

A COLORIMETRIC STUDY OF FLOUR IN RELATION TO ITS  
ASH CONTENT

by

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## INTRODUCTION

Flour color is one of the most difficult factors to adequately determine and record in flour analysis. Since the color and ash content of flour as milled by the modern process of milling, is influenced more by the presence of minute bran particles than by any other one factor, this study was undertaken to determine if a suitable routine laboratory method could be devised for the extraction of the reddish-brown pigment of bran. Furthermore, it was desired to ascertain if this extracted pigment could be accurately measured, numerically recorded, and the value obtained in such a form that it might be used as a measure of the amount of the bran particles remaining in the flour, which in turn is a measure of the degree of extraction. The latter is usually measured by the ash content of the flour.

Improved analytical methods for the determination of flour grade is one of the urgent needs of the milling industry. As the art of milling has improved, a need has been created for a measure of the quality and the purity whereby the miller, or the purchaser of the products of the milling industry, may determine whether or not the desired standards are being maintained.

It was not until 1890 that the present method of modern flour milling had definitely supplanted the more primitive methods. This change in milling technique permitted a more thorough separation of the endosperm of the wheat kernel from the bran and germ, consequently, the flour made under these conditions was considerably whiter and more free from foreign material than formerly.

Amos (1912), Kosmin (1917), Dedrick (1924), and Bellows (1924) have written very comprehensively upon the history of flour milling. An intimate acquaintance with the technical history and evolution of the art of milling paves the way preparatory to the understanding of the chemical and physical tests now used by the industry judging the products.

The invasion of science into practically all fields of industry also had its influence upon the standards of milling and the development of methods for testing flour. It has become the practice for bakers to buy on specifications, and for the millers to sell on guaranteed analysis. The housewives, too, have become more exacting in their demands when purchasing flour.

Those persons entrusted with the task of determining the quality of flour have found the problem exceedingly difficult because many of the factors which necessarily must

be considered could not be accurately measured or recorded. In describing any object, definite numerical terms are desirable. Lord Kelvin is reported to have said, "When you can measure what you are speaking about and express it in numbers, you know something about it; when you cannot measure it, when you cannot express it in numbers, your knowledge is a meager and unsatisfactory kind."

The ash test has been used by cereal chemists almost universally as one of the important criteria for flour grade. It became popular when Snyder (1904) suggested that the test might be so used. One of the reasons for the popularity of the ash test is that it can be easily determined and numerically expressed. In contrast, unfortunately, the status of the color value of flour has remained largely a matter of personal judgment.

The dislike of the consumer for dark flour is one of the survivals from the early days when wheat was threshed by treading with animals and consequently was contaminated with dirt and filth, which was not removed before the wheat was ground into flour. Since whiteness is usually associated with purity, the color of flour became of prime importance years ago. As early as 1857, Lawes and Gilbert published a paper on the chemistry of wheat and flour in which they said, "It is well known that the poorer classes (of people) almost invariably prefer the whiter bread----."

DISCUSSION OF EXISTING INFORMATION ON ASH  
AND COLOR IN FLOUR

It was the author's original intention to prepare a complete bibliography of the literature pertaining to color and ash in flour. There is no place in milling literature where such a bibliography can be found; therefore although the need for such a compilation is apparent, the task was beyond the resources of the author. Library facilities are not adequate for such a complete study since in important cases complete files of the milling, baking, or chemical journals are not available and obtaining periodicals from other libraries would prove to be a slow method of procedure even when librarians would be willing to offer their cooperation. Therefore, no claim is made for the completeness of this review, but the author is assured that the major papers published on color and ash in flour have been mentioned in this review of literature.

Meaning of Ash

When cereals are incinerated at a dull red heat, all the organic matter becomes volatile and thus disappears, but there always remains a small residue of the non-volatile inorganic material. This residual mineral matter is called ash. When wheat and flour are burned to constant weight it

has been determined that on the average about 1.8 per cent of wheat and 0.40 per cent of flour remain as a residue or ash.

#### Composition and Amount of Ash in Wheat and Mill Products

From available records Mayer (1857) is credited with publishing the first results on the composition of flour ash. He called attention to the fact that the ash content diminished with increasing refinement, but it is interesting to note that his "Superfine" wheat flour contained 0.58 per cent ash, while now our best grades of flour contain about 0.40 per cent, or less, ash.

Dempwolf (1869) was the first chemist to call attention to the composition of the ash of the various mill products. He reported the percentage output of the products, the total ash, and the percentage of  $\text{Fe}_2\text{O}_3$ ,  $\text{CaO}$ ,  $\text{MgO}$ ,  $\text{K}_2\text{O}$ ,  $\text{NO}_2$ , and  $\text{P}_2\text{O}_5$ . Furthermore, he showed that the  $\text{MgO}$  content varied directly with the flour grade, while the  $\text{CaO}$  varied inversely with the flour refinement.

Harding and Dysterheft (1927) have reported the quantity of iron, calcium, magnesium, phosphorus, total ash and protein in wheat, and also in various mill products.

Teller (1896) made a very thorough study of the composition of the ash of wheat and mill products to obtain further information concerning the distribution of the

various ingredients. He concluded that the ash was composed chiefly of the phosphates of potash, magnesia, and lime.

Levy (1910) utilized a microchemical method to determine variations in the quality of cereals. In this work, much importance is given to the relation which exists between the potash, phosphorus, and the gluten. Numerous ash analyses which were gathered from a variety of sources are also reported.

Although other articles appeared previous to that published by Lawes and Gilbert (1884), their paper "On the Composition of the Ash of Wheat Grain and Wheat Straw", represented the accumulation of data from 1844 to 1883 and must place them among the very first workers in this field of research. Table I of their paper records the highest, lowest, and mean per cent of potash and phosphoric acid contained in the pure ash of wheat and wheat straw grown in a series of sixteen consecutive seasons. It is pointed out that the proportion of ash is very much greater in the more branny portions, and that the proportion of potash is about ten-times as high in the dry substance of the bran as in that of the finer flours. The proportion of lime is determined to be four to five-times as high in bran as in flour; that of magnesia fifteen to twenty-times as great; and that of phosphoric acid more than ten-times as high.



