



# The microscopic structure of rye kernel and dough

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The microstructure of rye kernels and dough was studied by light microscopy. Cells of the rye endosperm contained both lenticular and spherical starch granules. The Calcofluor-induced fluorescence of the cell walls almost disappeared after the  $\beta$ -glucanase incubation of the cross-sections, while it only decreased after the xylanase treatment. The endosperm cell walls fragmented during baking.

## INTRODUCTION

In many countries rye is used chiefly as an animal feed. In Finland it is mainly used to bake rye bread. The light microscope is a useful tool for studying the microstructure of cereals. Two cell wall components, arabinoxylans and  $\beta$ -glucans, are of interest in rye baking. Our aim in the present work was to study the location of these components in the rye kernel and to determine the changes that occur in the rye cell wall polysaccharides during the baking process.

## MATERIALS AND METHODS

### Sample

The Canadian Muskate rye variety was used as the rye sample.

### Cryo-sections

Half of the rye kernel was fixed in 5% glutaraldehyde and frozen in liquid nitrogen (Fulcher & Wood, 1983). The sample was embedded in cryostat embedding medium (Jung, Germany) in a Leitz cryostat at  $-40^{\circ}\text{C}$  and the sections were cut  $10\text{ }\mu\text{m}$  thick at  $-25^{\circ}\text{C}$ .

### Plastic sections

The piece of the rye kernel was fixed in 5% glutaraldehyde (Fulcher, 1982), dehydrated with ethanol and embedded in Historesin Embedding Kit (Reichert-Jung, Germany), as recommended by the manufacturer.

The sections were cut  $5\text{ }\mu\text{m}$  thick with a Reichert-Jung microtome.

### Enzyme treatment

The plastic sections were incubated with purified xylanase and endo- $\beta$ -glucanase (produced by *Trichoderma reesei*) in a humid chamber at  $37^{\circ}\text{C}$  for 24 h. The sections were rinsed with distilled water and dried.

### Staining

For the bright field microscope the sections were stained with Lugol's solution and Light Green. For the fluorescence microscopic examinations the sections were stained with specific fluorochromes (Fulcher & Wong, 1980). Lipids of the cryo-sections were mounted in 0.01% Nile Blue. In the plastic sections, proteins were stained with 0.1% Acid Fuchsin and cell walls with 0.01% Calcofluor White M2R New for 1 min. The sections were rinsed with distilled water and dried.

### Microscopy

The samples were examined and photographed with an Olympus Vanox-T microscope.

## RESULTS AND DISCUSSION

Lipid droplets, which were stained yellow by Nile Blue, were mostly observed in the aleurone layer of the rye kernel. Lugol's solution stained starch granules violet and Light Green stained protein green. Cells of the

peripheral endosperm contained less starch than the cells in the starchy endosperm where both lenticular and spherical starch granules were observed in the protein matrix.

Cell walls were stained blue by Calcofluor. Compared with the reference section, fluorescence intensity of the endosperm cell walls had decreased after the xylanase treatment but the aleurone cell walls fluoresced intensively. Decreasing of fluorescence was strongest in the peripheral endosperm cell walls. The structure of the walls became more fragile during incubation.

After  $\beta$ -glucanase treatment the fluorescence of the aleurone and peripheral cell walls had disappeared and only the central endosperm cell walls fluoresced very weakly.

Since the rye dough was baked out of whole grain flour the structure of the dough was very complex. Acid Fuchsin stained the aleurone protein red and the

endosperm protein orange or light brown. Starch was unstained and it appeared black. The aleurone layers were resistant against breakage during flour milling and dough mixing, while a part of the endospermic cell walls broke down. Germs remained intact. The endosperm cell walls were highly fragmented in baked bread. The continuous matrix of rye bread is probably composed of several components.

## REFERENCES

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