Johnston, G. A. R., Triffett, A. C. K., Wunderlich, J. A., *Anal. Chem.* **40**, 1837 (1968).
Kapfhammer, J., Bischoff, C., *Z. Physiol. Chem.* **191**, 179 (1930).
Lim, F., Schall, E. D., *J. Assoc. Off. Anal. Chem.* **47**, 501 (1964).
Markley, K. S., Goss, W. H., "Soybean Chemistry and Technology", Chemical Publishing Co., Inc., Brooklyn, NY, 1944, p 13.
McCullough, T., *J. Chem. Educ.* **49**, 836 (1972).
McLean, W. F. H., Jewers, K., *J. Chromatogr.* **74**, 297 (1972).
More, C. A., Kenner, C. T., *J. Assoc. Off. Anal. Chem.* **53**, 588 (1970).
Schmidt, D. E., Speth, R. C., *Anal. Biochem.* **67**, 353 (1975).
Wang, F. L., Haubrich, D. R., *Anal. Biochem.* **63**, 195 (1975).

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Flocculants in the Separation of Green and Soluble White Protein Fractions from Alfalfa

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Fifty-four commercial flocculants were tested for their ability to improve the separation of the green chloroplastic protein fraction from alfalfa juice. From screening tests, it was determined that ten of the cationic flocculants improved the separation by centrifugation. Three of these flocculants were added at levels up to 1% without causing precipitation of the soluble white protein. At a flocculant level of 1%, a continuous high-speed centrifuge could be used to sediment the chloroplastic fraction although the processing rate was low (≤11.4 L/min). The flocculants also improved the separation of the chloroplastic fraction by membrane filtration. The permeate flux during ultrafiltration of flocculant treated juice was double that of untreated juice. The soluble protein concentration in the permeate from the treated juice was also higher than that from the untreated control.

Although leaf protein concentrates (LPC), prepared as described by Pirie (1971), have been fed to children in several trials with minimal acceptance problems (Kamalanathan and Devadas, 1975; Olatunbosun et al., 1972; Waterlow and Cruickshank, 1961), its green color and grassy taste has prevented its acceptance by the general population. A critical step in the preparation of a soluble white edible LPC is the removal of the green chloroplastic material. This material has been removed from the soluble white protein by centrifugation or ultrafiltration.

Centrifugal forces needed to separate the chloroplastic material from fresh alfalfa juice is very high (RCF_{max} 100000g for periods (>1 h). Machines capable of producing these conditions on a continuous large-scale basis are not available. Therefore, centrifugal separation on a large scale requires some type of pretreatment. Usually, a mild heat pretreatment (40–70 °C) is used to agglomerate the green fraction so that it can be easily separated from the soluble protein (Byers, 1967; Bickoff and Kohler, 1974; Cowlishaw et al., 1956; de Fremery et al., 1973; Edwards et al., 1975; Henry and Ford, 1965; Lexander et al., 1970; Subba Rau et al., 1969). The temperature necessary to agglomerate the green fraction and the amount of soluble protein which coagulates at those temperatures depend upon the type of plant (Bahr et al., 1977). When alfalfa extracts are heated to 56–65 °C, the green material can be separated in large-scale continuous centrifuges but, at these temperatures, 20–40% of the soluble protein is coagulated and separated as part of the green fraction (de Fremery et al., 1973; Edwards et al., 1975).

Ultrafiltration, which has been used to concentrate whey, skim milk, and extracts from seed and leaf tissue (Horton et al., 1972; Knuckles et al., 1975; Lawhon et al., 1973; McDonough et al., 1971; Peri et al., 1973; Porter and Michaels, 1971; Tragardh, 1978), has also been used to separate the chloroplastic and soluble protein fractions (Eakin, 1976; Eakin et al., 1978; Singh et al., 1974; Whitney and Bernardo, 1977). During ultrafiltration of fresh alfalfa juice, permeation rates decrease rapidly due to gel formation and concentration polarization. The permeation rate can be improved by use of polyelectrolytes (organic flocculants) (Eakin, 1976).

