Effect of Microwave Treatment on the Microstructure of Dehulled Rapeseed

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Cereal Chem. 58(5):381-384

ABSTRACT

The microstructures of *Brassica napus* c.v. Tower, *B. campestris* c.v. Candle, and *B. juncea* (oriental mustard), microwave-treated at 7, 10, and 13% moisture contents for durations of up to 2.5 min, were studied by scanning electron and light microscopy. The changes observed in the major cell inclusions, spherosomes (oil droplets) and aleurone grains, were a function of the extent of heating and included coalescence and loss of identity of spherosomes and distortion of aleurone grains. In samples in which myrosinase was completely inactivated by the microwave treatment, the membranes surrounding the spherosomes pulled away from the cell wall, cohered, and collected mainly in the center of oil-rich cells and around the aleurone grains in aleurone cells. Consequently, structureless areas filled with oil were formed near the cell walls. These microstructural changes provided an explanation for inactivation of enzymes and facilitation of oil extraction in rapeseed that may be applicable to other oilseeds heat-treated before crushing.

Rapeseed, like other oilseeds, is heat-treated before crushing to inactive enzymes such as lipase, lipoxygenase, and myrosinase (thio-glucoside glucohydrolase, EC 3.2.3.1) and facilitate the subsequent expression of oil by mechanical means. This heat treatment results in certain physical, chemical, and physicochemical changes that are not fully understood (Norris 1964). Since microstructure influences these changes, the objective of this study was to elucidate the alterations that occur in the microstructure of dehulled rapeseed upon treatment with microwave energy.

MATERIALS AND METHODS

Seed Samples

Canadian-grown seeds of *Brassica napus* c.v. Tower, *B. campestris* c.v. Candle, and *B. juncea* (oriental mustard) were obtained from the Saskatoon Research Station of Agriculture Canada and dehulled using the Palyi pneumatic small-seed dehulling unit (Stanley and deMan 1974).

Microwave Treatments

The dehulled seed samples were equilibrated to 7, 10, and 13% (± 0.15%) moisture levels and exposed to 2,450 MHz of microwave energy in a 1.25-kW Westinghouse microwave oven for periods of 1.0, 1.5, 2.0, and 2.5 min (Maheshwari et al 1980a, 1980b). The moist microwave-treated and untreated samples were air-dried to 5–6% moisture and stored at room temperature until required.

Sample Preparation

All dehulled seed samples (microwave-treated and untreated controls) were allowed to imbibe distilled water for 12 hr and then prefixed in 3% glutaraldehyde in phosphate buffer (0.05 *M*, pH 7.0) for 24 hr at room temperature. The samples were subsequently rinsed with three 10-min changes of the buffer and postfixed in 1% potassium permanganate in buffer for 4 hr at 4°C (Mills and Chong 1977, Stanley et al 1976). After being rinsed again in buffer, the samples were gradually dehydrated in an ethanol series and then divided into two portions, one of which was critical-point dried for examination by scanning electron microscopy and the other infiltrated and embedded in Epon 812 (Hayat 1972) for examination by light microscopy.

Scanning Electron Microscopy

Critical-point dried samples were sliced with a razor blade, glued to specimen stubs with carbon paint, coated with gold-palladium (20–25 nm), and then examined with an ETEC Autoscan microscope operated at 10 kV. Photomicrographs were taken on Polaroid 4 × 5 Land film.

Light Microscopy

Blue-green sections (about 1 μm thick) were cut with glass knives on a Sorvall Porter-Blum manual microtome and heat-fixed to glass slides at 65°C. After being stained with Sudan black B in ethylene glycol for fat and counterstained with nuclear fast red (Sheehan and Hrapchak 1973), the sections were examined with a Carl Zeiss photomicroscope. Photomicrographs were taken on Ilford Pan-F135 film.

RESULTS AND DISCUSSION

Observations of the three *Brassica* species with scanning electron and light microscopy showed them to be quite similar. Therefore, the micrographs presented are representative of these species. Tower, which is not shown, more closely resembled Candle.

The microstructures of dehulled seeds of *Brassica napus* c.v. Tower, *B. campestris* c.v. Candle, and *B. juncea* (oriental mustard) were similar with respect to the types of cell contents (Figs. 1a and b, 2a and b). However, some differences were apparent in the size and shape of the cells, the abundance of spherosomes, and the number and size of aleurone grains. The cotyledon cells of Tower and Candle were generally smaller in size than those of *B. juncea*; the relative abundance of spherosomes was greater in Tower and Candle cells; and the number and size of aleurone grains were bigger in *B. juncea*. These microstructural features demonstrated the relationship of abundance of spherosomes to higher lipid content and increased number and size of aleurone grains to higher protein content in these three *Brassica* species (Maheshwari et al 1980a).

