TYPE OF BREAKAGE OF CELL WALLS IN FLOUR MILLING

by

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INTRODUCTION AND REVIEW OF LITERATURE

There has been doubt in the minds of millers as to how cell walls in the starchy endosperm of the wheat kernel break in flour milling. Previous studies have not removed that doubt because they have not been definitive. The problem is a practical one, however. It is especially so at this time, when special methods of grinding and particle size classification are being used to produce flour fractions of low and high protein content, respectively, for special uses. What happens to cell walls in milling is then directly related to where the walls appear in classified fractions; this, in turn, affects the properties of the fractions. The purpose of the present study was to determine how cell walls are broken in milling.

The principle object of flour milling is to remove the starchy endosperm from the germ and bran layers of the wheat berry, and subsequently to reduce the endosperm to flour. The gross anatomy of the mature wheat kernel and the structure of its major parts have been described in detail by Bradbury, Cull and MacMasters (7) and Bradbury, MacMasters and Cull (8, 9, 10). The work of Bradbury and her co-workers clarified many points on structure of the wheat kernel for millers and cereal chemists; however, it was essentially limited to the structure of the unground grain. From the photomicrographs and the discussion in that work, it is obvious that the starchy endosperm of wheat has a cellular structure, as does most of the rest of the kernel.

The cellular structure of plants is basic to all elementary botany. A typical discussion is that in the text by Transeau, Sampson and Tiffany (37). However, a more detailed discussion can be found in the text by Esau (12). The main ideas to be obtained from the discussion by Esau and

other botanists are the following: the content of each cell is contained by a cell wall; the cell walls of adjacent cells are cemented to each other by an intercellular substance; the intercellular substance is composed chiefly of pectic compounds, whereas the cell walls are composed of cellulose, hemicellulose and pectic substances; and the intercellular substance, which is present as a thin layer, is called the "middle lamella". This is not a new concept, but one that has been established and presented to botany students for years. In addition, the middle lamella is so strong that chemical maceration is required to hydrolyse it. A careful study of the work by Bradbury, MacMasters and Cull (9) shows that the above is an adequate description of the cell structure of wheat starchy endosperm. Since flour milling ultimately amounts to reduction of the starchy endosperm, cell walls must be broken.

If the cell contents in the starchy endosperm are removed from a transection of the wheat kernel, the network of cell walls which remains has the appearance of a honeycomb (24). To the naked eye, and at low magnification (75X) with the microscope, each wall has the appearance of a single filament; however, from the brief discussion above, it is apparent that each wall is really the walls of two adjacent cells with the cementing middle lamella between them. It is these walls that must be broken in the milling process. There are two possible ways for the walls to break: a transverse break across the walls with the middle lamella between them, or along the middle lamella between the cell walls. If both possibilities occur as actualities during milling, possibly one type of breakage could be enhanced, if this should prove to be desirable. This might be accomplished by proper design of milling machinery or by control of the type of pretreatment of the wheat before it is ground. Such a change in processing could amount to

savings or to added cost to the mill, either as power requirement, new equipment to condition the wheat, or more expensive conditioning methods. Advantages gained might, however, offset any additional expense. There also is the possibility that the breaking of the walls occurs in only one way, independent of wheat variety or class, milling procedure, or pretreatment of wheat before milling.

There have been previous studies of the structure of flour particles, but with the exception of a very few, they have been concerned mainly with the starch and protein of the flour. The interest in protein and starch arises from the ability of the miller to prepare many flours by fine grinding and classifying a parent flour. For example, the flour from a wheat which is not suitable for either bread or cake production can be classified into two flours: one excellent for bread production, the other for cake production. Such a separation of one flour into several fractions on a large scale has been possible only since the development of a new milling process, described by Szasz (36), and the refinement of fine grinding mills and of classifiers (25). The application of fine grinding and classification is dependent on the physical characteristics of flour particles. There would always be the possibility of course that wheat of one type might show a different type of breakage of cell walls during milling to form flour particles than would occur in a wheat of another type. Seven classes of wheat are commonly known. Only hard, soft and durum classes are commonly milled.

The classes of wheat: hard red spring wheat, (amber) durum wheat, hard red winter wheat, and soft red winter wheat which are discussed in this paper are described and defined in the United States Department of Agriculture publication "Official grain standards of the United States" (1). Genetically,

durum wheat is different from the other three wheats listed, in that it has 28 chromosomes and the others have 42 chromosomes (3). A wheat belongs to a variety by heritage or it may be a new variety as a result of breeding. Each variety has been assigned to one of the classes, based on characteristics of the grain, which are the results of the genetic factors involved. When the terms "soft wheat" and "hard wheat" are used in the following discussion, the terms refer respectively to soft red winter wheat and hard red winter wheat.

Early in this century, Biffen (6) commented on the differences in types of particles obtained by crushing soft wheats and hard wheats between two iron plates. He described the particles from soft wheats as "a fine soft powder" and those from the hard wheats as "a gritty powder". His observations describe the obvious differences between the flour produced from soft and hard wheats, which even the novice can detect by rubbing a thin layer of each flour between his thumb and fingers. The flour from a hard wheat is characteristically more granular than that from a soft wheat. There are exceptions to this statement which are discussed in the section: Results and Discussion.

Berliner and Rueter (5) investigated these same phenomena, as did Greer and Hinton (18). Greer and Hinton found that when a dry transection of wheat was subjected to a crushing pressure between two glass plates, the ensuing fissures in the hard wheat generally followed the endosperm cell walls and granular particles shaped like the endosperm cells were produced. However, when the same treatment was applied to the soft wheat, the fissures followed indiscriminate paths and produced smaller particles. The photographs accompanying the text of their work are sufficient to convince a

novice of these differences.

Wichser and Shellenberger (40) pointed out that hard red spring wheat flour is more granular than hard red winter wheat flour, both being considerably more granular than soft red winter wheat flour.

Bell (4) commented that soft wheat flours contain many free starch granules and that the flour particles are irregular in shape, while in contrast, hard wheat flour has few free starch granules and the flour particles are prismatic in form. He further stated that known mixtures of hard and soft wheat flour can be used to estimate the comparative percentages of the two types in an unknown mixture of the same flours. The estimate is based on the apparent numbers of free starch granules and on the presence or absence of prismatically shaped flour particles.

