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MINERAL MIGRATION IN THE WHEAT KERNEL DURING MILL CONDITIONING

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Abstract

The structure and histology of the kernel govern migration of water during conditioning or drying. Studies by the energy dispersive x-ray system under the SEM have shown that during an increase of water content from 11.5 to 16.5 per cent, soluble elements migrated from the peripheral bran, accumulated in the aleurone cells and passed through its walls to the endosperm of the kernel if the water content was above 14.5 per cent. Results of study were compared with analyses of milling fractions obtained under the same conditions.

Introduction

The structure of the mature wheat kernel is well adapted to a progressive water absorption. Nevertheless, during the kernel development (Jenkins et al., 1975; Simmonds, 1974) teguments form a water-repellent protective zone. During the early stages of kernel development the first product of photosynthesis in the testa-pericarp is the C4 acid malate which could be a direct consequence of the close impermeability of its outer surface, even limiting the diffusion of atmospheric carbon dioxide (Duffus, 1979).

In the mature kernel, the bran contains a second cuticular wax which forms an impermeable and elastic layer during conditioning, the testa. It was demonstrated by Fulcher and Wood (1983) using Nile blue under blue light illumination (excitation 450-490 nm; transmission greater than 520 nm) showing strong yellow fluorescence, and by Hinton (1955) using a capillary tube in close contact with different layers of the bran. Water penetration was very low through the hyaline layer and lowest through the testa.

The threshed mature kernel is not quite impermeable. The cutinized seed coat is missing at the level of the tunicular scar where there is an area of pigmented strand type of cells. The inner layers of pericarp consist of these walled cells containing intercellular spaces through which water can move rapidly (Pomeranz, 1982).

Stenvert and Kingswood (1976) have shown, using autoradiography of wheat tempered by up to 15.5% moisture with tritiated water, that penetration of water was especially rapid near the top of the germ region where there could be seen natural lines of cleavage between the embryo and the endosperm and the embryo and the bran. The authors noted a preferential movement of water in the dorsal region and delayed movement into the central and crease regions.

Water absorption during wheat conditioning has been reviewed recently by Pomeranz (1982). Nuret and Willm (1961) noted that conditioning involved addition or release of water according to the intended use and a rest time to allow judicious distribution of water in the kernel. Conditioning results in a physical separation of the bran from the endosperm. In addition, physico-chemical transformations modify the storage conditions.
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Fig. 1. Distribution of ash in the wheat kernel (from Buré, 1938). Ash (% DM) is shown as function % of the tissue. Circled numbers represent, for each tissue the ash ratio as function of total kernel ash. Histograms width represent the % of tissue in the whole kernel; histogram heights are proportional to the tissue ash concentrations (% DM).

protein matrix from vitreous to floury (Moss et al., 1980).

Total minerals in parts of the wheat kernel have been calculated by MacMasters et al. (1971) according to the proportions of each tissue. About 61% of the total minerals are located in the aleurone layer, 20% in the endosperm, 7% in the pericarp and testa, 8% in the scutellum and 4% in the embryo. Not all the elements are distributed in the same way as the total minerals; thus, for example, 70% of Mg are present in the aleurone layer, 10 to 12% in the scutellum, and less than 10% in the endosperm; 50% of Ca and Na are in the endosperm and 25 to 30% in the aleurone layer.

Mineral material is often expressed by the ash content of samples. Buré (1938) has drawn a representative diagram showing the ash content (%) of the tissues of the soft wheat kernel (Fig. 1). The encircled number represents the ash ratio for each tissue.

The shift was followed by microanalysis (Energy Dispersive System) of sectioned kernels and confirmed by analyses of milling fractions.

Materials and methods

Wheat kernels
Soft wheat (var. Capitole, 1978) harvested at 14.3% moisture was desorbed to 11.1% at 20°C with P₂O₅, under vacuum, conditioned with water in closed bottles, and mixed in a conditioned room (4°C) for one week to produce series with water contents of 13.5 to 16.5%. Hydration was by sorption. Water content were determined using the French standard method NF - V/03-701.

Kernel hardness determination
Kernel hardness was determined by the Pohl grain cutter. At each water level, 100 kernels were sectioned to determine the rate of mealy, intermediate and vitreous kernels.

