Characteristics of Small-Granule Starch of Flour and Wheat[1]

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ABSTRACT

The objective of this study was to evaluate the fundamental and breadmaking properties of small wheat-starch granules and to compare them with those of regular starch. Regular wheat starches were isolated from commercially milled flours. From the same flours, a fraction (fines) containing principally small granules was separated by air classification and used as a source of small granules. To avoid the effects of starch damage from milling, regular and small-granular starches were isolated directly from wheat. Small granules were found to be lower in iodine affinity, indicating differences in amylose levels or some fundamental structural differences; the hot paste consistencies (Viscograph) of both starches were similar, but the small-granule starch was lower in hot paste stability and produced a cold paste consistency below that of the regular starch. Defatting of starch prior to testing did not remove these differences. Swelling powers were comparable from 65° to 95°C., except that at 95°C. the swelling power of small granules was higher. Solubilities were generally below those of regular starches. Gelatinization temperature ranges, water-binding capacities, and enzymic susceptibilities of small granules were higher than those of regular ones. Doughs made from starch-gluten systems had lower stabilities when small-granule starch was used than when they contained the corresponding regular starch. The acetic acid extractability and the release of gliadin from small-granule starch doughs were less than from the regular starch doughs. Baking tests using blends of different starches with a single gluten preparation showed that the small granules have a lower baking potential than the regular ones. All differences attributable to granule size were consistent in direction, regardless of the source or method of preparation of the starch.

The first starch granules deposited in the endosperm cells of wheat are often kidney-shaped; they later develop into large, lenticular granules (A type). About 14 days after the first deposit, new starch granules are formed in the same plastids, but these granules remain small (B type). According to Buttrose (1) the plastids produce evaginations resembling buds in which small granules are found. Eventually the buds are separated from the plastids by constriction. Wheat starch contains mainly granules of two distinct size groups, the small granules being as small as 3 nm. and the larger measuring up to 30 nm., with only a few granules of intermediate sizes. Stamberg (2) calculated the average distribution of starch granules from 17 wheat starches using the data of Grewe and Bailey (3). Of the total number of granules 81.2% were smaller than 7.5 nm.; 6% were intermediate (7.5 to 15 nm.); and 12.8% were of large size (15 to 30 nm.). By weight the proportions were 4.1% for small, 2.9% for intermediate, and 93% for large granules. Normal variations in ratios of starch granules did not appear to affect the baking properties of flours.

Medcalf and Gilles (4) reported pasting characteristics and mixing curves with small granules. Their data indicated that small granules have lower viscosities during pasting and shortened mixing times in starch-gluten systems. Hoseney et al. (5) isolated small-granule starch from a hard winter wheat flour and found that this starch fraction had baking characteristics nearly equal to those of the control flour. D'Appolonia and Gilles (6), in their study on the effects of various starches in baking, reported that the small-granule fraction from hard winter wheat flour was similar in baking properties to regular wheat starch when only gluten-starch systems were used, but in the presence of the water-soluble flour component its baking

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quality was lower than that of the control. Differences between small and large granules in their physical structures were suggested by Dronzek et al. (7) on the basis of differences in the mode of amylase attack on these two types of granules, indicated by means of scanning electron microscopy.

It is apparent from the foregoing that wheat starch is not uniform but consists of granules which may vary not only in size, but also in physical, chemical, and functional properties. The objective of this study was to evaluate the variations attributable to granule size and to measure their effects on the breadmaking potential of starch and parent flour.

MATERIALS AND METHODS

Starches

Regular wheat starches were isolated by the dough process (8) from commercially milled straight-grade hard red spring (HRS), hard red winter (HRW), and soft red winter (SRW), wheat flours. The small-granule starches were prepared by the same procedure from fines (a fraction containing principally small granules), obtained from these flours by air classification.

In addition to the starches from flours, one starch was isolated directly from hard red wheat (Trapper variety) to eliminate the possible interference of starch damage from milling. The granular starch was recovered by a previously detailed wet-milling procedure (8) and the small granules were then isolated from this starch by repeated sedimentations until microscopic examination indicated a high degree of purity of the preparation. This extra step was carried out at refrigeration temperature (2° to 5°C.), using toluene to control microbial growth.

All starches were exhaustively washed with distilled water and air-dried. The regular starches contained granules in normal proportions by size for wheat starch. The small-granule preparations contained only granules below 7.5 nm.

Defatting

Starches were defatting according to the procedure of Schoch (9).

Viscography

Pasting properties were determined by means of the Amyloviscograph, pasting 9 parts of starch solids per 100 parts of water (total volume being 420 ml.) (8). Heating was conducted to 95°C., kept at 95°C. for 60 min., then decreased to 35°C. and held at that temperature for an additional 60 min.

