# Carbohydrase Hydrolysis of Canola to Enhance Oil Extraction with Hexane[1]

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**Hydrolysis of three canola cultivars with carbohydrase 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 slurries 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 0.0625, 0.125, 0.250 and 0.500 g of purified Celluclast, Finizym, Pectinex, Enzeco and Novozym protein/100 g flakes. In the third experiment, Westar, Regent and Tobin flakes were incubated for 0, 3, 6

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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, extracted 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 hydrolysis of flaked seeds for only six hr and direct extraction 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 Goldfisch 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 duplicate.

## 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 temperature. 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 demonstrated to act as general carbohydrases. 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 concentration in the preparations (Table 1). While Celluclast 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 Celluclast 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 treatment used in the present investigation.

Initially, the crude enzyme preparations were diafiltered 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 contains more meal protein, and the samples used in this

**TABLE 1**

**Characteristics of Crude Carbohydrase Enzymes from Commercial Sources Used in the Study**

| Class of carbohydrase | Common name | Source | pH Optimum units | Temperature optimum (° C) | Protein concentration (mg/ml) | Cellulase activity (IU/ml) |
|---|---|---|---|---|---|---|
| Cellulase | Celluclast 150L | *Trichoderma reesie* | 5.5 | 55-60 | 10.7 | 85.2 |
| β-Glucanase | Finizym | *Aspergillus niger* | 5.0 | 60 | 5.6 | 6.4 |
| | Novozym 280 | *niger* | 4.5-5.0 | 60 | 16.7 | 45.2 |
| Hemicellulase | Enzeco | *niger* | 3.0-6.0 | 25-60 | 16.7 | 17.2 |
| Pectinase | Pectinex Ultra SP | *niger* | 5.0-6.0 | 40 | 9.8 | 16.7 |
| Mixed activity[a] | SP249 | *niger* | 4.0-5.0 | 30-60 | 9.6 | 14.8 |

[a]Cellulase, β-glucanase, hemicellulase, pectinase, cellobiase, arabanase, xylanase, α-galactosidase, protease activity.

**TABLE 2**

**Chemical Composition of Canola Cultivars of *Brassica napus* and *campestris*, % Dry Basis**

| Seed and meal constituents | *B. napus* | | *B. campestris* |
|---|---|---|---|
| | Westar | Regent | Tobin |
| Oil | 43.1 | 42.7 | 42.2 |
| Meal-protein | 42.7 | 44.5 | 43.2 |
| Meal-NDF | 24.3 | 25.5 | 24.1 |
| Meal-ADF | 17.9 | 20.0 | 17.7 |
| Meal-ADL | 10.7 | 9.8 | 5.6 |
| Meal-hemicellulose | 6.4 | 5.5 | 6.4 |
| Meal-cellulose | 7.2 | 10.2 | 12.1 |
| Meal-ash | 5.9 | 4.0 | 4.1 |

study reflected these differences (Table 2). Regent also contained more of the fiber components, NDF and ADF, while Westar had the most ADL. The Tobin sample was characterized by a high content of cellulose and hemicellulose but the least lignin.

*Enzyme and moisture concentrations.* After incubation with SP-249 for 12 hr, about 42% oil was extracted from Westar flakes within seven hr on the Soxhlet apparatus at seed moisture levels of 30 to 50%; the extractability decreased at 20% seed moisture (Table 3). Therefore, seed moisture during hydrolysis, including that provided in the enzyme slurry, was maintained at 30%, although more detailed study may show that even less moisture may be adequate for enzyme activity. For example, at 20% seed moisture, oil extractability was enhanced by increasing enzyme concentration whereas, at higher seed mois-

