The Inhibitory Effect of Neem (*Azadirachta indica*) Leaf Extracts on Aflatoxin Synthesis in *Aspergillus parasiticus*[1]

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The effect of neem (*Azadirachta indica*) leaf extracts on *Aspergillus parasiticus* growth and aflatoxin biosynthesis was investigated. The extracts were prepared by blending 50 g (wet weight) of fresh leaves in one l of 10 mM potassium phosphate (pH 7.0) or by boiling the leaves in the buffer. Extracts were added to fungal growth media at 1, 5, 10, 20 and 50% (vol/vol) concentrations prior to inoculation. The formulations did not affect fungal growth (i.e., mycelial dry weight) but essentially blocked (>98%) aflatoxin biosynthesis at concentrations greater than 10% (vol/vol). The inhibitory effect was somewhat diminished (60–70% inhibition) in heated leaf extracts. Volatile components of the extracts were analyzed using capillary gas chromatography/mass spectrometry; the major volatile component was 3-methyl 2-buten-1-ol. However, volatiles from blended leaf extracts did not affect either aflatoxin synthesis or fungal growth. The neem-mediated inhibition appears to involve regulation of secondary metabolism, because once secondary biosynthesis was initiated the inhibitory effect of the neem leaf constituents was lost.

Azadirachta indica Juss. (syn. *Melia azadirachta* L.) commonly known as "margosa," "neem" and "nim," is an ornamental tree of Asia and Africa. Neem components have reputed value for their medicinal, spermicidal, antiviral, antibacterial, antiprotozoal, insecticidal, insect repellent, antifungal and antinematode properties (1,2). Several active principles from different parts of the neem tree have been reported (3). The neem leaves are known to contain desactylimbin, quercetin and sitosterol (4,5). Neem oil yields various acids, sulphur, etc. (6, 8). The effects of these components on aflatoxin biosynthesis by either *Aspergillus parasiticus* or *Aspergillus flavus* have not been studied so far. Owing to the antimicrobial properties of the leaves of the neem plant, the current study assessed the fungicidal role of leaf formulations against aflatoxigenic strains of *Aspergillus parasiticus*.

EXPERIMENTAL PROCEDURES

Preparation of neem leaf extracts. Neem leaves were obtained from the ARS/USDA Subtropical Horticulture Research Station, Miami, Florida. After washing the leaves thoroughly with sterile distilled water, extracts were prepared by blending 50 g (wet weight) of fresh leaves in one l sterile 0.01 M potassium phosphate buffer, (pH 7.0) (blended). In another formulation (heat-extracted), leaves were boiled in sterile buffer for 30 min and the volume was adjusted to one l after cooling. Extracts were filtered through several layers of cheesecloth, and the filtrate was centrifuged for 15 min at 7,000 × *g*. Soluble extracts were sterilized by passing through a Millipore filter (0.22 μm pore size). In addition, one-half of both leaf formulations (blended and heat-extracted) were autoclaved to achieve sterilization.

Fungal growth conditions in submerged culture. The fungal strain used in this study was a wild-type aflatoxigenic isolate of *A. parasiticus* designated SU-1 (SRRC 143). The fungus was grown on growth medium (GM) (9) by inoculating with 0.1 ml of a spore suspension (10^6 spores/ml) to 100 ml of the medium containing 0 to 20% vol/vol neem leaf formulations in 250-ml flasks. The flasks were incubated for 2–4 days on a shaker incubator (Lab-Line Instrument Inc.) at 150 rpm and 28°C. Growth of the fungus was recorded after four days by harvesting the mycelial pellets through filtration on oven-dried Whatman No. 42 filter papers. The filter papers containing the fungal growth mass were oven-dried at 70°C for 24 hr, and the dry weight of the fungus was determined as an index of fungal growth. For the study of the conversion of secondary metabolites to aflatoxins, mycelia were washed thoroughly and transferred to low sugar replacement media (LSRM) (10).

Extraction and assay of metabolites and aflatoxins. After the desired incubation, mycelial pellets and media were extracted with aqueous acetone, followed by methylene chloride (10). Aflatoxins were separated on silica gel thin layer chromatographic (TLC) plates in ether:methanol:water (96:3:1). The toxins were quantitated by fluorometric scans (360 nm) of TLC plates containing the extracted samples and comparison with aflatoxin standards run on the same plate (10). Aflatoxin precursors from the mycelia were identified by the procedure described by McCormick et al. (11).

Determination of volatile components of neem leaves. Neem leaf extracts were placed in 1.2-l Kontes solvent storage bottles fitted with Teflon valves and an inlet tube that extended to two in. from the bottom of each bottle. Air-space above the extracts was purged with nitrogen for 30 min onto Tenax GC (60–80 mesh) tubes. Volatiles were analyzed with a Finnigan MAT GC/MS 4000 system interfaced with an external closed inlet device. The GC column was a 50-m capillary SE-54 column held at -30°C for loading. The temperature program used was -30°C (3 min) → 30°C (15°/min) → 150°C (2.5°/min) ∞ 250°C (10°/min). Data aquisition and analysis were accomplished with a Finnigan-Incos data system. Compounds were identified on the basis of computer-assisted library searches.

Effect of volatile components of neem leaves. Activity of volatiles from the blended neem leaf extracts on growth and aflatoxin production was determined by: (i) removal of two 10-mm diameter plugs from the outer edges of the PDA nutrient agar in Petri plates;

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TABLE 1

Effect of Neem Leaf Extracts on *A. parasiticus* Growth and Aflatoxin Synthesis in Submerged Cultures[a]

Treatment[b]	Mycelial dry Weight (g)	Aflatoxin (% of control)			
		B_1	B_2	G_1	G_2
Control	1.28 ± 0.19	100[c] ± 11	100 ± 14	100 ± 17	100 ± 9.0
Blended extract	1.32 ± 0.18	1.0 ± 0.1	1.4 ± 0.1	ND[d]	ND[d]
Blended extract (autoclaved)	1.34 ± 0.20	18.2 ± 2.7	13.5 ± 0.2	3.0 ± 0.4	3.8 ± 0.5
Heated extract	1.29 ± 0.13	7.6 ± 1.3	6.6 ± 0.7	ND[d]	ND[d]
Heated extract (autoclaved)	1.30 ± 0.08	30.1 ± 3.3	13.0 ± 0.7	15.8 ± 1.0	14.4 ± 0.2

[a]The results are the means of 4 experiments with 2 replicates each.
[b]The neem extracts were prepared as described in Materials and Methods.
[c]100% refers to 23.2 μg aflatoxin B_1, 5.2 μg aflatoxin B_2, 14.3 μg aflatoxin G_1 and 3.6 μg aflatoxin G_2 per g mycelial dry weight.
[d]ND = not detected.

an additional plug was removed from the center of the plate; and (ii) addition of a 0.5-ml *A. parasiticus* spore suspension (10^5 spores/ml) to the center opening and introduction of neem leaf extract in 1.5-ml beakers placed in the outer holes. The Petri plates were sealed and radial growth of the fungus was recorded each day over a four-day test period. At the end of the four days, Petri plate contents were extracted for aflatoxin.