Flocculants enhance the agglomeration of suspended solids in aqueous solutions. They have been used to separate or fractionate protein from plant extracts (Anelli et al., 1977; Horisberger and Olofsson, 1976). But they are most widely used in the removal of suspended solids from municipal water and waste water (Daniels, 1973; Cohen et al., 1958; Schaffer, 1963).

This paper reports the results of a laboratory study of the effect of commercially available flocculants on the separation of the chloroplastic and soluble protein fractions in alfalfa extracts. The study includes the effects upon centrifugal and ultrafilter separations.

EXPERIMENTAL SECTION

Preparation of Juice. Chopped alfalfa, treated with

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Table I. List of Flocculants Tested

company	designation	type
American Cyanamid	Magnifloc 570C	cationic
	577C	cationic
	1820A	anionic
	1906N	nonionic
Betz	Betz 1100	anionic
	1110	anionic
	1120	anionic
	1130	anionic
	1140	nonionic
	1160	cationic
	1170	cationic
	1180	cationic
	1190	cationic
Buchman Laboratories	Bufloc 30	cationic
	Bubond 60	cationic
	63	cationic
	64	cationic
	65	cationic
Calgon	Cat Floc 17B7C	cationic
	222LOA	cationic
	TO7B7D	cationic
Hercules Inc.	Hercofloc 812	cationic
	815	cationic
	818	anionic
	821	anionic
	827	nonionic
	828	nonionic
	831	anionic
Hercules Inc.	Hercofloc 831	anionic
	834	cationic
	849	cationic
	855	cationic
	860	cationic
	864	cationic
Nalco	Nalco 607	cationic
	609	cationic
	7120	cationic
	8101	cationic
	8102	cationic
	8113	cationic
	8114	cationic
	8184	anionic
	8182	anionic
	8171	nonionic
National Starch	Natron 88	cationic
	78-3711	cationic
	6082	amphoteric
	78-1717	amphoteric
Rohm and Hass	Primafloc C-3	cationic
	C-7	cationic
	A-10	anionic
Swift	TF Floc X100	cationic
	X400	anionic
	X700	nonionic

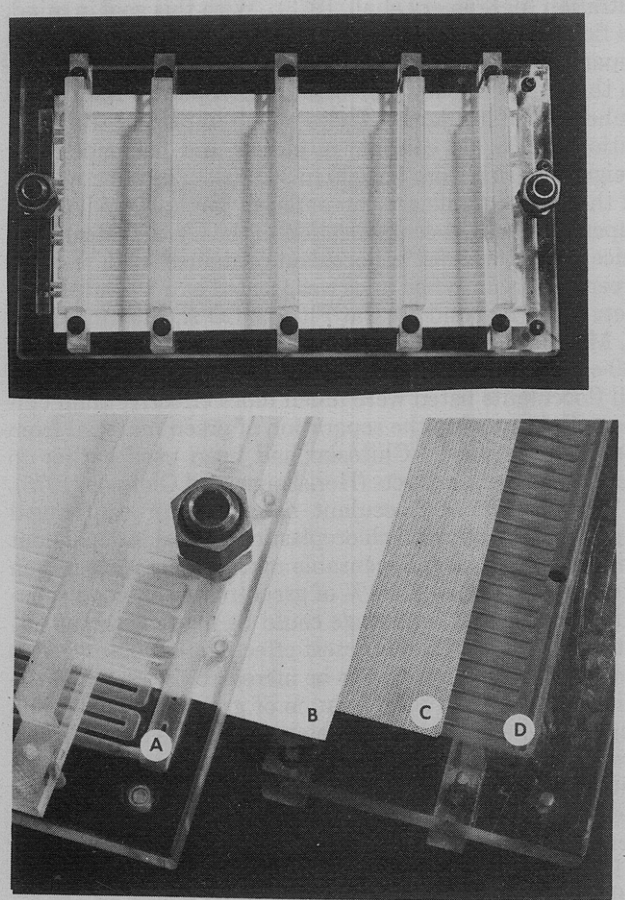

Figure 1. (a) Ultrafilter cell, assembled. (b) Ultrafilter cell, details of construction: (A) feed channels, (B) membrane, (C) perforated support plate, and (D) permeate channels.

a pH 6.2 bisulfite solution (<0.4 g of bisulfite/100 g of alfalfa) was ground in a screenless hammer mill (Owens Mfg Co., Verdon, NB) and pressed in a twin-screw press (Helipress No. 585, Bauer Bros. Co) in a manner similar to that described by Edwards et al. (1975). The juice (pH 6.2) for laboratory tests was passed through a 40-mesh screen to remove residual fiber.

