PROCESSING EFFECTS ON THE STRUCTURAL AND MICROCHEMICAL ORGANIZATION OF RAPESEED AND ITS PRODUCTS

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ABSTRACT: Rapeseed (Canola) samples from different processing stages were collected from a commercial crushing plant and studied by fluorescence microscopy. Frozen or glycol methacrylate-embedded sections were stained with a variety of fluorochromes with known specificities and examined microscopically. Results were based on known characteristics of the fluorescent markers, induced fluorescence and autofluorescence. The effect of processing on the structural and microchemical organization of rapeseed and its products were studied by examining samples collected after different processing procedures: (A) whole seeds, (B) flakes, (C) cakes, (D) meals after solvent extraction, (E) meals after desolventization, and (F) meals at storage. Cell walls were fractured though not completely destroyed due to the effect of mechanical crushing. After cooking, individual protein bodies fused to form one single mass still encompassing phytin-containing globoids and storage lipids (oil droplets coalesced into larger bodies). Most of the oil was removed after solvent extraction and was absent inside of the cotyledon after desolventization. The final product, meal, contained mostly hull and cotyledon fragments. The cotyledon fragments consisted of an amorphous protein matrix embedded with phytin globoids and supported by a network of broken cell walls. Structural and chemical components of the hull, however, remained unchanged after processing. Phenolic compounds, lignin, cell wall polysaccharides, chlorophyll, storage proteins and lipids were all detected by various fluorescent stainings. The ratio of hull to cotyledon fragments was roughly 1:2 based on microscopic examinations. The efficiency of processing was revealed by the presence of fragments having intact cotyledonary and aleurone cells full of oil and protein bodies.

INTRODUCTION: In rapeseeed processing, most quality control studies focussed on the chemical aspects of rapeseed oil and meal (Rutkowski 1970; Ohlson 1976; Sosulski and Zadernowski 1981). Processing effects on structural changes on the cellular level were also studied but to a lesser extent by means of electron microscopy (Hofsten 1974; Stanley *et al* 1976; Mills and Chong 1977; Smith 1979). Recently the technique of fluorescence microscopy has been adapted for analyzing the structural and microchemical organizations of cereal grains (Fulcher and Wong 1980) and rapeseed (Poon *et al* 1980; Yiu *et al* 1982). This method, being sensitive and simple, is most suitable for rapid screening of seed samples and related products. The present study investigated the effects of processing on the storage reserves of rapeseed and its products using the above technique.

The objectives of the project were: 1. To study the degree of cell wall rupture during crushing and flaking of different Canola products. 2. To investigate the effects of processing on the extractability of some of the chemical constituents including proteins, chlorophyll, oil, mucilage, phytate and phenolic compounds. 3. To test quality consistency of Canola products.

EXPERIMENTAL METHODS: Frozen (8-10 μm thick) and glycol methacrylate (GMA)-embedded (1-5 μm thick) sections of the samples were prepared, stained with different fluorescent reagents and examined microscopically. Storage proteins, lipids, phytin globoids, and cell wall polysaccharides were stained according to Fulcher and Wong (1980) and Yiu *et al* (1982). Phenolics and chlorophyll were detected by their autofluorescent characteristics under short and long wavelength excitation, respectively. Sections were examined with a Zeiss Universal Research Microscope equipped with a III RS epi-illumination condenser combined with a fluorescence system as described earlier (Yiu *et al* 1982). Photomicrographs were obtained using 35 mm Kodak Tri-X pan film, (ASA 400).

RESULTS AND DISCUSSION: Rapeseed samples were collected at different stages from four different runs between October and November, 1981 (Fig. 1).

A. Whole Seed: The whole seed samples collected from each processing run were used as a control for subsequent studies. Detailed fluorescence microscopic studies of the various structural and microchemical constituents of rapeseed were described previously (Poon *et al* 1980; Yiu *et al* 1982). Most of the Canola seeds collected for the present study came from dark seed-coated, unidentified varieties.

B. Flake: Transverse fractures of the cell wall were seen in many cotyledonary cells with protein bodies from one cell protruding into the cavity of the other (Fig. 2). Minute fluorescent fragments demonstrable by staining with Calcofluor white (or Congo Red) were detected within these cells (Fig. 2). Since they were not normally present in intact cells, these carbohydrate containing fragments could possibly derive from the broken cell walls. Oil bodies were no

longer confined within the cell. Instead, they were seen both at the interior and exterior of the cell due to the fractured cell wall. Phytin globoids inside each protein body were clearly visible after staining with Acriflavine-HCl. (Results not shown).