Sandstedt and Schroeder (33) have reported the same differences in the reduced endosperm of hard and soft wheats that other researchers have described.

Greer, et al. (19) in a continuation of their previous work, presented data demonstrating a fact that millers have known for years: the granularity of a hard wheat flour can be decreased by increasing the moisture content of the wheat being milled. They also indicated that molybdenum blue preferentially stained the endosperm cell walls a deep blue so that they could be seen on the surface of flour particles.

In a review article, Foerster (14) listed, with drawings, the possible types of flour particles which can arise from the wheat endosperm. These are: wedge protein, small starch granules, large starch granules, particles of endosperm cell wall, particles composed of protein and starch, broken endosperm cells partially covered by cell wall, and two or more endosperm

cells which were adjacent in the wheat kernel, with their adherent cell walls between them and with more or less endosperm cell wall covering the periphery of the particle. He mentions a large starch granule composed of small starch granules. Composite starch granules do not occur in wheat but do occur in oat and rice starch, as reported in a chapter on cereal starches by MacMasters and Wolff (26). The composite granule that Foerster reported may have been contamination by rice or oats, but probably was a severely damaged large starch granule.

Kent and Jones (23) had published illustrations of similar flour particles earlier than Foerster. However, Kent and Jones were more interested in the cellular structure of flour and the origin of the flour particle from the wheat kernel. A point of significance is that they used flour milled from Manitoba wheat, a hard red spring wheat. It is the class of wheat that is of importance at this point, since it has been previously indicated that flour from hard red spring wheat is more granular than flour from hard red winter wheat and soft red winter wheat. These authors reported that the prismatically shaped flour particles came from the back of the kernel (i.e., the dorsal side of the kernel, directly opposite the crease). Kent and Jones also found that the number of prismatic particles in the flour increased with increasing moisture content of the wheat sent to the mill, over the moisture content range of 12.3 percent to 16.9 percent.

Hence, there is little question about flours of differing granularities being produced from wheats of different classes. Even varietal differences may result in differences of granularity of flour from the wheats of a single class. The milling process, too, can cause differences in granularity; however, the economics of the industry tends to result in uniformity. Finally, the moisture content of the wheat going to the mill has an effect on the flour

granularity. The result is that flour usually has particles ranging in size from one or two microns in all three dimensions to particles having dimensions as great as 60 μ X 120 μ X 300 μ (19). It is not too unreasonable to attempt to separate a conventionally milled flour, with a range of particle sizes as that described above, into fractions of different sizes. As mentioned previously, a cake and a bread flour can be made from a medicare flour. This is what present classification processes accomplish.

Wichser and Shellenberger (41) studied various methods for determining flour particle size distribution. A few of the methods, microscopic analysis for example, obviously could not be used to separate a flour into several fractions commercially, but others like sieving and air elutriation could be so used. Wichser, Shellenberger and Pence must be credited for the majority of the early work which eventually resulted in some of the current commercial flour classification methods. Their work definitely indicated the possibilities, for they concluded their publication with this statement: "Air separation can eliminate the starchy, low protein, high ash material which tends to lower the baking quality of a flour." (42).

Wichser and Shellenberger (41) pointed out the drawbacks of using a sieving technique, mainly that the technique proved most useful when only one sieve rather than a stack of sieves was used at a time. Although the sharpness of the separation was best with one sieve, the process was time consuming and, as could be expected, rather low in capacity.

The air elutriation method is based on the terminal velocity of a falling particle. The formula for the calculation of the terminal velocity contains a term for the density of the particles being removed. In theory, this would be a good technique to employ in separating a flour into several

fractions, since materials composed chiefly of protein and those composed chiefly of carbohydrate would not be expected to have the same density. By successively subjecting the coarse fraction of a flour to a stream of air with subsequently increased velocity, Wichser and Shellenberger (41) separated a straight grade hard red spring wheat flour into six fractions. The separation of a flour into several fractions by an air classifier is based on particle density and other principles; but the separation is better than by sieving since the fractions theoretically cover a small specific range, like 18-38 μ, rather than 0-38 μ for a sieve (42).

In another study, Wichser, Shellenberger and Pence (42) separated a straight grade hard red winter wheat flour into eight fractions by using sieves. A very interesting result was that the fraction with the smallest particle size range, 0-38 μ, was lowest in protein content, even lower than the parent flour. This is easily explained, since most of the free starch would be included in this fraction, thereby diluting the protein. However in fractions of flour obtained by the use of air classifiers, the smallest particle size fraction is the richest in protein (15, 16, 17, 31, 34, 35).

Wichser and Shellenberger (41) also used a sedimentation procedure to study flour particles. The apparatus required a liquid medium of a definite specific gravity. Since wheat flour forms a dough when mixed with water, a non-aqueous medium was used. Wichser and Shellenberger used a mixture of carbon tetrachloride and naptha to separate a straight grade hard red spring wheat flour into eight fractions. Hess (20) used mixtures of chloroform-ether and chloroform-benzol to obtain a protein fraction and a starch fraction from a parent flour. Hess did not describe the type of wheat flour used, but since he worked in West Germany, it was probably a

soft wheat flour. The practical application of separation of a flour into fractions in a liquid medium has several disadvantages: the flour fraction must be recovered from the liquid medium, costs for the medium and its recovery for reuse are introduced into processing, and most important, at least here in the United States, Food and Drug Administration regulations do not permit toxic residues in foodstuffs destined for human consumption. Toxic residues could be expected from the use of solutions which contain carbon tetrachloride or chloroform. Classification of a flour by a sieving method, or by a method which employs an air stream, eliminates this hazard.

The commercial application of air classification to flour has attained great importance within the past decade. In general, a conventionally milled parent flour is subjected to air classification after fine grinding. Air classification is not the only method used to fractionate a flour. Since one company owns the patent rights to the method described by Sasse (36), it is unlikely that it will be used by the entire industry for a number of years. The result of classification procedures, in theory, is a "protein-rich" fraction and a "protein-poor" fraction. In actual practice, a series of fractions of varying compositions is obtained, grading from "protein-poor" to "protein-rich". These are recombined, as desired, to yield two, three, or more flours.