As a result of these various researches, our knowledge of the composition of the ash of wheat is quite complete. The following is typical of the results of the analysis of wheat ash: Phosphates ( $PO_4$ ), 43.1 per cent; potash  $K_2O$ , 30.2 per cent; magnesia, 12.9 per cent; lime, 3.0 per cent; sulphate,  $SO_4$ , 0.7 per cent; iron, 0.4 per cent; silica, 0.3 per cent; soda, 0.3 per cent; and chlorides, 0.1 per cent.

The location of ash in the wheat kernel is also important. On an average, the germ, or embryo, contains about 4.8 per cent ash, the bran, 8.6 per cent, and the endosperm 0.4 per cent. It is evident from these figures that even slight contamination of the endosperm by bran or germ particles during the milling process, will materially increase the ash content of the flour produced. The quantity of ash in flour can, therefore, become an index to the completeness of separation of the endosperm from the other portions of the wheat kernel.

#### Ash as a Measure of Flour Grade

Snyder (1904) (1908) was the first to direct the attention of the millers and chemists to the value of the ash test in determining the grade of flour. He contended that the ash is more constant in amount and composition than any other class of compounds found in wheat, the variation

being but slight from year to year. There is a definite relationship between the ash and grade of flour because the more completely the bran, shorts, and germ particles are removed, the smaller is the ash content.

The numerous criteria of flour grade that have been proposed by cereal chemists are enumerated by Bailey (1923). Among these are: ash content, pentosan content, fat, fiber, titreble acidity of water extract, specific conductivity, relative proportion of branny particles, number of wheat hairs per unit of flour, and viscosity. The ash test has proved to be by far the most valuable of any of these criteria.

#### Conditions Affecting the Amount of Ash Constituents of Wheat

Since the quantity of ash in flour has become one of the commonly used means of determining flour grade, it is useful to ascertain what factors influence the incorporation of mineral matter by the growing wheat plant. In general, it may be said that high ash wheat will mill into high ash flour, although many factors may alter this generalization. For example, Shollenberger (1922) has determined that the relative humidity in the flour mill has a decided effect on the ash content. High relative humidity has a tendency to lower the ash content.

However, the work of Sherwood and Bailey (1938) showed that the ash content of straight grade flour could be positively correlated with the ash content of the wheat from which the flour was milled. They also showed that the ash test could not be used as a measure of the percentage extraction of the flour unless something is known concerning the ash content of the wheat from which it was milled.

Lewis and Gilbert (1934) came to the conclusion that season was a much greater influence than fertilization on the quantity of ash constituents in wheat. Since that time, numerous investigators, such as Wiley (1901), Snyder (1908), Shutt (1909), Le Clerc and Leavitt (1910), Ames (1910), Le Clerc and Yoder (1913), and Shaw and Walters (1911), have conducted researches on the conditions affecting the composition of wheat. It would seem that climate will alter the ash content, but the soil on which the wheat is grown is a minor, or negligible, factor influencing the mineral constituents.

Numerous investigators, among whom are Alvey and Clark (1909), have observed that the mineral composition of cereals vary at different stages of growth, and, therefore, the degree of maturity of wheat affects its ash content.

Limitations of the Ash Test as a Grading  
Factor for Flour

It is interesting to note that the person who was first to recommend the use of the ash test to show its relationship to different flour grades, should also be one of the first to contend that the test had been overworked and misapplied. Snyder (1923) makes it clear that the Flour Standards Committee of the United States Department of Agriculture once considered the proposition of grading flour on its ash content but abandoned the idea when it was determined that a combination of various flours could be blended so as to give an ash test, equal to, or lower than, a confessedly better flour.

Snyder (1916) contends that the task of distinguishing between flours of various degrees of commercial quality is complicated by three sets of factors: (1) variation in wheat qualities not susceptible to chemical determinations; (2) lack of uniformity in the milling process; and (3) lack of sufficiently refined methods to distinguish differences in proportion to commercial value.

That the ash test has not been able to establish itself as a standard for flour grade in the courts of law, was also pointed out by Snyder (1914). He, however, states that the ash test when properly made and interpreted is very

valuable, but may also be misleading.

Goldtrap (1917) discusses some of the discrepancies which appear when ash determinations on the same sample of flour are compared, and Snyder (1920) lists several reasons for error in ash analysis. The sources of error are considered under three heads: (1) variations in moisture content of flour, (2) incomplete combustion, and (3) the tolerance present, or lack of standardization in analytical weights.

No one test is a reliable indication of flour grade, in the opinion of Lawellin (1923), but the ash test, when part of a complete analysis, is very important in judging the grade, value, and milling of any flour.

Juvind (1928) contends that the ash test is one of the most valuable, but it must not be used with too much emphasis when the history of the flour is not known.

From the opinions of these various workers in the field of cereal chemistry, one seems justified in concluding that the ash test is very useful, but that it has very definite limitations as a criterion of flour grade.

#### Methods of Determining Ash

Direct Burning. The direct burning of a weighed sample of flour is the oldest and most used method of determining ash content. When ash first became of importance as a test

of flour grade, no method of ash analysis was thoroughly adaptable for the work. The method of the Association of Official Agricultural Chemists was needlessly long, and the procedure outline was one formulated to cover a great variety of materials.

The mill chemist, in his desire to speed up the determination, began to decrease the size of the sample and increase the ashing temperature. This led to much controversy regarding the temperature to which the sample could be heated without fusion or volatilization of the mineral matter. Gamble (1902) in his work on ash constituents, in comparing the Shuttleworth and "Official" methods for ash in grain came to the conclusion that no loss occurred at dull red heat. Leavitt and Le Clerc (1903) report that there is no appreciable loss of phosphorus even though cereals are ashed at a red heat.

The Association of Cereal Chemists (1928) adopted as official the following method for ash. Incinerate 3 to 5 gm. of sample in a furnace at just below dull red heat until the ash becomes fluffy and white or gray-white in color, or until no further loss in weight occurs. Usually a temperature of 550°C. (1022°F.) will be sufficient.

To determine what effect temperature has on the speed of burning flour to ash, Wicks (1927) conducted a series of experiments using a high temperature. He found that it

was possible to obtain accurate results in as short burning time as sixteen minutes. The conclusion reached was that the best method for ashing flour was direct incineration at high temperature in platinum dishes without the addition of other substances.

