Enzyme-Aided *vs.* Two-Stage Processing of Canola: Technology, Product Quality and Cost Evaluation

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The investigation focussed on the use of carbohydrase enzymes to enhance oil extraction during pressing in a laboratory expeller. Enzyme-treated seeds at 6% moisture were pressed in the expeller set at full-press conditions. Control seeds were pressed at wider choke openings but at the same barrel pressures as enzyme-treated samples. Time of pressing and temperature and pressure inside the expeller barrel were used to calculate throughput and energy requirements per unit weight of processed material. Treatment with enzymes improved throughput of the expeller, increased oil flow rate and oil recovery. Material throughput was increased by 30–50%, depending on canola variety. The recovery of the oil was increased from 72% of the seed oil for control samples, to 90–93% for enzyme-treated samples. The average residual oil content in presscakes from enzyme-treated seeds was 7.4%. The oil quality was inferior to cold-pressed control but was much better than has been reported for solvent-extracted oil.

KEY WORDS: Canola processing, enzymes in canola processing, oil and meal quality, process cost.

Canola, like many other high oil content oilseeds, is primarily processed for its oil, and the commercial crushing process, pre-pressing and solvent extraction of residual oil from presscake, has been designed to maximize oil yield. The energy requirement of this process is high (1). The losses of hexane during extraction and subsequent solvent recovery from oil and meal can be 3 L/ton of processed seeds (2). The quality of oil recovered by solvent extraction is lower than that of pressed oil (3–6). In addition, canola meal tends to retain nearly twice the level of residual hexane found in soybean meal after treatment under similar desolventization conditions. Thus, more heat must be applied to remove the residual hexane from the meal, increasing the overall energy requirements of the process. Meal toasted for a prolonged time at high temperature has both a dark color and low nutrient availability.

A technique was recently developed for hydrolysis of canola seeds with carbohydrase enzymes prior to solvent extraction (7) or pressing in the expeller (8). Enzymatic pretreatment of canola, prior to pressing in the expeller, reduced the time of pressing but increased oil flow rate and oil recovery. The low level of residual oil in the presscakes might render further solvent extraction uneconomical.

The present paper summarizes the results from expeller pressing of enzyme-treated canola and rapeseed. The quality of oil and meal was evaluated. Also, a comparison was made in energy use for the commercial and enzyme-aided process.

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MATERIALS AMD METHODS

Materials. Commercial samples of pedigreed canola seeds, cultivars Westar *(Brassica napus)* and Tobin *(B. campestris),* were obtained from a local supplier. High-erucic acid rapeseed, cultivar Hero *(B. napus),* was also included to obtain data on industrial processing of a large-seeded cultivar. Seed of Hero was obtained from CSP Foods Ltd., Saskatoon, Saskatchewan, Canada.

Proximate analyses. Crude protein and fat were determined by an automated Kjeldahl procedure and a Soxhlet apparatus, respectively, according to the methods of the Association of Official Analytical Chemists (AOAC) (9). Moisture and ash contents of the seeds were also determined by AOAC methods. Composition of the fiber fraction was determined by the Goering and Van Soest procedure (10). Hemicellulose content was calculated as the difference between the neutral detergent fiber and acid detergent fiber (ADF). Cellulose was estimated as the difference between ADF and acid detergent lignin.

Enzymes. Samples of enzymes were obtained from commercial manufacturers. The SP-249 was provided by Novo-Nordisk Bioindustrial Inc. (Danbury, CT), and Olease was obtained from Biocon (U.S.) Inc. (Lexington, KY).

Enzyme treatment. Canola and rapeseed seeds were flaked to a thickness of 0.8 mm, autoclaved at 121°C for 5 min and treated with 0.1% vol/wt of SP-249 or 0.01% w/w of Olease. Enzyme hydrolysis was carried at 30% moisture and 50°C for 6 h. Samples were dried to 6% moisture prior to pressing in the expeller and were cold-pressed through the expeller. Controls were autoclaved, incubated in water under the conditions of enzyme treatment, dried and cold-pressed in the expeller.