Treatment of Juice. Fifty-five flocculants (Table I) from various companies were tested for their ability to effect the separation of chloroplastic protein fractions from alfalfa juice. Powdered flocculants were dissolved in water as described by the manufacturer at a level of 0.5%. Flocculants received as liquids were diluted so that the addition of juice gave the desired concentration (concentration ranged from 0 to 1.5% of juice). In some tests, a flocculant and a mild heat treatment were used in combination. The flocculant treated juice was pumped through a steam injection nozzle for heating to the desired temperature (Edwards et al., 1975). The temperature was maintained for 20 s by passing the juice through an insulated tube of appropriate length.

Centrifugation Tests. Centrifugal separation of the chloroplastic fraction was evaluated by using a GyroTester (DeLaval Separator Co.). This centrifuge is designed in such a way that a small protion of sample can be used to predict roughly the performance of large continuous centrifuges. The machine reaches maximum speed in 30 s and produces a RCF_{max} 9200*g*. Supernatant clarity was the suggested guide for evaluation. Ten-milliliter samples were centrifuged for 30 s, and the supernatant was decanted and centrifuged for 3 min at 9200*g*. The color and sediment volume of this second supernatant was used as the basis for comparison. Large-scale centrifugation studies on flocculant treated juice was limited to three continuous feed and discharge centrifuges. Two were decanter–types (P–660, RCF_{max} 4000*g* and P-3000 Super Decanter, RCF_{max} 3200*g*, Sharples-Stokes Div., Penwalt Corp). The P-660 had a nonstandard screw for solids discharge. The other centrifuge was a disc-type, high-speed, solids-discharging

Table II. Effect of Flocculants on the Centrifugal Separation of Chloroplastic Material from Alfalfa Juice

flocculant designation	concn, wt %	supernatant clarity[a] (residual sediment volume), vol %
Betz 1170	0.2	4.0
	1.0	1.0
1190	0.2	1.0
	1.0	0.3
Bubond 60	0.2	1.5
	1.0	0.4
65	0.2	3.0[b]
	1.0	1.0
Chitosan	1.0	5.0[c]
Cat Floc 22LOA	0.2	1.0[d]
	1.0	0.5
Magnifloc 570 C	0.2	1.5
	1.0	0.02
577 C	0.2	1.5
	1.0	0.2
Nalco 8113	0.2	2.0
	1.0	0.8
8114	0.2	2.0
	1.0	0.8
Primafloc C-3	0.2	1.0
	1.0	0.4

[a] Supernatant following centrifugation for 30 s was recentrifuged for 3 min. The sediment volume is expressed as percent of juice volume. Maximum sediment volume for the juice in this experiment was 10%. Unless indicated the supernatants after 3 min centrifugation were clear and yellow-brown. [b] Supernatant was hazy, greenish-yellow. [c] Supernatant was opaque, green. [d] Supernatant was cloudy, green.

centrifuge (Model BRPX-207S, RCF_{max} 14500g, DeLaval Separator Co). The juice was pumped through these centrifuges at 3.79 and 7.57 L/min. After an equilibration period, the liquid effluent was sampled over a 0.5–1-min period and tested in the Gyro-Tester for residual chloroplastic material.

Design and Operation of Ultrafilter. An ultrafilter unit (Figure 1) with 929 cm^2 active membrane area was constructed from 3.175 cm of clear plastic by routing inlet and exit manifolds and channels for juice flow. The bottom section was fitted with a stainless steel plate with 0.152-cm perforations for membrane support. The membrane (30 × 60 cm) was a cellulosic type having pores of 0.2 μm (DP 02, Amicon Corp.).

