# Ultrastructure of endosperm and quality

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# Ultrastructure of endosperm and quality

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SUMMARY - Scanning electron microscopic examinations of the endosperm of malting barleys reveal wide variabilities in the structure of the endosperm as regards compaction of the small starch granules and storage (matrix) protein. Mealy endosperms show lesser degrees of compaction than steely endosperms and, in contrast to steely endosperms, are more readily penetrated by endosperm-degrading enzymes during malting. The causes of these differences in degrees of compaction require urgent investigation because localized compaction of the endosperm can limit enzymic breakdown during malting. These localized under-modified areas are not readily detected by conventional malt analysis but may nevertheless release ß-D-glucan which retard wort separation and restrict beer filtration. In malted sorghum the mechanism of cell wall breakdown is different from that which occurs in malting barleys. In barley over 80% of the ß-D-glucan is broken down during malting, in sorghum very little change in the original level of ß-D-glucan occurs. However, portals (or holes) develop in the cell walls, through which enzymes migrate to hydrolyse protein matrix reserves and starch. The cell walls of malted sorghum are more soluble than are those of the unmalted grain.

*RESUME - "Ultrastructure de l'endosperme et qualité". Des analyses au microscope électronique de l'endosperme d'orge de malterie montrent une grande variabilité de la structure de celui-ci en ce qui concerne le tassement des petits granules d'amidon et de la protéine de réserve. Les endospermes farineux montrent un moindre degré de tassement que les endospermes durs, et les premiers sont, comparativement, plus facilement pénétrés par les enzymes de dégradation de l'endosperme lors du maltage. Il est donc nécessaire de rechercher les causes de cette différence de degré de tassement, car le tassement localisé de l'endosperme peut limiter la dégradation enzymatique pendant le processus de maltage. Ces zones localisées moins dégradées ne sont pas facilement détectables par l'analyse conventionnelle du malt, mais cependant elles libèrent du ß-D-glucane qui retarde la séparation du moût et limite la filtration de la bière. Chez le sorgho malté, le mécanisme de dégradation de la paroi cellulaire est différent de celui qui a lieu chez l'orge malté. Chez l'orge, plus de 80% du ß-D-glucane est dégradé lors du maltage, tandis que chez le sorgho il n'y a qu'une très légère modification par rapport au niveau initial de ß-D-glucane. Cependant, des trous se forment sur les parois de la cellule, les enzymes migrant au travers de ces trous afin d'hydrolyser la matrice protéique et l'amidon. Les parois cellulaires du sorgho malté sont plus solubles que celles des grains non maltés.*

## Introduction

Plant morphologists believe that structure quality usually reflects important aspects of function. One interpretation of this view is that the function of the endosperm cannot be understood until its anatomy and physico-chemical properties are known. In this context, the influence which the dead (i.e. non-living) tissue, the starchy endosperm of barley, has on the physiological processes which convert barley into malt, is of vital importance to those who wish to control the malting process.

The embryo and the aleurone layer of the barley grain are living tissues in which important physiological developments occur. For example, the germinated embryo produces and transports gibberellic acid to the aleurone layer which develops the enzymes which degrade the cell walls of starchy endosperm cells and release solubilized proteins and starch from them.

The rate of release, the degree of hydrolysis, the uniformity of hydrolysis and the yield of extracted substances from the starchy endosperm are linked to the physiological life processes of the embryo and aleurone layer but are not wholly controlled by them. In this regard, the structure of the starchy endosperm is an important aspect of the quality of the grain because it controls enzymic hydrolysis of endosperm tissue.

It is not clear how the structure of the starchy endosperm limits hydrolysis during malting. Notwithstanding the aim of this paper is to present microscopic and other evidence in support of the concept that endosperm structure is an important part of the quality of malting barley.

## Results and discussion

A generalised cross section of the starchy endosperm of barley endosperm is shown in Fig. 1. The section is horizontal, immediately above the crease or furrow. The crease tissue divides the section. In this micrograph it is noteworthy that the outer sub-auleurone tissue is more compact than the innermost tissue. Chemical analyses of the outer and inner endosperm of barley grains revealed that although the protein content of the whole grain can be 13%, the protein content of the outer endosperm can be 15%, middle endosperm 12% and inner endosperm 5%.

Fig. 2 shows more clearly that this gradation in storage protein distribution is associated with changing degrees of endosperm compaction. The outer endosperm tissue is more compact than the inner endosperm tissue and this compaction is caused by large deposits of protein matrix material which is mainly hordein and glutelin (Fig. 3). Fig. 4 shows cells of the inner endosperm. Note that the large starch granules are not embedded in dense protein matrix and that small starch granules are not present in great numbers.

These two extremes of the structure of the starchy endosperm could affect malting quality because a high protein content in the outer endosperm may delay optimal hydration during steeping and may retard the inward migration of those hydrolytic enzymes which are secreted by the aleurone layer during malting (Fig. 5). The prominent cell walls of these smaller cells of the inner endosperm may also escape adequate enzymic hydrolysis if steeping was sub-optimal.