**TABLE 3**

**Influence of Seed Moisture Level and SP-249 Enzyme Concentration on Oil Extractability from Westar Flakes, % Dry Basis[a]**

| Seed moisture level | Enzyme concentration | Oil extracted | Average oil extracted |
|---|---|---|---|
| 20 | 0.12 | 36.8 ±0.93[b] | |
| | 0.25 | 38.8 ±1.15 | |
| | 0.50 | 39.0 ±1.38 | 38.2 |
| 30 | 0.12 | 41.9 ±0.51 | |
| | 0.25 | 42.7 ±0.84 | |
| | 0.50 | 41.0 ±0.45 | 41.9 |
| 40 | 0.12 | 41.7 ±0.73 | |
| | 0.25 | 42.0 ±0.49 | |
| | 0.50 | 40.9 ±0.99 | 41.5 |
| 50 | 0.12 | 41.1 ±1.05 | |
| | 0.25 | 42.2 ±0.57 | |
| | 0.50 | 41.8 ±0.93 | 42.0 |

[a]Seeds were flaked before moisture adjustment and addition of enzyme slurry for 12 hr hydrolysis at 50 C followed by drying and hexane extraction for 7 hr.
[b]Standard deviation, n = 2.

**TABLE 4**

**Influence of Enzyme Concentration (g enzyme protein /100 g seed) on Oil Extractability of Regent Flakes, % Dry Basis[a]**

| Enzyme concentration | Celluclast | Finizym | Pectinex | Enzeco | Novozym | Average |
|---|---|---|---|---|---|---|
| 0.0625 | 33.4 ±1.26[b] | 40.2 ±1.80 | 37.5 ±0.74 | 33.2 ±1.44 | 38.4 ±1.55 | 36.5 |
| 0.125 | 34.2 ±0.50 | 39.1 ±0.89 | 40.7 ±1.34 | 39.7 ±1.47 | 41.7 ±0.78 | 39.1 |
| 0.250 | 39.3 ±0.74 | 38.7 ±0.80 | 37.8 ±0.93 | 41.7 ±1.81 | 42.2 ±1.27 | 39.9 |
| 0.500 | 25.6 ±1.75 | 35.5 ±2.29 | 38.0 ±1.05 | 38.1 ±1.47 | 40.3 ±2.17 | 35.5 |

[a]Flakes were adjusted to 30% moisture and hydrolyses were conducted for 12 hr at 50 C before drying and hexane extraction for 7 hr.
[b]Standard deviation, n = 2.

**TABLE 5**

**Effect of Hydrolysis Time at 50 C by SP-249 on Oil Extractability of Canola Flakes[a], % Dry Basis**

| Treatment | Hydrolysis time (hr) | Canola cultivar | | | Average |
|---|---|---|---|---|---|
| | | Westar | Regent | Tobin | |
| Control | 0 | 27.8 ±0.59[c] | 25.3 ±0.42 | 22.7 ±0.43 | 25.3 |
| Treated | 3 | 32.9 ±1.40 | 27.9 ±1.19 | 20.2 ±1.26 | 27.0 |
| Treated | 6 | 42.3 ±1.32 | 41.2 ±0.71 | 40.8 ±1.09 | 41.4 |
| Treated | 12 | 46.6 ±1.68 | 45.2 ±0.95 | 42.1 ±1.23 | 44.6 |
| Control[b] | 12 | 32.9 ±1.47 | 31.5 ±0.99 | 20.9 ±0.57 | 28.4 |

[a]At 30% moisture, 0.12% enzyme concentration, hexane extraction time of 7 hr.
[b]Incubated in water without enzyme.
[c]Standard deviation, n = 2.

ture levels, the 0.12% enzyme concentration of SP-249 appeared adequate for maximum oil extractability. Considering that the cost of enzyme and subsequent seed drying would be the major expense in the enzyme pretreatment, the conditions of moisture and enzyme concentrations should be minimized to enhance the economic feasibility of the process.

