature as rice grains developed.

Starch granules isolated from grains 4 days after anthesis varied in average diameter from 2.6 to 4.2μm and at maturity from 5.7 to 7.3μm depending on variety. Gelatinization temperatures fell during grain development and amylopectin average chain length remained constant. Starches from all samplings were fractioned into amylose and amylopectin. The amyloses had iodine binding capacities that ranged from 18.7 to 19.2% and were considered to be pure.

Table 12a. Properties of Developing Grain Starch

CALORO

Days from Anthesis	Average granule (μm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylose ml/g	Limiting Viscosity number Amylopectin ml/g
4	3.4	17.2	64.3	24	143	183
8	3.8	18.1	64.4	24	146	185
12	4.1	17.8	64.2	24	137	183
16	4.9	18.4	63.7	24	144	182
20	5.5	18.6	63.4	24	146	182
24	5.7	18.6	63.0	23	148	176
28	5.9	18.7	62.7	23	148	181
32	5.9	19.1	62.9	23	147	177
36	6.2	19.2	62.8	24	148	174
40	6.3	19.4	62.5	24	150	172
44	6.3	19.4	62.5	24	151	174
48	6.1	19.6	62.4	23	151	174
52	6.1	19.5	62.5	24	151	174

Table 12b. Properties of Developing Grain Starch

CALROSE

Days from Anthesis	Average granule (μm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylose ml/g	Limiting Viscosity number Amylopectin ml/g
4	3.2	17.4	68.8	23	163	134
8	4.0	17.4	68.8	23	163	132
12	4.8	17.6	68.2	24	165	132
16	5.3	18.4	68.4	24	166	127
20	5.5	18.5	68.3	24	163	126
24	5.7	18.4	67.9	24	168	127
28	5.8	18.7	67.7	23	168	124
32	6.0	18.7	67.6	22	174	124
36	6.2	18.9	67.3	23	167	125
40	6.4	19.2	67.3	24	173	124
44	6.3	19.2	66.8	24	174	125
48	6.2	19.1	66.7	24	174	125
52	6.2	19.2	66.8	24	174	125

Table 12c. Properties of Developing Grain Starch

KULU

Days from Anthesis	Average granule (µm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylose ml/g	Limiting Viscosity number Amylopectin ml/g
4	4.2	18.4	75.9	19	181	184
8	4.2	19.2	75.8	19	184	185
12	4.4	19.6	75.9	19	178	184
16	4.7	19.4	75.7	19	173	181
20	5.4	19.7	75.7	19	175	183
24	5.7	20.9	74.8	19	174	178
28	5.8	20.9	75.2	19	170	176
32	6.1	21.2	74.7	19	170	172
36	6.4	21.2	74.4	19	170	174
40	6.6	21.6	74.0	20	167	175
44	6.7	21.6	73.4	20	170	174
48	6.6	21.5	73.5	19	171	173
52	6.6	21.6	73.4	20	170	173

Table 12d. Properties of Developing Grain Starch

INGA

Days from Anthesis	Average granule (µm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylose ml/g	Limiting Viscosity number Amylopectin ml/g
4	3.0	17.3	74.2	23	182	183
8	3.6	17.8	74.0	22	182	181
12	4.1	18.2	74.2	23	186	183
16	4.2	18.0	73.8	23	187	182
20	4.2	18.6	73.7	23	189	175
24	4.8	19.1	72.8	24	201	177
28	5.2	19.3	72.1	23	194	173
32	5.3	20.4	72.1	24	196	168
36	6.1	20.9	71.9	24	208	167
40	6.4	21.7	71.4	23	208	168
44	6.4	22.0	71.6	23	211	162
48	6.3	22.4	71.5	23	209	164
52	6.3	22.4	71.6	23	214	164

Table 12e.

Properties of Developing Grain Starch

YR57

Days from Anthesis	Average granule (μm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylose ml/g	Limiting Viscosity number Amylopectin ml/g
4	2.6	17.6	76.3	24	189	184
8	3.2	18.4	75.2	24	187	184
12	4.1	19.3	75.0	24	194	192
16	4.4	21.7	74.1	24	193	187
20	4.4	21.5	74.4	23	199	184
24	4.7	22.8	74.0	24	201	175
28	5.1	23.1	73.8	24	198	166
32	5.2	23.5	73.7	24	206	172
36	5.7	24.9	73.5	24	206	167
40	5.8	25.4	73.3	24	210	158
44	5.8	25.2	73.5	23	209	161
48	5.7	25.2	73.5	24	207	163
52	5.6	25.3	73.4	24	208	160

Table 12f. Properties of Developing Grain Starch

ALI COMBO

Days from Anthesis	Average granule (μm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylose ml/g	Limiting Viscosity number Amylopectin ml/g
4	4.1	21.4	71.3	19	136	175
8	3.8	22.5	71.5	19	141	180
12	3.8	22.6	70.2	18	147	174
16	4.5	22.4	70.3	18	143	182
20	4.9	24.7	68.7	19	156	183
24	5.2	24.3	68.6	20	154	183
28	5.3	26.2	68.5	19	162	179
32	5.6	26.5	68.7	19	172	168
36	5.6	26.7	68.5	19	181	164
40	6.0	28.1	68.5	19	184	159
44	6.2	28.2	68.4	19	182	160
48	6.4	28.0	68.2	19	186	156
52	6.3	28.3	68.4	19	186	157

Table 12g. Properties of Developing Grain Starch

MADINIKA

Days from Anthesis	Average granule (µm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylose ml/g	Limiting Viscosity number Amylopectin ml/g
4	3.8	18.6	75.8	22	172	187
8	3.8	18.4	75.7	22	173	181
12	4.0	18.7	75.7	22	172	182
16	4.2	20.5	75.8	22	177	184
20	4.4	20.3	75.3	22	176	185
24	5.3	22.4	75.2	22	178	183
28	5.7	23.7	74.7	23	180	178
32	5.9	24.8	74.1	22	183	172
36	6.8	25.3	73.8	23	185	167
40	6.9	25.2	73.5	22	186	164
44	7.2	26.5	73.2	23	185	161
48	7.3	26.7	73.4	23	185	159
52	7.3	26.5	73.4	23	185	160
LSD (5%)	-	0.4	0.2	2.2	6.3	10.4

Table 12h. Properties of Developing Grain Starch

TARRA 140

Days from Anthesis	Average granule (µm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylopectin ml/g
4	3.3	5.4	76.1	19	211
8	3.7	4.2	75.2	19	211
12	4.2	3.0	75.2	19	210
16	4.8	1.2	74.5	19	213
20	5.4	0.8	74.6	19	213
24	5.7	0.4	73.8	18	210
28	6.0	0.4	73.1	18	203
32	5.9	0.6	72.4	18	197
36	6.2	0.4	71.5	19	204
40	6.3	0.5	71.7	18	186
44	6.3	0.4	71.9	19	187
48	6.2	0.4	71.6	19	184
52	6.2	0.3	71.8	18	186

Table 12i. Properties of Developing Grain Starch

KURO MOCHI

Days from Anthesis	Average granule (μm) diameter	Amylose Content %	Gelatinization temperature °C	Amylopectin average chain length (anhydroglucose units)	Limiting Viscosity number Amylopectin ml/g
4	3.2	7.1	66.2	23	134
8	3.4	5.2	66.4	23	134
12	3.6	3.3	65.8	23	135
16	3.8	2.1	65.3	23	133
20	4.3	0.7	64.7	22	135
24	4.4	0.6	64.6	23	130
28	4.8	0.6	64.4	22	132
32	5.1	0.5	64.5	23	131
36	5.5	0.6	64.5	22	126
40	5.7	0.6	64.7	23	124
44	5.7	0.7	64.6	22	117
48	5.8	0.4	64.6	23	119
52	5.7	0.6	64.5	22	118

The molecular size of isolated amyloses and amylopectins were estimated by viscometry in potassium hydroxyde solution (Greenwood 1964). Banks and Greenwood (1967) found that pure amylose binds 19.5± 0.2mg of iodine per 100mg of polysaccharide.

The limiting viscosity number $\{\eta\}$ of essentially unbranched molecules such as amylose has been shown to be related to the molecular weight (M) by the general equation $\{\eta\} = KM^{\alpha}$, where K and α are empirical constants for a given polymer solvent system. The viscosity method is not an absolute method but requires calibration by independent molecular-weight measurements. K and α are obtained by separating the polymer into fractions of narrow molecular weight distribution and determining the molecular weight of each fraction. The constants can then be determined from a graph of log (M) against log ($\{\eta\}$). Results for starch components by various workers are inconsistent. However, viscosity measurements by themselves provide a convenient method for determining relative molecular size (Greenwood 1964). The relation, number average degree of polymerisation = 7.4 (limiting viscosity number) for amylose in molar potassium hydroxide is commonly used (Cowie and Greenwood 1957a). For a highly branched molecule like amylopectin, limiting viscosity number and degree of polymerisation are not simply related. The limiting viscosity numbers of the amyloses isolated from developing grain increased throughout grain development (Table 12). This is in contrast to the results of Briones and co-workers (1968) who found that the limiting viscosity

of both amylose and amylopectin remained constant throughout grain development. The amyloses isolated by these workers were of lower purity based on their iodine binding capacities and this may explain the difference in results. The limiting viscosity number of rice amylopectins decreased for all varieties during maturity and this may evidence some change in amylopectins during ripening. The limiting viscosity values obtained for rice amylopectins are high as compared to values quoted for other starches (Greenwood and Thomson 1962). However, they are similar to the values obtained for rice amylopectin by Briones and co-workers (1968). The amylopectin chain lengths for all varieties investigated were lower than those reported by Briones and co-workers (1968).

The results show that the grains of rice varieties known to differ in endosperm starch properties develop in a very uniform way when grown together in southern New South Wales. The major changes in starch properties were that amylose content increased throughout grain development and gelatinization temperature decreased. High temperatures are known to increase the rate of grain ripening (Matsubayashi 1967). Variations observed in amylose content within a variety, between seasons, may in part be due to different ripening rates limiting the accumulation of amylose.