For nearly three decades the author has been studying differences in the starch-protein structure of the starchy endosperm with the objective of trying to relate structure to quality parameters such as uniformity of enzymic modification and speed of extract development. Although no attempt is being made to show the range of structural variation which can occur in the starchy endosperm, Fig. 6, 7, 8 and 9 show some of the differences which can be seen. Fig. 6 is an endosperm area with large deposit of matrix protein, Fig. 7 is a corresponding endosperm area showing lower levels of protein matrix deposition, Fig. 8 and 9 show even more reduced quantities of matrix protein in similar areas of the endosperm of barley grains.

In Fig. 6 and 7 the heavy deposit of protein has obscured the small starch granules. In Fig. 8 and 9,

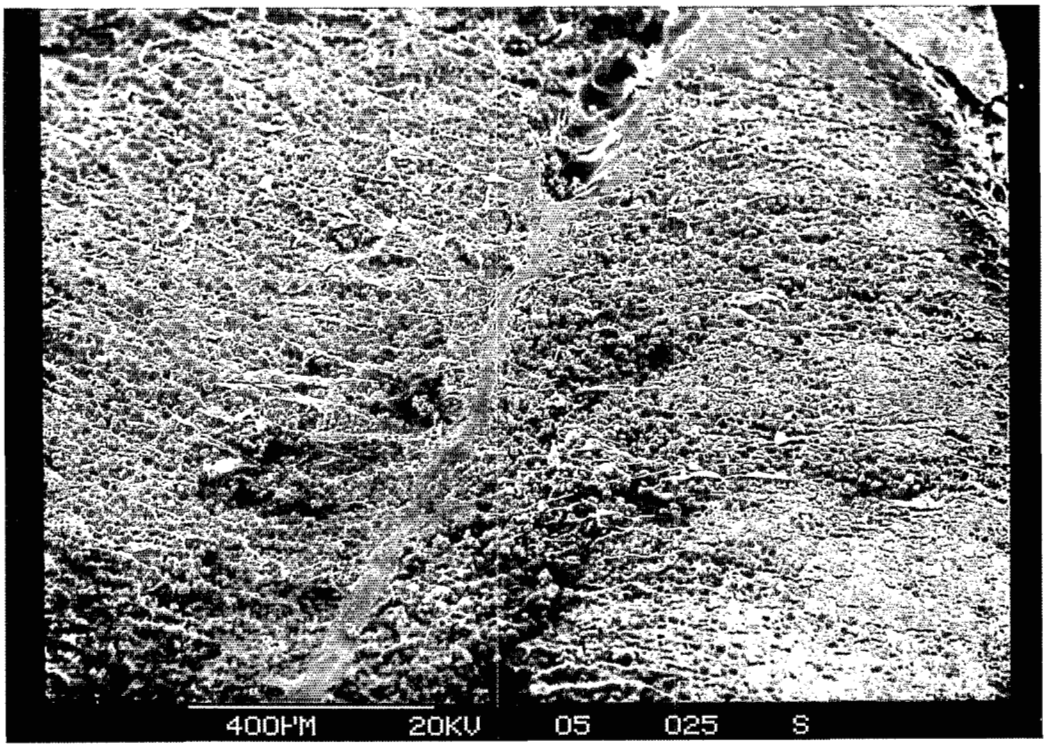


Fig. 1. Horizontal section through barley grain above the crease (furrow). The central crease line divides the grain into two halves. The walls of the endosperm cells are evident (left half). In this section protein content is highest at the periphery and lowest in the inner endosperm, near the crease line. The outer endosperm is compact, the inner endosperm is loosely packed.

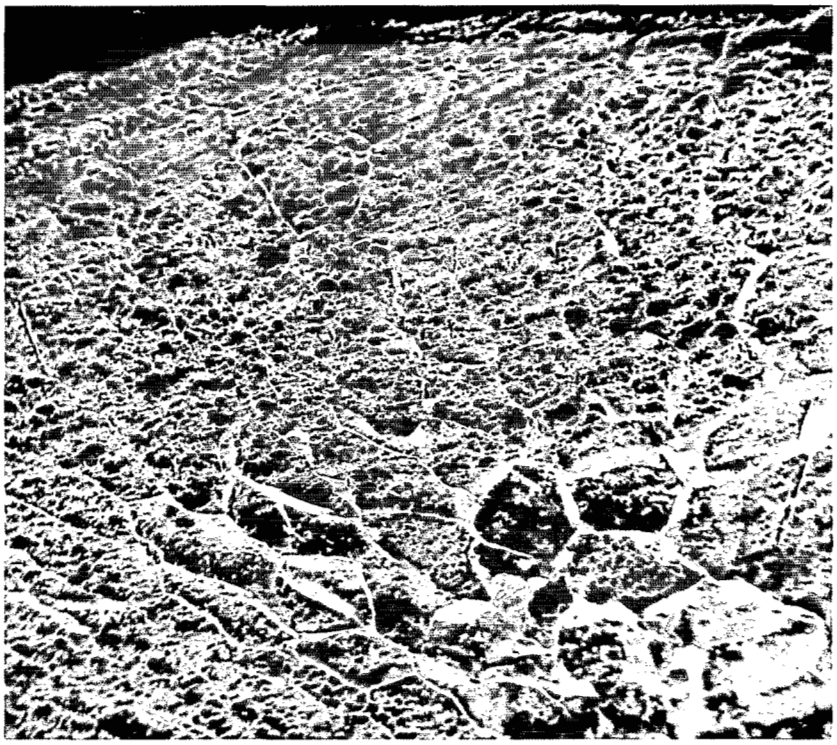

Fig. 2. Transverse (quarter) section across the starchy endosperm of barley. As is the case for Fig. 1 the outer compact endosperm contains more protein than the inner loosely packed endosperm. The cell walls of the starch and protein-containing cells of the starchy endosperm are present.