Henninger (29) and Auer (2), both employees of Miag, reported on the general distribution of finely ground flour particles after air classification: the 0-18 μ fraction contained small starch granules, wedge protein, and endosperm cell walls; the 18-45 μ fraction contained large starch granules, and particles of similar size which were lumps of protein and starch;

the fraction containing particles larger in diameter than 45 μ was the unreduced endosperm. It is only fair to point out that this was the description of an ideal situation, for Fig. 9 in Henninger's paper clearly includes a cell wall particle with the particles that are in the 24-39 μ size range. This also emphasizes the fact that the separation that is obtained is not perfect, and probably none ever will be.

Although research has been done with hard wheat flours, the main industrial application of fine grinding and air classification has been to soft wheat flours. It is customary to finely grind the parent flour before the classification steps if optimum results are desired, however fine grinding is not necessary to obtain flour fractions. It is much easier to disrupt soft wheat flour with less damage to the starch than it is to similarly treat a hard wheat flour. There is more to be gained by classifying soft wheat flours than hard wheat flours since soft wheat flours are generally lower in protein content, thus their relative protein content can be "increased" to levels suitable for bread production.

Greene (16) has air classified a conventionally milled soft wheat flour into protein-rich and protein-poor fractions without an intervening fine grinding of the flour. In a later study, he (17) air classified a hard red spring wheat flour without fine grinding it. He concluded that soft wheat flour was more responsive to air classification, and that the low protein fraction was most suitable for cake production. The low protein fraction of the spring wheat flour was best suited for pastry production and the high protein fraction for bread. It is possible that the classifier that Greene used was the same type as that described by Lykken and Lykken (25), which contains a pulverizer.

Sullivan, Engebretson and Anderson (35) subjected a hard red winter wheat flour to fine grinding before air classification. The results of these workers, in principle, was the same as that of previous workers, i.e., the fine flour fraction was much higher in protein content than the parent flour and the coarse fraction was richer in starch, thus lower in protein.

Pfeifer and Griffin (31) and Stringfellow, Pfeifer and Griffin (34) have studied an impressive array of flours milled from different classes of wheat, and the fractions obtained from the parent flours by fine grinding and then air classifying. They, like other workers, have always obtained a protein-rich fraction, of small particle size, and a starch fraction, whose particles were much larger. They report that a starch fraction with 3 percent or less protein content can be most easily obtained from a soft wheat flour. They add that this starch fraction can be used industrially as a source of starch.

More recently, Gehrig (15) has reported the high points of a conference on fine grinding and air classification of wheat flours. One of the conferees, D. B. Pratt, of Pillsbury Co., stated that the important role of air classification is to remove a fraction from a conventionally milled flour, a fraction which does not contribute to the final use for which the whole flour is intended. This means that two new flours can be made from one. Pratt termed air classification, in addition, a research tool.

British workers, Jones, Fraser and Moran (22), have explained that fine grinding of a hard wheat flour disrupts particles of scutellum (a part of the wheat germ) and aleurone layer (the outermost layer of endosperm cells), which are present in the parent flour, into small particles

which appear in the high protein fraction of the fractionated flour. Since the scutellum and aleurone layer contain relatively high amounts of the B-complex vitamins, this is said to explain the increase of niacin and thiamine content in the fine fraction obtained from a hard wheat flour. A similar increase in niacin is found in the fine fraction of a soft wheat flour which has been finely ground and air classified. Riboflavin content in the fine fractions of soft and hard wheat flours is also enhanced by fine grinding and air classification, while the pyridoxine and pantothenic acid contents are enhanced in the fine fraction of only the hard wheat flour.

The results of the workers cited above indicate that there are advantages to be gained by fine grinding and air classification of flour in some cases; however, the wheat endosperm must first be subdivided in some way to gain the greatest benefits. With the reduction of the wheat endosperm, cell walls must be broken from the cell contents. In regard to the classification of a flour, only Henninger (29) and Auer (2), as previously mentioned, have shown the presence of cell wall particles in the flour fractions. Photomicrographs published by Greene (16, 17) and Stringfellow, Pfeifer and Griffin (34) do not show the presence of cell wall material in any of the air classified fractions; this is somewhat surprising when compared with the results presented by Henninger (29) and Auer (2) but may be the result of selection of fields to be photographed. Although Kent and Jones (23) were studying only a conventionally milled flour, they presented some very good illustrations and photomicrographs of cell wall particles.

The discussion presented by Kent and Jones (23) contained two

suggestions for the breakdown of the starchy endosperm to form flour particles. As a result of either, a cell wall may be part of a flour particle, or may itself constitute a flour particle. Briefly stated, the break may be between the cell wall and its contents, i.e., the cell wall must first be broken transversely, and/or there may be a break down the middle lamella between the cell walls of adjacent cells. This latter possibility seems improbable in view of the difficulty of obtaining such a break in most plant material; however, it has been supported by some workers at technical conferences. Whitby and Sandstedt have strongly suggested this possibility in discussions following presentations of papers ((39) and (32), respectively), but they have not published their suggestions.

This unanswered question as to where the break occurs led to the present study. The present problem was to determine if the necessary breaking of wheat endosperm cell walls during milling is transverse or along the middle lamella. The approach was to exhaustively examine flour samples to determine the type of breakage of cell walls. Flour was examined from different mill streams, from wheat of differing geographic origin and class, and from wheat conditioned in different ways.

MATERIALS AND METHODS

Stains

Colloidal molybdenum blue ($Mo_3O_8 \cdot H_2O$), which has several synonyms, including blue molybdenum oxide and colloidal molybdenum tritoctoxide (27, 28), but is usually referred to as molybdenum blue, was prepared in the manner

described by Mellor (28). Two grams of molybdenum trioxide were added to twenty-five milliliters of distilled water in a dropping bottle with a bulb pipette (eyedropper) for a closure. One gram of metallic molybdenum was added to the aqueous suspension of molybdenum trioxide. The bottle was left undisturbed at the prevailing room temperature, approximately 25°C, to allow the reaction to occur as in the following equation:

$$Mo + 2\,MoO_3 + O_2 + H_2O \longrightarrow Mo_3O_8 \cdot H_2O.$$

The reaction proceeds slowly, but its occurrence can be detected by the formation of the dark blue complex which is colloidal molybdenum blue. After two weeks, the solution in the bottle was almost all blue, however the very deep blue colloidal solution was not entirely uniform in color intensity until almost six weeks after the reactants were placed in the bottle. The reaction is a slow one, but the stain is worth the long wait. The colloidal molybdenum blue solution was used to stain the cell wall particles without further dilution.

