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STRUCTURAL CHANGES OF WHEAT BRAN AFTER HUMAN DIGESTION

(with a summary in English)

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STRUCTURE OF BRAN AFTER DIGESTION

INTRODUCTION

The effects of coarse wheat bran on colonic function in humans are now well established (PAINTER et al., 1972; FINDLAY et al., 1974; CUMMINGS et al., 1976; KAY & TRUSWELL, 1977; for a review, see KELSAY, 1978). The changes reported in most studies include an increase of stool weight and water content, shortening of the intestinal transit time and a higher frequency of defaecation. Several hypotheses have been put forward to explain these effects, the two best known being the water adsorption theory (EASTWOOD, 1973) and the fermentation theory (HELLENDOORN, 1973).

Few studies, however, have dealt with the morphological changes in the bran due to in vivo digestion (reviewed by SAUNDERS, 1978) although it has been repeatedly stressed that the physiological effects of dietary fibre depend as much on the physical properties of the plant structures involved as on their chemical composition (see, e.g. EASTWOOD & MITCHELL, 1976). We thought it useful, therefore, to study these structural alterations, using samples from an experiment with human volunteers on the influence of dietary fibre on serum lipids, faecal lipids and colonic function (STASSE-WOLTHUIS et al., 1979). The main techniques used were light and electron microscopy, combined with histochemical and fluorescence methods.

MATERIALS AND METHODS

1. *Subjects and diets*

For a detailed description of the experiment we refer to STASSE-WOLTHUIS et al. (1979). Briefly, 62 human volunteers consumed a low-fibre diet for 17 days. After that period, a control group continued the low-fibre diet for another 35 days while a high-fibre group received the low-fibre diet enriched with bran. The coarse wheat bran was produced by Meneba (Rotterdam) from a grist of 65 per cent European and 35 per cent North American bread wheat (*Triticum aestivum*); total dietary fibre content was 50 per cent (wet weight). Of the total mean intake of 38 g per day for the high-fibre group 80–90 per cent was incorporated into bread and the rest into desserts. The low-fibre diet contained on average 14 g bran incorporated into bread.

For structural analysis samples were taken from the original raw wheat bran, from the bran after baking into bread and from faeces produced at the end of the experimental period by one subject in the high-fibre and one subject in the low-fibre group. Intestinal transit time (CUMMINGS et al., 1976) was measured over a period of 7 days and yielded a value of 48 h for the low- and 11 h for the high-fibre subject. All samples were kept frozen at –40 °C.

2. *Structural analysis of the samples*

a. Light microscopy

Samples were thawed and, after repeated washing in double-distilled water, pieces of bran were microscopically selected and air-dried. The appearance of the raw bran was very similar to what has been observed on fresh specimens, suggesting that the freezing and the washing had caused no important structural modifications.

The light microscopical study was carried out using microtome sections after safranin-fast green staining. Histochemical tests were carried out with unstained samples; the separate layers of the bran were qualitatively analysed for the presence of starch with I_2 dissolved in KI (IKI), cellulose using IKI-H_2SO_4, lignin with the phloroglucinol test and pectic substances with ruthenium red. Total polysaccharides were determined with periodic acid-Schiff's reagent, total proteins with ninhydrin and total lipids with Sudan III or Nile blue (for all methods, see e.g. JENSEN, 1962).

Autofluorescence was studied on untreated samples in double distilled water. A fluorescence microscope equipped with an Osram HBO 50 mercuryvapor lamp and exciter filters UG 1 and BG 38 was used. At an excitation wavelength of 366 nm the emission spectrum was recorded by measuring a sample area of 20 μm^2 with a RCA photomultiplier tube (type C 31034) operating at 1 kV. Maximal emission wavelength, intensity at maximal emission wavelength and decrease in intensity after 30 sec (fading) were measured (for details of the method, see WILLEMSE, 1972).

