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# Variation in Size Distribution of Starch Granules from Wheat Grain

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Starches, of wheat grains of diverse genetic origin, grown in New Zealand, were examined for granule size distribution using a Coulter Counter. A common bimodal distribution of starch weight versus granule diameter was shown by 46 out of 59 different cultivars. The remaining 13 cultivars were classified into two groups based on a negative or positive skew of weight distribution for granules with diameters from 10 to 28 µm. A further distinguishing feature within the positive skew group was the presence or absence of a significant weight of granules with diameters greater than 28 µm. The chemically determined starch content of the kernels was not significantly correlated with kernel weight. However, the proportion by weight of starch granules with diameter greater than 20 µm was significantly correlated with kernel weight. Analyses of starch from five cultivars of bread wheat, grown in three different years at six locations, confirmed that cultivar was the major determinant of starch granule size distribution, although location effects were also present. Drought appeared to decrease the proportion by weight of granules having a diameter greater than 20 µm.

## Introduction

Wheat grains contain two types of granular starch, type A granules of 10–35 µm diameter, which are the first formed, and type B granules of 1–10 µm diameter, which are formed later in development[1–8]. A small, distinct third population seen by Meredith[9] and Baruch *et al.*[10] may be present due to A-granule initiation mainly in peripheral cells[11]. However all granule sizes from 1 µm to 35 µm are represented[12]. The typical granule size distribution (GSD)† for wheat starch is bimodal when expressed in the differential form most familiar from sedimentation and sieve analyses; that is, proportions by weight of granules in equal diameter intervals, plotted against diameter. The significance of variation in the distribution of different granule sizes may go unrecognised if the results are determined or presented in an unsuitable form[13], or if there is poor recovery of starch. Thus, in early work, the contribution made by B-granules to total starch weight was badly underestimated, as noted by Hanssen *et al.*[14]. D'Apolonia *et al.*[15] concluded that B-granules contributed less than 10% by weight, but Evers[16] used micro-sieving to show that B-granules contributed 30% by weight. This was confirmed by Hughes and Briarty[17] using *in situ* stereology, and by Evers and Lindley[18].

† Abbreviations used: GSD = granule size distribution.

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With a good starch isolation procedure, GSD might be expected to reflect developmental patterns of the starch, although the determinants of such variation are not clear. Cunningham[19] used microscopic methods to find, with one exception, that neither cultivar nor locality had an influence on GSD for four cultivars grown in four locations over five successive years. However, Moss[20] reported that variations in weather conditions during the period of starch granule initiation, caused differences in the proportions of large starch granules in different cultivars. Brocklehurst and Evers[21] showed that the total number of starch granules is higher in 'large plump' kernels than in either 'small plump' or 'shrivelled' kernels grown under the same conditions. Yet the starch of all three types of kernel contained more than 30% by weight of B-granules. The work reported in this paper examines both the extent and cause of GSD variation in kernels from wheats of diverse genetic origins.

## Experimental

### *Materials*

A first set of 59 wheat samples, mainly bread wheats representative of the range of cultivars and advanced lines available in New Zealand, was grown in 1979 and harvested in 1980 at the Crop Research Division, D.S.I.R., Lincoln, N.Z. (43° 38′ S, 172° 28′ E). Cultivars Quern, Flavio and Konini were grown in the 1978–79 season. A second set, comprising 44 samples of bread wheat from five cultivars, was grown in New Zealand over three seasons at four North Island locations (approx. 40° S, 176° E), at Lincoln and at Gore (46° 06′ S, 168° 56′ E). The second set was incomplete owing to harvesting and storage mishaps, but it was sufficient to allow separate examination of effects due to cultivar, year and location. All wheat grain samples were held at −20 °C after collection.

