CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (r. 82/08)

Canada

PREPARATION OF LOW-PHYTATE RAPESEED PROTEIN

by

ROBERT DY SIY

Thesis presented to the School of Graduate Studies
of the University of Ottawa as partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Chemical Engineering

Ottawa, Ontario

ACKNOWLEDGEMENTS

The author is indebted to his research supervisor, Dr. F.D.F. Talbot, for guidance, support, encouragement, and aid throughout this work.

The author wants to express his appreciation to S. Sourirajan and T. Matsuura of Chemical Engineering, Division of Chemistry, National Research Council of Canada (Ottawa), for their valuable academic and technical advice; to J. Kramer of Agriculture Canada (Ottawa), for his interest and valuable ideas; to J.D. Jones and J. Holme of Agriculture Canada (Ottawa), and M.N. Radwan and B.C.-Y. Lu of the University of Ottawa for providing the rapeseed samples; and to G. Gasperetti, A. Bonaldo, and D. Lefebvre for their assistance in construction, and maintenance of the ultrafiltration cells and other equipment.

The author is also grateful to the Natural Sciences and Engineering Research Council of Canada for the financial support of this research.

ABSTRACT

A process for producing nutritionally rich low-phytate or phytate-free rapeseed protein meal, concentrate, and isolate was presented. The process consisted of the removal of the total phytate from the deoiled rapeseed meal or flour by aqueous extraction. This was then followed by the separation of the phytate from the aqueous extract by continuous diafiltration prior to the concentration of the dissolved protein by ultrafiltration.

The results of the extraction experiments showed that phytate was effectively and completely removed from the Tower and Candle variety rapeseed meal and flour using dilute aqueous sodium chloride solution. Single stage extraction experiments were carried out varying the solvent to rapeseed feed ratio (v/n) from 10 to 500 ml of extraction solution per gram of sample and using an extraction solution containing 0 to 10 (w/v) % sodium chloride. The initial pH of the extraction solution was varied from 3 to 10. The extent of phytate dissolution in aqueous sodium chloride solution was found to be independent of the type of the rapeseed and of the initial pH of the extraction solution, but was dependent on the sodium chloride concentration and on the v/n ratio. In the case of protein, it was found to be independent of the v/n ratio and of the initial pH of the

extraction solution, but was dependent on the sodium chloride concentration, and on the type of rapeseed and its processing history. Furthermore, it was demonstrated that the degree of phytate removal and protein loss in a multistage extraction process can be estimated graphically using the equilibrium diagrams constructed from the experimental data.

The feasibility of using ultrafiltration to eliminate phytate from the rapeseed extracts was studied. With a proper choice of casting solution composition and conditions, high flux cellulose acetate membranes suitable for the phytate-protein separation were effectively created. The efficiency of the cast cellulose acetate membrane on phytate-protein separation was greatly influenced by the solution pH. The presence of sodium chloride in the process solution was essential to obtain a high degree of phytate permeation. In addition, it was necessary to operate at a high feed flow rate and at a low operating pressure in order to obtain the highest possible phytate-protein separation. A complete removal of phytate from dissolved protein was attainable with a relatively low protein loss by continuous diafiltration.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Rapeseed is advancing to become one of the most important sources of vegetable oil. It is now Canada's second most valuable crop, next to wheat. The crop has expanded rapidly, reaching 3.4 million hectares in 1979 (Table I). The importance of the crop to the Canadian economy will continue to grow as new improved quality rapeseeds are being developed.

Rapeseed is used primarily for its oil. The oil is refined for use in the manufacture of margarine and shortenings, and salad and cooking oil. The remaining meal is used as a supplement in livestock and poultry feeds. The interest in developing the meal as a new source of food quality protein is due to the facts that it represents one of the most concentrated sources of plant protein; and it has a well-balanced amino acid composition. Rapeseed can therefore play an important role in alleviating the world's protein shortage for both nutritional and economic reasons. However, there are antinutrients associated with the rapeseed that must be reduced or eliminated namely, erucic acid in the oil, and glucosinolates and phytates in the meal. Glucosinolates and erucic acid are the antinutritional factors which are themselves physiologically deleterious. Phytates, on the other hand, are the antinutritional factors which induce physiological deficiency, i.e. , they affect the availability of minerals in the diet.

Table 1 Rapeseed area - yield, production and value (1)

Crop Year	Area (ha.)	Yield (t/ha)	Production (Tonnes)	Avg.Farm Price ($/t)	Total Value (Dollars)
1943-44	1,300	0.78	1,000	---	---
1944-45	4,400	0.63	4,000	---	---
1945-46	5,100	0.75	4,000	---	---
1946-47	9,500	0.62	6,000	---	---
1947-48	23,600	0.42	10,000	---	---
1948-49	32,400	0.90	29,000	---	---
1949-50	8,100	0.95	8,000	110.00	850,000
1950-51	200	0.34	50	84.00	4,600
1951-52	2,600	1.04	3,000	77.00	210,000
1952-53	7,500	0.84	6,000	75.00	472,600
1953-54	11,900	0.93	11,000	79.00	883,800
1954-55	16,200	0.81	13,000	73.00	953,700
1955-56	55,800	0.63	35,000	77.00	2,726,800
1956-57	142,400	0.95	136,000	77.00	10,493,700
1957-58	249,900	0.79	196,000	71.00	13,857,300
1958-59	253,300	0.70	176,000	55.00	9,702,500
1959-60	86,400	0.94	81,000	88.00	7,120,000
1960-61	308,800	0.82	252,000	73.00	18,348,000
1961-62	287,400	0.89	254,000	79.90	20,196,000
1962-63	150,200	0.89	133,000	90.00	12,013,000
1963-64	193,400	0.98	190,000	110.00	20,900,000
1964-65	320,100	0.94	300,000	112.00	33,604,200
1965-66	580,700	0.89	517,000	103.00	53,124,000
1966-67	617,100	0.95	585,000	109.00	63,760,000
1967-68	655,600	0.85	560,000	85.00	47,506,000
1968-69	425,700	1.03	400,000	81.00	35,472,000
1969-70	814,200	0.93	757,000	101.00	76,494,000
1970-71	1,639,000	1.00	1,637,000	103.00	168,179,000
1971-72	2,147,200	1.00	2,155,000	95.00	205,530,000
1972-73	1,323,300	0.98	1,300,000	161.00	208,570,000
1973-74	1,274,700	0.95	1,207,000	280.00	337,836,000
1974-75	1,278,800	0.91	1,163,000	319.00	370,753,000
1975-76	1,829,200	1.01	1,839,000	227.00	390,659,000
1976-77	719,500	1.16	837,000	289.00	241,705,000
1977-78	1,452,800	1.36	1,973,000	296.00	584,008,000
1978-79	2,824,700	1.24	3,498,000	280.00	979,400,000
1979-80	3,440,000	1.04	3,562,000		

The erucic acid and glucosinolate contents of rapeseed have been successfully reduced through selection processes. Improved varieties having these characteristics are in commercial production since 1975. In fact, over 80 percent of the rapeseed grown in Canada is now of these varieties. Methods to remove glucosinolates by physical means, i.e., heat treatment, leaching, and fermentation processes, are now no longer necessary. Unfortunately, the phytate content of rapeseed throughout the selection processes remained high. Phytates are the principal storage of phosphate and inositol in oilseed, and as such may not be easily removed through plant breeding programs. Moreover, in the removal of glucosinolates by leaching methods, phytates are mostly left intact with the rapeseed proteins. Since phytate is known as an antinutrient, its presence as a major impurity in the rapeseed proteins is of nutritional concern. A comparison of phytate content among oilseeds and protein products is given in Appendix D.

Nutritionally, phytate binds strongly with several essential minerals forming phytate - mineral or protein - phytate - mineral complexes. These complexes may be insoluble or unavailable under physiologic conditions, thus making the complexed minerals also unavailable. Phytate is therefore capable of inducing a wide variety of physiological deficiencies depending on whichever is the first limiting element in a specified diet. The effect of phytate

on mineral availability may be influenced by other minerals in the diet, nature of dietary protein, pH, processing condition, and possibly other factors.

Phytate, which is the metallic salt of phytic acid was encountered as early as 1872. However, the structure of phytic acid was only recently established as shown in Figure 1. The proper chemical designation of phytic acid is myo - inositol 1,2,3,4,5,6 - hexakis (dihydrogen phosphate). For simplicity , the common terms phytic acid and phytate are used for myo - inositol hexaphosphate and its salts, respectively. The chemical composition and structure of phytate as existing in nature have not yet been defined. It has been suggested that phytate exists in seeds as phytin, the calcium - magnesium - potassium salt of phytic acid.

To date, there has been no published work on the removal or elimination of phytate from the rapeseed protein, and there are only very few reports on its removal from other plant proteins. It is therefore the objectives of this project (1) to determine a most favourable condition for aqueous extraction of phytate from deoiled rapeseed meal, and (2) to develop a suitable membrane process and the corresponding conditions to effectively remove the extracted phytate from the dissolved proteins prior to the recovery of low - phytate protein isolates.

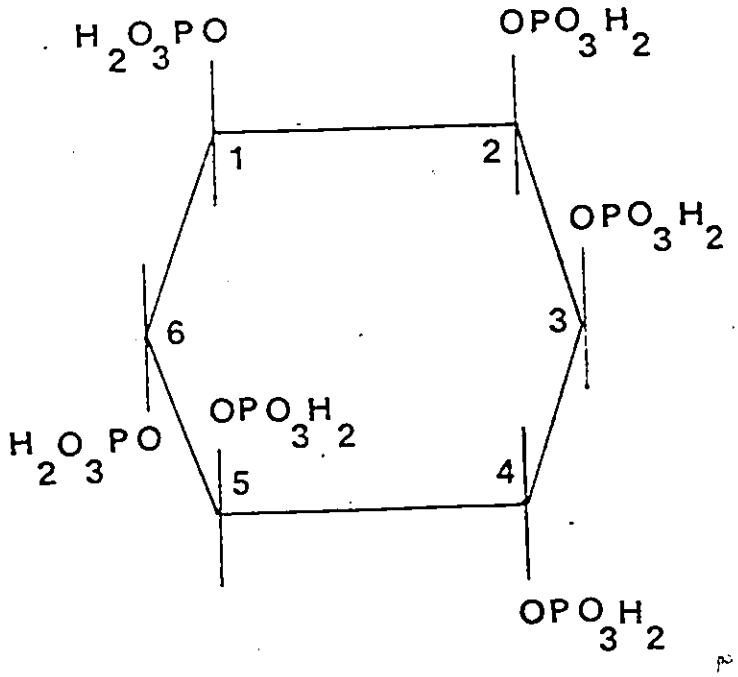


Figure 1 Structure and configuration of Phytic acid, myo-inositol 1,2,3,4,5,6 - hexakis (dihydrogen phosphate)

LITERATURE SURVEY

Origin and History of Rapeseed Industry in Western Canada

The root word " rape " in rapeseed is derived from the Latin word " rapum " meaning turnip. Turnip, cabbage, brussel sprout, and mustard are some of the close relatives of rapeseed. Rapeseed is an oil - bearing seed from the plants of the Brassica genus. It grows well in the cooler areas of the world's agricultural regions, such as Western Canada. Some rapeseeds are developed from a mixture of two or more types of rapeseed species. A detail of the botany of rapeseed is available (2).

Ancient civilization in Asia and along the Mediterranean had recorded the use of rapeseed oil as illuminating oil and cooking oil. The crop was only introduced to Europe in the 13th century. With the development of steam power , rapeseed oil was used as a lubricant since it clinges to water and steam washed surfaces better than the other known lubricants.

In 1943, rapeseed was commercially produced in Western Canada for the war effort as a source of lubricating oil for the Allied Navies. It was in 1956 that rapeseed oil was commercially extracted in Canada for used as an edible oil. This marked the beginning of a fast growing western-based industry. Presently, it has become the most widely used edible

oil in the domestic market, and Canada has become the world's largest producer and exporter of rapeseed (Table 2).

Throughout the short history of rapeseed in Western Canada, the quality and nutritional aspects of the rapeseed oil for human consumption and the rapeseed meal as a livestock supplement have been questioned. Primary concerns have been the presence and amount of erucic acid in the oil and glucosinolates in the meal. In response to challenges to save the industry, enormous amount of research and development work has been carried out to improve the rapeseed quality through extensive breeding programs and to improve the rapeseed products through better processing techniques.

Table 2 (1)

Rapeseed production in major producing countries ('000 Tonnes)

	1974	1975	1976	1977	1978	1979*
NORTH & SOUTH AMERICA						
Canada	1,163	1,839	837	1,973	3,498	3,562
Chile	33	61	64	70		
Estimated Total N.& S. America	1,198	1,810	1,002	1,846		
EUROPE						
France	691	500	561	421		490
W. Germany	310	199	222	282		305
Netherlands	45	37	34	30		18
Denmark	120	130	80	90		155
United Kingdom	55	61	109	142		198
Estimated Total E.E.C.	1,228	930	1,011	969		
Finland	17	25	22	35		
Sweden	340	330	250	233		251
Switzerland	28	20	24	21		
Estimated Total West Europe	1,616	1,330	1,307	1,258	1,570	1,490
Czechoslovakia	93	130	131	134		100
E. Germany	300	364	318	330		260
Poland	524	700	983	672		450
Hungary	50	60	68	70		80
Eatimated Total East Europe	961	1,284	1,505	1,246	1,360	730
AFRICA						
Estimated Total Africa (mostly Ethiopia)	20	20	20	20		
ASIA						
China	1,075	1,300	1,300	1,350	2,030	2,650
India	1,692	2,252	1,936	1,562	1,880	1,800
Pakistan	305	248	267	293	260	260
Bangladesh	100	110	120	115		
Korea (estimated)	34	33	33	35		
Estimated Total Asia	3,392	3,995	3,673	3,376		
AUSTRALIA						
Estimated Total Australia	11	9	20	8		
ESTIMATED WORLD TOTAL	7,210	8,454	7,525	7,969	10,880	11,010

* Estimated production

Research and Development

Oil Quality Improvement

Originally, rapeseed oils were unique among the oil seeds because of their high levels of erucic acid, (C22:1), which made up from 33 - 50 per cent of the fatty acids in rapeseed oil triglycerides. The possible health hazard of rapeseed oil due to its high content of erucic acid was suggested in the 1950's (3). In 1968, the rapeseed breeders responded with the release of Oro, Canada's first low erucic acid rapeseed (Lear) variety.

In 1970, experimental tests disclosed that animals fed a diet containing high erucic acid rapeseed oil caused fat accumulation in the heart muscle (cardiac lipidosis) in short term feeding trials, and some necrotic cardiac lesions in extended feeding trials (4, 5). Thus, in the same year the Canadian government recommended the switch over to Lear production and in 1973 decreed that all rapeseed oil for edible purpose should have no more than 5 % erucic acid content (5a).

In 1972 , the Canadian rapeseed breeders responded with the development of two Lear varieties, Zephyr and Span. Two other Lear varieties, Midas and Torch, were introduced in 1973. By 1977, the average erucic acid content of the Canadian crop was less than 2 per cent (Figure 2).

The reduction of erucic acid in rapeseed oil has corrected the cardiac lipidosis problem in laboratory rats (7).