In preparation of a pH 8 phosphate buffer (21), 0.68 grams of potassium dihydrogen orthophosphate (KH_2PO_4) was dissolved in fifty milliliters of distilled water. Forty milliliters of a solution of sodium hydroxide (NaOH), which contained 0.20 grams sodium hydroxide in 50 milliliters of distilled water, was added to the phosphate solution. With the aid of a Beckman pH meter, the phosphate solution was brought to pH 8 by adding sodium hydroxide solution dropwise with continuous stirring.

A 0.025 percent solution of Congo red in pH 8 phosphate buffer was prepared by adding 0.10 grams of Congo red to 400 milliliters of phosphate buffer, the preparation of which is described above.

Microscopic Mounts

Microscopic mounts of flour particles were prepared by adding as much flour as would adhere to 1/8" of the end of a dissecting needle to a drop of stain which had been placed on a clean dry microscopic slide. Care was taken to avoid contact of the stain with the dissecting needle. The mount was completed by covering the flour particles and stain with a clean dry glass cover slip. Forceps were occasionally used to carefully place the coverslip on the stain and flour particles to help decrease the amount of air entrapped between the coverslip and the slide. The mount was then observed microscopically. All cell wall particles which could be found in a mount were scrutinized. Depending on the number of particles present in a mount, from 3 to 60 mounts were prepared from each flour sample. At least 50 flour particles composed of or containing cell wall were selected in this random fashion from each sample and examined.

Photographic Equipment

The optical system of a Bausch & Lomb series R model RCL 88 research microscope with monocular body served as the lens for the two cameras used in this study. An Exakta model VX IIa single lens reflex 35 mm camera was used for the color photomicrography. A Bausch & Lomb model L photomicrographic camera with its accessories was used for the black and white photomicrography. An American Optical model 350 microscope lamp was used as a light source for all photographic work. Kodachrome professional film Type A and Type F were used in the 35 mm camera. Supply determined which film was

used. For this study, the results obtained from the two types of film were equal. Kodak Panatomic-X sheet film, a slow speed, fine grain film, was used in the black and white camera. Film exposure was determined by trial and error. A commercial firm processed the color film, while the black and white film was developed in the laboratory with Kodak Microdol-X developer to obtain maximum fine grain development. The University Illustrations Department prepared all black and white photographic prints.

Flour Samples

Samples of flour which had been milled for other research studies were obtained for microscopic examination. Tables 1-5 contain summaries of the flours studied and the milling process involved. The exact details pertaining to all of the flours are not known, in particular, the varieties of some of the wheat, because the wheat came from a commercial source where the varietal identity was lost. The milling process is known for the majority of the flours, but as will be seen in the discussion of the observations, a complete description of a flour's past can almost be pieced together from observing particles of the flour under the microscope.

The Ponca wheat flour designated in Table 1 was used for the majority of the basic studies in this investigation. It and the Bison wheat were milled as follows, with the Ponca sample used here as the example. Flour was milled in the laboratory from a sample of No. 1 Ponca variety hard red winter wheat grown at Stillwater, Oklahoma in 1960. The grain was dry cleaned in a Carter dockage tester and a Forster dry scourer and was then cold conditioned. Sufficient water was added, as the grain was tumbled in

s drum, to raise the moisture content to 15 percent. When all water had been taken up by the wheat, it was allowed to rest for 24 hours in a closed can. The grain was then milled. A flow of four breaks and five reductions on Allis laboratory roller mills and a plane sifter with an 11XX flour cloth was used. Flour of 74.4 percent extraction was obtained. The term "cold" conditioning means that the moisture content of the wheat was adjusted without supplying heat from an external source. The term "straight grade" means all of the flour produced from the wheat. The Bison wheat was grown at Hays, Kansas, in 1960.

Table 1. Straight grade flour experimentally milled from cold conditioned hard red winter wheat.

Sample Number	Variety	Crop Year	Geographic Origin
1	Ponca	1960	Stillwater, Oklahoma
2	Bison	1960	Hays, Kansas

The flours listed in Table 2 were milled by Eustace, and are completely described in his thesis (13) on milling flour from wheat which was conditioned in different ways. Briefly, the milling process for these samples was different from that of the samples in Table 1 by the following: a coarser flour cloth (10XX) was used; Ross laboratory roller mills were used for five breaks and eight reductions; the total grain conditioning time was exactly twelve hours before the wheat was milled; and a Mieg laboratory conditioner was used to heat the grain when the conditioning process included a heat treatment. When the Mieg conditioner was used, timing began when the grain attained the desired temperature. At the end of the heating period, the wheat was

cooled, removed from the conditioner and was placed in a closed can for the remainder of twelve hours when applicable. The wheat was not a cross of the two varieties listed, but the crop harvested from an intentional planting of a mixture of the two wheat varieties mixed in equal amounts before planting.

Table 2. Straight grade flour experimentally milled from Kaw and Ottawa (1:1) varieties hard red winter wheat grown at Manhattan, Kansas, in 1959.

Sample Number	Conditioning Method
3	Cold
4	1/2 hour at 40°C
5	1 hour at 40°C
6	2 hours at 40°C
7	12 hours at 40°C
8	1/2 hour at 50°C
9	1 hour at 50°C
10	2 hours at 50°C
11	12 hours at 50°C
12	1/2 hour at 60°C
13	1 hour at 60°C
14	2 hours at 60°C
15	12 hours at 60°C
16	1/4 hour at 70°C
17	1/2 hour at 70°C
18	1/12 hour at 90°C
19	Wheat immersed in water for 1 hour, excess water drained off, and wheat dried with 50°C air for 3 hours.
20	Wheat immersed in water for 1/12 hour, excess water drained off, and wheat dried with 50°C air for 1 hour.

Flour from different mill streams, listed in Table 3, was obtained while the Kansas State University pilot flour mill was in operation on February 23, 1962. Mill mix A, a mixture of hard red winter wheat from a commercial source, was being ground on this date. The wheat was dry cleaned, washed and cold

conditioned two days before it was milled. A sample of flour was obtained from each flour stream in the mill.

Table 3. Flour milled from cold conditioned 1961 crop hard red winter wheat (mill mix A) on the Kansas State University pilot flour mill.