Determinations

The iodine affinity values were obtained by amperometric (10) and potentiometric (11) procedures. These values were converted to apparent amylose content by assuming the iodine affinity to be 19.24 g. per 100 g. of pure amylose (10). The gelatinization temperature ranges were measured microscopically using a Kofler hot stage (12). Swelling power and solubility determinations were carried out for the temperature range from 60° to 95°C., making measurements at 5°C. intervals (13). Enzyme susceptibility was estimated by the procedure of Leach and Schoch (14) as follows: 15 g. of starch was incubated with 0.25 g. of a commercial fungal amylase (Rhozyme 33) in a water suspension at 30°C. for 24 hr. using a shaker-water bath, and then the solubilized portion was determined. The water-binding capacity was determined according to the method of Medcalf and Gilles (15).

Farinography

Farinograms were prepared using a 50-g. bowl according to the conventional constant flour weight procedure (16). Gluten-starch blends were used in place of flour. Each starch preparation was combined with sufficient gluten to produce a protein level in the mixture of 12.5% (N X 5.7, moisture-free basis). A single source of gluten isolated from a good quality patent flour (HRW wheat) was used for all mixtures.

Acetic Acid Extractability

Doughs were mixed in the farinograph for 7 and 17 min., freeze-dried, and hammer-milled to pass a 0.10-mesh sieve. The samples were then extracted with 0.05M acetic acid and the extracts separated using Biogel P-150 (17).

Baking Tests

Bread of pup-loaf size was prepared according to the remix procedure of Irvine and McMullan (18) as adapted for bread preparation from flour fractions (8). Gluten was added to produce 12.5% protein (N X 5.7) in each gluten-starch mixture, dry basis. A common gluten (single preparation) was used with each starch. The formula and the method of preparation were used as reported (8).

RESULTS AND DISCUSSION

Iodine-Affinities of Starches

The iodine affinities and apparent amylose values are given in Table I. In all cases, the amylose content of the small granules was appreciably lower than that of the corresponding regular starch granules, regardless of the source of starch. Obviously, to attribute the differences in iodine affinity between the small and large granules to different levels of amylose may be an oversimplification, since the iodine affinity values may reflect a more fundamental structural variation. This possibility was recognized by Maywald et al. (19) and Matheson and Whestley (20), and was also pointed out in our study of the changes of starch during maturation of wheat (21). A careful fractionation and characterization of the starch polymers are necessary to resolve this question.

Pasting Properties

The viscograms shown in Fig. 1 indicate differences in the pasting characteristics between the small and regular granules, derived from various sources. They vary somewhat depending on the type of parent wheat, but the general trend is consistent, indicating definite pasting differences between the small and regular granules.

Generally the temperature of the initial rise in consistency was about 10°C. less for regular starch than for the small granules; the hot paste consistencies were similar for the comparable regular starch and small-granule fractions; the stabilities of the small-granule hot pastes were significantly below those of the pastes of the regular starches; further, the consistencies of small-granule cold pastes were substantially less than those from normal starches. There was no appreciable difference in these indices between the flour and wheat starches.

TABLE I. IODINE AFFINITY VALUES OF STARCHES

Starch from	Amperometric Procedure I_2 affinity %	 Amylose %	Potentiometric Method I_2 affinity %	 Amylose %
Winter wheat[a]				
Regular granules	4.77	24.4	4.16	21.3
Small granules	4.18	21.4	3.26	16.7
Winter flour				
Regular granules	4.67	23.9	3.97	20.3
Small granules	3.83	19.6	3.12	16.0
Spring flour				
Regular granules	4.47	22.9	4.08	20.9
Small granules	3.86	19.8	3.22	16.5
Soft flour				
Regular granules	4.43	22.7	3.75	19.2
Small granules	3.75	19.2	2.93	15.0
Average				
Regular granules	4.59	23.48	3.99	20.43
Small granules	3.91	20.00	3.13	16.05

[a] Starch isolated directly from wheat.

Although the granules of both sizes were similar in protein content, the small ones contained, on the average, 1.1% lipid material and the large ones 0.45% (dry basis). It is known that in many cases this minor component affects the pasting properties of starches. To determine whether this could explain our results, the starches were retested after defatting. The viscograms given in Fig. 2 show that while the removal of lipids altered the pasting patterns of both types of granules, the basic differences between the small and large granules were retained. This demonstrated that the differences in the pasting properties could not be attributed to lipid-starch complexes, but were due to the carbohydrate structure of the starch matter itself.

Gelatinization Temperature Ranges

As is evident from Table II, the small starch granules gelatinized consistently within higher ranges than did the regular granules. The underlying cause of this behavior is the higher degree of crystalline order in the small than in the regular granules, attributable to the different physical structure and chemical composition.