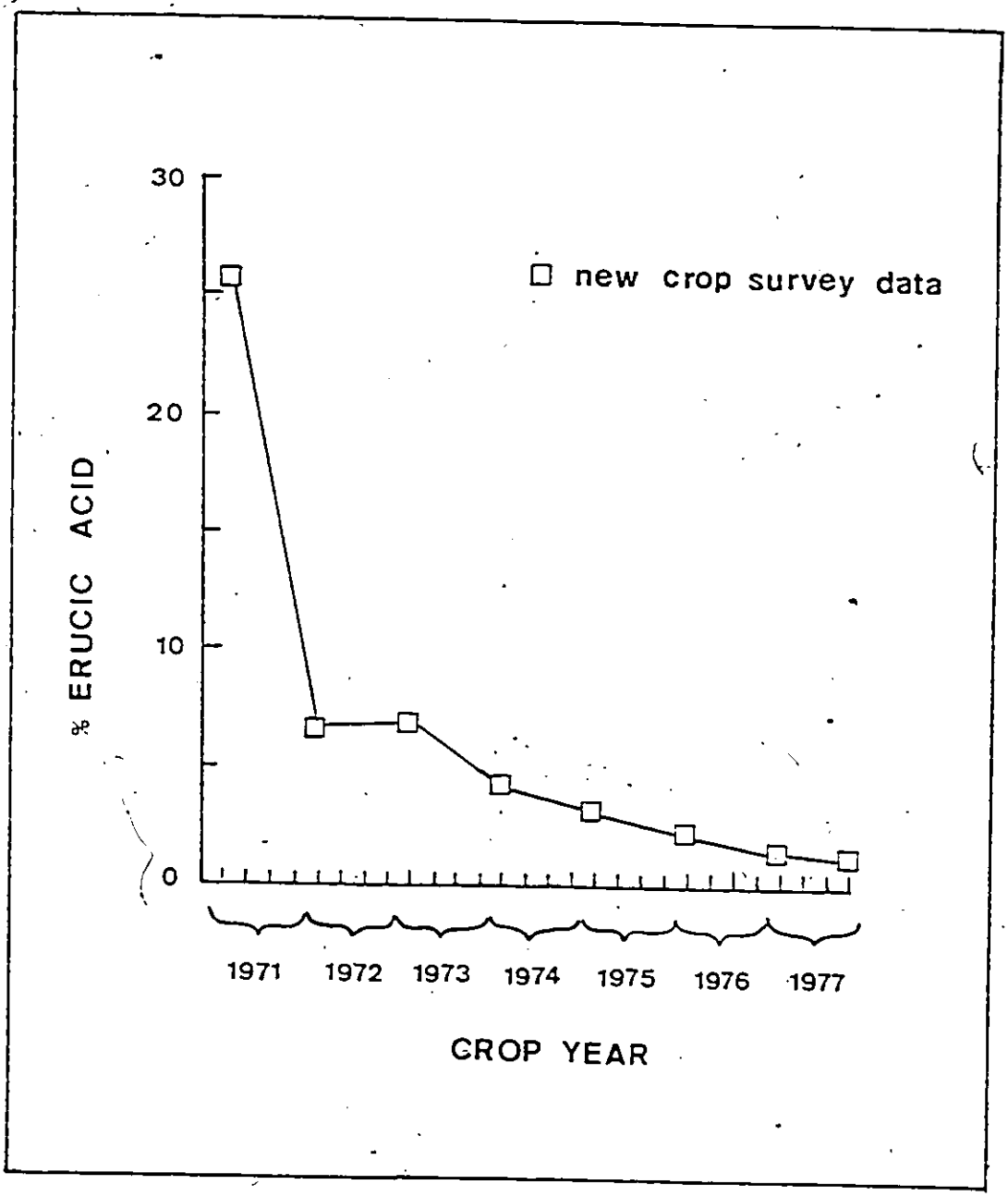


Figure 2. Erucic acid content of Canadian rapeseed crops (6)

The incidence and severity of necrotic lesions in long term feeding studies using male rats were also abated (8). Male rats had been reported to be susceptible to the development of cardiac lesions even when fed with diet containing low erucic acid rapeseed oil (9). Kramer et al. (10) questioned the physiological significance of these heart lessions in rats, since they also occured in rats fed control diets. The authors further pointed out that 26 monkeys after being fed with a diet containing low erucic acid rapeseed oil for 6 months, showed no evidence of cardiac lesions. Furthermore, swine showed no difference in heart lesion incidence whether their diet contained rapeseed oil or not (11). The suggestion made was that the cardiac lesions in rats are due to a fatty acid imbalance (10).

Meal Quality Improvement

The toxicological effects of rapeseed meal was disclosed in 1944 when it was observed that rapeseed meal fed to poultry caused thyroid enlargement (1). The toxicological problems associated with the meal were found to be due to the presence of glucosinolates (thioglucosides). Under the influence of moisture and the enzyme myrosinase (thioglucoside glucohydrolase E.C.3.2.3.1), glucosinolates can be reduced to glucose, potassium persulfate, isothiocyanates, nitrile, and thiocyanates (Figure 3). The resulting products from glucosinolates are determined by the condition of seed storage, the treatment during the defatting operation

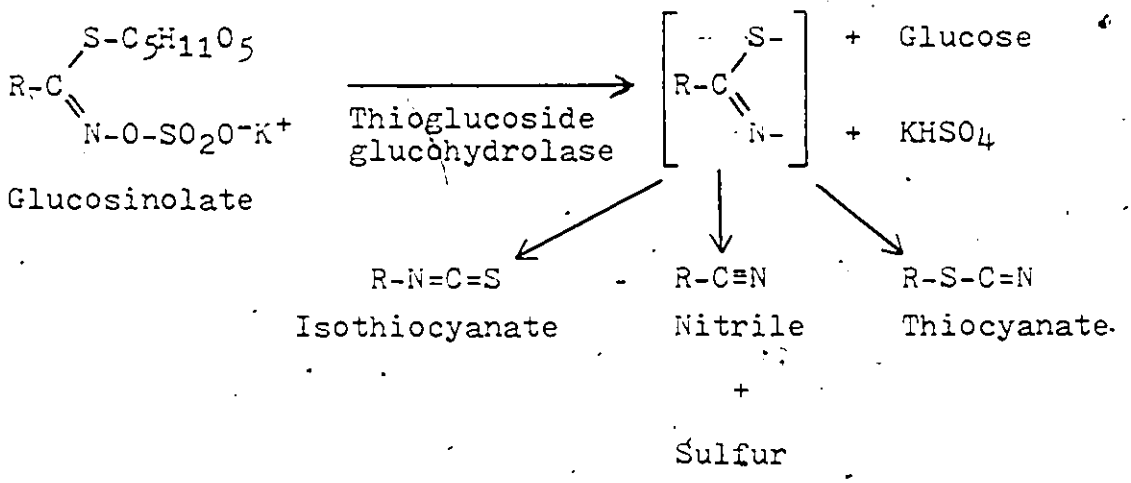


Figure 3 The general structures of glucosinolates and products formed by enzymatic hydrolysis, followed by chemical rearrangement reaction. The thiocyanates are generally not formed in the autolysis of Brassica seed meals. (12)

and the conditions at the time of enzymatic activity. The resulting products can be either unsaturated nitriles or isothiocyanates, known to have goitrogenic activity. The hydroxy-

isothiocyanates undergo cyclic rearrangements to form the compounds called goitrin because of its goitrogenic activity. The goitrins cause mild hyperplastic goiter with slight reduction in weight gain and mild liver enlargement (13 - 16) . The nitriles tend to have greater acute toxicity than the goitrins, and they can cause pathological kidney and liver lesions. The elimination of glucosinolates from the rapeseed meal is therefore a prerequisite to its use as a source of protein for animal feed and human food. Attempts were therefore made to modify the glucosinolates chemically or to leach them out of the crushed seed (17 - 22). Most of these detoxification processes consist of some variation of heat inactivation and/or aqueous extraction.

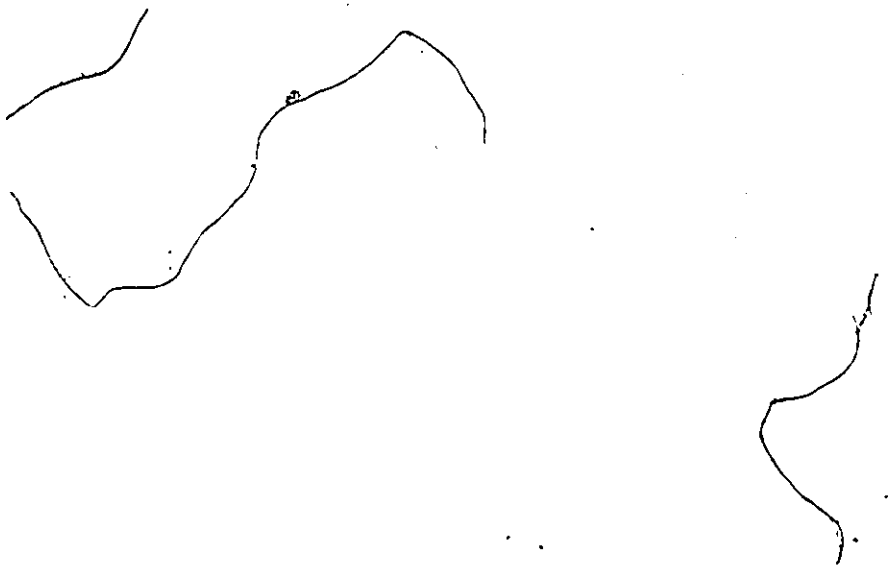
With the discovery of a very low glucosinolate Polish summer rape variety, " Bronowski ", in 1967, plant breeders started to incorporate these low glucosinolate characteristics into varieties with suitable agronomic features. Since it was found that the level of glucosinolates is mainly controlled by the genotype of the maternal plant, and that the levels of erucic acid and glucosinolates are inherited independently of each other (23), it is therefore possible to breed the so-called " double low " rapeseed varieties. These varieties are characterized by very

low levels of erucic acid and glucosinolates. Remarkable advancements have been achieved in the development of these " double low " rapeseeds. Rapeseed meal and oil from these newly developed varieties have improved to such as extent that they bear little resemblance to their predecessors. The currently grown rapeseed varieties in Canada are of the Argentine (Brassia napus) and Polish (Brassis campestris) species. Present cultivars within these species are low in both erucic acid and glucosinolates, and are now referred to as " Canola ". The term is used to denote oil, meal, protein extractions, seed and seed hulls from/of rapeseed varieties with 5 per cent or less of erucic acid in the oil and 3 milligrams per gram or less of normally measured glucosinolates in the meal.

New Rapeseed Varieties (Canola)

Tower, the first " double low " rapeseed of the Argentine type variety, was introduced in 1974. Among the newer " double low " Argentine type rapeseeds introduced are Regent and Alt ex. Regent and Tower are of late maturing varieties and Alt ex is an early ripening variety. Early maturing Polish type Candle variety was developed as a companion to the late maturing variety in order to help meet the increasing demand of " double low " rapeseeds. Candle, besides being " double low ", has a thinner seed coat. Instead of the usual black or brown hull, it has a yellow-brown hull which is an indication of higher

oil and protein content and less fibre in the meal. These are highly desirable characteristics. Candle is presently one of the common rapeseed crops grown in the Prairie. A historical outline of the development of improved rapeseed varieties is presented by Nagy and Furtran (3) and is available in Appendix C.



Nutritional Value of Detoxified Rapeseed Protein

Nutritional evaluation of detoxified rapeseed protein concentrate indicated that its protein efficiency ratio (PER) is equal to that of methionine enriched casein (23). Campbell and Eggum (24) reported the nutritional value of three canola meals using standard rat balance-trial technique. Their results showed that the three canola are of excellent nutritional quality as indicated by their biological value of greater than 90 per cent. However, due to the relatively low digestibility of the protein, the net worth of the canola meals as protein source is reduced. They attributed the low protein digestibility of the canola meals to high fibre and high phytate content.

Earlier toxicological feeding trials using detoxified rapeseed concentrate as the sole source of dietary protein for young male and female rats showed no adverse effects either in survival, growth, organ weights, blood analysis or at the histopathological examination of organs such as liver, kidneys, heart, gonads, thyroid gland, and the hypophysis (25). However, when detoxified rapeseed proteins were fed to female rats in reproduction studies, severe toxic symptoms developed during pregnancy (26, 27). The symptoms were reported to appear only at the last trimester of the gestation. Prior to this period, the female rats appeared completely normal. The toxic symptoms were very

similar to those observed in the reproductive studies involving zinc deficiency which includes slow and suffering delivery, still births, fewer and smaller pups, retention of pups in the uterus and poor survival of the offspring. In addition, other toxic symptoms include loss of appetite, wasting, apathy, conjunctival bleeding, and even death of the female rats just prior to or during parturition. There was conclusive evidence of a zinc deficiency as demonstrated by low levels of zinc in the blood serum of these rats. This adverse effect on zinc metabolism was attributed to the high levels of phytate in the rapeseed protein concentrates (27, 28). The zinc deficiency syndrome was overcome by proper supplementation of the diet with zinc (28).

Chemistry and Occurrence of Phytate

Phytate was encountered as early as 1872 by Pfeffer. The term " la Phytine " was used by Posternak (29) in 1903 to describe the major organic phosphate component of seeds which was later identified as an inositol hexaphosphate. Inositol phosphates containing fewer than six phosphate groups are commonly found in the complex lipids of both animals and plants as well as in the soil. However, inositol hexaphosphate (phytate) is known to occur exclusively in plant tissues, primarily in seeds and whole grains.

Early chemical studies suggested that phytate was an " inosite hexaphosphate " (30). However, controversy had arisen concerning its structure. The controversy centered around the structure proposed by Neuberg (31) and that by Anderson (30). Several other structures had also been proposed (32). Evidence using nuclear magnetic resonance (33) and X-ray crystallography (34, 35) supported the structure suggested by Anderson (Figure 1).

Phytate is the principal storage form of phosphate and inositol. It is ubiquitously distributed in cereal grains and legume seeds. It is also found in significant quantities in roots and tubers (36, 37). Its concentration in typical whole grain cereals and oilseeds ranges from approximately 1 per cent (dry basis) for corn, rice and wheat to approximately 5 per cent for defatted sesame meal (38).

The distribution of phytate in oil seeds and cereal grains varies with type. For examples, endosperm of wheat and rice kernels is almost devoid of phytate while the germ and outer layers contain almost 90% of the phytate phosphorus (39). In corn, almost 90% of its phytate phosphorus occur in the germ (39, 40). Oilseeds, which generally contain little or no endosperm, have their phytates distributed throughout the kernel and rarely have them occured in the hull (41). Researchers using corn grains and cereal grains, have observed that the synthesis of phytate in seeds began at about three weeks after pollination and that phytate concentration increased with seed maturation (36, 37, 42, 43). The amount and concentration of phytates in plant protein diet will therefore depend on the type of seed, the portion of the seed consumed, and the maturity of the seed at harvest.

Cytological evidence supports the premise that the major portion of phytate in wheat and oilseeds is located within the specific subcellular particles called aleurone grains or protein bodies (44-47). The aleurone grain which is surrounded by a classical unit membrane, is imbedded and surrounded by the cytoplasm of the cell and cell wall. The access to these phytates in the aleurone grains is therefore limited by the permeability of a series of structures, by the degree to which these structures are disrupted during processing, by the solubility characteristics of the surrounding proteins, and by the solubility of phytate salt itself.

Formation of Phytate - Metal Complexes

The structure of phytic acid suggests chelating characteristics. This notion is supported by the chemical analysis of plants showing that phytic acid exists as mixed salts. Numerous publications have reported that phytic acid is capable of forming a wide variety of salts with several heavy metals. The formation of a particular metal - phytate complex is dependent on the surrounding pH and on the secondary cations. The synergistic effect of the simultaneous presence of two or more cations in increasing the precipitation of metal-phytate complexes has been demonstrated for zinc and calcium (48-50) and for copper and calcium (51). At pH 7.4 and in the presence of a single type of cations, the affinity of phytic acid to form complexes with the metal ions is in the following decreasing order: $Cu^{++} > Zn^{++} > Co^{++} > Mn^{++} > Fe^{++} > Ca^{++}$ (51, 52). Some important observations made on the effect of pH on metal - phytate complexes are as follows: phytate quantitatively precipitates zirconium, thorium, titarium, and uranium in 6N acid (53, 54); calcium, magnesium, and barium phytate form best at slightly alkaline conditions (49, 50); zinc and copper phytates are least soluble at pH values between 4 and 7; ferric and scandium phytates are least soluble in dilute acid but dissociate in concentrated acid or dilute alkali (55).

Effect of Phytate on Nutritional Availability of Dietary Minerals

It is generally recognized that phytate is a poor source of inositol, inorganic phosphate and minerals to monogastric animals. Man and monogastric animals either lack or have low intestinal phytase activity. Consequently, they are unable to digest (hydrolyze) phytate effectively or to cleave the bound minerals from the phytate complexes.

On Calcium Availability

In 1925, Melanby first reported anti - calcifying and rachitogenic properties of certain cereals fed to dogs (56). After further studies, he attributed these effects to phytic acid binding minerals in cereals (57). Since then much attention has been given to the effect of phytate in foods in decreasing calcium absorption in the intestine (58, 59). McCance and Widdowson (60) showed that phytate decreased the absorption and urinary excretion of calcium. The effect of dietary phytate on calcium absorption was observed to be influenced by vitamin D, lipids, and other dietary factors. When vitamin D is limiting in the diet, calcium absorption will be less efficient and the effect of phytate on decreasing calcium absorption will be more pronounced (61, 62). Foods having sufficient amount of calcium, phosphate, and vitamin D, and a calcium: inorganic phosphate ratio between 1:1 and 2:1, are unlikely to be rachitogenic though much calcium may be bound by phytate.

On Zinc Availability

Early investigations showed that rats developed zinc deficiency symptoms when the diet contained less than 2 ppm of zinc (63). Due to the ubiquituous occurrence of zinc in food sources, the probability for man and animal to develop zinc deficiency was henceforth considered to be remote. The evidence of zinc deficiency developed in animals fed with natural product diet based chiefly on corn and soybean meal was observed by Tucker and Salmon in 1934 (64). The symptoms were characterized by poor growth and severe skin lesions. They further observed that parakeratosis in pigs can be cured or prevented by zinc supplementation. The human counterpart of zinc deficiency was reported by Prasad et. al. (65) in Egyptian boy patients whose diets were mainly of bread and beans. The patients were characterized by dwarfism and hypogonadism. Their symptoms were alleviated after given zinc supplemented diet. Chemical analyses of the diets showed sufficiency of zinc content in both cases. The common factor in both cases was the consumption of food largely of plant origin - cereals and legume seeds.