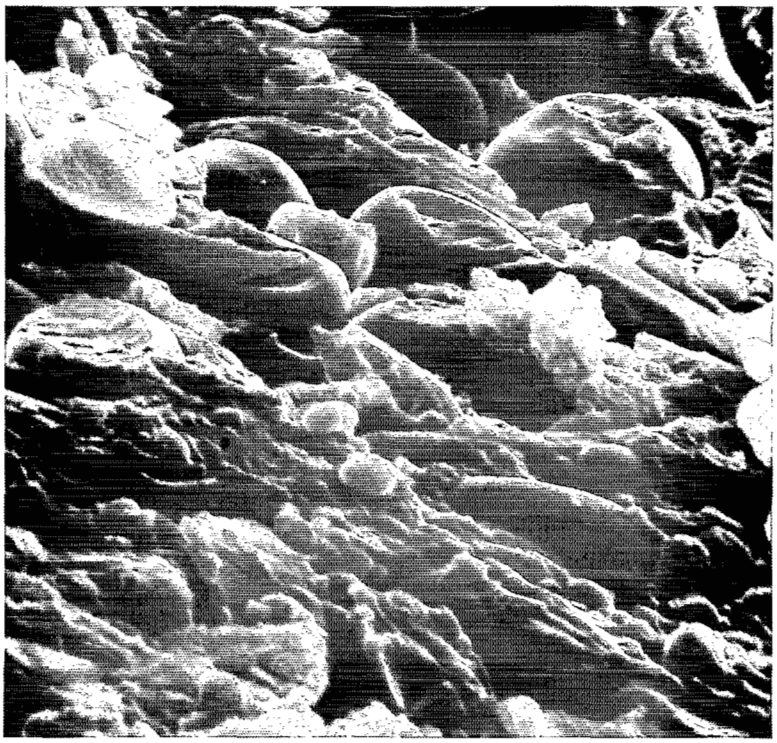

Fig. 3. High power micrograph of outer endosperm, beneath the aleurone layer. Note that small starch granules are obscured by heavy deposit of protein matrix material in the cell. Large (25μm) granules can be seen.


Fig. 4. Inner endosperm tissue of the starchy endosperm of barley. In some grains this area of the endosperm can have mainly large starch granules and very low levels of storage proteins. The cell walls of the endosperm cells are clearly seen.

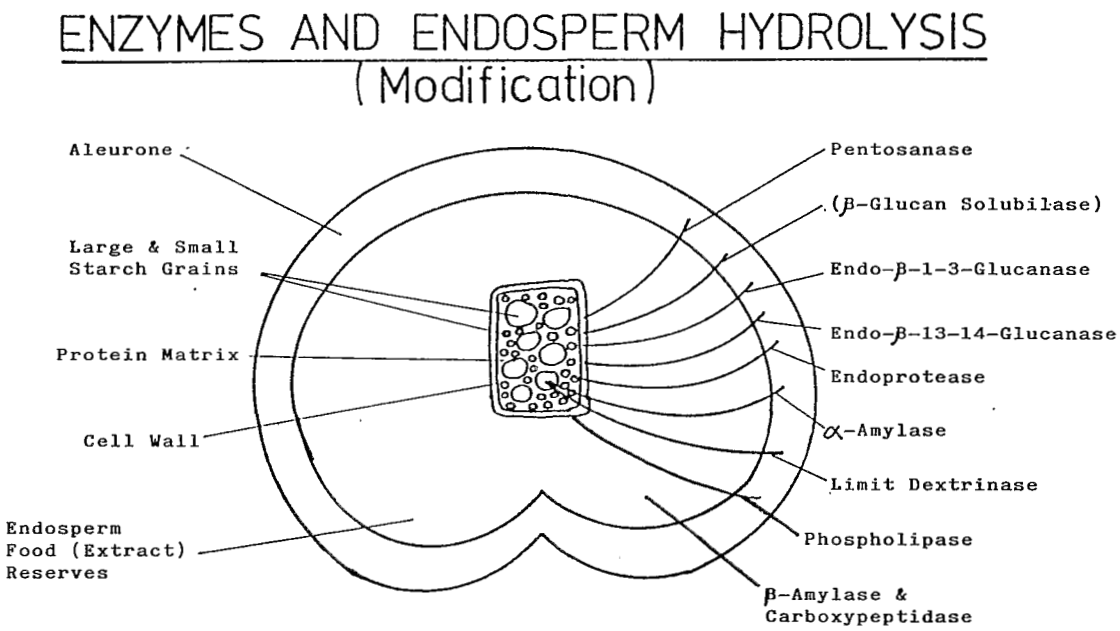


Fig. 5. Transverse section of barley endosperm showing aleurone and one endosperm cell.