Swelling Powers and Solubilities

The swelling curves, represented in Fig. 3, indicate comparable swelling power values for both granule sizes at all temperatures except at 95°C., where the swelling power of small granules increased above that of the regular granules. This trend was consistent for all starches, including those derived directly from wheat (Fig. 3, upper right). The high swelling of small granules at 95°C. was, as expected, inversely correlated with the reduced stability of the hot paste. This type of relationship was previously established by Schoch and Maywald (22) for potato and tapioca starches, and was also confirmed with immature wheat starches (21). It

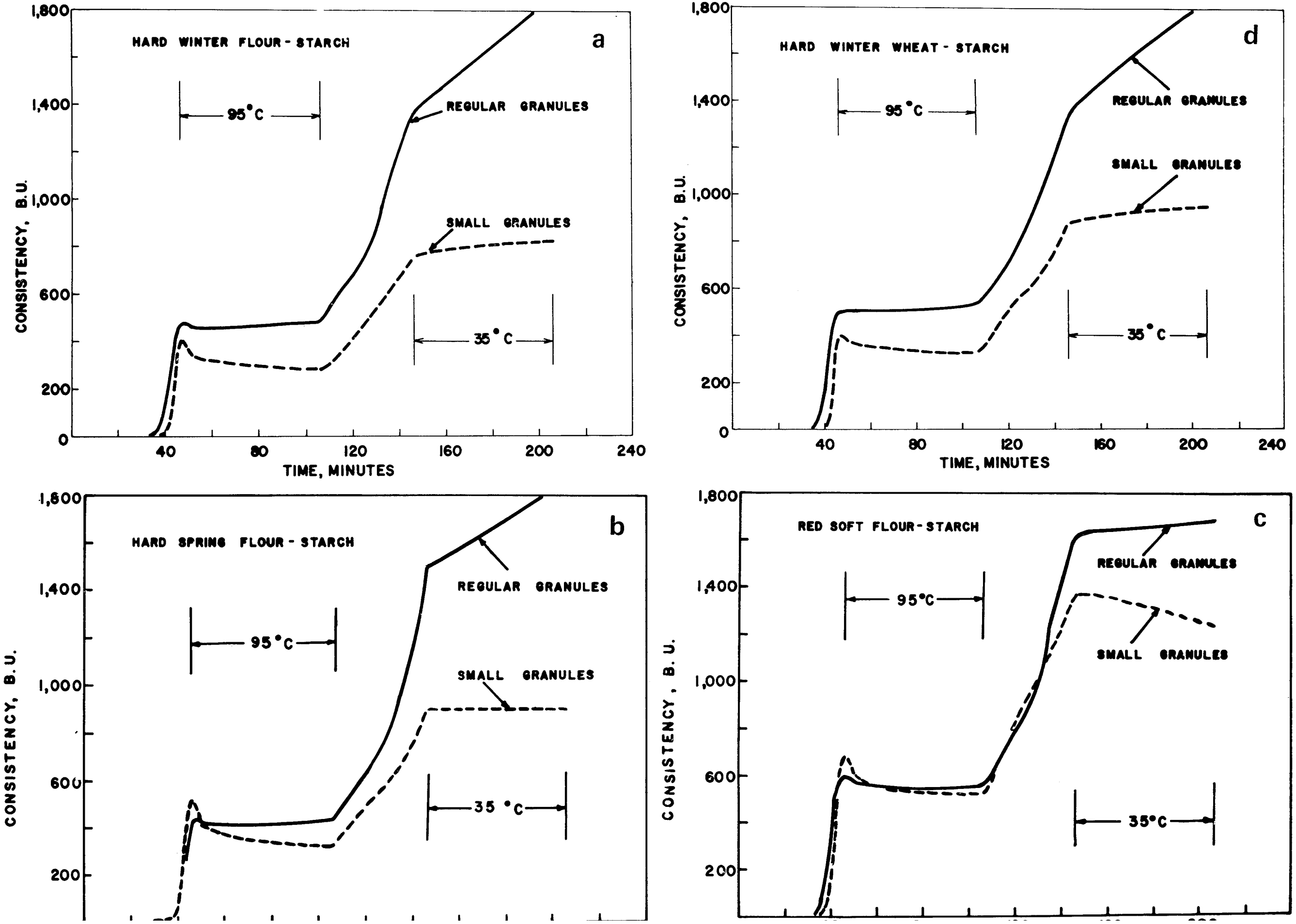

Fig. 1. Viscograms of regular and small-granule starches from HRW flour (a), HRS flour (b), SRW flour (c), and HRW wheat (d).