In 1957, it was reported that the zinc in the soybean protein was less available to chicken than that in the casein (66). Similar observations were reported for pigs (67, 68) and rats (69). Rats fed soybean protein required 50 % higher in dietary zinc than those fed casein. The appar-

ent absorption of zinc for rats fed casein was 84 per cent and for those fed soybean protein was 44 per cent. Similar results were obtained for growing chicks (70). Soybean protein was observed to cause a lower percentage absorption of zinc from the intestine (70, 71). These and many other similar findings suggested that zinc and other minerals in plant protein products are bound so that they are absorbed less effectively than those from the animal source such as casein and egg whites.

One major difference between animal and plant sources of protein is the presence of phytate in plant protein products, particularly in oilseeds and cereal grain products. In view of the experimental facts that oilseed protein binds phytate tenaciously and that phytate is liable to complex mineral elements; it is reasonable to hypothesize that phytate associated with the plant protein is responsible for the lower availability of zinc and other essential minerals of plant protein products. Numerous dietary tests have been undertaken to verify this hypothesis. Tests conducted on chicks fed casein based diets with added phytic acid showed that phytic acid decreased the availability of zinc in the casein diets and produced symptoms similar to those fed soybean protein containing a comparable level of phytate (72). Similar results were obtained by the addition of phytic acid based on free amino acids (73). The growth depressing effect of

phytic acid when added to a low zinc casein based diet was reported to be directly proportional to the dietary level, up to 1.0 % (74). The binding of zinc by phytate was observed in pigs fed casein diet containing 0.7 and 1.4 % of added phytic acid (68) and in rats fed casein diets with added 0.4 - 2.0 % phytic acid (50, 75). Proper zinc supplementation has shown to overcome the adverse effects on growth rate and prevented skin lesions in all cases. However, Oberleas pointed out that under present nutritional policy in which zinc cannot be supplemented to human foodstuffs, zinc can readily become the first limiting nutrient in plant - seed protein products (76).

Interrelationship of Zinc and Calcium

The addition of calcium salts in peanut meal ration accentuated the skin lesions and other signs of zinc deficiency in pigs (64). Since then studies have been carried on to investigate the relationship between calcium, zinc, and phytate. This interrelationship of zinc and calcium was reviewed by Forbes (77).

O'Dell et.al. (78) found that the addition of excess calcium to a chick diet based on soybean protein (15 ppm Zn) depressed growth rate unless it was supplemented with zinc. In one study, young pigs were fed a casein ration to which 1.4 per cent phytic acid was added (68). The ration contained 14 ppm zinc and 1.5 per cent calcium. The results showed

that excess calcium depressed growth rate in the presence of added phytic acid but not in its absence. A slightly better growth was observed when the calcium level was reduced from 1.5 to 0.8 per cent. The depressing effect of excess calcium in the presence of phytic acid on growth rate was also reported for rats (50, 75) and chicks (74). There was clearly an interaction among calcium, zinc and phytic acid.

In order to determine the mechanism whereby those two minerals interacted with phytic acid, solutions of zinc or calcium salts were added to a solution of sodium phytate (79). When the molar ratios of calcium and zinc were 1:1 or 2:1, calcium decreased the absorption of zinc by the phytate. When calcium to zinc ration was 100:1, the incorporation of zinc to phytate increased to 99 per cent. In the absence of calcium, no zinc was incorporated into phytate at zinc concentration of 0.25 mM. However, only 9 per cent of the zinc was bound to the phytate when zinc concentration was increased to 0.5 mM.

It was therefore postulated that calcium initiated a co-precipitation with zinc to form insoluble phytate at high calcium to zinc ratio. At low calcium to zinc ratio, calcium competes with zinc for the phytate since there is not sufficient amount of calcium to initiate the co-precipitation. According to this theory, the adverse effect of phytic acid could be overcome by adding enough zinc to the ration and by a " mass action law " effect overcome the action of phytic

acid (79).

On Iron Availability

Reports on the effect of phytate in decreasing iron availability were inconsistent. The controversy exists primarily because iron absorption is dependent upon many complex and often interrelated factors. The intrinsic factors that influence the physiological absorption of iron includes the amount and chemical form of iron ingested, the body - iron stores, the protein intake, the amino acid composition of dietary proteins, the organic acid content (e.g., ascorbic, citric, and lactic) and the bacterial flora of the gut. Food iron is normally less absorbed than administered iron salts. In normal adults, only 5 to 10 per cent of food iron is absorbed (80). Ascorbic acid may enhance absorption of dietary iron especially in iron deficient subjects (81). A dietary protein of less than 15 to 18 per cent resulted in impaired iron absorption in rats (82).

In contrast to North America and Europe where most of the dietary phosphorus is derived from animal sources, the common diet in India is mostly of vegetable origin. Hence as much as 50 per cent of the total dietary phosphorus may be of phytate. Typical Indian diet is high in bulk, high in carbohydrate content, low but adequate in protein content, low in calcium and high in iron. Inspite of high iron intake, the incidence of iron deficiency anemia in India is abnormally high. The poor

absorption of iron and the high incidence of iron deficiency anemia has been attributed to high dietary phytates.

Experimental studies have been conducted to elucidate the effect of phytate on iron absorption. McCance et.al. (83) reported that the incorporation of sodium phytate into white bread interfered with the intestinal absorption of ferrous and ferric iron. Sharp et.al. (84) found that adding sodium phytate decreased the absorption of ferric chloride by fifteenfold. However, they observed no correlation between the phytate content of foods and the reduction of iron absorption. Sathe and Krishnamurthy (85) investigated the effect of naturally occurring phytates in milled rice on hemoglobin regeration in anemic rats. These investigators observed that the hemoglobin and stored iron levels increased as the phytate phosphorus decreased with increased polishing of the rice. However, Liebman and Driskell (86) found no inhibitory effect on iron absorption with naturally occurring phytate diet as measure by hemoglobin regeneration and liver iron repletion in the rats. Similarly Cowan et.al. (87) observed no effect on iron absorption with added sodium phytate by measuring the total hemoglobin regeration in nutritionally anemic rats.

Cosgrove (88) and Foy et.al. (89) reported an increased need for iron when phytate phosphorus levels were more than 40 per cent of the dietary phosphorus. Welch and Camper (90) found that intrinsic labelled iron in mature soybeans

was more available than iron in immature soybeans even though mature soybeans contained three times more phytic acid. Davies and Nightingale (91) observed considerable reduction in whole body retention of iron when sodium phytate was added to the diet of rats. Studies conducted by Koepke and Stewart (92), Murray and Stein (93,94), and Multani et.al. (95), indicated that phytate decreased iron availability by decreasing the complexing of iron with gastroferrin in the stomach.

The reduced absorption of iron in whole grain products as compared to that of animal sources was partially attributed to the inhibitory effect of dietary phytate (96, 97). Whole wheat flour is rich in iron, zinc, calcium and most minerals but also contains high levels of phytic acid (36). Elwood et.al. (98) reported inhibition of iron availability in wheat by phytate. Kent - Jones and Amos (99) noted that phytate can interfere with the association of calcium and iron in human and animal nutrition, particularly when long extraction flour or the fibrous part of wheat constituted an important portion of the diet. Widdowson and McCance (100) found that more iron was absorbed from a white bread diet than from a brown bread with a 50 per cent higher intake of iron. Wozenski and Woodburn (101) pointed out that any evaluation of food containing high levels of phytic acid must also take into account the phytase activity. Although wheat, rye, oats, and corn are similar in phytate phosphorus content, both wheat and rye have sufficient phytase activity to

hydrolyze large portion of their phytate during the leavening of bread (102), but oats and corn have low levels of phytase, and can only hydrolyze a small portion of their phytate during breadmaking (103). Some of the factors affecting phytic acid hydrolysis during breadmaking include the type of flour used, the form of phytic acid complexes, the levels of phytase activity, the presence of phytase inhibitors (positive or negative), the pH of the dry ingredients and of the resulting breads, the time of fermentation, baking temperature, and baking time. These help to explain the conflicting findings of other investigators on brown bread.

Interrelationship of Iron and Calcium

The absorption of iron, calcium, and phosphorus seemed to be interrelated. Dietary iron absorption may be reduced to less than 3 per cent in a diet with a low calcium, high phosphorus and high phytic acid content (104). Excess dietary iron and/or calcium may reduce the effect of phytate in decreasing iron absorption (105, 106). Excess dietary calcium and a low phosphorus intake may decrease intestinal iron absorption, whereas a high intake of both calcium and phosphate may increase iron absorption (106).

On Magnesium, Copper, and Manganese Availability

Magnesium seems to be more poorly absorbed from diets which are rich in phytate, such as brown bread, brown rice, oatmeal, or refined bread to which phytate was added. McCance and Widdowson (60, 108) and Walker et al. (107) studied the influence of phytate content of diet on magnesium balance for human subjects. Their results showed that the fecal excretion of magnesium ranged from 41 to 83 per cent of the magnesium intake with low phytate intake, and ranged from 67 to 94 per cent with high phytate intake. A positive balance was observed with high phytate intake only in men whose daily magnesium intake exceeded 10 mg per Kg. Improved magnesium absorption was observed after several weeks of experimental diet. In addition, McCance and Widdowson (60, 108) showed that the addition of sodium phytate to white bread increased intestinal excretion of magnesium and that the removal of phytate from brown bread greatly improved intestinal uptake of magnesium.

Hathaway (109), reported on the experiment with young women that they excreted more magnesium while on an oatmeal-rich, phytate - rich diet than while on an amino acid - rich diet. Cullumbine et al. (110) reported that Ceylonese students kept on brown rice diets for eighteen weeks showed improved magnesium retentions at the end of the experimental diet. Magnesium availability in soybean meal was reported by

Guenther and Sell to be equal to skim milk and eggs, while its availability in processed cereal food is lower than that of the whole grain from which it is derived (111). Seelig (112) also reported that phytate decreases magnesium availability and that a deficiency can be developed under appropriate conditions.

Davies and Nightingale (91) found that phytates reduce the whole body retention of iron, copper, manganese and zinc in rats, whether or not the diet was supplemented with zinc. Davies et al. (113) showed that an isolated soybean protein that contains phytate reduced the availability of manganese and copper in the chick.

These numerous results clearly indicated that food containing phytic acid - mineral complexes have low bioavailability of mineral nutrients. In view of the fact that phytate complexes with such a broad spectrum of metals, it is therefore capable of producing a wide variety of mineral deficiencies depending on which element first becomes limiting under a specific dietary conditions. The results also point out the fallacy of depending only on chemical concentrations to specify levels of trace elements needed in a nutritionally adequate diet.

Other Dietary Factors Affecting the Bioavailability of Dietary Minerals

Among the dietary factors that affect the bioavailability of dietary minerals are fibers and mineral complexing agents such as phytate and other naturally occurring compounds as well as synthetic chelators. Some complexing agents decrease mineral availability while others increase it. Rackis et al. stated that some plant foodstuffs may contain oxalate, phenolic compounds, and other unknown constituents in sufficient quality to reduce mineral absorption (114).

Dietary Fibers

Foodstuffs rich in phytate content such as whole meal bread, tanok, and bran - based products also contain high concentration of fibre. Reinhold et al. (115) reported clinical deficiencies of zinc, iron, and calcium in segments of Iranian population consuming up to 75 per cent of their energy intake with unleavened whole meal breads. After bread feeding trials, they concluded that both fibre and phosphorus complexes decrease retention of calcium, magnesium, and zinc in human. Moreover, there were suggestions that dietary fibre in wheat bread accounts for most of the poor availability of minerals (116, 117). For essential minerals bound to the insoluble polysaccharides in bread are unavailable for absorption since these polysaccharides are not digested.

Studies done by Davies and Nightingale showed that the inclusion of purified sodium phytate in a fibre - free rat diet at a concentration commonly encountered in soyabean or cereal bread diets can fully account for the retention of available zinc (91). Davies et al. later reported that rats consuming bran or phytate exhibited reduced growth and food intake and that these symptoms were corrected when additional zinc was supplemented (118). However, they observed that rats consuming extracted bran fibre had growth rates and food intake similar to those with zinc - adequate controls. Furthermore, they observed that the inclusion of pectin (the soluble fibre components that are known to act as cation exchanger) in a diet of marginal zinc adequacy did not affect growth rates or food intakes. They therefore concluded that phytate rather than fibre is the major determinant of zinc availability.

Chelating Agents

Kratzer et al. (119) were the first to report that ethylenediaminetetraacetate (EDTA) decrease the zinc requirement of an isolated soybean protein diet. An EDTA level of 100 ppm in the diet was reported to be approximately equivalent to 8 ppm of zinc, which is about the amount of zinc bound by the soybean protein (69). The addition of EDTA to an isolated protein diet was shown to increase weight gain and zinc absorption in rats (120). Chelating agents with

stability constants for zinc in the range of 13 - 17 were found to be effective in increasing zinc availability (121, 122). EDTA has a stability constant for zinc of 16.5.

There are naturally occurring amino acids and peptides that aid in the absorption of zinc. Cysteine, for example, complexes with zinc and makes it more available. Lease (123) showed that two varieties of sesame meal differ markedly in regards to the availability of their zinc. One with greater availability of zinc contained a soluble component that facilitated zinc absorption in the presence of phytate. These evidences suggested that EDTA and the other effective chelating agents in some way compete with phytate for the zinc ion. Experiments were conducted to test the effect of EDTA on the availability of zinc in the presence of phytic acid. Chicks (74) and rats (50) were fed casein based diets with added phytic acid. The results indicated that EDTA counteracts the deleterious effect of phytate on growth rate. However, the mechanism by which one complexing agent (EDTA) increases while another (phytate) decreases zinc availability is not known for certain. It seems reasonable to believe that EDTA (or other effective complexing agent) complexes zinc in competition with phytate and the soluble complex formed is available to the intestinal mucosa either for exchange of the zinc ion or to be absorbed intact.

External Factors Responsible for the Inconsistent Findings on the Nutritional Effects of Phytic Acid

Much work was done to study the effects of phytate in plant protein products on animal nutrition. It is sad to say that some of the contradictory findings could have been avoided by the investigators. The following reasons may have contributed to the present confusion.

(1) Investigations were conducted on different sources and different forms of plant protein product that underwent different processing conditions and different extent of enzymatic hydrolysis of the phytate; (2) Feedstuffs of different compositions and concentration were consumed by different kinds of animals, and man; (3) Investigators utilized different types of experimental designs and techniques, and different length of feed trial periods; (4) Some studies were done on the effect of sodium phytate while others on that of naturally occurring dietary phytates. The problem of resolving these conflicting data is further complicated by the investigators' frequent failure to provide adequate information to enable one to identify the type of protein product used and the conditions of its production. It was well-worded that " results obtained in studies of the influence of phytates.....are of no value unless the many complex and often interrelated factors influencing absorption are known and controlled " (81).

Effects of Food Processing on Phytic Acid Content

Different processing operations and conditions can affect the nutritional quality of plant protein product, particularly its phytate content. Ferrel et al. (124) noted that much of the phytate in wheat went into the by- products of flour milling. Hence, phytate can become concentrated in the wheat protein concentrate prepared for human consumption through remilling of the selected by - production fractions. Kennedy and Schelstraete (125) studied the properties of rice flour obtained by the abrasive milling of commercial rice. The results of their sample analyses showed (1) that the first - pass flour, which constituted only about 2 per cent of the rice kernel, contained 23 times as much phytic acid as did the original rice, and (2) that the flour from the three milling passes, which made up about 6 per cent of the original rice kernel, accounted for nearly all of the phytic acid in the whole kernel.

The phytate concentration and distribution in oilseed protein products will depend upon the manner in which the seed is fractionated and processed (126). Wolf and Briggs (127) reported that the cold - insoluble fraction of soybean meal protein precipitated by cooling after the addition of calcium chloride contained 1.40 per cent of phosphorus as compared to 0.17 per cent of phosphorus when the cold - insoluble fraction was precipitated in the absence of cal-

cium ion. Nakayama et al. (128) found that large quantity of phosphorus precipitated with protein at pH of about 5.7 by calcium salt, and that practically 70 - 90 per cent of the water extractable phosphorus transferred into soybean curd (tofu) during the coagulation process. It has been demonstrated that phytate - protein complexes are formed in the production of soy isolates (129). Phytic acid reacts with proteins in the water extract of raw soy flakes during acidification, hence insoluble protein - phytate complexes are formed upon the neutralization of the precipitated proteins. Since evidence indicated that phytate is associated with protein (130, 131), Oberleas (76) stated that any process that utilizes solution and isoelectric reprecipitation would concentrate the phytate into the resulting product.

Proposed Methods for Phytic Acid Elimination

Food process techniques proposed to reduced or eliminate phytate or phytate-mineral binding include autoclaving, enzymatic hydrolysis, selective dissolution and precipitation methods, ion exchange processes, dialysis and ultrafiltration.

