

EFFECT OF ENZYME PRETREATMENT OF CANOLA ON YIELD AND
QUALITY OF EXPELLER OIL AND MEALK. Sosulski (1), F.W. Sosulski (2)

- (1) Saskatchewan Research Council, 15 Innovation Blvd.
Saskatoon, Canada S7N 7W9
- (2) University of Saskatchewan, Saskatoon, Canada S7N 0W0

INTRODUCTION

Canola seeds contain numerous constituents which are extracted into the oil during the traditional process of pre-pressing and solvent extraction. However, the portion of the oil pressed from seeds on the expeller is reported to be superior in quality to solvent-extracted oil from presscake (Diosady et al. 1983; Usuki et al. 1984). 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 and decreasing nutrient availability.

It appears that elimination of solvent extraction of the residual oil in presscake could simultaneously improve overall quality of oil and meal.

Enzymatic pretreatment of canola, prior to pressing on the expeller, modifies the cell walls of cotyledons and hulls, reduces the time of pressing and increases oil recovery from 75% to 92% of the total oil (Sosulski and Sosulski, 1990). The low level of residual oil in presscake might render further solvent extraction uneconomical. However, only preliminary data are available on oil quality, ease of refining and oil stability. Also, no information is available on the quality of the presscake obtained from enzyme-treated seeds.

Therefore, the objectives of the study were:

1. To determine the quality of oils and ease of refining of oils pressed from flaked, enzyme-treated canola seeds.
2. To determine the chemical quality, availability of nutrients, feed value and presence of toxic constituents in presscakes from enzyme-treated canola.

MATERIAL AND METHODSSamples

The investigation included canola cultivars of B.napus and B.campestris.

Enzymes

Two mixed activity enzymes were used in this study: SP-249, provided by Novo-Nordisk A/S, Bagsvaerd, Denmark, and its Canadian representative, Novo Laboratories, Lachine, Quebec, and Olease, obtained from Biocon (US) Inc., Lexington, KY.

Treatments

Flaked seeds were autoclaved and hydrolysed with enzymes at 30% moisture and at 50°C for 6 hr, dried to 5.5% moisture and cold pressed on an expeller. Control flakes were autoclaved, incubated in water, dried and cold pressed on the expeller. Mass and oil balances were calculated for each treatment.

Oil Quality

Oils collected during pressing of enzyme-treated seeds were separated from fines by centrifugation. Free fatty acid (FFA) contents were determined in oils immediately after pressing, following the AOAC (1984) procedure. Chlorophyll and carotenoids were also determined immediately after pressing of oil from seeds, using modifications of Levadoux et al. (1986) and De Ritter and Purcelli (1981) procedures, resp. Oils were degummed, refined and bleached with diatomaceous earth, following the procedure of Gunstone and Norris (1983). Colour of the oils was measured using the Hunter Lab colour meter. Phosphorus content in crude and degummed oils was determined by modified methods of Raheja et al. (1973) and Totani et al. (1982). Peroxide value (PV) was analyzed in crude and bleached oils using the modified method of Swoboda and Lea (1985).

Presscakes Characteristics

Presscakes were evaluated for their chemical and antinutritional composition. Total nitrogen and fat contents were determined by the Micro-Kjeldahl and Soxhlet procedures of AOAC (1984), resp. Total amino acid content in presscake protein and the availability of lysine were determined on the Beckman Amino Acid Analyzer. Fiber fractions and composition were determined by the procedure of Goering and Van Soest (1970). Hemicellulose content was calculated as the difference between the Neutral Detergent Fiber and the Acid Detergent Fiber (ADF). Cellulose was calculated as the difference between the ADF and the Acid Detergent Lignin. In vitro digestibility of presscakes was determined with rumen fluid, as described by Troelson and Hannel (1968). The intact glucosinolates were determined according to the procedure of Sosulski and Dabrowski (1984).

RESULTS AND DISCUSSION

Enzyme treatment of canola seeds, flaked to a thickness of 0.8 mm, improved throughput on the expeller and the rate of flow of oil (Table 1). The SP-249 enzyme was more efficient as the treatment of B.napus but B.campestris canola was more susceptible to Olease. Material throughput was increased by 57% and the rate of oil flow increased by 91% in B.napus. The corresponding values for flakes treated with Olease were only 12% and 38%.

Enzyme treatment, prior to the expelling process, increased the recovery of oil from 73-78% to 91-92% (Table 1). The residual oils in presscakes decreased from 14.5-17.9% to 6.9-7.8%.