RESULTS AND DISCUSSION

Effect of neem leaf formulations on A. parasiticus *growth and aflatoxin production in submerged culture.* The effect of 20% (vol/vol) neem leaf extracts on fungal growth and aflatoxin synthesis was determined after four days of fungal growth in the presence of the extract. Addition of extracts did not alter the pH profiles of the growth medium from that of controls during the four-day incubation. The results (Table 1) demonstrate that the neem leaf constituents do not affect fungal growth in submerged culture but do inhibit aflatoxin production by the mycelia. A gradual increase in inhibition of aflatoxin biosynthesis was observed with increasing concentration of neem leaf extracts (Table 2). Presence of 10% extract in the fungal growth medium was sufficient to obtain maximal (>98%) inhibition of toxin production. However, the inhibitory property was considerably reduced after heating the leaf extracts by either boiling the leaves in the buffer or autoclaving the leaf extracts (both blended and heat-extracted) (Tables 1 and 2). The observations suggested that the inhibitory factor(s) are unstable to heat and might be volatile. The differences in the volatile components of the blended and heat-extracted leaf formulations were, therefore, determined.

Effect of neem leaf volatiles on fungal growth and aflatoxin production on agar. The content of 10 of 13 major volatile components was significantly higher in the blended neem leaf extract than in the autoclaved heated extract. The major volatile component present, 3-methyl-2-buten-1-ol, was nearly 400-fold greater in the blended extract than in the heated extract. Observing these differences in the volatile components of the two extracts, the effect of neem leaf volatiles on fungal growth and aflatoxin production was investigated. The bioactivity of the neem leaf volatiles was assessed by measuring the fungal growth and aflatoxin production by the fungus grown on agar medium and exposed to an atmosphere containing volatiles from neem leaf extracts (Table 3). The ratio of neem extract to the agar medium was nearly 1:5, and the amount of inoculum added in the bioassay was in the same ratio as that added in submerged cultures. However, volatiles from blended neem leaf extracts did not affect either aflatoxin synthesis or fungal growth during a four-day incubation of the fungus on agar medium. The radial fungal growth was 3.2 ± 0.6 and 5.6 ± 1.5 cm in two and four days, respectively, for the controls and 3.4 ± 0.8 and 6.1 ± 1.9 cm for the same duration for the blended neem extract. The total aflatoxin content after four days of fungal growth was determined to be 21.3 ± 2.8 μg and 20.4 ± 4.0 μg for the control and neem extract treatment, respectively.

Localization of the inhibition of aflatoxin biosynthesis by neem leaf extracts. To elucidate the factor(s) responsible for the inhibition of aflatoxin synthesis

TABLE 2

Effect of Concentration of Neem Leaf Extract in the Incubation Medium on Aflatoxin B_1 Biosynthesis

Concentration of extract (vol/vol)	Aflatoxin B_1 (% of control)[a]			
	Blended extract	Blended extract (autoclaved)	Heat extracted	Heat extracted (autoclaved)
0	100[b]	100	100	100
1	15.1	48.9	52.3	60.2
5	6.5	36.2	35.2	43.4
10	2.6	24.6	18.7	35.2
20	2.0	17.8	9.2	29.8
50	1.8	16.3	6.4	31.2

[a]The pooled mean standard error in the results was ± 12.4% (n=3).
[b]100% refers to 20.6 μg aflatoxin B_1 produced/g mycelial dry weight.

TABLE 3

Ability of *A. parasiticus* Mycelia Grown in the Presence of Neem Extract to Utilize Aflatoxin Precursors

	Aflatoxin B_1 produced in LSRM (μg)[a]					
	Control mycelia			Neem-treated mycelia		
Incubation (hr)	No precursor	+20 μg AVN	+10 μg ST	No precursor	+20 μg AVN	+10 μg ST
0	0	0	0	0	0	0
24	4.8 ± 0.6	7.9 ± 1.0	9.6 ± 1.3	0.2 ± 0.06	3.5 ± 0.4	5.9 ± 0.3
48	6.3 ± 0.6	10.0 ± 0.8	12.6 ± 1.6	0.4 ± 0.10	4.2 ± 0.7	6.8 ± 0.4

[a]One-g fractions of wet 48-hr-old mycelia were obtained from fermentations carried out either in the absence (control mycelia) or presence (neem-treated mycelia) of 20% (vol/vol) of neem blended extract in GM. Mycelia were thoroughly washed in distilled water and transferred to 10 ml LSRM for assay. Secondary biosynthesis was determined by production of aflatoxin B_1 after addition of pathway precursors AVN (averantin) or ST (sterigmatocystin).

by neem leaf extracts, the effect of the aqueous leaf extracts on initiation/inhibition of secondary metabolism was investigated. The ability of resting mycelia to carry out secondary biosynthesis was used as a test system to monitor the effects of leaf extracts on the process. Mycelia were grown in GM containing 20% neem extract (Table 3) and the aflatoxin content measured after transferring the mycelia to a low sugar resting medium. The results demonstrated that aflatoxin biosynthesis was irreversibly inhibited in *A. parasiticus* mycelia by neem leaf constituents; removal of mycelia from exposure to leaf extracts did not restore aflatoxin synthesis. Enzymes required for aflatoxin biosynthesis were, however, apparently intact in the treated mycelia because the aflatoxin precursors [averantin and sterigmatocystin, (10)] fed to these mycelia were converted to aflatoxin B_2 at the same rate as control mycelia not exposed to leaf extracts (Table 3). Norsolorinic acid and averantin, both early precursors in aflatoxin biosynthesis (10), were not detected in mycelial extracts of treated mycelia, but the compounds were present in extracts of the control mycelia. Therefore, norsolorinic acid and averantin apparently were not synthesized in mycelia grown in the presence of neem extracts, whereas the enzymes required for the conversion of averantin and sterigmatocystin to aflatoxin B_1 were present in the mycelia (Table 3). Inhibition of aflatoxin biosynthesis by neem extracts in fungal cells appears to occur in the very early stages of the biosynthetic pathway because after the initiation of secondary metabolism, the inhibitory effect of the neem leaf consitutents was lost (Table 4). The enzymes involved in the latter stages of aflatoxin synthesis were not affected by the neem extracts.