Heating is reported to overcome the physiological effects of phytate particularly with respect to the raw form of soybean meal (132). Autoclaving reduced the chicks' and poults' need for added zinc on isolated soybean protein or sesame diets (119, 133, 134). This reduction has been associated to phytic acid destruction (74). O'Dell found that 4 hours autoclaving reduced the phytic acid of isolated soybean protein to 13 per cent of its original amount (135). In contrast, Lease reported that 4 hours autoclaving reduced the phytic acid of sesame meal only to 78 per cent of its original amount (134).

Phytate can be removed by enzymatic hydrolysis. Phytase, an enzyme that is present in most seeds, catalyzes the hydrolysis of phytate into inositol and inorganic phosphate. Nelson et al. (136, 137) observed that the addition of phytase in chick diets increased the utilization of phytate phosphorus by chick. Chang et al. (138) demonstrated that phytate can be eliminated from whole dry beans by enzymatic hydrolysis and diffusion. By incubating the presoaked beans at different pH and temperature, they observed that the most

effective condition was to incubate them in water at 60 °C for 10 hours. Under such conditions, the phytate content was found to be lower by 90 per cent. Their study suggests that it was the destruction of heat-sensitive cell membranes during incubation that led to the activation of phytase.

Fontaine et al. (130) observed that the solubility of the phosphorus compounds in the water extract of soybean meal varied with the pH of the solution, and that it was very similar to that of the major protein components except at pH just above 4.0. It was suggested that this difference in solubility could be used to reduce the phytate content of soy isolate. McKinney et al. (139) reported that up to 80 per cent of the phosphorus of the basic solution of soybean meal extract can be removed by precipitation with calcium and barium ions, followed by centrifugation of the precipitated phytate. They also showed that phosphorus - free soy protein could be prepared from wet acid curd by dialyzing the curd against 1N NaCl solution. Ford et al. (140) proposed a process to remove phytic acid from full - fat flour. Their study showed that phytic acid removal is best attained either at the combination of low pH levels (3.5 - 4.0) and high calcium concentration (0.04 M) or at the combination of high pH levels (5.0 - 5.5) and low calcium levels. Under appropriate conditions, 90 per cent of the phytic acid was eliminated in the acid - precipitated curd.

In the preparation of soybean protein isolates, Smith and Rackis (129) proposed the elimination of phytate and inorganic phosphorus (1) by dialysis, (2) by an acid - exchange process, and (3) by a combination of these two processes. Their results indicated that the most satisfactory way of removing phytate was by using a combination of dialysis followed by treatment with an anion - exchange resin with both steps at pH 7.0. The combination process removed 75 to 78 per cent of the phosphorus. An ion - exchange process has also been proposed for phytin isolation (141, 142) and determination (143). Okubo et al. (144) reported the removal of phytate from the soybean meal extract by equilibrium dialysis at 25 °C. They found that phytate permeability at this pH follows closely the ideal behaviour of a fully permeable substance. It was also demonstrated that the presence of 0.01 M EDTA facilitated the membrane permeation of phytate by both ultrafiltration and dialysis.

Hartman (145) described a " three steps " process for preparation of low phytate soy protein. The first step was the water extraction of soy flake at pH 9 - 10. On the second processing step, the phytate was precipitated at pH between 11 and 12 and was removed by centrifugation or vacuum filtration. The soy extract was then quickly adjusted to pH 7.0 to prevent protein degradation and was pasteurized at 220 - 230 °F for 1 minute to reduce the microbiological load

prior to the final ultrafiltration and diafiltration step. The phytate and phosphorus contents of the resulting soy protein isolate were 0.1 and 0.2 per cent respectively, as compared to 2.6 and 0.8 for a typical commercial soy protein isolate. The corresponding PER value was reported to be higher than that of the commercial soy isolate or that of an acid isolate prepared from the same soy flakes.

Based upon the results of their studies on the solubility of phytate and protein in soy extracts as influenced by pH, sodium chloride, calcium and EDTA, de Rham and Jost (146) successfully derived and pilot plant tested three processes for preparing low - phytate soy protein products. The main steps of the three proposed processes as compared to the classical process are summarized in Table 3 . A comparison of their yield and phytate levels is also included. The low - phytate soy protein products obtained from the three proposed processes were similar in composition, except for the high residual salt content with the proposed process III, and the low ash content of the isolates precipitated with the proposed process I.

Omosaiye and Cheryan (147) evaluated the feasibility of eliminating phytic acid by ultrafiltration in the production of soy protein isolate or full - fat soy concentrates. Soy extract containing 3.5 - 3.9 per cent total solid was processed in a pilot scaled hollow - fiber ultrafiltration unit. When

Table 3 Isolate production processes performed at the pilot plant (146).

Classical	Extraction of the defatted soy flour with water at pH 8.2. Centrifugation, washing of the residue, agidification to pH 4.5, washing of the precipitate, lyophilization.
I	Identical to classical, except the precipitation at pH 5.5, and the omission of the washing steps.
II	Identical to classical, except an extraction pH of 11.5 (NaOH), precipitation at pH 5.5, and the omission of the washing steps.
III	Extraction of the defatted soy flour with 10 % NaCl solution, centrifugation (first juice), washing of the residue with water, centrifugation, addition of NaCl to 10 % (second juice). Separate filtration and ultrafiltration-diafiltration of the first and second juice, lyophilization of the ultrafiltration concentrates.

				III	
Process	Classical	I	II	First juice	Second juice
Nitrogen yield (%)	78	60	72	60	19
Phytate level (%)	1.84	0.60	0.18	0.14	1.5

the soy extract was concentrated to a volume concentration ratio of 5, the elimination of phytic acid at pH 6.7, 8, and 10 was 65, 43, and 27 per cent respectively. Following this, dilution of the respective solution back to their original volume at their respective pH and reultrafiltration, about 92 per cent of the total phytic acid was eliminated at pH 6.7 and more than 80 per cent at pH 8.0 and 10.0.

It must be reemphasized that there has been no work done on the elimination of phytate from rapeseed protein. The only available relevant information is the dissolution and precipitation of rapeseed phytate phosphorus which was incidentally included in the dissolution and precipitation studies of rapeseed protein by Gillberg and Törnell (148).

GENERAL CHARACTERISTICS OF REVERSE OSMOSIS AND ULTRAFILTRATION

The phenomenon of natural osmosis involves the spontaneous transport of solvent across the membrane from a region of lower concentration to a region of higher concentration (Figure 4a). The driving force for the flow of solvent molecules is the difference in chemical potential on the two sides of the membrane. The permeation of solvent will continue until the fluid pressure developed in the more concentrated solution is sufficiently high that the chemical potentials are equalized. At equilibrium (Figure 4b), the excess pressure is called the osmotic pressure difference.

However, if a pressure in excess to the osmotic pressure difference is applied to the more concentrated solution, solvent will permeate towards the region of lower concentration (Figure 4c). This transport process is known as reverse osmosis. It must be emphasized that the transport of fluid across the membrane for osmosis and reverse osmosis is not restricted only to pure solute. In other words, both processes are not restricted to 100 per cent solute separation.

Ultrafiltration is generally referred to that membrane separation process in which the membrane is permeable to solvent as well as to certain microsolute species, but impermeable to macrosolutes. Consequently, the osmotic pressure difference across the membrane will generally be very low, thus

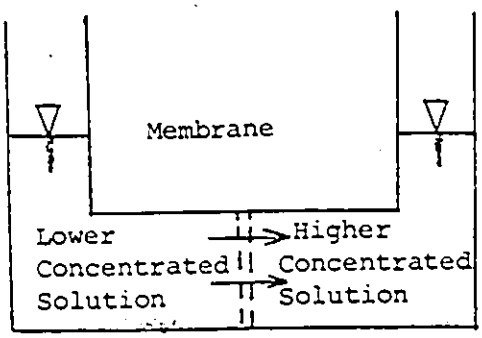


Figure 4a Natural osmotic flow

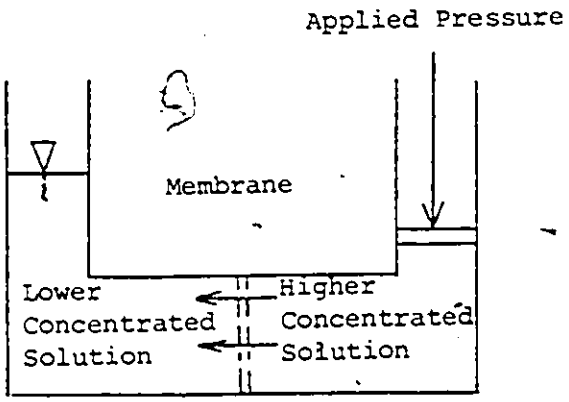
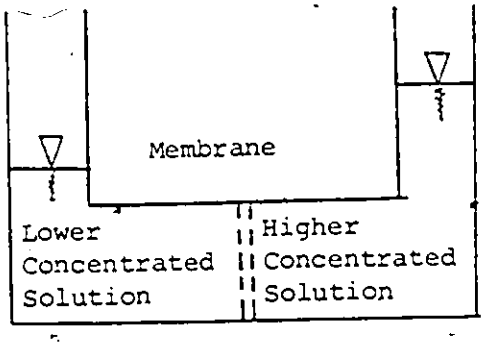


Figure 4c Reverse osmosis



Osmotic Pressure Difference

Figure 4b Equilibrium situation

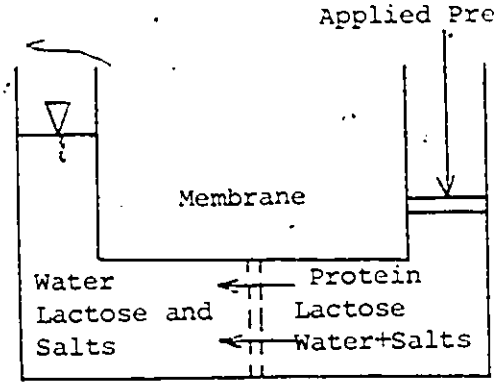


Figure 4d Ultrafiltration

permitting the ultrafiltration system to be operated at low hydrostatic pressure.

The operation of ultrafiltration consists of pumping a mixture to be separated, across a selectively permeable membrane at elevated pressure. The description of membrane selectivity is a complex function of thermodynamics and transport properties of the solutes, the solvent, and the membrane materials. In the separation of protein solution, the protein brought to the membrane surface by convection is generally rejected by the membrane. In effect, the surface concentration increases until a concentration gradient is established. the accumulation of solute at the interface region is referred to as " concentration polarization " (Figure 5). This phenomenon is of primary concern since it leads to a rapid reduction in permeate flux.

Under steady state operating conditions, the membrane rejected solute is diffused back to the bulk solution at the same rate that it is brought up to the membrane by convection. This can be expressed mathematically as follows:

$$\mathcal{D} \frac{\partial C_s}{\partial z} = V_w C_s$$

The equation also expresses the basic difference between conventional filtration and ultrafiltration. Conventional filtration is never operated at the " steady-state " condition.

CONCENTRATION
POLARIZATION

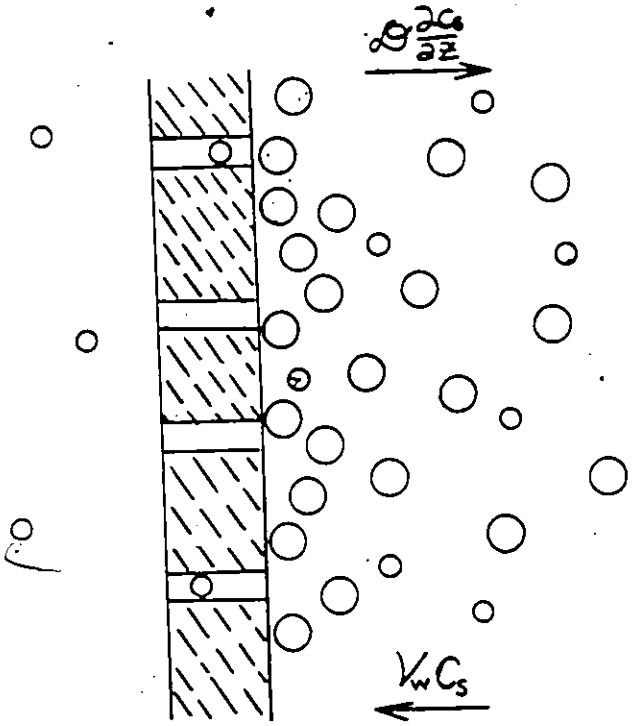


Figure 5 Ultrafiltration of protein solution

Instead, the unit is operated at unsteady high permeation rate with solute accumulating into a cake at the filter surface.

EXPERIMENTAL METHOD AND MATERIALS

Rapeseed Samples

The three rapeseed samples used in the study were deoiled commercial Tower meal with hull, dehulled deoiled Tower meal and Candle flour. The deoiled commercial Tower meal FRI-75-1 and the Candle flour FRI-77-3/4/5 were obtained from Dr. J.D. Jones at the Food Research Institute of Agriculture Canada, Ottawa. The dehulled deoiled Tower meal was provided by the researchers of our own Chemical Engineering Department and was prepared by Dr. J.D. Jones. The preparation procedure was reported elsewhere (149). Briefly the Tower rapeseeds were cracked and the hulls were separated from the meats. A single disc attrition mill was used in the dehulling step. The hulls and the meat fines, which constituted about 30 per cent of the seed mass, were removed by air classification. The hull-free meat fraction was deoiled by hexane extraction for 23-30 hours in a Soxlet extractor. The extracted oil was approximately 42 per cent of the initial meat mass. The resulted meal was air dried and any remaining solvent was completely removed under vacuum (1 mm Hg) at room temperature for 1 hour. The nitrogen, total phosphorus and phytate phosphorus contents of the three rapeseed samples were analyzed and are given in Table 4 on an air dried basis.

Table 4 An analysis of the total nitrogen, total phosphorus, and phytate phosphorus contents of the rapeseed samples.

Type of samples	Weight % of air-dried deoiled meals		
	% total nitrogen	% total phosphorus	% phytate phosphorus
Commercial Tower meal	6.68	1.13	0.84
Candle flour	6.66	1.40	1.16
Tower meal	7.53	2.03	1.54

Rapeseed Extraction Procedure

The extraction experiments were carried out in a mechanical shaker at 24 ± 1 C. The pH of the extraction solution was adjusted with reagent grade hydrochloric acid and sodium hydroxide when applicable. A weighed amount of rapeseed meal or flour and 100 ml. of prepared extraction solution together with glass beads were placed in a stoppered 200 ml Erlenmeyer flask and mechanically shaken for 3 hours. The insoluble material was removed by centrifuging at 2500 rpm for 10 minutes followed by filtering through ashless filter paper of medium retentivity. The supernatant liquid and the insoluble solid (when applicable) were analyzed for nitrogen, total phosphorus, and phytate phosphorus content. The amount of solute dispersed was calculated as:

$$\% \text{ solute dissolution} = \frac{\left[\begin{array}{l}\text{Concentration}\\ \text{of solute in the}\\ \text{supernatant}\\ \text{liquid}\end{array}\right] \times \left[\begin{array}{l}\text{Initial}\\ \text{volume of}\\ \text{extration}\\ \text{liquid}\end{array}\right]}{\left[\begin{array}{l}\text{Initial weight of solute in the}\\ \text{original rapeseed sample}\end{array}\right]} \times 100$$

The rapeseed extract used in ultrafiltration was prepared by placing a weighed amount of rapeseed sample and a measured volume of prepared extraction solution in a stoppered 6 liter extraction flask and was mechanically mixed with a magnetic stirrer for 3 hours. The insoluble material was removed by vacuum filtration using qualitative filter paper of fast filtering speed. The extraction was conducted at laboratory temperature of 24 ± 2°C. The pH of the rapeseed extract was adjusted with reagent grade hydrochloric acid and sodium hydroxide when applicable.

Membrane Casting Procedure

The membrane casting solution, which consisted of cellulose acetate, acetone, water, and magnesium perchlorate, was homogeneously mixed using Burundum cylinders in a covered glass jar placed on a rotating jar mill for at least 24 hours. The prepared casting solution was properly sealed and stored at 4°C. The composition of the membrane casting solution and the membrane casting conditions are given in Table 5.

Twenty ml. of prepared casting solution was poured onto a 15 cm. x 25 cm. glass plate, and was cast into a film of uniform thickness using a glass rod. After casting, the solvent was allowed to evaporate from the film surface for one minute. The film was then immersed first in an ethanol - water gelation bath for 15 minutes to control its porosity (150 - 152) and then in a water bath for one hour for washing and for ensuring complete gelation. During the immersions, the film sets to a gel from which the acetone and the magnesium perchlorate were leached out. The resulting membrane sheet was easily separated from the glass plate. The finished membrane sheet was cut to size and stored in 20 (v/v) % ethanol solution at 4°C. The thickness of the cast membrane was approximately 0.4 mm. The membrane should always be kept in wet condition since dehydration will lead to irreversible shrinkage and breakdown of its porous structure. If desired, the above membrane can be store dry with-

Table 5 Membrane casting details

A. Composition of the membrane casting solution (wt %)

Cellulose acetate (Eastman grade 400-25)	= 14.8 %
Acetone	= 63.0 %
Deionized distilled water	= 19.9 %
Magnesium perchlorate	= 2.3 %

B. Membrane casting condition

Temperature of the casting solution	= 4 ± 1 °C
Temperature of the casting atmosphere	=22 ± 2 °C
Solvent evaporation time	= 1 minute
Temperature of the ethanol gelation bath	=22 ± 2 °C
Gelation time	= 15 minutes
Temperature of the leaching bath	=22 ± 2 °C
Leaching time	= 1 hour

out change of properties by pretreatment with surface active agents as demonstrated by Merten et. al. (153).

