below that for the other meats examined. To determine whether this low ratio represented incomplete development of the cured meat pigment, a sample of this sausage was dipped briefly in a solution containing 0.2% nitrite and 0.2% ascorbic acid (pH adjusted) and stored several days in the refrigerator. After this treatment, the sample was a pinker color than the original meat and the ratio had increased to 2.51. The average extinction ratio 570/650 mμ of all the meats examined was 2.56. With the improved color of liver sausage the average was 2.59 and without the liver sausage 2.61. It is possible that the ratios would be increased for some of the other meats by treatment with excess nitrite and reducing agents. Ratios below 2.4 on freshly exposed cured meat surfaces would indicate that full cured meat color had not been developed during curing. The diagnosis is confirmed if the ratio is increased by treating with nitrite and reducing agent.

Table I also gives the extinction ratios for the oxidized pigment. The average value of these ratios is 1.56. The difference between the two average extinction ratios is highly significant.

Absorption ratios of light-faded meats were all lower than those of the fresh product and approached those of the ferricyanide-treated samples with increased color loss. There was a close correlation between the extinction ratios and the visual score of the faded meats. The correlation coefficient of all samples tested was 0.90.

From the given data it is possible to calculate roughly the percentage of the original pigment in a light-faded product which is oxidized to the ferric form. A more accurate calculation can be made if the extinction ratios for the fully developed color and for the completely oxidized form of the meat under scrutiny are known. For example, the extinction ratio of fresh picnic ham is 2.50; after complete oxidation of the pigment the ratio is 1.63. After exposure to light of 200 foot-candles intensity for 1 hour the ratio is 1.92. This represents a pigment oxidation of 66%. Exposure to 15 foot-candles for 4 hours results in oxidation of 23% of the pigment (ratio 2.30).

Porphyrin Ring Destruction. The extinction ratios of the hydrogen peroxide–treated samples (Table I) are invariably lower than those of the reversibly oxidized samples of the same meat. The average value is 1.33, which differs significantly at the 1% level from the average ratio of the ferricyanide-treated samples.

Use of this ratio, however, to establish porphyrin ring destruction in cured meats is of questionable value, as iron oxidation and porphyrin ring destruction may go on simultaneously, so that at intermediate values a fall in this ratio could be ascribed to either type of chemical change in the heme pigment.

The ratio between the extinction at 540 and 500 mμ, however, provides the desired distinction between the two fading processes (Table I). The average value of the fresh and the ferricyanide-treated samples are close together. Formation of metmyoglobin does not result in a significant change in this ratio. When porphyrin ring destruction takes place, the value drops considerably. The average value for the extinction ratio at these wave lengths is 1.02 for the fresh surface, 1.04 for the oxidized pigment, and 0.89 for the hydrogen peroxide–treated samples. The difference between the averages of the fresh and the oxidized pigment on the one hand and the hydrogen peroxide –treated on the other hand is highly significant at the 1% level. From these data the amount of ring destruction can be determined in a similar way as for the ferrous-ferric oxidation.

Determination of the two extinction ratios given here makes it possible to establish the extent and the type of reaction taking place in a fading meat product.

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Received for review July 23, 1956. Accepted August 16, 1956. Research financed in part by a grant from Swift & Co. to the Florida State University. Part of a dissertation submitted by A. M. Erdman to the Graduate School, Florida State University, in partial fulfillment of the requirements for the degree of doctor of philosophy.

STARCH IDENTIFICATION

Microscopic Characteristics of Starches in the Identification of Ground Cereal Grains

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Observations made during various projects on starch and cereal grain characteristics are given to aid feed microscopists in the identification and quantitative estimation of cereal grain constituents in feeds. The microscopic appearance of ungelatinized and gelatinized starch granules can be used to distinguish the presence of most of the cereal grains. Microscopic structure of kernel parts can provide final distinction between grains that have similar starches, such as corn and sorghum.