In conclusion, nonvolatile neem leaf constituents irreversibly and almost totally inhibit aflatoxin biosynthesis in *A. parasiticus*, but they do not affect fungal growth. Inhibition of aflatoxin biosynthesis appeared to occur in the early stages of the biosynthetic pathway. If the inhibitory factor(s) could be effective in field studies, neem leaf extracts might be used in controlling the preharvest aflatoxin contamination of food and feed commodities.

TABLE 4

Direct Effect of Neem Leaf Extract on Aflatoxin B_1 Synthesis by Resting *A. parasiticus* Mycelia

	Aflatoxin B_1 produced (μg)[a]	
Incubation (hr)	Control mycelia	Neem-treated mycelia
2	2.7 ± 0.4	2.7 ± 0.4
24	5.8 ± 0.7	5.5 ± 0.6
48	7.1 ± 1.1	6.8 ± 1.0

[a]One-g fractions of wet 72-hr old mycelia were obtained from fermentations in GM and transferred to 10 ml LSRM containing either 0 (control mycelia) or 20% (neem-treated mycelia) vol/vol of neem-blended extract. Aflatoxin content was determined after 2, 24 and 48 hr of incubation.

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Mechanics of Oil Expression From Canola

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A laboratory-scale oilseed screw press was used to investigate the effects of shaft speed and choke opening and of seed pretreatments, including moisture conditioning, flaking and preheating, on the canola pressing performance. Maximum pressure increased, and press throughput and residual oil (RO) in presscake both decreased, with a reduction in choke opening and with lowering of shaft speed. When either whole seed or flakes were preheated in the range 40–100 C, the pressure and throughput increased, and RO decreased. Press throughput and oil output both achieved maxima at a 5.0% moisture content in seed, while the RO showed a continuous increase with increasing seed moisture contents. The observed effects of choke opening and shaft speed on pressure, throughput, RO and press temperature could be explained with the aid of a simple equation representing the axial flow within the press. The same equation also served to explain the changes in pressure and throughput corresponding to the various seed pretreatments, when changes in viscosity of the oilseed mass were postulated. It was inferred that any seed pretreatment which increased viscosity would also increase throughput of the press. A further examination of the individual components of viscosity might explain the changes in the residual oil content and facilitate the development of improved or novel pretreatments of oilseeds.

Screw presses have been used universally for the expression of oil from oilseeds for over 80 yr. During this period, great strides have been made in improving their capacity and energy efficiency. Oil mill operators have determined the optimum operating conditions for each type of seed being crushed by experimentation. Although the process conditions and pretreatments used by the operators are widely known (1, 2), there is a lack of systematic data in the published literature on the quantitative effects of individual parameters on press performance. Until recently, due partly to the unavailability of small, laboratory-scale presses, published data on press performance has come mostly from press manufacturers (3–5), who provide qualitative justifications for the optimum process conditions recommended for their presses.

With a growing interest in producing oil on the farm as an alternative fuel for farm machinery, small presses have been actively marketed by at least two major manufacturers during the past two decades (6, 7). These presses have been used for several systematic studies on oil expression (8–13). Most of these recent studies were performed with the objective of determining the optimum operating conditions for on-farm pressing. They have focussed either on cold pressing, i.e., pressing of unheated whole seed, or on the pressing of heated whole seed. The cause-effect relationships between input and output parameters of a press have been discussed in the literature but only to a limited extent.

The objective of the present study was to investigate changes in press throughput and oil output for a range of press settings, including choke opening and speed of wormshaft rotation, and for seed pretreatments including moisture conditioning, flaking and heating. Pressure and temperature measurements were made during each experiment near the discharge end of the press so as to obtain insights into the mechanics of its operation. It was expected that a simple equation representing the flow of seed mass would aid in the investigation of interrelationships between the input and output parameters of the press.

MATERIALS AND METHODS

Laboratory press. A small scale oilseed press, the Mini 40 Screw Press manufactured by Simon-Rosedowns Ltd. of Hull, England, was used for the experiments (Fig. 1). The press has a 15.0-cm × 6.0-cm barrel made up of 12 vertical rings, each of which is grooved on the inner surface to facilitate the flow of softer seeds such as canola. The wormshaft has a continuous helical worm with 10 turns, the worm depth and pitch both decreasing continuously after the seventh turn so that the overall compression ratio of the shaft is 14 to 1. The detailed dimensions of the shaft, worm flights and the choke are given elsewhere (14).

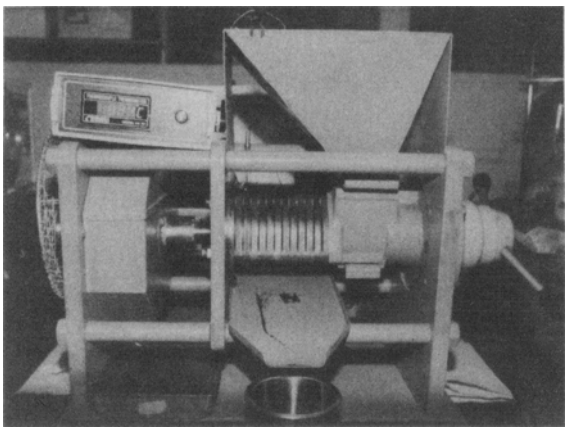

FIG. 1. Photograph of the laboratory press, a Simon-Rosedowns Mini 40 Screw Press.

Oilseed. Canola seed of the black-coated Westar variety (*Brassica napus* L.), with an oil content of 46.3% on an ambient moisture (3.9%) basis, was used for the experiments.

Cold press experiments. About 90 kg of unheated whole seed was pressed at three shaft speeds, 120 rpm, 90 rpm and 70 rpm. At each speed, five settings of choke opening, 0.23 mm to 0.80 mm, were used. Before the start of each run, the press was preheated to 45 C

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by means of a heating pad wrapped around the barrel. One kg of seed was then pressed at an opening of 0.60 mm to heat the press to its working temperature range above 80 C. The experiment sample, two kg of seed, was then poured into the hopper. The seed flowed freely, under gravity, into the barrel, the flowrate being determined by the shaft speed and choke opening. Each experiment was conducted in duplicate.