A membrane made from the above procedure is asymmetrical in form. It has a very thin surface layer of dense microporous structure on the side which was exposed to the atmosphere during film casting. This side of the membrane is called the " surface layer side ". Riley et al. (154, 155) and Michael et. al. (156) estimated that the dense surface layer has a thickness of about 0.25 micron. However, estimation given by Kesting et al. (157) was a few microns. Certainly, the thickness of the dense surface layer will depend on the casting procedure and condition. Underneath the dense surface layer is a spongy porous mass usually about 100 microns in thickness. The macroporous spongy substructure serves merely as a built-in support for the surface layer. It is the microporous structure of the surface layer that governs the level of solute separation and hence, is held in contact with the feed solution during the ultrafiltration operation. The membrane asymmetry was displayed by the fact that overall membrane thickness has very little effect on the trans-membrane solvent flux. Most of the resistance to trans-membrane fluid flow occurs in the dense surface layer while the macroporous spongy mass underneath offers relatively insignificant resistance to fluid flow. The membrane asymmetry is depicted in Figure 6.

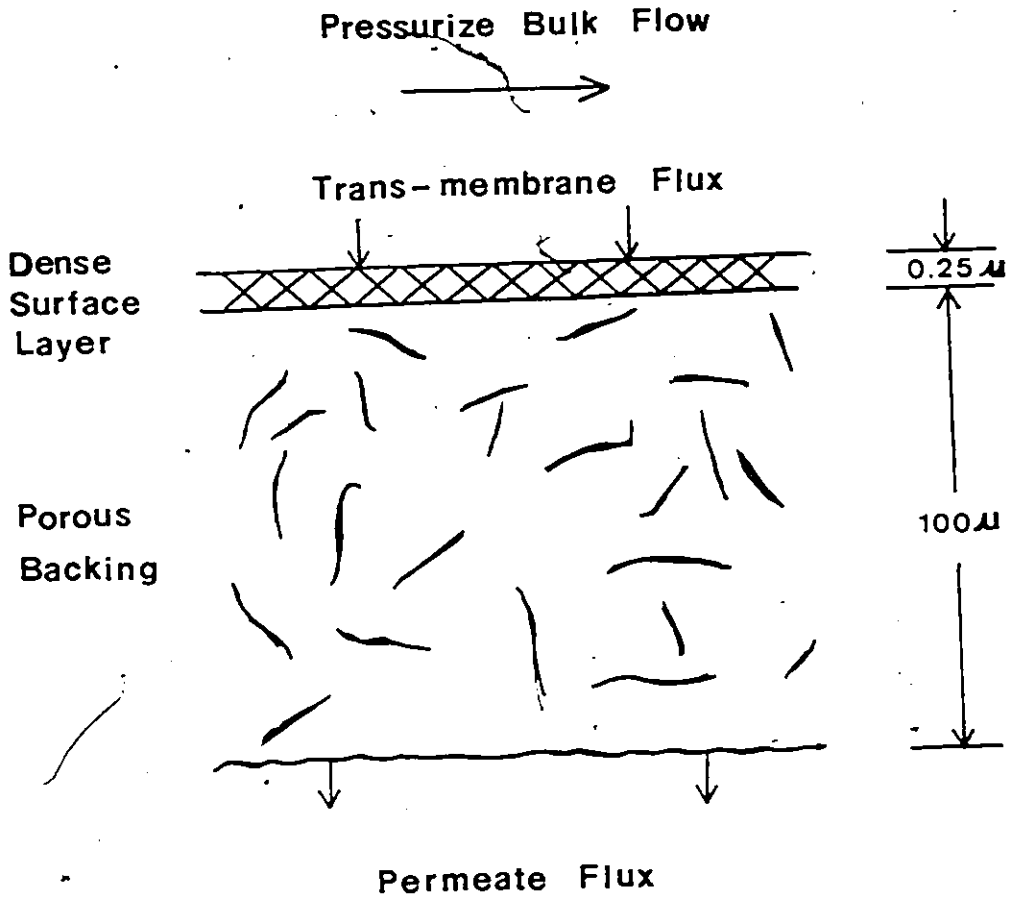


Figure 6 Asymmetric cellulose acetate membrane

Description of the Ultrafiltration Cell Unit:

The design of the ultrafiltration cell unit used in this study was obtained from the National Research Council of Canada (Ottawa) Chemical Engineering group of the Division of Chemistry. The cell unit has a thin channel typed flow pattern to provide higher fluid velocity and to minimize concentration polarization. A detailed description of the cell unit cannot be presented due to the condition of patent application. Instead, the cell unit reported earlier by Sourirajan (158) is presented in Figure 7.

Each cell unit consists of two detachable parts. The lower part is a high pressure region provided with inlet and outlet for the flow of the feed solution under pressure. The upper part is a membrane supporter embedded with a stainless steel porous plate and is provided with an outlet for the withdrawal of the permeated product. The wet membrane was mounted in the upper part of the cell. The effective area of the membrane in the cell unit was 14.30 sq. cm. The upper and the lower parts of the cell unit were set in proper alignment and sealed with the rubber O-rings. A pressure tight joint was secured by clamping the cell unit tightly together.

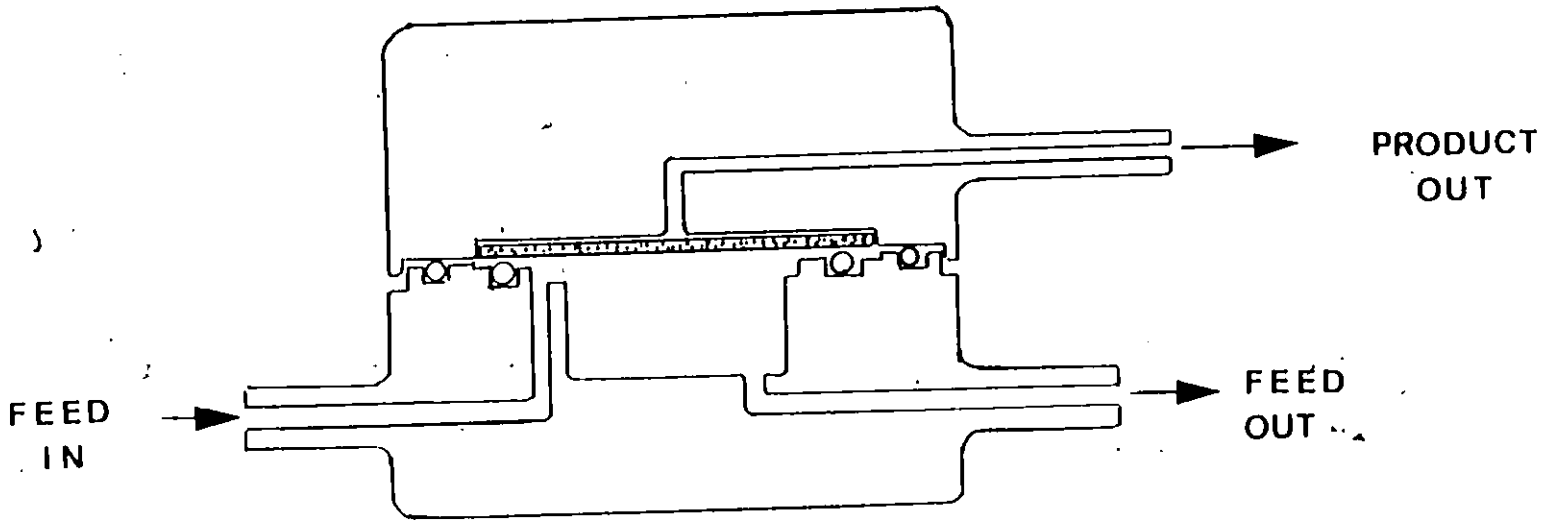


Figure 7 Reverse osmosis cell unit

Description of the Apparatus and Operation

A schematic flow diagram of the ultrafiltration system is presented in Figure 8. The system was simple and easy to operate. A high capacity Pulsa 7120 diaphragm-metering pump was used to pump the feed solution under pressure through a series of six cells. Note that only one cell unit is shown in the diagram. A Pulsatrol pulsation dampener pressurized with nitrogen gas was employed to minimize the pressure fluctuations in the cell units. The needle valve downstream regulated the operating pressure. The operating pressure was measured with pressure gauges located before and after the cell units. Six cells were used in series in order to permit six membranes to be tested simultaneously. The entire system was constructed from 316 stainless steel.

All experiments on the ultrafiltration system were carried out at a laboratory temperature of 24 ± 2 C. The new membranes were pretreated in the apparatus with distilled water at a pressure of 344.7 KPa for 30 minutes in order to stabilize their porous structure. The reported permeation rates are those adjusted to 25 C using the relative viscosity and density data for pure water. The solute separation was defined as:

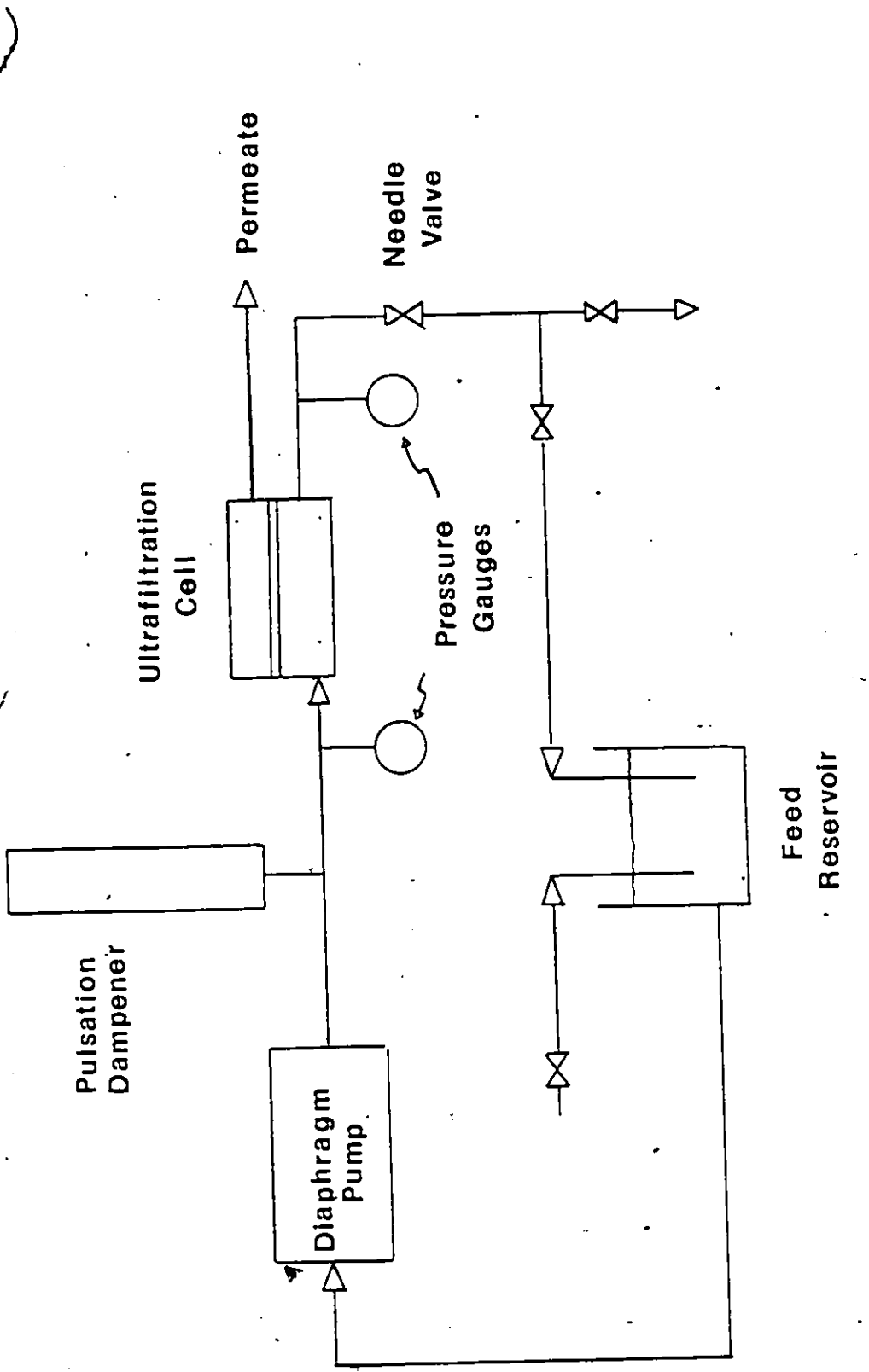


Figure 8 Schematic flow diagram of the UF system

$$\% \text{ solute retention} = \left[1 - \frac{\text{concentration of permeate (ppm)}}{\text{concentration of feed (ppm)}}\right] \times 100$$

$$\% \text{ solute removal} = \left[1 - \frac{\text{concentration of final solution (ppm)}}{\text{concentration of initial solution (ppm)}}\right] \times 100$$

Unless otherwise stated, the conditions for UF experiments were as follows : The feed solution flow rate was 2.5 L/min and the operating pressure was 137.9 KPa . The feed solution pH was 6.3, and the permeate samples were collected at the steady state condition, generally after 45 minutes of total reflux operation. It was assumed that a steady state condition is attained when constant results were obtained over a period of at least 30 minutes.

Analytical Techniques:

The determination of Kjeldahl nitrogen content was done according to Lang (159) and the protein assay was determined by the Coomassie Brilliant Blue dye binding method of Bradford (160). The total phosphorus and phytate phosphorus were determined according to Pons et al. (161) with modification on digestion step. Briefly, the digestion was done as follows: A given weight/volume of sample was placed into a 100 ml Kjeldahl flask together with two glass beads and 3 ml of concentrated sulfuric acid. The mixture was heated until the organic matter charred and a homogeneous solution was obtained. Thirty minutes of heating was usually sufficient. Ten drops of 30 per cent hydrogen peroxide were added and the heating continued for another one hour until the solution was colorless. The addition of 30 per cent hydrogen peroxide was repeated followed by another one hour of heating. The sample was allowed to cool down and 20 ml of distilled water was added. Finally, the sample was boiled for 10 minutes to remove any remaining peroxide and to ensure conversion of the phosphorus to the ortho form. The sample was then transferred quantitatively to a 100 ml volumetric flask and diluted to volume with distilled water. The one hour heating time after the addition of hydrogen peroxide was found to be critical for complete digestion of the organic sample.

RESULTS AND DISCUSSION

Protein can be extracted from deoiled rapeseeds using a dispersing agent such as water, salt solution, alkaline or acid solution. There are several methods proposed for the separation of protein from rapeseed meal. Sosulski and Bakal (162) isolated protein from three varieties of deoiled rapeseed meals by a successive extraction procedure using distilled water, 5 per cent sodium chloride, 70 per cent ethanol and 0.7 per cent sodium hydroxide. Finlayson et al. (163) extracted the salt soluble protein from eight varieties of rapeseed using 0.01 M. sodium pyrophosphate (pH 7.0) and 1.0 M. sodium chloride. Owen and Chichester (164) prepared a nontoxic rapeseed protein isolate by extracting press cake meal with 6 per cent sodium chloride followed by exhaustive washing which caused a great loss in protein. Their net yield of protein was only 18 per cent. Lo and Hill (165) prepared a protein concentrate with reduced crude fiber and glucosinolates by extracting rapeseed meal with 10 per cent sodium chloride and followed by dialysis. However, there was an increase in ash content during processing. Kodagoda et al. (166) developed a successive three stage extraction procedure for the separation of nutritionally and functionally distinct protein fractions from rapeseed flour. The yield of protein after isoelectric precipitation for the three

stage extraction using water, 0.1N hydrochloric acid, and 0.02N sodium hydroxide was 11, 13, and 42 per cent respectively. Keshwarz et al. (167) modified the three stage extraction process as developed earlier by Kodagado et al. for application to the commercial oil-extracted rapeseed meals.

In view of these reports on rapeseed protein extraction, studies were conducted to investigate the effects of these commonly known dispersing agents on the dissolution of phytate in the deoiled rapeseed samples. The extraction solutions used included aqueous solution of hydrochloric acid, sulfuric acid, sodium hydroxide, sodium sulfate, sodium chloride, and ethanol.