IDENTIFICATION OF GROUND CEREAL GRAINS alone or in mixtures is a problem of control and regulatory laboratories of the feed, food, and other industries. Because cereal grains are similar in chemical constitution, identification is made microscopically.

Some microscopic characteristics of cereal grain starches that have proved sufficiently distinctive to be useful in identification of the grains in mixtures are presented. Attention is limited to the starches of the common commercial grains—viz., corn, wheat, grain sorghum, barley, oats, rye, and rice. Much

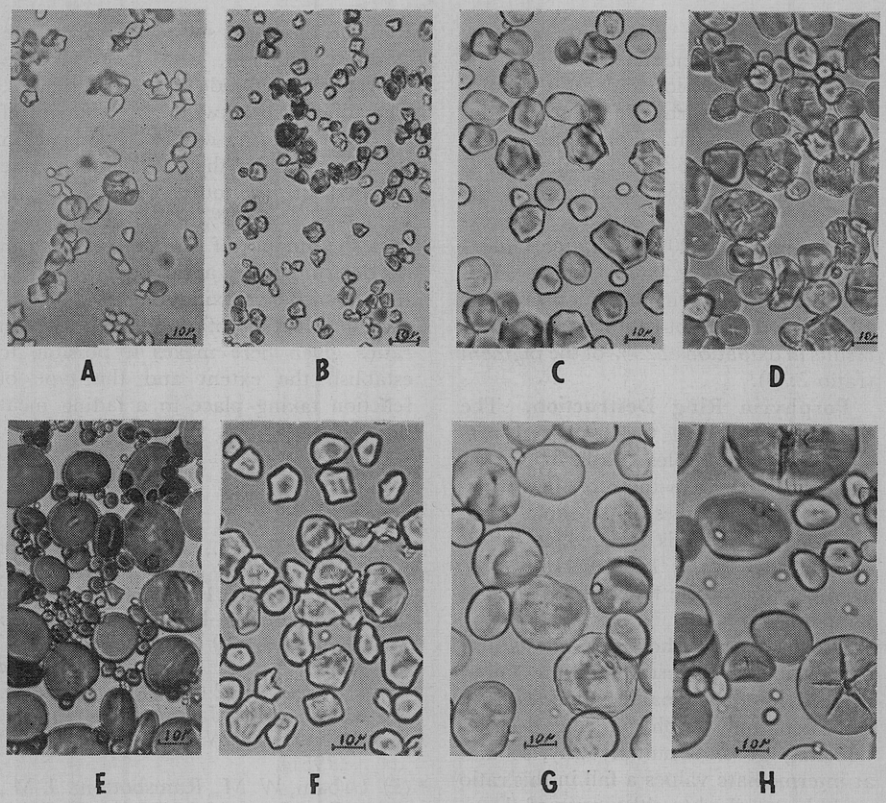

Figure 1. Ungelatinized starches

A, oat; B, rice; C, corn; D, sorghum; E, wheat; F, waxy corn; G, barley; H, rye

information of use to feed microscopists will also be found in the recent article by Schoch and Maywald (*1*).

Starch granules are always apparent in ground whole cereal material, even when other fragments are too small to be useful for identification. If free granules are not numerous, they may be increased by gently manipulating, in water, the grain fragments composed of cells or parts of cells in which the granules lie. The arrangement and appearance of the granules in the cells are sometimes useful indications of the kind of grain that is present.

Ungelatinized Granules

Materials containing ungelatinized starch may be mounted directly in water or in a mixture of water and glycerol. The addition of glycerol to the water used for mounting, helps to keep starch granules from moving about between larger particles. If the material is mounted directly in commercial glycerol, without dilution, the liquid penetrates the granules slowly. The hilum and any cracks or fissures radiating from it are easily observed as the liquid passes from the periphery to the edge of the hilum and any related fissures.