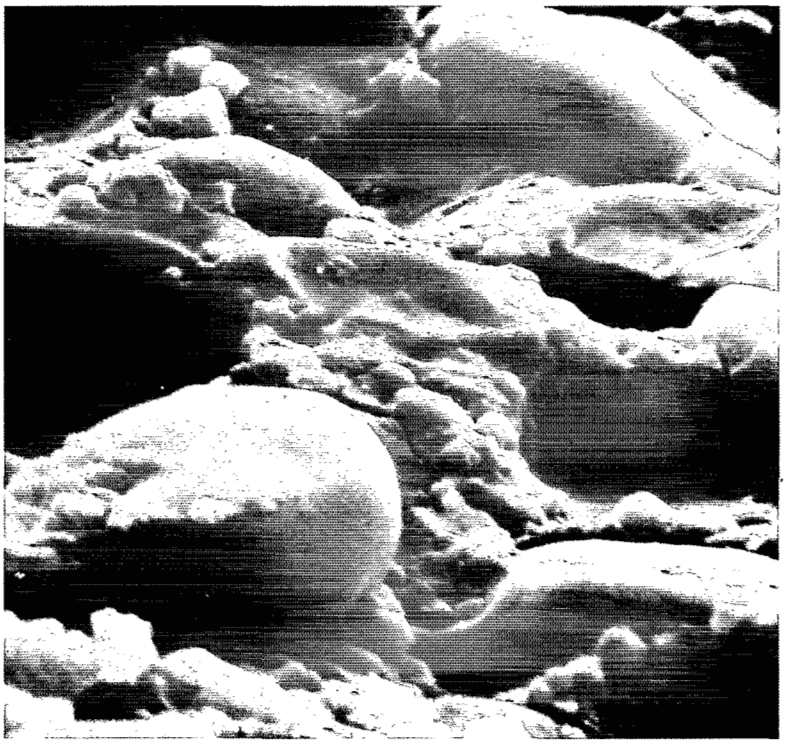

Fig. 6. Shows less compact association between matrix protein and small starch granules than shown in Fig. 3. Large starch granules (25μm in diameter) are present.

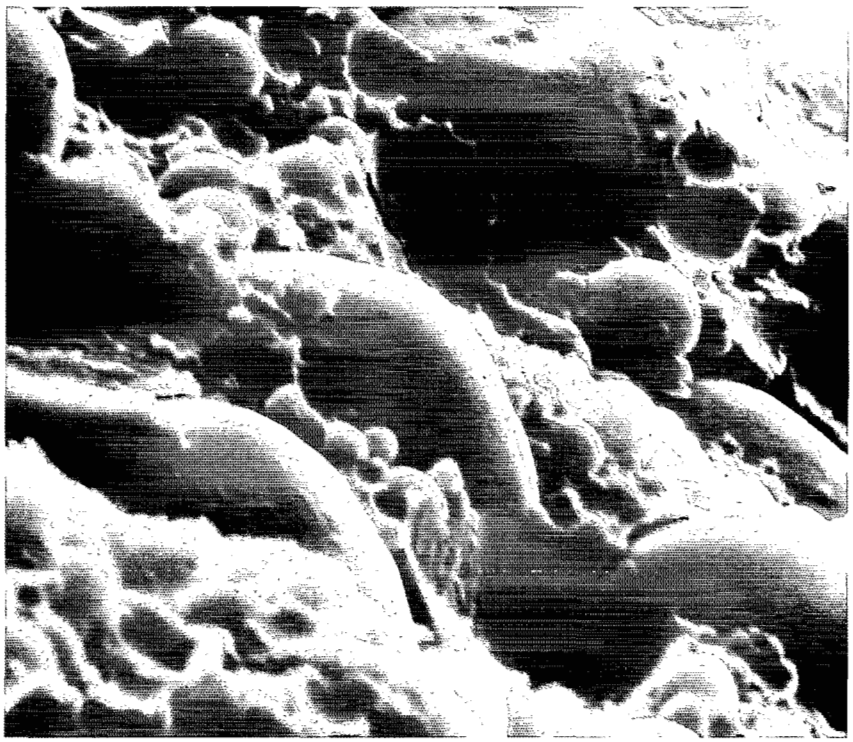

Fig. 7. Shows even less compaction between small starch granules and protein matrix than shown in Fig. 6. The small starch granules and matrix protein lies between the large starch granules (25μm in diameter).

Fig. 8. Note reduction in matrix protein as indicated by clear appearance of the small starch granules. Note large starch granules.