Oil quality. Oils collected during pressing of enzyme-treated seeds were separated from fines by centrifugation. Free fatty acid contents of the oils were determined immediately after pressing, following the AOAC (9) procedure. Free fatty acids were expressed as % oleic acid. Chlorophyll determination was based on a modification of the Levadoux *et al.* (11) procedure. Oil, diluted with a mixture of petroleum ether and ethyl alcohol, was scanned in a spectrophotometer. The maximum absorbance at 660 nm and readings at the base line on both sides of the maximum were used to calculate the absorbance of chlorophyll. The calibration curve for chlorophyll was constructed by using chlorophyll A (Sigma Chemical Co., St. Louis, MO) in acetone (1 mg/mL). The procedure of De Ritter and Purcelli (12) was used to determine the carotenoid contents of oils. The absorbance of oil in hexane was measured in a spectrophotometer, scanning from 300 to 600 nm against hexane. Net absorbance was calculated as A_{446} nm less A_{600} nm, and ppm of carotenoids were calculated as lutein. Oils were degummed, refined and bleached with diatomaceous earth (13). Color of the oils was measured with a Hunter Lab color meter. Phosphorus contents in crude, degummed and bleached oils were determined by the modified methods of Raheja *et al.* (14) and Tbtani *et al.* (15). A calibration curve was constructed with

phosphatidylcholine (Sigma Chemical Co.). Peroxide value (PV) was analyzed by a modified method of Swoboda and Lea (16). Sulfur compounds in oils were converted to hydrogen sulfide by nascent hydrogen released from glacial acetic acid with a magnesium catalyst (17). The hydrogen sulfide was removed from the oil with a stream of nitrogen and passed over filter paper saturated with lead acetate. The darkness of the resulting lead sulfide deposits corresponded to the sulfur content of the oil. Standards were prepared with dibenzyl disulfate in corn oil over the range of 50–300 ppm. Sulfur contents in canola and rapeseed oils were determined by visual comparison with standards. The oxidative stabilities of oils were determined at 60°C, according to the modified Schaal oven test (18).

Presscake quality. Presscakes obtained by pressing of enzyme-treated canola flakes were evaluated for their chemical and antinutritional composition. Available lysine was determined by measuring both total lysine in each sample and the lysine remaining in the sample after treatment with 1-fluoro-2,4 dinitrobenzene (19). The difference between these two values represented the available lysine. *In vitro* digestibility of presscakes was determined with rumen fluid, and digestible organic matter (OMD) was calculated in percent of the total organic matter (20).

RESULTS AND DISCUSSION

The characteristic differences in seed composition of oil, protein and fiber are evident in the data presented in Table 1.

Flaked and autoclaved Westar seeds, pressed at the rate of 9.2 kg/h, yielded 3.1 kg/h of oil, which constituted 72% of the total oil (Table 2). Residual oil in presscake of 17.7% compared favorably with those of commercial presscakes. Treatment with SP-249 and Olease enzymes improved throughput of the expeller and the rate of oil flow. The SP-249 enzyme was more efficient than Olease when used as the treatment for the black-seeded Westar. Material throughput was increased by 51% and oil flow was improved by 84%, as compared to the control. The corresponding values for flakes treated with Olease were only 14 and 39%, respectively. The yellow-seeded Tobin and the high-erucic acid rapeseed were more susceptible to Olease than the SP-249 enzyme were. However, the improvements in the throughput of the expeller and the increases in the oil flow rates were much less than those observed for *B. napus* and the SP-249 enzyme. The average throughput and oil flow rate calculated for all samples were slightly better for Olease.

TABLE 1

Proximate Composition of Canola and Rapeseed

Seed and meal constituents	*Brassica napus* Westar	*B. campestris* Tobin	Rapeseed Hero
Oil	43.1	42.2	44.1
Meal-protein	42.7	43.2	40.0
Meal-hemicellulose	6.4	6.4	18.3
Meal-cellulose	7.2	12.1	5.2
Meal-lignin	10.7	5.6	7.8
Meal-ash	5.9	4.1	5.1

Enzyme treatment, prior to the expelling process, increased the recovery of oil from 72–78% of the seed oil for control samples to 90–93% (Table 2). The residual oils in presscakes decreased from 14–18% in control samples to 6.4–8.0% in samples treated with enzymes. The average residual oil content in presscakes from samples treated with the SP-249 and Olease enzymes was 7.5%.