4. Mature Grain Starch

At the completion of the grain development study the trials were header harvested using a small plot header, the grain cleansed

through a Simon/Carter dockage tester, hulled and milled to white rice using Satake laboratory rice mills. The yield and protein content of the plots is presented in Table 13. The local short grain varieties gave best yield followed by local long grain and then introduced varieties. All varieties had a similar brown rice protein content.

Starches were isolated and fractioned from the milled grains. Mature starches were all polyhedral in shape (e.g. plate 5). The properties of the mature endosperm starches and starch fractions are presented in Table 14. Milled rice was also ground to flour and the paste viscosities of the flours and starches determined using a Brabender Viscograph. Viscogrammes for flour and starch were very similar. Flour viscogrammes for each of the varieties are presented in figures 8 to 16. The viscograph data is summarised in Table 15a. Milled grain was cooked under standard conditions (Blakeney 1979) and cooked grain texture measured in a Kramer cell on an Instron Model 1140 Universal testing machine. The results are presented in Table 15b. The properties of mature grain starch from this set of varieties grown in southern New South Wales are similar for those reported for tropical rices (Reyes *et al* 1965; Juliano 1972). The local commercial long grained varieties, Kulu and Inga, are both soft and slightly sticky cooking. They have amylose contents of about 22% and cooked rice textures of about 100 units. The three higher amylose varieties YR57, Ali Combo and Madinika all had

Table 13.

Grain Yield and Brown Rice Protein Content

Grain Starch Trial 1972/73

	Grain Yield (Paddy Kg/ha)	Brown Rice Protein Content[a] (%)
Caloro	8740	7.8
Calrose	9860	8.2
Kulu	8650	8.3
Inga	8280	8.4
YR57	7630	8.2
Ali Combo	4280	8.6
Madinika	6490	9.3
Tarra 140	8340	8.5
Kuro Mochi	7650	8.7

[a] N X 5.95 (Juliano 1972) 13% moisture basis

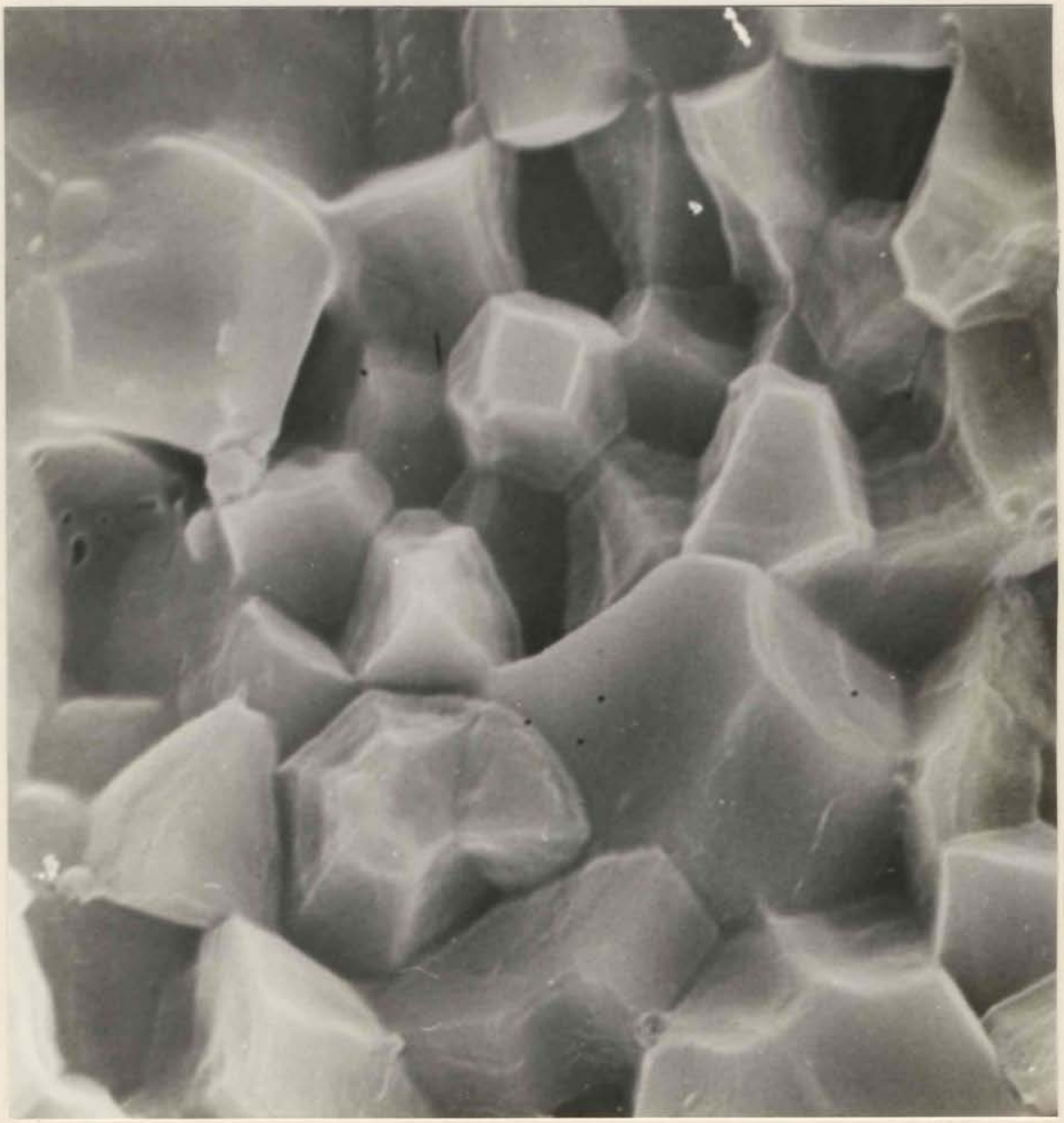


Plate 5. Scanning electron micrograph of individual granula within an amyloplast of Kulu endosperm (X 8000)

Table 14.

Properties of Starch and Starch Fractions isolated from Mature Milled Grain

	Amylose Content %	Gel. temp. [a] °C	β amylolysis limit [b] A	Ap	Chain length [c] Per.	Pull.	Limiting Viscosity Number [d] A	Ap
Caloro	19.5	62.5	88	52	24	19	153	172
Calrose	19.2	66.4	84	49	21	20	176	125
Kulu	21.8	73.2	83	54	23	21	170	169
Inga	22.3	71.7	84	53	21	20	210	162
YR57	25.2	73.2	81	53	24	22	204	164
Ali Combo	28.4	68.4	89	57	18	17	186	154
Madinika	26.7	73.4	84	54	22	22	186	159
Tarra 140	0.4	71.2	-	49	18	19	-	187
Kuro Mochi	0.6	64.3	-	52	23	22	-	120
LSD (5%)	0.3	0.2	-	-	2.1	1.4	5.2	9.6

[a] Gelatinization temperature

[b] β amyloiysis limit (% maltose) A = Amylose; Ap = Amylopectin

[c] Amylopectin average chain length (anhydroglucose units)
Per. Periodiate oxidation method; Pull. Pullulanase method

[d] Limiting Viscosity number ml/g A = Amylose; Ap = Amylopectin

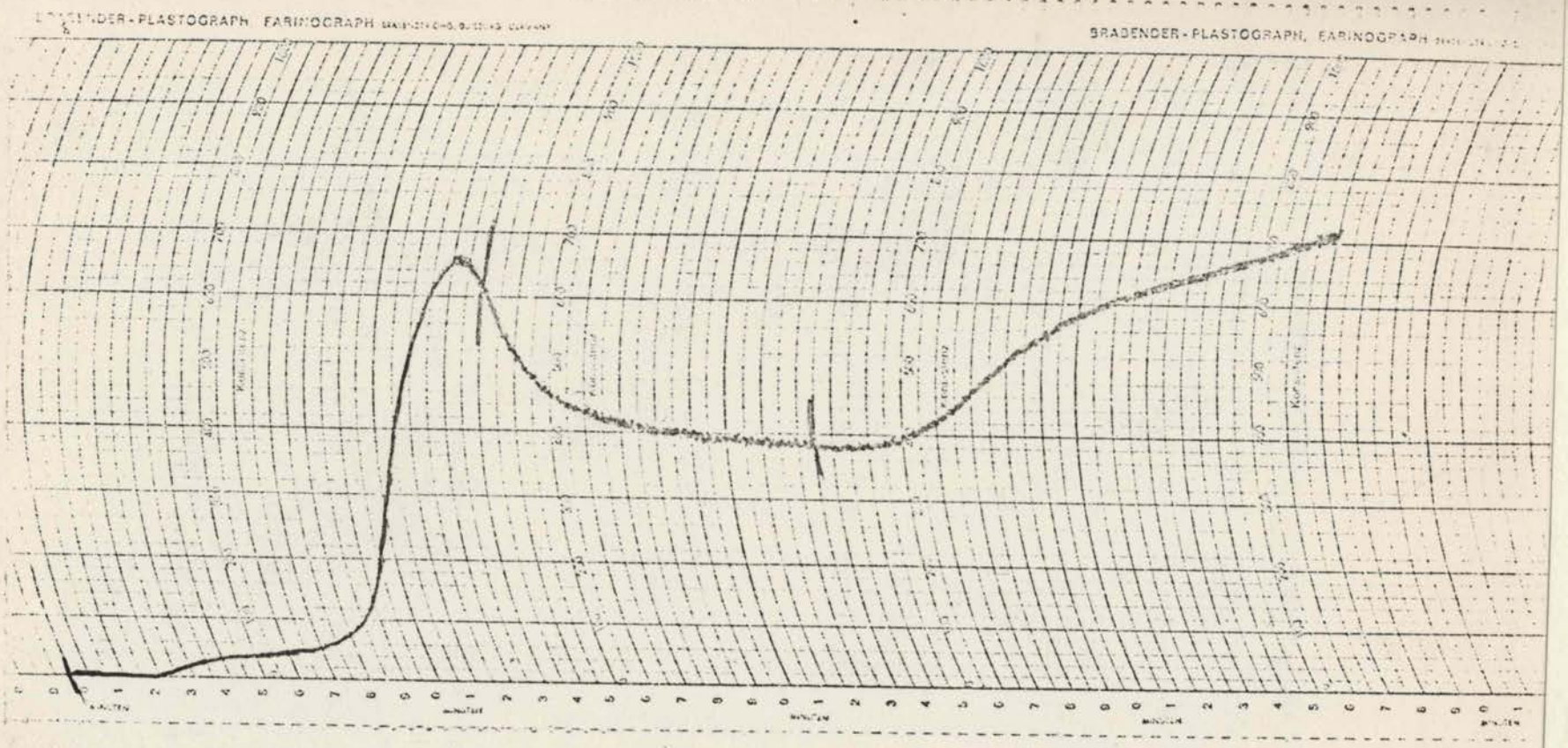


Figure 8.