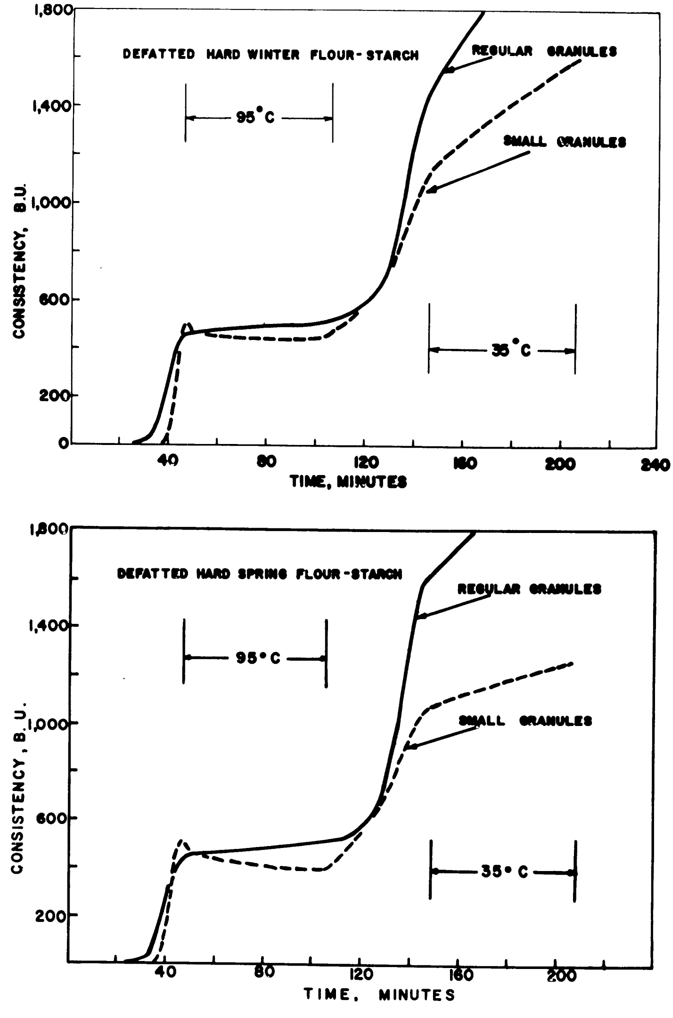

Fig. 2. Viscograms of defatted regular and small-granule starches from HRW (upper frame) and HRS flours (lower frame).

appears that small granules become overextended at the hot peak, producing fragile, swollen granules which set to a weaker cold paste than that obtained with the comparable regular starches. A contributing factor to this behavior of small granules may be the lower content of amylose.

The solubility values (Table III) of both types of granules were similar at the lower temperatures. With rising temperatures the solubility of large granules increased above that of small granules. Since amylose is the predominant starch fraction being leached out of the granules, one may speculate that the low release of this component from small granules may contribute to the low stability of the hot paste and the low consistency of the cold paste.