Admixture of Substances to the Sample. Hartwell and Kellogg (1906) were the first to recommend a variation of the ordinary incineration method for the determination of ash. They used acetic and oxalic acid to extract the charred mass after burning at low heat. By this procedure, all the sodium and potassium compounds are supposedly retained. Many other methods have been proposed as improvements on the original procedure since those recommended by Hartwell and Kellogg, and all of them include the adding of some substance to the sample. The material added has been of three general types: (1) inert material such as alundum ( $Al_2O_3$  ground to pass through a 60 mesh sieve) which prevents the sample from packing and thus increases the burning area; (2) oxidizing agents, such as superoxyl (30 per cent hydrogen peroxide), and the passage of oxygen gas into the furnace to hasten the incineration; and (3) the addition of material to prevent fusion, such as glycerol-alcohol mixture.

The following articles deal with the modified methods as previously named.

Hertwig and Bailey (1924) recommend that the sample to be ashed be mixed with a glycerol-alcohol solution to hasten the process. By using this procedure, a flour can be ashed in two hours as compared to five hours as required of the Association of Official Agricultural Chemists method. Eight collaborators compared the Bailey-Hertwig method with the Association of Official Agricultural Chemists method. The results were included in the report of the committee on methods of analysis of the American Association of Cereal Chemists, submitted by Bailey (1924). The findings were that the glycerol-alcohol mixture tended to run over the sides of the ash crucibles. This method also gave slightly higher results.

Since the chief criticism of the above determination seemed to be that the wetted flour mixture tended to boil over the sides of the crucibles, Hertwig and Bailey (1925) published a more adequate description of their procedure.

Hertwig (1925) recommended further study of the glycerol and calcium acetate methods. He also reported that the addition of fine asbestos fiber to a flour very markedly shortens the ashing period, so that a white ash is obtained in about forty-five minutes.

Farper (1922) warns the cereal chemists that regardless of the method used, care in sampling and care of apparatus



used is essential to accurate ash analysis.

Coleman and Christie (1925) note that there is no uniform method in use for making ash determinations on flour, and that the official method is used mainly as a guide for the preparation and development of other methods. The size of sample, time of ashing, and temperature are varied to suit the desires of the individual. How much these variables will influence the final test is the subject of their paper.

Mangels (1925a), as chairman of the committee on methods for the American Association of Cereal Chemists, circularized members of the Association with a questionnaire to obtain information and opinions regarding the methods in use for determining the ash content of flour. He reports (1925b) that the methods now generally followed by cereal chemists give accurate results but that such methods require too long a period of time for practical purposes. The result of this investigation was that the glycerol-alcohol method was no longer recommended. Since the addition of calcium acetate to the sample prevented fusion of the ash, even at high temperature, this procedure was recommended.

Some collaborative work on ash was reported by Coleman (1926). Samples of flour were sent to different laboratories with the request that the ash be determined by the following methods:

1. Method usually employed in the collaborator's own laboratory.
2. Method of the American Association of Cereal Chemists.
3. Method of Bailey and Hertwig.
4. Glacial Acetic Acid Method.
5. Use of Alundum.

Analysis of the reports of the collaborators show that method 4 was considered the most difficult to carry out, while methods 2 and 3 appeared to be the most dependable. The time element involved and the attention necessary for the preparing of the sample for the muffle seemed to be the most serious objection to the use of method 3, although Rogers (1926) contends that the Bailey and Hertwig method is just as accurate as the official method and much faster.

A study of the calcium acetate and the calcium acetate-acetic acid procedures were reported by Mangels (1926). According to the work of Brendel (1926), by the use of his oxygen-acetate method, the ash value of a flour could be reported within forty-five minutes after the sample was received. These methods, however, have never received wide application.

Sullivan and Near (1927) report that in the commercial testing of flours for their ash content erroneous results

are often reported because all the samples are ashed in the same manner regardless of the quantity, or composition, of the ash. They further state that the single standardization of the ash method as to exact temperature and time is unscientific and inaccurate, as conditions of time and temperature which will produce a clean ash fit to weigh for one flour often cause a fused or insufficiently burned ash with another sample, the ash of which has a different composition. In their work on the relation of magnesium in the ash and the lipid-protein ratio to the quality of wheats, the flour was ashed with the aid of Merck's super-oxyd which is thirty per cent hydrogen peroxide.

In an article on the relation between the fusibility of flour ash and its mineral constituents, Johnson and Scott (1928) have noted that under apparently identical conditions of incineration some flours always yield white, fluffy ashes, other partially fused, while still others yield completely fused ashes. Fused ash, of course, is considered to give inaccurate results.

The collaborative studies for the year 1927 (reported by Coleman) of the American Association of Cereal Chemists were confined to the two methods adopted by the association at its 1926 convention, namely the Association of Official Agricultural Chemists and glycerol-alcohol methods. The average percentage of ash determined by use of either of

these two methods as reported by 52 collaborators was nearly the same.

Spalding (1930) contends that by the use of magnesium acetate in 95 per cent ethyl alcohol added to flour makes it possible to determine ash in fifteen minutes.

Recently the use of certain salts of earth metals occurring in the third and fourth groups of the periodic system, particularly the more basic elements, such as zirconium, lanthanum, yttrium, cerium, and thorium, have been used to accelerate combustion of flour ash and render it non-hygroscopic. (Walters 1930) It is only necessary to incinerate the sample for about thirty minutes when the salts of these elements are used as aids.

Although the addition of certain substances to the flour sample has proven to be of considerable value as aids in burning the flour to ash, yet all are open to the objection that the method is thereby complicated, creating a greater potential error. A correction for the ash in the material added must always be applied before the true ash content of the flour can be reported.

Direct Weighing of Ash. The use of the direct weight method for determining ash was first recommended by Klepfstein (1920) (1930) and further elaborated by Liddel

(1929) and Spalding (1930). The direct weight method merely involves a special way of handling the ash obtained by any of the previously discussed procedures. Direct weight in this case means the transference of the ash from the crucible to the balance watch glass, thus obtaining the weight of ash without regard to the weight of the container. It is contended that this method is less susceptible to error than the indirect procedure.

To show the present trend in regard to methods for determining the ash content in flour, reference is made to the report of the 1930 meeting of the Association of Official Agricultural Chemists. Le Clerc (1931) states that the following recommendation in regard to ash was adopted: "That the study of rapid methods of ashing flour, bread, and other baked products be further made. That the study of the nature of the losses which take place on fusion of ash be temporarily discontinued."

#### The Present Use and Value of the Ash Test

The value of the ash test to the miller, baker, and salesman is discussed by Lawellin (1922). He concludes that the test is more important to the miller than to any one else, and it is the baker who receives the least value from the test, but who does the most complaining.