C. Cake: The effect of cooking on oil bodies was clearly demonstrated by the emergence of large bodies within the cells (Fig. 4). Cooking not only coalesces the oil into larger droplets but also makes the cell walls permeable to oil. The decreased intensity of fluorescence after staining with Nile Blue A indicated that part of the oil had been removed. Additional fractures of the cell wall were observed. Some of the walls were channeled (Fig. 3) indicating that cooking might have dissolved some of the cell wall components. Although phytin globoids remained intact within the storage proteins, individual protein bodies were no longer recognizable. They fused together to form single masses (Fig. 5). The intensity of fluorescence after staining was reduced indicating that the effect of cooking might have altered the affinity between certain functional groups of the protein and the fluorescent reagents (1-anilino-8-naphthaline sulphonic acid (ANS) and Acid Fuchsin).

D. Meal after Solvent Extraction: The meal was mostly devoid of oil droplets. Occasionally, a small amount of oil could be seen trapped between cells. Additional fracture of the cell wall was observed leaving behind a matrix composed mainly of protein masses still containing phytin globoids.

E. Meal after Desolventization: The only oil present in the meal was seen within the aleurone cell layer of the endosperm which remained attached to the hull after flaking. The structural components of the hull remained relatively unchanged. Storage proteins of the cotyledon fragments were further compressed to form a protein matrix embedded with intact phytin globoids (Fig. 6).

F. Meal at Storage: The meal at storage contained mostly hull fragments mixed with cotyledon pieces at a ratio of 1:2. In general, storage lipids were no longer detected within the cell except in fragments containing the aleurone cell layer. Most of the seed coat layers and walls of the aleurone cells remained intact after processing. The autofluorescent characteristic of some of the seed coat layers (Yiu *et al* 1982) was also retained indicating the presence of phenolic compounds (Fig. 7). Red autofluorescent bodies could be detected within cells of the aleurone layer demonstrating the presence of chloroplasts . Normally, chlorophyll was found in cells of the aleurone layer and the cotyledon of green rapeseeds. The absence of chlorophyll in the cotyledon fragments of rapeseed meal suggested that either very few green seeds were processed for these particular runs or it had been removed by processing. The removal of chlorophyll was closely related to the structure of the cell wall. Hence, the most likely location where chloroplasts could be detected in the meal would be inside cells having intact walls,

i.e. those of the aleurone layer.

CONCLUSION: Mechanical crushing induced fractures in the cell wall facilitating subsequent oil extraction procedures. The effect of cooking had altered the structures of oil and protein bodies. Solvent extraction had no effect on the extractability of proteins, phytin, cell wall carbohydrates, lignin and other phenolics. The components of the hull remained unscathed. The persistence of the structural components contributes to the hull's high fibre content. Development of new processing techniques, either mechanical or chemical is necessary to obtain a Canola meal of better palatability and higher nutritive values.

The efficiency of processing could often be revealed by the structural and chemical composition of the meal fragments. Cells with intact walls encompassing their contents were still present in some fragments. Most noticeable, some cotyledonary cells and most of the aleurone cell layer still contained oil and protein bodies. The ratio of intact cells to broken cells estimated in the meal samples was not high but could be sufficient enough to cause a lower yield of Canola oil and a reduced bioavailability of certain nutrients. Microscopic examination of the meal fragments on a continual basis would help to assess processing efficiency, leading to better quality of Canola products.

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Seed Storage → Cleaning (A) → Crushing Rolls (B) → Cooker → Expeller (C) → Flaking Rolls → Solvent Extractor (D) → Desolventizer (E) → Meal Cooler → Storage (F)

Fig. 1. Scheme of rapeseed meal processing (Clandinin, 1981) showing stages from which samples (A) → (F) were collected.

Fig. 2. Rapeseed flake section stained with ANS and Calcofluor White demonstrating fractured cell walls with protein bodies (*) protruding from one cell into another and fluorescent cell wall fragments (arrows). X 1120.

