# THE SPECIFIC SURFACE OF FLOUR AND STARCH GRANULES IN A HARD WINTER WHEAT FLOUR AND IN ITS FIVE SUBSIEVE-SIZE FRACTIONS[1]

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## ABSTRACT

The specific surface of starch granules in a Minnesota hard winter wheat patent flour, as obtained from the particle size distribution data using the Whitby centrifuge after proteolysis by Bromelin, was measured to be 0.7708 $m^2/g$. If a correction is applied for the swelling in aqueous medium on the basis that the density decreases by the voluminal addition of water, the specific surface figure reduces to 0.7272 $m^2/g$. This latter figure is about 3 to 4 times larger than that reported formerly on samples prepared either by dough washing in water, drying and resuspension in water, or by digestion in pepsin and dilute hydrochloric acid with drying and resuspension in pentane before microscopic measurement.

The specific surface of the parent flour was determined similarly to that of the starch granules. The value obtained was 0.1249 $m^2/g$. (i.e. about 4.5 times smaller than the total starch granule surface in the same flour).

The ratio of total starch granule surface to the total flour surface in the five subsieve-size fractions of the parent flour increased from 1.34 in the finest fraction to 5.45 in the coarsest fraction, indicating larger starch granule surface exposed to atmospheric conditions with flour of smaller particle size.


The size distribution and specific surface of starch granules within a flour, or rather, their measurements, have intrigued many investigators in the field. Grewe and Bailey (8) measured the amount of starch granules between 0 and 7.4, 7.4 and 14.8, and 14.8 and maximum micron sizes, using a water suspension and the 3.7$\mu$ gridding of a hemocytometer under the microscope. They counted about 700 granules in each of their highly purified starch samples which were obtained by washing in water, drying, deagglomeration, and sieving. Using the data of 17 parent flour samples ranging from 9.73 to 14.45% in protein content, Stamberg (15) calculated the specific surface to be between 0.1780 and 0.2339 $m^2/g.$, assuming spherical granule shape and 4.7, 10.1, and 24.9$\mu$ as average sizes in the three quoted size ranges respectively.

Hanssen *et al.* (10) determined the size distribution of starch granules in 100 flour samples, measuring 250 granules of each sample under the microscope. Samples were proteolyzed with pepsin and dilute hydrochloric acid. After drying, the samples were resuspended

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in pentane for microscopic measurements to eliminate swelling in an aqueous medium. Using Stamberg's method of calculation, they obtained an average of 0.1974 $m^2g.$ for the specific surface of starch granules for the 100 samples.

In a primitive approach Gracza and Norris (7) washed out starch granules from a Kansas hard winter wheat flour in a Waring Blendor. The centrifuged residue was vacuum-dried and gently deagglomerated in an agate mortar. Size distribution measurements were made following Whitby's method (16), using benzene for the sedimentation liquid. Based on the Fisher average particle size for the determination of specific surface as suggested by Kamack (13), the specific surface of starch granules was 0.307 $m^2/g.$ on the basis of size distribution data (7) as suggested by Herdan (11), 0.2676 $m^2/g.$ specific surface was obtained. In the calculation of the specific surface by Fisher the equation

$$S_w = \frac{6}{d\,\rho} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

was used. The surface figure obtained by the size distribution data was calculated using the equation

$$S_w = 6/\rho\ (w_1/x_1 + w_2/x_2 + w_3/x_3 + \ldots) \quad (2)$$

where $S_w$ = weighted specific surface;

$d$ = average particle size by Fisher;

$\rho$ = density of starch granules;

$w_1, w_2, w_3 \ldots$ = weight percentages of the measured individual size ranges of the samples; and

$x_1, x_2, x_3 \ldots$ = mean particle sizes of the corresponding size intervals calculated as median of the respective size ranges.

The size intervals were chosen between 0, 5, 10, 20, 30, 40, 60, and 80 SED micron sizes (16). For starch density $\rho = 1.500$ g./ml. was used.