Mitchell (1915) (1921) also emphasizes the value of the ash test to the baker and miller. He contends that the test

is the best indication of the uniform operation of the mill and is therefore of value to the miller but not the baker. Braun (1913) is also of the opinion that the baker is not justified in using the ash content of a flour as a basis of its cash value, although he does admit that the test is useful.

White and Beard (1913) noted the relation of the phosphorus content of wheat flour to the baking qualities. Willard and Swanson, in the same year (1913), studied the influence of various substances, some mineral salts included, on the baking qualities of flour. From this work it would seem that mineral salt content, although small, may not be without its influence on the baking qualities of flour.

Swanson (1925) (1926) states that the test weight of wheat and the percentage of ash in the wheat vary inversely. He considers high ash in flour to be traceable to two sources; first, the bran specks fine enough to pass through flour bolting cloth, and second very fine material high in ash. The color of flour is affected by the former, so no doubt that is the greatest objection to high ash flour since ash itself does not seem to be harmful.

Bailey (1913) undertook to ascertain how closely the per cent of ash and color score paralleled one another. The color score was determined by judging with the eye the relative depth of color in the crumb of the finished loaf.

The conclusion drawn from the data presented was that there is a more or less definite relation between the average percentage of ash and the color score in a large number of samples. A more rapid way of determining the same characteristic which ash gives to flour is, according to Bailey (1921), the determination of the specific conductivity of the water extract. As time and temperature, if varied, affect the result, a standard method is recommended.

Jacobs and Rask (1920) contend that the ash content of the wheat as received at the mill should be determined. They state that the flour-mill chemist ordinarily confines his activities to tests on the finished products, but would increase the value of his services appreciably by including more tests on the raw material.

The correlation between ash and gluten was worked out by Weaver (1921). There is nothing to indicate that a high ash and high gluten content accompany each other, but the presence of the salts contained in the ash does help to stabilize the gluten as well as furnish yeast food during fermentation. Considering these facts, some chemists have claimed that the higher the ash the more valuable the flour as a food and the better the fermentation of the dough.

Forbes, Beagle, and Mensching (1913) call attention to the deficiency of flour in the quantity of mineral material necessary for growth. However, if our entire diet were comprised of whole wheat, it would still be inadequate to fur-

nish the body with sufficient minerals.

#### Determination of Color of Flour

An accurate determination of flour color is one of the most difficult problems confronting the cereal chemist, since it is difficult to express the color of flour numerically.

Micros (1927) states that the main tests for the determination of color of flour are the Pekar test, tintometer, and the gasoline color value. We may now also add the methyl alcohol value. To date the method most generally employed by flour millers and chemists is the so called Pekar or "slick" test. It is not known when Pekar devised this test, but it has been used extensively for many years. The most serious objection to the test is that it gives no numerical data that can be recorded for future reference.

The Lovibond tintometer was originally designed to measure the color of beer by transmitted light and appears to have been first employed by Shepard (1905) to judge the color of flour. It is necessary to modify the apparatus for flour work because reflected light must be used.

Jago (1921) also used the tintometer in flour color determinations, but the results were not very satisfactory due, primarily, to the difficulty of obtaining a uniform reflecting surface, or matching the flour color with the



standard tinted glasses.

The gasoline color test for flour was developed by Winton and Shanley (1909), Winton (1911), and Coleman (1927). This test is of some value on unbleached flour but has never been in general use, so the Association of Official Agricultural Chemists, Coleman (1930), concluded that the method was not of sufficient importance to justify further study.

Kent-Jones and Herd (1927) published an article on the numerical expression of flour color in which they used both gasoline and methyl alcohol as extractives for the pigments of flour. Hoofst and de Leeuw (1928) and Kent-Jones and Herd (1929) have further criticized and studied the methyl alcohol color value.

According to Bailey (1925), the five factors that combine to produce flour color are: first, the purely physical phenomena caused by the effect of granulation or size of particle; second, the moisture content; third, the reddish-brown pigment xanthophyll which is present in the bran from red wheats; fourth, dirt and foreign matter, such as soil, weed seeds, and smut spores; and fifth, the carotinoid pigments, or the yellow coloring matter of flour.

## EXPERIMENTAL

## Wheat Hair Count and Ash Content

The first problem undertaken was to determine what use could be made of the microscope as a means for determining the relation of the wheat hair count to the ash content of flour. Since the miller's problem is to separate the endosperm of the wheat kernel from the bran and germ, it occurred to investigators, early in their research work, that a direct method of determining the thoroughness of the milling operation would be to ascertain the quantity of bran, or germ, remaining in the flour. Several efforts have been made to grade flours on this basis. Keenan and Lyons (1922) have suggested that the best method for the designation of flour grade is by the use of the microscope.

Methods. The method, as recommended by these authors, is to weigh out on an assay balance a 5 mg. portion of flour, transfer to a slide of ruled area, and add 3 or 4 drops of chloral hydrate. The slide is then heated over a small flame until the starch is dissolved. The number of bran particles per unit area are then counted. Since the essential purpose of milling is to obtain from the finely ground endosperm of wheat a flour which is as free as possible from bran particles, hairs, and germ tissue, the amount of such

material found upon microscopical examination will, therefore, be a measure of the flour grade.

It was found that the identification of bran particles by the untrained eye was very difficult; therefore Keenan (1923) recommended that the wheat hairs only be counted instead of counting also the bran particles. He showed that hair count alone could be used to classify a flour.

Keenan and Lyons (1920) examined the mill streams which went to compose a straight grade of flour. The following table is reproduced from their work and illustrates the great variation in the number of bran and wheat hair particles in different mill streams.

Table I Bran particles and wheat hairs in flour streams.

Flour Stream	Bran Particles	Wheat Hairs
First break	113	38
Second break	75	38
Third break	131	53
Fourth break	228	106
Fifth break	368	173
First middlings	21	8
Second middlings	48	27
Third middlings	26	7
Fourth middlings	29	2
Fifth middlings	55	12
Sixth middlings	60	18
Seventh middlings	143	23
Eighth middlings	264	38

A study of these figures indicates that a wheat hair count would be as useful as the bran count in classifying flour, and is far easier to make.