### *Methods*

*Starch isolation procedure*. Starch was isolated under conditions giving minimal mechanical damage and amylase activity by an acid-steeping and squashing method[22]. Prime starch was isolated from the second set of wheats by reserving only clean white material from the centrifuge cake and discarding upper discoloured material without further treatment. To inhibit microbial growth, thiomersal (0·1 mM) was added to the wet starch preparations, which were stored at 5 °C as briefly as possible (maximum of 24 h) before analysis.

*Measurement of granule size distribution (GSD)*. As time-dependent, inter-granular disaggregation was observed with dilution of starch slurries, a dilute ($<$ 0·1% w/v) suspension of the starch under test was stirred gently in water for 30 min at 20 °C and then stirred for a further 30 min in aqueous NaCl solution (0·9% w/v) at the dilution most suitable statistically for counting ($<$0·01% w/v; 20,000 granules $ml^{-1}$). A Coulter Counter Industrial Model D equipped with a 140 μm orifice counted granule numbers at successive 2 μm intervals of hydrated sphere-equivalent diameter. Reduction to the form of proportion by weight of starch within each diameter interval used trapezoidal quadrature. Calibration was achieved using polystyrene latex granules (12·90 μm), paper mulberry pollen (15·20 μm), and lycopodium spores (26·44 μm).

*Kernel weight*. The average kernel weight was obtained by counting manually the number of kernels in 10·0 g of grain. Dry basis results are presented.

*Starch content*. The starch content of whole wheat kernels was determined by the semi-micro chemical method of Adkins *et al.*[23], after the whole kernels had been finely ground and shaken with glass beads in a McCartney bottle for 30 min. Complete conversion of starch to glucose was assumed. The precision of the method was ±0·5% starch, dry weight basis.

## Results and Discussion

*Variation with cultivar*

The mean and standard deviation for the weight proportion observed in each diameter interval for 59 cultivars is shown in Fig. 1. Results for distribution of A-granules within some individual cultivars are shown in Table I. The values obtained for total A-granule content of the starch ($69{\cdot}8 \pm 4{\cdot}5\%$) confirmed that starch, with the expected complement of B-granules, had been extracted successfully from wheat. Thus, 30% of the area under the differential weight versus diameter curve (Fig. 1) lay within the B-granule region. Two major size classes, and a minimum in the range 10–12 µm, were observed

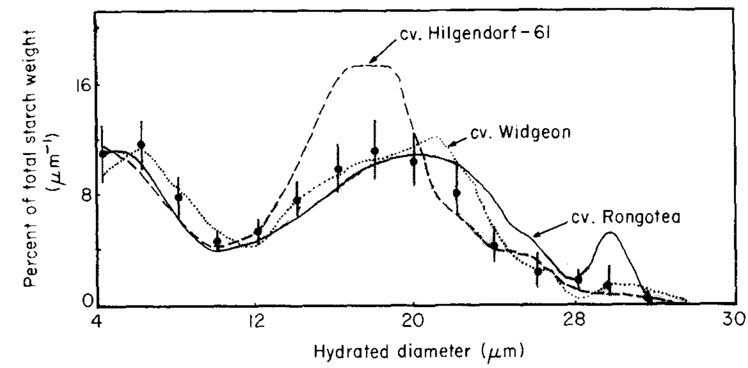


FIGURE 1. Granule size distribution determined by Coulter Counter for starch extracted from 59 wheat cultivars. The shaded area represents the mean ± one standard deviation for all starches. The skewed modes of A-granule distribution are exemplified by appropriate cultivars: Hilgendorf-61 – negative skew mode; Rongotea – positive skew plus mode; Widgeon – positive skew mode.

in every cultivar. The most frequent weight-average A-granule size varied in diameter between 16 µm (cv. Tainui) and 22 µm (cvs. Rongotea, Isis (brown), Widgeon). Also present in nearly every cultivar was a third granule class comprising very large A-granules with a mean diameter about 30 µm but ranging as high as 36 µm (cvs. Gamenya, Olympic). Both Gamenya and Olympic are cultivars of Australian parentage and they contained the largest granules encountered (38 µm). These large A-granule classes were present with a numerical frequency of about six or seven per 10,000 granules; their presence was accentuated by the choice of differential weight versus diameter axes. A shoulder in the GSD curve was frequently noticed between 12 and 14 µm; this was probably due to immature A-granules[11].