The conclusions, drawn from each technique, were based on observations on 3 to 5 replicate samples.

b. Electron microscopy

For transmission electron microscopy samples were stained for 2 h with 5 per cent $KMnO_4$ in double-distilled water at room temperature. Alternatively the samples were prefixed in 5 per cent glutardialdehyde in 0.1 M Na-cacodylate buffer, pH 7.2, with 0.5 per cent $CaCl_2$ added, and then stained with 1 per cent OsO_4 in buffer for 12 h at room temperature. The samples were rinsed in buffer and dehydrated using a graded alcohol series. Poststaining took place during 60 min in the 70 per cent ethanol step using 1 per cent uranylacetate. Thin sections of Epon-embedded samples were studied using a Philips 301 electron microscope at 60 kV.

For scanning electron microscopy the samples were coated by gold evaporation and studied with a Jeol scanning electron microscope.

RESULTS

The structure of unprocessed wheat bran is schematically represented in fig. 1. This scheme is based on our own observations combined with descriptions of

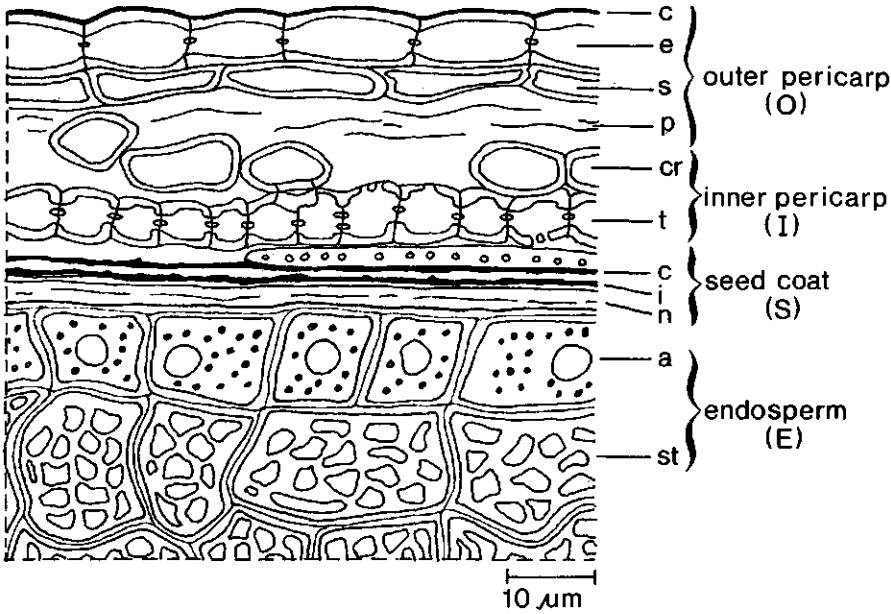


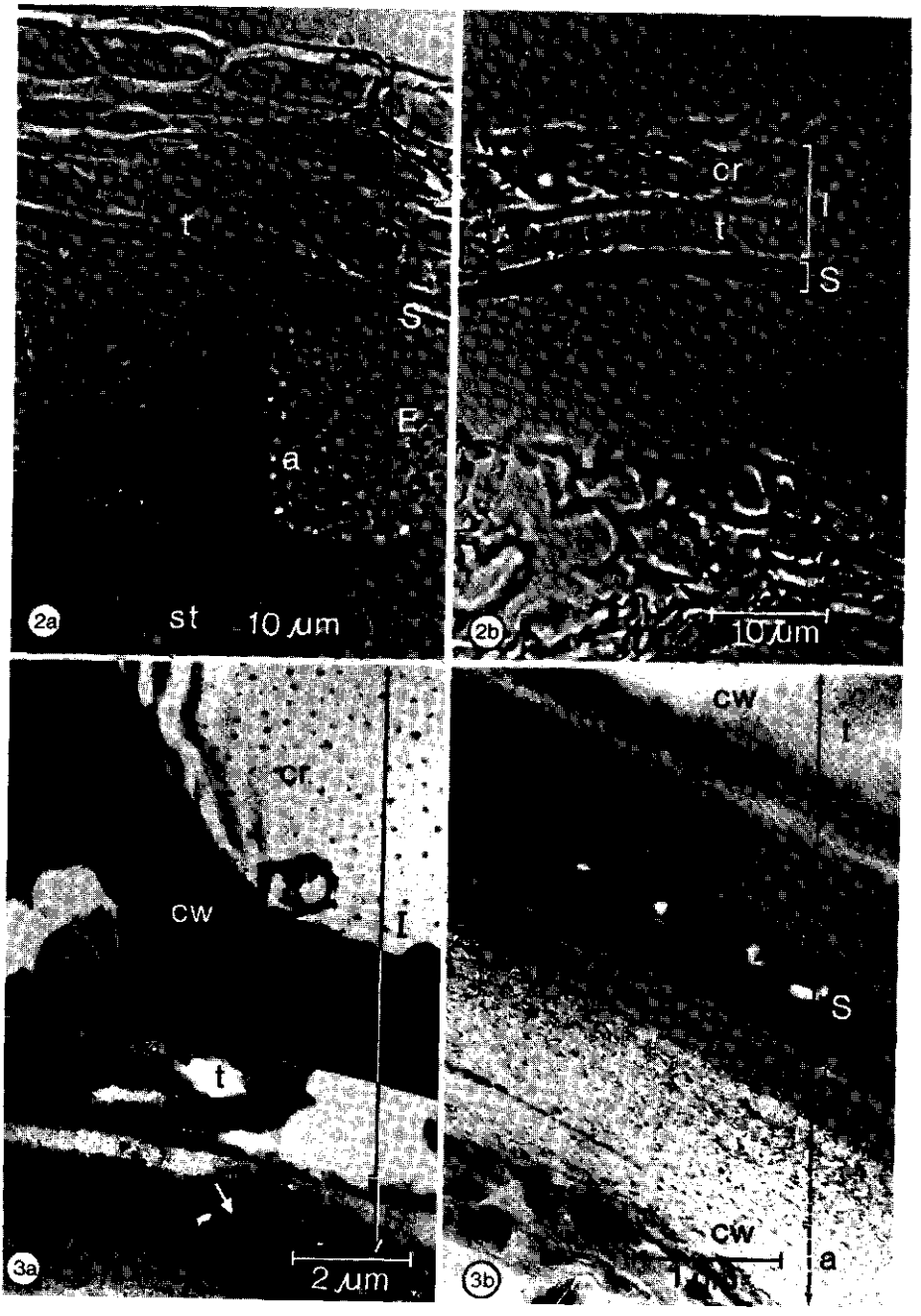
FIG. 1. Schematic representation of the structure of raw wheat bran. Abbreviations: O, outer pericarp; I, inner pericarp; S, seed coat; E, endosperm; c, cuticula; e, epidermis; s, subepidermal layer; p, crushed parenchyma cells; cr, cross cells; t, tube cells; i, integumental part; n, nucellar part; a, aleuron layer; st, starch containing cells.

others (PERCIVAL, 1921; KAUSSMANN, 1963; ESAU, 1977; SAUNDERS, 1978). In the scheme the separate layers of the bran are indicated. From outside to inside, the following layers are observed: the waxy cuticula, epidermis, subepidermal layer and crushed parenchyma cells, which together make up the outer pericarp; the cross cells and tube cells which form the inner pericarp; the seed coat, consisting of several crushed cell layers and the starch-containing endosperm with its outer layer of aleuron cells. The seed coat is made up of the former integumental and nucellar cells which have been compressed during growth of the wheat grain, so that only the cell walls remain, forming firm layers. The exact borders of these several layers, therefore, are difficult to determine.