Returning again to the consideration of ash in flour, Table I becomes of even greater interest when we consider that bran particles are high in ash, and consequently there is a direct relation between the number of bran particles, or wheat hairs, and the ash content of the flour. The following two problems now present themselves: (1) Would it be possible to use the hair count and ash content to prepare a graph such as is used in many microscopical examinations to determine the percentage of adulteration? If so, the wheat hairs may be considered as the adulterant in flour and the approximate ash content determined from the hair count in a manner to be described later. (2) Would such a determination be practical?

To prepare a graph that would be useful in this problem, it is necessary to select at least three samples of flour, determine the ash content in the usual manner, and also count the wheat hairs. The hair count values are then plotted as abscissas and the ash content, expressed in per cent, as ordinates. The points thus determined are joined by a straight line as shown in Figure 1. Having once made this graph, it would only be necessary to make a hair count of the flour samples obtained from any flour stream, and then

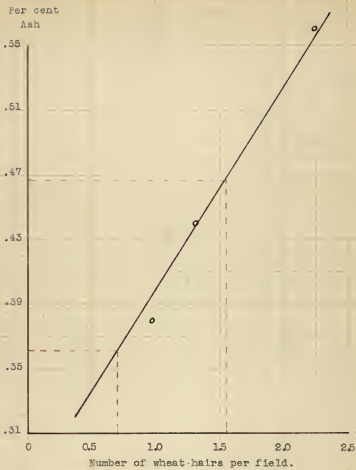


Figure 1. Chart for Determining Ash Content from Wheat Hair Count.

follow this value up the graph ( as indicated by the dotted red lines) to the intersection with the curve, which would indicate the approximate ash content of the flour.

To try this method, two samples of flour, one a patent flour, and the other a clear flour, were obtained and some synthetic samples of known proportions were made up and the per cent of ash determined in the usual manner. (Official Method) The method utilized in determining the hair counts was altered from that employed by Keenan (1923), because, in this case, it was desired to avoid the time and effort required to accurately weigh out a sample and transfer it to a slide. It can be readily seen that if the sample must be weighed the ash may as well be obtained by the incineration of the flour, unless, of course, the sample had been specially treated, such as phosphated flour, in which case the ash content would be no indication of the flour grade.

It was determined that with a little practice it was possible to balance approximately the same quantity of flour on the end of a small flattened platinum wire each time. In preparing slides, the same quantity of flour, as nearly as could be estimated, was placed on a flattened platinum wire and mixed thoroughly on the slide with a drop of glycerine and water, 1:1. By means of a mechanical stage, the slides can be examined and the hair counts per field readily made.

The results secured by this procedure are given in Table II.

Table II Comparison of wheat hair count and ash content of flour.

Number:	Mixture	Per cent: ash	No. of wheat hairs per field.	Ash per cent as determined from chart
:	:	: Actual	:	:
1	: Patent	: .38	: 0.95	:
2	: 75% Patent, 25% Clear:	.40	: 0.68	: .362
3	: 50% " 50% " :	.44	: 1.37	:
4	: 75% Clear, 25% Patent:	.51	: 1.52	: .465
5	: Clear	: .56	: 2.25	:

Samples No. 1, 2, and 3 were used to make the graph. The number of wheat hairs per field were counted on no less than three slides per sample, making twenty-five counts on each slide. The points on the chart represent the average of seventy-five counts.

Results. To determine if this data could be used in a practical way to obtain the ash content of unknown flour mixtures, counts were made on samples No. 2 and 4, which gave results as reported in the table. By use of the chart, we find the probable per cent of ash in sample No. 2 to be 0.362, and for No. 3 it is 0.465. This is a difference of 0.038 and 0.045 from that found by using the official method.

Although insufficient data has been gathered from which to reach any conclusion as to the accuracy of this indirect method of deriving the ash content of flour, the gathering of further data seemed unnecessary since the procedure proved to be too long and tedious for practical application, even if it were desirable in other respects.

#### Flour Pigment Extraction

Discussion. The two pigments of flour which are of importance, because of their influence on flour color, are carotin and xanthophyll. Carotin is a hydrocarbon to which the formula  $C_{40}H_{56}$  is generally assigned. This pigment is responsible for the yellowish color of wheat, or flour. The fact that flour whitened with age, or whitened when it was subjected to the action of bleaching agents was discovered by early experimenters, but it remained for the researchers of various workers, such as Winton (1911), Monier-Williams (1912) and Palmer (1922), to demonstrate that this color change was due to the oxidation of the highly unsaturated carotin molecule. Since the development of the chemical treatment of flour, the carotin pigments present in wheat have largely lost their significance for the reason that it is a simple process to render them colorless by bleaching.



Properties of Carotin and Xanthophyll. The method of determining the quantity of carotin in flour was first suggested by Winton (1911), and has since undergone many refinements. The method is one of the "official" methods of the Association of Official Agricultural Chemists and, therefore, need not be reported here other than to say that carotin is soluble in gasoline, hence this solvent is used in its extraction; therefore, the term, "gasoline color value."

Xanthophyll, on the other hand, is an extremely vital factor in flour color consideration because it cannot be removed by bleaching. This pigment is what gives the reddish-brown color to bran particles, and, therefore, the flour color is affected by the proportion in which the bran particles are present. The formula assigned to xanthophyll is  $C_{40}H_{56}O_2$ , but the structural arrangement of the molecule has not as yet been determined. The solubility of xanthophyll is essentially different from that of carotin.

Willstatter and Stoll (1928) prepared in tabular form, Table III, which compares the properties of carotin and xanthophyll.

Table III Comparison of carotin and xanthophyll.

	: Carotin	: Xanthophyll
Formula	: $C_{40}H_{56}$	: $C_{40}H_{56}O_2$
Typical crystal form	: rhombic plates	: swallow tail prisms.
Color by transmitted light.	: red	: yellow
Solubility in petrol- cum ether.	: quite soluble	: insoluble
Solubility in alcohol	: difficulty sol.	: quite soluble
Solubility in carbon disulphide.	: exceedingly sol.	: difficulty sol.

From the above table, it can readily be deduced that a separation and measurement of these two pigments might be made on the basis of their respective solubilities. This is exactly as was recommended by Kent-Jones and Herd (1927). In brief their method for the extraction of xanthophyll is as follows:

Kent-Jones Method. Weigh 20.0 gm. of flour into a wide mouth 8 oz. bottle; add 50 cc. of water plus 5 cc. of normal NaOH solution, shake and let stand for one hour. Then add 100 cc. of methyl alcohol, and shake for 16 hours. Allow to settle, decant the supernatant liquid into a beaker, and by means of  $N/8$  HCl and the indicator brom-thymol blue used externally, precipitate the glutenin at its isoelectric point,

which is pH 6.4. Centrifuge, filter, and add one cubic centimeter of caustic soda to reproduce the pigment color which is destroyed in acid solution. The solution can then be compared with a standard solution, such as dilute potassium chromate, in any suitable colorimeter.