By overlaying Fig. 1 with individual GSD curves, it was possible to suggest four GSD modes, depending upon the distribution of A-granule weight. We have not demonstrated physiological significance for these GSD modes.

The *common mode*, shown by 46 of the 59 cultivars, displayed no deviations beyond the shaded area of Fig. 1.

Four cultivars (Hilgendorf-61, Kolibri, Ranger and Zg 4240-73) displayed a *negative skew*, with a very high A-granule peak at about 17 µm and a corresponding reduction in the proportion of weight found above 30 µm. It appears as though the A-granules had not developed fully, or as though their growth had been terminated at an earlier point than in other cultivars, perhaps by earlier initiation of B-granule growth.

TABLE I. Granule size distribution data for starch preparations from 59 wheat cultivars grown in 1979–80 at Lincoln, N.Z.

| GSD mode / cultivar | Kernel weight (mg, d.b.) | Starch content (% d.b.) | GSD (% by weight) 10–20 μm I | 20–34 μm II | 10–34 μm III | Ratio I/II |
|---|---|---|---|---|---|---|
| Common[a] (46 cultivars) | | | | | | |
| Average ($n = 46$) | 43 | 80 | 49·3 | 20·1 | 69·4 | 2·56 |
| S.D. | 4 | 3 | 3·6 | 3·8 | 3·9 | 0·63 |
| Minimum | 33 | 74 | 41·6 | 12·0 | 60·0 | 1·69 |
| Maximum | 52 | 85 | 58·6 | 27·6 | 76·8 | 4·27 |
| Negative skew | | | | | | |
| Hilgendorf-61 | 41 | 80 | 54·6 | 19·2 | 73·8 | 2·84 |
| Kolibri | 37 | 82 | 61·2 | 15·0 | 76·2 | 4·08 |
| Ranger | 54 | 81 | 60·0 | 20·4 | 80·4 | 2·94 |
| Zg 4240-73 | 34 | 78 | 52·6 | 10·2 | 62·8 | 5·16 |
| Positive skew plus | | | | | | |
| Rongotea | 48 | 80 | 43·0 | 30·0 | 73·0 | 1·43 |
| Kavkaz | 51 | 79 | 44·0 | 31·6 | 75·6 | 1·39 |
| Isis (brown) | 42 | 81 | 36·8 | 36·0 | 72·8 | 1·02 |
| Isis (white) | 53 | 77 | 38·0 | 28·2 | 66·2 | 1·35 |
| Glenlea | 51 | 80 | 40·2 | 22·4 | 62·6 | 1·79 |
| Likafen | 40 | 83 | 42·8 | 20·0 | 62·8 | 2·14 |
| Positive skew | | | | | | |
| Iona | 51 | 82 | 40·8 | 33·6 | 74·4 | 1·21 |
| Widgeon | 47 | 84 | 47·4 | 23·6 | 71·0 | 2·01 |
| Favorit | 45 | 78 | 53·6 | 25·0 | 78·6 | 2·14 |

[a] Names of cultivars giving a common mode of GSD: 946·01, Federation (A), Aotea, Armada, Atlas 66, Bella, Carifen, Cledor, Cross 7-35, Cross 7-61, Dove, Flavio, Fortuna, Gaines, Galiafen, Gamenya, Heima Desprez, Heurtebise, Hilgendorf-47, Huntsman, Justin, Karamu, Koga II, Konini 2655·01, Kopara, Magali, Olympic, Opal, Oroua, Pahau, Quern, RBS/63-112-66-2, Raven, Ring, S3830, Selkirk, Sentry, Sirius, Skemer, T.sphaerococcum, Tainui, Takahe, Wakanui, Wizard, Zg 887-73.