Sample Number	Mill Stream Represented
21	All
22	Pre-break
23	First break
24	Second break
25	Third break
26	Fourth break
27	Fifth break
28	Fine sizings top
29	Fine sizings bottom
30	Coarse sizings top
31	Coarse sizings bottom
32	First middlings top
33	First middlings bottom
34	Second middlings top
35	Second middlings bottom
36	Third middlings
37	Fourth middlings
38	Fifth middlings
39	Sixth middlings
40	Second quality
41	First tailings
42	1-2-3 break reduct
43	Suction
44	Bran and shorts duster

Samples of a soft red winter wheat flour and the fractions obtained from it by air classification were supplied by DCA Food Industries. The samples are enumerated in Table 4. Sample DCA-1 was a straight grade soft wheat flour from which the other samples were obtained by making seven passes on a Mlag-Walther laboratory classifier. The numbers 30, 25, etc.,

refer to the setting of the air flow valve on the classifier. The coarse fraction was removed in each pass and the residual material became the feed material for the succeeding pass. The flour dust was collected seperately.

Table 4. Flour fractions air classified from a commercially milled soft wheat flour using a Mieg-Walther laboratory classifier.

Sample Number	Sample Identity and Mieg Setting
DCA-1	Parent straight grade soft wheat flour
DCA-2	First pass on Mieg, 30 coarse
DCA-3	Second pass on Mieg, 25 coarse
DCA-4	Third pass on Mieg, 20 coarse
DCA-5	Fourth pass on Mieg, 15 coarse
DCA-6	Fifth pass on Mieg, 10 coarse
DCA-7	Sixth pass on Mieg, 5 coarse
DCA-8	Seventh pass on Mieg, 3 coarse
DCA-9	Residue from seventh pass on Mieg, 3 fines
DCA-10	Flour dust from first pass on Mieg
DCA-11	Flour dust from second and third passes on Mieg
DCA-12	Flour dust from fourth and fifth passes on Mieg
DCA-13	Flour dust from sixth and seventh passes on Mieg

The samples listed in Table 5 were diverse in nature and geographic origin. They were obtained from the Pillsbury Milling Co. The terms "patent", "1st clear" and "2nd clear" refer to the manner in which the total flour produced during milling is subdivided into three flours by selecting the flour produced from certain mill streams and combining these streams. A patent flour contains the flour milled from the prime middlings, and may be from 70 percent to 76 percent of the flour produced from the wheat. The 1st clear flour would be the next 15 to 20 percent, and the 2nd clear the balance of the flour produced. The total adds to 100 percent,

Table 5. Flour milled from cold conditioned wheats of different classes by Pillsbury Milling Co.

Sample Number	Variety or Code	Wheat[a] Class	Crop Year	Where Grown	Milled	Type of Flour
45	Ponca	HRW	1960	Kansas	Pilot mill	Straight grade
46	XT6989	Durum	1961	North Dakota	Commercial mill	Unknown
47	Selkirk	HRS	1961	Minot, N. Dakota	Pilot mill	Patent
48	Selkirk	HRS	1961	Minot, N. Dakota	Pilot mill	First clear
49	Selkirk	HRS	1961	Minot, N. Dakota	Pilot mill	Second clear
50	Unknown	HRS	1961	Russia	Pilot mill	Patent
51	Unknown	HRS	1961	Russia	Pilot mill	First clear
52	Unknown	HRS	1961	Russia	Pilot mill	Second clear
53	Unknown	SRW	1961	Illinois and Indiana	Commercial mill (Turbo milling process)	"Beevo", a fraction of an air classified flour

a HRW - hard red winter, HRS - hard red spring, SRW - soft red winter.

or all of the flour produced. Clear flours are not considered to be the best flour for bread production.

RESULTS AND DISCUSSION

Inconclusive Methods

Plant cell walls are complex structures. They contain mainly cellulose with some hemicellulose and maybe some pectic material, depending upon their origin (12). Norman (30) and Nsau (12) have reported that the middle lamella is chiefly pectic material. Based on the questionable assumption that the middle lamella contains pectic material, the following approaches were attempted but yielded limited success. A pectinase enzymic digestion of flour particles yielded complete solution, as did a chemical digestion with Jeffrey's fluid (10 percent aqueous chromic acid and 10 percent aqueous nitric acid solutions 1:1) (11). Attempts to use I_2KI and H_2SO_4 (11) to stain cell walls were unsuccessful, although this is the conventional stain for cellulose. By this method, Wolf, et al. (38) detected cellulose, sometimes only a trace, in the cell walls of Pacific Northwest wheat.

When flour particles are mounted in water and viewed microscopically, the cell walls are extremely difficult to distinguish, even by an experienced microscopist, because they are very transparent. Molybdenum blue proved to be useful only in detecting the presence of cell wall material. Flour particles sectioned with a microtome, by means of the freezing technique, were less useful for the study than the original flour particles. Attempts to dissect flour particles with glass needles proved to be hopeless.

Photographs of flour particles which were obtained by using a Polaroid Land camera were of limited value since critical sharp focusing was not possible. When black and white photomicrographs were prepared by using the 35 mm camera, a detailed enlargement of the tiny negative was blurry. Kodak Contrast Process Ortho film produced too much contrast when it was used in the sheet film black and white camera.

Specific Stains

Norman (30) has reported that ruthenium red in dilute ammonia, methylene blue, and the safranin stains will stain pectic material, however he cautions that these stains are not necessarily specific. Each of the above stains was tried. If they stain pectic material, and if the middle lamella of the wheat endosperm contains pectic material are questions that are still pending. However, in all cases when the above stains were used, the middle lamella was clearly visible in a cell wall particle viewed in cross section. But the stains produced only a small amount of contrast. When the stain used was Congo red, the resulting contrast between the middle lamella and the cell walls was sufficient to show clearly in photomicrographs.

Congo red stains just about everything in a flour sample (Figs. 1 and 2), in contrast to molybdenum blue which stains only damaged starch granules and cell walls (Fig. 3). As previously mentioned, molybdenum blue stains the cell walls so dark (Figs. 3 and 4d) that its use is limited to detecting the presence of cell walls in a flour sample.

Cell walls were found in flour either as free particles, like those in

Figs. 1, 4b and 4d, or attached to a clump of protein and starch, like those in Figs. 2, 3, 4a, 4c, and 5. Sometimes the entire cell contents had dropped out during milling and the wall resembled a cylinder, like the particles in Figs. 4b, 4c, 4d and 5. All had a middle lamella between the cell walls of two endosperm cells which were adjacent in the wheat kernel.