Effect of Hydrochloric Acid and Sulfuric Acid Concentration on the Dissolution of Phytate

Earlier studies done on the extraction of phytate from the commercial Tower meal with hydrochloric acid and sulfuric acid showed that high phytate dissolution occurred only at a very low pH. The results are tabulated in Appendices A1 and A2. At 0.1N hydrochloric acid, which corresponds to a pH of 1.0, the dissolution of phytate for the commercial Tower meal is 26 per cent. In comparison, at 0.1N sulfuric acid, which corresponds to a pH of 1.23, the dissolution of phytate was 68.5 per cent. This indicates that sulfuric acid is a better dispersing agent than hydrochloric acid for phytate extraction. However, extraction under extremely low pH condition causes protein denaturation, both hydrochloric acid and sulfuric are therefore not suitable dispersing agents for the extraction of phytate from the rapeseed meal. In order to avoid extraction at a low pH, the effect of their respective sodium salts on phytate dissolution was examined.

Effects of Sodium Sulfate Concentration on the Dissolution of Phytate

The extent of phytate dissolution at different sodium sulfate concentrations for the commercial Tower meal is presented in Figure 9 . A liquid-to-solid ratio, v/n, of 200 milliliter of extraction solution per gram of rapeseed sample was used. The pH of the extraction solution was not adjusted. The tabulated results are presented in Appendix A3.

Figure 9 shows that the introduction of sodium sulfate into the extraction solution resulted in a marked increase in the extraction of phytate. By increasing the sodium sulfate concentration in the extraction solution from 0 to 0.5 (w/v) %, the dissolution of phytate increases sharply from 10.5 to 71.8 per cent. However, further increase in sodium sulfate concentration only increased the phytate extraction slightly. The phytate solubility curve finally approaches 100 per cent at 10.0 per cent (w/v) of sodium sulfate.

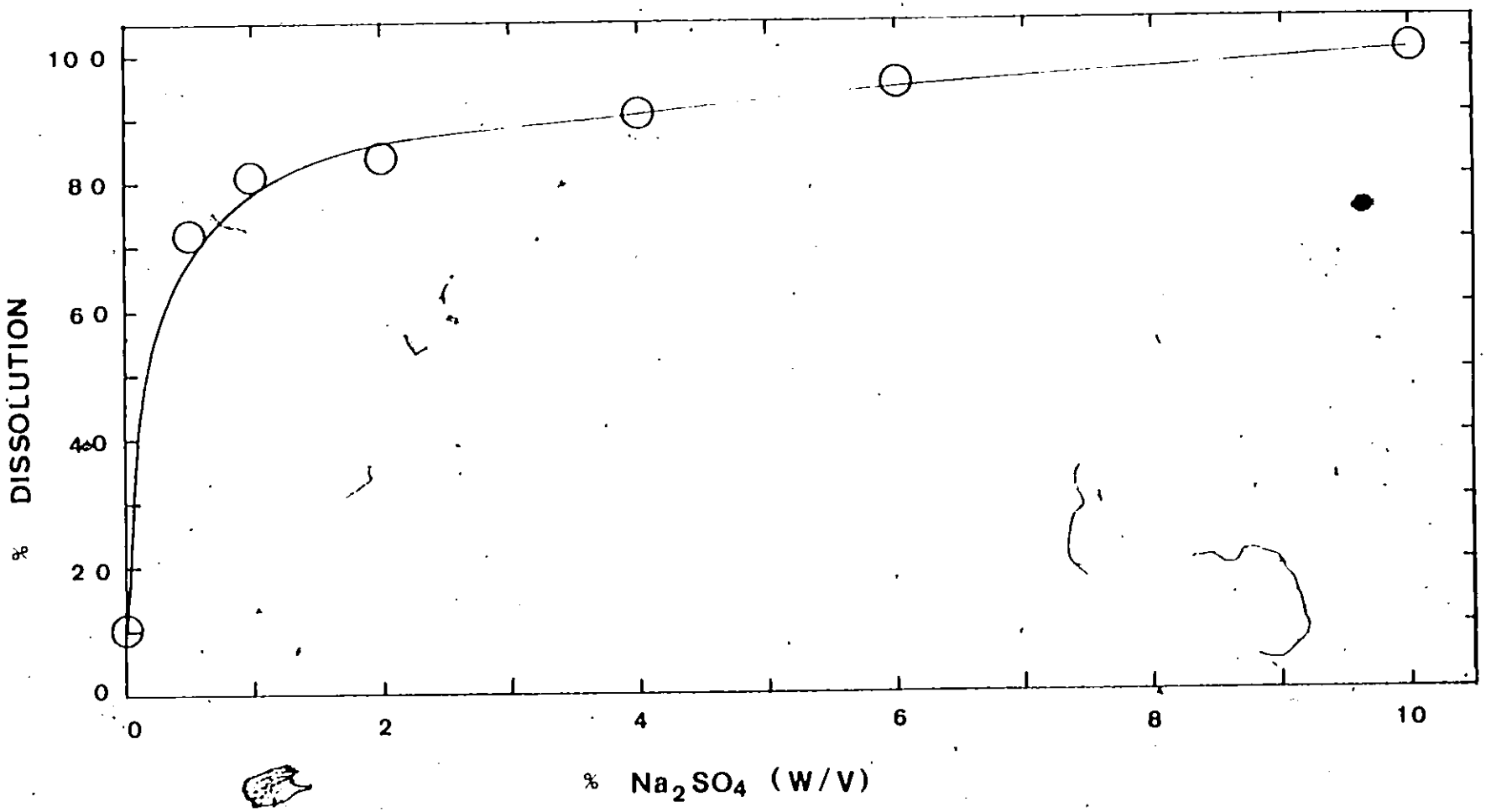


Figure 9 Effect of sodium sulfate concentration on the dissolution of phytate from the commercial Tower meal (v/n = 200)

Effect of Sodium Chloride Concentration on the Dissolution of Phytate and Nitrogen-Containing Substances

The effect of sodium chloride concentration on the dissolution of phytate and nitrogeneous protein compounds gave similar types of curves for all three rapeseed samples. The experimental results are tabulated in Appendix A4, and are presented in Figure 10 . The liquid-to-solid ratio, v/n, used was 200 ml/g and the pH of the extraction solution was not adjusted.

In Figure 10 , it is observed that with a 4 (w/v) % sodium chloride extraction solution, the dissolution of phytate for the commercial Tower meal was 90 per cent and for the Tower meal and the Candle flour was 95 per cent. A single phytate solubility curve is drawn for all three rapeseed samples. The curve increases sharply with sodium chloride concentration and rapidly approaches 100 per cent dissolution at 4 (w/v) % sodium chloride. The results show that sodium chloride is an excellent dispersing agent for phytate extraction and is preferred over sodium sulfate. Furthermore, the use of sodium chloride is more economical. However, the sodium content of the protein product should be of acceptable level.

The extent of dissolution for the nitrogen-containing substances was quite different in the three samples, 27 per cent for commercial Tower meal, 40 per cent for Tower meal, and 75 per cent for the Candle flour. Lower nitrogen solubility is

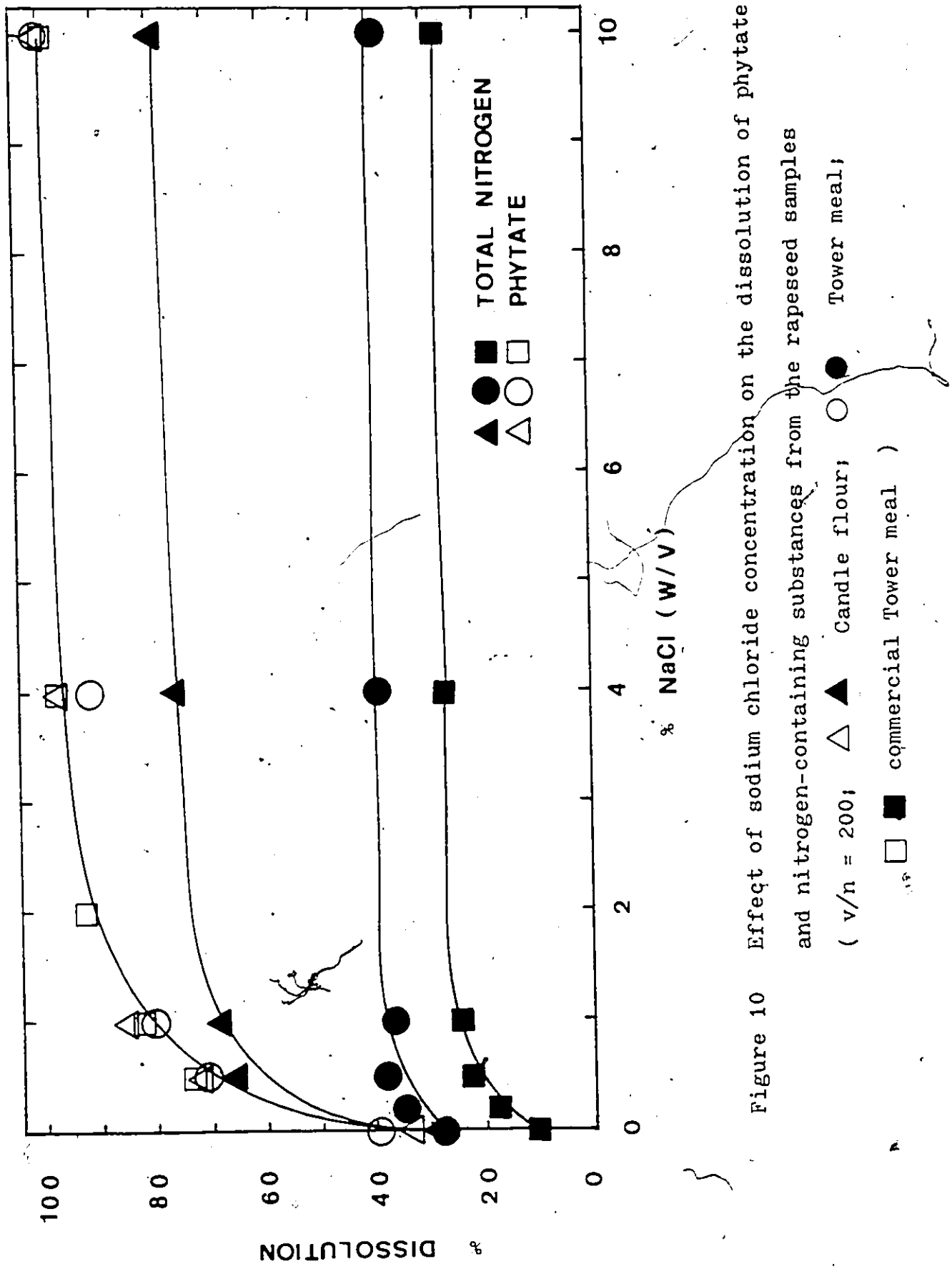


Figure 10 Effect of sodium chloride concentration on the dissolution of phytate and nitrogen-containing substances from the rapeseed samples (v/n = 200; △ ▲ Candle flour; ○ ● Tower meal; □ ■ commercial Tower meal)

associated with higher extent of protein denaturation. This could explain the low solubility of the commercial Tower meal which had received the most severe heat treatment for the inactivation of the enzyme myrosinase. On the other hand, Candle flour and Tower meal were specially prepared to minimized protein denaturation. However, the Candle sample, in addition, had been ground into flour, and hence a more efficient extraction would be expected. Figure 10 shows the limited solubility of the phytate and nitrogen compounds of the samples, and the dramatic increase in phytate solubility when sodium chloride is added as a dispersing agent.

Effect of Ethanol Concentration on the Dissolution of Phosphorus- and Nitrogen- Containing Substances

Becker et al . (168) employed low molecular weight alcohols to inhibit the solubility of proteins in the extraction solvent while effectively extracting carbohydrates from the deoiled soybean flakes.

Jones et al . (169) used an aqueous isopropanol solution in the multistage extraction of glucosinolates and phenolic compounds from the deoiled rapeseed flour with only a minimal amount of nitrogeneous materials extracted. In view of these reports, extraction experiments were done to determine if low molecular weight alcohols could effectively extract phytate in preference to protein. The rapeseed samples were extracted using an aqueous ethanol solution and the v/n ratio was 50 ml/g. The effects of the ethanol concentration on the dissolution of nitrogen and phosphorus compounds are shown in Figures 11 and 12, respectively. The data are tabulated in Appendix A5.

In Figure 11, the nitrogen solubility curves indicate that ethanol inhibited the solubility of proteins in all three rapeseed samples. Their nitrogen dissolution decreased to less than 10 per cent at 46.40 (v/v) % ethanol solution. Likewise, their dissolution of phosphorus compounds was also decreased to less than 10 per cent at 46.40 (v/v) % ethanol

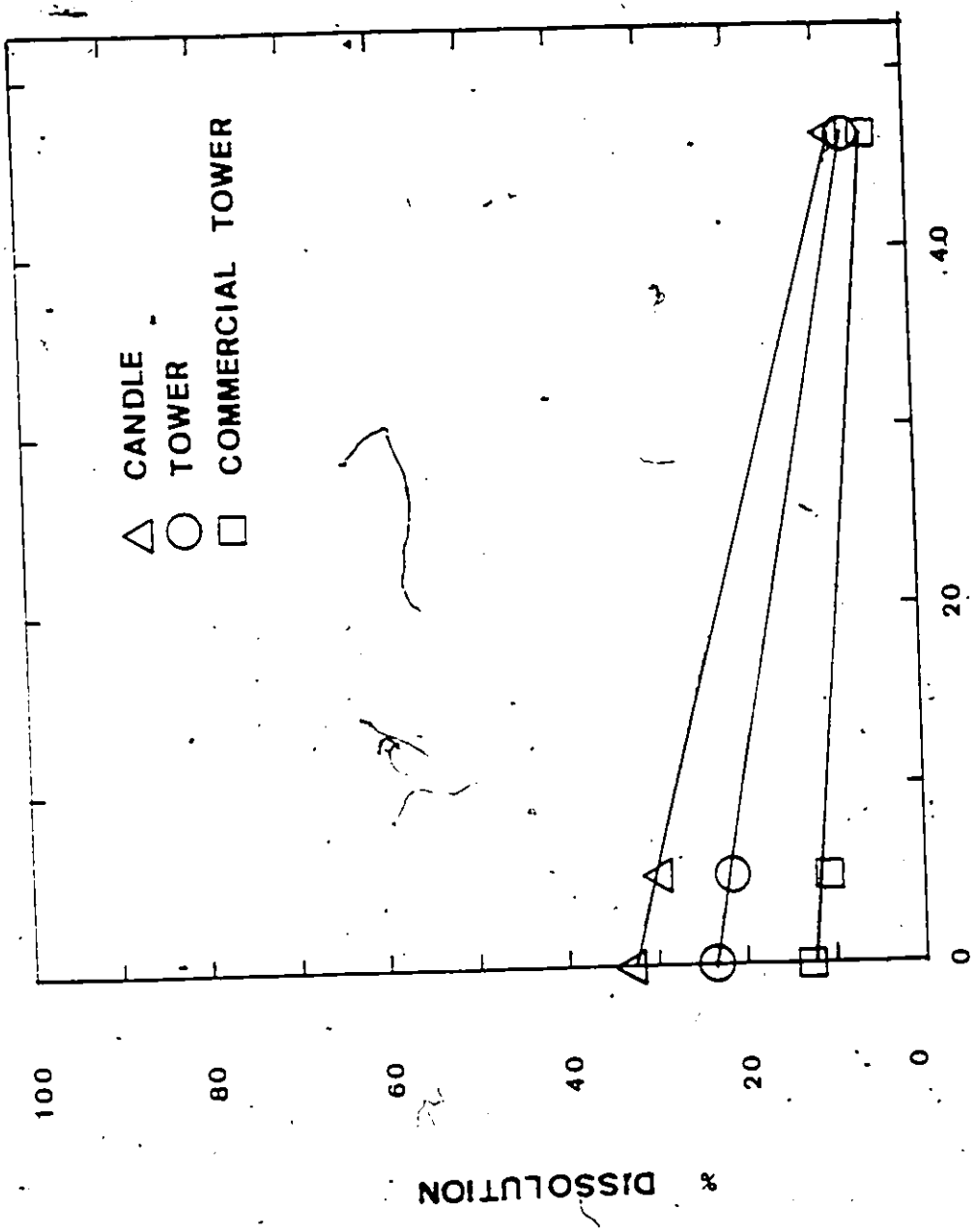


Figure 11 Effect of ethanol concentration on the dissolution of nitrogen-containing substances (v/n = 50)