Gasoline Color Values as Related to Ash Content. To determine the relation between the yellow flour pigments and ash content, the following experiment was tried. Five samples of flour, the same as those upon which the wheat hair counts were made, were extracted with gasoline. The regular method was used; that is 20 gm. of flour were shaken with 100 cc. of colorless gasoline and allowed to stand for 16 hours, after which the solution was filtered and the color compared with that of a 0.005 per cent potassium chromate solution. A Duboseq colorimeter was used with the standard set at 10 mm. The calculation of the gasoline color value then becomes:

$$\frac{\text{Reading of standard}}{\text{Reading of Unknown}} = \text{Gasoline Color Value.}$$

These values are given in Table IV.

It is clearly evident that there is no correlation between gasoline color value and ash content of a flour sample.

Table IV Gasoline color values.

Number: Sample	:Reading:	Value	:Per cent Ash
1 : Patent	: 6.5 :	1.54 :	0.38
2 : 75 Patent-25 Clear	: 11.9 :	.84 :	0.40
3 : 50 Patent-50 Clear	: 10.4 :	.96 :	0.44
4 : 25 Patent-75 Clear	: 5.7 :	1.76 :	0.51
5 : Clear	: 12.8 :	.78 :	0.56

Kent-Jones Method as Related to Ash Content. The next step was to obtain the methyl alcohol values of these same flours by the method recommended by Kent-Jones and Herd (1927) as previously described. The only difficulty experienced with any of the procedure came in precipitating the glutenin. Blish and Sandstedt (1925) have reported that the glutenin is completely precipitated at pH 6.4 and Kent-Jones and Herd likewise endeavored to bring their solutions to this pH value, but in our work it was found, after repeated checks in which the electrometric hydrogen-ion apparatus with quinhydrone electrode was used, that the glutenin was more easily separated when the pH value was about 6.1.

Apparently the samples of flour examined by Kent-Jones and Herd contained a greater quantity of xanthophyll than

the flour samples selected for this experiment because the standard solution as recommended by them was found to be too strong. They made up a standard solution by taking 5 cc. of a 0.5 per cent potassium chromate solution plus 2.0 cc. of 10 per cent anhydrous cobalt nitrate solution and diluting to 100 cc. with distilled water. Ten cc. of this standard made up to 70 cc. with water proved to be about the proper strength for this work. The standard was set at 20.0 mm. and the calculation for the methyl alcohol value made as for the gasoline color value. The values obtained are given in Table V.

Table V Methyl alcohol values.

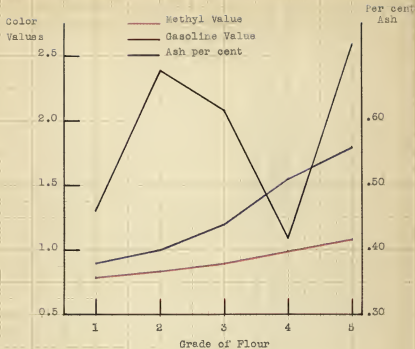
Number:	Sample	:Reading:	Value	: Per cent Ash
1	:Patent	: 24.7	: .81	: 0.38
2	:75 Patent-25 Clear	: 24.1	: .83	: 0.40
3	:50 Patent-50 Clear	: 22.3	: .90	: 0.44
4	:25 Patent - 75 Clear:	20.1	: 1.00	: 0.51
5	:Clear	: 18.6	: 1.08	: 0.56

The above table shows clearly that there is a correlation between the methyl alcohol value and the ash content of flour.

In Figure 2 the relationship of the gasoline color value, the methyl alcohol value and the ash content for five flours are given in graphic form. An analysis of these curves shows that the carotin content of bleached flour is not related to the ash or xanthophyll values. This was to be expected on the bleached flour samples, since most of the carotin had been rendered colorless by oxidation. As a matter of fact the gasoline, after having been used to extract the carotin, was so nearly colorless that the variations shown may well be within experimental errors in reading the colorimeter.

However, a very good agreement between the ash content and color as extracted with methyl alcohol was observed. This likewise might be expected, since xanthophyll is the pigment of bran and bran contains about twenty-times as much ash as does the endosperm of the wheat kernel; consequently, the more bran particles in the flour the higher the ash and the greater the quantity of reddish-brown pigment.

This method is valuable in that it gives a numerical expression for flour color. It has, however, the following disadvantages: (1) It requires too long a time; (2) Methyl alcohol is a rather expensive extractive; and (3) The use of a colorimeter for this type of work is very tiresome on the eyes. It seemed desirable to try to eliminate, if possible, some of these unfavorable factors.



1 = Patent

3 = 50% Patent-50% Clear

2 = 75% Patent-25% Clear

4 = 75% Clear-25% Patent

5 = Clear

Figure 2. A Comparison of the Ash, Gasoline and Methyl Alcohol Color Values on Five Flours.

## Modification of the Kent-Jones Method

Use of the Solvents. To determine just how specific the solvent action of methyl alcohol is for the extraction of xanthophyll from wheat products, several other solvents were tried. The extraction experiments were conducted on samples known to millers as tailings stock. This material was selected because the tailings streams are known to be high in ash, fat, and fiber; therefore, there would be greater color intensity in the extractive solution and differences in solvents could be detected more readily. The ratio of solvent to tailings stock was in each case five cubic centimeters of solvent to each gram of stock. The results of the use of several organic solvents are given in Table VI.

Table VI Solubility of xanthophyll in several solvents.

Number	Material	Solvent	Color of solution
1	: Tailings stock	: Methyl alcohol	: Strong color, much the best.
2	: " "	: Ethyl "	: Slightly colored.
3	: " "	: N-Propyl "	: " "
4	: " "	: Butyl "	: Very slight.
5	: " "	: Acetone	: Colorless
6	: " "	: Ether	: Slight color
7	: " "	: Formaldehyde	: " "



From the above results, it seems improbable that a better solvent for xanthophyll than methyl alcohol would be found since neither the common ethers, aldehydes, or ketones are of value as solvents and the higher alcohols, too, have less action on the reddish-brown pigment than methyl alcohol. Furthermore, unless ethyl alcohol or acetone proved to be useful for this work ( which they did not) very little would be gained as far as cost is concerned by using other solvents. It was, therefore, decided that methyl alcohol was the best solvent for xanthophyll.