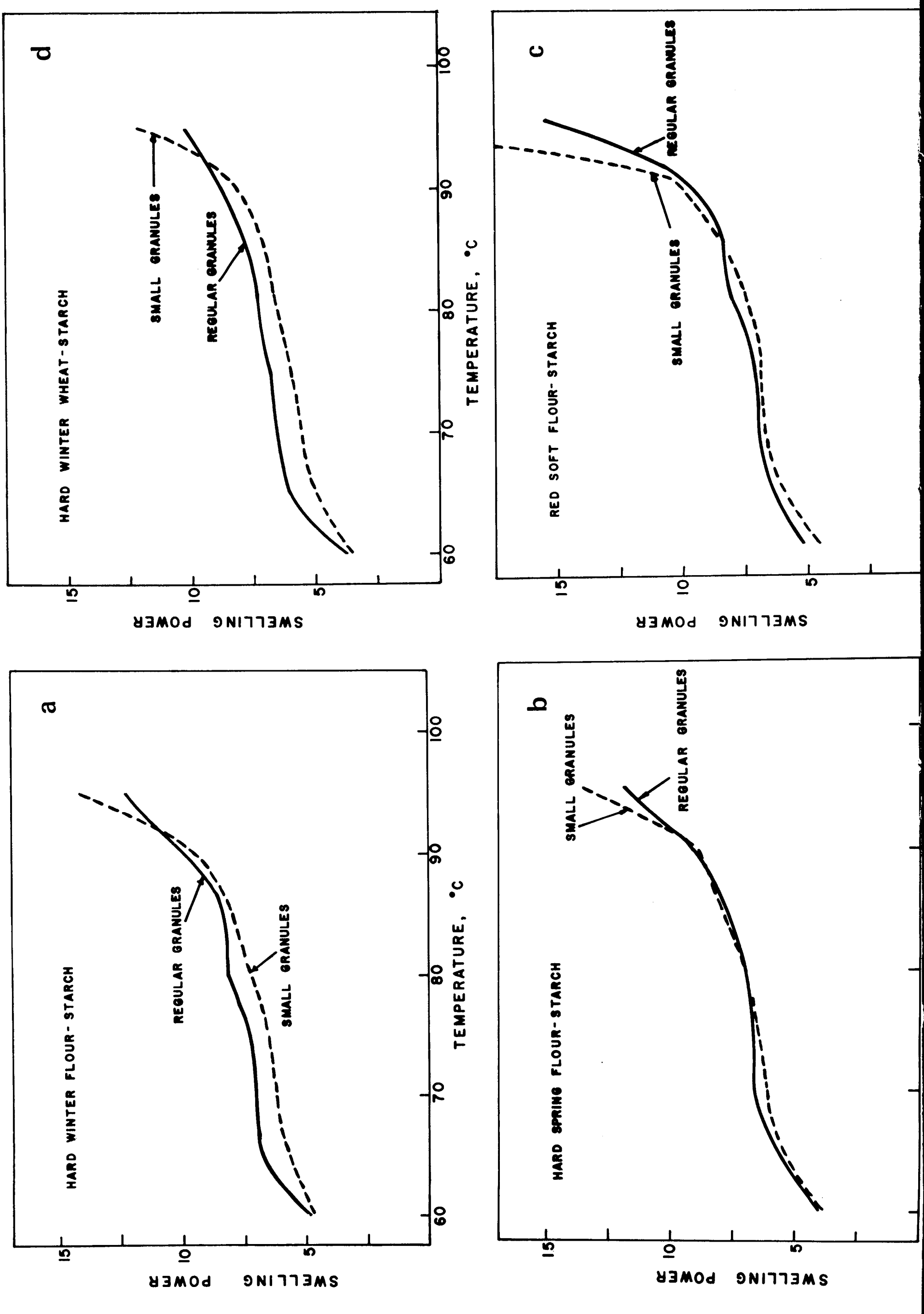

Fig. 3. Swelling powers of regular and small-granule starches from HRW flour (a), HRS flour (b), SRW flour (c), and HRW wheat (d).

Enzyme Susceptibility

Small granules were degraded more readily by amylases than regular-size granules whether the starches were derived from milled flour or directly from wheat (Table IV).

It is well known that enzyme susceptibility is a function of starch damage and this fact is commonly utilized as an index of starch damage in milled products. The damage inflicted by milling undoubtedly accounts for the increased susceptibility of both small and regular starches from flour over the values for starches derived from wheat, but it is doubtful that differences in damage alone can explain the observed difference in enzyme susceptibility between small and regular starches. For the starches from flour, one would have to assume greater damage to the small granules than the regular ones during milling, which is contrary to the microscopic observations of Moss (23), who found the large granules to be generally more damaged by milling than the small ones. For starches derived from wheat one would have to assume that the small granules exhibited greater inherent imperfections than the larger ones to account for the difference in susceptibility. Evidence of inherent imperfections of starch granules in the form of cracks was found not only in isolated starches (24,25) but also in granules of living plants as early as 12 days after fertilization (24). Badenhuizen (26) attributed occurrence of these types of cracks to internal stresses within the granules.

Other factors which might contribute to the higher enzyme susceptibility are the differences in the mode of enzymic attack of amylases on the small and large granules and also the higher surface area of the small granules exposed to the action of the enzymes. According to the results of Dronzek et al. (7), using scanning electron microscopy, the large granules are attacked at the groove and at localized sites of the surface. Once the surface is eroded, the degradation seems to move

TABLE II. GELATINIZATION TEMPERATURE RANGES

	°C.		
Starch from	Initiation	Midpoint	Final
Winter wheat[a]			
Regular granules	56.0	58.0	61.0
Small granules	55.0	64.0	67.0
Winter wheat flour			
Regular granules	55.0	59.5	63.0
Small granules	59.0	62.0	64.0
Spring wheat flour			
Regular granules	54.0	58.0	61.5
Small granules	53.5	61.0	64.0
Soft wheat flour			
Regular granules	54.0	58.0	62.5
Small granules	56.0	59.0	64.0
Average			
Regular granules	54.75	58.38	62.00
Small granules	55.88	61.50	64.75

[a] Starch isolated directly from wheat.

TABLE III. SOLUBILITIES OF STARCHES, PERCENT

Starch from	Temperature, °C. 60	70	80	90	95
Winter wheat[a]					
Regular granules	0.4	1.5	3.8	7.8	11.4
Small granules	0.9	2.1	3.6	4.8	9.3
Winter flour					
Regular granules	1.0	3.1	5.0	9.9	17.0
Small granules	2.0	3.3	3.8	5.5	11.6
Spring flour					
Regular granules	1.4	3.1	4.3	8.1	14.6
Small granules	1.7	2.8	3.5	5.4	10.0
Soft flour					
Regular granules	1.5	3.0	5.0	9.4	23.6
Small granules	1.0	2.6	3.7	6.4	19.8
Average					
Regular granules	1.08	2.68	4.53	8.80	16.65
Small granules	1.40	2.70	3.65	5.53	12.68

[a]Starch isolated directly from wheat.