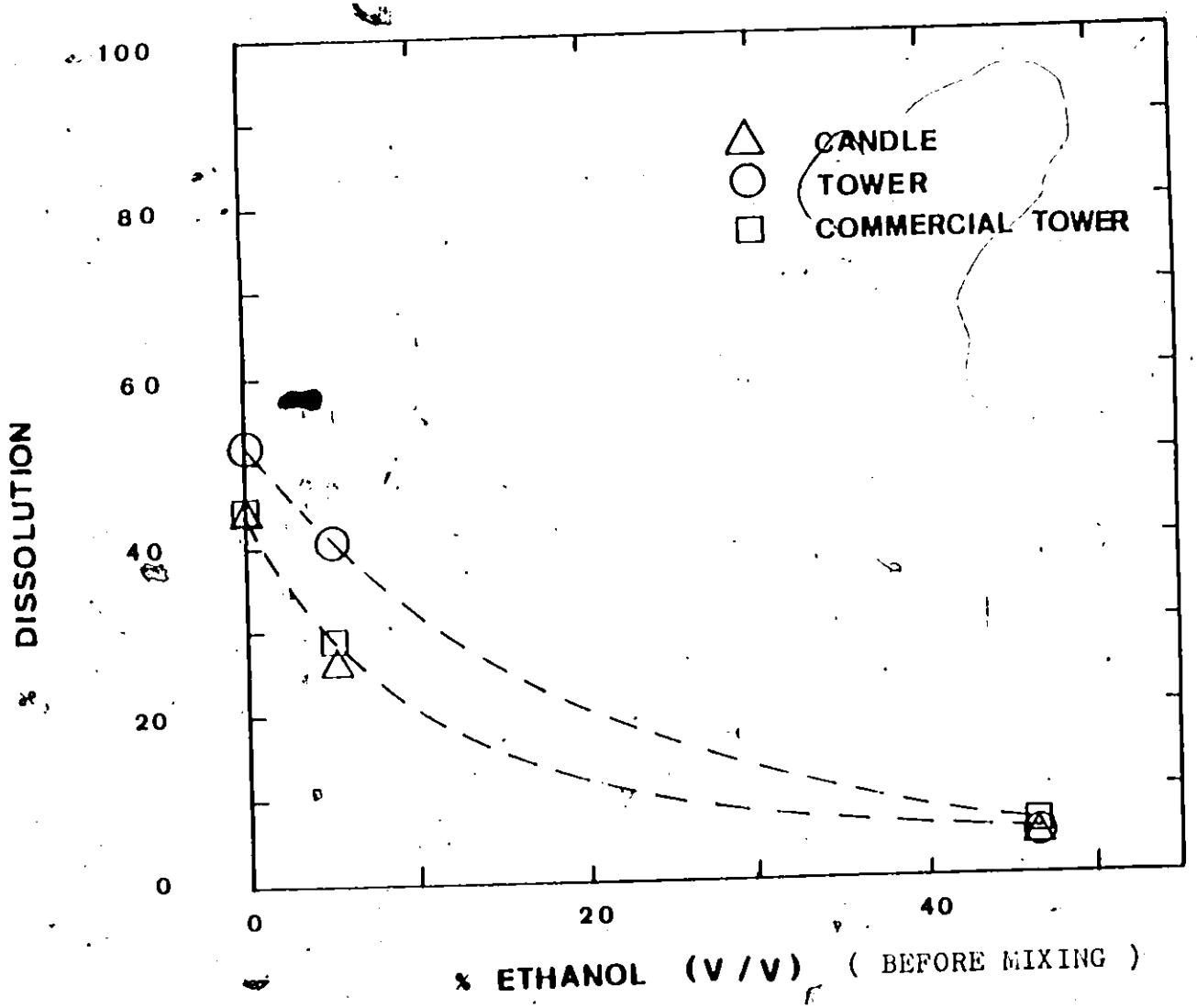


Figure 12 Effect of ethanol concentration on the dissolution of phosphorus-containing substances (v/n = 50)

as shown in Figure 12. The solubility data obtained for the total phosphorus compounds can be used as a good approximation for that of phytate since phytate phosphorus accounted for more than 75 per cent of the total phosphorus in the rapeseed samples as shown earlier in Table 4. These findings showed that ethanol-water solution cannot selectively extracted phytate from the rapeseed samples.

Since sodium chloride promotes while ethanol inhibits the extraction of both phytate and protein from the rapeseed samples, extraction experiments were done to examine the combining effects of sodium chloride and ethanol. The effect of ethanol concentration on phytate extraction was investigated in the presence of 4 (~w/v) % sodium chloride at a v/n ratio of 50 ml/g. The results obtained from these extraction studies are presented in Figures 13 and 14, and in Appendix A6.

A single phosphorus solubility curve was obtained for all three rapeseed samples as shown in Figure 14. The curve showed that while the presence of sodium chloride greatly enhanced the dissolution of phosphorus compounds, the introduction of ethanol into the extraction solution quickly diminished its effect. The phytate dispersing ability of the sodium chloride solution became ineffective at higher ethanol concentration. This is illustrated by the rapid decrease in

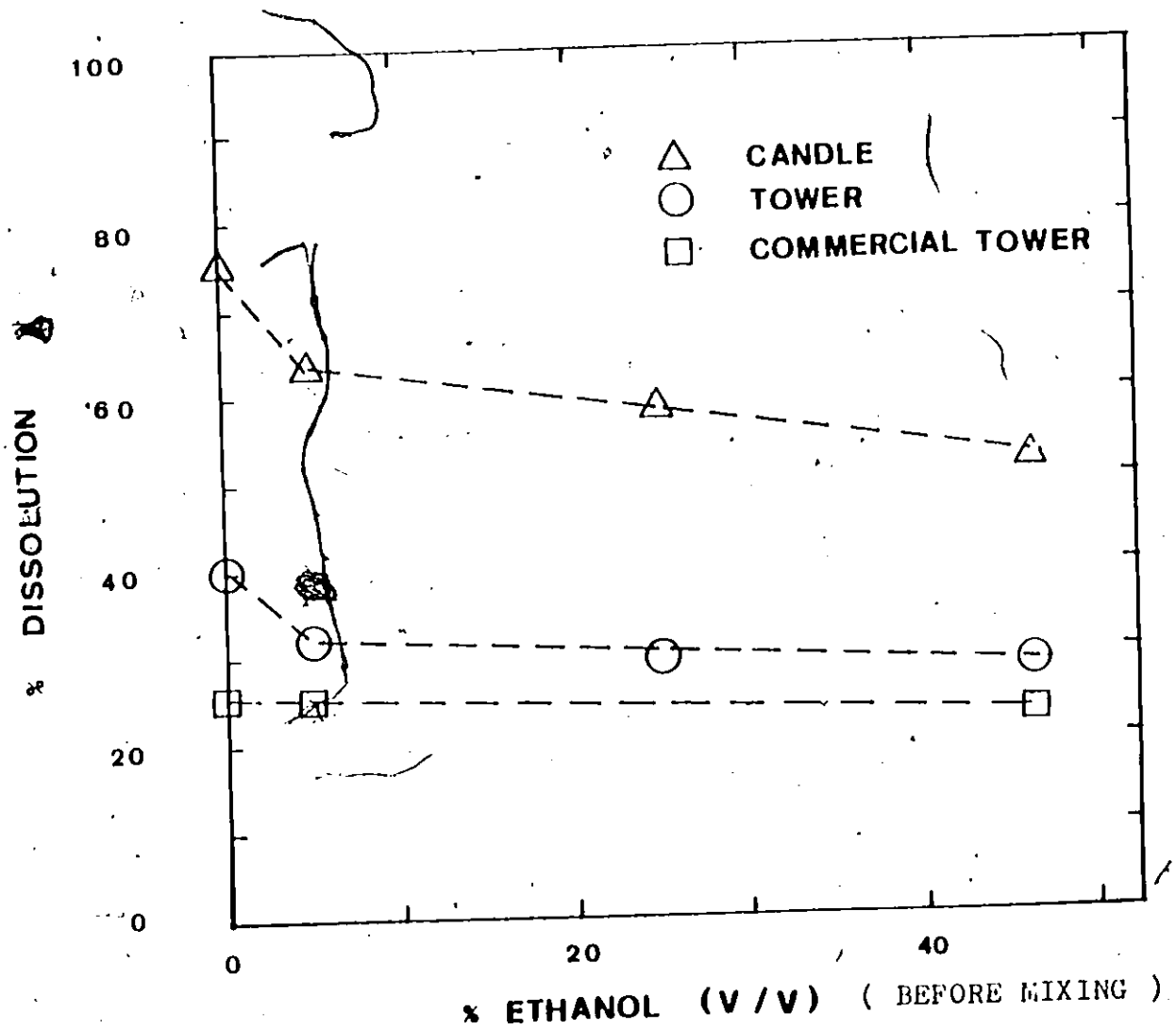


Figure 13 Effect of ethanol concentration on the dissolution of nitrogen-containing substances in the presence of 4(w/v)% NaCl (v/n = 50)

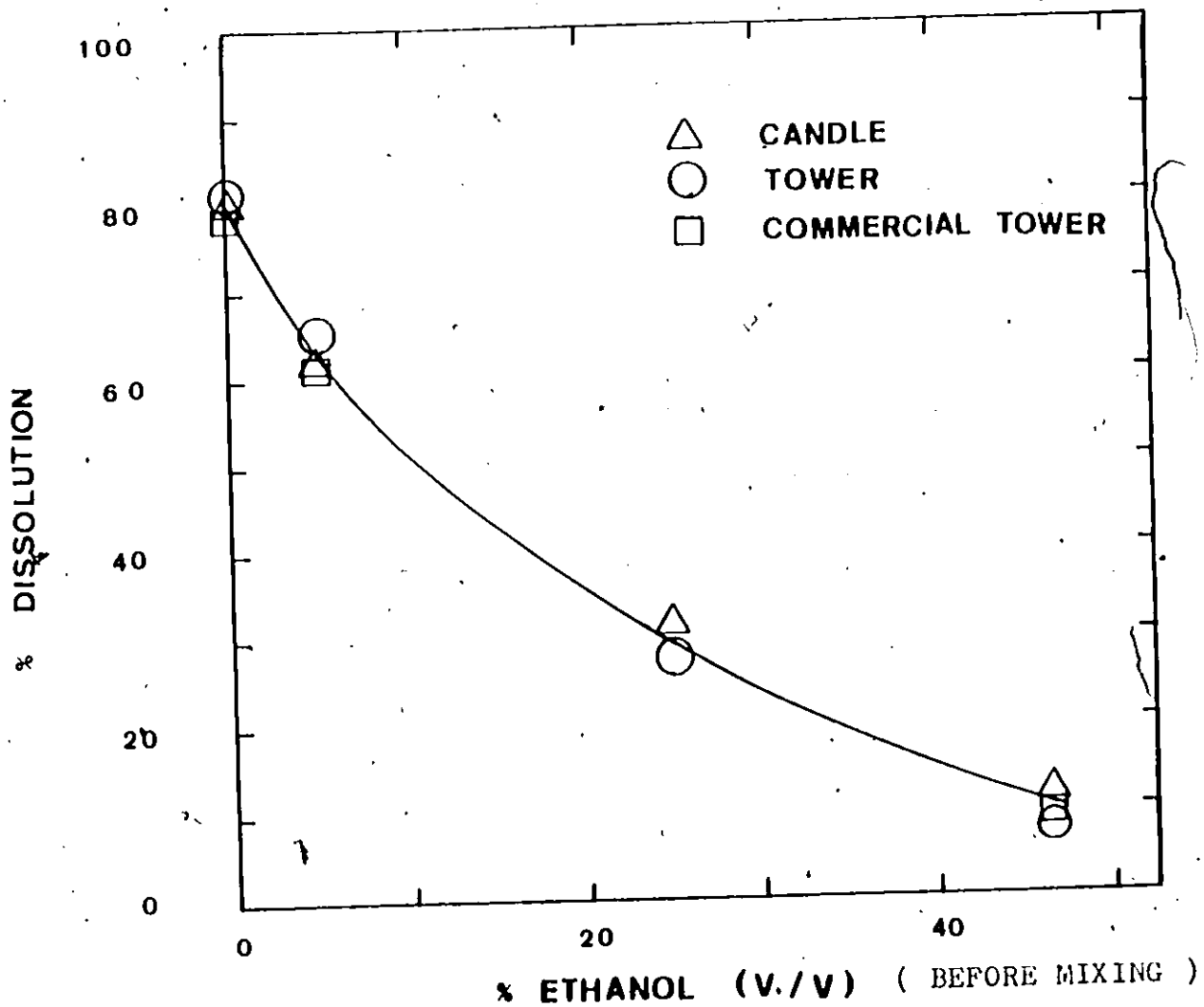


Figure 14 Effect of ethanol concentration on the dissolution of phosphorus-containing substances in the presence of 4(w/v)% NaCl (v/n = 50)

phosphorus solubility curve to less than 10 per cent at 46.40 (v/v) % of ethanol, which is nearly equal to that obtained previously in Figure 12 in the absence of sodium chloride. Hence, a sodium chloride - ethanol - water solution is ineffective for phytate extraction.

On the other hand, the protein dispersing ability of the sodium chloride was suppressed by the ethanol to a lesser degree as indicated in Figure 13. The denaturation of the rapeseed protein appears to be prevented in the presence of sufficient amount of salt. This extraction result is opposite to our extraction objective, i.e., to extract the phytate in preference to protein. However, this finding can provide an excellent alternative method for the preparation of low - phytate rapeseed isolates by which the protein from the rapeseed sample can be effectively extracted into the solution while most of the phytate will be retained in the meal.

Effect of v/n Ratio on the Dissolution of Phosphorus- and Nitrogen- Containing Substances

The foregoing extraction experiments showed that sodium chloride is an excellent dispersing agent for phytate extraction. Further studies were conducted to determine the effect of v/n ratio on the extraction of phytate and protein from the rapeseed samples using 4 (w/v) % sodium chloride solution. The pH of the extraction solution was unadjusted and was 6.3. The data obtained are plotted in Figure 15 and are tabulated in Appendix A7.

Figure 15 shows that the soluble nitrogen compounds dissolved readily at all tested v/n ratios. Increasing the v/n ratio had no effect on the dissolution of nitrogen compounds for either the Tower meal or the Candle flour. The dissolution of nitrogen compounds for the Tower meal and the Candle flour was about 40 and 74 per cent respectively. The dissolution of the phosphorus compounds increased rapidly with increasing v/n ratio and approached completion at a v/n ratio of 200 ml/g. Selective dissolution is possible at v/n ratio of less than 200 ml/g.

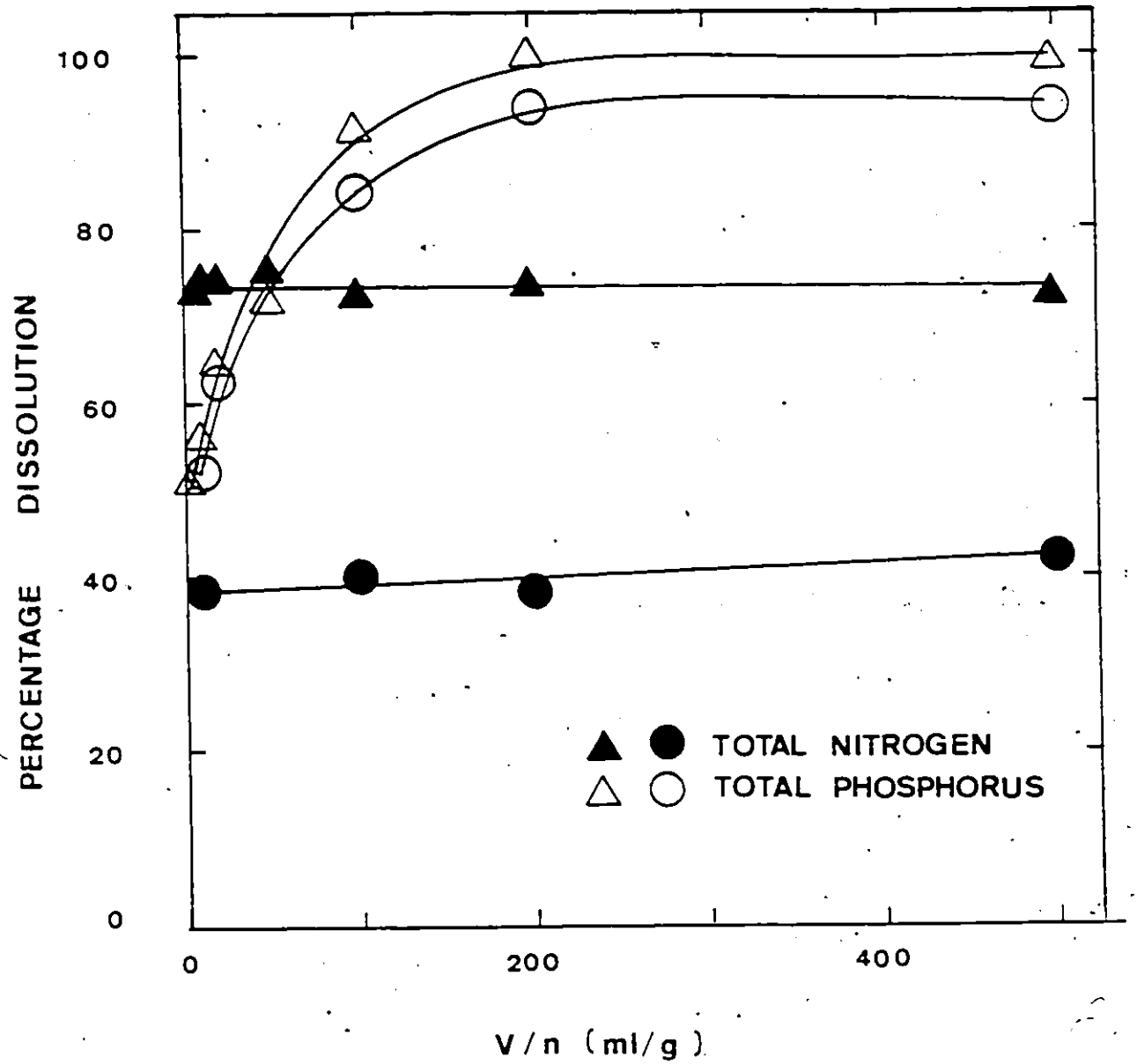


Figure 15 Effect of v/n ratio on the dissolution of phosphorus- and nitrogen-containing substances (pH = 6.3; 4(w/v)% NaCl solution)

▲ △ Candle flour ● ○ Tower meal

Effect of the Initial pH of the Extraction Solution on the Dissolution of Phosphorus- and Nitrogen- Containing Substances

The influence of the initial pH of the extraction solution on rapeseed extraction was investigated using sodium chloride concentrations of 0.0, 0.5, and 1.0 (w/v) % and v/n ratios of 50 and 20 ml/g. The solution pH was unadjusted during the course of extraction. The results showed that within the pH range of 3.0 to 10.0, the initial pH of the extraction solution had no significant influence on the dissolution of nitrogen- and phosphorus- containing substances under all test conditions. The results are presented in Tables 6 and 7. Gillberg and Törnell (148) have reported that the dissolution curves of nitrogen- and phosphorus- containing substances vary in a very complicated manner with pH when rapeseed is extracted with deionized water. However, they used a different variety of rapeseed and a different extraction procedure. Their extraction was carried out for thirty minutes at constant extraction pH and did not represent equilibrium data. They studied the effect of the extraction pH while we investigated the effect of the initial pH of the extraction solution. Not unexpectly, differences were observed in the solubility characteristics between these two sets of results. As shown in Table 6 for the commercial rapeseed sample, only at the extreme high and low pH values did the initial solution pH affect the extent of dissolution.

