Microscopic View of the Mashing Process: Starch and Protein[1]

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ABSTRACT

Described here are some microchemical techniques we have found useful for studying changes in starch and protein during mashing. The IKI-NaCl stain provides a convenient differential staining for intact starch grains, amylose, amylopectin, and protein coagula. The fluorescent stain 1,8-ANS (1-anilino-8-naphthalene sulfonic acid, Mg salt) is also useful in mash studies because it is specific for protein and reveals the protein present in starch grains and also protein present in finely divided form. The polarizing optics of the microscope are useful in detecting "omega grains" in spent grains. Omega grains are grains of starch that have survived the mashing process with such little change that they still display the black cross when viewed in the unstained condition with a polarizing microscope.

Key words: *Mashing, Microscopy, Protein, Staining, Starch.*

The economics of brewing have resulted in an examination by many breweries of the ratio of adjunct to malt in the materials balance, and to the investigation of alternate adjuncts, such as barley. Current material costs have further prompted an interest in higher ratios of adjunct to malt than previously compatible with American brewing practice. With such increased adjunct use, and particularly where barley is the adjunct, the use of microbial enzymes (both proteases and amylases) in mashing may be indicated. These many changes have necessitated close controls of the mashing operations to ensure that they proceed on schedule, that the raw materials are used efficiently, and that the quality of the resulting worts and beers is not diminished.

One of the simplest, most direct, and most expeditious means for following the mashing operation is that of light microscopy coupled with staining techniques and microchemical tests. In this present paper are described some microscopical techniques we have found useful for studying changes in starch and protein during the mashing process.

STAINING TECHNIQUES

The IKI-NaCl Stain (3)

Iodine Solution. Place about 10 ml H_2O into a 100-ml volumetric flask; add 1 g KI; agitate to dissolve. Add 0.5 g I_2; agitate to dissolve. Make up to the mark with additional H_2O. Store this solution in the dark, well-stoppered.

NaCl Solution. Prepare about 100 ml of a saturated solution of NaCl in H_2O.

Stain Solution. Add 5 ml of the iodine solution to 50 ml of clear supernatant of the saturated NaCl solution, add 10 grams crystalline NaCl, mix well. Store this mixture in the dark, well-stoppered.

Method

Place a drop of sample (e.g., mash) on a clean microscope slide, add 2 drops of the stain solution, mix the drops with a fine-tipped glass rod, apply a clean cover glass, and examine.

This stain precipitates amylose and colors it blue; it precipitates amylopectin and colors it rose. Intact starch grains are colored purple or nearly black. Particles of protein are stained golden brown. Do not add an excess of stain solution to a sample; this will produce a muddy color reaction and the desirable differential staining will be lost.

The 1,8-ANS Stain (2, p. 26)

Stain Solution. Prepare a small volume (*e.g.*, 50 ml) of a 0.05% solution of 1-anilino-8-naphthalene sulfonic acid Mg salt in H_2O. This stain is best when freshly prepared.

Method

Place a drop of the sample (*e.g.*, mash) on a clean microscope slide, add 2 drops of the stain solution, mix the drops with a fine-tipped glass stirring rod, apply a clean cover glass, and examine with a fluorescence microscope. Proteinaceous particles fluoresce brilliantly.

The Safranin-O Stain

Stain Solution. Prepare a small volume (*e.g.*, 50 ml) of a 0.1% solution of Safranin-O in H_2O. This stain is best when freshly prepared.

Method

Place a drop of sample (*e.g.*, mash) on a clean microscope slide, add a drop of the stain solution, mix the 2 drops with a fine-tipped glass stirring rod, apply a clean cover glass, and examine. This stain lacks specificity and sometimes produces murky images, but it is often useful in revealing particles of protein. Safranin-O is a chloride; the colored moiety is a cation. It is thus useful in staining particles having some anionic surface properties (*e.g.*, protein particles, fragments of husk, and vascular tissue).

Polarized Light (2, pp. 29, 110)

A microscope equipped with polarizing elements can provide an optical staining that is useful in following the behavior of starch grains during the mashing process. When the polarizing elements are crossed, the field of view becomes dark and any intact starch grains in the specimen shine brightly and display distinct black crosses. This behavior with polarized light is a consequence of the orderly internal structure of intact starch grains, a characteristic which is lost as mashing progresses. Thus an examination of a mash or spent grains with a polarizing microscope permits one to detect and examine those starch grains that have not succumbed to the mashing process.