Microanalysis of kernel cross sections
Sections (1 mm thick) were placed into similarly sized cavities drilled into pure carbon cylinders (Fig. 2a) in such a way that they were horizontal and at the level of the cylinder plane. Spaces between the periphery of the sections and the carbon holder were carefully filled with a thick carbon glue, slightly diluted with ammonia. A very thin layer of carbon was deposited onto the grain using the JEOL JEE 4B evaporator.
Mineral migration in wheat kernel

Fig. 2. Preparation of samples for microanalysis: a) wheat kernel cross section in pure carbon cylinder; b) milling fraction or ash in pure carbon cylinder.

Fig. 3. Microanalysis spectra of the teguments (1 to 5), aleurone layer (6 to 10) and mealy endosperm (11 to 15) as function of the water content of the wheat kernel; 1, 6 and 11: 12.5%; 2, 7 and 12: 13.5%; 3, 8 and 13: 14.4%; 4, 9 and 14: 15.4%; 5, 10 and 15: 16.5% water content.

Analyses were performed using the JEOL SEM 50A. For analyses, homogeneous tissue parts were selected for scanning.

Microanalysis of milled fractions and ash

Samples were pressed into small cavities (Fig. 2b) drilled into pure carbon cylinders as described previously for the preparation of kernel samples.

Conditions of microanalysis

Analyses were performed using the EDAX System 711F. The detector pipe angle was tilted to 45°. The resolution was 150 eV for Mn (KA) at 20 keV. Time of analysis was 200 seconds. The scanned areas at 1000X magnification were 150 square micrometers for the tissues and 20,000 square micrometers at 300X for all fractions and ash samples. SEM accelerating voltage was 20 keV and absorbed current 1 to 5×10^-11A. A computer was used for the ZAF corrections. Angle of sample tilt was 0°; X-ray emergence angle was adjusted to 34.3°. Quantitative analyses were performed using comparative spectra of BaCl₂, 2H₂O crystals obtained each time under the same conditions of analyses as for the samples.

Results and Discussion

Influence of hydration on the kernel structure

Hardness tests showed (Table I) a decrease of the vitreous and an increase of the mealy kernels as the water content increased. The percentage of intermediate kernels remained almost constant. Grain of intermediate texture had small, dot-like floury zones at the lowest water content (12.5%). As the water content increased,
Table 1: Influence of hydration on kernel texture

<table>
<thead>
<tr>
<th>Water content (%)</th>
<th>Mealy</th>
<th>Intermediate</th>
<th>Vitreous</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>11</td>
<td>84</td>
<td>5</td>
</tr>
<tr>
<td>13.5</td>
<td>12</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>14.4</td>
<td>13</td>
<td>86</td>
<td>1</td>
</tr>
<tr>
<td>15.4</td>
<td>15</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>16.5</td>
<td>16</td>
<td>84</td>
<td></td>
</tr>
</tbody>
</table>

floury areas enlarged and finally merged. There were no completely vitreous kernels in grain with 16.5% moisture.

Mineral changes during damping
Analyses were conducted by scanning five areas ( tegument, aleurone layer, mealy and vitreous endosperms, scutellum) of the intermediate kernels. Figure 3 shows EDS spectra for the accumulated in the aleurone layer until a maximum of 4.3% at 14.4% water content, and decreased to 1.8% at 16.5% water.

In the vitreous and starchy endosperms, concentration of potassium was stable (about 0.06%) for 11.1 to 14.4% water content. After that, potassium increased to 0.13%. In the scutellum, changes were very irregular at the beginning of
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Fig. 5. SEM and potassium X-ray images of wheat kernel sections also function of the grain water content. a: 11.1%; b: 12.5%; c: 13.5%; d: 14.4%; e: 15.4% and f: 16.5%.

wetting; from 14.4% water content, concentration of potassium increased.

Phosphorus: is the second major mineral component. Phosphorus was high in the aleurone layer and in the scutellum. It changed in a manner that was similar to that of potassium, a decrease in the tegument, and an increase until 14.4% water content followed by a decrease in the aleurone. The changes were inversed in the endosperm. In the scutellum, changes paralleled those of potassium.