Brabender viscogramme of Caloro rice flour.

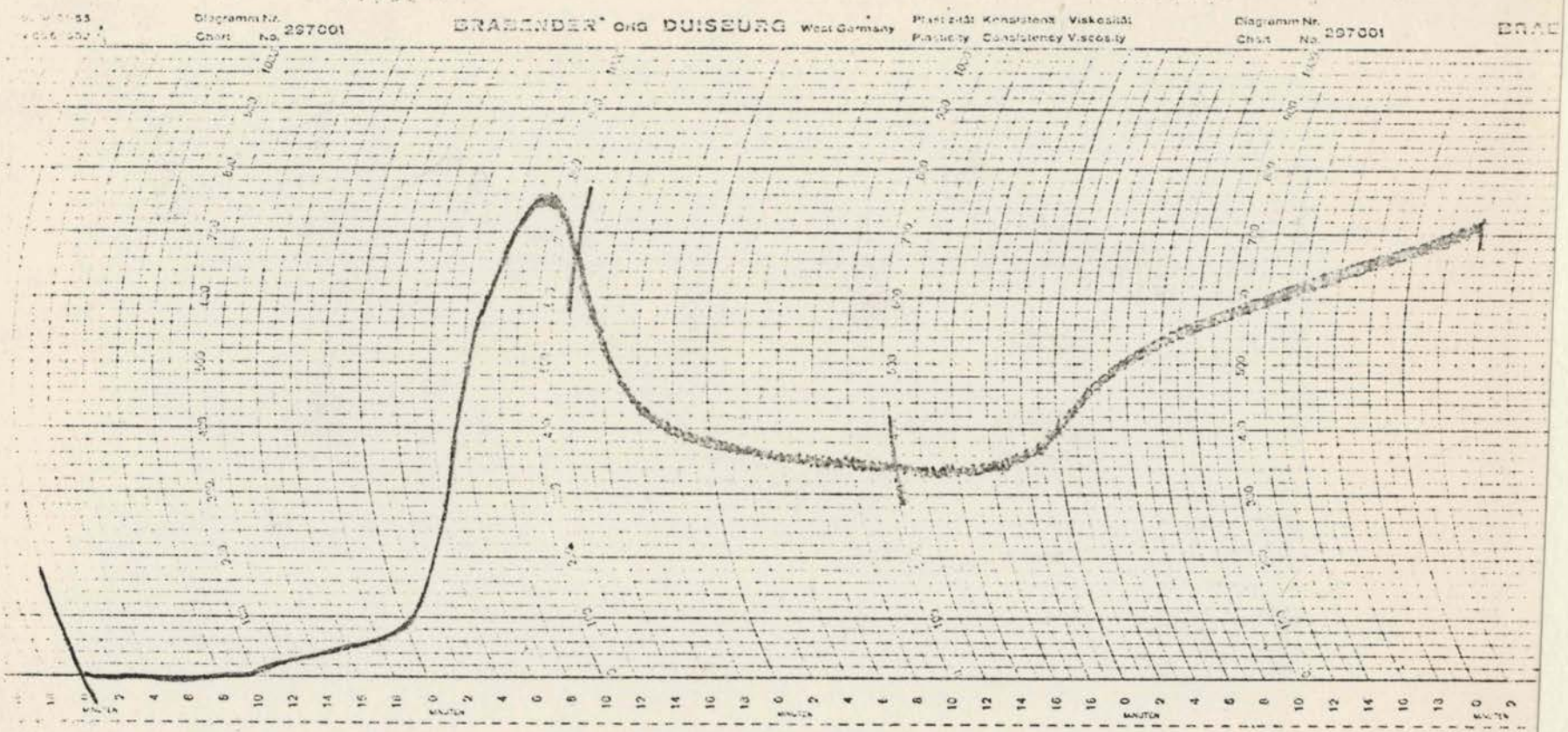


Figure 9.

Brabender viscogramme of Calrose rice flour.

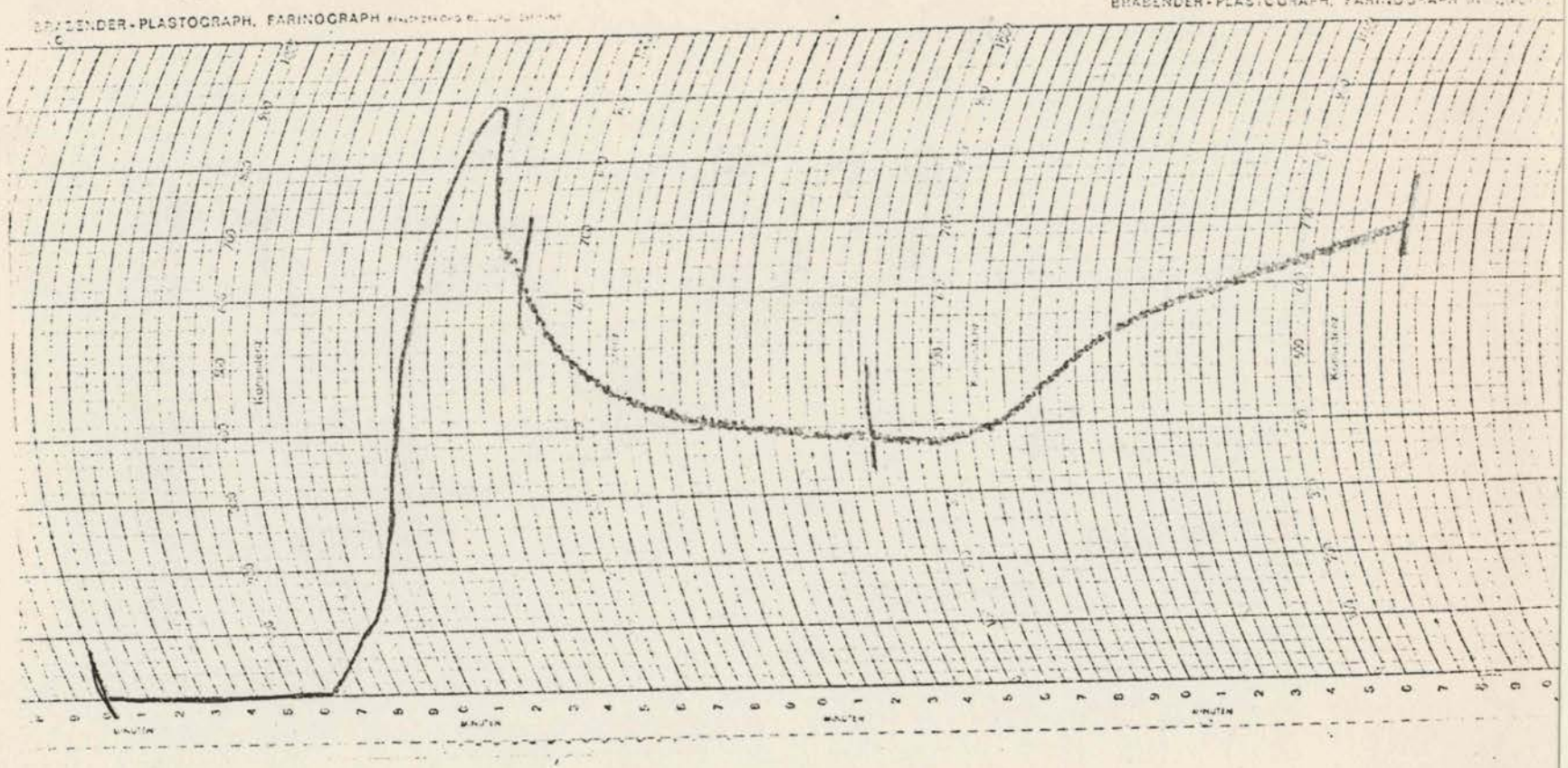


Figure 10.

Brabender viscogramme of Kulụ rice flour.