The ultrafilter unit was operated by recirculating juice through the system at a rate of 95 L/min. At this flow rate the inlet and outlet pressures were 1.4 and 0.35 kg/cm^2, respectively. The juice volume (90–100 L) was sufficiently large that the effect of concentration could be ignored in measurements of permeation rate.

Analytical Methods. Nitrogen was determined by standard Kjeldahl methods (AOAC, 1975). Protein nitrogen was assumed to be that nitrogen insoluble in 10% trichloroacetic acid. Protein was calculated as 6.25 × N. Total soluble protein in juice was measured by centrifuging at RCF_{max} 100000g for 2 h at 1 °C (Model L2, Beckman Instrument) and analyzing the clear supernatant for total and nonprotein nitrogen, the difference being protein nitrogen. To measure soluble protein in flocculant treated juice, only an RCF_{max} of 40000g (Model RC2-B, Sorvall) was needed to produce a clear supernatant. Dry matter was determined by removing most of the moisture by evaporation on a steam bath followed by final drying at 110 °C for 2 h in a force draft oven. The relative amounts of pigment present in supernatants were compared by measuring absorbance at 430 nm under standard conditions. Samples of appropriate dilution (5 mL) were mixed with an equal volume of Tris–HCl buffer (pH 8.0), and the absorbance was measured.

Table III. Soluble Protein Remaining in Supernatants following Flocculant and Heat Treatment

Magnifloc 577 C concn, wt %	heat treatment[a]	soluble protein in solution % of original
0	none	100[b]
0.5	none	100
1.0	none	98.9
0	45 °C, 20 s	83.5
0.5	45 °C, 20 s	82.0
0	50 °C, 20 s	79.9
0.5	50 °C, 20 s	79.5
0	55 °C, 20 s	76.6
0.5	55 °C, 20 s	76.3
0	60 °C, 20 s	69.4
0.5	60 °C, 20 s	67.5

[a] Juice was heated by steam injection. [b] Value for protein in supernatant following centrifugation at 100000g for 2 h. All other values were for protein in supernatants following centrifugation at 48,000 g for 20 min. All solutions were clear yellow-brown.

RESULTS AND DISCUSSION

Centrifugation Studies. The removal of the chloroplastic fraction is accomplished by centrifugation and filtration. Requirements for centrifugation are that it must reduce the green fraction to a level where subsequent filtration rates are high with a minimum quantity of filter aid. It has been suggested that 0.8 vol% is an acceptable amount of residual chloroplastic material to remove via filtration (deFremery et al., 1973). With this goal in mind, 54 flocculants were tested for their effect on the centrifugal separation. Only a few of the flocculants caused a change in the sedimentation rate of the chloroplastic fraction in unheated alfalfa juice. Those which improved the separation are highly cationic in nature and had molecular weights ranging from 100 000 to 300 000. The effectiveness of these flocculants are compared in Table II. All of the supernatants (30-s centrifugation in the Gyro-Tester) from juice treated with 0.2% flocculant contained >1% residual green material. When juice was treated at a 1% level with Betz 1190, Bubond 60, Cat Floc 22 LO A, Magnifloc 570 C, Magnifloc 577 C, and Primafloc C-3, the supernatants (30-s centrifugation) contained <0.5% residual sediment. All flocculants listed were much more effective than chitosan in improving the separation of green material from fresh alfalfa juice. Chitosan had been used earlier to fractionate plant extracts (Horisberger and Olofsson, 1976).

The amount of flocculant necessary for centrifugal separation of the green chloroplastic material is dependent on the amount of sedimentable material present. When the juice contained 5 vol% of green material, good separation in the test centrifuge could be made with the addition of 0.2% of the four most effective flocculants. The amount of flocculant had to be increased to 1% when the juice contained 20% by volume of green material. The heat treatments were kept below 60 °C, the temperature previously used to obtain separation in continuous centrifuges (Edwards et al., 1975). The use of heat decreased the requirement for flocculant but losses of soluble protein were observed.

In the following paragraphs, flocculant refers to Magnifloc 577 C unless otherwise noted.