The pressure just before the choke end was continuously monitored with a piezoresistive transducer, mounted on barrel ring no. 12, connected through an amplifier and a low-pass filter to a two-channel recorder. The temperature of the inside barrel wall near the choke end was continuously monitored with a thermocouple connected to the other channel of the recorder.

The press throughput was calculated by dividing the sample weight, two kg, by the time required to press each sample as observed from the pressure chart, which showed a sharp increase in pressure from its zero-level at the beginning of each experiment and a sharp fall to zero-level at the end. The expressed oil was separated from 'foots' (solids expressed with oil) by centrifugation and weighed. The weight of oil trapped in foots, in excess of the residual oil content in presscake, was added to the weight of the separated oil, and the combined weight divided by the pressing time, to calculate the oil output. The presscake from each experiment was ground, mixed and the residual oil content determined on a Goldfisch extractor by AOCS methods (15).

Seed pretreatments. These experiments were conducted at a shaft speed of 120 rpm and a choke opening of 0.42 mm on two-kg samples at a pressing time of 10–15 min. The seed hopper was covered during the experiments to minimize the loss of heat and moisture. Each experiment was performed in duplicate.

Seed heating. Seed samples were placed in closed containers which were stored in an air oven at predetermined temperatures for 40 min with intermittent shaking.

Flaking. The seed was preheated to 38–40 C and then flaked to 0.25–0.30 mm thickness using a pair of smooth rolls. The preheating was done to minimize shattering of seed during flaking. The flakes were then heated by the same procedure as for whole seed.

Moisture conditioning. The moisture content of the Westar seed exposed to ambient air for three days was 4.0 ± 0.2% on dry basis. Four additional samples were prepared by the addition of water to bring the moisture content to 5.0, 6.0, 8.0 and 10.0%, respectively. The samples were stored in sealed containers for two days to allow for the uniform absorption of water.

RESULTS AND DISCUSSION

Effect of press settings: choke opening. As the choke was tightened progressively, the maximum pressure in the barrel increased and press throughput decreased (Fig. 2). Pressure increased slowly when the choke was tightened from 0.80 mm to 0.61 mm and 0.42 mm, but sharply at narrow openings of 0.32 mm and 0.23 mm. Similarly, the decrease in throughput between the choke openings of 0.80 mm and 0.42 mm was less than at narrower openings, especially at lower shaft speeds. Oil output remained unchanged until the intermediate opening of 0.42 mm, but decreased when the opening was narrowed further to 0.32 mm and then to 0.23 mm (Fig. 3). Residual oil in presscake (RO) decreased with the narrowing choke. It should be noted that RO would be inversely related to oil output and directly related to seed throughput. At a constant oil output, a higher throughput would lead to a lower oil recovery per unit

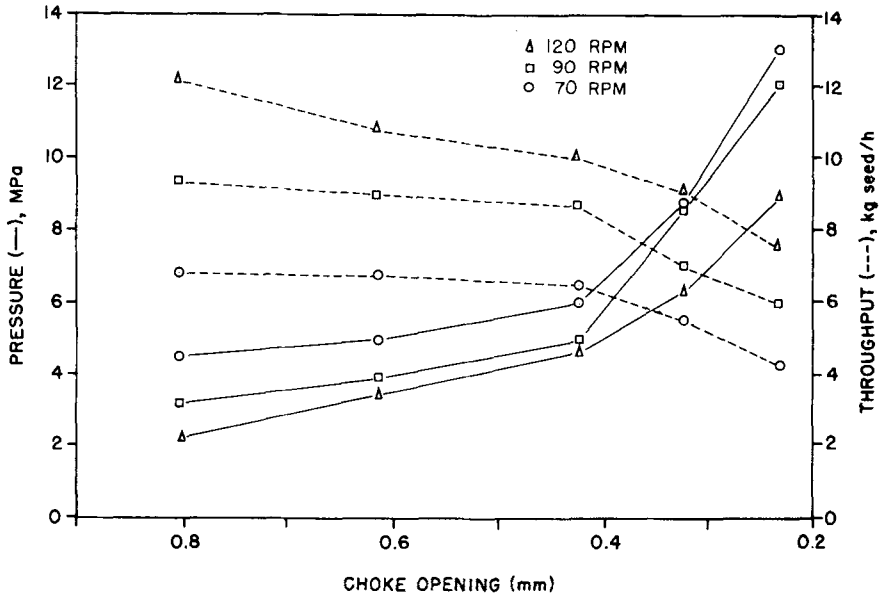

FIG. 2. Effect of choke opening and shaft speed on the maximum pressure in the barrel and press throughput during cold pressing of canola. The deviations in pressure readings were ±10.0% of the mean values. The average standard deviations in throughput were ±1.03, ±0.60, ±0.54, ±0.24 and ±0.21 units at choke openings of 0.80 mm, 0.61 mm, 0.42 mm, 0.32 mm and 0.23 mm, respectively.

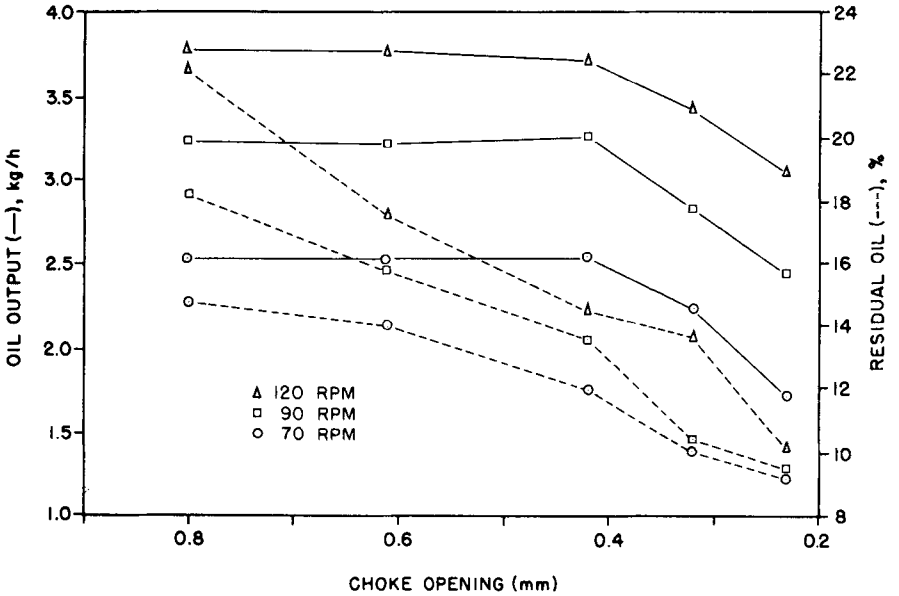

FIG. 3. Effect of choke opening and shaft speed on oil output and residual oil content during cold pressing of canola. The average standard deviations in the oil output were ±0.30, ±0.18, ±0.18, ±0.08 and ±0.07 units, and in residual oil were ±0.76, ±0.56, ±0.46, ±0.45 and ±0.38 units, at choke openings of 0.80 mm, 0.61 mm, 0.42 mm, 0.32 mm and 0.23 mm, respectively.