At the 30% moisture level, the five enzymes with specific activities were evaluated at concentrations of 0.0625 to 0.500% on Regent flakes (Table 4). Cellulase, Enzeco and Novozym performed best at 0.250% concentration, whereas Finizym showed the highest activity at 0.0625%. On the average, the 0.125% concentrations of the five enzymes resulted in 39.1% oil recovery after oil extraction with hexane for seven hr, which was nearly as effective as 0.250 g enzyme protein/100 g flakes. However, it was apparent that the optimum concentration differed widely from enzyme to enzyme, and that excess enzyme was as detrimental as inadequate concentration, possibly due to lack of available substrate. The optimum would also depend on seed moisture (Table 3), temperature, presence of inhibitors, etc., and these conditions would need to be carefully evaluated for any enzyme system adopted for commercial use. Since 0.125% enzyme was satisfactory for all enzymes tested here, except Celluclast, this concentration was adopted for the remainder of the study.

*Hydrolysis time.* Duration of hydrolysis was assessed on each cultivar using incubation times of 3, 6 and 12 hr (Table 5). When untreated flakes were extracted for seven hr on the Soxhlet apparatus, only one-half of the seed oil was extracted, with Westar showing greater ease of extraction than Tobin in particular. Hydrolysis with SP-249 for three hr improved oil extractability only marginally in two cultivars, but six hr of incubation with the enzyme brought oil yields to over 40% in each cultivar. Incubation and hydrolysis for 12 hr brought oil yields to over 45% in Westar and Regent flakes. A control sample of each cultivar was incubated in water for 12 hr to show that the soaking and drying operation alone enhances oil extractability to a limited degree.

All of the enzymes were then tested on each cultivar to assess the effects of treating intact seed, with and without post-incubation grinding, as compared to hydrolysis and extraction of flaked seed (Table 6). Control samples of each cultivar, after autoclaving and incubation at 30% moisture at 50 C for 12 hr, were extracted, without grinding, for 14 hr on the Soxhlet apparatus. Almost no oil was released from intact seeds, but grinding to 40-mesh before oil extraction permitted a fairly complete oil extraction of each cultivar, the average yield being 42.7%. Extracting the ground samples for only seven hr yielded an average of 37.2% oil. Solvent extraction of flaked seed after incubation for six hr yielded only about 22.3% of oil from the three cultivars.

The enzyme treatments for the intact seed hydrolysis were of 12-hr duration and release of oil subsequently by hexane extraction was minimal, i.e. about 2% oil yield from Westar and Regent. Tobin showed much higher yields, an average of 7%, in contrast to its poorer comparative performance when flaked

**TABLE 6**

**Effects of Type of Enzyme on Oil Extractability of Intact or Flaked Seeds With and Without Grinding before Oil Extraction, % Dry Basis**

| Seed treatment enzyme | Hydrolysis time[a] (hr) | Oil extraction time (hr) | Intact seed hydrolysis and extraction | | | | Intact seed hydrolysis and ground seed extraction | | | | Flaked seed hydrolysis and extraction | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Westar | Regent | Tobin | Average | Westar | Regent | Tobin | Average | Westar | Regent | Tobin | Average |
| Controls | —[b] | 14 | 0.1±0.03[d] | 0.1±0.03 | 0.3±0.03 | 0.2 | 43.1±0.40 | 42.7±0.25 | 42.2±0.30 | 42.3 | 23.1±0.71 | 22.8±0.90 | 20.9±0.53 | 22.3 |
| | — | 7 | — | — | — | — | 37.7±0.49 | 37.1±0.70 | 36.9±0.57 | 37.2 | — | — | — | — |
| Celluclast | 12[c] | 7 | 1.9±0.08 | 0.9±0.08 | 5.8±0.13 | 2.9 | 38.6±1.05 | 35.1±1.47 | 39.5±1.66 | 37.7 | 38.3±1.29 | 32.8±0.99 | 32.3±1.33 | 34.5 |
| Finizym | 12 | 7 | 2.0±0.15 | 1.2±0.04 | 6.3±0.13 | 3.2 | 38.2±1.32 | 36.9±1.63 | 37.4±1.44 | 37.5 | 40.9±2.13 | 39.2±2.15 | 38.7±1.69 | 39.6 |
| Pectinex | 12 | 7 | 1.9±0.02 | 2.1±0.11 | 7.2±0.13 | 3.7 | 40.9±2.03 | 37.9±1.88 | 39.2±1.98 | 39.3 | 39.2±3.18 | 40.9±1.70 | 38.9±2.03 | 39.7 |
| Enzeco | 12 | 7 | 1.1±0.09 | 1.3±0.06 | 5.3±0.14 | 2.6 | 39.9±1.93 | 40.3±2.00 | 40.3±2.57 | 40.2 | 39.5±2.21 | 39.2±1.59 | 39.7±1.81 | 39.5 |
| Novozym | 12 | 7 | 2.1±0.05 | 2.1±0.14 | 6.6±0.14 | 3.6 | 40.2±1.75 | 42.9±1.91 | 43.8±1.49 | 42.3 | 38.8±1.98 | 39.3±1.81 | 37.8±1.99 | 38.6 |
| SP-249 | 12 | 7 | 2.9±0.11 | 3.7±0.14 | 10.4±0.16 | 5.7 | 43.1±2.02 | 43.3±1.43 | 42.2±2.48 | 42.8 | 42.3±2.12 | 41.2±1.00 | 40.8±1.19 | 41.4 |
| Enzyme average | — | — | 2.0 | 1.9 | 6.9 | | 40.2 | 39.4 | 40.4 | | 39.8 | 38.8 | 38.0 | |

