Hydrolysis of three canola cultivars with carbohydrate reduced oil extraction time and increased oil yield. The optimum pretreatment before hexane extraction of oil was flaking, autoclaving, adjustment to 30% seed moisture including 0.12% enzyme concentration (g enzyme protein/100 g flakes), and incubation for 12 hr at 50 C, followed by drying to 4% moisture. Hexane extraction was enhanced by grinding the flakes. The relative order of enzyme efficiency in enhancement of oil extraction was mixed activity enzyme > β-glucanase > pectinase > hemicellulase > cellulase.

Mechanisms for the rupture of cell walls usually are used as preliminary steps prior to prepress or direct solvent extraction of oil-bearing plant materials. Rupture of cell walls is achieved, in part, by crushing and flaking the seeds between single- or multiple-stage rollers (1,2). The conditioning of oilseeds in multiple stage cookers also renders the cell walls permeable to the coalesced, fluid oil droplets. Modified shaft arrangements including an elongated, notched worm and restrictive orifices at critical points in the prepress barrel have been used to masticate the seed mass when uncrushed cold seeds are fed directly to the prepress (3). The necessity for complete rupture of cell walls in canola to facilitate rapid solvent extraction of oil has been demonstrated by Diosady et al. (4) using the Szego mill.

Fullbrook (5) used proteolytic and cell-wall degrading enzymes to release protein and oil in finely-ground slurry of melonseeds, soybean and rapeseed. Addition of hexane to the aqueous slurry enhanced oil extractability up to a maximum of 2 to 3% enzyme concentration (w/w basis), at which 90% of soybean oil was extracted in the organic phase. Although greater oil yields were achieved with rapeseed, the hexane-water:seed meal extraction system separated a maximum of 70 to 72% of the Soxhlet-extractable oil.

The objective of the present investigation was to develop the optimal conditions for enzymatic hydrolysis of cell walls in canola seeds for enhanced oil recovery during subsequent oil extraction. This initial study was concerned with hydrolytic enzyme pretreatments to hexane extraction; in a following paper, the effects of enzyme pretreatments to full press expelling are described.

MATERIALS AND METHODS

Materials. Seed of spring-sown Brassica napus (cultivars, Regent and Westar) and yellow-seeded B. campestris (cultivar, Tobin) were provided by CSP Foods Ltd. and the Department of Crop Science and Plant Ecology, Saskatoon, SK. All cultivars were of the canola type, having low erucic acid and glucosinolate contents.

During preliminary investigations not reported here, over 40 crude enzymes, primarily carbohydrases, supplied by commercial manufacturers were evaluated for their abilities to hydrolyze one or another of the above cultivars and to enhance oil extraction. The most effective crude enzyme preparations of four classes of carbohydrase enzymes, and a mixed enzyme preparation, were selected for comparative evaluation and optimization in the present study. Cellulase (Celluclast 150L), β-glucanases (Finizym and Novozym 280), Pectinase (Pectinex Ultra SP) and mixed activity enzyme (SP-249) were provided by Novo Industri A/S, Bagsvaerd, Denmark, and their Canadian representative, Van Waters and Rogers Ltd., Lachine, PQ. The hemicellulase (Enzeco Hemicellulase) was obtained from Enzyme Development Co., New York.

In initial experiments, enzymes were purified on a Pelicon Ultra-filtration Cassette System (Millipore Corp., Bedford, Massachusetts) fitted with 10,000 mw cut-off point membrane. Diafiltration was conducted at constant volume in order to maintain the enzyme concentration.

Analyses. Proximate analyses of the canola samples were conducted by AACC (6) procedures using a Soxhlet apparatus for lipid extraction and the N × 6.25 conversion factor for protein content. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (7). Hemicellulose content was calculated as the difference between NDF and ADF. Cellulose content was taken as the difference between ADF and ADL.

Cellulase activity was determined by the filter paper assay of Mandels and Weber (8). One international unit of activity was defined as one μmol glucose released/min/ml. All determinations were carried out in duplicate.

Treatments. In the initial experiments, canola cultivars were flaked between cold rolls spaced at 0.8 mm to ensure 99% seed coat rupture but not to the thinness of commercial flakes (<0.15 mm). The flakes were autoclaved at 120 C for one min to inactivate myrosinase and other enzymes (4 min total time in autoclave). The first study involved adjustment of Westar samples to 20, 30, 40 and 50% moisture and incubation for 12 hr at 50 C with purified SP-249 enzyme at 0.12, 0.25 and 0.50 g enzyme protein/100 g flakes. In the second experiment, Westar flakes were adjusted to 30% moisture and incubated for 12 hr at 50 C with pure purified Novozym 280 protein/100 g flakes. In the third experiment, Westar, Regent and Tobin flakes were incubated for 0, 3, 6
and 12 hr with 0.12% SP-249 at 50 C in comparison 
with an incubated control.

After each of the above incubations, the hydrolyzed 
flakes were dried to 4% moisture at 70 C in an air 
oven. Dried samples were defatted with hexane (bp, 
60 C) on a Soxhlet apparatus for seven hr, less than 
half the recommended extraction period of 16 hr at 
2-3 drops/sec (6), in order to differentiate the effects of 
the various treatments.