Sulfur: sulfur decreased in the tegument as the water content increased. Concentration of sulfur in the aleurone cells decreased to half at 13.5% water and increased to the original level at higher water contents. The decrease of sulfur took place earlier in the mealy endosperm than in the vitreous one, probably due to the facility of water to move faster into the mealy endosperm which is porous and whose protein matrix is discontinuous. Changes in sulfur in the scutellum were similar to the other components.

Magnesium: traces were observed in the tegument until 13.5% water content; it could not be detected at higher levels. The concentration in the aleurone layer was very high (between 0.3 to 0.6%). As with potassium and phosphorus, concentration of magnesium increased between 13.5 and 14.4% water content and decreased to around 0.1% at higher levels. Migration of magnesium was confirmed by its increase in the mealy and vitreous endosperms.

Chlorine: at the 11.1% water content, chlorine was detected only in the scutellum and endosperm. In the scutellum, concentration of chlorine, gradually decreased until 13.5% water content, could not be detected as in the aleurone layer and was present at the 16.5% water content. This scheme was inversed in the endosperm where after 13.5% water content, chlorine gradually decreased. Chlorine concentration was higher in the mealy than in vitreous endosperm.

Figure 5 shows the potassium X-ray images obtained under the same detection conditions for different water contents. Proteins of the aleurone cells showed particularly strong emission; they were more diffuse in the tegument below 13.5% water content and were weaker in the subaleurone layer. The emission increased in the aleurone until 14.4% water content. Subsequently the emission was stronger in the subaleurone layer. No emission was shown in the thick cell walls of the aleurone layer below 15.4% water content.

According to Butcher and Stenvert (1973), the rapid movement of water through the grain occurs initially in the germ area, the remainder of the kernel being protected by the tegument cuticular layers which act as physical barriers against rapid hydration. Still, the aleurone layer is a barrier to water migration, probably due to water binding by the high protein content of this layer.

Hemicellulose also contributes to the rate of water penetration in the tegument. At the beginning of dampening, tissues rich in open porous or hydrophilic structures are also rich in water
Fig. 6. Longitudinal section of the wheat kernel: a) its tissue content (after Bure, 1938), b) SEM of peripheral part of the kernel, and c) corresponding milling fractions.

Fig. 7. Microanalysis spectra of the bran (l and 5), shorts (2 and 6), semolina (3 and 7) and flour (4 and 8) as function of two water levels of the wheat kernel: 1 to 4 (12.5%) and 5 to 8 (16.5%).

content. At the end of a long rest time, there is an equilibrium in moisture content. Differences in the rate of water penetration between mealy and vitreous endosperms could be explained by the absence of open structures and high concentrations of proteins in the vitreous endosperms which exhibit slow rates of water movement (Butcher and Stenvert, 1973). During wheat conditioning free water is absorbed fast; subsequent moisture movement is by diffusion from both the tegument layers and the germ region. If potassium can be considered as a marker for water migration, hydration takes place in the tegument layers during damping up to about 14.5% water.
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During hydration, mineral components accumulate outside of the aleurone cell walls which act as a barrier. When hydration is high, water passes into the subaleurone layer and the starchy endosperm and carries along the soluble elements.

Mineral changes in milling fractions

Milling yields: Figure 6 depicts a longitudinal section of the wheat kernel and indicates the corresponding milling fractions. Theoretically, semolina and white flour are obtained from the starchy endosperm, bran includes the pericarp and the aleurone layer, and shorts are obtained by regrind and twice bolted bran and include the inner part of the bran, the aleurone layer and some subaleurone layer. We milled 500 g of wheat for each moisture level. Resulting fractions are described in Table 2. As the water content increased, there was a progressive increase in the flour extraction from 26 to 36% and the percentage of mealy kernels increased from 11 to 16% (Table 1). At the same time, percentage of semolina decreased from 48 to 38 and the amount of highly vitreous kernels gradually decreased. The amount of kernels with intermediate texture was unchanged but areas of mealy endosperm increased gradually in size and merged. Bran yields after bolting remained basically constant and amounts of shorts increased from 0.3 to 0.9%. The yield of bran plus shorts remained fairly constant (25.3 to 27.0%) and was not affected consistently by the increase in moisture content from 14.4 to 16.5%.