In the cells containing aleurone grains (ie, aleurone cells) these electron-dense grains were surrounded by spherosomes (Fig. 1a). The aleurone grains were globose, with smooth outer surfaces, and were suspended within a tightly packed array of spherosomes. Examination of cut surfaces could not distinguish the aleurone grains with crystalloid inclusions and those with globoids. Although the smaller protein bodies within the aleurone grains were generally indistinguishable from the amorphous mass of storage proteins, these were sometimes seen in heat-damaged aleurone grains. This configuration of the aleurone grains, ie, smaller protein bodies packed in an amorphous mass of protein and surrounded by a membrane, has previously been reported for soybean (Wolf and Baker 1972).

Spherosomes were membrane-bound and distributed throughout the cytoplasm. The spherosomes were more numerous near the cell walls in the aleurone cells (Fig. 1a), and they almost filled the cells devoid of aleurone grains (oil-rich cells, Fig. 1b). This arrangement of spherosomes and aleurone grains created some

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0009-0352/81/05038104/$03.00/0

difficulties in proper fixation and sectioning of the seeds. The slices of unheated seeds invariably showed minor disruptions of the microstructure near the cell walls and some smearing of the cut surfaces from the released oil (Fig. 1a).

Preconditioning of dehulled seeds was not observed to have any significant effect on their cellular structure. At 7% moisture content, exposure of samples to microwave energy for 1 min did not produce any marked changes in the microstructure of any of the three species. As the exposure period was increased to 1.5 min and beyond, significant disruption of intracellular structure was observed, the disruption being greater with increasing exposures to microwave energy. For any given duration of microwave treatment, the changes in the seed microstructure were greater at 10 and 13% moisture contents. This indicated that alterations in the microstructure were dependent on the extent of heating, which in turn was dependent on the moisture content of the samples and the length of exposures to microwave energy (Maheshwari et al 1980a).

Both scanning electron and light microscopic examinations of all preparations from the three species indicated that the microstructural changes caused by microwave heating in aleurone and oil-rich cells of *B. juncea*, Tower, and Candle were similar. On microwave treatment of samples of 7% moisture content, the disruption of spherosomes (coalescence due to the rupture of their surrounding membranes) was evident earlier (1.5 min) than that of aleurone grains (2.5 min) in the aleurone cells. The aleurone grains were considered disrupted when their smooth outer surfaces appeared jagged and irregular. In samples with 10 and 13% moisture content, the disruption of both these cellular inclusions occurred even upon microwave treatment for 1 min (Fig. 1c). Microwave treatments of 1.5 min and longer at these moisture contents resulted in complete disruption and loss of identity of spherosomes (Fig. 1d) and severe distortion of aleurone grains (Fig.