In the principal investigation, autoclaved seed of 
the three cultivars was incubated with each crude 
enzyme at a concentration of 0.12 g enzyme protein/
100 g seed and treatment time of 12 or 6 hr at 50 C. 
Hydrolyses were conducted for 12 hr on intact seeds 
which were then dried and, without grinding, extract-
ated with hexane for seven hr. In a second series of 
experiments, the above hydrolyzed seeds were ground 
to 40 mesh in a coffee grinder before oil extraction 
with hexane for seven hr on the Soxhlet extractor, in 
contrast with untreated controls extracted for 7 and 
14 hr. A third series of treatments involved hydroly-
sis of flaked seeds for only six hr and direct extrac-
tion of oil from the hydrolyzed flakes.

In a final study, Regent was flaked, autoclaved, 
adjusted to 30% moisture and hydrolyzed with 0.1% 
SP-249 enzyme for 12 hr at 50 C. After drying, the 
flakes were extracted with hexane directly and after 
grinding. Oil extraction was conducted on a Gold-
fisch fat extractor for the recommended four hr (6) 
and for two hr to assess oil extraction rates based on 
this rapid solvent extractor (5-6 drops solvent/sec).

All enzyme treatments were carried out in duplic-
ate.

RESULTS AND DISCUSSION

Enzymes. The suppliers of four enzymes used in this 
study indicate that the optimum activity occurred at 
60 C (Table 1) but, as demonstrated by Boyce (9), the 
enzyme stability would be greater at lower tempera-
ture. Thus, 50 C was selected as a more functional 
temperature for the 12-hr hydrolytic treatments used 
in this study.

Each of the commercial enzymes was characterized 
as having a specific activity, but most can be demon-
strated to act as general carboxydrases. For example, 
the six enzymes were assayed for cellulase activity 
and the range of values was from 6.4 to 85.2 IU/ml, 
with no correction being made for protein concentra-
tion in the preparations (Table 1). While Cellulact 
150L exhibited the highest cellulase activity, it was of 
interest that, among the β-glucanases, Finizyn 
showed little cellulase activity but Novozym 280 had 
over half the activity in Cellulact 150L. In effect, 
each enzyme preparation was likely a mixed activity 
carbohydrase with a range of temperature optima.

The pH of ground canola dispersed in water and 
equilibrated for 30 min was 5.65, within the range of 
pH optima for the enzymes used in the study (Table 
1).

Due to the wide range in protein concentrations 
among the preparations (Table 1), enzymes were 
compared on the basis of the protein concentrations, 
rather than on a volume or weight basis. Previous 
investigations were conducted using 2-3% enzyme 
concentration (w/w basis) (5), whereas the enzyme 
solution of SP-249 was 12.5% (v/w) or 15.2% (w/w 
basis) for the 0.12 g enzyme protein/100 g seed treat-
ment used in the present investigation.

Initially, the crude enzyme preparations were dia-
filtered as a purification step to enhance hydrolytic 
activity. It was found that crude enzymes were as 
effective as the purified enzymes in hydrolysis of 
canola seeds, and so the diafiltration procedure was 
discontinued in later experiments.

Seed composition. Among the spring-sown canola 
cultivars grown in Western Canada, Brassica napus 
cultivars contain more oil than B. campestris, even 
though the latter cultivars have thinner seed coats 
associated with their yellow seed color (10). Westar is 
characteristically higher in oil, whereas Regent con-
tains more meal protein, and the samples used in this

<table>
<thead>
<tr>
<th>Class of carbohydrate</th>
<th>Common name</th>
<th>Source</th>
<th>pH Optimum units</th>
<th>Temperature optimum (°C)</th>
<th>Protein concentration (mg/ml)</th>
<th>Cellulase activity (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulase</td>
<td>Celluclast 150L</td>
<td><em>Trichoderma reeseis</em></td>
<td>5.5</td>
<td>55-60</td>
<td>10.7</td>
<td>85.2</td>
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<tr>
<td>β-Glucanase</td>
<td>Finizym</td>
<td><em>Aspergillus niger</em></td>
<td>5.0</td>
<td>60</td>
<td>5.6</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Novozym 280</td>
<td><em>Aspergillus niger</em></td>
<td>4.5-5.0</td>
<td>60</td>
<td>18.7</td>
<td>45.2</td>
</tr>
<tr>
<td>Hemicellulase</td>
<td>Enzeco</td>
<td><em>Aspergillus niger</em></td>
<td>3.0-6.0</td>
<td>25-60</td>
<td>16.7</td>
<td>17.2</td>
</tr>
<tr>
<td>Pectinase</td>
<td>Pectinex</td>
<td><em>Aspergillus niger</em></td>
<td>5.0-6.0</td>
<td>40</td>
<td>9.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Mixed activitya</td>
<td>SP249</td>
<td><em>Aspergillus niger</em></td>
<td>4.0-5.0</td>
<td>30-60</td>
<td>9.6</td>
<td>14.8</td>
</tr>
</tbody>
</table>

*aCellulase, β-glucanase, hemicellulase, pectinase, cellobiase, arabanase, xylanase, α-galactosidase, 
protease activity.

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