TABLE IV. ENZYME SUSCEPTIBILITY AND WATER HYDRATION CAPACITIES OF STARCHES

Starch from	Enzyme-Solubilized %	Hydration Capacity %
Winter wheat[a]		
Regular granules	0.2	72.5
Small granules	3.0	78.5
Winter flour		
Regular granules	3.2	84.5
Small granules	10.1	94.2
Spring flour		
Regular granules	7.0	84.8
Small granules	13.6	95.5
Soft flour		
Regular granules	4.5	90.5
Small granules	6.6	103.9
Average		
Regular granules	3.73	83.08
Small granules	8.33	93.03

[a]Starch isolated directly from wheat.

through the layers of the granules toward the center. The center is completely digested, whereas only portions of the radial starch are broken down. The erosion of small granules proceeds differently. In these granules small circular spots, randomly distributed over the surface, are attacked. In view of the difference in the mechanism of the enzyme action, one may postulate that the surface is more critical for the small than for the large granules. It is also possible that the milling and inherent damage, even if equal to that of the large granules, resulted in a higher enzymolysis of the starch matter of the small granules, the underlying reason being the difference in the surface area. The viscographic data, solubility patterns, and the gelatinization ranges presented in this study do not support the conclusion that the enzymolysis differences are a result of starch damage only. In view of the characteristics of these indices for starches derived from flours and the corresponding native starches (8) one would expect, if the differences were the result of starch damage only that a) the viscosity characteristics of a small-granule hot paste would be lower than those of the regular starch; b) the solubilities of small granules would exceed those of the large granules; and c) the gelatinization temperature ranges would be depressed.

Thus, we believe that the differences in enzymolysis of the small and large granules cannot be explained simply by the imperfections of starch granules (mechanically produced or inherent) but are due to a combination of factors which are, in addition to the condition of granules, the surface area and the mode of enzymic attack.

Water-Binding Capacities

Data in Table IV demonstrate that the small-granule starches are higher in water-binding capacity than the comparable regular ones. The starches from flours were generally higher in this property than those from wheat, reflecting the effect of milling damage. Nevertheless, the differences attributable to granule size were consistent. These differences were further confirmed by farinography of starch-gluten systems as shown in Fig. 4. The blends containing small granules required higher water of absorption than those with regular starches to produce normal consistency doughs (500 B.U.); furthermore, the doughs with small granules

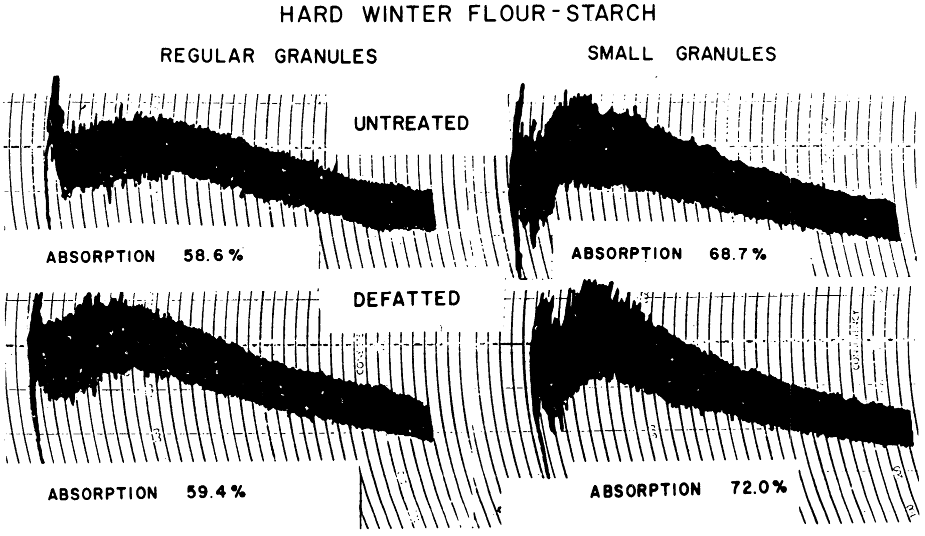

Fig. 4. Farinograms of gluten-starch blends. Upper frame, untreated regular and small-granule starches from HRW flour; lower frame, same starches, defatted. Gluten constant.

had a lower stability than those with regular ones. The mixing times, however, were not affected by the granule size. These results indicate that the dough stability may depend not only on the quality of gluten, but may also be affected by a gluten-starch interaction in which the properties of the starch component assume importance.

TABLE V. EFFECT OF GRANULAR SIZE ON THE ACETIC ACID EXTRACTABILITY OF PROTEIN OF STARCH-GLUTEN DOUGHS

Starch Isolated from	Mixing Time min.	Protein Extracted, %	
		Starch-gluten	Starch-defatted gluten
Control flour	7	92.1	89.1
	17	92.5	93.2
Small granules from spring wheat flour fines	7	84.7	81.9
	17	83.2	87.1
Small granules from winter wheat flour fines	7	85.7	77.4
	17	85.5	80.3
Small granules from soft red wheat flour fines	7	73.2	74.3
	17	82.8	87.5