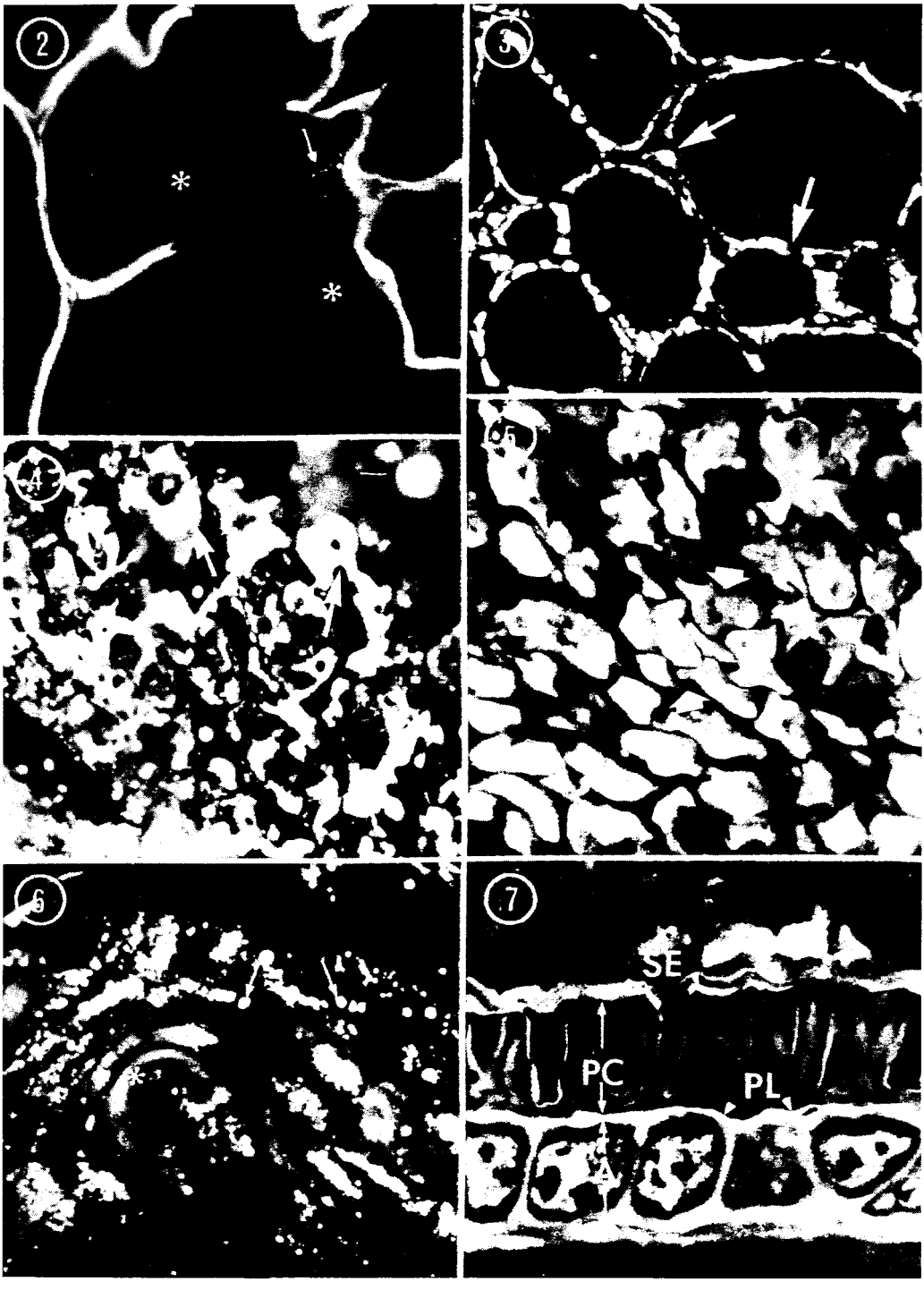
Fig. 3. Calcofluor White-stained rapeseed cake section demonstrating the presence of channeled cells walls (arrows). X 630.

Fig. 4. Rapeseed cake section stained with Nile Blue A showing large oil bodies (arrows). X 840.

Fig. 5. Rapeseed cake section stained with ANS to show fused protein masses (arrows). X 798.

Fig. 6. Acriflavine-HCl stained rapeseed meal section demonstrating the presence of phytin globoids (arrows) within the protein matrix. X 828.

Fig. 7. Unstained rapeseed meal section showing the presence of autofluorescent sub-epidermis (SE) and pigment layer (PL) as well as the palisade (PC) and aleurone (A) cells. X 642.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