Changes in Method. Having decided on Methyl alcohol as the best extractive to use, the next investigation undertaken was to shorten the Kent-Jones method. As previously described, this procedure involves extracting the flour with both alkali and about 70 per cent methyl alcohol. The added alkali, normal in this case, would saponify the fat and also dissolve the alkaline soluble protein glutenin, while the alcohol would act as a solvent for the alcohol soluble protein gliadin. It is, of course, then necessary to precipitate these proteins before the true color of the dissolved pigment can be determined, but this precipitation is not an easy task. The elimination of several of these steps is highly desirable.

Since the prolamines, or alcohol soluble proteins, are practically insoluble in alcohol stronger than 80 per cent,

the use of strong alcohol would not dissolve appreciable quantities of proteins but would be just as effective for removing the pigment. Likewise, a small quantity of sodium hydroxide solution would saponify the fat without dissolving the alkali soluble protein glutenin. Accordingly, the following method was tried.

Weigh 20 gm. of sample into a wide mouth 8 oz. bottle, add 150 cc. of 95 per cent methyl alcohol plus 0.5 cc. of normal NaOH, shake and filter off the alcohol.

The pigment removed in a unit time by this method from the same sample of flour compares favorably with the quantity removed by the Kent-Jones method, and it is no longer necessary to precipitate the dissolved proteins. When the methyl alcohol solution, used as described above, is brought to a pH of 6.1, there is no precipitation, which indicates that there has not been sufficient solubility of proteins to cause any interference in the color comparison of this solution with a standard.

Extraction Time. From the experimental work conducted on the length of time necessary to extract the xanthophyll pigment from flour, it appeared that after a period of continuous agitation for one hour, nothing further was gained by longer extraction. After a few hours, the color value actually decreased because of the fading of the pigment. Reference to Figure 3 indicates the relation between extrac-

tion time and color value. Extraction time in this case means constant agitation for the period indicated.

Since the yellowish pigment extracted with methyl alcohol is found to be rather unstable when exposed to light, it is very essential that the color comparison be made as soon as possible after the flour has been separated from the solution as otherwise a low result is obtained due to the fading of the color. Figure 4 illustrates the tendency for the color value to drop as the solution is allowed to stand in the sunlight.

Results with the Modified Method. Using the same five samples of flour as were used in experimenting with the Kent-Jones method, the modified method was tried. The results obtained are recorded in Table VII.

Table VII Methyl alcohol values (Modified Method).

No.: Samples	per cent:		Color Value		
	: ash	: 30 min.	: 1 hour	: 2 hours	
1 :Patent	: .38	: 1.44	: 1.00	: 0.77	
2 :75 Patent-25 Clear:	.40	: 1.38	: .91	: 0.80	
3 :50 " 50 "	: .44	: 1.35	: 1.05	: 0.87	
4 :25 Patent-75 Clear:	.51	: 1.44	: 1.00	: .90	
5 :Clear	: .56	: 1.64	: 1.06	: .94	

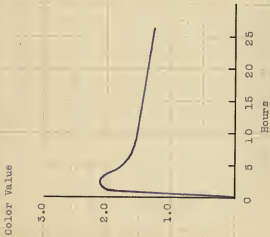


Figure 3. Relation Between Extraction Time and Color Value.

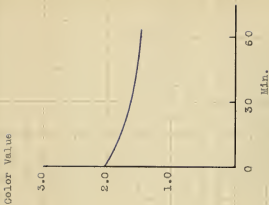


Figure 4. Fading of Color with Time.

From the above results, it is evident that the only consistent values obtained are from the two-hour extraction period.

The standard solution used for comparison in this work contained 0.005 per cent potassium chromate. One hundred cc. of the 0.005 per cent  $K_2CrO_4$  was diluted with 20 cc. of water and 2 drops of 10 per cent cobalt nitrate solution added.

Twenty grams of flour and 150 cc. of methyl alcohol were found to be unnecessarily large quantities of material to use; hence, experiments were conducted in which the proportion of sample to solvent were varied. Since the preliminary trials with the modified method gave satisfactory results with the ratio of one to ten for sample and solvent, it seemed advisable to use this proportion. Therefore, 5 gm. of sample to 50 cc. of methyl alcohol was tried. Samples of break flour from the experimental mill were obtained and the per cent of ash determined as well as a determination made of the color value. The results are reported in Table VIII. This was a 2 hour extraction with the standard solution set at 20 mm.

In this case the results were fairly satisfactory, but further work revealed that active agitation was obtained even when 10 gm. of flour were extracted with 50 cc. of alcohol. The colorimeter can also be read with greater

Table VIII Methyl alcohol values on 5 gm. samples.

Sample	Reading	Value	Ash
First Break	9.0	2.23	.55
Second Break	10.7	1.87	.55
Third Break	9.9	2.02	.60
Fourth Break	8.5	2.35	.79
97 Per Cent Flour.	20.1	1.00	.48

accuracy when the color of the solution examined is more intense. All further data collected was on the basis of 10 gm. samples.

Effect of Gasoline Extraction on the Methyl Alcohol Value. Although xanthophyll is considered to be insoluble in petroleum ether and carotin likewise insoluble in methyl alcohol, an interesting fact was observed that whenever the flour samples were first extracted with petroleum ether, dried, and then extracted with methyl alcohol, the color intensity of the alcohol solution is considerably less and greater fluctuations occur in the colorimeter readings. At first it was thought that the removal of the lipoids by the petroleum ether might account for this difference in color, but after determining the crude fat in a number of samples, it seems as though this is unlikely. The fat content of

flour is small and quite constant in amount. The result of this work is summarized in Table IX.

Table IX Color value on flour before and after being extracted with gasoline.

Sample	Methyl alcohol value		Fat	Ash	Mois- ture
	Before extrac- tion with gas- oline.	After extrac- tion with gasoline.			
First Break	3.34	2.00	.97	.57	13.25
Second Break	3.15	1.61	1.16	.52	13.88
Third Break	3.20	3.28	1.47	.59	13.97
Fourth Break	4.35	3.92	1.41	.83	11.77
97 Per cent Flour	1.44	.83	1.18	.45	13.50

Judging from this data, it would seem as though carotin must be to a considerable extent soluble in methyl alcohol.