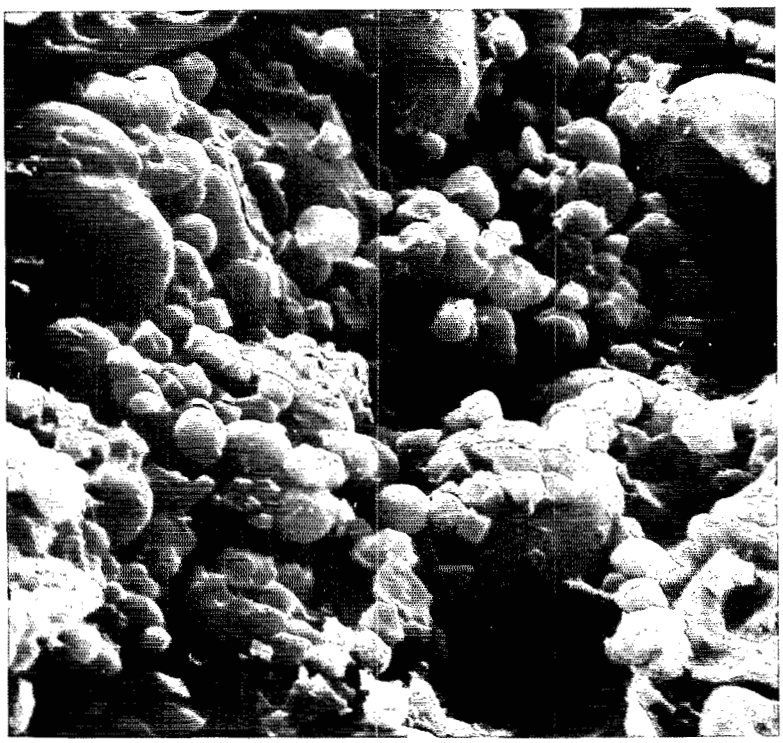

Fig. 9. Endosperm cell containing small starch granule and low content of protein matrix material. Small starch granule are about 5μm in diameter.

**Table 1. Analysis of malt that retarded wort separation and beer filtration.**

| | |
|---|---|
| Hot water extract (L°/kg) | 304.0 |
| Fine/coarse difference (L°/kg) | 4.0 |
| Total nitrogen % | 1.6 |
| Total soluble N % | 0.6 |
| Frialbility % | 89.0 |
| Homogeneity % | 98.0 |
| Dextrinizing units | 35.0 |
| ß-Glucanase IRV | 500.0 |
| Wort viscosity cP | 1.6 |
| ß-D-Glucan % | 0.6 |

where matrix protein deposition is limited, the small starch granules are clearly seen. These micrographs illustrate that the cells of the endosperm can vary in the quantites of matrix protein deposited and in the number of small starch granules which are found in different endosperm cells.

Recent studies of the endosperms of malting barleys have revealed that malt samples which gave acceptable malt analyses (Table 1) nevertheless gave brewhouse problems especially as regards retarded wort separation and beer filtration.

Detailed microscopic analyses of these malts showed that localized areas of the starchy endosperms were undermodified (Fig. 10). Examination of these localized under-modified areas revealed that they contained undegraded cell walls, and compact matrices of small starch granules and partly degraded storage protein (Fig. 11 and 12). In acceptably (enzymically) modified areas of the endosperm (Fig. 13), the cell walls were extensively degraded and significant degradation of the small starch granules and associated protein matrix had occurred.

The undegraded cell walls have been observed to associate with the small starch granules and protein matrix materials in the mash bed. This complex retarded wort separation and during sparging ß-D-glucans were extracted into the wort which slowed down beer filtration. Brewers who have encountered these problems have had to use ß-glucanases to solve them.

The concept currently under investigation is that the endosperm of barley can contain discrete areas of compaction or nodules, not detected by routine analyses (Table 1), which resist steeping and enzymic hydrolysis during malting. In order to remove this resistance, steeping had to be optimised in terms of increased hours of submersion steeping. It is not clear how or why these compact localized areas of endosperm compaction develop in the grain. However, this may be related to

