DEHULLING OF RAPESEED

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The method of dehulling of rapeseed described in this paper is based on pneumatic attrition and was developed by Mr. L. Palyi, a Canadian milling consultant. The rapeseed is introduced in the feed hopper and through an air-lock enters into a stream of air. The air flows at 283 cubic feet per second and carries the seed into the dehulling tubes. The dehulling tubes are circular sections of pipe lined with sets of inclined saw teeth. Through impact of the fast moving seeds the hulls are loosened. From the dehulling tubes the material enters a cyclone and an air-separator. In the air separator two fractions are obtained, one consisting of dehulled rapeseed (meats) and the other consisting of hulls and fines. The latter fraction is further separated in a plan sifter to yield hulls and fines. The pilot plant installation can dehull about 150 lbs of seed per hour. The material balance gave the following results.
Meats obtained represented 50 % by weight of the seed processed, 15,5 % hulls were obtained and 4,5 % fines.

Analysis of the chemical composition of meats, hulls and fines indicates that both moisture and ash contents of the hulls were higher than those of the meats. The fiber content of the hulls was 39,0 % and of the meats 9,56 %. This means that about half of the fiber of the rapeseed is removed in the dehulling process. The hulls contained 12,44 % of oil, much less than the seed of meats. Protein content of the hulls was 14,04 %, which is also lower than that of the meats.

To become familiar with the rapeseed kernel and the changes it undergoes during dehulling;the gross and detailed structure of experimental material was examined by scanning electron microscopy. This technique has been applied with success to cereal and oilseeds and offers the advantage of obtaining three-dimensional images of the sample surface with minimal preparation.

Whole and sectioned seeds of Brassica campestris or turnip rape and the less common Brassica napus or oilseed rape were prepared by the procedures outlined below, coated with carbon and 200-300 Å of gold and examined at a normal accelerating voltage of 20 kv with an ETEK Autoscan SEM.

In general, fixation, a process in which the tissue is killed, hardened and preserved, is necessary for structural studies by electron microscopy. Unfortunately, many of the common fixation procedures available to the electron microscopist are not adequate for the preservation of seed tissues. The major problem is the rate of penetration of the fixative through the dense cell walls of the dry (approximately 10 % moisture) tissue.

The preparative methods used in this study included:

1) Untreated material. This is a rapid and easy technique which is adequate for examining external features but is useless for looking at internal structures. For studying internal aspects the following procedures were employed:

2) The easiest technique used consisted of dissecting the seed with a razor blade, passing the slice through a standard ethanol series (25, 35, 50, 75, 85, 95 and 3 x 100 %) and air drying. This proved satisfactory for fairly rigid structures such as the hull or where not much detail was required but it produces varying degrees of distortion to cell walls and cell contents that was visible at higher magnifications.

3) A more satisfactory procedure consisted of quench freezing unfixed whole seeds in isopentane cooled by liquid nitrogen and fracturing the seed with a razor blade under isopentane to which 8 % cyclohexane had been added to depress the freezing point. The seed fractured along cell boundries and in some cases through the cell. The material was subsequently vacuum dried which caused a slight degree of shrinkage.

4) To stabilize the cells and prevent shrinkage the method of MOLLENHAUER and TOTTEN (1971) was employed. Seeds were sliced with a razor blade under 3 % glutaraldehyde buffered to pH 7.0 with phosphate buffer and pre-fixed in this solution for 4 h at room temperature. The tissues were then rinsed in five 8 min. changes of buffer at room temperature and post-fixed in 1 % $KMNO_4$ at 0-5° C for 30 min. The tissues were then rinsed in several changes of distilled water and dehydrated in seven 8 min. changes of 15, 25, 50, 75, 85 and 95 % ethanol and three 10 min. changes of 100 % ethanol. Although this method has been reported to give good results in TEM studies of seeds, we observed much distortion of cell contents from its use.

5) Consistently better results were obtained by imbibing whole seeds in distilled water for 24 h followed by the above glutaraldehyde fixation, again for 24 h. The seed were then treated as above and, after air drying, sliced with a razor blade.

The following methods were used in the histological studies of rapeseed:

1) Fixation - Seeds were fixed in a solution of 2.5 % gluteraldehyde in 0.1M Mg O_4 buffer (pH 7.0) for seven days.

2) Dehydration - Water was removed by gradual displacement with tertiary butyl alcohol (TBA). The material was past through an increasing series (50, 70, 85, 95, 100 %) of TBA, remaining 14 h at each concentration. This was followed by two 4 h soaks in 100 % TBA.

3) Paraffin embedding - Seeds were embedded in paraffin for 7 days to insure complete infiltration.

4) Sectioning - A rotary microtome was used to produce 10 µm sections.

5) Staining - Iodine stain - Paraffin was removed from the sections and the tissue partially rehydrated. The IKI solution was applied for 1-3

hours.
Millons reaction - As before but stained in Millons reagent at 50° C for 40 min.
Orcinol - As before but stained in 10 % orcinol in concentrated HCl for 30 min. at room temperature.

As well gathering information on the cellular structure of rapeseed with the SEM, histochemical studies were undertaken that were directed at locating myrosinase, the enzyme responsible for the release in rapeseed meal of isothiocyanate and oxazolidinethione compounds which have been shown to be toxic. Older literature describes the myrosinase containing cells as "having grains without globoids, the whole cell being more refractile than adjoining cells". A more precise knowledge of the site of myrosinase is obviously needed;

- Original magnification X256, stained with orcinol. Orcinol is specific for hexoses and pentoses and will react with glucose, a reaction product of glucosinolates and myrosinase. This stain has been reported to identify myrosinase in idioblasts of cabbage. The cells that take up the stain are generally close to the periphery of the embryo and appear larger than adjacent ones.

- Original magnification X1000, stained as above. A higher magnification showing that the stain is absorbed by the protein bodies.

- Original magnification X160, stained with Millons reagent. Millons reagent is specific for tyrosine and tryptophan containing compounds, i.e., proteins, and it has been reported that myrosin cells preferentially take up this stain although all proteins will react to some degree. Again differential staining is observed with these preparations in the same area as seen previously. Higher magnifications reveal that while all protein bodies are stained, certain cells appear to give a stronger reaction with this stain.

- Original magnification X160, stained with iodine. Iodine is a specific histochemical test for polysaccharides. With this stain nonpreferential staining is seen. Although this may seem to contradict the previous results, the method used was taken from a 1906 reference in which the technique was not fully described and the mechanism by which myrosin cells should differentially take up iodine was not made clear.

From the above structural data we conclude that rapeseed is a much more complex source of protein than commonly thought and more research is needed to further elucidate the relation of seed microstructure to behaviour during dehulling and oil extraction procedures. For example, dehulling may produce cell injury leading to myrosinase activity. We tentatively conclude that myrosinase may be differentially distributed in the rapeseed embryo. Confirmation of this finding could have important implications in processing and detoxification steps.

The authors would like to acknowledge the financial support and continuing encouragement given to this project by the Canada Department of Industry,

Trade and Commerce. Also we are grateful for the advice of Dr. R. L. Peterson, Department of Botany and Genetics, University of Guelph, and Dr. John Lott, Dept. of Biology, McMaster University.

Reference

MOLLENHAUER and TOTTEN (1971):
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