Because we observed that incorporation of bran into bread altered its structure a short description of these changes is also given.

a. The structure of the raw bran

Fig. 2.a. shows a section through a raw bran sample as seen through the light microscope. Many of the layers described in fig. 1 are visible. The endosperm cells showed a positive reaction for starch content; the aleuron layer was strongly ninhydrin-positive. After ruthenium red staining pectin was clearly visible in the pericarp walls, while lignin, as determined by phloroglucine/HCl was located only in the walls of the cross cells and tube cells of the inner pericarp. All cell walls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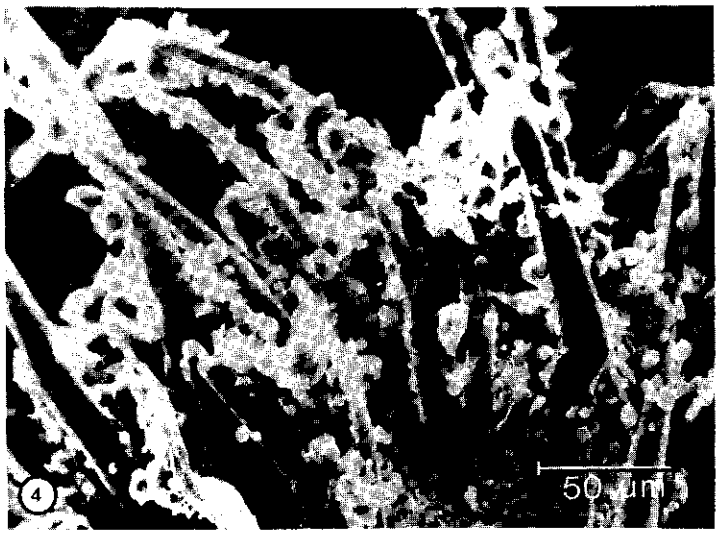

FIG. 4. Scanning electron micrograph of a raw wheat bran sample. The apex of the bran is clothed with a number of short stiff hairs, forming the 'brush' of the former grain.

contain cellulose. PAS-staining revealed the presence of polysaccharides in all cell walls; in addition, the cytoplasm of the aleuron cells was stained, together with the starch grains in the adhering remnants of the endosperm. The location of lipids was difficult to determine: only a faint reaction was visible in the pericarp with either method.

The autofluorscence study showed for the raw bran a maximal emission at 460 nm. This value was found also in investigations on wood autofluorescence (WILLEMSE, in prep.) and might therefore confirm the presence of lignin or lignic components in the raw bran sample as was indicated by the phloroglucinol test.

Fig. 3.a. shows some details of the seed coat (S) and the adjoining tube (t) and cross cells (cr) at a high magnification. The composition of the seed coat is complex. As can be seen, only some remnants of cells – probably derived from the former inner integument – remain (white arrow). All other layers of the seed

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FIG. 2. a. Light microscopical view of a section through a raw wheat bran sample, stained with safranin-fast green. For abbreviations, see fig. 1.

b. Light microscopical view of a section through a faecal wheat bran sample. For abbreviations, see fig. 1. Note, in this extreme case, the disappearance of both outer pericarp and whole endosperm.

FIG. 3. a. Transmission electron micrograph of a raw bran sample. $KMnO_4$-fixation. Abbreviations: I, inner pericarp; S, seed coat; cr, lumen of a cross cell; t, tube cell; cw, cell wall material. Note the presence of several smooth layers in the seed coat (black arrow) and of cellular remnants (white arrow).

b. Transmission electron micrograph of a faecal bran sample. $KMnO_4$-fixation. Abbreviations: s, seed coat; t, tube cells; a, aleuron cells; cw, cell wall material. In general, the cell walls are thinned, while, attached to the bran, bacteria are present (arrow).

coat have a rather smooth appearance. Cell remnants were not observed there.

The raw bran often bears some short stiff hairs at the top of the former grain (fig. 4; see also SAUNDERS, 1978).

b. The structure of the bran baked into bread

As compared with the raw bran these samples showed some modifications. The endosperm cell walls showed some cracks and ruptures, while the starch inside these cells was less prominent or completely invisible. Where it is still present, iodine staining showed a shift to a more red-brown colour as compared with the raw bran, indicating a break-down of amylose molecules into shorter fragments (REES, 1967). In the aleuron cells the aleuron granules had partially disappeared while the cytoplasm stained more intensely with ninhydrin. This suggests a break-up of the granules and a redistribution of their contents over the cytoplasm. Pectin staining in the pericarp was slightly stronger. The cell walls showed a more pronounced layered composition. No changes were observed in the seed coat.