Table III shows that at room temperature the addition of flocculant at levels up to 1% caused almost no decrease in soluble protein. The soluble protein content decreased with increasing temperature. When flocculant and heat treatments were combined, the amount of soluble protein

Table IV. Residual Chloroplastic Material in Alfalfa Juice following Addition of Flocculant and Large-Scale Centrifugation

centrifuge type and model	Magnifloc 577 C concn, wt %	feed rate, L/min	residual sediment in supernatant[a] vol %
decanter, P-660	0	1.9	20
	0.5	1.9	20
	1.0	1.9	10
decanter, P-3000	0	1.9	20
	0.5	1.9	20
disc, BRPX-207	0	3.8	10
	0.5	3.8	2
	1.0	3.8	0.5

[a] Residual chloroplastic material as determined by centrifuging in the Gyro-Tester for 3 min. Expressed as percent of sample volume. Maximum sediment volume was 20%. Twenty percent values indicate no separation.

in solution was the same as that following heat treatment alone. The decrease in soluble protein content was probably due to the heat treatment and not the flocculant.

Experiments with the continuous centrifuges showed that the decanter types were not as effective as the disc type in removing the chloroplastic material from flocculant treated juice (Table IV). This was expected from the relative *g* force developed by each type of machine. At flocculant levels below 1%, there was almost no sedimentation of chloroplastic material in the decanter centrifuges. The residual sediment was reduced to 0.1 vol% in the P-660 if juice treated with 0.5% flocculant was also heated to 62.5 °C. However, in this case, 42% of the soluble protein was lost. The disc type centrifuge could reduce the residual sediment to 0.5% when 1% flocculant was added but the feed rate is too low for economical processing. These centrifuges did not perform as well as expected from the laboratory data. Possibly this is due to the effects of the high shear rates experienced by the feed as it is accelerated up to the peripheral bowl speed. These forces, which are not present when tested with the Gyro-Tester, can reduce effective floc size.

Prevention or removal of brown color is important in the preparation of an edible protein concentrate from plant extracts. It was visually observed during the centrifugation tests that there was less brown color in the supernatants from the flocculant tested juice than in the control samples. Subsequent tests showed the 430-nm absorbance for juice treated with 0, 0.25, 0.5, and 1.0% cationic flocculant to be 0.18, 0.17, 0.15, and 0.13, respectively. The improvement at 1% flocculant was about 28%. Normally, reducing agents or polyvinylpyrrolidone are used to inhibit or to remove precursors of brown pigments (Anderson and Rowan, 1967; Bickoff and Kohler, 1974; Edwards et al., 1975; Loomis and Battaile, 1966).

Ultrafiltration Studies. Flocculants (Magnifloc 577 C and Betz 1190) improved the permeation or flux of liquid through the ultrafilter membrane (Figure 2). The initial flux for flocculant treated juice was 43–56% greater than that for the control. The flux rates for treated juice remained higher throughout the 3-h processing period. At 0 and 0.5% flocculant, flux rates dropped off very rapidly during the first 30 min, in agreement with earlier work (Eakin, 1976). However, much lower rates of decrease were found when Betz 1190 was added at the 1% level. At the end of 3 h the flux rate was still over twice that obtained with untreated juice.

Soluble protein flux was also improved by the addition of flocculants. The improvement was due to both higher-liquid permeation rates and to higher protein concentrations in the permeate. Figure 3 shows the soluble protein concentration in permeates collected at intervals over a 3-h period. The soluble protein concentration in permeate from treated juice was twice that in permeate from untreated juice probably because of changes in the formation and characteristics of the gel layer, a phenomenon described by Porter and Michaels (1971). The soluble protein fluxes, calculated from the 3-h average for protein concentration and liquid flux, were 2177 g/m^2 day for juice treated with 1% flocculant and 639 g/m^2 day for untreated juice. This is a significantly larger improvement than that reported by Eakin (1976) for juice with 0.25% flocculant.