MASH STUDIES

To provide an example of the application of these stains in the study of the mashing process, we shall describe our observations of a laboratory mash. The schedule of the mash was as follows:

Cooker Mash

Mash in at 50° C; hold at 50° C for 30 min; raise the temperature 2° C/min until boiling starts; boil for 30 min; transfer the contents of the cooker to the main mash.

Main Mash

Mash in at 50° C; hold at 50° C for 30 min with only occasional stirring; add the contents of the cooker and, with constant stirring, raise the temperature to 65°C within about 15 min. Hold the mash

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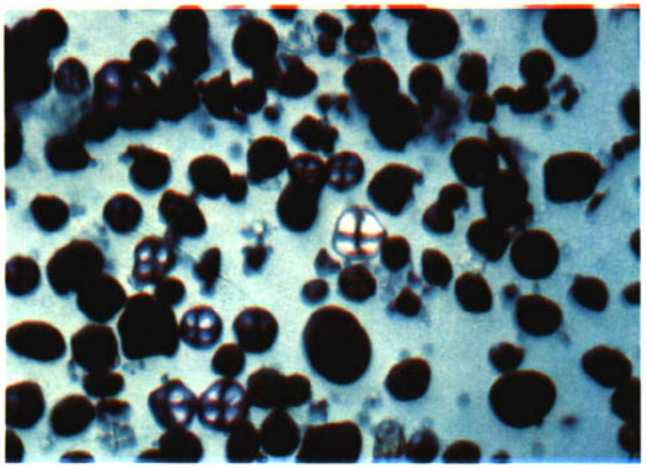

Fig. 1. A cooker mash at the start, containing common yellow corn grits with 10% added malt as an enzyme source. Stained with IKI-NaCl, polarizing optics. The starch grains are largely intact. Those that have not stained display typical black crosses. 220× magnification.

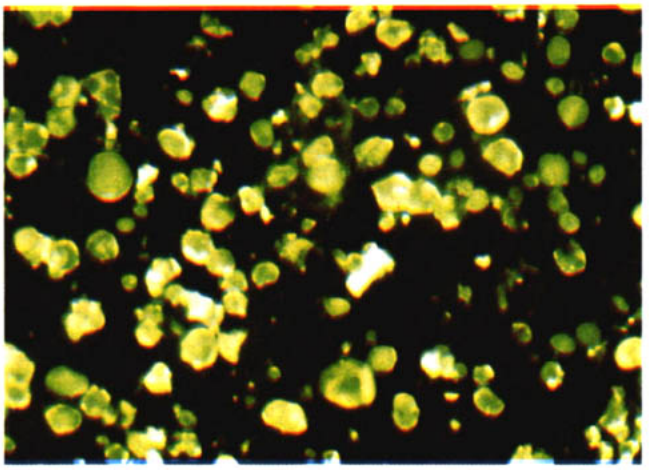

Fig. 2. This is the same sample as in Fig. 1, but stained with 1,8-ANS. The protein of the starch grains fluoresces brightly. There is no protein free in the liquid. 220× magnification.

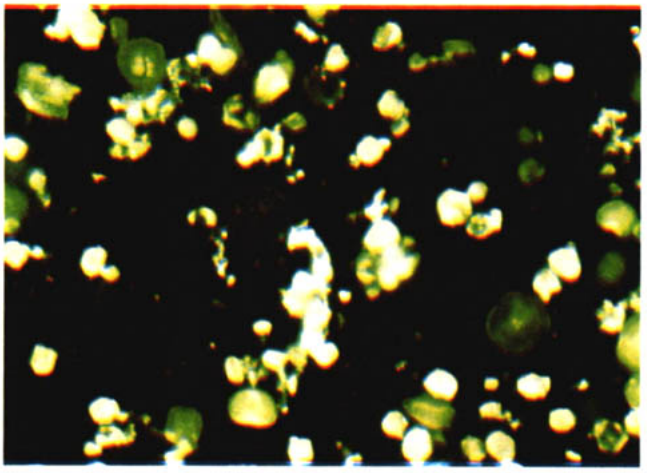

Fig. 3. The cooker mash at 65° C. Stained with 1,8-ANS. The grains of malt starch have lost some of their protein; small protein coagula have formed; the background is still quite clear, indicating that there is no protein free in the liquid. 220× magnification.