[a]At seed moisture of 30%, enzyme concentration of 0.12%, for 12 hr at 50 C.
[b]Controls were treated to autoclaving and 12 hr at 30% moisture without enzyme.
[c]Flaked seed hydrolysis was for 6 hr.
[d]Standard deviation, n = 2.

(Table 5). This different response was attributed to the thinner seed coat and lower composition of lignin in the seed coat and, possibly, other seed tissues. Another result of this experiment was the clear superiority of SP-249 among the various classes of enzymes. The mixed activity enzyme released about 50% more oil than the more specific enzyme sources.

Grinding the hydrolyzed seeds before oil extraction released about 40% oil from each cultivar (Table 6). Celluclast and Finizym treatments resulted in an average yield of 37.6% oil from the three cultivars, whereas Novozym and SP-249 treatments yielded ca. 42.5% oil. The latter yield was fully comparable to the control sample extracted for 14 hr rather than the seven hr used for the enzyme treatments.

Based on the results in Table 6 it was apparent that hydrolysis and extraction of flaked seed would give greater yields of oil than the intact seed procedure. Therefore, flakes were incubated for only six hr in order to observe, more critically, the differences between enzymes. However, average oil yields were still high, varying from 40% for Westar to 38% for Tobin. Under these conditions, Celluclast performed relatively poorly but Finizym, Pectinex and Enzeco appeared superior to Novozym. Again, SP-249 was clearly the most suitable enzyme for hydrolysis of canola cultivars prior to hexane extraction.

The final experiment was designed to combine the optimum treatments apparent from the previous experiment but using the more rapid Goldfisch oil extractor (Table 7). The maximum oil extracted from the control flakes was 40.1%, whereas enzyme treatment increased oil extractability to 41.7% in two hr and 43.2% after four hr extraction. Grinding the flakes increased oil extractability to 44.6 and 45.9%, respectively, which clearly demonstrated the efficacy of hydrolytic enzymes to release the total lipids in canola.

Evaluations of the quality of oil and meal from these treatments are underway.

**TABLE 7**

**Oil Extractability of Westar Flakes after Enzyme Hydrolysis with 0.12% SP-249 for 12 hr at 50 C, % Dry Basis**

| Treatment of flaked seeds before oil extraction | Goldfisch extraction time | |
|---|---|---|
| | 2 hr | 4 hr |
| Control flakes incubated, dried, ground | 39.9 ±0.28[a] | 40.1 ±0.21 |
| Enzyme-treated flakes dried | 41.7 ±1.13 | 43.2 ±0.56 |
| Enzyme-treated flakes dried, ground | 44.6 ±0.56 | 45.9 ±1.83 |

[a]Standard deviation, n = 2.

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