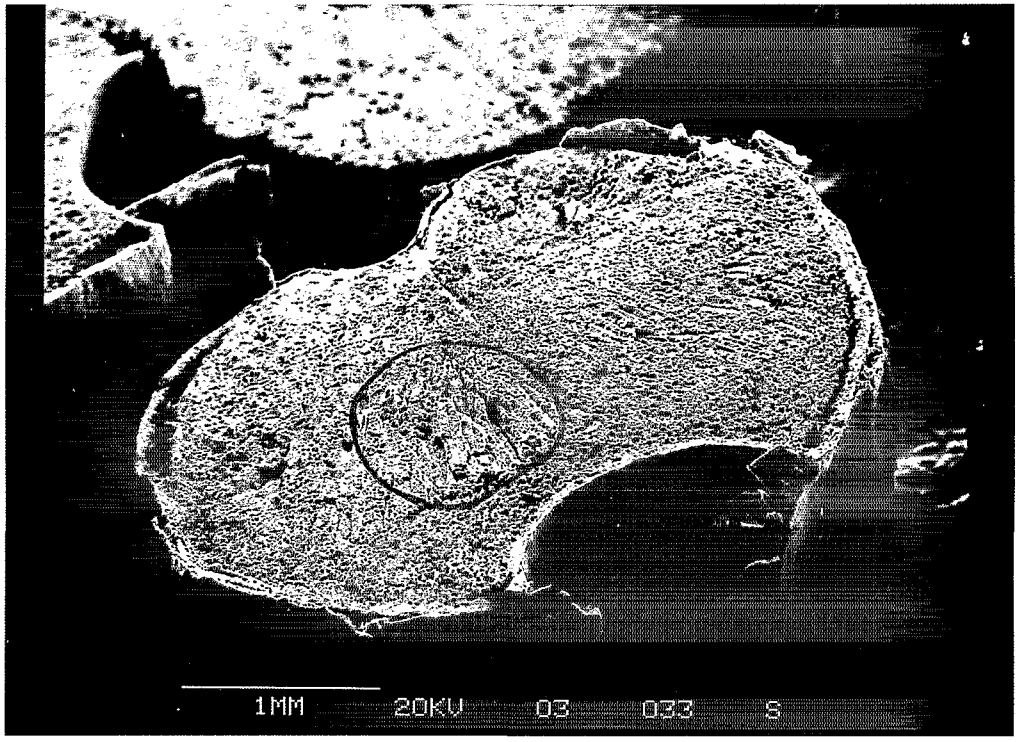


Fig. 10. Transverse section through unevenly modified endosperm of malted grain. Ringed area is undermodified (Fig. 11 and 12). Sub-aleurone area of the starchy endosperm shows the greatest degree of endosperm modification.

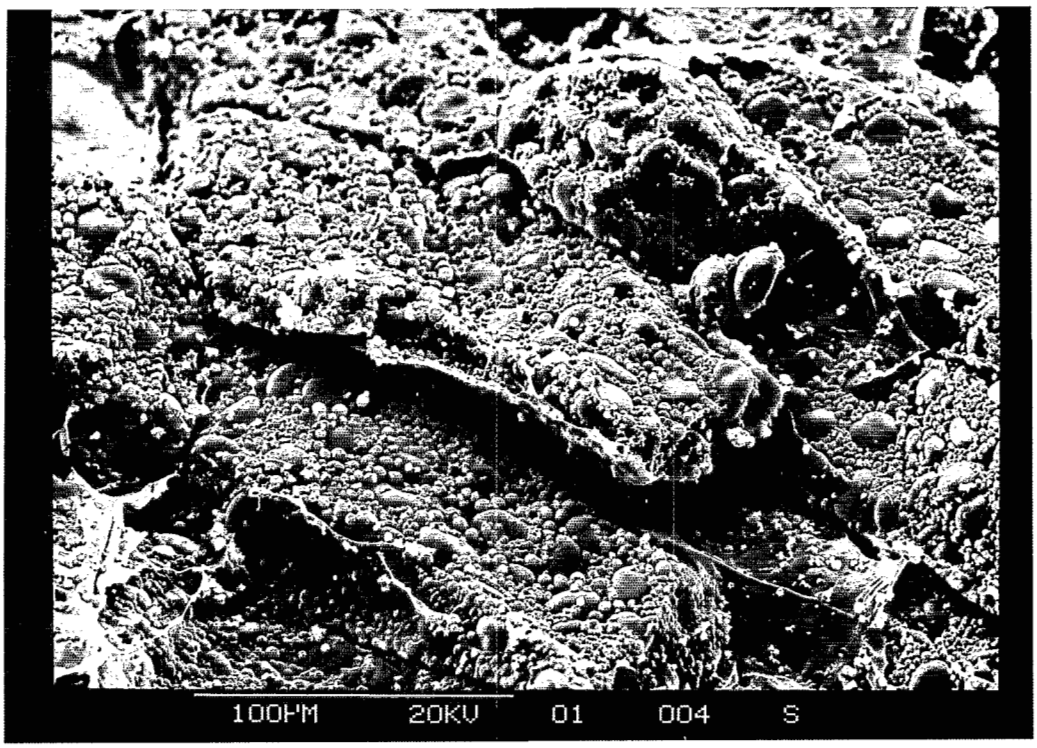


Fig. 11. High power micrograph of the localized undermodified endosperm area of Fig. 10. Note undermodified cell walls and excess of small starch granules.