Protein Extractability and Size Distribution

The farinogram differences indicated an interaction between the starch and gluten. To gain insight into these changes, the acetic acid extractability and size distribution patterns of the proteins extracted from starch-gluten doughs prepared from small granule and regular starch-gluten systems were studied. As is evident from Table V, the doughs with small granules yielded less soluble protein than those with the regular starch, at comparable mixing times. Further, the column chromatograms (Fig. 5) revealed that, on an equal protein basis, less gliadin was present in the extracts from doughs containing small starch granules than from those with regular starch. According to Tsen (17) a disaggregation of the protein complex takes place during the mixing of flour doughs which is evidenced by a gradual increase in acetic acid-soluble protein, especially in the gliadin component. The present evidence suggests that disaggregation may be influenced by the type of starch present: in the case of regular granules the release of gliadin and of solubles proceeded more readily than when gluten was mixed with the small granules; on the other hand, a higher dough stability resulted from the interaction of gluten with regular than with small-granule starch.

Baking Properties

The data in Table VI indicate a lower baking potential for small granules as compared with the regular starches. The doughs with small granules, as expected from water-binding capacities and farinogram data, required a higher absorption than the regular starches. Otherwise the properties of the doughs were normal. The breads prepared with the blends containing small granules were appreciably lower in volume and poorer in quality than the controls made with the comparable regular starches. The poor baking performance was also confirmed with starch preparations

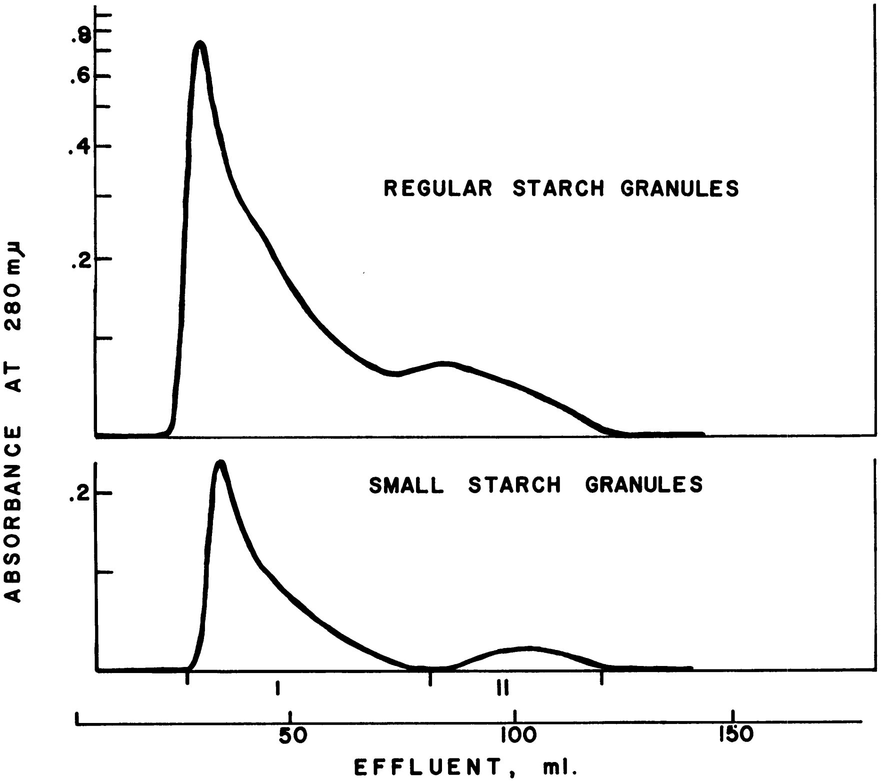

Fig. 5. Elution curves for protein components of gluten-starch blend, mixed 7 min. Upper frame, regular; lower frame, small-granule starch. Gluten constant; I = gliadin fraction.

TABLE VI. BAKING TESTS

	Bread Volume, cc.	
Starch from	As-is	Defatted
Winter wheat[a]		
Regular granules	770	...
Small granules	660	...
Winter wheat flour		
Regular granules	820	840
Small granules	565	560
Spring wheat flour		
Regular granules	820	790
Small granules	790	560
Soft wheat flour		
Regular granules	585	530
Small granules	275	300
Average		
Regular granules	749	720
Small granules	573	473

[a] Starch isolated directly from wheat.

from wheat rather than from flour. This observation excluded the possibility that milling starch damage was an interfering factor in these experiments. A similar trend was observed when these baking tests were conducted with defatted starches, thus also eliminating lipids as a factor responsible for these differences.

GENERAL CONCLUSIONS

The results presented support the conclusion that the starch granules of wheat flour are not uniform in their physical structure and chemical composition. Variations in these properties had concomitant effects on their functional and technological characteristics. Comparisons of regular and small–granule starches derived from various sources demonstrated consistent variations. The small granules differed from the regular ones in the following indices: they were lower in iodine affinity, reflecting lower amylose levels or some intrinsic structural differences; produced pastes similar in hot paste consistency; swelled more and became less soluble at high temperatures than the regular starches; were attacked more readily by amylases; and had higher water-binding capacities. Finally, small granules produced doughs of reduced stability due to different degrees of protein-starch interaction as suggested by acetic acid extractability data and disaggregation patterns. The baking potential of small-granule starch was found to be inferior to the regular starch.