weight of seed, causing a higher RO in presscake. The changes in pressure, throughput, oil output and RO followed similar patterns at the three shaft speeds investigated.

Jacobsen and Backer (13) reported similar effects of choke opening on the throughput and RO content of sunflower. Prinsloo and Hugo (8) found that choke opening had no effect on throughput of sunflower seed at wider openings than used in the present study. In the present experiments, the change in throughput over the range 0.80 mm to 0.42 mm was less than that observed at the two narrowest openings. In preliminary studies, when additional experiments were conducted at openings 0.99 mm and 1.18 mm (setting 14 and 16 on the press, respectively) at 120 rpm and 70 rpm, no changes in throughput were observed from those at 0.80 mm.

Temperature of the barrel increased progressively as the choke was tightened, and ranged from 104 ± 2 C at the widest opening of 0.80 mm to 133 ± 4 C at the narrowest opening of 0.23 mm when shaft speed was maintained at 120 rpm (Table 1). Similar changes in barrel temperature were observed at the three speeds of shaft rotation. These results matched those reported by Prinsloo and Hugo (8).

Effect of press settings: shaft speed. Lowering the shaft speed increased pressure and reduced throughput, oil output and RO (Figs. 2 and 3). These results were similar to those observed when the choke was narrowed. Throughput decreased in a uniform pattern at each choke opening (Fig. 2), and so did oil output (Fig. 3). Throughputs at the shaft speed of 70 rpm were lower by 35-45% than those at 120 rpm at the respective openings of the choke, while oil outputs were lower by 33-43%. Reduction in RO with decreased shaft speed was generally larger at wider openings and smaller at narrower openings (Fig. 3). Barrel temperatures were a little lower at slower shaft speeds, the difference between 120 rpm and 70 rpm ranging from 3 C at the widest opening of 0.80 mm to 9 C at the narrowest opening of 0.23 mm (Table 1). The trends observed in all the parameters described above, except pressure, were in agreement with those reported for sunflower pressing by Prinsloo and Hugo (8). Effect of speed on pressure development had not been reported previously in the literature.

TABLE 1

The Effect of Shaft Speed and Choke Opening on Barrel Temperature During Canola Cold Press Experiments[a]

Choke opening (mm)	Shaft speed		
	120 rpm	90 rpm	70 rpm
0.80	104	102	101
0.61	108	105	104
0.42	112	108	105
0.32	116	116	112
0.23	133	128	124

[a]Average deviation in temperature readings ± 3 C.

The optimum press settings for cold press operation, such as for on-farm crushing or for village level crushing as practiced in industrially less-developed countries, would depend on the performance criterion used. If maximum oil extraction, i.e. minimum RO, were the objective, the narrow opening of 0.32 mm (choke setting 7 on the press) would be preferable. The narrowest opening of 0.23 mm (choke setting 6) would not be advisable because the high pressure may lead to excessive wear of the barrel and shaft surfaces, and also

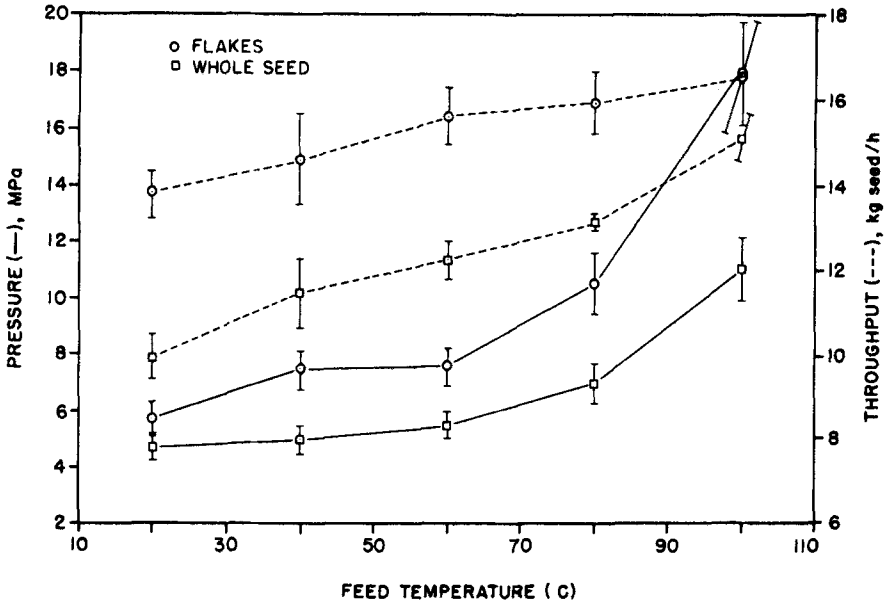

FIG. 4. Effect of flaking and preheating on the maximum pressure and press throughput at shaft speed of 120 rpm and 0.42-mm choke opening.