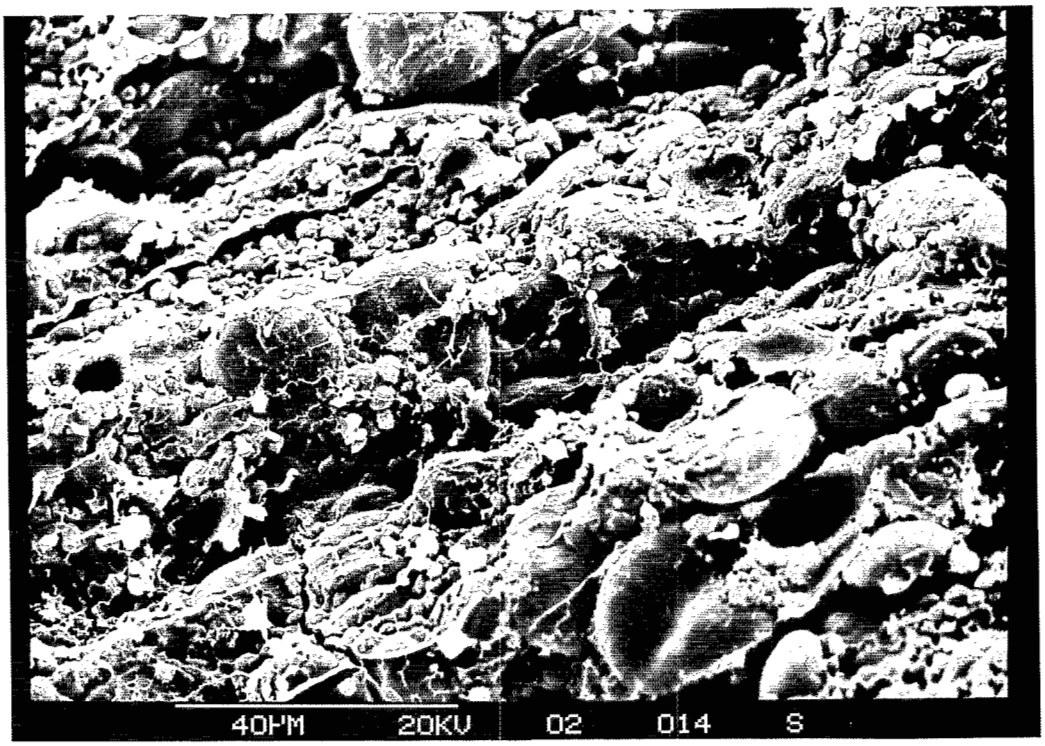


Fig. 12. High power micrograph of the undermodified area shown in Fig. 10. Note cell walls, small starch granules and compaction of the undermodified endosperm of the malted grain.

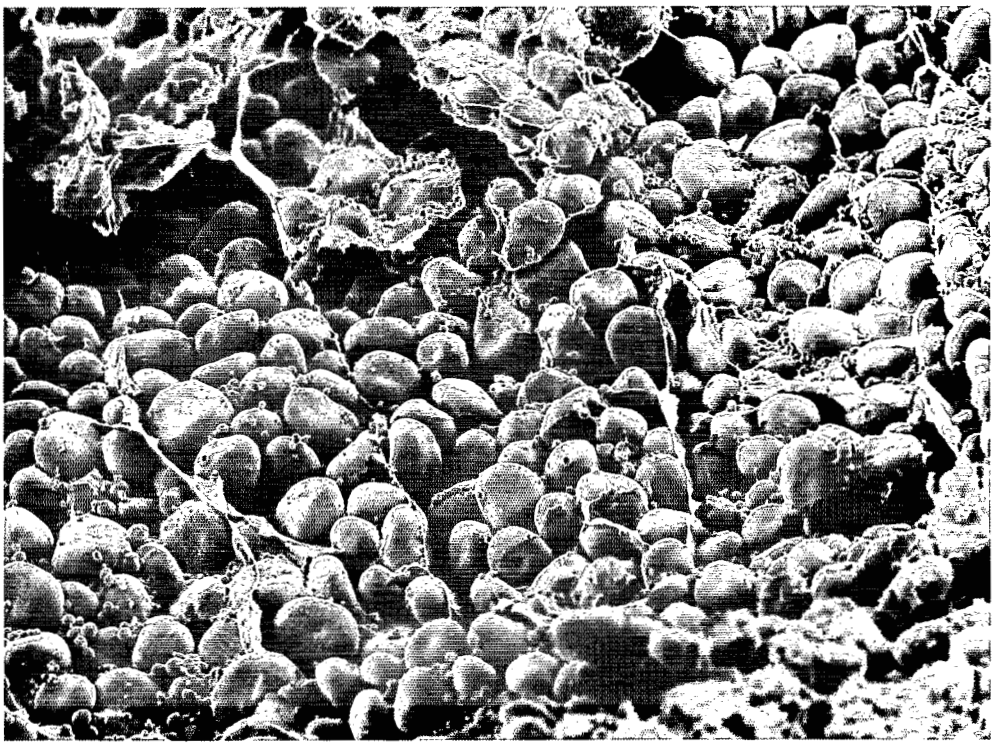

Fig. 13. High power micrograph of modified area of the endosperm shown in Fig. 10. Although residues of cell walls are present the small starch granule-protein matrix has been effectively degraded by enzymes during malting. This area of the malt should not cause wort separation or beer filtration problems.

