The Application of Light and Scanning Electron Microscopy During Flour Milling and Wheat Processing

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ABSTRACT

Light and scanning electron microscopy have been employed as part of an on-going study on the effect of the milling system on flour composition and quality. Examples are given of some areas where microscopy has been particularly useful in understanding the functional changes that take place during milling or the subsequent processing of the flour. The use of heavy reduction roll pressures was shown to modify gluten protein quality as well as produce the desired increase in starch damage. The use of in-line impact machines not only disrupted flaked flour particles but also fragmented wheat germ and allowed it to enter the flour. This reduced the dough mixing time and influenced the improver requirement. Microstructural studies also assisted in establishing production methods for two Middle-Eastern wheat products and formed an integral part of a study that investigated the factors influencing noodle quality.

INTRODUCTION

At the Bread Research Institute of Australia, microscopy is regularly used as a part of the flour milling and baking research programs, in addition to being used as a trouble-shooting tool. Light and scanning electron microscopy are usually used in conjunction with each other as one technique complements the other. Scanning Electron Microscopy (SEM) is particularly useful when hydration associated with light microscopy may produce significant artifacts due to swelling, e.g., the relationship between wheat morphology and grain hardness, or where the porosity of a dry, highly gelatinised baked food has to be determined. However, SEM mainly provides information based on the shape, or location of the individual components of the system, whereas light microscopy can, via the use of selected histochemical reagents, provide more information about the chemical composition of the components.

Microscopy is always used in conjunction with other techniques and may only provide confirmatory or supplementary evidence. However, in some cases, it can provide a unique insight into the system or process due to its ability to provide information on location and distribution, in addition to chemical composition. Examples of the research and trouble-shooting applications of microscopy in flour milling and related industries are illustrated and implications are discussed.

MATERIALS AND METHODS

Microscopic Techniques

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was gradually raised to 4°C over a 24 hour period. The freeze-dried samples were then coated and examined as previously described. (Moss et al. 1980).

Flour Milling

Previous work (Moss et al. 1980) has concentrated on the relationship between wheat morphology and grain hardness, and how this influences conditioning requirement and bran clean-up during milling. In addition, hard wheats produce more granular stocks which flow and sieve more easily in the mill, and facilitate the production of starch damage in the mill. A controlled level of starch damage is required in bread flours to regulate water absorption as damaged starch absorbs more water than undamaged starch. In a hard wheat the continuous protein matrix firmly binds together the starch granules and they are damaged by the shear and pressure that the smooth reduction rolls apply to the endosperm particles. The air spaces in the endosperm of a soft wheat grain cause it to fall apart very easily and the reduction rolls cannot effectively apply the necessary forces to sufficiently damage starch granules. Many commercial mills increase the pressure on the reduction rolls to such an extent that large flakes of endosperm, containing damaged starch, are produced (Fig. 1). The damage sustained by many of the starch granules is not apparent until the flakes are hydrated. This causes the starch granules to swell and lose birefringence. Another consequence of producing flakes that is frequently not appreciated, is that the functional properties of the protein can also be adversely affected. In Figure 1 it is difficult to clearly recognise the individual starch granules because the protein has been smeared over the surface of all the granules. This protein has lost much of its vitality, as a cohesive, elastic gluten cannot be washed out from such material. The farinograph mixing curves of gluten from flaked and non-flaked semolina are shown in Figure 2 and the effects on bread quality are depicted in Figure 3. (N.B. "semolina" equivalent to American sizings or first middlings)

To prevent these endosperm flakes from being sieved out with the coarse bran fractions, impact machines are currently used in the reduction systems of almost all mills. These machines not only disrupt flakes, but also break up wheat germ very effectively and to a lesser extent, also release bran particles into the flour (compare Figs. 4 & 5). The amount of wheat germ in flour can be measured (Stenvert et al. 1981) but the presence of any appreciable amount of bran can interfere with the analysis and hence microscopy can be used in these situations to give an indication of the amount of wheat germ present in a sample.