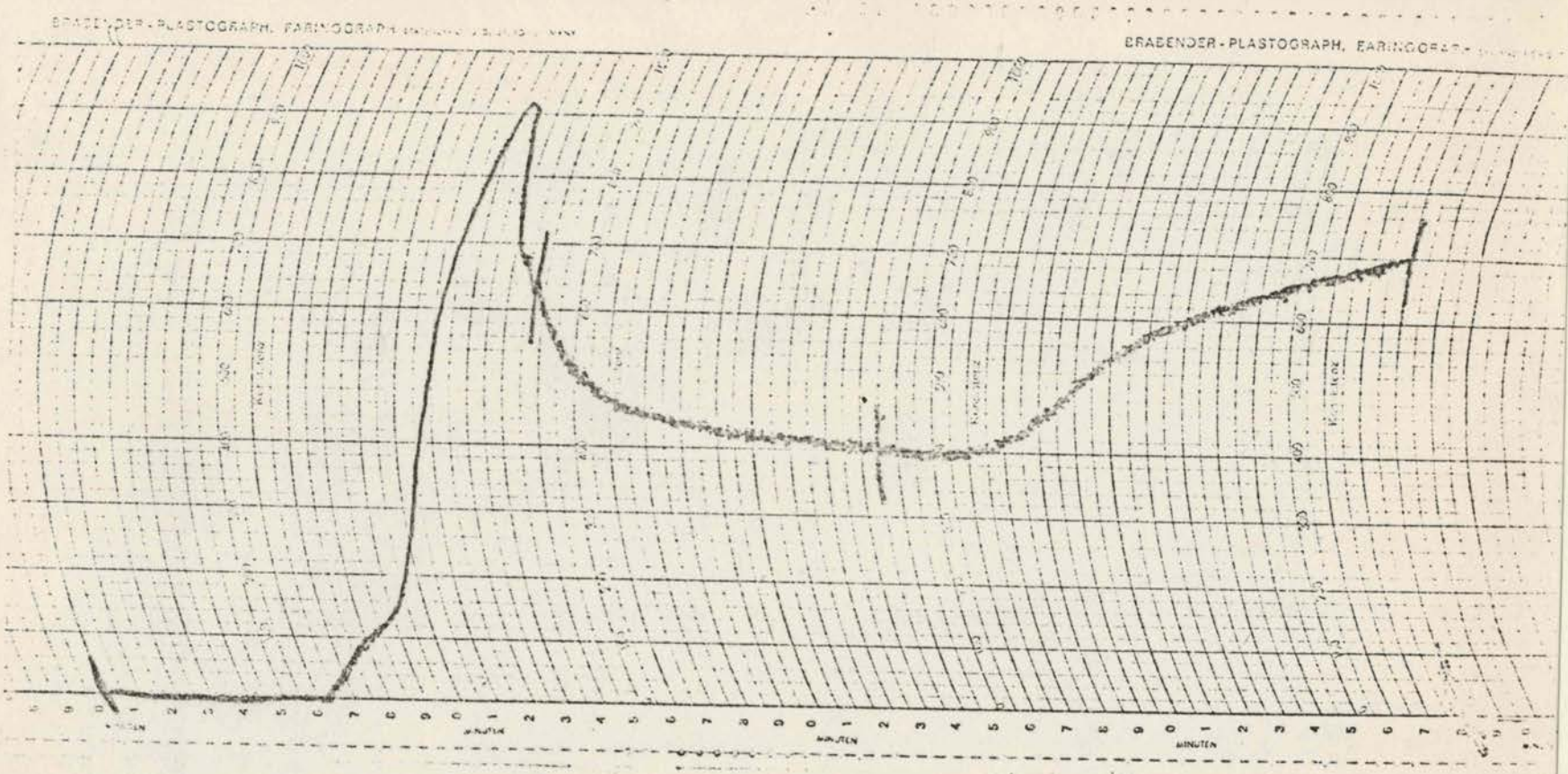


Figure 11.

Brabender viscogramme of Inga rice flour.

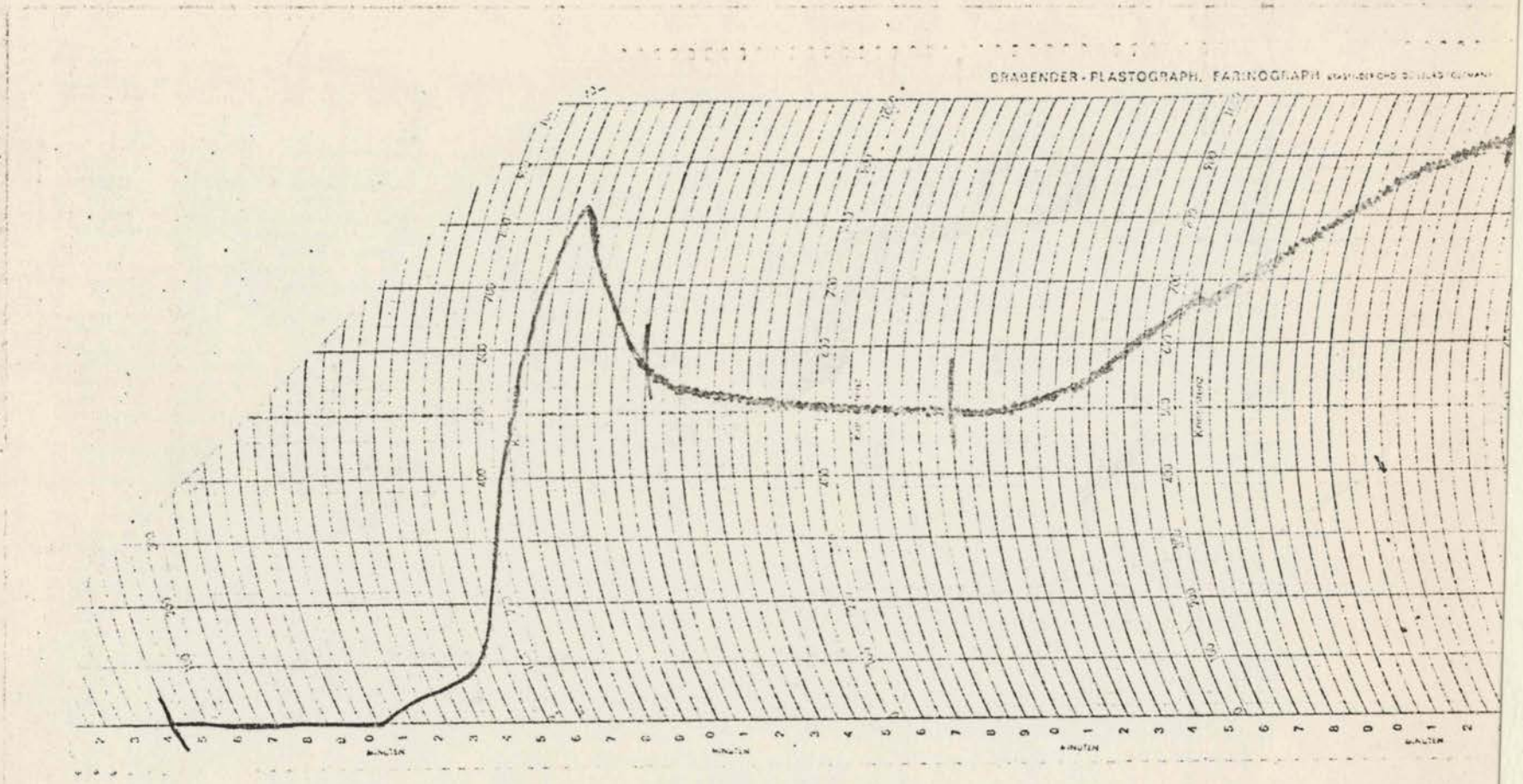


Figure 12.

Brabender viscogramme of YR57 rice flour.

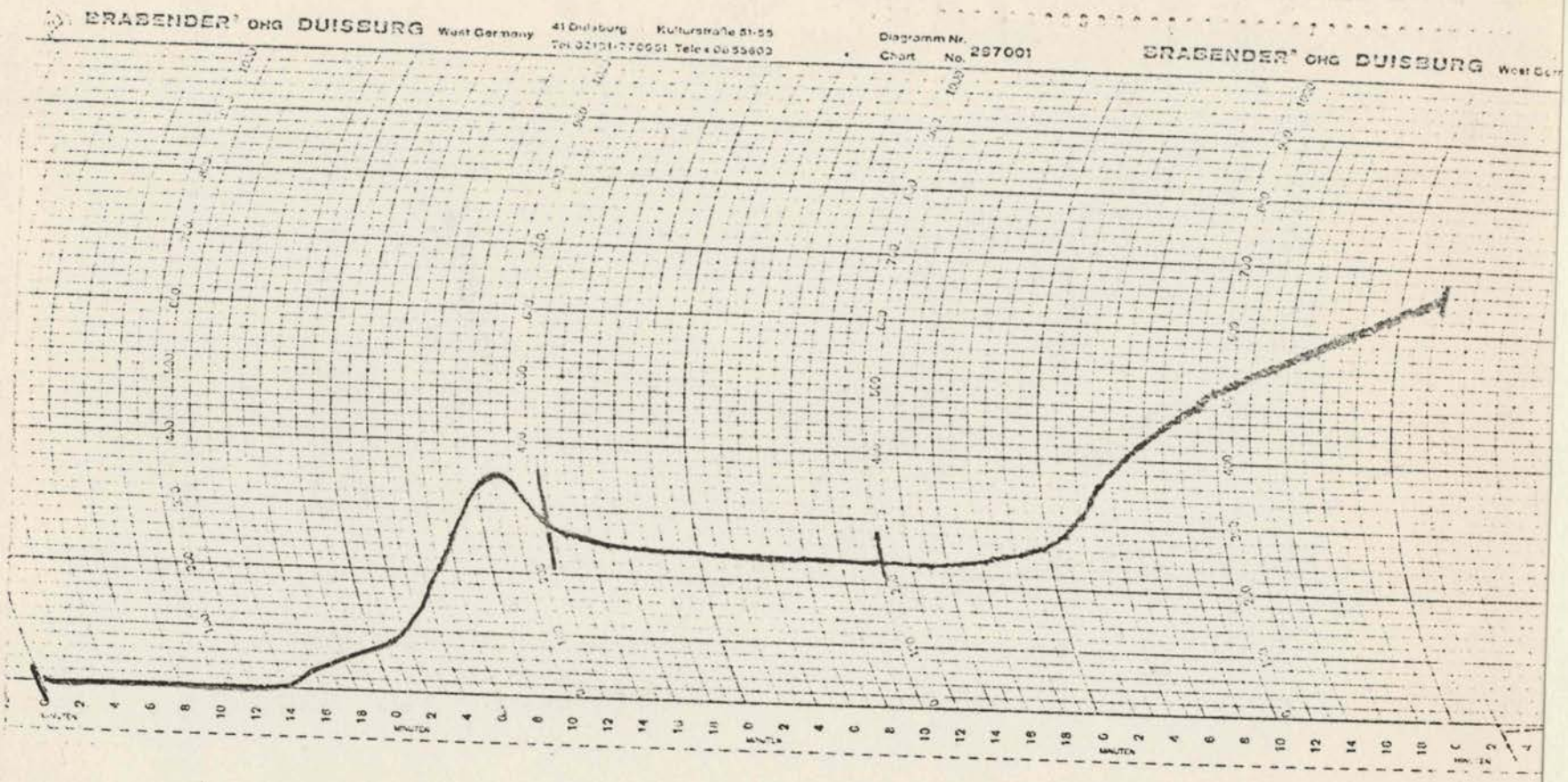


Figure 13.