The methods discussed for the determination of specific surface of starch granules within a flour are rather lengthy. Moreover, there are some details in the procedures which may be questioned, such as loss of smaller starch granules in the washing procedure; the arbitrary character of microscopic measurement in selecting the granules and their dimension for the measurement; the increase of granule size by swelling if aqueous suspension is used in the measurement; the possible size reduction of granules in the Waring Blendor and/or in the deagglomeration procedures; insufficient deagglomeration of granule clusters stuck together in the drying procedure if nonaqueous suspension is used in the measurement; and creation of new surfaces in the grinding of the dried starch sample different from the original

granule surfaces.

Following is a presentation of a method and measurements made to determine the specific surface of starch granules of a hard wheat parent flour and its five subsieve-size fractions. The method is relatively fast and eliminates the major potential sources of error listed above.

## Materials and Methods

An unbleached patent flour having 11.0% protein and 0.40% ash content on 14% moisture basis was milled from Minnesota hard red winter wheat and was subjected to four consecutive air-classification steps yielding five fractions, namely, A, B, C, D, and DD, in order of increasing coarseness (5.).

The Fisher average particle size figures were averages of two runs and were obtained according to the instructions in the manual of the instrument (4).

The protein and starch contents were determined as described in *Cereal Laboratory Methods* (1) and identified by the numbers 67.1 and 86.1 respectively. The latter was somewhat modified: instead of a 5-g., only a 2-g. sample was used, and the dextrinization of malt was replaced by acid hydrolysis. The size distributions of the flour samples were obtained by the use of the Whitby sedimentation method (16).

The size distribution of starch granules within the flours was determined as follows: 0.25 g. of Bromelin concentrate[4] was dissolved in 40 ml. distilled water using a 250-ml. Erlenmeyer flask. After dissolving, the Bromelin was added to 10 g. of flour of about 10% moisture content in a 600-ml. beaker. Additional distilled water, 50 ml., was used to rinse the flask and the sides of the beaker while the flour was stirred into a smooth slurry. The beaker containing the slurry was placed in a 45°C. water bath and the contents were gently stirred. After a 1-hour digestion time the slurry was transferred to a 250-ml. centrifuge tube and centrifuged for 15 minutes at 1,600 r.p.m. in an International Centrifuge, size 2, model V. The supernatant was discarded. The residue was resuspended in water to almost fill the centrifuge tube and then stirred into a homogeneous mixture to eliminate the effect of size and slight protein gradation which occurred in the centrifuge tube. A 500-mg. sample of this paste was suspended in 45 ml. of ethyl alcohol in a Waring Blendor and stirred for 105–110 seconds. A sample was immediately withdrawn with a long eyedropper, and placed in the feeding chamber of the sedimentation tube. Distilled water was used for the sedimentation liquid.

[4] Undiluted concentrate of Takamine Laboratories, Clifton, N. J. Lot 14-1; 1225 GDU.

The determination of the size distribution of the digested sample followed the method described by Whitby (16). The following schedule was calculated, using the viscosity of water at 24°C. and 1.297 g./ml. for the density of the wet Bromelin-extracted residue as measured by the use of a pycnometer.

| *Size* | | *Time* |
|---|---|---|
| *SED micron* | *r.p.m.* | *minutes:seconds* |
| 120 | Gravity | :17 |
| 100 | Gravity | :24 |
| 80 | Gravity | :36 |
| 60 | Gravity | 1:07 |
| 40 | Gravity | 2:29 |
| 30 | Gravity | 4:27 |
| 20 | 300 | :33 |
| 10 | 300 | 3:51 |
| 5 | 600 | 4:08 |
| 0 | 1200 | ... |

The sedimentation data thus measured starch granules which became slightly swollen during the digestion and sedimentation procedures. The starch granule distribution data presented are averages of three determinations of a single suspension on each sample.