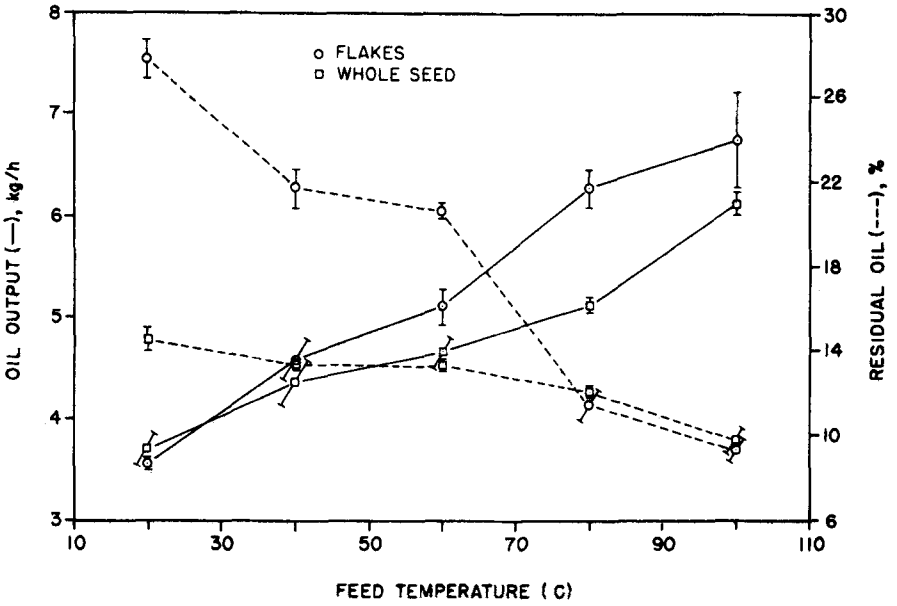

FIG. 5. Effect of flaking and preheating on oil output and the residual oil in presscake at shaft speed of 120 rpm and 0.42-mm choke opening.

high power consumption. Additionally, the high temperature at narrowest setting may cause excessive discoloration of oil. The slower speeds would give better extractions at moderate barrel temperatures. However, power consumption would be somewhat higher due to the higher pressures at slower speeds. If high throughput and moderate oil extraction were the objective, a higher speed and intermediate choke opening of 0.42 mm or 0.61 mm (i.e. choke settings 8 and 10) would be preferable.

Effect of seed heating and flaking. As the degree of heating of whole seed was increased the pressure, throughput and oil output increased, while RO decreased (Figs. 4 and 5). This pattern was in contrast with the results from cold press experiments where any decrease in RO was associated with a decrease in throughput. The pressure recorded while pressing the hottest seed (100 C) was nearly 2.4 times that recorded for the unheated seed, the throughput being 51% higher; oil output was greater by as much as 65%. Barrel temperature increased with the degree of seed heating and reached 130 C while pressing the hottest (100 C) seed.

The results available in literature on the effect of seed heating on press performance were generally sup-

portive of the above results. Ramsey and Harris (10) obtained maximum oil output from soybeans when the beans were preheated in the range 70-90 C, above which the oil output slowly decreased. Peterson et al. (11) reported that oil extraction from peanuts increased to 88% of the seed oil content at 60 C from being negligible at 22 C. They also observed, however, a slightly negative correlation between the percent oil extraction and the preheat temperature of winter rapeseed, in the range 25 to 85 C. Sivakumaran et al. (12) reported that, at an optimum moisture content, the RO was maximum when peanuts were heated to between 90 and 100 C. In experiments on sunflowerseed, Jacobsen and Backer (13) observed a steady rise in the percent oil extraction up to 75 C. The latter authors also reported that the throughput nearly doubled when the seed was heated from ambient temperature to 50 C. No further increase in the throughput was observed, however, at higher temperatures up to 75 C. In the present study, the throughput and oil output did not decrease or did not remain constant above an optimum feed temperature. Temperature treatments above 100 C might indicate optimum points for canola seed.

When the seed was flaked and pressed without heating, the throughput increased by 39%, from 10.0 kg/hr for the unheated seed (20 C seed) to 13.9 kg/hr for the unheated flakes (Fig. 4). The maximum pressure increased by 21%. Oil output was a little lower than that from whole seed (Fig. 5). The higher throughput of flakes meant a much lower residence time in the press, which was reflected in higher RO in presscake. In one experiment, the presscake from flakes with high RO was fed into the press for a second pass. Under the conditions of this run, only a little additional oil was extracted but the yield of foots increased markedly, to 15% solids in the oil from ca. 6% in oil obtained from first pressing.

As the flakes were heated, maximum pressure, throughput and oil output increased and RO decreased (Figs. 4 and 5). The results were similar to those obtained for heated whole seed. Throughput of 100 C flakes was greater than that of unheated flakes by 20%, while the oil output was greater by as much as 90%. The throughput of flakes continued to be higher than that of whole seed at corresponding heating temperatures (Fig. 4). Also, all the heated flake samples (40-100 C) gave higher oil outputs than the heated whole seed samples (Fig. 5). RO in the presscake obtained from flakes decreased at a greater rate than for seed with the increased degree of heating.

Barrel temperatures were lower than those observed during whole seed pressing up to 60 C seed temperature, but rose above those for whole seed at 80 C and 100 C (Table 2). RO values showed exactly the reverse trend; the flakes RO values were higher than seed RO values up to 60 C, but became lower at 80 C and 100 C.

The highest pressure of 18 MPa was recorded while pressing flakes heated to 100 C. This was much lower than those reported for commercial pressing operations where similarly heated flakes were pressed (1, 3). The commercial full press machines are designed to exert a maximum pressure in the range 100-150 MPa (15,000-20,000 psi), and the commercial prepresses normally exert about 30-40 MPa (5,000 psi) pressure. The high level of oil extracted in the present study at even 18 MPa was due to the small thickness of the layer of oilseed material that flowed through the laboratory press. The depth of the worm channel near the choke end of the Mini 40 press was about three mm. In the larger presses the depth would be in the range 8-15 mm. The pressure in the larger presses would have to be higher by the same order of magnitude to achieve similar levels of oil extractability and low RO with moderate residence times.

In commercial crushing plants, canola seed is flaked and then heated to 85-90 C prior to prepressing (5), or to 105-110 C prior to full pressing (16). As seen from the present results, these treatments would allow for high throughput together with a high oil output. The use of 100 C or higher temperatures in commercial processing has been reported to result in a marked reduction in the quality of oil and cake of rapeseed (16, 17) which may, in part, explain why the seed is heated to only 90 C for prepressing operations.

For a small scale operation a convenient heat source may not be available. In such a case it may not be advisable to flake the seed prior to pressing, as losses of residual oil in the presscake would be prohibitive. Also, second pressing of the cake is not likely to yield much oil but would increase the percentage of foots.