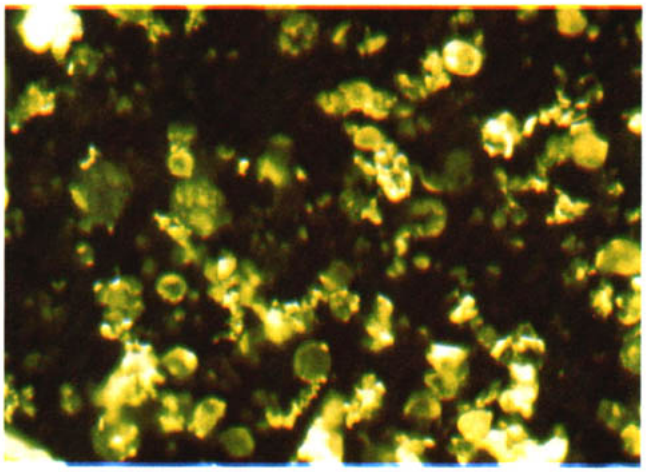

Fig. 4. The cooker mash at 70° C. Stained with 1,8-ANS. The starch grains have lost much of their protein; numerous protein coagula have formed; the background is murky and full of fluorescence, indicating that there is much protein dispersed in the liquid. 220× magnification.

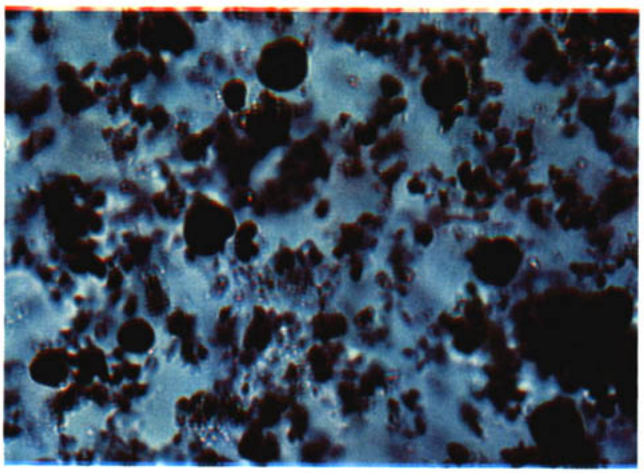

Fig. 5. This is the same sample as in Fig. 4. Stained with IKI-NaCl. Most of the starch grains are disintegrated. There is much free amylose. 220× magnification.

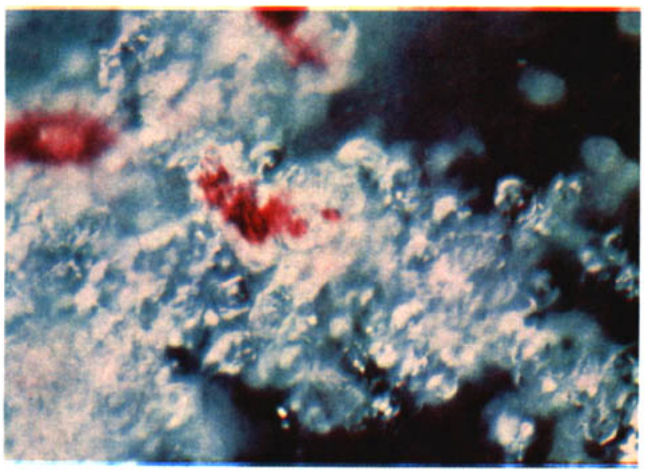

Fig. 6. The boiling cooker mash. Stained with Safranin-O and viewed with darkfield illumination using a dark blue filter at the light source. The protein coagula, shown in red, are surrounded by masses of amylose. 220× magnification.