Wheat Germ and Bread Quality

Previous work at the Bread Research Institute indicated that the wheat germ content of bakers' flour can vary on a day-to-day basis, and that this variation can influence improver requirement and bread quality (Moss et al. 1984). A range of flours was produced where the only variable was the amount of germ in the flour. Microscopy was used to study the effect of wheat germ on gluten protein development during mixing or molding. When the wheat germ content of a flour was increased, the gluten developed more quickly (Figs. 6 & 7) and hence if this situation arose commercially there is a risk that the gluten protein would be overdeveloped, i.e., spread out into very thin films of protein (Fig. 8) which coat the surface of all the starch granules. This results in sticky doughs and the thin films of protein allow the gases to escape during proofing and baking. This results in bread composed of round thick-walled cells which produce a coarse texture and low volume. At a similar level of addition, bran has a negligible effect on mixing time, gluten development or the structural characteristics of the bread.

Flour for Starch-Gluten Production

Microscopy has been a useful aid in a number of trouble-shooting investigations undertaken by the Institute for those of its members who are involved in starch-gluten separation. In one case, comparisons were made of the material removed from the starch suspensions in two factories. Factory 1 was experiencing difficulties due to screens becoming blocked at more frequent intervals than occurred at factory 2. Light microscopy was particularly useful in the investigation, due to the extra compositional information available from histochemical techniques. Examination of the material removed by the screens at factory 1 indicated that the major component was endosperm cell wall material. This material was in the form of thin, relatively structureless, flexible sheets and was readily identified using polarised light. In contrast, the material removed by the screens at factory 2 was mainly larger fragments of bran and germ. Further discussions with the member...
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Figure 3. Bread produced from flour obtained from a single sample of semolina that had been milled to starch damage levels (L-R) of 10 Farrand units (non-flaked) and 73 Farrand units (flaked). Note the poor volume and coarse texture of the loaf from the flaked semolina.

Figure 4. SEM of Stock from B reduction roll to in-line impact machine. The germ particles (P) are partially covered with starch due to the oily nature of the germ.

Figure 5. Stock from B in-line impact machine. Note how many of the larger germ particles have been disrupted into small particles (S) which can enter the flour. Bar = 200 μm. Inset is a higher powered micrograph of the small germ particle S. Bar = 40 μm.

Figure 6. SEM of dough from flour containing no added wheat germ (Germ Index 0.11). The dough was mixed for 3½ minutes and the gluten was not fully developed as indicated by the coarse gluten strands (G). Five minutes were required to optimally develop the dough and provide a continuous gluten matrix.
company indicated that the design of the screens in factories 1 and 2 differed, and that the unwanted improved removal of endosperm cell wall material at factory 1 was the cause of the problem. Examination of the A and B starch samples produced by the two factories confirmed this speculation. The B starch (small granules) from factory 1 contained principally starch, whereas that from factory 2 contained appreciably more material of endosperm cell wall origin.

Wheat for Middle-Eastern Foods

Jerish. This food is made from cracked wheat and is cooked and eaten like rice. The cracked grains have a vitreous appearance similar to durum wheat. Samples were received from Iraq, Syria, India and Bahrain and the Iraqi sample was reported to be the best in quality. The Institute was requested by the Australian Wheat Board to determine how these products were made, and to suggest a process whereby a premium quality product could be made from Australian Standard White (A.S.W.) wheat. The samples were analysed for protein content and the values ranged from 9.0 to 14.0%. Microscopic examination (Fig. 9) indicated that the starch in the Iraqi sample was completely gelatinized as indicated by the deformed, folded starch granules and that in one Syrian sample there was a mixture of heat-treated and non heat treated grains. All the other samples had not received any heat treatment. The samples were also subjected to electrophoretic analysis which indicated that they did not contain any durum wheat. Microscopic examination was most useful in this case as the supplier was not aware of the heat treatment process and it was felt that durum wheat might have to be used to produce a good quality vitreous product. However, by fully gelatinizing the starch in A.S.W. wheat prior to air drying and cracking, a premium quality product was produced with the desired vitreous appearance. (Compare figures 10 & 11).

Harriss. Harriss is another Middle-Eastern food prepared from pearled wheat. Microscopic examination indicated that both hard and soft wheats were present in the reference samples and that the wheats had not received any heat treatment. The wheat had been pearled in such a manner as to leave most of the aleurone layer intact (Fig. 12) but to remove most of the embryo and a small amount of the scutellum. This suggested that a suitable machine would be a rice pre-whitener as a barley pearler would tend to remove all the germ if satisfactory bran removal was to be achieved. Further trials indicated that abrading to a 9% loss in weight gave a more satisfactory product than to a 7% loss in weight.