The increase in granule size due to swelling was estimated by comparison of densities of the Bromelin-extracted residue and that of the starch in the flour. In the estimation it was assumed that the decrease in density upon hydration of the starch granules is caused by addition of water to the granules having 1.500 g./ml. density at about 10% moisture level.

Using the symbols:

$d_1$ = size of starch granule in flour before swelling (cm.),
$d_2$ = size of swollen starch granule in the residue (cm.),
$\rho_1$ = density of starch granule before swelling (g./ml.),
$\rho_2$ = density of swollen granule (g./ml.),
$\rho_3$ = density of water (g./ml.),
$w_1$ = weight of granule having $d_1$ size (g.),
$w_2$ = weight of granule having $d_2$ size (g.), and
$w_3$ = weight of water absorbed in swelling (g.),

four equations can be staged for the weights of the granule before swelling, after swelling, and for the weight of absorbed water.

$$w_1 = \pi/6 \cdot d_1^3 \cdot \rho_1 \quad (3)$$
$$w_2 = \pi/6 \cdot d_2^3 \cdot \rho_2 \quad (4)$$
$$w_3 = \pi/6 \, (d_2^3 - d_1^3) \, \rho_3 \quad (5)$$
$$w_3 = w_2 - w_1 \quad (6)$$

The equations can be solved for the relation of granule densities and sizes using 1 g./ml. for the density of water

$$d_1 = d_2 \cdot \sqrt[3]{\frac{(\rho_2 - 1)}{(\rho_1 - 1)}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

Substituting for $\rho_1 = 1.297$ g./ml. and for $\rho_2 = 1.500$ g./ml.,

$$d_1 = 0.842\, d_2$$

i.e., a swelling factor of 0.842 is obtained which represents a 19% increase in the diameter of a spherical granule upon hydration to the condition described. This compares with 30.5% as measured by Hanssen and co-workers (10), who determined this increase in granule size using absolutely dry, water-washed starch suspended in water at 20°C.

The specific surface of the flours and proteolyzed flours were calculated using equation 2, the median sizes of the 0–5, 5–10, 10–20, 20–30, 30–40, 40–60, 60–80, and 80–100 SED micron size ranges, 1.440 g./ml. density for flour, and the 1.297 g./ml. density for the swollen granules in the residue. The specific surface corrected for swelling was calculated from the medians of the same size ranges, adjusted, however, by the $d_1 = 0.842\, d_2$ swelling factor as explained above.

The nitrogen content of the Bromelin-extracted residues varied with different flours and fractions from 0.54 to 1.88% in terms of protein content on 14% moisture basis. Microscopic observation of the homogenized paste suspended in ethyl alcohol, however, could detect neither a gluteninaceous matrix on the surface of the individual starch granules nor starch granules stuck together in clusters.

## Results

Table I compares the particle size distribution data of the hard wheat *flour* and its five subsieve-size fractions with the size distribution of the *starch granules* in the same parent flour and flour fractions. It is shown that the starch granules of a flour yield a finer size distribution than the flour particles of their individual parent flours. The amount of granules in the 0–5 SED micron size range decreases in the coarser fractions, A, B, and C, and increases in the coarsest fractions, D and DD. These latter fractions contain increasingly more endosperm chunks and consequently slightly more of the smaller starch granules with the endosperm chunks. These observations are expected from morphological studies on flours and flour fractions (5).