Six cultivars, referred to as *positive skew plus* (Rongotea, Kavkaz, Isis (brown), Isis (white), Glenlea and Likafen), displayed an A-granule peak displaced to 22 μm and a high proportion of large A-granules at about 30 μm. It appears as though A-granule growth had proceeded relatively unchecked in these cultivars.

The fourth mode of GSD, which was less clearly distinguished, was referred to as *positive skew*, (cvs. Widgeon, Iona and Favorit). This mode was similar to the positive skew plus distribution in the 22 μm range but it lacked the higher proportion of large A-granules.

*Significant sizes of granules*

GSD results were correlated with other properties of the starches by generating a sliding-window linear correlation matrix. Thus, the proportion of starch weight found within each 2 μm interval or window was correlated with kernel weight. The window width was then increased to 4 μm and correlated, and so on. The maintenance of statistical significance with increasing window width served to delineate clearly the GSD regions of importance (Fig. 2). Significance was established for the regions 10–20 μm (I) and 20–34 μm (II); the ratio I/II was adopted as a measure of skew of the A-granule size distribution. Kernel weight was positively correlated ($r = +0{\cdot}39$, $P < 0{\cdot}01$) with II and negatively correlated with I/II ($-0{\cdot}45$, $P < 0{\cdot}01$). There was, however, no significant correlation between kernel weight and total starch content (% dry weight basis) of kernels, nor between kernel weight and the proportion of A-granules in the starch (III). Physiologically, these results suggest that ultimate kernel weight depends on factors other than kernel-filling with either A- or B-granules.

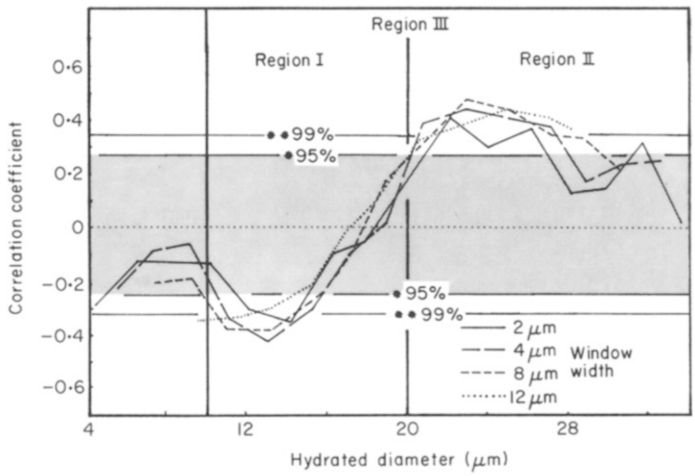


FIGURE 2. The correlation between kernel weight and the proportion of starch weight within a particular diameter range, the diameter range being increased successively from 2 μm to 12 μm. Starch was extracted from 59 wheat cultivars. Regions I (10–20 μm) and II (20–34 μm) together cover the A-granule size range (III). range (III).

An examination of starches, other than those of the common mode, showed that different modes of GSD were not distinguished by kernel weight or starch content. Of particular interest, as may be seen in Fig. 1, was the fact that there was no reduction in the proportion of B-granules that might be ascribed to an increase in the extent of development of the A-granules. For instance, the proportion by weight in the region 10–20 μm (I) was negatively correlated ($r = -0{\cdot}60$, $P < 0{\cdot}001$) with the proportion by weight in the region 20–34 μm (II). As GSD curves represent proportionality, an increase in the proportions of I or II must result in a decrease elsewhere in GSD; however, the decrease did not occur in the proportion of B-granules, but occurred entirely *within* the A-granule distribution. Thus, the distribution of weight within the A-granule portion of the GSD curve was variable, but as a proportion of total starch distribution it appeared to be fixed. This distribution was probably under genetic control but was also affected by growing conditions as discussed below.