Effect of seed moisture content. Maximum pressure dropped slightly as the seed moisture was increased

TABLE 2

Effect of Seed Pretreatments on Barrel Temperature while Pressing Canola[a]

Heated whole seed		Heated flakes		Moisture conditioned seed	
Seed temp[b] (C)	Press temp[b] (C)	Flakes temp[b] (C)	Press temp[b] (C)	Seed moisture (%, d.b.)	Press temp[b] (C)
20	112	20	94	4.0	112
40	115	40	96	5.0	103
60	116	60	106	6.0	94
80	119	80	125	8.0	86
100	130	100	140	10.0	82

[a]Shaft speed, 120 rpm; choke opening, 0.42 mm.
[b]Average deviation in temperature readings ± 3 C.

from 4.0% to 5.0% and sharply thereafter (Fig. 6). Barrel temperature decreased steadily as moisture increased from the ambient 4.0% to 10.0% (Table 2). The pressure of 1.2 MPa and temperature 82 C, corresponding to the 10.0% moisture seed, were the lowest recorded during the study. Throughput and oil output data showed maxima at 5.0% moisture content in seed (Figs. 6 and 7). As the moisture content increased from 4.0% to 5.0%, throughput increased by nearly 20%, and then decreased steadily at the higher moisture levels. The throughput of 10.0% moisture seed was reduced to 68% of the maximum level at 5.0%, while the output was reduced to only 32% of its maximum. Changes in RO were opposite to those of pressure; RO increased slightly as the seed moisture increased from 4.0% to 5.0% and sharply thereafter.

Blake (9) reported that throughput of canola seed decreased and RO increased when moisture content was increased above 7%. No results were reported for moisture contents below 7%. Sivakumaran et al. (12) determined the optimum moisture content of peanuts to be 5.4% for achieving the minimum RO in presscake. No optimum moisture for minimum RO was found in the present study.

Formerly, the canola seed crushing industry adjusted seed moisture to 4.0–5.0% during full pressing where

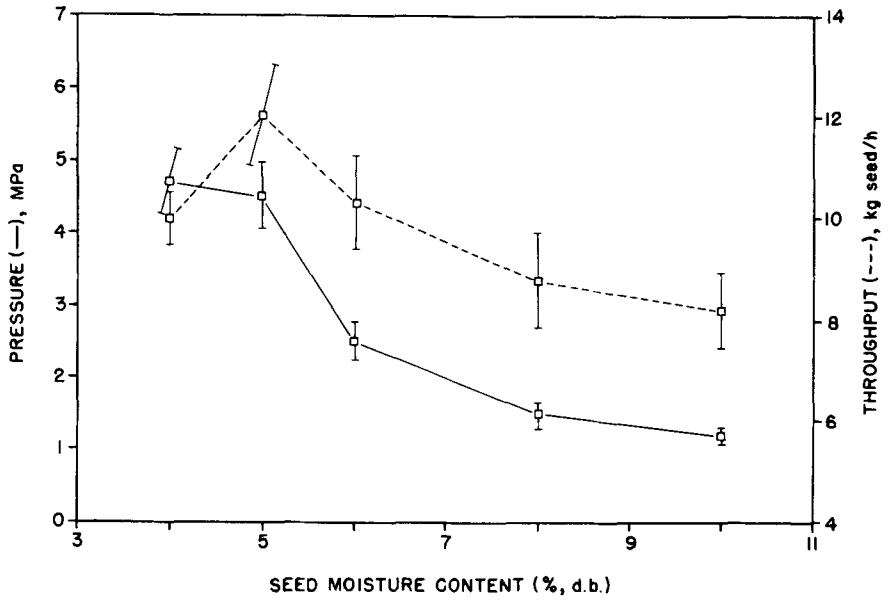

FIG. 6. Effect of seed moisture content on maximum pressure and press throughput at shaft speed of 120 rpm and 0.42-mm choke opening.

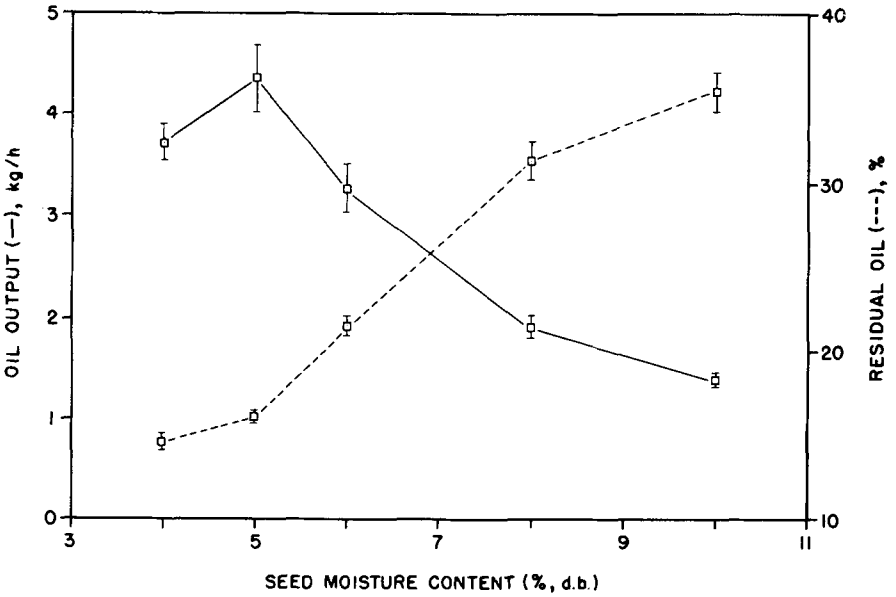

FIG. 7. Effect of seed moisture content on oil output and on the residual oil in presscake at a shaft speed of 120 rpm and 0.42-mm choke opening.

maximum press performance was the main objective (16). In the present study, the optimum moisture content for maximum throughput and oil output was found to be 5.0%. However, RO was lowest at 4.0% seed moisture. Thus, the moisture range of 4.0–5.0% would be optimum for overall press performance. It should be noted that the seed was not heated prior to pressing. A past study on peanut pressing (12) would indicate that the optimum moisture content for minimum RO may be lower at a higher seed temperature.

Interrelationships between input and output parameters of press: a simple axial flow equation. The quantitative effects of press setting and seed pretreatments observed on the various press performance parameters are summarized in Figure 8. The interrelationships between the various parameters could be explained by means of the following equation:

$$Q' = G_1 * N - (G_2/\mu) * P \quad [1]$$

where Q′ is the mass flowrate of seed through the press (i.e., throughput); G_1 and G_2 the geometric parameters of the press, multiplied by seed density; N the speed of rotation of the wormshaft; μ the apparent viscosity of the seed mass, and P the maximum pressure in the barrel, as measured near the choke end.