All of the particles that are shown in Figs. 1 through 5 were found in straight grade flours. The flour milled from the Ponca and Bison wheats contained similar types of cell wall particles as those shown in Figs. 1-5. However, extensive searching was required to find a cell wall particle which had lost its cell contents during milling. Since these two wheats had been cold conditioned before they were milled, it was thought that other milling pretreatments might affect the nature of the cell wall particles.

When each of the flour samples listed in Table 2 was observed microscopically, it was found that the cell wall particles were always composed of a middle lamella between two cell walls. However, an interesting point was that the apparent size of the cell wall particles, which were produced during milling, decreased as the time of heating increased. The difference was most noticeable when the heating time was 12 hours instead of 2 hours. Also, as the heating temperature was increased from 40°C upward to 90°C the cell wall particle size decreased. Even after conditioning at 90°C, the cell wall particles contained a middle lamella between two cell walls. The most interesting samples though were the two that were soaked in water and then dried before milling (Nos. 19 and 20, Table 2). There was a very great number of cell wall particles which were totally free of protein and starch in these two flours. From the appearance of the cell wall particles and the many prismatic shaped flour particles, which lacked a cell wall covering,

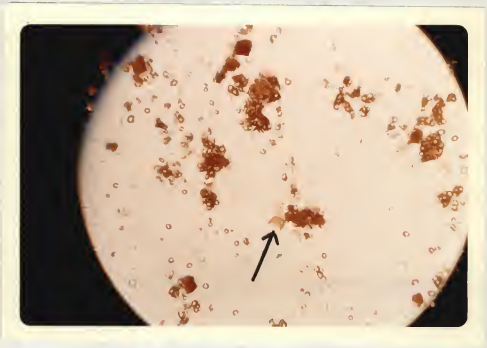

Figure 1. Flour particles of Kaw and Ottawa wheat flour (sample number 3, Table 2) stained with Congo red. The arrow points to a sheet-like cell wall flour particle. Exposure time was 1/25 second on Type A Kodachrome. 300X.

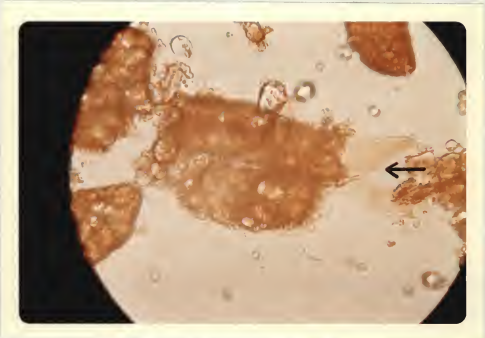

Figure 2. Flour particle of Kaw and Ottawa wheat flour (sample number 3, Table 2) stained with Congo red to show the middle lamella between two cell walls (arrow). Note the filamentous nature of the cell wall projecting to the right, and that the middle lamella is visible only in that portion of the cell wall which is seen in cross section. Exposure time was 1/2 second on Type A Kodachrome. 1335X.

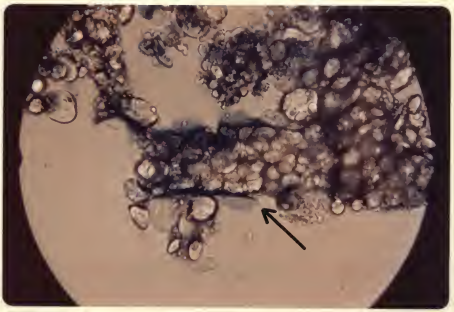

Figure 3. Prismatic shaped flour particle milled from Bison wheat (sample number 2, Table 1) stained with molybdenum blue. The arrow points to the cell wall that is being pealed away from the cell contents, which explains how the cell contents lost part of its cell wall covering (just above the arrow). The exposure time was 8 seconds on Type F Kodachrome film. 1280X.

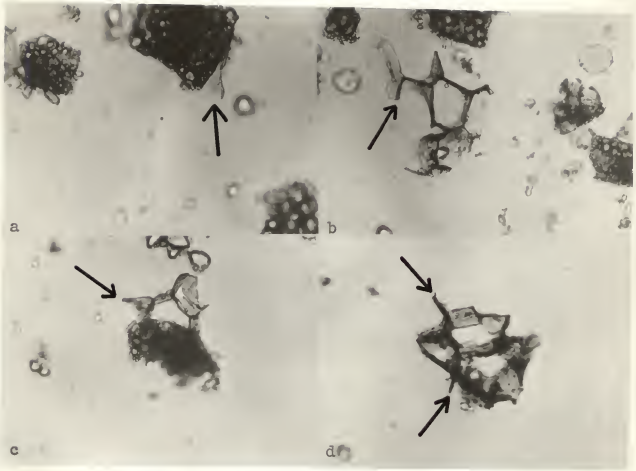

Figure 4. Diverse types of cell wall particles found in Ponca wheat (sample number 1, Table 1). The particles in a, b, and c were stained with Congo red, while the particle in d was stained with molybdenum blue. The arrow in a points to a flap of cell wall that is attached to the contents of an endosperm cell. The particles in b, c, and d have lost their contents during milling; they are referred to as compound cell wall particles in the text. The arrows in b, c, and d point to stumps that remain as a result of the breakage in milling. The magnification (at the negative) is not sufficient to show the middle lamella between two cell walls. The exposure time was 8 seconds on Panatomic-X film. 215X.

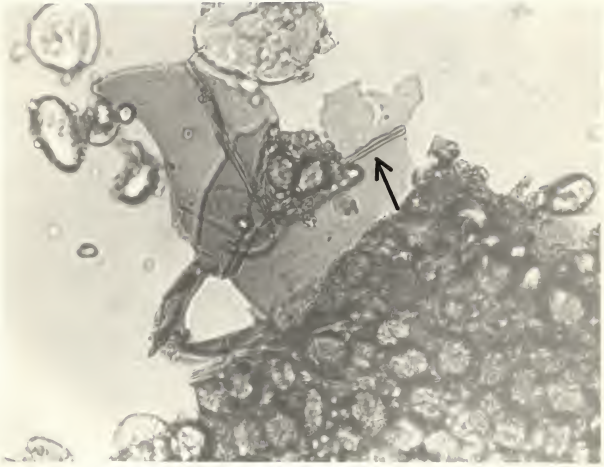

Figure 5. Cell wall flour particle milled from Ponca wheat (sample number 1, Table 1) stained with Congo red. The arrow points to the middle lamella between two cell walls which are viewed in cross section. The exposure time was 16 seconds on Panatomic-X film. 760X.

the impression was that the cell walls had been "peeled" away from the cell contents during milling.