*Effect of growth conditions*

As variability within the A-granule range appeared more significant than that between proportions of A- and B-granules, only prime starch was isolated from the second sample set. The prime starch contained between 1·9 and 26·5% by weight of B-granules. This level of contamination did not appear to be important, as results for analyses of variance using the ratio I/II had significances equivalent to those for I and II expressed separately.

Examination of GSD curves showed that in each year at each location cv. Rongotea displayed the positive skew plus mode, with a most frequent weight–average A-granule diameter displaced to 22 μm and a high proportion of larger A-granules at around 30 μm. Less clearly, cv. Karamu tended to display a negative skew, with the A-granule peak at

TABLE II. Granule size distribution (GSD) data for 44 starch preparations from five cultivars of wheat grown at six locations in three harvest years

| Location[a] Year Cv.[b] | Kernel wt. (mg, d.b.) | GSD (% by weight) I[c] | GSD (% by weight) II[d] | Ratio I/II | Location[a] Year Cv.[b] | Kernel wt. (mg, d.b.) | GSD (% by weight) I[c] | GSD (% by weight) II[d] | Ratio I/II |
|---|---|---|---|---|---|---|---|---|---|
| L 79 Ro | 50·9 | 47·6 | 49·7 | 0·96 | H 80 Ro | 40·4 | 49·6 | 45·8 | 1·08 |
| Or | 44·4 | 54·6 | 43·0 | 1·27 | Or | 36·3 | 56·7 | 41·1 | 1·38 |
| Ar | 44·7 | 59·6 | 28·4 | 2·10 | Ka | 35·9 | 56·2 | 35·3 | 1·59 |
| Ka | 44·6 | 59·5 | 30·5 | 1·95 | 81 Ro | 42·1 | 44·9 | 48·7 | 0·92 |
| Ko | 41·7 | 58·0 | 15·5 | 3·74 | Or | 35·4 | 53·6 | 36·2 | 1·48 |
| 80 Ro | 46·6 | 47·3 | 44·6 | 1·06 | Ka | 38·0 | 61·3 | 30·6 | 2·00 |
| Or | 42·4 | 64·9 | 29·9 | 2·17 | T 80 Ro | 33·6 | 47·5 | 48·7 | 0·98 |
| Ar | 46·6 | 62·0 | 28·4 | 2·18 | Or | 30·2 | 60·1 | 32·2 | 1·87 |
| Ka | 39·7 | 63·5 | 25·1 | 2·53 | Ka | 25·2 | 70·3 | 22·1 | 3·18 |
| Ko | 42·5 | 60·9 | 25·1 | 2·43 | 81 Ro | 46·2 | 40·7 | 52·7 | 0·77 |
| 81 Ro | 46·6 | 52·2 | 38·8 | 1·35 | Or | 35·3 | 48·6 | 35·4 | 1·37 |
| Or | 40·2 | 64·7 | 31·9 | 2·03 | Ka | 38·1 | 53·7 | 35·9 | 1·50 |
| Ar | 45·0 | 64·6 | 22·8 | 2·83 | W 80 Ro | 42·9 | 47·4 | 50·7 | 0·93 |
| Ka | 40·6 | 64·7 | 31·2 | 2·07 | Or | 36·5 | 51·1 | 38·3 | 1·33 |
| Ko | 38·9 | 60·3 | 19·8 | 3·05 | Ka | 37·2 | 61·0 | 33·2 | 1·84 |
| G 80 Ro | 43·8 | 48·2 | 39·9 | 1·21 | 81 Ro | 33·6 | 50·5 | 41·2 | 1·23 |
| Or | 36·4 | 53·6 | 39·9 | 1·34 | Or | 29·6 | 54·4 | 33·4 | 1·63 |
| Ka | 28·4 | 71·3 | 19·7 | 3·62 | Ka | 32·5 | 59·8 | 34·3 | 1·74 |
| 81 Ro | 47·2 | 47·9 | 39·5 | 1·21 | | | | | |
| Or | 30·8 | 68·4 | 26·9 | 2·54 | | | | | |
| K 80 Ro | 44·0 | 38·8 | 53·9 | 0·72 | | | | | |
| Or | 38·1 | 48·3 | 48·6 | 0·99 | | | | | |
| Ka | 36·4 | 57·1 | 24·6 | 2·32 | Mean | 39·3 | 55·5 | 35·8 | 1·74 |
| 81 Ro | 41·9 | 42·9 | 50·1 | 0·86 | Minimum | 25·2 | 38·8 | 15·5 | 0·72 |
| Or | 40·6 | 55·8 | 40·6 | 1·37 | Maximum | 50·9 | 71·3 | 53·9 | 3·74 |
| Ka | 36·2 | 58·5 | 29·3 | 2·00 | Repn Cv. % | 3 | 2 | 7 | 8 |