Enzyme treatment increased the content of free fatty acids (FFA), on average, from 0.40 to 0.61% and the PV, on average, from 0.29 to 0.40 nM/kg (Table 3). Both enzymes were similar. The changes due to the enzyme treatments, however, were less severe than those observed when seeds were stored for a prolonged period (21). According to Appelqvist and Loof (21), FFA in rapeseed stored for up to 33 months increased to 7%. Similar levels of FFA were found in cracked, germinated or heat-damaged seeds.

The phospholipid phosphorus contents in oils pressed from enzyme-treated flakes were increased to levels that were almost double those in controls (Table 3). Olease released slightly more phosphorus from seeds than did SP-249. The phosphorus contents determined in this study for canola were, on average, 120 ppm, which was

TABLE 2

Influence of Treatment with Hydrolytic Enzymes on Pressing of Canola and Rapeseed Flakes[a]

Variety	Treatment	Seed throughput (kg/h)	Oil flow rate (kg/h)	Oil recovery (% of seed oil)	Presscake oil (%)
Westar	Control[b]	9.2	3.1	72.0	17.7
	SP-249	13.9	5.7	91.8	6.5
	Olease	10.5	4.3	92.6	6.4
Tobin	Control	13.8	5.0	77.9	14.5
	SP-249	15.4	6.4	91.2	7.7
	Olease	18.9	7.5	91.0	7.8
Rapeseed	Control	9.2	3.1	75.0	18.3
	SP-249	12.0	4.7	90.2	9.0
	Olease	12.8	5.2	91.1	7.8
Average	Control	10.7	3.7	74.9	16.8
	SP-249	13.8	5.6	91.1	7.7
	Olease	14.1	5.7	91.6	7.3

[a]Flaked seeds were autoclaved and hydrolyzed with enzymes at 30% moisture and 50°C for 6 h, dried to 5.5% moisture and pressed on expeller.
[b]Control flakes were autoclaved and incubated in water.

TABLE 3

Quality of Oils Cold Pressed from Enzyme-Treated Canola and Rapeseed Flakes[a]

Treatment	FFA (%)	PV (mM/kg)	P (ppm)	S (ppm)	Chl. (ppm)	Carot. (ppm)	Color		
							L	a	b
Westar									
Control	0.30	0.37	55.5	180	9.8	80.0	40.0	1.0	26.8
SP-249	0.60	0.34	81.7	200	24.2	89.3	42.4	8.8	28.6
Olease	0.50	0.38	120.3	180	29.9	92.7	29.7	7.6	18.7
Tobin									
Control	0.42	0.26	20.5	200	2.8	35.7	75.1	8.4	29.2
SP-249	0.72	0.37	107.7	200	5.8	40.6	26.7	9.9	35.2
Olease	0.67	0.40	132.3	180	7.9	39.7	24.5	8.7	33.8
Rapeseed									
Control	0.49	0.26	80.2	300	10.9	79.9	42.7	2.1	27.6
SP-249	0.57	0.47	143.3	300	21.3	84.1	44.4	5.4	31.7
Olease	0.63	0.43	137.3	350	22.7	80.4	40.2	3.3	23.2
Average									
Control	0.40	0.29	52.1	227	7.8	65.2	52.6	3.8	27.9
SP-249	0.63	0.39	110.0	233	17.1	71.3	37.8	8.0	31.8
Olease	0.60	0.40	129.9	237	20.1	70.9	31.5	6.5	25.2

[a]PV, peroxide value; FFA, free fatty acids; P, phosphorus; Chl., chlorophyll; Carot., carotene.

comparable to those reported in the literature. The phosphorus contents were influenced by processing conditions and increased with an increase in temperature of seed flaking (21), cooking of seeds (22) and increase in seed moisture and temperature of the press (23,24).

Sulfur contents in oils pressed from canola controls, incubated in water, and samples treated with enzymes were comparable (Table 3). The SP-249 enzyme gave slightly higher values on average than did Olease. Rapeseed oils from control and enzyme-treated samples were higher in sulfur than the corresponding canola oils. According to Persmark (25), sulfur content in oil was influenced more by processing conditions, *e.g.,* moisture content and temperature during cooking or conditioning, than by the initial glucosinolates level in seeds. In this study, canola, on average, had a lower sulfur content in oil than rapeseed, which was in agreement with the data reported by Persmark (25).