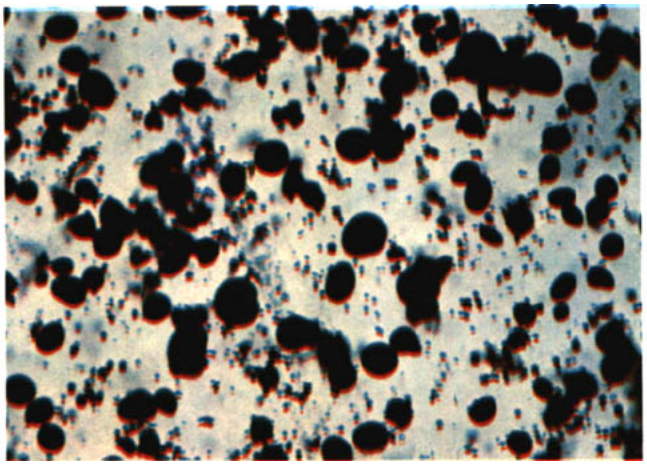

Fig. 7. The main mash at the start, stained with IKI-NaCl. The two sizes of grains of malt starch are plainly evident. 220× magnification.

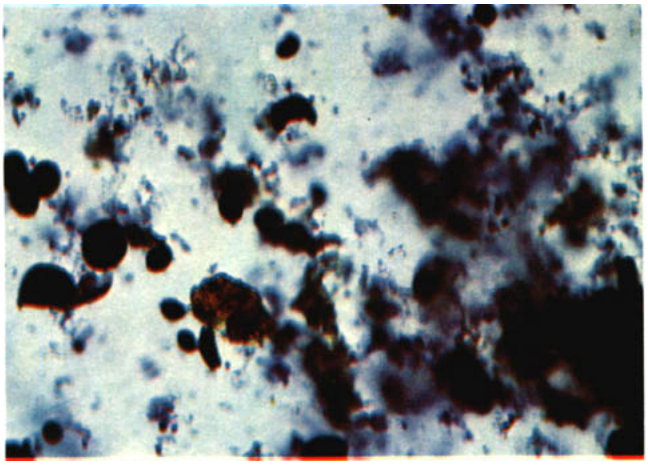

Fig. 8. The main mash shortly after the addition of the cooker. Stained with IKI-NaCl. Note the particles of amylose and amylopectin from the cooker mash; the grains of malt starch of the main mash appear little changed. 220× magnification.

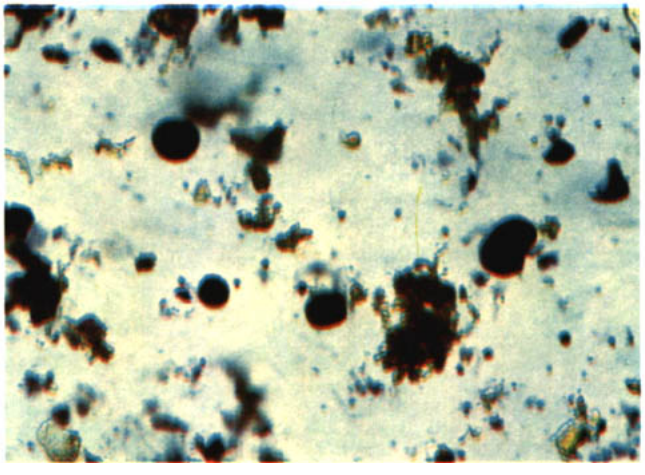

Fig. 9. The main mash at the end of the 65° C holding period. Stained with IKI-NaCl. The amylose and amylopectin from the cooker are almost gone; the grains of malt starch of the main mash appear little changed. 220× magnification.

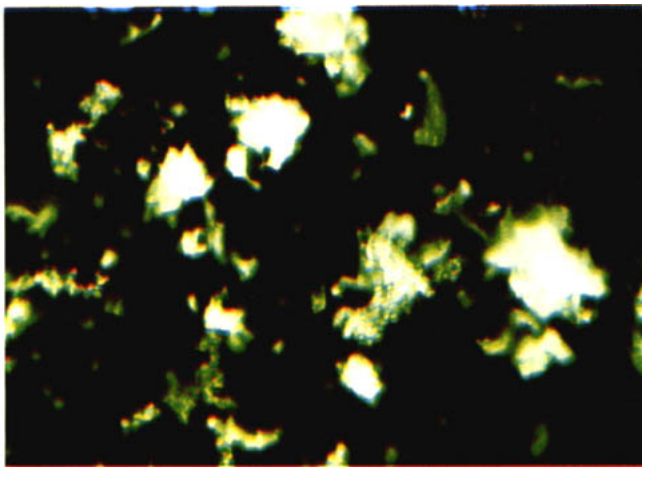

Fig. 10. The fine-particulate matter of the spent grains. Stained with 1,8-ANS. The protein coagula are large and they fluoresce intensely. Note that the background is clear and dark; the finely dispersed protein that was present earlier (*cf.*, Fig. 4) has gone, either into the wort or into the large coagula. 220× magnification.