Compared with the raw bran sample, autofluorescence also showed a maximal emission at 460 nm, suggesting still the presence of lignin substances. The intensity, however, was significantly higher, which might have been caused by a change in polymerisation of lignin molecules, possibly induced by the baking process.

c. The structure of bran from faecal samples

The same structural changes were observed in bran from faeces of the low- and of the high-fibre subject as compared with the raw bran. In both cases the starch-containing endosperm cells had disappeared totally; the layer of the aleuron cells was heavily attacked or absent too. Cell walls locally showed ruptures or were partially absent. In some cases even the outer pericarp layers were lost together with the nucellar remnants from the seed coat (fig. 2.b.). In general, cell walls were thinned, while aleuron grains, if present, were much reduced in size. Bacteria were often observed attached to the bran, mainly at the side of the former endosperm (fig. 3.b., arrow).

Histochemical analysis still showed a positive reaction for lignin and cellulose; however, the PAS-reaction for total polysaccharides was less intensive. In a few cases a slight positive reaction for pectin was observed within the pericarp. Compared with the raw bran, the intensity of autofluorescence – with also a maximal emission near 460 nm – notably decreased while the speed of fading increased, possibly indicating a loss of lignin components.

DISCUSSION

Dietary fibre is, by definition, resistant to hydrolysis by the digestive enzymes of man (TROWELL et al., 1976). A variable part of its components is, however, not recovered in the faeces because of the action of colonic bacteria. Dietary pectin is

completely broken down; the major part of the hemicelluloses, which make up most of the dietary fibre in cereal products like bran, is similarly affected. Only lignin and, to a lesser extent, cellulose resist bacterial action and are recovered with the faeces (see e.g. HOLLOWAY et al., 1978; VAN SOEST, 1978; CUMMINGS et al., 1979).

Although our results are qualitative and are based on only two subjects, they illuminate these findings and show that the structure and composition of bran is radically altered after digestion. The chewing of the food, the action of digestive juices, peristaltic movements and bacterial attack may be the main reasons for bran destruction. The bran is distorted at both sides. In most cases the pericarp and seed coat remain structurally intact and retain their positive reaction for cellulose and lignin. However, the whole structure of these layers has been softened, probably due to a loss of pectin, hemicellulose and cellulose. All other layers were lost, chemical attack probably being the predominant factor. These effects were found in both subjects, although they had widely differing diets, and intestinal transit times of 48 h and 11 h respectively. It suggests that, in spite of the extremely rapid intestinal transit in the high-bran subject, the intestinal passage time was still long enough to allow bacterial break-down of all susceptible structures. However, this suggestion is only tentative and should be confirmed by investigating larger number of subjects.

The importance of the physical structure of dietary fibre as opposed to its chemical composition has been stressed previously (EASTWOOD & KAY, 1979). This view is supported by the observations that grinding a coarse bran preparation to smaller particles will diminish many of the effects on colonic function in man (see KIRWAN et al., 1974). The physiological effects of bran, therefore, strongly depend on the structural integrity of the bran used. Our results indicate that seed coat and pericarp are the dominant structural factors which might act upon these processes. Possibly the hairs remaining on the bran might have an influence on the speed and intensity of digestion too.

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SUMMARY

Key words: dietary fibre / bran / structure / human nutrition.

The structure of raw wheat bran was compared with that of bran baked into bread and that of bread-bran after passage through the digestive tract of two

human subjects with widely different intestinal transit times. Changes were measured qualitatively using light and electron microscopy combined with histochemical and fluorescence methods.

Baking of the raw bran into bread causes structural modifications. Moreover, after digestion the composition of the bran was radically altered. In both subjects the main change was the partial or even complete disappearance of pericarp and endosperm.

The results support the suggestion that various physiological effects – seen after addition of bran to food – may greatly depend on changes in bran structure which occur during preparation and consumption of the food.

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