Size, shape, and structural details, such as prominence of fissuring from the hilum, identify the granules. Rice and oat starches are easily distinguished by their small, comparatively uniform, granule size and by the sometimes frequent observation of large compound granules, or of parts of them (Figure 1). The individual, simple oat starch granules of which the compound granules are composed are larger than those of rice. Granules of the other common cereal starches occur in a wide range of sizes within any one sample, but always some are larger than any of oat and rice starches (Figure 1).

Granules of corn and sorghum starches are roughly spherical or polyhedral in shape (Figure 1). They are of approximately the same size range, but sorghum starches have more large granules than cornstarch, a characteristic by which the experienced microscopist can distinguish separate samples. It would be difficult or impossible to distinguish them in mixtures.

Wheat, barley, and rye starches are similar, as all contain both small spherical and large lens-shaped granules (Figure 1). Wheat starch, however, has a large number of small granules, and rye starch has the prominent hilum with radiating fissures in many of the larger granules.

Rice, corn, sorghum, and barley occur in the so-called waxy or glutinous as well as in commonly known varieties. In waxy cereals, the endosperm starch (not that of the germ or of the hull) is composed wholly or largely of amylopectin, the branched component of starch. Ordinary starches are composed of a mixture of amylopectin, which predominates, and amylose, the linear component. Because of this difference in composition, waxy and nonwaxy starches from the same cereal species may be distinguished by their coloration with iodine. The material is mounted as usual and a drop of Lugol's or other iodine solution is applied at the edge of the cover slip. As the iodine enters the mount, a concentration gradient is set up and the coloration of the starch can be observed at any desired concentration of iodine. When the iodine concentration is too low, all starches appear uncolored or only faintly violet and when it is too high, all starches appear black in color. At the proper concentration, the common nonwaxy starches are colored blue or violet and waxy starches appear red to red-brown, except waxy barley starch, which is mottled with red and blue areas.

Gelatinized Granules

Gelatinization is defined at this laboratory, as at many others, as the loss of birefringence. It is occasioned by a decrease in molecular organization of the spherocrystal (starch granule), which occurs in cereal starches heated in water to about 58° to 78° C. and is accompanied by slight swelling. Subsequent swelling of the granule, when heating is continued to higher temperatures, is much greater and more characteristic of the starch variety. The gelatinization temperature ranges of the common cereal starches are so similar that their observation is of but little use in identification of the source of the starch. The appearance of the granules during subsequent swelling in hot water is more useful to the microscopist.

The preparation and examination of swollen starch granules are relatively easy. About 1 to 2% of the material is suspended in distilled water in a test tube and is placed in a water bath. The temperature of the bath is then slowly raised and a thermometer in the suspension can be used to stir it gently. At each temperature at which an observation is to be made, a drop of the suspension is removed and placed on a microscope slide under a cover slip. A return to room temperature will have little effect on the appearance of the starch granules.

In preparations that have been heated to 90° C. or above, the granule outlines become faint, on cooling, and observation is facilitated by adding a little dilute iodine–potassium iodide solution or by mounting the drop in a small drop of a solution of iodine in water. Enough iodine to color the granules faintly is sufficient, as too strong coloration obscures outlines and details. A heating stage can be used with identical results if the heating is adjusted to a slow rate.