Brabender viscogramme of Ali Combo rice flour.

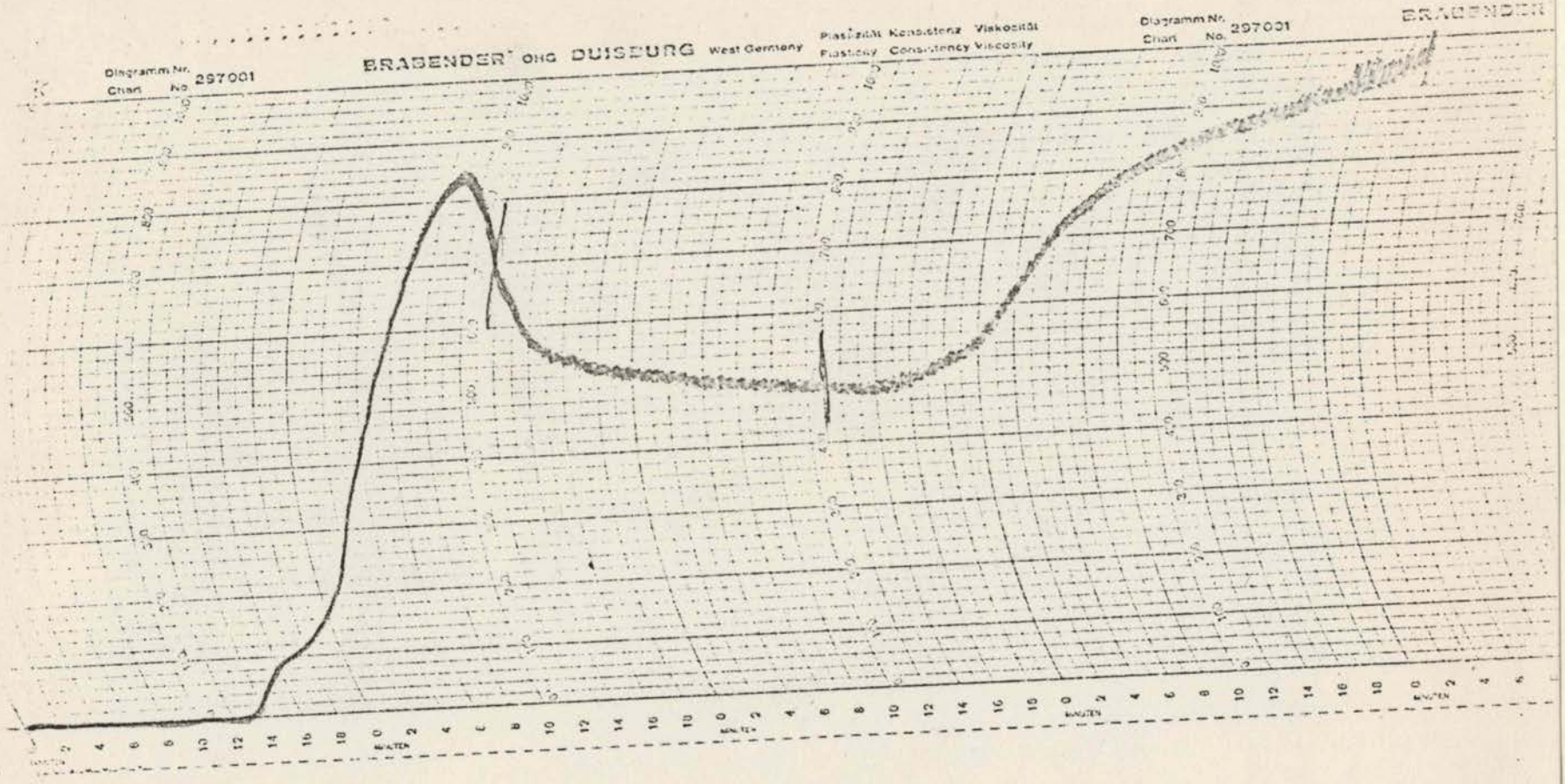


Figure 14.

Brabender viscogramme of Madinika rice flour.

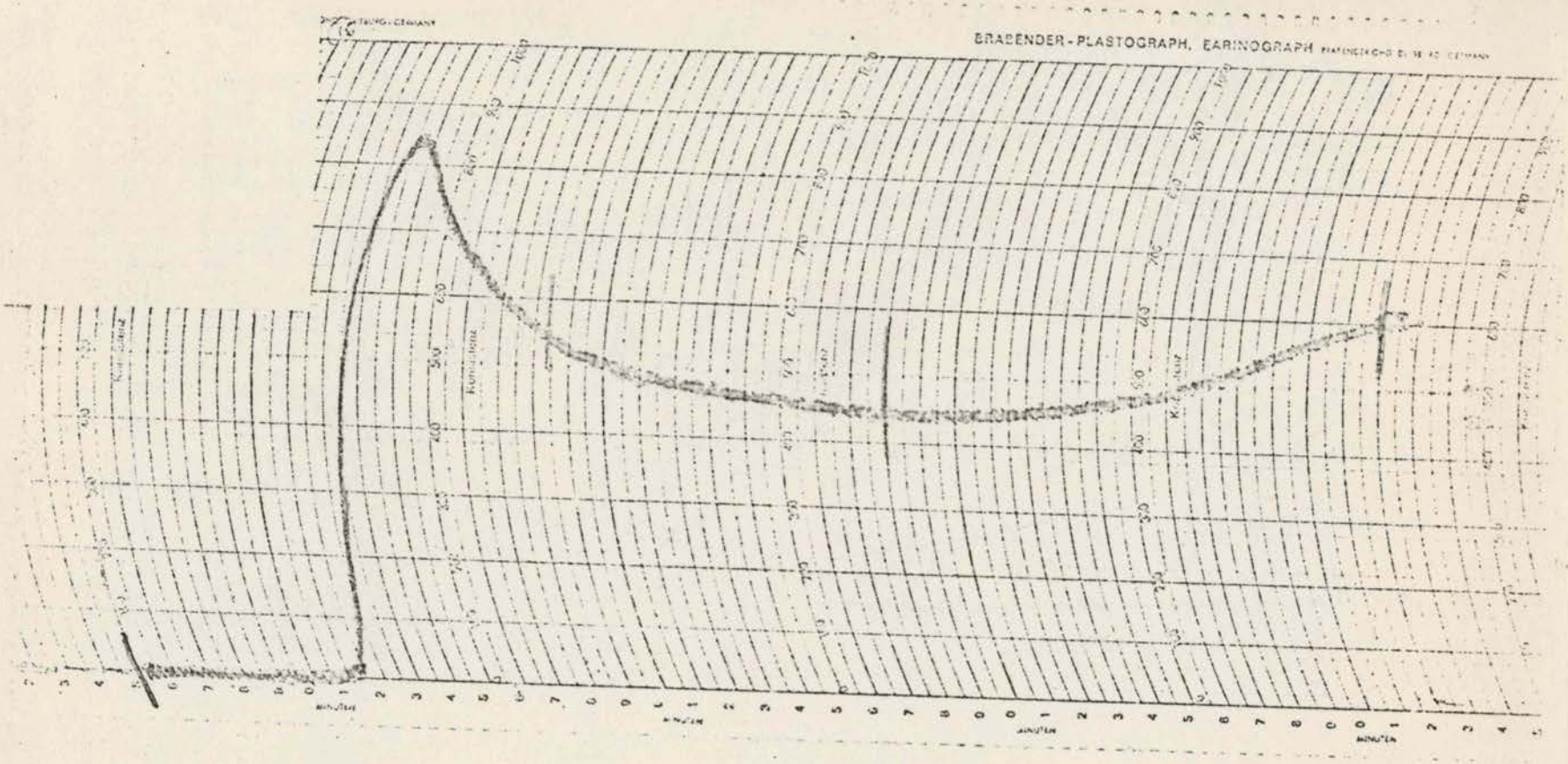


Figure 15.

Brabender viscogramme of Tarra 140 rice flour.