The Relation Between Ash and Color Value. Although, as is evident from the preceding tables, there is a definite relationship between the methyl alcohol color value and the ash content of a series of flour samples, the correlation is not sufficient to be of much practical use as a substitute for the ash test. To further determine what might be expected of this method, samples were obtained from the various mill streams of the experimental mill. The method followed in determining the color value of these samples

involved, as previously stated, the use of 10 gm. of sample and 50 cc. of alcohol plus two drops of normal sodium hydroxide solution. The extraction time was two hours, and the readings were made immediately after filtration of the solutions. Table X gives the results.

Table X Methyl color values and ash on various mill streams.

Sample	:Reading:	Color	:Ash
		Value	
First Break Stock	: 4.5	: 4.4	: 1.96
Second " "	: 3.3	: 6.1	: 2.61
Third " "	: 2.9	: 6.9	: 4.29
Fourth " "	: 2.6	: 7.7	: 5.90
First Middlings Stock:	8.1	: 2.5	: 0.42
Second " "	: 8.7	: 2.3	: 0.41
Third " "	: 8.3	: 2.4	: 0.39
Fourth " "	: 8.5	: 2.3	: 0.43
First Tailings	" : 1.8	: 11.0	: 1.47
Second " "	" : 7.5	: 2.6	: .82
Sizings Stock	: 8.0	: 2.5	: .47
97 per cent Flour	: 14.0	: 1.4	: .48
Shorts	: 6.1	: 3.3	: 1.80
Bran	: 2.1	: 9.5	: 7.05



The general agreement between color value and ash content is apparent, but as a further comparison of these two factors, the five samples of flour on which wheat hair count and Kent-Jones color value had been determined were again used. This time 20 gm. of flour was extracted for two hours with 50 cc. of methyl alcohol. Table XI records the data which are given in graph form in contrast to the Kent-Jones values and ash content on Figure 5.

Table XI Comparison of Kent-Jones and modified method color values.

Sample	Wheat : hair count	Kent-Jones : color values.	Modified : method values.	Per cent : Ash
Patent Flour	: 0.95 :	.81	: 1.43 :	.38
75 Patent-25 Clear	: 0.68 :	.83	: 1.54 :	.40
50 Patent-50 Clear	: 1.37 :	.90	: 1.72 :	.44
25 Patent-75 Clear	: 1.52 :	1.00	: 2.13 :	.51
Clear Flour	: 2.25 :	1.08	: 2.59 :	.56

As is indicated, the two methods compare very favorably.

#### Presence of the Xanthophyll in White Wheat

The next phase to be considered was in regard to the presence of xanthophyll in white wheat. Since this pigment gives the reddish-brown color to bran, it would, therefore,

Color Values

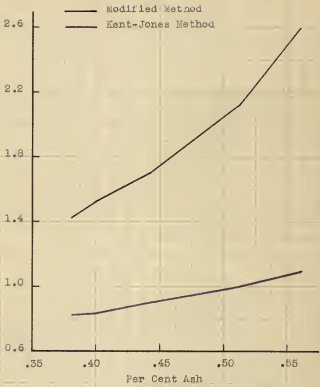


Figure 5. Comparison of Kent-Jones and Modified Method Color Values.

be expected to be present in a smaller amount in bran from white wheat. Some samples of white wheat flour milled on the experimental mill proved to yield sufficient pigment to be measurable in a colorimeter. However, when one of the very best grades of pastry flour was extracted with methyl alcohol, the solution remained practically colorless, showing that there was little, or no, soluble pigment present.

Some white wheat kernels were soaked in water until they became plastic, but not too soft, a treatment which required several days. They were then cut open and a part of the inner endosperm removed. In this manner, a sufficient amount of endosperm that was free from germ and bran was collected to extract with both gasoline and methyl alcohol. It was found that carotin, the gasoline soluble pigment, was present even in the pure endosperm, but xanthophyll was not present. It, therefore, seems that the methyl alcohol color value is truly a measure of the contamination of flour by bran particles.

#### DISCUSSION OF RESULTS

The method for extraction of the reddish-brown pigment of bran, as outlined by Kent-Jones (1927), has been materially shortened and simplified. In place of extracting for 16 hours, 2 hours is sufficient, and by the modified pro-

cedure, it is no longer necessary to regulate the pH value of the solution before filtration.

Since xanthophyll is truly the pigment of the bran coat of the wheat kernel and is not found in the endosperm, a measure of the quantity of this pigment in flour becomes an indication of the amount of bran contamination. The ash test has generally been used for the same purpose. Naturally then, the ash content of flour and the methyl alcohol value should be related. The data submitted in this paper shows the extent of this relationship as indicated by the methods now in use.

The greatest source of error in the determination of the color value lies in the comparison of the extracted pigment with a standard solution. This is particularly difficult when there is but a small amount of pigment present in the solution. Although the method is sufficiently accurate when using numerous samples to show a very close agreement with the ash content, yet in any one particular case it would be impossible to predict the ash content from the color value. If an improved color comparator were devised, undoubtedly the correlation between the color of the extracted pigment and the ash content of flour would be even greater.

One of the most useful purposes to which the wheat hair count and methyl alcohol value could be applied is in the numerical recording of the quantity of bran fragments and

the color in certain grades of flour. These values could then be used to compare the flour produced from season to season. With such recorded data as bran or wheat hair counts and a numerical expression for flour color, the miller or chemist could quickly tell at the beginning of a new wheat crop whether it was a case of faulty milling or of high ash wheat, if the flour differed in ash content from the previous year.

#### CONCLUSIONS

1. A simple method has been devised to obtain and record flour color as effected by pigments.
2. The carotin content of flour as determined by the gasoline color value cannot be correlated with the ash content of the flour.
3. The number of wheat hairs per unit of flour can be used to predict the approximate ash content of a flour.
4. Xanthophyll, as determined by extraction with methyl alcohol, is present in the bran coat of both red and white wheat. This pigment is not found in pure endosperm.
5. The color intensity of the pigment extracted from flour, by use of methyl alcohol as the solvent, varies directly with the ash content of the flour.
6. The methyl alcohol color value of a flour can be quite easily determined and the value numerically expressed.

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