When it is necessary to examine cooked cereal products in which the

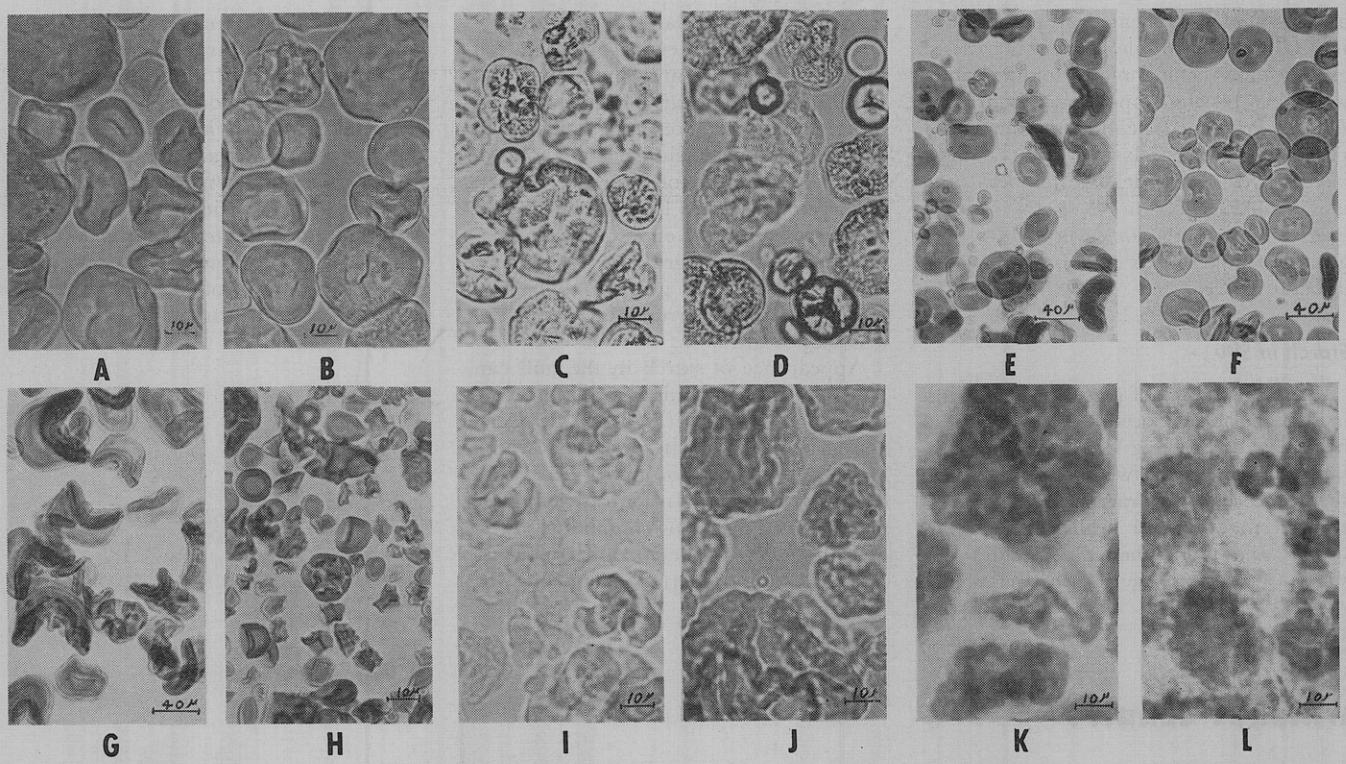

Figure 2. Starches heated in distilled water

A, corn; B, sorghum; C, waxy corn; D, waxy sorghum; heated to 70°C.; E, wheat; F, barley; G, rye; H, oats; heated to 80°C.; I, wheat; J, corn; K, waxy corn; L, waxy corn (after 1 hour); heated to 97°C.

starch has been gelatinized and more or less swollen, it is usually possible to wash some starch granules out of the larger pieces of the sample. According to most textbooks, starch granules gelatinize, swell, break, and disperse as they are heated in water to boiling. This is true of the cereal starches only under certain conditions. In an excess of water and with only moderate jostling and crowding, the granules merely swell and wrinkle. Some starches break more readily than others when swollen, and all break to some extent if severely agitated. Cereal starches are less subject to breakage than root starches and some of the waxy cereal starches break more easily than the common kinds.

Rice and oat starches swell without much change in shape of the granules. Angular forms, caused by compression of the individual granules in the naturally occurring large compound granules are evident during swelling.