Table II presents a comparison of the particle size and specific surface relations of the *flour* samples and those of the *starch granules*

TABLE I

PARTICLE SIZE DISTRIBUTION DATA OF A LONG-PATENT HARD RED WINTER WHEAT FLOUR AND ITS FIVE SUBSIEVE-SIZE FRACTIONS COMPARED TO THEIR BROMELIN-DIGESTED RESIDUES

(Showing size distribution of starch granules within individual samples)

| | SIZE | PARENT | SUBSIEVE-SIZE FRACTIONS | | | | |
|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | DD |
| | *SED μ* | *% finer than size* | *% finer than size* | *% finer than size* | *% finer than size* | *% finer than size* | *% finer than size* |
| Flour series[a] | 100 | 100.0 | | | | | 100.0 |
| | 80 | 95.1 | | | | 100.0 | 96.2 |
| | 60 | 68.2 | | | 100.0 | 99.6 | 69.6 |
| | 40 | 37.7 | | 100.0 | 98.7 | 94.9 | 25.4 |
| | 30 | 28.0 | 100.0 | 99.6 | 89.2 | 72.7 | 12.7 |
| | 20 | 15.8 | 95.4 | 89.7 | 47.3 | 25.8 | 3.8 |
| | 10 | 2.4 | 58.4 | 45.5 | 9.5 | 2.3 | 0.0 |
| | 5 | 0.0 | 16.9 | 17.6 | 4.1 | 0.0 | 0.0 |
| Bromelin-digested flour series[b] | 30 | 100.0 | | | 100.0 | 100.0 | 100.0 |
| | 20 | 89.7 | 100.0 | 100.0 | 89.9 | 82.6 | 87.0 |
| | 10 | 43.8 | 90.7 | 61.5 | 36.5 | 33.7 | 39.1 |
| | 5 | 27.7 | 61.8 | 38.0 | 18.3 | 20.1 | 23.1 |

[a] Sedimentation liquid, benzene; 1.440 g./ml. density used in the calculation of time scheduled for flour particles.

[b] Sedimentation liquid, distilled water; 1.297 g./ml. density used in the calculation of time scheduled for slightly swollen starch granules.

isolated from the same flour samples by proteolysis. The size and surface data employ different modes of expression which have been recently cultivated in the art.

The specific surface of the starch granules within the selected hard winter wheat flour is 0.7708 $m^2/g.$, if it is calculated from the size distribution data of the starch granules which became slightly swollen in the digestion and sedimentation procedure. If the sizes of the granules are corrected, in the measured ranges, by the 0.842 swelling factor as derived from density measurement, this figure reduces to 0.7272 $m^2/g.$, or about 3 to 4 times larger than reported by previous workers (10,15). Such discrepancies are believed to stem mainly from the selection of procedures for sample preparation, but also from the different measuring principles involved (microscopics vs. sedimentation). While in the water-washing procedure a considerable amount of starch granules could not be recovered, in the present method care was exercised not to lose any, since a small amount of little granules may account for considerable surface loss.

The total surface of starch granules in the flour is calculated from the corrected specific surface and starch content of the flour on a dry basis, as the use of starch content in flour on 14% moisture basis

## TABLE II

ANALYTICAL, PARTICLE SIZE, AND SURFACE DATA OF A LONG PATENT HARD RED WINTER WHEAT FLOUR AND ITS FIVE SUBSIEVE-SIZE FRACTIONS
(Using various expressions for the size as recently cultivated in the art)

| | | Yield | Protein Content on 14% Moisture Basis | Starch Content on Dry Flour Basis | Specific Surface[a] Based on Median Size of Size Ranges | | | Total Starch Surface[a] on Dry Flour Basis | Ratio of Starch Granule Surface to Flour Surface |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | (a) | (b) | (c) | (c) | |
| | | % | % | % | $m^2/g.$ | $m^2/g.$ | $m^2/g.$ | $m^2/g.$ | |
| Parent flour | | 100.0 | 11.0 | 78.0 | 0.1249 | 0.7708 | 0.7272 | 0.5672 | 4.55 |
| Fraction | A | 3.5 | 19.0 | 66.3 | 0.6249 | 1.3448 | 1.2652 | 0.8388 | 1.34 |
| | B | 8.7 | 13.1 | 72.1 | 0.5870 | 0.9640 | 0.9069 | 0.6539 | 1.12 |
| | C | 17.1 | 7.3 | 77.9 | 0.2853 | 0.6324 | 0.6055 | 0.4717 | 1.65 |
| | D | 9.7 | 7.2 | 79.3 | 0.1866 | 0.6374 | 0.5895 | 0.4675 | 2.51 |
| | DD | 61.0 | 10.9 | 77.2 | 0.0940 | 0.6957 | 0.6658 | 0.5140 | 5.46 |
| Sum of fractional surfaces | | | | | 0.1977 | 0.7252 | 0.6837 | 0.5221 | |