[a] Locations: L = Lincoln, G = Gore, K = Kairanga, H = Halcombe, T = Tikokino, W = Westmere.
[b] Cultivars: Ro = Rongotea, Or = Oroua, Ar = Arawa, Ka = Karamu, Ko = Kopara.
[c] I: diameters 10–20 μm.
[d] II: diameters 20–34 μm.

16 μm and few of the larger A-granules. Kernel weight was again correlated negatively with I ($r = -0{\cdot}41$, $P < 0{\cdot}01$) and positively with II ($r = +0{\cdot}33$, $P < 0{\cdot}05$). Thus, wheats with a high kernel weight also exhibited a high proportion by weight of A-granules with diameters greater than 20 μm.

Results presented in Table II allow analyses of variance to be made on two bases. The first utilises three cultivars harvested in two successive years from six locations with one missing sample, and the second basis utilises those samples taken from the Lincoln location, representing five cultivars harvested in three successive years. Results are given in Table III. The proportion of smaller A-granules, from 10–20 μm (I), was highly significantly influenced by cultivar, and significantly influenced by location, with a significant interaction of year with location. The weight of starch in the range 20–34 μm (II) exhibited similar analyses of variance to those for I, due to the inverse relationship between I and II as shown above.

The observed interaction of location and year was due to specific edaphic effects at two locations. The location, Tikokino, experienced a drought in 1980, followed by a normal year in 1981. The Westmere location experienced normal conditions in 1980, but was exposed to poor cultural conditions in 1981. The effect of drought (Fig. 3) was to

TABLE III. F-ratios and levels of significance[a] obtained for analyses of variance made upon granule size distributions of 44 starches

| GSD (% by weight) | CY[b] | | CYL[c] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cv. (C) | Year (Y) | Cv. (C) | Year (Y) | Location (L) | Y.L | L.C | Y.C |
| 10–20 μm (I) | 20·1*** | 8·2* | 60·4*** | 0·1 | 7·4*** | 4·5** | 1·6 | 0·4 |
| 20–34 μm (II) | 10·3*** | 1·1 | 71·9*** | 0·6 | 6·8** | 2·1 | 2·0 | 3·6* |
| Ratio I/II | 7·1** | 0·4 | 49·7*** | 0·0 | 7·0** | 2·5 | 2·5* | 2·4 |

[a] * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
[b] CY: five cultivars harvested in three years at one location.
[c] CYL: three cultivars harvested in two years from six locations, with one missing sample.