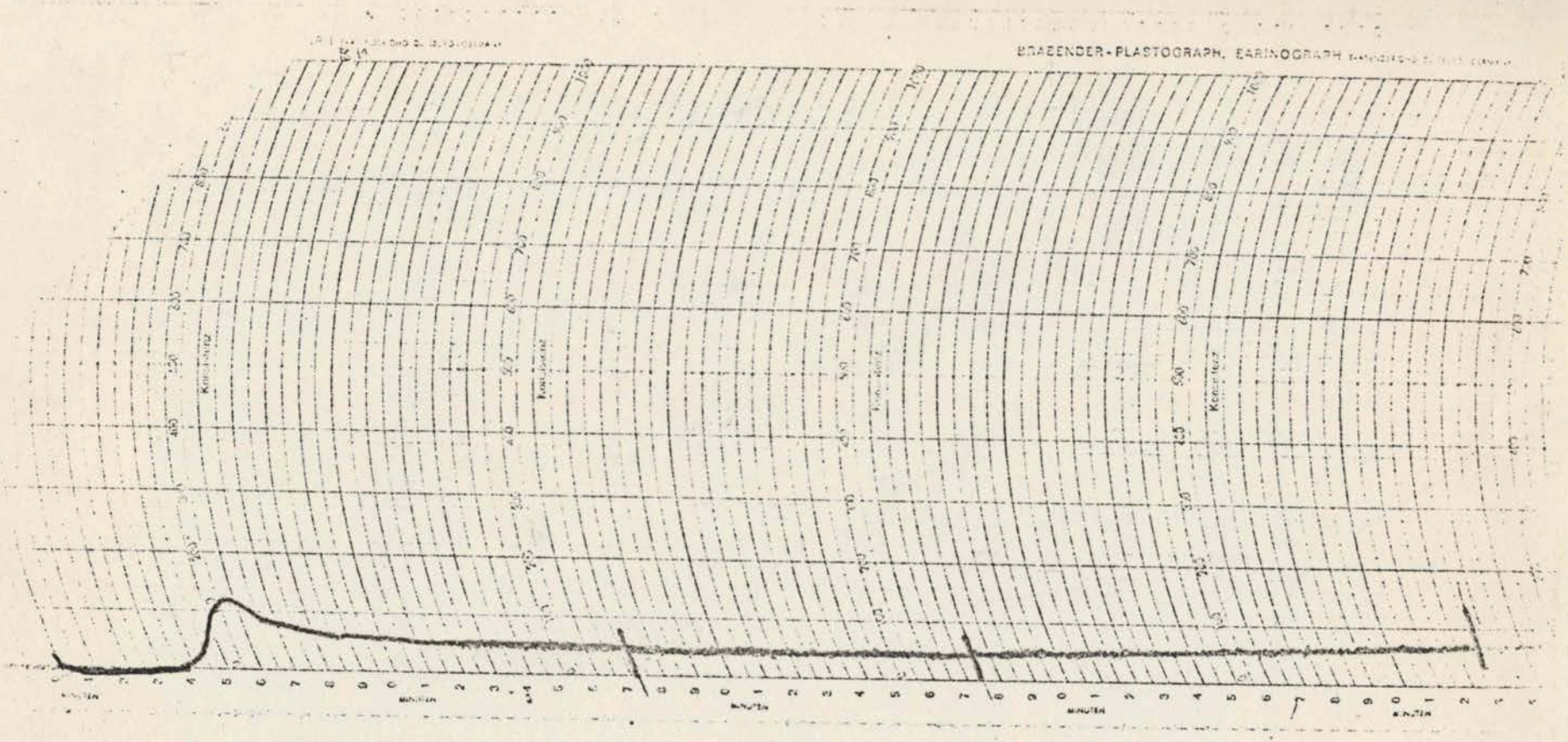


Figure 16.

Brabender viscogramme of Kuro Mochi rice flour.

Table 15a.

Rice Grain Flour and Starch Brabender Viscograph Data

	Gelatinisation temperature °C		Viscograph paste viscosity (B.U.)[a]					
			Peak		After 20 minutes at 95°C		Set Back[b]	
	Flour	Starch	Flour	Starch	Flour	Starch	Flour	Starch
Caloro	62	62	680	550	390	390	+40	+30
Calrose	67	65	750	710	340	360	-40	-10
Kulu	73	71	880	800	380	390	-200	-160
Inga	72	71	910	820	400	430	-210	-180
YR57	74	74	820	730	500	510	+100	+120
Ali Combo	68	66	350	320	240	260	+310	+340
Madinika	71	72	840	770	490	460	+120	+150
Tarra 140	71	71	840	760	440	420	-240	-200
Kuro Mochi	64	63	120	100	70	60	-60	-40

[a] B.U. Brabender Units. Flour samples 50g/450ml, Starch samples 40g/460ml

[b] Set back is calculated by subtracting the peak viscosity from the viscosity after cooling to 50°C.

Table 15b.

Cooked Rice Grain Texture

	Peak Resistance (compression) Kg.
Caloro	86
Calrose	101
Kulu	96
Inga	104
YR57	121
Ali Combo	110
Madinika	132
Tarra 140	83
Kuro Mochi	59
LSD (5%)	2.4

significantly firmer textures (Table 15b) and were dry cooking. YR57 and Madinika have undesirably firm cooked rice. However, Ali Combo is dry cooking but still relatively soft textured. This type of rice is considered preferable on the basis of subjective taste tests. Methods for the sensory assessment of cooked, milled rice have recently been reviewed by Del Mondo (1979). The chemical properties of starch determined were not apparently related to this character and the direct measurement of cooked rice appears the best way of selecting dry cooking, soft textured rices. The Brabender viscograph also identified Ali Combo as different from the other higher amylose varieties (Table 15a; Fig. 12, 13, 14). All these varieties have a positive set back. However, the paste viscosity of Ali Combo was lower throughout the cooking cycle. The amylopectin limiting viscosity numbers of the two waxy varieties are reflected in their Brabender viscogrammes (Fig. 15, 16; Table 14). Tarra 140 has a high limiting viscosity number and a high viscograph viscosity. Kuro Mochi has a lower limiting viscosity number and a very low viscograph viscosity.

5. Chalky Grain

(a) Grain morphology

The milling quality of rice is based primarily on the yield of whole grain obtained. Grain breakage during processing reduces yields which vary with variety and environment. Two major causes of breakage during milling are suncracking and chalky cored

grain. Chalky core is the white opaque region that occurs in otherwise clear translucent grain (plate 6). It is doubly undesirable because, as well as contributing to grain breakage, it also detracts from the visual appearance of milled rice.

To investigate grain breakage and possible causes of chalky core, a series of rice grain samples representing varieties that suncracked easily and others that were resistant, as well as chalky and translucent varieties, were examined by both light and scanning electron microscopy. These grain samples came from the variety collection and included all the varieties used in other parts of this study. The variety Kulu is particularly susceptible to chalky core and has been used in the micrographs to illustrate the problem.

The cracked surface of grains that had naturally cracked in half were examined. It was observed that the majority of the cracks run along the lines of the endosperm cell walls (plate 7). Differences in the wall patterns were observed between varieties. In some, the walls of adjacent cells ran in radial lines while in others the pattern was more random. The structures these patterns reflect could explain the inherent differences that exist in the suncracking potential of varieties; a random structure would offer more strength than a radial one. Where the cracks passed through chalky areas of the endosperm, the cracks no longer occurred along the lines of the cell walls but cracked through them. This is typical of the behaviour of soft endosperm and is a well known

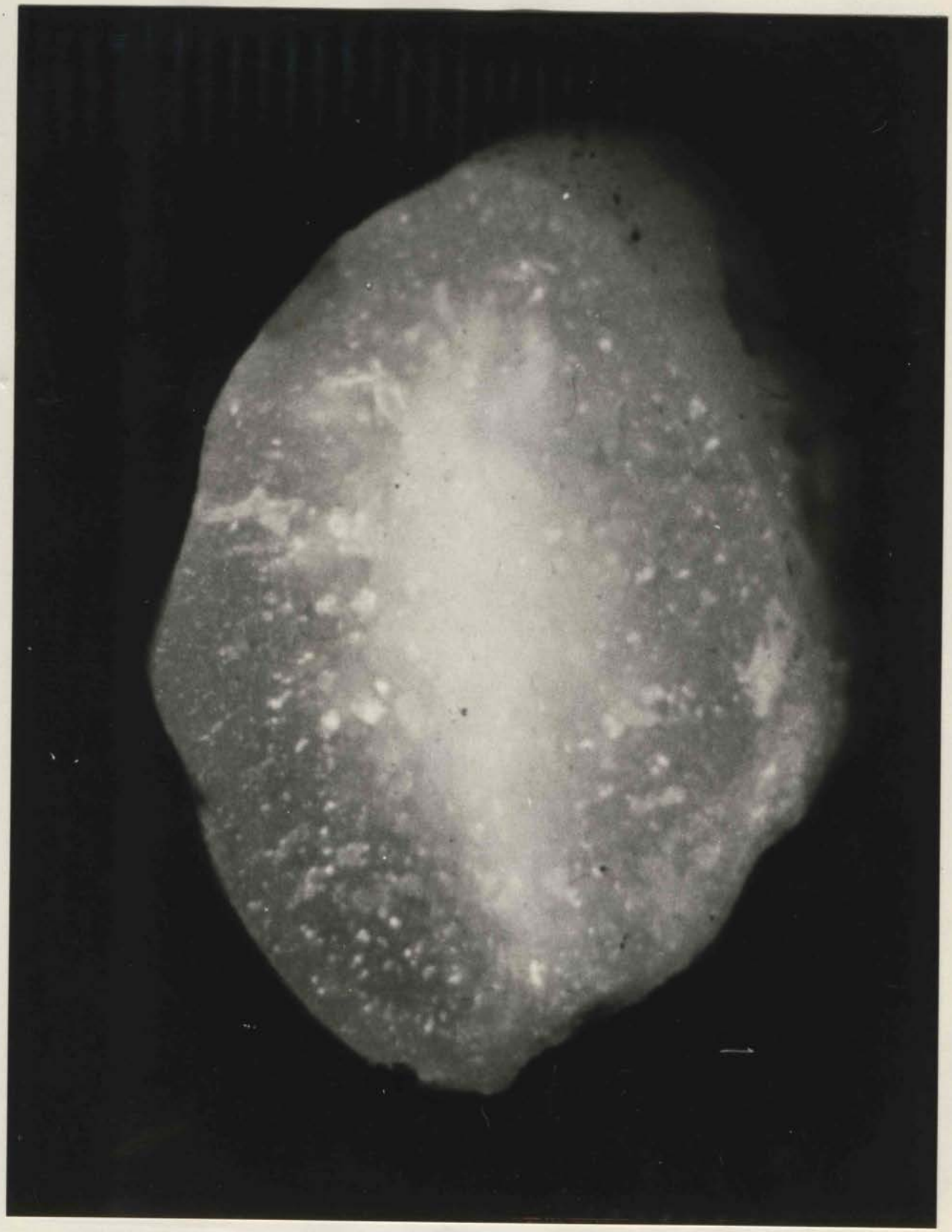


Plate 6. Light micrograph of a broken milled grain of Kulu rice illustrating chalky core (X 110)