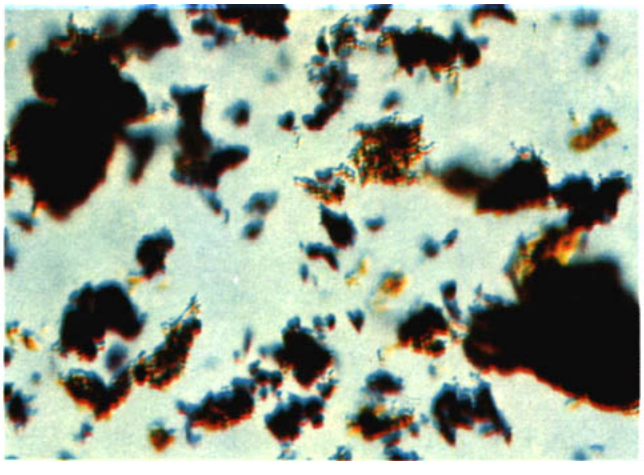

Fig. 11. The fine-particulate matter of the spent grains. Stained with IKI-NaCl. Note the protein coagula and the fragments of amylose and amylopectin. This mash was successful in terms of yield obtained. 220× magnification.

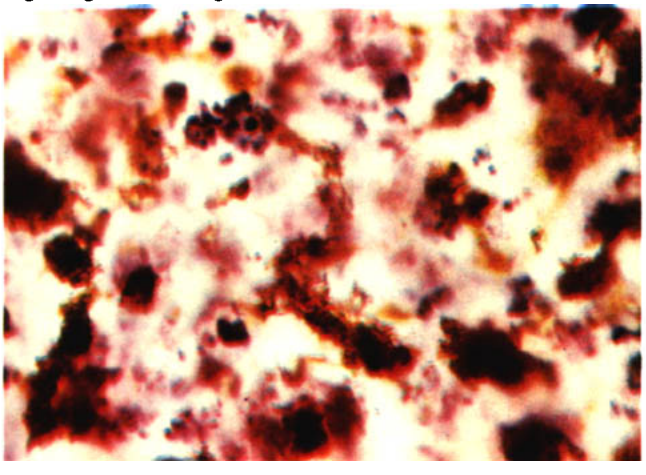

Fig. 12. The fine-particulate matter of the spent grains from a mash that employed a 25/75 malt/corn ratio. IKI-NaCl stain. Note the abundance of amylose and amylopectin. This mash was a failure in terms of extract yield. 220× magnification.

at 65°C for 30 min with constant stirring. Raise the temperature to 75°–76° C within about 10–12 min. Hold the mash at this temperature until conversion is complete (*i.e.*, a negative test for starch in a spot-plate test). The mash is then filtered through paper to collect the wort.

The mash we shall describe is a 55/45 malt/corn mash yielding about 3 liters of 12° Plato wort. A small charge of malt (10% of the weight of the adjunct) is added to the cooker to facilitate liquefaction of the adjunct in the cooker.

MICROSCOPIC OBSERVATIONS (See illustrations)

Cooker Mash

At the start of the cooker mash the starch grains of the corn and the malt stain darkly with the IKI-NaCl. They display distinct black crosses when viewed with the polarizing optics of the microscope in the unstained condition. A little amylose is free in the liquid; this probably originated from fractured starch grains. The protein of the mash is present chiefly as components of the starch grains; a few irregular protein granules are visible; these are usually closely adherent to the starch grains. There is no protein finely dispersed in the liquid at the start of the cooker mash.

There is no apparent change in the starch and the protein during the 30-min holding period at 50°C. Change becomes apparent, however, as the temperature of the cooker mash is raised. At about 62°C the grains of malt starch begin to lose their ability to fluoresce with the 1,8-ANS, an indication that they are losing some of their protein. This protein appears to precipitate immediately as irregular coagula or nuggets which fluoresce brilliantly with the 1,8-ANS stain. There is apparently no soluble or finely dispersed protein in the liquid at this time, as evidenced by the lack of fluorescence in the liquid between the starch grains.