The understanding of the physicochemical reasons for this behavior must await evaluation of the fine structure of amyloses, amylopectins, and of intermediate fractions of small and large granules along with the corresponding physical implications. This can be accomplished by careful fractionation of starches, followed by chemical and physical studies of the components. The correlation of the presented indices with breadmaking quality of starch is equally difficult at this time, but the data suggest a close relationship between the baking quality of starch and its physicochemical structure.

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Literature Cited

1. BUTTROSE, M. S. Untrastructure of the developing wheat endosperm. Aust. J. Biol. Sci. 16: 305 (1963).
2. STAMBERG, O. E. Starch as a factor in dough formation. Cereal Chem. 16: 769 (1939).
3. GREWE, E., and BAILEY, C. H. The concentration of glutenin and other proteins in various types of wheat flour. Cereal Chem. 4: 230 (1927).
4. MEDCALF, D. G., and GILLES, K. A. The function of starch in dough. Cereal Sci. Today 13: 382 (1968).
5. HOSENEY, R. C., FINNEY, K. F., POMERANZ, Y., and SHOGREN, M. D. Functional (breadmaking) and biochemical properties of wheat flour components. VIII. Starch. Cereal Chem. 48: 191 (1971).
6. D'APPOLONIA, B. L., and GILLES, K. A. Effect of various starches in baking. Cereal Chem. 48: 625 (1971).
7. DRONZEK, B. L., HWANG, P., and BUSHUK, W. Scanning electron microscopy of starch from sprouted wheat. Cereal Chem. 49: 232 (1972).
8. KULP, K. Physicochemical properties of starches of wheat and flours. Cereal Chem. 49: 697 (1972).

9. SCHOCH, T. J. In: Methods in carbohydrate chemistry, ed. by R. L. Whistler et al. Vol. IV, p. 56. Academic Press: New York and London (1964).
10. LARSON, B. L. GILLES, K. A., and JENNESS, R. Amperometric method for determining the absorption of iodine. Anal. Chem. 25: 802 (1953).
11. SCHOCH, T. J. In: Methods in carbohydrate chemistry, ed. by R. L. Whistler et al. Vol. IV, p. 161. Academic Press: New York and London (1964).
12. SCHOCH, T. J., and MAYWALD, E. C. Microscopic examination of modified starches. Anal. Chem. 28: 534 (1959).
13. LEACH, H. W., McCOWEN, L. D., and SCHOCH, T. J. Structure of the starch granule. I. Swelling and solubility patterns of various starches. Cereal Chem. 36: 534 (1959).
14. LEACH, H. W., and SCHOCH, T. J. Structure of the starch granule. II. Action of various amylases on granular starches. Cereal Chem. 38: 34 (1961).
15. MEDCALF, D. G., and GILLES, K. A. Wheat starches. I. Comparison of physicochemical properties. Cereal Chem. 42: 558 (1965).
16. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC. The Association: St. Paul, Minn. (1962).
17. TSEN, C. C. Effects of oxidizing and reducing agents on changes of flour proteins during mixing. Cereal Chem. 46: 435 (1969).
18. IRVINE, G. N., and McMULLAN, M. E. The "remix" baking test. Cereal Chem. 37: 603 (1960).
19. MAYWALD, E., CHRISTENSEN, R., and SCHOCH, T. J. Development of starch and phytoglycogen in golden sweet corn. J. Agr. Food Chem. 3: 521 (1955).
20. MATHESON, N. K., and WHESTLEY, J. M. Diurnal-nocturnal changes in starch of tobacco leaves. Aust. J. Biol. Sci. 16: 70 (1963).
21. KULP, K., and MATTERN, P. J. Some properties of starches derviced from wheat of varied maturity. Cereal Chem. 50: 496 (1973).
22. SCHOCH, T. J., and MAYWALD, E. C. Preparation and properties of various legume starches. Cereal Chem. 45: 564 (1968).
23. MOSS, R. A study of the microstructure of bread doughs. CSIRO Food Res. Qtrly. 32: 50 (Sept. 1972).
24. MELCHIOR, H., and FEUERBERG, H. Structure of the starch grains of cereals. Ber. Deut. Bot. Ges. 67: 394 (1954).
25. GUILBOT, A., and LAVAVASSEUR, G. Microscopie electronique structure submicroscopique de l'amidon: Examen de grains en coupes minces au microscope electronique. C. R. Acad. Sci. Paris 249: 2636 (1959).
26. BADENHUIZEN, N. P. Chemistry and biology of starch granules. In: Protoplasmatologia. Springer-Verlag: Vienna (1959).

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