| | | Fisher Average Particle Size[a] (a) | Mass Median Size[a] (MMS) in SED Micron[a] | | | Specific Surface[a] Based on Average Particle Size Based upon: | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | (a) | (b) | (c) | Fisher (a) | MMS[b] (a) | MMS (b) | MMS (c) |
| | | $\mu$ | SED $\mu$ | SED $\mu$ | SED $\mu$ | $m^2/g.$ | $m^2/g.$ | $m^2/g.$ | $m^2/g.$ |
| Parent flour | | 18.4 | 48.5 | 11.2 | 9.4 | 0.2264 | 0.0859 | 0.3571 | 0.4920 |
| Fraction | A | 4.6 | 8.7 | 3.9 | 3.3 | 0.9057 | 0.4788 | 1.0256 | 1.4018 |
| | B | 7.2 | 10.7 | 7.5 | 6.3 | 0.5786 | 0.3793 | 0.5333 | 0.7343 |
| | C | 11.4 | 20.1 | 11.8 | 9.9 | 0.3654 | 0.2073 | 0.3380 | 0.4673 |
| | D | 16.6 | 25.0 | 12.8 | 10.8 | 0.2596 | 0.1666 | 0.3125 | 0.4283 |
| | DD | 27.0 | 51.0 | 11.7 | 9.8 | 0.1543 | 0.0817 | 0.3419 | 0.4720 |

[a] (a), Flour sample; (b), proteolyzed residue of flour; (c), proteolyzed residue, size corrected for swelling.
[b] MMS, mass median size.

would assume that no moisture is present within the architecture of starch granules. A comparison of the 0.5672 $m^2/g.$ and 0.1249 $m^2/g.$ values indicates that the total starch granule surface within the tested hard wheat flour is about 4.5 times larger than the total surface of the flour.

While the specific surface of the flour fractions decreases with increasing average flour particle size, the specific surface of the starch granules within the flours follows the presence of smaller starch granules. They are responsible for the large total starch surface in the fine fraction and for the slightly larger total starch surface in the coarsest fraction, DD, as compared to D fraction, which is relatively finer but has also smaller total starch surface.

The ratio of total starch granule surface to the total flour surface in the flour fractions increases from 1.34 to 5.46 with increasing flour particle size. This would indicate less starch granule surface exposed to atmospheric conditions with larger flour particle sizes.

The Fisher average particle size and another average, namely the mass median size (MMS), and the derived specific surface values are given in Table II. They are presented to demonstrate by simple comparison the errors introduced by these vague, rather invalid approaches. Data indicate that the errors introduced into the specific surface measurements by Fisher increase the surface figure as compared to those obtained by sedimentation and summation of the surface values in the measured size ranges. If the average size parameter of MMS in SED micron size is used, the figures are lower, as is expected from the definition of the mass median size.

The accuracy of specific surface measurements of starch granules within a flour sample is dependent on definitions, assumptions, sample preparations, measuring, and calculating methods. Some of the errors entering into the method presented may be estimated by the sum of the fractional surfaces proportional to their yield. This sum, in an ideal case, should be equal to the specific surface of the parent stock. In the flour fraction series (a), the calculated sum of proportionate fractional surfaces is 0.1977 $m^2/g.$ This is 58.2% larger than measured in the parent flour; 0.1249 $m^2/g.$ This is explained in that considerable size reduction occurred within the classification procedure. In the proteolyzed series (b), in the proteolyzed swelling-corrected series, (c), and in the starch granule series on flour basis, the sum of the proportionate fractional surfaces is 0.7252 $m^2/g.$, 0.6837 $m^2/g.$, and 0.5221 $m^2/g.$ respectively; that is, 5.9, 6.0, and 8.1% smaller than measured in their respective parent stocks.

