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Cytochemical study of intact and processed barley grain

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SUMMARY - Barley grain was submitted to various industrial treatments and examined by fluorescence microscopy. Either ground in a hammermill or crushed in a roller mill, barley grain particles retain their original structure: when only partially comminuted, husks are the main cause of pellet brittleness. After heat treament, proteins are stretched in elongated sheets and starch granules more or less gelatinised.

RESUME - "Etude cytochimique de grains d'orge intacts et traités". L'analyse microscopique par fluorescence est un outil précieux d'étude de l'orge soumis à divers traitements technologiques. Il met en évidence le rôle original des produits du broyage de la graine (péricarpe, couches à aleurone, endosperme) sur les caractéristiques physiques des agglomérés. Il montre aussi comment se modifient, sous l'effet de la chaleur, les principaux composants du grain d'orge : selon l'intensité du traitement, les protéines s'étirent en lames minces et les grains d'amidon gonflent ou se gélatinisent progressivement.

Introduction

Contrary to wheat, husk of barley kernel is strongly attached to the seed. When observed on a cross section, barley kernel shows a rounded outline and a ventral crease. As a consequence, husk which is closely attached to the tegument may not be removed at the crease level without eliminating a large part of the endosperm.

Endosperm presents a mosaic of dull and translucent areas, corresponding to floury and horny endosperms respectively. When observed on a longitudinal section, embryo, which appears rather small in comparison with the whole kernel, is attached to the endosperm by an intermediate tissue, the scutellum, whose role is essential during kernel germination.

Methods

Cytochemical studies (Fig. 1a to 1h) are very useful in improving our knowledge of the kernel structure and composition as well as their modifications under processing. The most usual and easiest technique (Fig. 1a) consists of a chemical fixation by a mixture of formalin, acetic acid and alcohol (10:5:85), sections being coloured with fast-green (the amines materials are dyed green) and iodine (the starchy material is dyed blue to purple).

As recently shown, fluorescence microscopy is one of the most original ways which can be used for the study of cereal kernels (Fulcher, 1982; Fulcher *et al.*, 1989). Some kernel components show primary fluorescence but numerous non-fluorescent compounds may also become fluorescent after being linked to specific and very sensitive fluorescent dyes. Kasten's technique (Kasten *et al.*, 1959), though not commonly used, is interesting because it is very close to the well known PAS (periodic acid-Schiff) reaction and perfectly fitted to the fluorescence techniques. It consists of a mild oxidation of some α-glycols groups (the linked C2 and C3 "-HCOH-" groups of the anhydroglucose units in starch and cellulose) to produce reducing groups to which the acriflavine dye is bound. Kasten's method recommends the excitation of the stained sections at 400