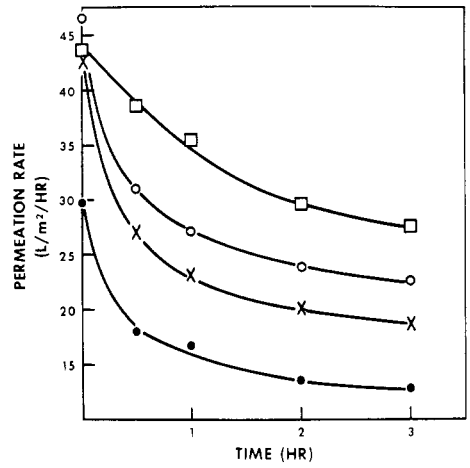

Figure 2. Effect of flocculant on permeation rate during ultrafiltration of alfalfa juice. Operating parameters, temperature = 24 °C, linear velocity = 3.76 m/s. No flocculant (●–●), Magnifloc 577 C (0.5%) (○–○), Betz 1190 (0.5%) (x–x), Betz 1190 (1.0%) (□–□).

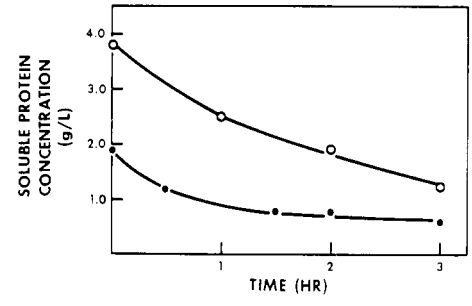

Figure 3. Effect of flocculant on soluble protein in ultrafilter permeates. Soluble protein in supernatant (100000*g*) was 10 mg/mL. Operating parameters, temperature = 24 °C, linear velocity = 3.70 m/s. No flocculant (●–●), Magnifloc 577 C (1%) (○–○).

CONCLUSIONS

The use of cationic flocculants improves the separation of chloroplastic material from alfalfa juice by either centrifugation or by membrane filtration. Further work is needed to determine if the use of flocculants is economically justified.

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LITERATURE CITED

Anelli, G., Fiorentini, R., Massignan, L., Galoppini, C., *J. Food Sci.* **42**, 1401 (1977).

Anderson, J. W., Rowan, K. S., *Phytochemistry* **6**, 1047 (1967).

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, DC, 1975.

Bahr, J. R., Bourque, D. P., Smith, H. J., *J. Agric. Food Chem.* **25**, 783 (1977).