## Discussion

Individual investigators had different goals when they pursued the measurement of size and/or specific surface of starch granules within the cereal endosperm or in the flour manufactured from it.

Naudain (14) intended to characterize the quality of wheat flour by the number and size of the starch granules: the larger portion of small starch grains indicated to him a good grade of flour. Stamberg (15) wished to index baking quality of a flour by its starch granule size distribution. He succeeded in showing higher absorption at constant protein and dough mobility levels with smaller starch granules. Dadswell and Wragge (3) measured the "average" diameter of starch granules for four different varieties grown at four places. They found that "the variation in volume distribution of the granules due to environment is of approximately the same magnitude as that due to variety."

Hanssen's motivation for the measurement of the starch granule surface (10) may have been his conviction (9) that the interaction between water and flour plays a determining role in the baking procedure; thus he suggested investigation of the two most important components of flour; namely, starch and protein. The interest of Bradbury *et al.* (2) was the histological description of wheat endosperm. Gracza and Norris (7) demonstrated that flour fractions air-classified from the same flour stock and having equal protein content but different specific surface and/or specific starch granule surface had different rheological properties (mobility at constant absorption level and extensigraph properties) and consequently different baking characteristics.

From this short review it appears that the use of starch granule size and/or surface measurement in flour is in the field of flour hydration or dough and batter rheology, which ultimately leads to the characterization of flours for baked goods.

The importance of surface relations in the hydration phenomena of a flour needs to be assessed in view of its heterogenic character. Histological studies (2,5) suggest at least two major factors for this heterogeneity, namely, protein matrix and starch granules. Generally, the former loses its identity upon water addition and mixing and becomes part of the continuous phase of the dough. Here the relative position of the constituent molecules constantly changes. Upon water addition and mixing, the starch granules keep their identity and form the solid phase of the dough. Generally, within the individual granules the constituent molecules do not change their relative position; i.e.,

they form a coherent structure which, depending on its size, surface, and other conditions, is adapted to deplete water from the continuous phase, or vice-versa, changing the consistency of a flour-water mixture. The magnitude and rate of such changes depends upon the specific surface of the flour and/or the size, condition, and specific surface of the starch granules in the flour (7).

It was shown that the lipid content on protein basis extracted from an air-classified low-protein fraction containing mainly larger starch granules is about twice as large as the lipid content on protein basis in an air-classified high-protein fraction containing mainly smaller starch granules (6,12). Such observation upon flour fractions of common parental origin may indicate that the lipids are not homogeneously distributed in the protein matrix; i.e., lipids are concentrated in proteins associated with larger starch granules, or there is more lipid associated with a unit weight of smaller starch granules than with a unit weight of larger starch granules, or the 5.7 protein factor is doubtful in characterizing the volume of the protein matrix of the endosperm, or perhaps something else.

Past efforts for using surface indices may have failed or succeeded only in a limited manner because of crude measuring techniques which introduced many artifacts into evaluations such as those listed in the introduction, and especially by the combination of water-washing, drying, and deagglomerating. However, the interest of flour producers and flour users is attached primarily to the starch granules which are not denatured but possess the condition in which they occur in the endosperm of the mature wheat kernel in its virgin state or in the flour which is milled from the wheat.

Improved, reliable measuring methods, together with proper assessment of importance order for the role of starch granules in dough rheology, place the specific surface of flour and/or that of starch granules in a flour among the advantageous flour indices which are amenable for interpretation not only by the biologist, chemist, physicist, and others in the laboratory, but also by the practical manipulators in the plant.

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