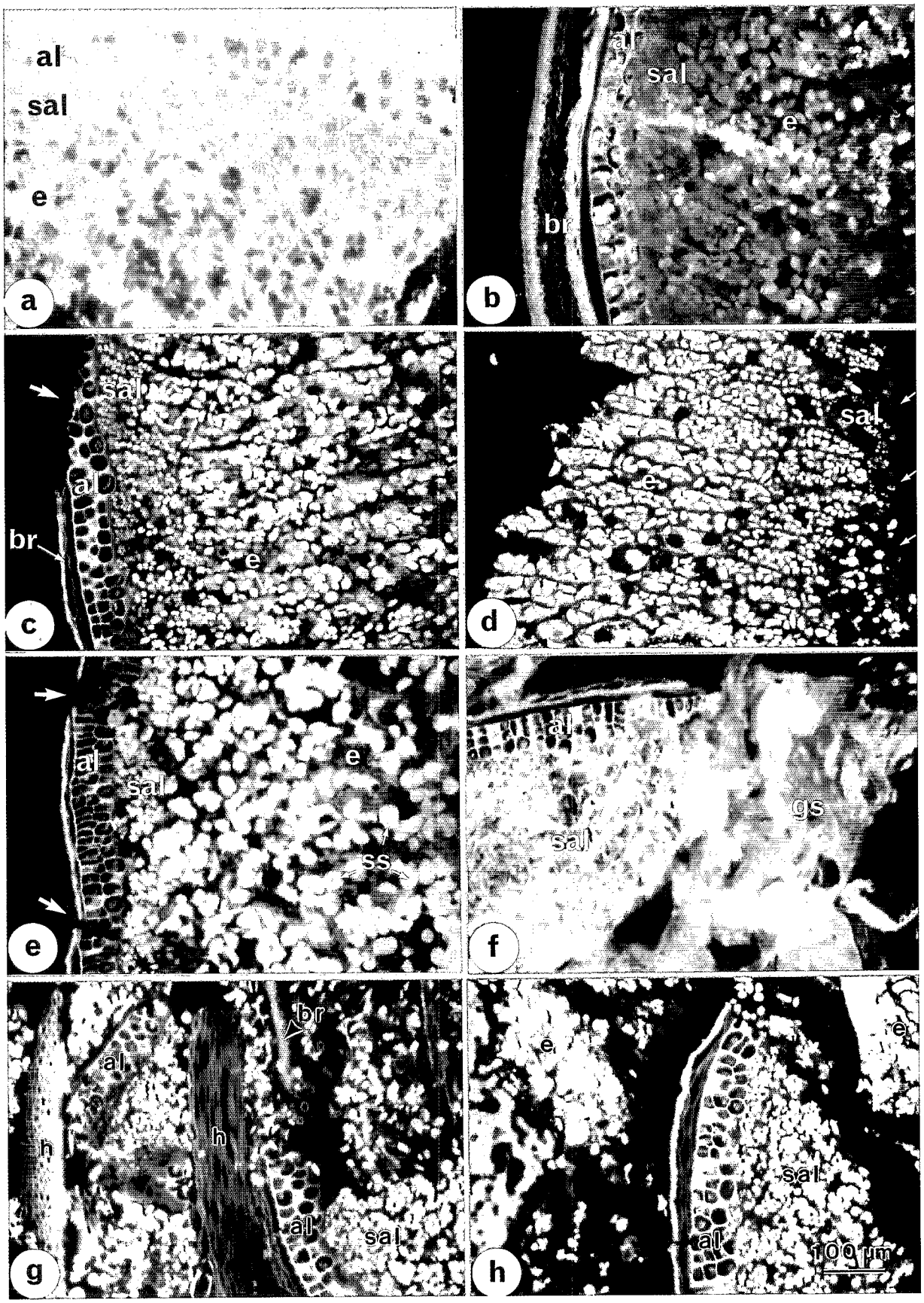
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Fig. 1. **(a)** Cross section of barley kernel coloured with fastgreen/iodine mixture. Several layers of aleuron cells (al) are seen with dense protein content (green) as a result of the stained protein bodies. Sub-aleuron tissue (sal) is protein rich (green), but contains numerous small starch granules (purple). Endosperm (e) shows radial elongated cells with large amount of starch (purple) and lower protein content. **(b)** Cross section of barley kernel under fluorescent microscope (excitation and barrier filters at 350 and 400 nm, respectively). Under such conditions, images show differentiated structures: large lenticular starch granules (type A starch) appear yellow to green; small polygonal starch granules (type B starch) appear orange; cell walls are coloured light green and proteins dark-green. (al: aleuron layers; br: bran; e: endosperm; sal: sub-aleuron layer). **(c)** Cross section of hulled barley kernel (same conditions as in b) showing that husk has been entirely cleaned but an important part of the kernel's outer layers is also locally removed (arrow). (al: aleuron layers; br: bran; e: endosperm; sal: sub-aleuron layer). **(d)** Cross section of pearl barley (same conditions as in b) shows that hull has been completely removed (arrows). (e: endosperm; sal: sub-aleuron layer). **(e)** Cross section of flaked barley kernel (same conditions as in b) showing numerous fissurations and cracks (arrows) across bran, aleuron layers, sub-aleuron and endosperm tissues. (al: aleuron layers; e: endosperm; sal: sub-aleuron layer; ss: swollen starch granules). **(f)** Cross section in a strand obtained after cooking-extrusion of barley flour (same conditions as in b) showing a multiphasic gel with starchy (yellow to brown) and proteins (green) compounds, into which some solid compounds such as cell wall fragments (husk, bran and aleuron layers) are scattered. Proteins (green emission) are mostly observed as very thin and elongated sheets. (al: aleuron layers; e: endosperm; sal: sub-aleuron layer). **(g)** Cross section in a pellet obtained after milling raw barley kernel with a rotary hammermill (Forplex). Observed under same conditions as b, pellet appear homogeneous, with fragments of husk and bran material, small endosperm agglomerates and isolated starch granules. (al: aleuron layers; br: bran; e: endosperm; hull: h; sal: sub-aleuron layer). **(h)** Cross section in a pellet obtained after milling raw barley kernel in a roller mill (SOCAM) and observed under the same conditions as in b. Meal appears coarser, hulls are not fragmented; the final product is very heterogenous. (al: aleuron layers; e: endosperm; sal: sub-aleuron layer).

nm and sample emission filtered at 515 nm. Oxidized groups linked with acriflavine emit in yellow bright to green. This mode of observation favours structures such as the cell walls or starchy materials (which appear intense and deep yellow) at the expense of protein or other structures. With another band of wavelengths (excitation in UV light at 350 nm and emission filtered at 400 nm), images show much more differentiated structures.

For the microscopic examination of pellet samples and in order to prevent artificial segregation during sample preparation, pellet samples are included in agar gel before their sequential treatment of fixing and paraplast embedding prior to be sectioned. Agar gel keeps then the flour particles in their initial position but, after sectioning, it cannot be removed. Consequently, agar gel takes also a part of the fluorescent emission. As a result, a shifting in the wavelength of acriflavine emission is observed, thus reducing the specific intensity of the fluorescent dye.

Microstructure of barley grain

Below the pericarp, barley kernel shows several superposed layers of aleuron cells, instead of one in wheat kernel. Aleuron cells show dense protein content (dark green) as a result of the stained protein bodies, whereas the nucleus of the cells is light green. Sub-aleuron tissue, the area immediately under the aleuron layers, still rich in protein, contains numerous small starch granules. From the outside to the inside of the kernel, cells appear elongated. Their composition may vary according to their floury or horny structure but, on the whole, the starch/protein ratio increases dramatically.