To this point, all flours which had been examined microscopically were straight grade flours. The individual mill stream flours, listed in Table 3, permitted a closer inspection of the possible source of certain types of cell wall particles. Flours from the break system were characterized by sheet-like particles of cell walls, otherwise rather nondistinctive in appearance. (The break flours also contained a considerable amount of bran particles and beards; neither type is considered as a "true" flour particle since neither comes from the starchy endosperm which is the source of "true" flour particles.) The cell wall particles in the break flours were always composed of a middle lamella between two cell walls.

The sizings flours were very granular and contained two particular types of cell wall particles: cell wall attached to an endosperm cell, like that in Fig. 4a, and particles consisting of cell walls of two or more adjacent endosperm cells which had lost their contents during milling, like the particle in Fig. 4d. The latter type may be conveniently referred to as a compound cell wall particle. The flour from the first, second, third and fourth middlings mill streams also contained compound cell wall particles, just as did the flour from the 1-2-3 break redust sifter. The presence of compound cell wall particles in the 1-2-3 break redust flour is not surprising, since the stock going to this sifter is eventually separated into stock for the fine sizings, coarse sizings, 1st middlings and 2nd middlings rolls, where more compound cell wall particles are produced. But, the compound cell wall particles in the 1-2-3 break redust flour were formed in the break system of the mill, not the reduction system, for the flour is

"put in the sack" as soon as it is made. The compound cell wall particles, just as all of the other cell wall particles observed, were composed of a middle lamella between two cell walls. They are particularly difficult to photograph at high magnification, because the depth of field at 356X and higher magnifications is extremely shallow (2 or 3 μ), and the edge of the particle tends to "go over the mountain and down into the valley". This effect can be seen in Fig. 5 very easily. Only a small portion of the middle lamella which was photographed could be brought into sharp focus at one time.

The flour from the fifth middlings, sixth middlings, second quality and first tailings mill streams contained sheet-like cell wall particles. These cell wall particles were like all of those previously discussed, in that they were composed of a middle lamella between two cell walls. However, these flours were not tremendously interesting because by the time the flour had been formed from the stock sent to their respective rolls, it was so pulverized that it looked more like a soft wheat flour than one from a hard wheat. This is typical of the tail-end flours on a hard wheat mill; they are less granular than those flours from the head of the mill.

The bran and shorts duster flour is another tail-end flour, but it is an interesting flour even though it is strongly contaminated by bran particles. It contains a large amount of endosperm cell wall particles which were formed by the vigorous treatment, probably the most severe grinding step in any mill, applied to the bran and shorts by their respective dusters. If wheat were a pig, bran and shorts dusters could be called the machines which remove the "squeal". The endosperm cell wall particles in this flour were like all of the others previously described, i.e., a middle lamella

between two cell walls. In addition, a great number of aleurone cell layer particles were present in this flour. Anatomically, the aleurone cell layer is a part of the endosperm (9), but due to its high ash and non-gluten protein contents, and lack of starch granules, this cell layer is considered to be part of the bran. When a sample of this flour was chemically macerated with Jeffrey's fluid and the process was viewed microscopically, slow solution occurred. The starch and protein dissolved first and any surrounding or attached cell wall material was left so that particles similar to those in Figs. 4b, 4c, 4d and 5 remained. With more time, particles of the starchy endosperm cell wall dissolved and only particles of the aleurone cell layer remained. Finally the middle lamella between the aleurone cells dissolved and the aleurone cells were separated from one another by applying gentle pressure to the glass coverslip. Simultaneously, pericarp particles which were present were also macerated.

Suction flour is obtained by sifting the stock collected from the pneumatic lifts and the purifier dust collectors. The purifiers are the obvious source of many of the cell wall particles found in the suction flour which is also a tail-end stream. In a bucket elevator flour mill, the suction flour would generally come from the dust collected from the purifiers, rolls, and elevator leg suction system. Many of the cell wall particles in the suction flour were sheet-like; these particles probably came from the purifiers. Like all of the other cell wall particles, these were composed of middle lamella between two cell walls. Since cell wall particles were found in all mill stream flours, it was reasonable to assume that similar particles might occur in any or all fractions of an air classified flour. An air classified flour was investigated to confirm this.

The parent soft wheat flour and its fractions are listed in Table 4. The parent flour had the appearance of any soft wheat flour. The flour particles were very irregular as to outline and many free starch granules were present. The edges of the cell wall particles were ragged, but each particle still was composed of a middle lamella between two cell walls. Sample DCA-2 was similar in appearance to DCA-1, the parent flour. It, too, contained cell wall particles composed of the middle lamella between two cell walls, but the surprising observation about DCA-2 was the very small amount of damaged large starch granules in the flour. Similarly, DCA-3 contained predominantly large undamaged starch granules with very few particles of protein and starch in one clump. But the familiar cell wall particles with the middle lamella between two cell walls were present. Large starch granules were predominent in DCA-4 and -5, with some tiny starch granules and cell wall particles like those previously described. There were more of the tiny starch granules in DCA-6 than in any of the previous samples in this series. The cell wall particles in DCA-6 were generally free of adhering protein and starch, and always consisted of a middle lamella between two cell walls.

It was hoped that one of the fractions would have a particularly high concentration of cell wall particles; DCA-7 proved to be the sample in this series which had a particularly large quantity of cell wall particles, whose character was like that of all the other cell walls seen in this study, a middle lamella between two cell walls.

Samples DCA-8 through -13 were very fine, almost like face powder by feel, and to the naked eye they had a dirty appearance. Tiny starch granules and wedge protein were most prominent in DCA-8, with some cell wall particles and mainly tiny starch granules. The protein and starch seemed to be present in almost equal amounts in DCA-9, but cell wall material was present too.