Equation 1 is usually used to represent the flow of material through a screw extruder (18). For the purpose of this discussion, it may be assumed to apply to axial flow through an oilseed press, or throughput, although Q′ would change continuously along the axis of the press as more and more oil is removed from the oilseed mass.

The first term on the right hand side of Equation 1, G_1*N, represents the flow due to the conveying action of rotating shaft and is called 'drag flow'. The second term, (G_2/μ)*P, is the 'back flow' caused by the pressure within the barrel. In actuality there is no back flow in the press; the material is churned within the channel instead of advancing uniformly forward, which causes a net reduction in the axial flow.

The viscosity of oilseed mass can be represented by means of a power-law model (18):

$$\mu = K*(\gamma)^{n-1}*\exp(a*F)*\exp[b/(T+273)] \quad [2]$$

where:

$$\gamma = \pi*D*N/H \quad [3]$$

and n is less than unity.

In Equations 2 and 3, K is the consistency coefficient of the seed mass; γ the rate of shear of seed mass; n the power-law index of seed mass; a and b are constants; F the oil content of the seed mass in the barrel; T the temperature of the inside barrel wall or of the seed mass in the barrel; D the inside diameter of the barrel, and H the depth of worm flights.

Press settings. It can be shown that, for an annular flow path such as the material path through choke, the pressure required to maintain the flow rate of a material varies inversely with the width of the annulus (19). This would explain the rise in maximum pressure when the choke opening was narrowed (Fig. 8). It may be seen from Equation 1 that higher pressure would reduce the throughput, Q′, when the other terms in the Equation were unchanged. The higher pressure and the longer residence time, resulting from lower throughput, would both contribute toward more oil extraction and thus would account for the lower RO at narrower openings. Also, as noted in the discussion of the two flow terms in Equation 1, the higher pressure would cause more churning of the material within the worm channel, which would lead to the generation of more heat. This would account for the higher temperatures at narrower openings.

VARIABLE	P	Q′	RO	T
PRESS PARAMETERS				
CHOKE OPENING ↓	↑	↓	↓	↑
SHAFT SPEED ↓	↑	↓	↓	↓
SEED TREATMENTS				
HEATING ↑	↑	↑	↓	↑
FLAKING	↑	↑	↑	↓
FLAKING + HEATING ↑	↑	↑	↓	↑
MOISTURE ADDITION ↑	↓	↓	↑	↓

FIG. 8. Summary of qualitative effects of press settings and seed pretreatments on pressure (P), throughput (Q′), residual oil content (RO) and barrel temperature (T).

It can be seen from Equation 3 that at lower shaft speeds, γ would be lower. A lower γ would increase the viscosity if n were less than unity (Equation 2). The value of n for the crushed canola mass was determined to be 0.13 (14). Thus, at a lower shaft speed, viscosity of the canola mass would be higher. A more viscous material would offer more resistance to flow through the press and thereby give rise to higher pressure; this phenomenon is explained in some detail in the following section. The churning of material would be much less due to the lower shaft speed, leading to lower temperatures in spite of the corresponding higher pressures (Fig. 8). From Equation 1 it was apparent that, at a lower value of N, the drag flow term would be lower. The second term would not change much as the higher P and higher μ values would tend to counter each other. As a result, the throughput would be lower at a lower speed. As explained above, for the case of results of the experiments on effect of choke opening, the higher P and lower Q′ would both contribute to a lower RO in presscake at a lower shaft speed.

Seed pretreatments. When the seed was preheated, pressure and throughput both increased (Fig. 8). This was in contrast with the results from experiments on press settings when higher pressures were associated with lower throughputs. This difference could be explained if certain changes in material viscosity were postulated. The heating of seed has been reported to bring about the coagulation or fusion of protein bodies within the individual cells of an oilseed (5, 20). This fusion could cause an increase in the viscosity of oilseed

mass. From Equation 1 it can be shown that an increase in the viscosity of oilseed mass would cause an increase in both the barrel pressure and the press throughput. If the value of μ is increased, Q′ would increase correspondingly if P were held constant, or P would increase if Q′ were held constant. The increases in P or in Q′ would be of the same order of magnitude as in μ. Because P and Q′ are interdependent in a pressing operation, any increase in μ would actually cause simultaneous increases in both P and Q′, although both would be lower in magnitude than the increase in μ.

It follows from the discussion of results on press settings that the higher pressure associated with the heated seed samples would have a favorable effect on oil extraction, but the higher throughput, i.e., lower residence time, would have an adverse effect. The heating treatment has been reported to release the oil droplets from their tiny inclusions within the individual cells, and reduce the oil viscosity, thereby rendering the oil more separable from the oilseed mass (5, 20). The combined effect of the above factors would account for the lower RO of heated samples (Fig. 8). The higher pressure and the higher internal temperature would account for the increase in barrel temperature.

When the unheated canola flakes were pressed, the pressure and the throughput both were higher than that for the unheated whole seed (Fig. 8). As discussed above, these changes could be explained by means of Equation 1 if an increase in the material viscosity due to the flaking action were assumed. It was not possible, however, to predict the relative increases in throughput and pressure which would determine the changes in RO.

The results corresponding to the heating of canola flakes, i.e., the flaking + heating treatment (Fig. 8), could be explained in the same manner as for the heating of whole seed.

The simultaneous decrease in pressure and throughput at increased moisture content could be explained on the basis of a reduction in oilseed viscosity using arguments reverse to those used above to explain the higher pressure and higher throughput at a higher viscosity. It would be of interest to note that the moisture content of several food materials has been correlated negatively with the viscosity (18). The lower pressures would account for the lower temperatures and the higher RO at higher moistures (Fig. 8).

In this discussion, material viscosity, as a single entity, has been examined as a factor affecting the performance of a press with changes in press settings and with seed pretreatments. As is seen from Equation 2, the viscosity can be represented as a composite of various terms representing the material structure (K, n, a and b) and composition (F), and the external environment factors (γ and T). It is very likely that any seed pretreatment would change most of the structural components of viscosity. The relative changes in these components might well determine the relative changes in these components might well determine the relative changes in pressure and throughput. Thus, there is a need to determine how the various viscosity components are affected by various pretreatments, and how they affect the performance of a press. Such an examination could suggest improved or novel seed pretreatments which could be used to improve the press performance. In addition, there is the consideration of how the various pretreatments affect the filtration characteristics of oilseed matrix, which would affect the outward flow of oil. This aspect was not considered in the present study.

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