The degree of extraction of color compounds from the seed cotyledon into the oil depended on cultivar and initial chlorophyll content. Although enzymes increased chlorophyll levels in all oils, these changes were much greater for *B. napus* than for *B. campestris* (Table 3). On average, more chlorophyll was released by Olease than by SP-249. Carotenoid contents were also slightly increased in oils from enzyme-treated samples than from controls. Color of oil could also be evaluated in the Hunter Lab color meter as L (white), a (−a = green, +a = red) and b (−b = blue, +b = yellow) values. Higher L and a values determined in oils indicated their lighter and less green colors (Table 3). On average, both enzymes decreased the "whiteness" of oils but also decreased their greenness. Additionally, SP-249 increased the yellowness of oils.

The release of the color compounds into the oil increases when poor-quality or immature seeds are pressed (21). Solvent extraction yields oil that is inferior in color as compared to pressed oil (3,4). Color of oil and its phospholipid phosphorus content are major problems faced by industry in the oil refining process. Removal of phospholipids during the degumming process, and of color during bleaching with diatomaceous earth, result in major losses of oil during the refining process. Adverse colors are often removed only by repeated bleaching, which increases the losses of oil absorbed on the clay. Removal of phosphorus is conducted with acid to aid recovery of both hydratable and nonhydratable phospholipids.

Water degumming of control oils did not change the FFA value and only minimally decreased the phospholipid phosphorus and chlorophyll (Table 4). It appeared that most of the phospholipids pressed into the oil from control samples were nonhydratable. However, their level was reduced to 8–14% of those originally present in Westar and Tobin oils, respectively, during the acid refining step. Degumming of oils from enzyme-treated canola with water removed 25–53% of the phospholipids. Greater reductions in phospholipid contents (*i.e.,* water-hydratable

TABLE 4

Changes in Oils During Water and Acid Degumming

Method of degumming	Westar			Tobin		
	FFA (%)	P (ppm)	Chl. (ppm)	FFA (%)	P (ppm)	Chl. (ppm)
Control[a]	0.30	55.5	9.8	0.42	75.1	1.6
Water	0.30	53.9	8.0	0.38	53.0	1.3
Acid	0.24	4.5	8.1	0.38	10.6	1.7
SP-249	0.72	173.4	24.2	0.72	205.2	5.1
Water	0.49	81.2	23.2	0.67	143.2	5.1
Acid	0.48	24.5	22.6	0.68	39.6	5.1
Olease	0.77	164.8	19.5	0.72	201.1	4.9
Water	0.55	124.0	18.9	0.67	131.1	4.8
Acid	0.58	56.3	18.8	0.72	61.2	4.9
Average	0.60	131.2	17.8	0.62	160.6	3.9
Water	0.45	83.4	16.7	0.57	109.1	3.7
Acid	0.43	28.4	16.1	0.59	37.1	3.9

[a]Flaked and autoclaved seeds. Abbreviations as in Table 3.

TABLE 5

Peroxide Values of Oils During Storage at 60°C

Variety	Treatment	Peroxide values (mM/kg) at day 0	3	6	9	12	15
Westar	Control	0.90	2.59	3.91	5.11	7.22	8.19
	SP-249	0.99	2.64	4.47	5.29	7.92	8.89
	Olease	0.84	3.01	4.19	5.71	8.04	8.97
Tobin	Control	0.79	3.12	4.84	5.92	6.93	7.99
	SP-249	0.83	3.26	5.50	6.63	7.24	8.92
	Olease	0.79	2.82	4.75	6.92	8.62	9.17
Rapeseed	Control	0.49	2.11	3.89	5.29	6.91	8.07
	SP-249	0.61	2.47	3.51	5.40	7.02	8.11
	Olease	0.74	1.90	3.18	5.02	6.89	8.03
Average	Control	0.73	2.61	4.21	5.44	7.02	8.08
	SP-249	0.81	2.79	4.49	5.77	7.39	8.64
	Olease	0.79	2.58	4.04	5.88	7.89	8.72

phospholipids) were obtained for oils from the SP-249-treated canola than for those treated with Olease. Acid degumming removed 86 and 81% of phospholipids from the SP-249-treated Westar and Tobin, respectively, but only 66 and 69% from Olease-treated samples. The FFA and chlorophyll contents were slightly decreased during water or acid degumming.