Sections of bran fractions showed us expected release of the subaleurone layer; occasionally we saw a separation between the epiderm and

Table 2:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Flour</th>
<th>Semolina</th>
<th>Bran</th>
<th>Shorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel moisture (% DM)</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>12.5</td>
<td>26.0</td>
<td>48.3</td>
<td>25.2</td>
<td>0.3</td>
</tr>
<tr>
<td>13.5</td>
<td>28.7</td>
<td>45.0</td>
<td>25.6</td>
<td>0.6</td>
</tr>
<tr>
<td>14.4</td>
<td>30.7</td>
<td>42.4</td>
<td>26.0</td>
<td>0.7</td>
</tr>
<tr>
<td>15.4</td>
<td>33.0</td>
<td>40.2</td>
<td>25.8</td>
<td>0.8</td>
</tr>
<tr>
<td>16.5</td>
<td>35.9</td>
<td>37.9</td>
<td>25.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Fig. 8. Histograms of the K, P, S, Mg and Cl concentrations (% DM) in milling fractions (semolina, flour, bran and shorts). Numbers under histograms correspond to the water content of the samples (1: 12.5%; 2: 13.5%; 3: 14.4%; 4: 15.4%; 5: 16.5%). Chlorine is represented by hatching.
the mesocarp or between the pericarp and the seed coat.

Analysis of milling fractions: Figure 7 shows spectra of bran, shorts, semolina and flour for the 12.5 and 16.5 water contents. There were considerable decreases of minerals in the bran and the shorts in agreement with the spectra of bran and aleurone in Figure 4.

Figure 8 shows that the release of elements in the peripheral fractions was not compensated by their recoveries in fractions from the central part of the kernel, especially at the 16.5% water content where all the elements showed a deficit. The decrease was particularly conspicuous in the shorts.

Some fluctuations were noted for potassium in the bran fractions, which were compared to the aleurone layer. About half of the potassium in the aleurone layer was present in the bran, because of the heterogeneity of the sample and resulting dilution of the X-ray emission. Histograms of semolina and flour, even though two times smaller, were comparable to those of the meal and the vitreous endosperms.

Some of the discrepancies between the results of testing tissues and milling fractions can be attributed to heterogeneity of the milling fractions, loss and migration of water during milling, differences between the crystalline structure of the BaCl₂ reference crystal and the porous structure of the powdered milled particles (which may disperse the X-ray emission).

Analysis of ash: An experiment was also conducted on milling fractions and ash of Capitole wheat harvested from 1981 (Figs. 9 and 10). Potassium was highest in bran, but there were inexplicable variations and decreases in the other fractions. Similar variations were observed for the other elements. There were significant decreases in sulfur and especially chlorine, during ashing.

Total ash and individual minerals were higher by up to one-third than those reported by Pomeranz and Dikeman (1983) using atomic absorption spectrometry (Dikeman et al., 1982). The differences could be due to the analytical methods and/or extraction rates of the flours (Peterson et al., 1983).

Conclusions

EDS, while not as precise as in atomic absorption spectrometry or EDXRF, is well suited for localization of minerals in various tissues. Migration and distribution of mineral components are affected by the texture of the wheat, water level, length of conditioning and degree and type of milling.
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References


Discussion with Reviewers

R. Moss: How were the grain samples dried prior to carbon coating?

Authors: Dehydration using acetone series and critical point or freeze-drying in such cases of very low moistures (no free water) seemed unadaptable and able to cause artifacts of mineral relocation. Just after removing the samples from their conditioned bottles and after sectioning, cross kernel sections were transferred into the evaporator. Low vacuum (10⁻³ torr) was obtained between 1 and 2 min.

R.G. Fulcher: Is there any possibility that changes in moisture levels during preparation may have influenced the apparent relocation of minerals?

Authors: It may be that changes in moisture levels during preparation influence the apparent relocation of minerals. We discussed that possibility before this study. In fact, moistures of samples were very low and migrations took a long time. On the periphery of the sections, cells were cut and opened towards the top, limited by their cell walls. The probability for horizontal shift from cell to cell was then negligible, in contrast minute vertical shifts could probably occur, which cannot be detected according to the area scanned for microanalyses.

R. Moss: Do the authors feel that this work has commercial relevance? In milling, lying times are much shorter than the seven days used here (usually times range from 8 to 24 hours). Milling behaviour is sensitive to lying time, is mineral distribution?