The free fatty acid (FFA) content in oils, pressed from enzyme treated flakes was increased as compared to controls, on average from 0.4 to 0.7% (Table 1). The peroxide values (PV) increased only slightly. The phospholipid phosphorus in crude oils, pressed from flakes treated with the SP-249 enzyme, doubled as compared to control oils. The Olease treatment removed even more phosphorus than SP-249. The Olease enzyme also released more chlorophyll, from both B.napus and B.campestris, than SP-249. Carotenoids contents were minimally affected by enzyme treatment. The B.napus contained almost twice as much carotenoids as B.campestris. When colour of oils were evaluated as L (white), a (-a=green, +a=red), and b (-b=blue, +b=yellow) on the Hunter Lab colour meter, on average, both enzymes decreased "whiteness" of oils but also decreased

their greeness. Colour of oil and its phospholipid phosphorus content are major problems faced by industry in the process of oil refining. Removal of phospholipids during degumming process, and colour during bleaching with the diatomaceous earth, result in major losses of oil during the refining process. Adverse colours are often removed only by repeated bleaching which increases the losses of oil absorbed on the clay. Removal of phosphorus can be conducted with acid to aid recovery of both hydratable and non-hydratable phospholipids.

Water degumming of control oils only minimally decreased the phospholipid phosphorus and chlorophyll contents (Table 1). It appeared that most of the phospholipids pressed into the oil from control samples were non-hydratable. However, their level was reduced to 8-20% of those originally present in B.napus and B.campestris oils, resp., during the acid refining step.

Degumming of oils from enzyme treated canola with water removed 5-17% of the phospholipids. Greater reductions in phospholipid contents (= more of water hydratable phospholipids) were obtained for oils from the SP-249 treated canola than for those treated with Olease. Acid degumming removed 75% of phospholipids from the SP-249 treated canola of both genotypes, but only 58 and 67% from Olease treated samples.

Presscakes from both enzyme-treated canola samples contained one-half to one-third of the oil contents of controls (Table 1). Decreased oil content in presscakes increased their protein levels. The hemicellulose fraction of fibre was most affected by enzymes. Cellulose and lignin were only marginally changed. The digestibility of the organic matter in presscakes from both types of canola were improved by enzyme pretreatment and the values were greater than for controls by 8 to 21%. The greatest improvement in digestibility was obtained for B.campestris, a variety of canola that was rich in cellulose, intermediate in hemicellulose and poor in lignin, as compared to B.campestris. The effects of SP-249 enzyme on digestibility were superior over Olease.

Presscakes from cold pressed samples contained 94-95% of available lysine (Table 1).

Glucosinolates in presscakes were determined in their intact form, after inactivation of the myrosinase enzyme. The controls, flaked and autoclaved seeds contained 23.2 and 24.8 $\mu\text{M/g}$ of glucosinolates (Table 2). Incubation in water decreased the intact glucosinolates to 14.3 and 17.2 $\mu\text{M/g}$, which were comparable to those obtained in enzyme-treated samples.

CONCLUSIONS

1. Enzyme treatment of canola flakes, 0.8 mm thickness, improved throughput on an expeller, increased the oil flow rate and enabled recovery of 92% of seed oil, during a single cold pressing on an expeller.
2. Oils pressed from enzyme-treated canola had slightly increased free fatty acids and peroxide values. Chlorophyll and carotenoid levels were also increased minimally; however, colour evaluation on the Hunter Lab colour meter indicated a reduction in green colour of treated oils as compared to control.
3. Phospholipid phosphorus contents, in oils from enzyme-treated samples, were more than double those of the controls. An acid refining step removed 70-75% of phosphorus.

4. Presscakes from enzyme treated canola contained reduced oil, by 50-60%, as compared to controls. Protein contents were slightly increased and lysine availability remained high. Fiber contents were also reduced, by 1.1-2.6 fiber units, the hemicellulose fraction exhibiting the greatest change. Digestibility of organic matter of treated presscakes was improved over that of control by 8-21 digestibility units.
5. Enzyme treatment created a marked increase in plant throughput and oil yield. The oil quality was inferior to cold-pressed control but would be much better than solvent-extracted oil. The SP-249 enzyme appeared to be superior to Olease.