The Effect of Alkali on Chinese Noodle Quality

In the manufacture of Chinese noodles (Moss 1985) the doughs are made alkaline both to develop flour, and also a yellow colour in the raw noodles due to the reaction with flour flavone glycosides. However the eating quality is also influenced by the choice of alkali. Sodium hydroxide (1% of flour weight) produces a softer noodle with a slightly sticky or "slimy" surface, whereas the same level of Kan Sui (a mixture of 90% Na2CO3 and 10% K2 CO3 produces a firmer noodle with a non-sticky surface. Light microscopy indicated that the sodium hydroxide impaired gluten development and endosperm particles were apparent at the final reduction stage. The gluten protein matrix was also less uniform and coherent (Fig. 13) when compared with the corresponding Kan Sui noodle (Fig. 14).

The noodles were then pre-cooked in boiling water for 40 seconds, cooled in water (15°C), oiled and re-cooked the following day in boiling water for 2½ minutes. The re-cooked noodles were then cooled in water and a portion was placed into fixative and the remaining portion was frozen in liquid nitrogen and freeze dried. The surface of the freeze-dried noodles was then examined in the scanning electron microscope. The surface of the noodle containing sodium hydroxide had a more delicate, thin walled, open appearance compared to that of the noodle containing Kan Sui (compare Figures 15 & 16). The greater surface disruption of the noodle containing sodium hydroxide was responsible for the slimy surface characteristics. When transverse sections of the noodles were examined using the light microscope it was apparent that internally the starch-protein matrix of the sodium hydroxide noodle was also highly disrupted and voids were present (Fig. 17), whereas that of the Kan Sui noodle was more compact and continuous (Fig. 18), resulting in a firmer eating quality.

CONCLUSION

The foregoing examples illustrate how microscopy can be used in both research and trouble-shooting applications. In some instances microscopy is mainly used to suggest what additional quantitative analytical data should be obtained. In other cases it provides the majority of the information one requires to establish the cause of a problem or suggest the nature of the processing that a particular product has received.

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References

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Figure 7. SEM of dough from flour containing added wheat germ (Germ Index 0.46). The dough was mixed for 3.5 minutes and was fully developed as indicated by the uniform distribution of the gluten.

Figure 8. SEM of dough from flour containing added wheat germ (Germ Index 0.46). The dough was mixed for 5 minutes and was grossly overdeveloped as indicated by the extensive, thin films of protein (F) adhering to the surface of the starch granules.

Figure 9. SEM of a fracture surface of Iraqi Jerish. Note the folded appearance of the starch granules in the central endosperm, which indicates that the product has been cooked.

Figure 10. SEM of a fracture surface of Jerish from Australian Standard White wheat. Note the extremely vitreous appearance of the sub-aleurone endosperm which is due to the cooking process prior to drying.

Figure 11. SEM of a fracture surface of uncooked durum wheat. Note the inherent, vitreous appearance of the sub-aleurone endosperm.

Figure 12. SEM of a cut surface of Harriss. Note the removal of most of the bran layers whilst retaining the aleurone layer (AL).
Discussion with Reviewers

E. A. Davies: Were the noodles in Figures 15 and 16 wet or dehydrated prior to SEM preparation?
Author: The noodles were frozen in liquid nitrogen and freeze dried. The reported surface differences were consistent and the same microstructural characteristics were also observed when samples were frozen in iso-pentane cooled by liquid nitrogen prior to freeze drying.

D. B. Bechtel: Is the gluten damage that occurs during the flaking process a result of the intense shearing or due to the heat produced by the shearing process?
Author: It is difficult to precisely answer this question as the grinding process itself generates heat in the stock. However, we do know that heavy grinding with cold (15°C) rolls will produce flakes which have modified gluten properties - commercially, reduction rolls operate with roll surface temperatures up to 75°C. The gluten is toughened during the production of flakes, depending on the degree of toughening, this need not be deleterious. Some weak gluten can benefit from a slight degree of toughening. Further work is being undertaken in this area.