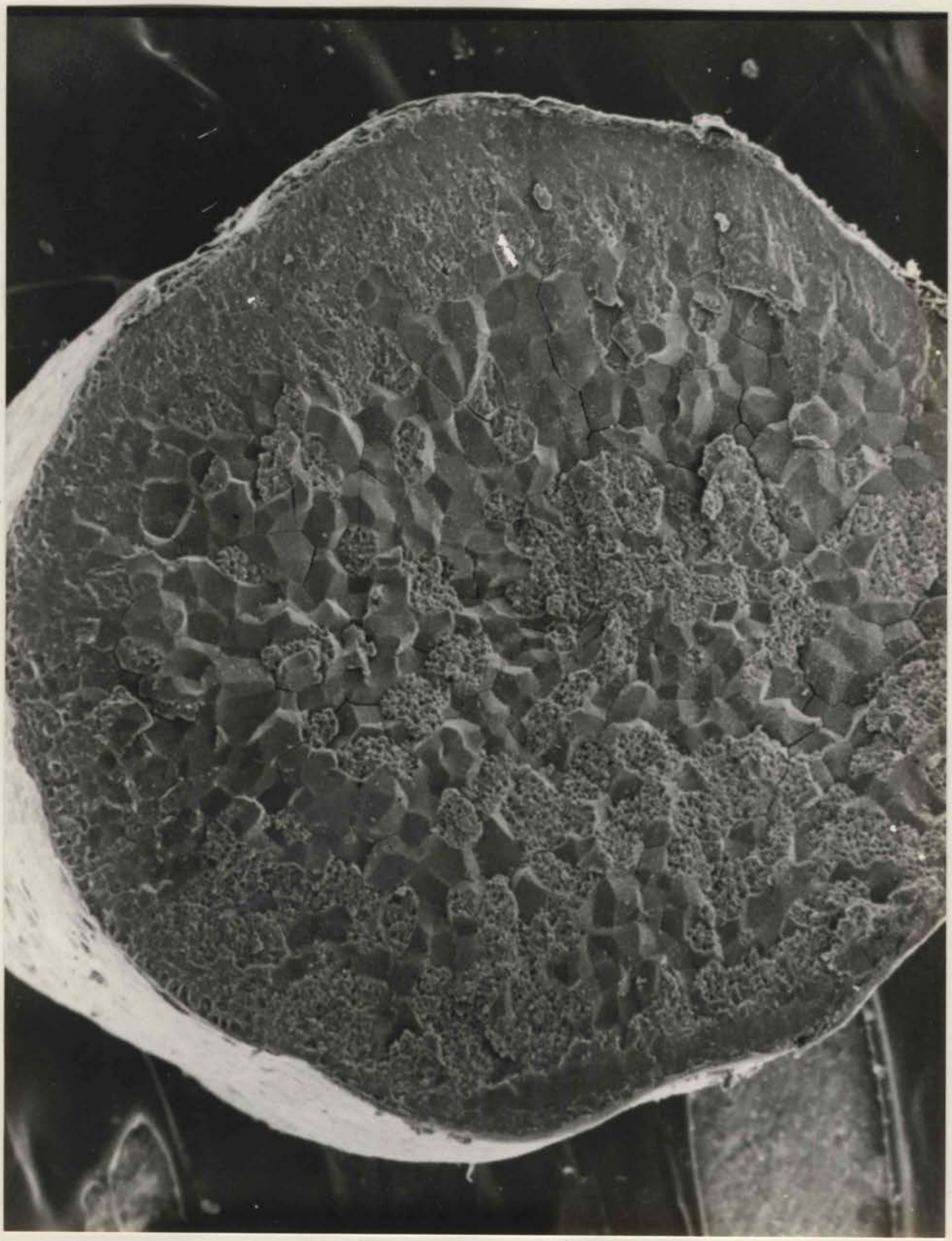


Plate 7. Scanning Electron Micrograph of the naturally suncracked surface of a chalky cored grain of Kulu rice (X 135)

phenomenon in soft non-vitreous wheats. In the chalky area of rice endosperm the cell contents constitute zones of weakness which readily break under impact. In normal hard translucent endosperm the cell contents are hard and the cell wall - cell contents interface is the area of weakness. Light micrographs of rice endosperm stained for starch revealed a lower starch packing density in chalky areas. These results confirm similar observations made by IRRI researchers some years ago (del Rosario *et al* 1968). Staining endosperm for protein (Flint and Moss 1970) revealed that the starch grains of the rice endosperm are embedded in a near continuous protein matrix, except in chalky areas where small voids between the granules are noticeable.

The difference between translucent endosperm and chalky endosperm is striking. Plates 8 and 9 are scanning electron micrographs taken at the same magnification in the translucent and chalky regions of a grain of Kulu rice. The translucent endosperm in plate 8 contains few recognisable structures. All the tissues have collapsed during grain desiccation and are compressed together so tightly that the amyloplasts are no longer individually identifiable. Bechtel and Pomeranz (1978) recently commented on the poorly preserved central endosperm area of translucent rice grains. In contrast, chalky endosperm is well preserved. In plate 9, the amyloplasts, their constituent starch granules and the air spaces between amyloplasts are easily identified.

Plate 8. Scanning electron micrograph of an area of translucent endosperm in a chalky cored grain of Kulu rice (X 4300)

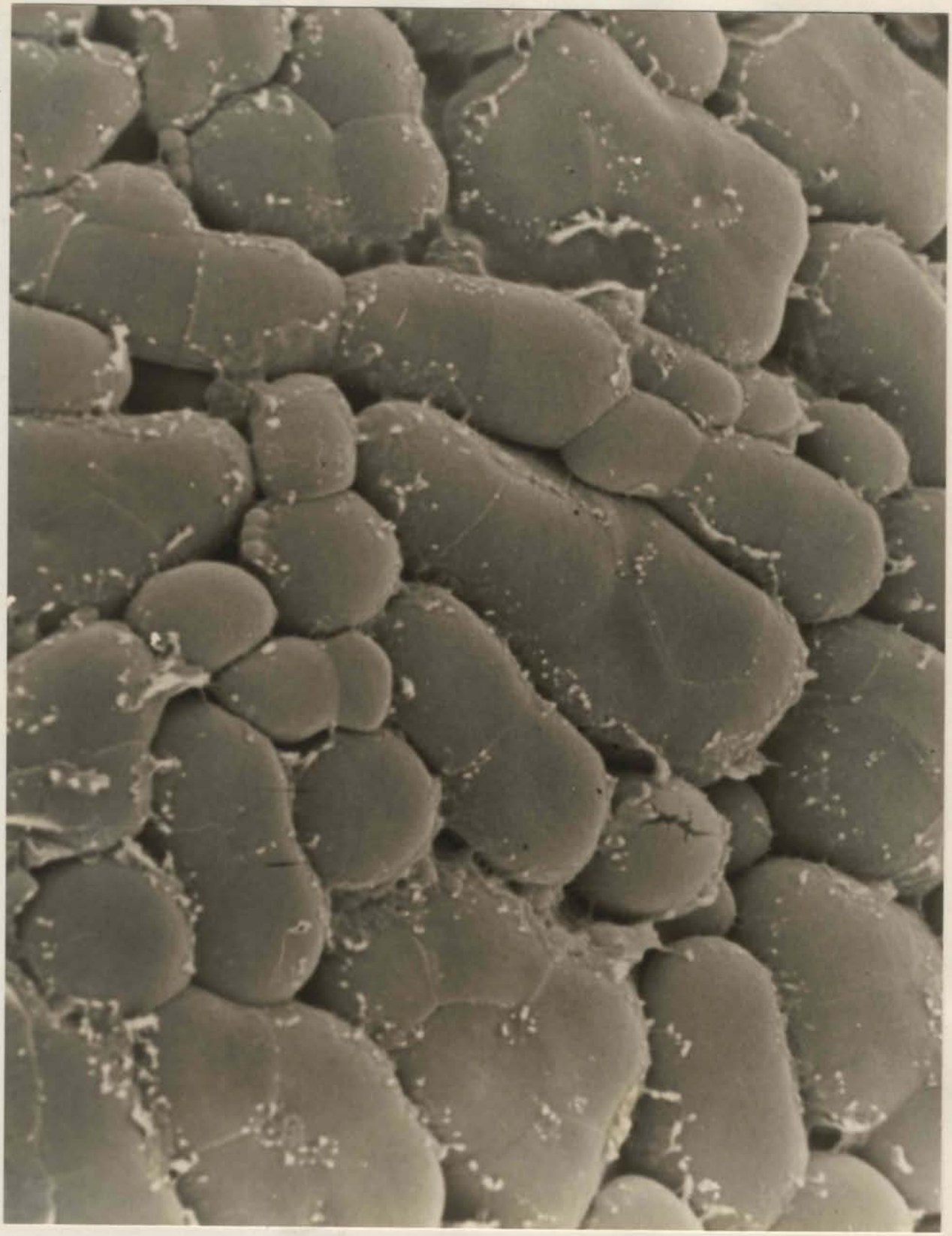


Plate 9, Scanning electron micrograph of an area of chalky endosperm in a chalky cored grain of Kulu rice (X 4300)

Ando and Ichikawa (1974) have commented upon the random, irregular packing of chalky endosperm cells. Using electron microscopy they found that single starch granules were massed loosely together so that large irregular openings could be seen. They attribute the opaque appearance of chalky endosperm to the inclusion of minute air spaces. These air spaces probably also contribute to the softness of chalky endosperm as they reduce the area available for starch-protein adhesion.

Using the scanning electron microscope, the edges of chalky regions were followed on samples from several varieties. All samples showed an abrupt change from chalky to translucent tissue. Often one endosperm cell would be chalky and the adjacent cell translucent (plate 10). Counting and measuring of chalky endosperm cell contents showed they usually contained fewer but larger amyloplasts.