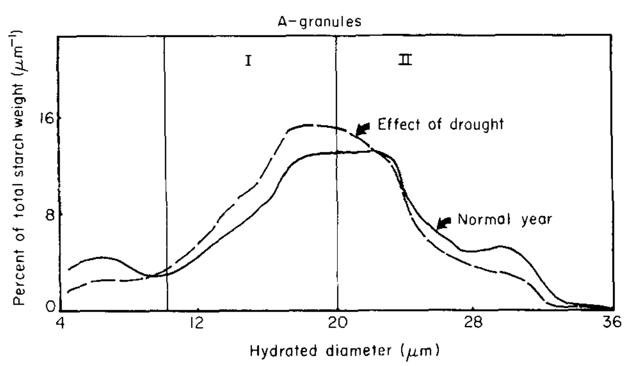


FIGURE 3. The mean granule size distributions determined by Coulter Counter for prime starch extracted from three cultivars grown at Tikokino in two consecutive years. Tikokino experienced a drought in 1980, followed by a normal year in 1981.

decrease the proportion of large A-granules (II, 20–34 μm). Poor cultural practice had less but similar effect. This finding concerning changes due to edaphic factors is more specific than previous work[20, 21] has suggested.

## *Conclusion*

Variation of GSD in wheat starch appears to be largely under genetic control, although extreme edaphic factors may decrease the proportion of 'mature' A-granules with diameters greater than 20 μm. This finding may have significance for the wheat starch industry, in which sedimentation of B-granules is sometimes a problem.

These experiments have been restricted to crops grown in New Zealand, where conditions are conducive to prolonged growth before ripening. We would hesitate to assume similar conclusions for crops grown where there are climatic limitations on growth and where, consequently, cultivars have been developed that are adapted to such limitations.

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## References

1. Buttrose, M. S. *Aust. J. Biol. Sci.* **16** (1963) 305–311.
2. Badenhuizen, N. P. The biogenesis of starch granules in higher plants. Appleton-Century-Crofts, New York (1969).
3. Evers, A. D. *Starch* **21** (1969) 96–99.
4. Evers, A. D. *Ann. Bot.* **34** (1970) 547–555.
5. Evers, A. D. *Starch* **23** (1971) 157–162.
6. Evers, A. D., Greenwood, C. T., Muir, D. D. and Venables, C. *Starch* **26** (1974) 42–46.
7. Gallant, D. and Guilbot, A. *Starch* **25** (1973) 335–342.
8. Baruch, D. W., Meredith, P., Jenkins, L. D. and Simmons, L. D. *Cereal Chem.* **56** (1979) 554–558.
9. Meredith, P. *Starch* **33** (1981) 40–44.
10. Baruch, D. W., Jenkins, L. D., Dengate, H. N. and Meredith, P. *Cereal Chem.* **60** (1983) 32–35.
11. Hoshikawa, K. Translation RTS 6636, National Lending Library for Science and Technology, Boston Spa (1961).
12. MacMasters, M. M. and Waggle, D. H. *Starch* **15** (1963) 7–11.
13. Meredith, P., Baruch, D. W. and Jenkins, L. D. *Starch* **29** (1977) 217–223.
14. Hanssen, E., Dodt, E. and Niemann, E. *Kolloid Z.* **130** (1953) 19–31.
15. D'Appolonia, B. L., Gilles, K. A., Osman, E. and Pomeranz, Y. In 'Wheat Chemistry and Technology' (Y. Pomeranz, ed), American Association of Cereal Chemists. St Paul, MN (1971) pp. 301–392.
16. Evers, A. D. *Starch* **25** (1973) 303–304.
17. Hughes, C. E. and Briarty, L. G. *Starch* **28** (1976) 336–337.
18. Evers, A. D. and Lindley, J. *J. Sci. Food Agric.* **28** (1977) 98–102.
19. Cunningham, R. N. Report 4, Wheat Industry Research Council, Melbourne, Australia (1963) pp. 1–67.
20. Moss, H. J. *J. Sci. Food Agric.* **18** (1967) 610–612.
21. Brocklehurst, P. A. and Evers, A. D. *J. Sci. Food Agric.* **28** (1977) 1084–1089.
22. Dengate, H. N., Baruch, D. W. And Meredith, P. *Starch* **30** (1978) 80–84.
23. Adkins, G. K., Banks, W., Greenwood, C. T. and MacGregor, A. W. *Starch* **21** (1969) 57–61.