D. B. Bechtel: Which is more important to the miller? Production of damaged starch or reduction of loaf volume by damaged gluten?
Figure 3 would indicate that a very good loaf of bread could be produced without the flaking process and damaged starch.

Author: In practice a compromise has to be reached between the benefits of achieving the desired level of starch damage and the possible adverse effects on gluten caused by the production of excessive amounts of endosperm flakes. In many cases the mill can be set up in such a way that the desired level of starch damage can be achieved without any adverse baking effects being observed. However, in certain circumstances problems can arise e.g., due to a lack of roller surface or if large amounts of semolina are being removed from a bakers grit for pasta manufacture. This is particularly important if the wheat is not in the hard category. In such cases very heavy roller pressures have to be used to produce starch damage and gluten damage can then become significant.

D. B. Bechtel: What criteria do you use to measure starch gelatinization by SEM? Our experience with parboiled rice indicates that complete gelatinization results in loss of starch granule integrity yet your Fig. 9 shows distorted but intact starch granules. Could these granules be only partially gelatinized?
Author: We relate starch gelatinization to the loss of birefringence as observed using the light microscope, and frequently apply a qualifying description to indicate the extent of gelatinization. The latter depends on the combination of time, temperature and water availability. In Jerish there was a range of gelatinization but no loss of starch granule integrity. However, the sample illustrated in Figure 9 contained no birefringent starch granules. When starch granules completely lose their integrity this indicates that the availability of water was not restricted, but in many other foods, such as Jerish, this is not the case.

P. Resmini: What are the temperature and moisture conditions that were used to produce the Jerish from ASW wheat?
Author: The wheat was steeped for 18 hours in cold (15°C) water to allow the central endosperm to fully hydrate. The soaked grains were then covered, heated in a small excess of water to 100°C, and held at this temperature for 20 min. The grains were then drained, spread out in a thin layer and air dried at 37°C.

P. Resmini: Can the author formulate any hypothesis on the different "mechanisms" and effects promoted by these two alkaline media on the gluten matrix?
Author: This aspect is currently under investigation but it is well known that sodium hydroxide has a marked effect on gluten, even causing solubilisation of the protein (Batey and Gras, 1981). This denaturation of gluten, due to sodium hydroxide, reduces the continuity of the gluten protein matrix. The resultant lack of continuity appears to soften the cooked noodle due to greater expansion on cooking. This may be due to a number of factors: a) more rapid water penetration due to the lack of continuity of the protein matrix; b) weakening of the protein due to the alkali; c) enhanced gelatinisation of the starch due to the alkali.

P. Resmini: Did the author make any attempts to differentiate the proteins and the starch in the SEM images of cooked Chinese noodles (Figs. 15 and 16)?
Author: It was not possible to differentiate between the starch and the protein in the SEM. However from the stained sections prepared for the light microscope it was apparent that the major constituent of the noodle surface was grossly expanded, gelatinised starch. In both alkali treatments the protein matrix at the surface was extremely disrupted due to the expansion of the starch, but the protein matrix appeared to be more disrupted when sodium hydroxide was used.

Additional Reference

Figure 13. Light micrograph of a transverse section of a raw sodium hydroxide noodle sheet prior to the final reduction stage. Note the lack of continuity in the gluten protein matrix and the undeveloped endosperm particles.

Figure 14. Light micrograph of a transverse section of a raw Kan Sui noodle sheet prior to final reduction stage. Note the continuity of the protein matrix and the lack of underdeveloped endosperm particles.

Figure 15. SEM of surface of a fully cooked sodium hydroxide noodle. Note the disrupted, delicate appearance of the surface.

Figure 16. SEM of surface of a fully cooked Kan Sui noodle. Note the thicker walls and the smaller voids in the surface when compared to the sodium hydroxide noodle after cooking.

Figure 17. Light micrograph of a transverse section of a fully cooked sodium hydroxide noodle. Note the disruption of the protein matrix resulting in a softer texture. (The gelatinized starch is unstained and is therefore not readily apparent.)

Figure 18. Light micrograph of a transverse section of a fully cooked Kan Sui noodle. Note the more compact appearance of the noodle and the more continuous protein matrix.