Endosperms of grasses are characterized as being of the nuclear type: that is, the early mitoses occurring in the developing endosperm are not accompanied by cytokinesis. Endosperm mitosis begins with the single, large, uninucleate, membrane-limited cell, the central cell or primary endosperm cell. Divisions of the central cell nucleus are initiated, resulting in a multinucleate cell consisting of a peripheral zone of cytoplasm surrounding a large central vacuole. Subsequently cytokinesis is initiated in the multinuclear endosperm and the cellular endosperm typical of the maturing grain is formed (Mares *et al* 1975). If rice endosperm

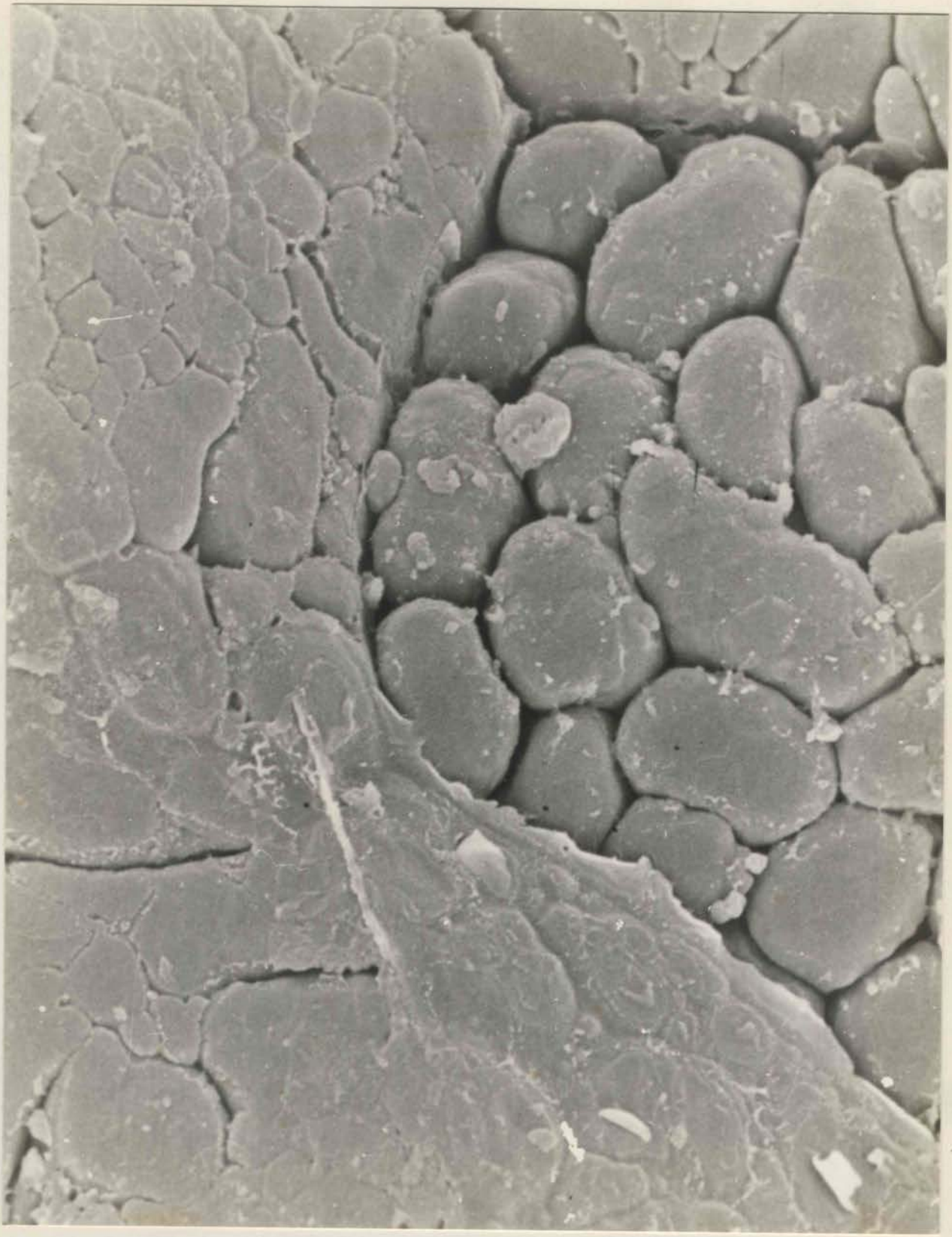


Plate 10. A section of a Kulu grain showing where chalky core starts. The chalky cell on the right contains loosely packed amyloplasts and numerous air spaces (X 2400)

develops in this manner as the published photomicrographs of Ueda and Ota (1958) suggest, the sudden change from chalky to normal translucent cells may be due to an environmental or nutritional influence on amyloplast formation in the original multinucleate cell.

(b) Starch Structure

Starches were isolated from the chalky and translucent areas of chalky samples of three varieties of Australian rice. Rice samples were obtained from growers crop appraisal samples kept by the Rice Marketing Board for the state of New South Wales and were from the 1975/76 rice season. Samples were selected that were high in chalky core and after test milling, were hand sorted into chalky and translucent grains.

Translucent grains were processed directly into starch. Chalky grains were cut in half longitudinally and hand dissected. A clamp with rubber jaws was carved from a rubber eraser as described by Cobb (1904). Chalky grains were placed in the rubber clamp and the clamp held shut in a small vice. With the aid of a binocular microscope, portions of chalky and translucent endosperm tissue were dissected out using flattened needle probes. A large number of grains were sectioned and only a small amount of each type of endosperm taken from each to minimise contamination. The tissue portions of each type of endosperm from each variety were bulked and processed into starch.

The properties of the starches and starch fractions isolated from translucent and chalky endosperm are presented in Tables 16 and 17. The starches from all varieties are very similar. Chalky starches appear to contain a little less amylose and have a slightly lower gelatinization temperature. The isolated amyloses were all of reasonable purity based on their iodine binding capacities. Chalky amyloses had higher limiting viscosity numbers.

These results show that the chalky core problem is principally a physiological one. Chalky core is caused by the central endosperm cells failing to initiate enough amyloplasts and the tissue developing to be loosely packed and containing air spaces. The starches present in the two types of endosperm differ only slightly in their properties.

Table 16. Properties of Starches Isolated from translucent and Chalky Endosperm

			Amylose Content [a] (% whole starch)	β amylolysis limit (as % maltose of whole starch)	Amylopectin average chain length [b] (anhydroglucose units)	Gelatinization temperature °C
1	Kulu	translucent grain	20.7	55	23	72.6
2	Kulu	translucent endosperm from chalky grain	19.8	54	24	71.4
3	Kulu	chalky endosperm	19.6	55	23	69.2
4	Inga	translucent grain	21.6	54	23	71.5
5	Inga	translucent endosperm from chalky grain	22.4	53	22	71.8
6	Inga	chalky endosperm	21.3	54	22	71.2
7	Calrose	translucent grain	19.5	55	24	66.3
8	Calrose	translucent endosperm from chalky grain	19.8	54	25	66.8
9	Calrose	chalky grain	19.4	54	25	64.9

[a] Calculated from iodine binding capacity assuming that pure rice amylose binds 19.5g iodine per 100g.

[b] Assuming that the contribution of amylose to formic acid production is negligible.

Table 17.

Properties of Starch Fractions isolated from Translucent and Chalky Endosperm Starches

			Iodine binding capacity amylose	Limiting Viscosity number amylose [a]	Limiting Viscosity number amylopectin [a]	β amylolysis limit of amylose (as a % maltose)
1	Kulu	translucent grain	18.66	187	148	86
2	Kulu	translucent endosperm from chalky grain	18.90	186	152	85
3	Kulu	chalky endosperm	18.93	193	146	87
4	Inga	translucent grain	19.0	184	143	89
5	Inga	translucent endosperm from chalky grain	18.72	192	147	90
6	Inga	chalky endosperm	19.10	194	145	89
7	Calrose	translucent grain	18.82	183	152	85
8	Calrose	translucent endosperm from chalky grain	18.93	182	153	87
9	Calrose	chalky grain	18.77	188	149	88

[a] In 0.5M potassium hydroxide; ml/g

CONCLUSION

Starch is the reserve carbohydrate at all stages of rice growth. Unlike other cereals rice accumulates large amounts of starch in its stems. The properties of stem starch are unrelated to endosperm starch and would be of no use in predicting endosperm starch properties in a rice breeding programme. The contribution that translocated stem starch makes to yield needs further study.

Pollen starch properties are related to endosperm starch properties and may be of use in a breeding programme. The starch iodine/iodide staining test has already been used as a check on homogeneity in waxy rice pure seed schemes.

As rice grains ripen, their starch increases in amylose content and decreases in gelatinisation temperature. Environmental conditions, particularly temperature, influence ripening rate. The relationships between ripening rate, starch amylose content and gelatinisation temperature require further study. This would establish if the variability observed in amylose content and gelatinisation temperature within a variety, between seasons, can be attributed to the rate of ripening.

Current New South Wales long grained rice varieties are, when cooked, sticky and soft textured. The breeding programme at Yanco Agricultural Research Centre aims at producing dryer cooking (non sticky) varieties that are still relatively soft textured.

Hard textured varieties become unpalatable as they cool. A rice with a starch amylose content of about 27% and which is soft when texture tested, would be ideal. None of the starch properties investigated in this study could account for cooked grain texture; direct texture testing and past viscosity testing using the Brabender viscograph are still necessary. The chalky core character is due to small air spaces that are present in chalky tissue but absent from translucent tissue. Starches isolated from chalky and translucent parts of chalky cored rice grains were similar; chalky core appears to be a physiological problem. The chalky core problem is best overcome by breeding translucent varieties. These can be selected by absence of chalky core when they are grown in a range of environments; possibly in a controlled environment glasshouse.

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