By examination under fluorescent light of samples treated by the Kasten's technique, large lenticular starch granules (type A starch) appear yellow to green; small polygonal starch granules (type B starch) appear orange; lipids appear bright yellow; cell walls are coloured green and proteins blue-green. Compared with fast-green which stains uniformly the proteins, modified Kasten's method shows some differences in the emission wavelengths of proteins in the different tissues, e.g. between aleuron and sub-aleuron tissue. In the endosperm cells, type B starch granules are also much more visible than when stained with iodine.

Treatments of barley grain

Barley is used in breadmaking, malt and brewing technologies, in some dietetic foods for humans and in animal feeding. In France, barley is much less used than wheat in human nutrition. Some dietetic foods use a special variety of barley (called biological barley) showing smaller sized kernels than for brewing. Hulled barley is prepared by abrasive milling, by which husk is almost completely removed, producing milled or polished barley and the byproducts. Abrasive milling is visually controlled by the technologist as far as greyish kernels showing glossy aspect are obtained. Observation under the microscope shows that husk has been entirely cleaned (Fig. 1c) but an important part of the kernel's

external layers is also locally removed, by attrition of the pericarp and tegument and of the aleuron layers. Conversely, at the crease level, the aleuron layers, pericarp and even husk which are sticking to the endosperm and penetrating inside it show hull remnants. Abrasive milling is then not uniform and corresponds to an extraction rate of about 70%.

Another mechanical treatment is used in order to remove all the brans. Such hulled barley much more completely cleaned is called pearl barley (Fig. 1d) and is equivalent to the polished rice. Kernels appear as small floury balls, like translucent pearls. Under the microscope, it can be observed that even when the outer tissues seem entirely removed from barley kernel and even when extraction rate reaches 90% and more, bran remnants can be seen at crease level.

Flaked barley (Fig. 1e) is prepared from good quality hulled barley. Long steaming time (45 min.) of the kernels is applied in a steam cooker, followed by flaking between two heated rolls. Flaked kernels are flat, show numerous fissurations and cracks which are from place to place deeply cutting up bran, aleuron layers and subaleuron tissues. During this process, kernels are subjected to high pressures which induce structural disorders, such as the protrusion of the crease area and the elimination of the remnant bran. These observations are in agreement with Farber and Gallant results (1976) who also noticed starch granules swelling and fissuration.

Puffed barley is obtained from hulled milled kernels, which are fed in a puffing-gun, preheated during a few minutes cooking time until superheated steam builds up pressure which is suddenly released. Puffed kernels show blistered and alveolated structures. At periphery of the kernels, aleuron layers do not seem modified in their structure by such hydrothermic treatment although this tissue is broken in many places, but a shift in the emission wavelength shows that chemical denaturation has occured. Endosperm and subaleuron layer are dramatically modified and show quite numerous and large alveoles. In these modified areas, starch is gelatinized and proteins are gathered together and appear in stretched sheets.

In animal feeding, barley is used whithout dehulling; after milling, it can be submitted to pelleting or to extrusion cooking. Pellet quality depends for a large part on how kernels are milled. For instance, two pellets which were made in our laboratory after being milled by two milling procedures are shown in Fig. 1g and 1h.

Kernels crushed and milled between rotating cylinders in a roll crusher mill (SOCAM process) give a coarse flour mill. During this process, husk are easily removed from the kernels and only partially fragmented. Because of the large differences in size and specific gravity between husk and kernel fragments, large segregation occurs between particles. Pellets of barley meal give samples where particles are facing each other (Fig. 1g). But, this meal being relatively coarse, the high particle size prevents very good adhesion between them, especially when particles show hydrophobic cuticular layer.

Mechanical disruption of the whole kernels by hammermilling is more severe. In this case, the final product is a very homogeneous flour constituted by husk and bran material divided into minute fragments, small endosperm agglomerates and isolated starch granules. Pelleting of this flour gives pellets with a higher cohesion between the more resistant endosperm particles; but the presence of numerous flat husk fragments, which are hydrophobic by their cuticular layer and which are oriented perpendicularly to the direction of pellet axis, renders it somewhat crumbly.

Cooking-extrusion of ground kernels give rise to a spongy product made of a multiphasic gel with numerous gas bubbles, as shown on Fig. 1f. Multiphasic gel shows an heterogeneous composition with starchy (yellow to brown) and proteins (green) components, into which some solid elements such as cell wall fragments (husk, bran and aleuron layers) are scattered. Proteins are mostly observed as very thin and elongated sheets (green emission), more or less stretched, as previously seen in the subaleuron tissue of puffed barley. Starchy material gives rise to two different wavelength emissions (a bright yellow emission and a light brown one), a still unexplained phenomenon. Their careful examination shows that, when the yellow emission occurs, starch granules are intact or starting to swell (solid phase), whereas, with the brown emission, starch granules have already been swollen (gel phase). This starchy gel determines the elasticity and cohesiveness of the extruded strand.

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