The presence of cell wall material in DCA-10 through DCA-13 was only occasional, in fact it was necessary to use molybdenum blue to identify absolutely the cell wall particles, for they were generally so small that they were difficult to detect and to distinguish from wedge protein with Congo red. Even though considerable searching was required to find cell wall particles in these last four samples which were almost entirely wedge protein and tiny starch granules, cell wall particles were present in all and the particles always consisted of a middle lamella between two cell walls. Pillsbury Beevo (sample number 53, Table 5) is a commercial product whose microscopic appearance was similar to that of samples DCA-9 through DCA-13. The Pillsbury Beevo was found to be a similar mixture of small starch granules, wedge protein and cell wall material. The cell wall particles were just as difficult to find in the Pillsbury Beevo as in samples DCA-10 through DCA-13. The cell wall particles in the Pillsbury Beevo always represented a middle lamella between two cell walls.

All of the studies of the previously discussed samples indicated that cell wall particles are formed during the milling process of soft red winter wheat and hard red winter wheat. Spring wheat flour and amber durum flour, which are listed in Table 5, were observed to obtain information on still other classes of wheat. The samples of Selkirk variety hard red spring wheat flour were extremely granular. They contained an array of cell wall particles: sheet-like, compound, and attached to a particle of flour. The spring wheat flour from Russia was completely different. Its microscopic appearance was more like that of a soft wheat flour rather than that of a flour from hard spring wheat or even of a hard winter wheat flour. It is difficult to know if this Russian spring wheat flour is typical or atypical

of the flour milled from spring wheats grown in Russia. It has been recognized for a long time that environment can change the characteristics of the flour milled from a wheat. If a typical hard wheat is grown in a soft wheat area, the hard wheat will eventually acquire soft wheat characteristics. The flour which is milled from a wheat grown in a new environment could be extremely difficult to classify according to its true wheat class under these circumstances. The Selkirk and Russian spring wheat flours contained cell wall particles, composed of a middle lamella between two cell walls.

The Ponca wheat flour was used early in this study to determine if commercial milling also produced the same type of cell wall particles as does laboratory milling. The answer was, yes.

The flour from the amber durum wheat was particularly granular. Most of the cell wall particles were attached to endosperm particles like the one in Fig. 4a. These cell wall particles were always composed of a middle lamella between two cell walls.

It was found that an absolute requirement for observing the middle lamella was that the cell wall particle must be viewed in cross section or the middle lamella will not be visible at any magnification. In Fig. 5 this is very clear, for the middle lamella is not visible in the portion of the cell wall particle that is not oriented for cross sectional viewing. A cell wall particle can be correctly oriented in a microscopic mount by applying pressure to the cover slip, thus causing the mounting medium to move, and simultaneously tumbling the particles in the mount. The manipulation must be done with caution, or the entire mount may be destroyed.

When sheet-like cell wall particles and compound cell wall particles are formed during milling, since these cell wall particles do not have protein or

starch adhering to them, there are flour particles formed which contain little or no cell wall material. Figure 3 contains a particle of this type. Kent and Jones (23) also observed particles of this type. The arrow in Fig. 3 points to the cell wall material as it is being peeled away from the cell contents. Similar flour particles were observed when Congo red was used as the stain. The Congo red stained the middle lamella in the cell wall of these particles, too. It was quite clear that the middle lamella and the two cell walls that it was between were being peeled away as a unit from the cell contents. If Kent and Jones had used Congo red instead of molybdenum blue, perhaps they would have made the same observation. At any rate, Congo red stains the flour particles so that what has happened during milling can be easily observed.

SUMMARY AND CONCLUSIONS

All endosperm cell wall particles which were found in each of the flour samples observed in this study, when correctly oriented, were composed of a middle lamella between two cell walls. Cell wall particles were found in wheat flour regardless of the geographic origin of the wheat, the wheat class, the milling procedure used, the mill stream from which the flour was obtained, or the subsequent subdivision of the flour into several fractions. Every cell wall particle was always a middle lamella between two cell walls.

It is concluded that cell walls are broken transversely during milling so that each particle of cell wall is always composed of a middle lamella between two individual cell walls.

Perhaps when classification methods are further refined, fractions of

flour may be obtained which do not contain particles of cell walls.

SUGGESTIONS FOR FUTURE WORK

The primary purpose of this investigation was to answer the basic question concerning the breakage of the endosperm cell walls. The results obtained have suggested possible areas for future investigations with regard to the practical application of the knowledge to air classification.

The determination of the density of endosperm cell walls might lead to further refinements in flour classification apparatus and thus an improved technique might be obtained.

A determination of the percentage, by actual counting, of cell wall particles in classified flour fractions would help to define the accuracy of classification.

The effect of soaking and drying wheat before milling might be investigated to determine if a method could be developed for preparation of flour free of cell walls.

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TYPE OF BREAKAGE OF CELL WALLS IN FLOUR MILLING

by

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Millers have used machines for scores of years to reduce the starchy endosperm of the wheat kernel to flour without knowing how the breakage of endosperm cell walls occurred. Previous workers have discussed the occurrence of cell wall particles, and suggested how they may be formed. This study was undertaken to establish definitely how the endosperm cell walls are broken in milling. With the advent of modern milling methods, knowledge of particle formation may be used to advantage in the milling process or in the design of new machinery.

At least 50 flour particles containing or composed of endosperm cell wall, for each sample studied, were viewed microscopically to determine how the cell walls were broken during milling. The flours studied included samples conventionally milled from soft red winter, hard red winter, hard red spring, and durum wheats, and the air classified fractions of a soft red winter wheat. A solution of pH 8 phosphate buffer saturated with Congo red was used to stain the flour particles in microscopic mounts so that the middle lamella in the cell wall of a flour particle could be observed. Molybdenum blue was used to detect the presence of cell wall material in some of the flour samples.

Cell wall particles were found in the flour milled from hard red winter wheat, soft red winter wheat, hard red spring wheat, and amber durum wheat. Cell wall particles were found in the flour of all mill streams milled from a hard red winter wheat, and in all fractions of an air classified soft red winter wheat flour.

Every cell wall particle that was scrutinized microscopically was composed of a middle lamella between two cell walls, as could be demonstrated by proper staining.

From the unanimous results obtained, it was concluded that cell wall particles are formed in only one way during milling: the break is transverse across the cell wall and between the cell contents and the cell wall, so that a cell wall particle always is composed of a middle lamella between two cell walls.