ESEM Study of the Effects of Hydrolytic Enzymes on Wheat Bran Structure

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INTRODUCTION
Wheat bran is used in the human diet as a major source of dietary fibres (50% of the wheat bran components). These fibres are polymers (hemicelluloses and cellulose) located in the walls of wheat bran cells. Wheat bran, as the residual part of the wheat kernel after flour milling, is composed of several tissues which are structurally different. The outer pericarp tissue is made of three layers: epicarp, mesocarp and endocarp, made of longitudinal cells. Tubular cells from the endocarp are directed perpendicularly to the other outer layers. Pericarp cell walls are made of 60% gluconorobinolylanxs, 30% cellulose and miscellaneous compounds such as lignin [1]. On the inner side of the wheat bran, the aleurone layer is a monocellular layer, made of square cells surrounded by thick walls made of 65% arabinoxylan and 29% β-glucans [2]. Many starch granules are located on the inner face of the aleurone layer. Our aim was to visualise modifications of wheat bran after different enzymatic treatments. Wheat bran fibres can be hydrolysed by enzymes such as cellulases and hemicellulases. Enzymatic treatment occurs with cellulases and cellobiohydrodrolase, xylanase, α-arabinofuranosidase, α-L-xyllosidase, α-amylase, β-amylase, amylglucosidase, and α-glucosidase with tissue structure modifications. When cellulse or xylanase were used alone or in association with other enzymes, separation of outer layers (epicarp and mesocarp) from the endocarp and aleurone layers was observed. Starch granule removal was observed only with a cocktail of enzymes.

KEYWORDS
wheat bran, cellulases, hemicellulases, fibres, hydrolysis, ESEM

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MATERIALS AND METHODS

Wheat bran
Coarse wheat bran (Markal, France) was made of 50% fibre, distributed in 12% cellulose, 34% hemicelluloses (arabinoxylans and gluconorobinolylanxs), 3% lignin and traces of β-glucans. It contained starch (10%), proteins (16%), ash (6%), phytate (3%) and lipids (3%). The moisture content was 12%.

Enzymes
All enzymes were obtained from Megazyme or Sigma, and were: cellulase (Megazyme ref. E-CELTR), cellobiohydrolase (E-CBHIII), β-glucosidase (E-BGLUC), xylanase (E-XYTR3), arabinofuranosidase (E-FAFSE), β-xyllosidase (Sigma, ref. X3501), α-amylase (E-BAAM), β-amylase (E-BCBM), amylglucosidase (E-AMGDF), and α-glucosidase (E-TSAGL).

Enzymatic treatment
For each enzymatic treatment, 10% wheat bran was suspended in water. 0.24 % 2-bromo-2-nitro-1,3-propanediol (bronopol), a large spectrum biocide, was added to the mixture to avoid bacterial and fungal contamination. Each enzyme solution was tested separately, as well as in combination with all the enzymes. The enzymes were added at a concentration of 1 U/ml. The reaction mixture was incubated for 14 h at 30°C under agitation (150 rpm, Aerotron, Infors). A blank without enzyme was also incubated. The mixture was then centrifuged at 4000 g for 10 minutes (Eppendorf Centrifuge, 5810R) and the pellet was kept for observations.

RESULTS AND DISCUSSION

Control
In the ESEM, examination of wheat bran fibres revealed the longitudinal cells of the outer layers of the pericarp, with the perpendicular orientation of the endocarp cells (Fig 1). The large aleurone cells layer was associated with damaged endosperm cells containing starch granules.
Wheat bran after enzymatic treatment

Enzymatic treatments such as cellulase and xylanase treatments were the most effective at separating the wheat bran layers. The outer layers of pericarp (epicarp and mesocarp) dissociated from the endocarp and the aleurone layer (Fig 2). Pieces of the outer layers totally separated from the inner layers could be observed (Fig 3). The treatment with the cocktail of enzymes led to a dissociation of the outer layers from the endocarp and the aleurone layer and also removed the starch granules (Fig 4).

The enzymatic treatments of wheat bran with \( \alpha \)- and \( \beta \)-amylase, glucoamylase, cellobiohydrolase, \( \beta \)-glucosidase, \( \beta \)-xylosidase, arabinofuranosidase, and \( \alpha \)-glucosidase used alone did not show any structural modifications of the wheat bran tissues. The amylases could not achieve starch degradation individually, and did not attack arabinoxylans and cellulose. Enzymes such as \( \beta \)-glucosidase and \( \beta \)-xylosidase acted on short-chain oligosaccharides, whose hydrolysis could not be observed, whereas cellulase and xylanase could start the depolymerisation of whole chains of cellulose and hemicelluloses, leading to destruction of the wheat bran layers.

Contrary to the observations of Tervilä-Wilo et al. [6] and Benamrouche et al. [7], the aleurone layer was not removed by the enzymatic treatments. This may be due to the low concentrations applied (1 U/ml), chosen with the objective of future comparison to Lactobacillus plantarum and Saccharomyces cerevisiae culture broth, where similar levels of enzyme activities were measured.

CONCLUSIONS

Wheat bran is resistant to hydrolysis and thus a poor substrate for fermentation by microorganisms. By using ESEM, we were able to observe wheat bran solubilisation by low levels of enzymes such as cellulases and hemicellulases. We could not observe any structural modification when osidases were used, although they could release soluble components from the bran matrix. The low concentrations of enzymes we used were representative of what could occur during wheat bran fermentation by microorganisms. Coarse wheat bran was only partially modified by these soft treatments, so that it may keep its functional properties but could be easier to introduce into a manufactured product after fermentation.

REFERENCES


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