REFERENCES

- AOAC, 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, D.C.
- De Ritter, E. and Purcelli, A. E. 1981. Carotenoid Analytical Methods. In: Carotenoids as Colorants and Vitamin A Precursors. J.C. Bauernfeind (ed.). Accademic Press, New York. p. 841.
- Diosady, L.L., Sleggs, P. and Kaji, T. 1983. Degumming of canola oils. In: 7th Progress Report. Research on Canola Seed, Oil, Meal and Meal Fractions. Publ. No. 61. Canola Council of Canada, Winnipeg, Man. p. 186.
- Goering, H.K. and Van Soest, P.J. 1970. Forage Analysis. USDA Handbook No.379, Washington, D.C.
- Gunstone, F.D. and Norris, F.A. 1983. Lipids in Foods. In: Lipids. Chemistry, Biochemistry and Technology. Pergamon Press, Oxford.
- Levadoux, W.L., Kalmokoff, M.L. and Pickard, M.D. 1987. Pigment removal from canola oil using chlorophyllase. JAACS, 64, 139.
- Raheja, R.K., Kuar, C., Singh, A. and Bhatia, I.S. 1973. New colorimetric method for quantitative estimation of phospholipids without acid digestion. J. Lipid Res. 14, 695.
- Sosulski, F.W. and Dabrowski, K.J. 1984. Determination of glucosinolates in canola meal and protein products by desulfation and capillary gas-liquid chromatography. J. Agric. Food Chem. 32, 1172.
- Sosulski, K. and Sosulski, F. 1990. Enzyme Pretreatment to Enhance Oil Extractability in Canola. In: Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology. F. Shahidi (ed.). Van Nostrand Reinhold, New York. p. 277.
- Swoboda, P.A.T. and Lea, C.H. 1958. Determination of the peroxide value of edible fats by colorimetric iodometric procedures. Chem. Ind. 2, 1090.
- Totani, Y., Pretorius, H.E., DuPlessis, L.M. 1982. Extraction of phospholipid from plant oils and colorimetric determination of total phosphorus. JAACS. 59, 161.

Table 1. Influence of treatment with enzymes on pressing of canola flakes and on quality of oil and presscake.

Parameter	<u>B. napus</u>			<u>B. campestris</u>		
	Control	SP-249	Olease	Control	SP-249	Olease
<u>Press Characteristics</u>						
Seed flow, kg/hr	10.2	15.8	11.4	13.8	15.4	18.4
Oil flow, kg/hr	3.4	6.5	4.7	5.0	6.4	7.5
Oil yield, % of total	73.0	90.8	91.9	77.9	91.2	91.0
Presscake oil, %	17.9	7.0	6.9	14.5	7.7	7.8
<u>Oil Quality</u>						
FFA, %	0.4	0.6	0.6	0.4	0.7	0.7
PV μ M/kg	0.4	0.5	0.5	0.3	0.4	0.4
Phosphorus, ppm	59	98	131	54	107	132
Chlorophyll, ppm	5	15	18	3	6	8
Caroteneids, ppm	68	74	76	36	41	40
Colour L	48	46	41	75	27	25
a	3	9	8	8	10	9
b	33	31	27	29	35	34
<u>Water Degummed Oil Quality</u>						
Phosphorus, ppm	54	81	124	50	90	110
Chlorophyll, ppm	8	23	19	2	6	8
<u>Acid Degummed Oil Quality</u>						
Phosphorus, ppm	5	25	56	11	27	43
Chlorophyll, ppm	8	23	19	2	6	8
<u>Presscake Quality</u>						
Oil, %	18	7	7	15	8	8
Protein, %	42	44	44	38	39	39
Hemicellulose, %	7	5	5	6	4	6
Cellulose, %	9	9	10	11	10	10
Lignin, %	10	9	9	5	5	5
OMD, %	63	72	67	61	73	71
Avail. lysine, %	95	95	94	94	94	94

Table 2. Effect of enzyme pretreatments on intact glucosinolate content ($\mu\text{M/g}$ presscake).

Glucosinolates	Untreated Control	Treated ¹ Control	SP-249	Oleace
<u>B. napus</u>				
3-Butenyl	4.15	3.36	3.21	3.42
2-Pentenyl	0.47	0.48	0.43	0.47
2-OH-3-Butenyl	7.99	7.99	7.49	7.71
2-OH-4-Pentenyl	-	-	-	-
3-Indole Me	0.59	0.39	0.55	0.33
4-OH-3-Indole Me	9.98	2.08	3.43	2.43
Total	23.18	14.30	15.11	14.36
<u>B. campestris</u>				
3-Butenyl	5.20	4.96	3.38	4.42
2-Pentenyl	2.65	1.65	1.82	2.41
2-OH-3-Butenyl	9.96	6.62	5.36	6.93
2-OH-4-Pentenyl	0.98	0.94	0.56	0.74
3-Indole Me	-	-	-	-
4-OH-3-Indole Me	5.92	3.07	2.60	3.41
Total	24.71	17.24	13.72	17.91

¹ Seeds flaked, autoclaved, incubated in water, dried and cold pressed on the expeller.

REFERENCES (continued)

Troelsen, J.E. and Hannel, D.J. 1969. Ruminant digestion *in vitro* as affected by donor, collection day and fermentation time. Can. J. Anim. Sci. 46, 149.

Usuki, R., Suzuli, T., Endo, Y., Kaneda, T. 1984. Residual amounts of chlorophylls and pheophytins in refined vegetable oils. JAOCS. 61, 785.