Bichkoff, E. M., Kohler, G. O., U.S. Patent 3 823 128, July 9, 1974.
Byers, M., *J. Sci. Food Agric.* **18**, 33 (1967).
Cohen, J. M., Rourke, G. A., Woodward, R. L., *J. Am. Water Works Assoc.* **50**, 463 (1958).
Cowlishaw, S. J., Eyles, D. E., Raymond, W. F., Tilley, J. M. A., *J. Sci. Food Agric.* **7**, 775 (1956).
Daniels, S. L., *AIChE Symp. Ser.* **70**, 266 (1973).
de Fremery, D., Miller, R. E., Edwards, R. H., Knucles, B. E., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **21**, 886 (1973).
Eakin, D. E., M.S. Thesis, University of California, Davis, 1976.
Eakin, D. E., Singh, R. P., Kohler, G. O., Knuckles, B. E., *J. Food Sci.* **43**, 544 (1978).
Edwards, R. H., Miller, R. E., De Fremery, D., Knuckles, B. E., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **23**, 620 (1975).
Henry, K. M., Ford, J. E., *J. Sci. Food Agric.* **15**, 425 (1965).
Horisberger, M., Olofsson, M., British Patent 1 430 566, March 31, 1976.
Horton, B. S., Goldsmith, R. L., Zall, R. R., *Food Technol.* **26**, 30 (1972).
Kamalanathan, G., Devadas, R. P., Presented at the Tenth International Congress on Nutrition, Kyoto, Japan, Aug 3–9, 1975.
Knuckles, B. E., de Fremery, D., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **23**, 209 (1975).
Lawhon, J. T., Lin, S. H. C., Cater, C. M., Mattil, K. F., *Food Technol.* **27**, 26 (1973).
Lexander, L., Carlsson, R., Schalen, V., Simonsson, A., Lundborg, T., *Ann. Appl. Biol.* **66**, 193 (1970).
Loomis, W. D., Battaile, J., *Phytochemistry* **5**, 423 (1966).
McDonough, F. E., Mattingly, W. A., Vestal, J. H., *J. Dairy Sci.* **54**, 1406 (1971).
Olatunbosun, D. A., Adadevok, B. K., Oke, O. L., *Niger. Med. J.* **2**, 195 (1972).
Peri, C., Pompei, C. Rossi, F., *J. Food Sci.* **38**, 135 (1973).
Pirie, N. W., in "Leaf Protein: Its Agronomy, Preparation, Quality, and Use", IBP Handbook 20, Blackwell Scientific Publications, Oxford, 1971.
Porter, M. C., Michaels, A. S., *Chem. Technol.* **1**, 56 (1971).
Schaffer, R. B., *Eng. Bull. Purdue Univ., Eng. Ext. Ser. Purdue* **115**, 447 (1963).
Singh, R., Whitney, L. F., Chen, C. S., Presented at the Annual ASAE Meeting, Oklahoma State University, Stillwater, OK, June 23–26, 1974.
Subba Rau, B. H., Mohadeviah, S., Singh, N., *J. Sci. Food Agric.* **20**, 355 (1969).
Tragardh, C., in "Leaf Protein for Human Consumption" (investigations of some unit operations included in solvent extraction process), Div. Food Eng. Chemical Center, Lund University, Lund Institute of Technology, Alnaup, Sweden, June, 1978.
Waterlow, J. C., Cruickshank, E. K., in "Meeting Protein Needs of Infants and Children" (preliminary trials in refeeding malnourished infants with LPC), N.A.S.-N.R.C., Publ. 843, 1961, p 69.
Whitney, L. F., Bernardo, M. C., Presented at the Annular ASAE Meeting, North Carolina State University, Raleigh, NC, June 26–29, 1977.

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Evaluation of Rapeseed Protein Concentrate as a Source of Protein in a Zinc Supplemented Diet for Young Rats

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In the first experiment 32 male Wistar weanling rats were divided into four equal groups on the basis of their body weights. The control group (D1) was fed 20% protein from casein and the other three groups received diets D2, D3, and D4 containing the same level of protein from Tower (*Brassica napus*) rapeseed protein concentrate (RPC). The amount of zinc added as $ZnSO_4$ to the four diets was 13, 100, 150, 200 μg/g, respectively. From the body weights and zinc levels in the liver and femur of the rats after a 4-week feeding period, the optimal level of zinc supplementation was found to be 150 μg/g. In the second experiment 48 weanling rats were allocated equally to three diet groups. The casein diets D5 and D6 contained 13 and 150 μg/g of zinc, respectively, and the RPC diet D7 had 150 μg/g of zinc. At the end of feeding for 8 and 16 weeks, there was no consistent and significant effect of feeding RPC on body weight, levels of zinc, iron, copper, manganese, and calcium in serum, liver, testes, and femur. Only the magnesium concentration in serum and femur was elevated but it did not result in any gross adverse effects. The thyroids of the RPC-fed rats were larger than those of the controls.

The optimal level of zinc supplementation of a diet for young rats, containing 20% protein from Tower (*Brassica napus*) rapeseed protein concentrate (RPC), was suggested to be 150–200 mg/kg diet (Shah et al., 1979). Using a 10% protein diet, Anjou et al. (1978) did not find any improvement in the PER (protein efficiency ratio) value for Span (*Brassica napus*) RPC by adding more than 70 mg/kg zinc to the diet of young male Sprague-Dawley rats. Moreover, they reported that the PER value (3.5) for RPC was much higher than other protein sources including textured soy flour, soy protein isolate, ground meat mixture, and casein. This level of zinc supplement is in general agreement with the above suggestion for a 20% protein diet. It is necessary, however, to determine the exact level of zinc supplement required in a rat diet containing 20% protein from RPC and to determine in a long-term study

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