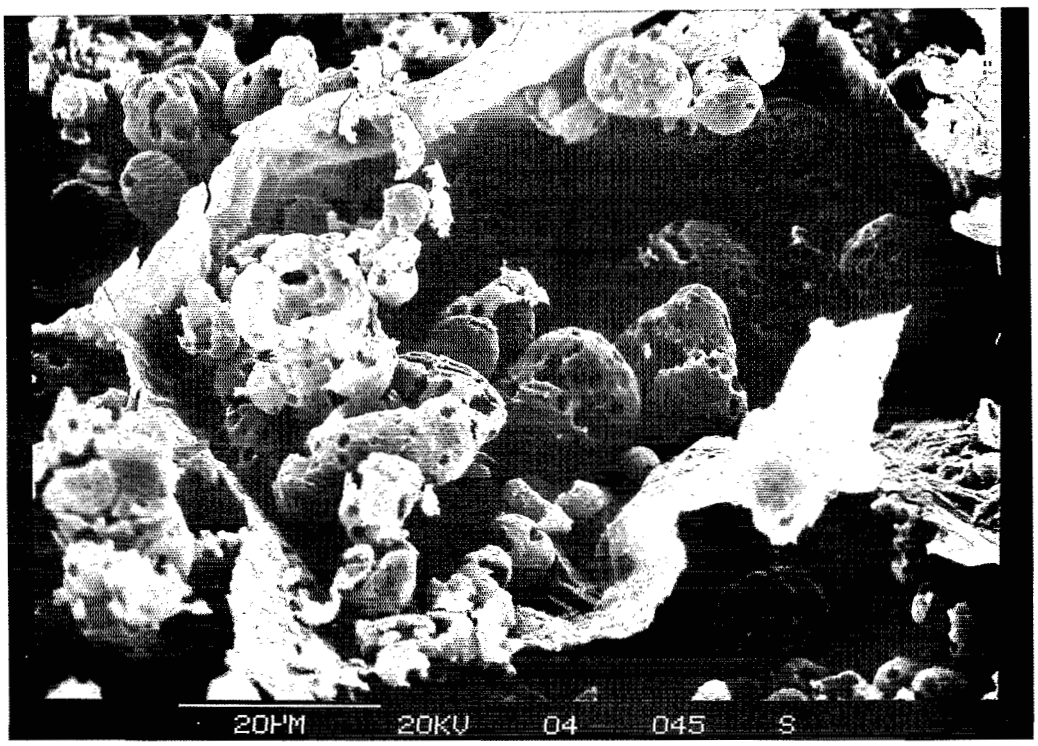


Fig. 14. Shows that in sorghum malt extensive degradation of starch can occur in cells which apparently have "intact" cell walls. This is different from barley where cell wall breakdown occurs during malting.


Fig. 15. The mechanism of endosperm breakdown in sorghum malt is different from that of barley (Fig. 13 and 14) because during malting the cell walls of sorghum develop distinct portals through which amylolytic and proteolytic enzyme pass to effect breakdown of starch and protein.

erratic availability of nitrogen and moisture during the grain filling process.

The cell walls of the endosperm may also limit hydration of the endosperm and enzyme distribution and action during malting. Very little is known about the structural organisation of the cell walls of the endosperm of barley. It has been suggested that the pentosans are mainly on the outside of the walls whilst the ß-D-glucans are mainly on the inside. A recent report by the author indicated that these cell walls are traversed by plasmodesmata and therefore phospholipases may be involved in the digestion in the walls. Although "thick" cell walls may resist enzymic breakdown, it has not been observed that the endosperms of slow-malting malting barley contained cell walls which were thicker than those of barleys which malted faster and more uniformly.

## Conclusion

Although a high protein content in the grain can limit enzymic modification of the endosperm, microscopic evidence suggests that the physico-chemical properties of the small starch granule-protein matrix of poor quality barley may be different from those of better quality barleys. However, at this stage of this study all that can be said with certainty is that localized deposition of small starch granules and protein matrix materials can prevent cell wall degrading enzymes from hydrolysing (modifying) endosperm cell walls satisfactorily during malting. Although extra periods of steeping under water can accelerate the modification of these resistant endosperm areas, care, in terms of adequate air-rests, should be taken in order not to damage the germinative potential of the steeped grain.

The use of the electron microscope to give a structural dimension to endosperm quality is increasing. Small localized differences in structure may be important but may escape detection in milling or staining tests which may not be specific enough. Indeed, the underlying concept of the microscopic approach is that providing all other parameters such as gibberellic acid levels, enzyme development and secretion and moisture percentages are found equally in different samples of malting barleys, gross differences in malting rates and evenness of enzymic modification must be related to differences in the structural qualities of the starchy endosperm.

Current work on the mechanism of endosperm breakdown in sorghum malt illustrates and confirms the importance of the microscopic technique in the study of grain quality.

Fig. 14 shows an enzymically modified cell of the endosperm of sorghum malt. Unlike barley malt, it is evident that extensive internal digestion of starch (and protein) can occur despite the presence of the cell walls which appear to be intact. Mindful that the starch and protein-degrading enzymes must enter the cell to effect such dramatic hydrolysis of starch and associated protein, the apparently intact cell walls were examined microscopically. Fig. 15 clearly shows that in malting sorghum, the cell walls develop portals through which amylolytic and proteolytic enzyme can migrate. The partly modified cell walls of the endosperm of sorghum malt may present more brewing problems than the corresponding walls of the unmalted grain.

Finally, these microscopic studies have not only highlighted differences between the malting properties of barley and sorghum, they have illustrated that some problems of barley and malt may require even more detailed microscope examination to provide the technological solutions required to improve quality.

## References

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