Authors: As written earlier, such migrations have been described by Rohrlíčka and Hopp (1961). The present study was to prove this and precisely what happened at the tissular level. Results show that in the regulating of laws care of possible shifting must be taken. For example, an increase in the ash content may not always be due to the presence of peripheral parts in flour. Actually, conditioning and lying time take usually one day or less. But in that case heterogeneity in moisture at the tissular level can be seen. We considered that 7 days lying time gave more uniform moisture and then more confident results in mineral distribution.

R. Moss: An indication of whether the differences in mineral composition are statistically significant should be given. How many samples were examined and what level of significance (e.g. p < 0.991)?

Authors: The study was mainly exploratory and the aim was to show the evolution of some mineral changes. Procedure of analysis took a very long time and the difficulty was to analyse under the same conditions a long series of samples in a few days. Data were the mean of 2 areas of each tissue for 2 kernels of the same moisture. When data differed, a third and fourth kernel were also analysed. So, it cannot be said that mineral
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composition was statistically significant because only a few kernels were investigated but, in total, results were homogeneous.

On the other hand, analysis of a scanned area, which represented the analyses of very high number of individual points, was statistically significant.

A.T. Marshall: You state that the detector angle was 45° and that the specimen tilt angle was 0°. It is not clear how you arrive at an X-ray emergence angle (take off angle) of 34.3°. Please, explain in more detail what is meant?

G.M. Roomans: The description under "Microanalysis conditions" is not clear. If the sample tilt is 0°, and the X-ray emergency angle was 34.3° then the detector must have been tilted 34.3° too.

Authors: This take off angle has been calculated according to geometry data of the specimen stage of the microscope (JEOL 50A) and using the "Take off angle program" published in Edax Editor 1978, 8, (4) p. 15.

Note that our samples were 3.5 mm higher than the goniometer stage, which explains this data.

G.M. Roomans: A ZAF correction is no longer considered the best method for analysis of biological bulk specimens (Boekestein A., Stols A.L.H., Stadhouders A.M. (1980). Quantitation in X-ray microanalysis of biological bulk specimens. SEM 1980; II: 321-334; Boekestein A., Stadhouders A.M., Stols A.L.H., Roomans G.M. (1983). Quantitative biological X-ray microanalysis of bulk specimens: an analysis of inaccuracies involved in ZAF-correction. SEM 1983; II: 725-736) where it is demonstrated that the EDAX ZAF programs are not accurate for analysis of low-Z specimens. To make matters worse, the authors have used a standard that does not resemble the specimen in any respect. This will increase the inaccuracies in the ZAF correction and, in addition, differences in mass loss between specimen and standard are introduced. Although this does not affect the relative values of the measured concentrations, the absolute values may not be correct.

A.T. Marshall: You present your data as concentrations by weight percent and refer to the Edax program with ZAF corrections for your particular matrix. Does the ZAF correction program allow you to insert C, O and N concentrations? What did you use for standards for the other elements and what is the significance of the BaCl₂ standard? Is the program a no standards program?

Authors: When the study was done, 3 years ago, ZAF program was used as a useful and routine program. Since then, we have also heard of the new program for analysis of biological bulk specimens, by Boekestein et al.

Different tissues of the wheat kernel are heterogeneous in structure and composition, some being porous (bran, aleurone layer), others being more compact, as the endosperms. The best standard to be used has never been described and seems still unknown for such a case. We have chosen the BaCl₂, crystals being flat and clean, stable under electron beam and because they did not provoke shifts. Note that the ZAF program allows us to insert C, O and N concentrations, except for the ash where the program allows us to insert O only. It is possible that the BaCl₂ was not the best standard to be used for this study. Maybe this explains the differences we noted with AAS (atomic absorption spectrometry).

G.M. Roomans: Are the differences in dot density (X-ray maps) not due to differences in local mass?

A.T. Marshall: In showing X-ray maps, a map of continuum counts should also be shown, since it is possible to obtain the type of maps you show in Fig. 5 simply by virtue of an increase in background counts under the K peak. This increase could be a result of an increase in density. It is therefore important to show that this is not the case by including a map using a peak free band of continuum.

Authors: A map using a peak free band of continuum shows very weak, scarce and uniform dot density.

The map of potassium shows high dot density at the aleurone layer level. So, the dots show differences in potassium concentration and not differences in local mass or density.