At about 70°C the grains of corn starch begin to lose their ability to fluoresce with the 1,8-ANS; this loss of protein from the grains of corn starch is accompanied by the development of a great many coagula of protein which fluoresce brilliantly with the 1,8-ANS. There is also the development of considerable fluorescence in the liquid of the mash; this indicates that much protein is present in finely dispersed form. The grains of malt starch are by now mostly disintegrated; the grains of corn starch are greatly swollen; there is much amylose dispersed in the liquid.

At about 75°C the 1,8-ANS staining reveals that the protein coagula have increased in size and that there is much finely dispersed protein in the liquid. Practically no starch grains now fluoresce with the 1,8-ANS; this indicates that they have lost practically all of their protein. A staining with the IKI-NaCl reveals that only a few intact starch grains remain; there is much amylose and amylopectin free in the liquid.

During the boiling of the cooker mash the particles of amylose and amylopectin tend to aggregate in large masses around the protein coagula. The protein coagula become very large; there is much finely dispersed protein free in the liquid.

Main Mash

The main mash is an all-malt mash at the start; a staining with IKI-NaCl reveals the typical large and small grains of malt starch. A small amount of amylose is free in the liquid. A staining with 1,8-ANS indicates that virtually all of the protein in the mash is contained in the starch grains. Some small proteinaceous granules are adherent to the larger starch grains.

During the 30-min holding period at 50°C, the starch grains tend to give a slightly more reddish staining reaction with the IKI-NaCl. This indicates that some of the amylose has been lost from the starch grains and that the starch grains are now relatively richer in amylopectin than before. There is only a little increase in the amount of amylose free in the liquid. This indicates that the amylose which left the starch grains must have been digested away about as rapidly as it became free.

The addition of the cooker mash introduces the many protein coagula and the masses of amylose and amylopectin that were developed during the cooker operation. These masses of amylose and amylopectin begin to diminish with surprising quickness as the temperature of the main mash approaches 65°C. The grains of malt starch become swollen during the climb to 65°C.

During the holding period at 65°C, the masses of amylose and amylopectin that come from the cooker continue to diminish and then disappear from view. The protein coagula from the cooker remain essentially unchanged. The grains of malt starch continue to swell; some are disintegrating.

The grains of malt starch disintegrate rapidly as the temperature of the main mash is raised to 75°–76° C. There is little or no accumulation of amylose or amylopectin during this temperature rise; these substances are apparently digested as rapidly as they are released.

The protein coagula are the most conspicuous particles in the mash after conversion. These coagula have apparently survived the mashing with very little change. A few faint wisps of amylose and amylopectin also remain; these may even be found in mashes that give a negative starch reaction in a simple spot-plate test.

Spent Grains

The mash is next filtered through paper (Reeve Angel No. 802, 32-cm diameter). The fine-particulate matter of the spent grains consists chiefly of protein coagula with occasional wisps of amylose and amylopectin. Some intact starch grains may also be present. Starch grains that have survived the mash with such little change that they still display the black cross when viewed in the unstained condition with a polarizing microscope are termed "omega grains" because they have persisted to the end. These are usually smaller grains of malt (or barley) starch, although larger grains of malt (or barley) starch may also be present. In view of the reported 8:1 ratio of smaller grains of malt starch to larger ones (1), it is not surprising that the smaller ones are more likely to occur as omega grains.

Mashes employing exaggerated ratios of adjunct to malt (*i.e.*, more than 50% of the extract coming from adjunct) sometimes fail to utilize the available starch efficiently and, as a result, the spent grains of such mashes may contain numerous particles of amylose and amylopectin as well as numerous omega grains of malt starch.

Mashes that appear entirely successful from the standpoint of extract yield may contain some omega grains of malt starch.

Excessive numbers of omega grains may be indicative of inadequate milling of the corn, rice, barley, or malt. In such instances a microscopic examination may reveal clusters of omega grains trapped in bits of tissue debris. It is generally a simple matter to determine the origin of the omega grains by microscopic examination. The starch grains of corn, rice, and malt (or barley) are quite different from each other and are easily distinguished microscopically.

Excessive numbers of particles of amylose and amylopectin in spent grains may be indicative of an enzyme-substrate imbalance in the mash. This may be corrected by the application of commercially available mashing enzymes. Microscopic examinations of the spent grains are a convenient means for monitoring the changes wrought by such enzyme additions.

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