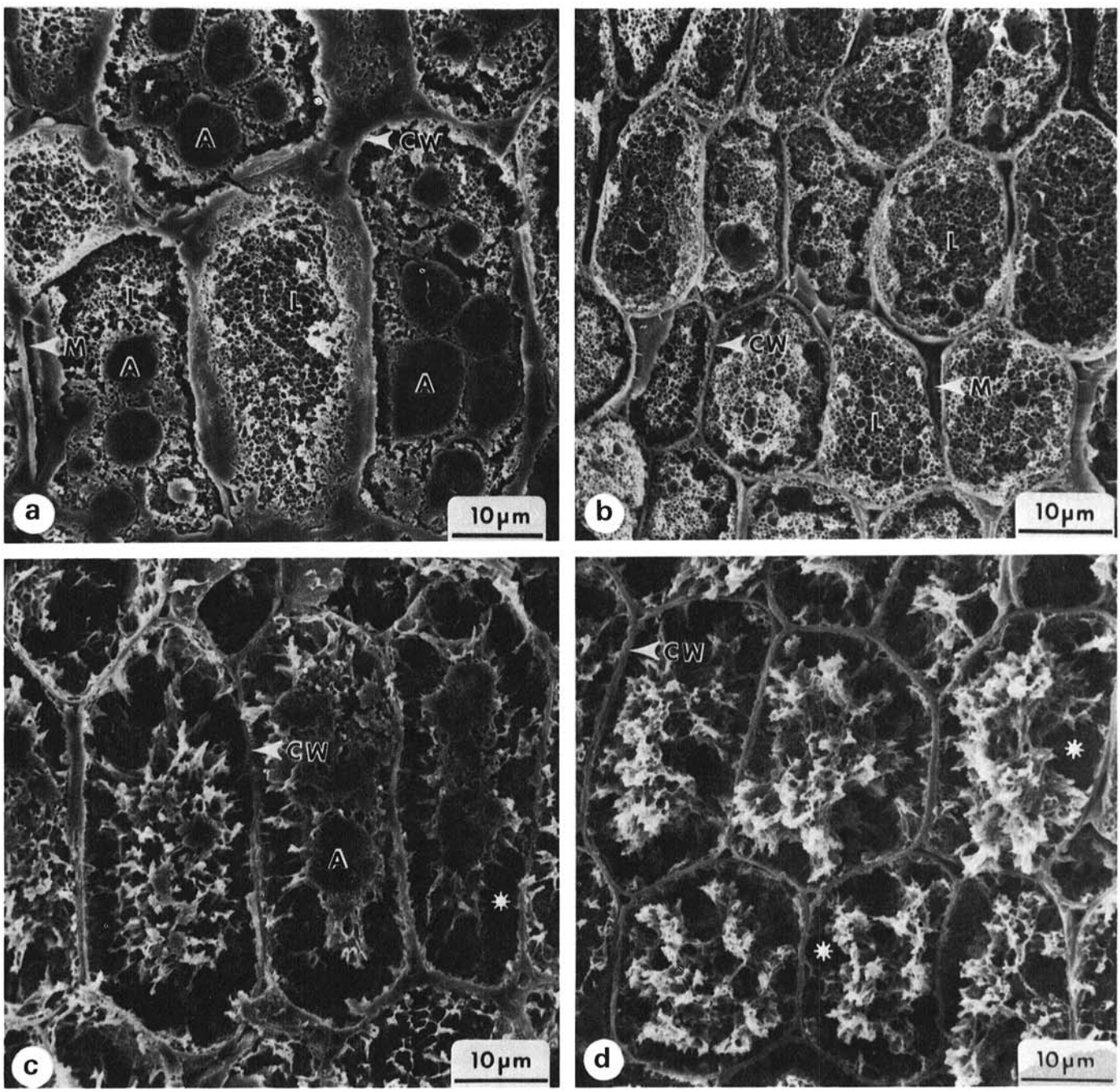

Fig. 1. Scanning electron micrographs showing the microstructure and microstructural changes in: **a,** unheated aleurone cells of *B. juncea*; **b,** unheated oil-rich cells of Candle; **c,** aleurone cells of *B. juncea* microwave-treated at 13% moisture content for 1.0 min; **d,** oil-rich cells of Candle microwave-treated at 10% moisture content for 1.5 min. **A** = aleurone grain, **CW** = cell wall, **L** = spherosomes (oil droplets), **M** = middle lamella, * = structureless area in cell.

2c and d). Mills and Chong (1977) have also observed similar structural changes in severely heat-damaged, elevator-stored rapeseed.

Myrosinase activity in dehulled seeds of Tower, Candle, and *B. juncea*, which is largely associated with the plasmalemma and present in a majority of cotyledon cells (Maheshwari et al 1981), was destroyed completely upon microwave treatment of samples at 10% moisture content for a minimum of 1.5 min. At 13% moisture content, the exposure requirement was reduced to 1.0 min only for *B. juncea*. Samples at 7% moisture content required longer exposures to microwave energy, and a 2.5-min treatment completely destroyed the enzyme in Tower and *B. juncea* (Maheshwari et al 1980a). In all adequately microwave-treated samples, ie, those in which myrosinase was completely inactivated, the membranes surrounding the spherosomes and presumably the plasmalemma pulled away from the cell wall, cohered, and collected as an electron-dense mass of poorly defined shape mainly in the center of oil-rich cells and around the aleurone grains in aleurone cells. No appreciable changes were detected in the cell walls and the middle lamella as a result of microwave treatment.

In the adequately microwave-treated *Brassica* seed samples, the coalescence of spherosomes resulted in separation of oil from surrounding membranes and formation of structureless areas filled with oil near the cell walls. These microstructural changes in rapeseed along with the obvious thermal denaturation of proteins and consequent alterations in their functionality were considered responsible for facilitating the expression and extraction of its oil by mechanical and other means upon adequate heat treatment. This explanation may also be applicable to other oilseeds that are heat-treated before being crushed.