Heating in water to 70° C. will swell both ordinary and waxy corn and sorghum starches considerably (Figure 2, *A*, *B*, *C*, *D*). At this temperature, corn and sorghum starches are difficult to distinguish although corn starch granules are usually more regularly swollen than most sorghum starch granules. Waxy corn and sorghum starch granules are easily distinguished from those of the corresponding non-waxy starches at this temperature; the waxy starch granules often show a characteristic rosette-like appearance.

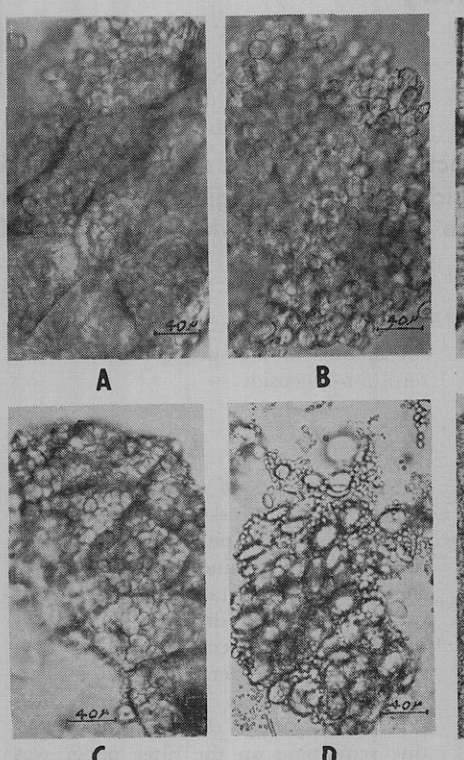

Figure 3. Endosperm particles

A, corn (horny); B, corn (floury); C, kafir var. sorghum (horny); D, hard wheat

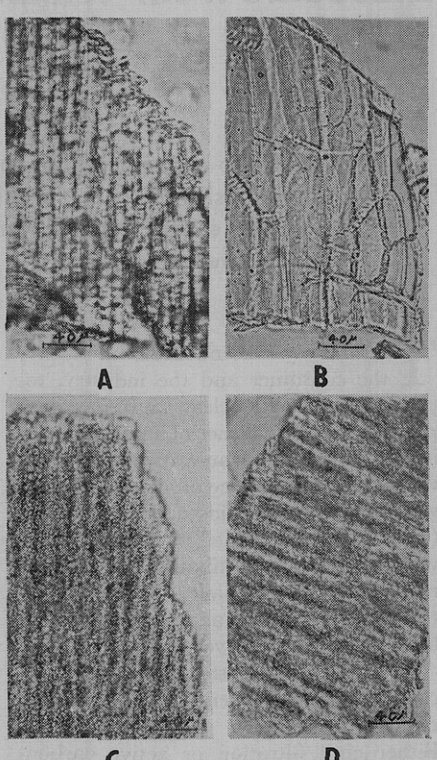

Figure 4. Pericarp particles

A, corn; B, wheat; C, Milo var. sorghum; D, kafir var. sorghum

They also exhibit the same difference in coloration with iodine as do the ungelatinized granules.

Wheat, barley, and rye starches, when heated in water to 80° C., are easily distinguished from the other common cereal starches because the larger granules have been swollen and twisted into characteristic saddle-shaped structures (Figure 2, *E*, *F*, *G*).

The appearance of most starch granules changes as the water in which they

are suspended is heated from 80° C. to boiling. Granule size increases greatly and much more folding and wrinkling becomes apparent (Figure 2, *I, J*). Prolonged heating at one temperature produces relatively little change beyond that seen when the temperature is first attained (Figure 2, *K, L*); however, after heating for several days the granules often shrink and become very difficult to break.