The stability of oils at the elevated temperature of 60°C was measured for up to 15 d, and the rancidity was monitored chemically as an increase in the PV (Table 5). The development of rancidity followed the same path in controls and in oils from enzyme-treated canola and rapeseed. Enzyme treatment appeared to have minimal effect, if any, on oil stability at 60°C. These observations were not surprising because the stability of refined oils were reported to be directly related to the level of unsaturated fatty acids, mainly linolenic acid (26).

Presscakes from enzyme-treated canola contained one-half to one-third of the oil content of controls (Table 6). The lowest residual oil contents were in Westar presscakes, 6.5 and 6.4% of dry matter, for SP-249 and Olease-treated samples, respectively. Tobin and rapeseed presscakes were considerably higher in residual oil. Decreases in oil content in presscakes were associated with increases in protein content.

Enzyme treatment is thought to improve pressing in an expeller by the hydrolysis of the cell walls of cotyledons and, to a lesser degree, that of hulls. Results in Table 6 indicate that the hemicellulose fraction of fiber was most affected by enzymes. Cellulose and lignin were only marginally decreased. The decrease in fiber content was paralleled by improvement in the digestibilities of organic matter in presscakes from both cultivars of canola and rapeseed. The effects of the SP-249 enzyme on digestibility were superior to those of Olease.

Presscakes from cold-pressed samples contained 94–95% available lysine (Table 6). Their glucosinolate contents, determined in the intact form, of controls incubated in water were comparable to those of enzyme-treated samples.

Power consumption for the enzymatic process was extrapolated from the published data on energy use in a two-stage process (27,28) and temperature/time and pressure/time conditions used in the experimental, enzyme-aided process. It was estimated that rolling seeds to 0.8 mm flakes in the experimental process (Table 7) will require

TABLE 6

Composition and Nutritional Quality of Presscakes from Enzyme-Treated Canola and Rapeseed (dry basis)

Variety	Treatment	Oil	Protein	Hemicel[a]	Cellulose[a]	Lignin[a]	OMD[b]	Available lysine (%)	Intact glucosinolates (μM g)
Westar	Control	17.7	41.7	7.4	9.0	9.9	62.6	94.9	14.3
	SP-249	6.5	44.2	5.3	9.0	8.9	72.3	95.1	15.6
	Olease	6.4	42.5	5.2	9.6	9.2	67.4	95.0	14.4
Tobin	Control	14.5	37.6	5.5	10.9	4.8	60.5	93.0	17.2
	SP-249	7.7	39.3	4.3	10.2	4.8	73.3	94.2	13.7
	Olease	7.5	38.9	5.9	10.4	4.9	70.9	94.0	17.9
Rapeseed	Control	18.7	47.0	4.9	8.7	11.1	59.7	95.0	n/a
	SP-249	9.0	48.3	2.8	8.3	11.2	66.9	94.9	n/a
	Olease	8.7	47.6	4.2	8.6	10.8	65.2	95.0	n/a
Average	Control	17.0	42.1	5.9	9.5	8.6	60.9	94.3	15.7
	SP-249	7.7	43.9	4.1	9.2	8.3	70.8	94.7	14.6
	Olease	7.5	43.0	5.1	9.5	8.3	67.8	94.3	16.1

[a]Total fiber = hemicellulose + cellulose + lignin. [b]OMD, digestible organic matter.

TABLE 7

Power Consumption (kWh/ton)

Process	Prepressing plus solvent extraction	Enzyme pressing
Flaking	10[a]	4
Cooking/enzyme/drying	65[a]	79
Pressing	22[b]	15
Solvent extraction	1[a]	—
Desolventizing	50[a]	—
Transport, other	50[a]	5
Total	198	148

[a]Reference 27. [b]Reference 28.

6 kWh/ton less energy than flaking to 0.1 mm (28). Enzyme treatment and drying would require more energy than cooking alone, but there will be energy saving on pressing, due to increased throughput of enzyme-treated seeds. Additionally, 51 kWh/ton of seed will be saved due to elimination of hexane use and the associated need for desolventization of oil and meal. The total energy requirement for an enzymatic process should be 50 kWh/ton lower than for the commercial, two-stage process.

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