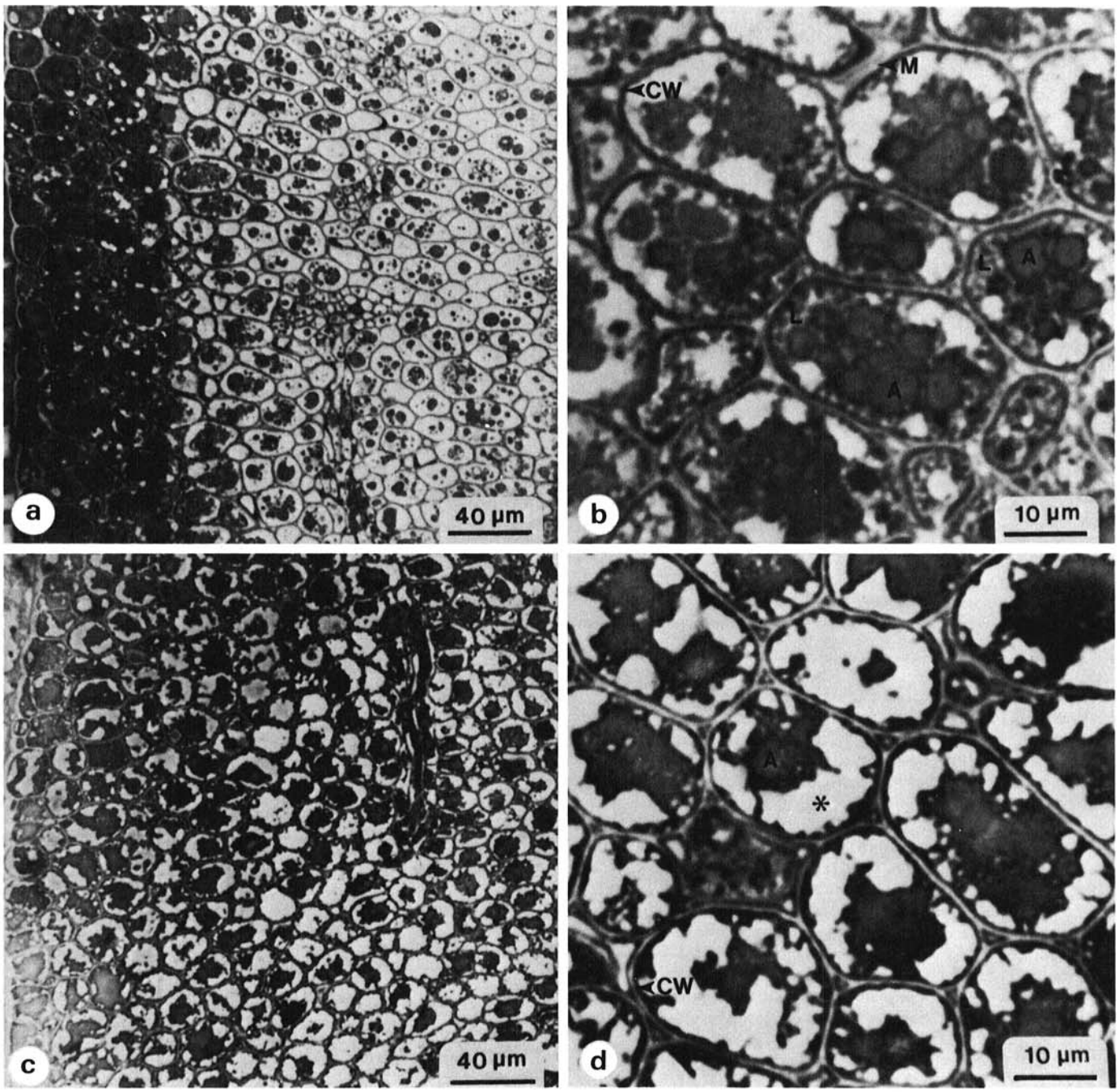

Fig. 2. Light micrographs at low and high magnification showing the microstructure and microstructural changes in cotyledon cells of Candle. **a** and **b** = Unheated, **c** and **d** = microwave-treated at 13% moisture content for 1.5 min. **A** = aleurone grain, **CW** = cell wall, **L** = spherosomes (oil droplets), **M** = middle lamella, * = structureless area in cell.

ACKNOWLEDGMENTS

We wish to thank H. J. Swatland, Department of Animal and Poultry Science, and C. L. Duitschaever and Alexandra K. Smith, Department of Food Science, University of Guelph, for their assistance in the light and scanning electron microscopical examinations of samples. The Canadian Commonwealth Scholarship and Fellowship Administration provided a Commonwealth Scholarship to P. N. Maheshwari. This research was supported in part by the Ontario Ministry of Agriculture and Food and the Natural Sciences and Engineering Research Council of Canada.

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[Received November 12, 1980. Accepted January 26, 1981]