Starch in Situ

The appearance of pieces of the grain in which starch occurs is sometimes helpful in identifying the grain. Corn, for example, gives two types of endosperm pieces. In fragments of floury endosperm, the cell walls are rarely visible and the spherical granules of starch appear to be loosely arranged without any particular order (Figure 3). In pieces of horny endosperm, the cell walls are obvious and the polyhedral starch granules appear to be packed in an orderly mosaic. Sorghum endosperm fragments appear similar to those of corn. Fragments of barley, wheat, and rye endosperms look somewhat like the pieces of floury endosperm of corn, but the two sizes of granules are evident, the small spherical granules lying between and often surrounding the large lens-shaped granules.

Appearance of starch in the hull can be used to distinguish sorghum from corn and wheat (Figure 4). Corn pericarp, like wheat pericarp, which forms the major portion of the hull, contains no starch. In contrast, sorghum pericarp includes cells containing starch granules much smaller than those occurring in the endosperm, which give the fragment of sorghum pericarp a somewhat stippled appearance. Large granules may be washed in from endosperm as the material is mounted.

Familiarity with known mixtures of cereal starches will aid the microscopist to recognize similar mixtures in samples of unknown composition. Sometimes it is possible to estimate proportions of the different starches present and so to gain some indication of the percentage composition of the sample.

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Received for review April 14, 1956. Accepted November 5, 1956. 1956 Annual Meeting of the American Association of Feed Microscopists, Terre Haute, Ind., April 9 to 11, 1956.

WHISKEY CLARITY

Isolation and Identification of a Sterol Glucoside from Whiskey

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A hitherto unrecognized factor in the clarity of whiskey has been identified as sitosterol β-D-glucoside. The glucoside is of plant origin and originates in the finished beverage as a barrel extractive. From 60 pounds of white oak wood, 0.20 gram of the glucoside was isolated.

THE DISTILLER, endeavors to satisfy the consumer and the industry, to produce a visually clear and sparkling product free of any trace of haze or cloudiness. The major cause of turbidity in whiskey is the presence of high molecular weight, free fatty acids and their esters (*1*). These are carried over in the high wine during the distillation of the fermented grain mash and originate from the oils in the grains and from the metabolic products of the yeast.

At least two successful, though empirical, methods have been developed for the removal of these bothersome fats. Either chill filtration or active carbon treatment of whiskey is used today by most distillers to produce a generally satisfactory product.

Some bonded whiskies and more especially the lower proof straight whiskies, even after such treatments, sometimes develop a slight precipitate on storage in the bottle. This paper presents the isolation and the physical and chemical data of this material, and proves the identity of this substance to be sitosterol β-D-glucoside.

Experimental

Several bottles of carbon-treated 86-proof straight whiskey containing the characteristic precipitate were filtered in an effort to determine the nature of this material. The retained matter was washed with cold diethyl ether to determine the presence of any fatty substances. The ether on evaporation yielded no residue, indicating the absence of fatty acids or esters. The residue remaining on the filter paper was then continuously extracted with diethyl ether and after several hours a white floc collected in the lower flask of the Soxhlet apparatus. The floc was filtered from the ether, air dried, and then vacuum dried. Infrared absorption spectra and a melting point of about 282–92° C., with decomposition, was obtained for the compound. It also gave a positive and classical response to the Liebermann-Burchard test and a positive Salkowski-Hesse reaction for sterols. The quantities obtained, however, were insufficient for complete and positive identification.

To obtain a sufficient quantity of material for further investigation, the complete filter cake (diatomaceous earth) and precoat from the filtration of 2500 gallons of 86-proof straight whiskey (chilled to 17° F. and maintained for 48 hours) was airdried for several days on large copper trays. The dried material was then placed in cloth bags and charged in the side extraction chamber of a large modified Soxhlet extractor and continuously extracted with diethyl ether for at least 48 hours. All ether extracts from 15 such extractions were combined, and most of the ether was evaporated, leaving a volume of 800 ml. This solution was filtered, and the residue was washed with 50 ml. of cold ethyl alcohol, which removed most of the coloring matter. The residue, gray in color, consisted largely of siliceous filter aid, not