Table 6 The effect of pH on the dissolution of nitrogen- and phosphorus-containing substances using 1 % (w/v) NaCl solution and at a v/n ratio of 50 ml/g

pH	Commercial Tower Meal			Tower Meal			Candle Flour		
	Total N	Total P	Phytate P	Total N	Total P	Phytate P	Total N	Total P	Phytate P
0.57	30.87	97.73	100.00	-----	-----	-----	-----	-----	-----
1.04	25.72	79.12	87.12	-----	-----	-----	-----	-----	-----
1.43	24.41	61.49	64.81	-----	-----	-----	-----	-----	-----
2.03	20.31	71.43	79.84	-----	-----	-----	-----	-----	-----
2.73	27.54	87.67	100.00	-----	-----	-----	-----	-----	-----
3.44	30.44	66.30	79.83	-----	-----	-----	-----	-----	-----
3.52	-----	-----	-----	37.73	70.69	78.68	72.63	77.61	61.25
4.00	-----	-----	-----	38.47	70.12	72.59	69.92	74.12	63.07
4.46	32.32	64.38	77.32	-----	-----	-----	-----	-----	-----
5.05	-----	-----	-----	37.90	67.42	69.17	72.08	73.01	59.99
5.34	30.52	-----	-----	-----	-----	-----	-----	-----	-----
5.63	35.43	67.41	77.12	-----	-----	-----	-----	-----	-----
6.33	-----	-----	-----	39.92	68.50	74.80	70.63	75.07	58.48
6.38	28.11	62.40	-----	-----	-----	-----	-----	-----	-----
6.53	27.19	61.02	-----	-----	-----	-----	-----	-----	-----
6.68	28.21	63.01	69.01	-----	-----	-----	-----	-----	-----
8.35	31.97	62.60	69.17	-----	-----	-----	-----	-----	-----
9.46	-----	-----	-----	36.28	65.90	70.93	70.98	74.94	61.76
9.83	29.87	60.44	65.14	-----	-----	-----	-----	-----	-----
10.53	30.10	54.48	58.48	-----	-----	-----	-----	-----	-----
11.20	27.92	31.31	12.34	-----	-----	-----	-----	-----	-----
11.74	32.09	-----	-----	-----	-----	-----	-----	-----	-----
11.93	30.21	14.98	13.34	-----	-----	-----	-----	-----	-----
12.82	55.78	55.14	47.44	-----	-----	-----	-----	-----	-----
13.86	97.80	100.00	100.00	-----	-----	-----	-----	-----	-----

Table 7 The effect of pH on the dissolution of nitrogen- and phosphorus-containing substances in Tower meal using distilled water and 0.5 (w/v) % NaCl solution.

pH	% Dissolution (v/m = 20 ml/g)					
	Distilled Water			0.5 (w/v) % NaCl Solution		
	Total N	Total P	Phytate P	Total N	Total P	Phytate P
3.3	-----	-----	-----	40.93	56.61	55.23
3.4	34.79	48.90	43.13	-----	-----	-----
4.0	33.34	47.54	44.50	39.67	54.92	53.33
4.5	34.39	47.17	43.50	-----	-----	-----
4.9	-----	-----	-----	39.53	51.36	52.19
5.4	-----	-----	-----	39.64	53.35	52.42
5.5	32.93	47.18	42.30	-----	-----	-----
6.1	-----	-----	-----	43.28	55.40	52.46
7.0	33.21	47.43	41.72	-----	-----	-----
9.9	-----	-----	-----	38.60	52.68	51.24
10.0	34.50	46.58	41.97	-----	-----	-----

As suggested by Gillberg and Törnell (148), the complex variation of the phytate solubility at both extreme pH's may be due to phytic acid reacting with metal ions as well as protein during extraction. However, extreme pH values were outside the interest of our extraction criterion, since it was our intent to minimize protein denaturation.

Multistage Crosscurrent Extraction of Rapeseed Meal

The use of a single extraction stage has its limitations with regards to the design of a processing sequence. If the processing objective is to produce a low phytate residue from a deoiled meal such as the commercial Tower meal used in this study, a single extraction using a 4 (w/v) % sodium chloride solution and a v/n ratio greater than 200 ml/g would suffice. However, it is prudent to reduce the volume of extraction liquid and use a multistage extraction procedure. This is desirable if the supernatant liquid is to be processed to recover a low phytate protein isolate.

A multistage crosscurrent extraction of Tower meal was done using deionized distilled water at a v/n ratio of 10 ml/g per stage. The amount of the original solutes remaining in the meal after each stage of crosscurrent extraction was measured for phytate phosphorus and total nitrogen compounds. The results are plotted in Figure 16. The pH of the extraction solution was at 6.3. The tabulated data are presented in Appendix A8. The nitrogen extraction curve as shown in Figure 16 levelled off after the fourth extraction stage, and the total nitrogen compounds extracted after eight extraction stages was about 40 per cent. The total extraction of phytate after eight extraction stages was only 30 per cent. These results show that a low and limited phytate extraction was obtained with deionized distilled water.

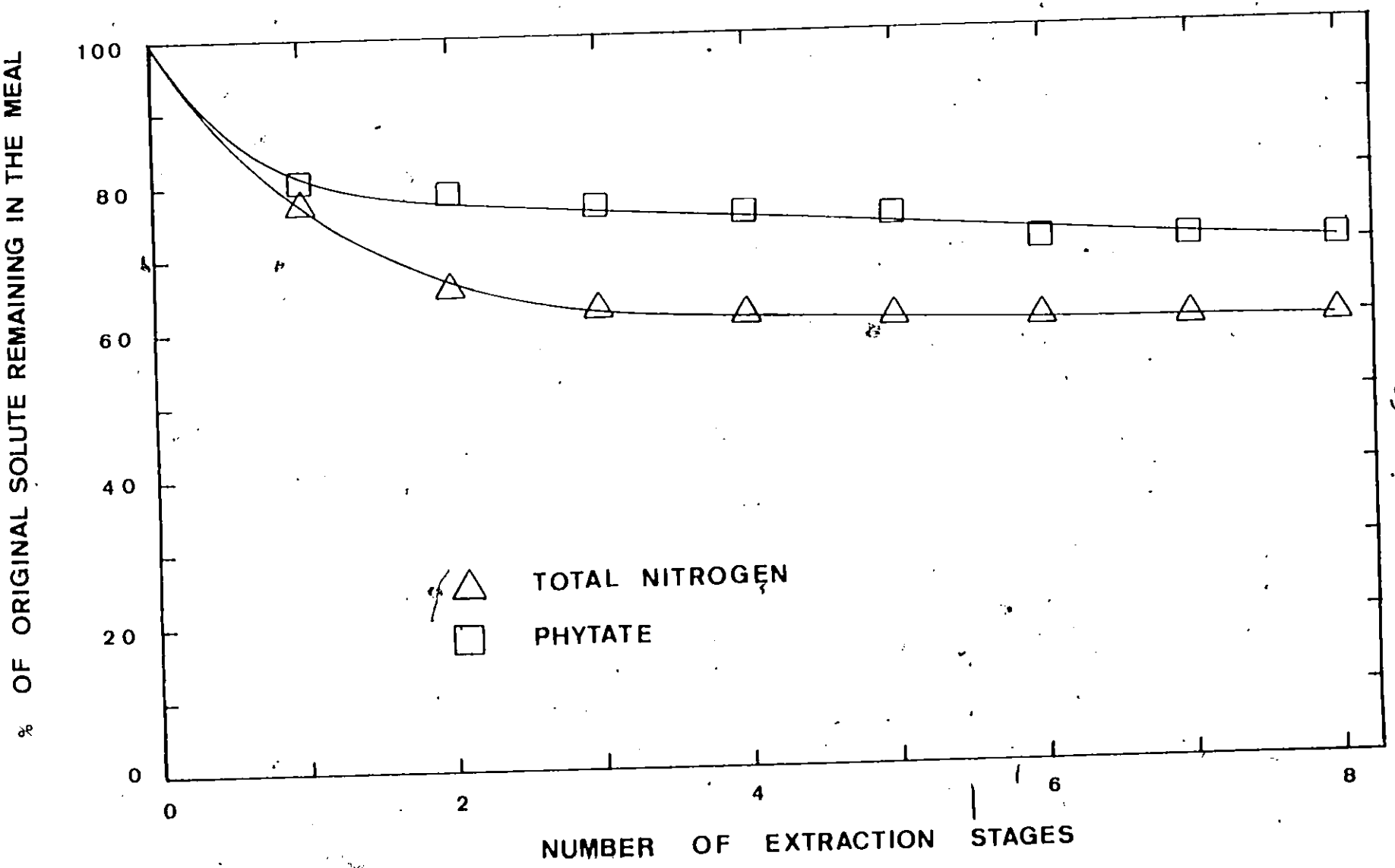


Figure 16 Multistage crosscurrent extraction of Tower meal

(v/n = 10; Deionized distilled water)

A similar multistage crosscurrent extraction of Tower meal was conducted using 4 (w/v) % sodium chloride. The v/n ratio was 10 ml/g per extraction stage. The results are tabulated in Appendix A9, and are presented in Figure 17.

The percentage of the original solutes remaining in the meal after each stage of crosscurrent extraction was measured for the total nitrogen compounds, the total phosphorus compounds, and the phytate phosphorus. The nitrogen extraction curve levelled off after the fourth extraction stage and the total amount of nitrogen compounds extracted was about 50 per cent after the eighth extraction stage. The phytate extraction curve follows the phosphorus extraction curve very closely. About 90 per cent of the total phosphorus and 95 per cent of the phytate phosphorus from the Tower meal were extracted with eight processing stages producing an overall v/n ratio of 80 ml/g. This study demonstrates that phytate can be effectively and completely removed from the deoiled rapeseed meal with aqueous sodium chloride solution using the standard extraction technique. This example is by no means optimum as the ultimate extraction sequence will depend on the processing objectives.

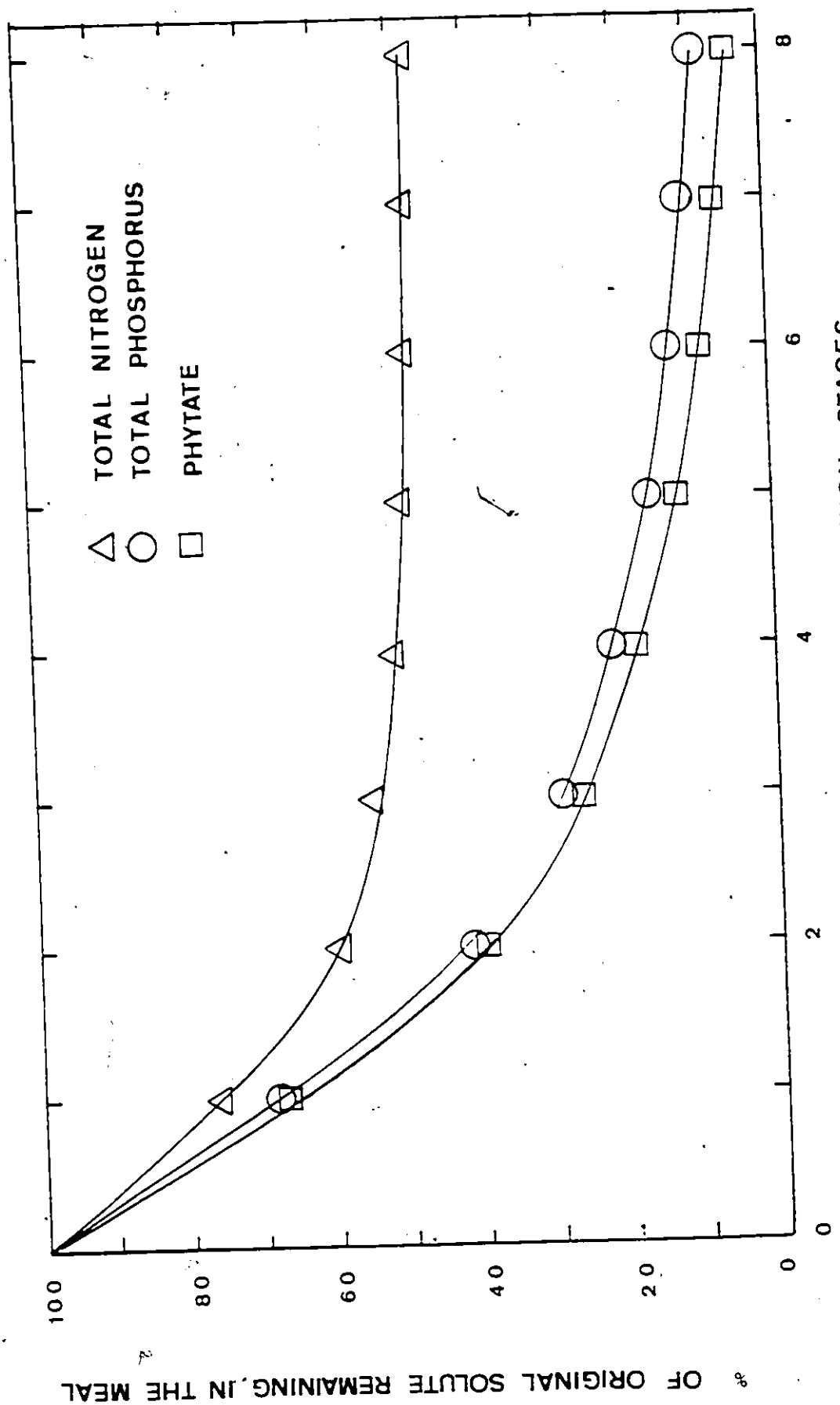


Figure 17 Multistage crosscurrent extraction of Tower meal
(pH = 6.3; v/n = 10; 4(w/v)% NaCl solution)

Graphical Calculation for Multistage Phytate Extraction

In the studies of extraction processes, it is frequently necessary to estimate the number of equilibrium stages required to bring about a specified degree of extraction. Alternatively, information may be required on the performance of a system with a specified number of stages. Such information may be obtained by stage-to-stage calculations.

Generally, it is simplest and easiest to do stagewise calculations graphically. This will require graphical representation of the equilibrium conditions. For ease of calculations, it was decided to deal only with the three component systems containing solvent (A), insoluble carrier solid (B), and soluble solute (C). Furthermore, for simplicity purposes, it was assumed that the only extractable solutes were phytate and protein. In order to deal with three component systems during the graphical calculations, the two solutes, phytate and protein, were treated separately. Two interrelated equilibrium diagrams were separately constructed for solute phytate (C_1) and solute protein (C_2).

The equilibrium diagrams for the phytate and the protein extraction from the Tower meal using 4 (w/v) % sodium chloride solution were separately plotted in Figures 18 and 19. Sample calculations for the equilibrium data are found in Appendix B1, and the calculated equilibrium data are tabulated

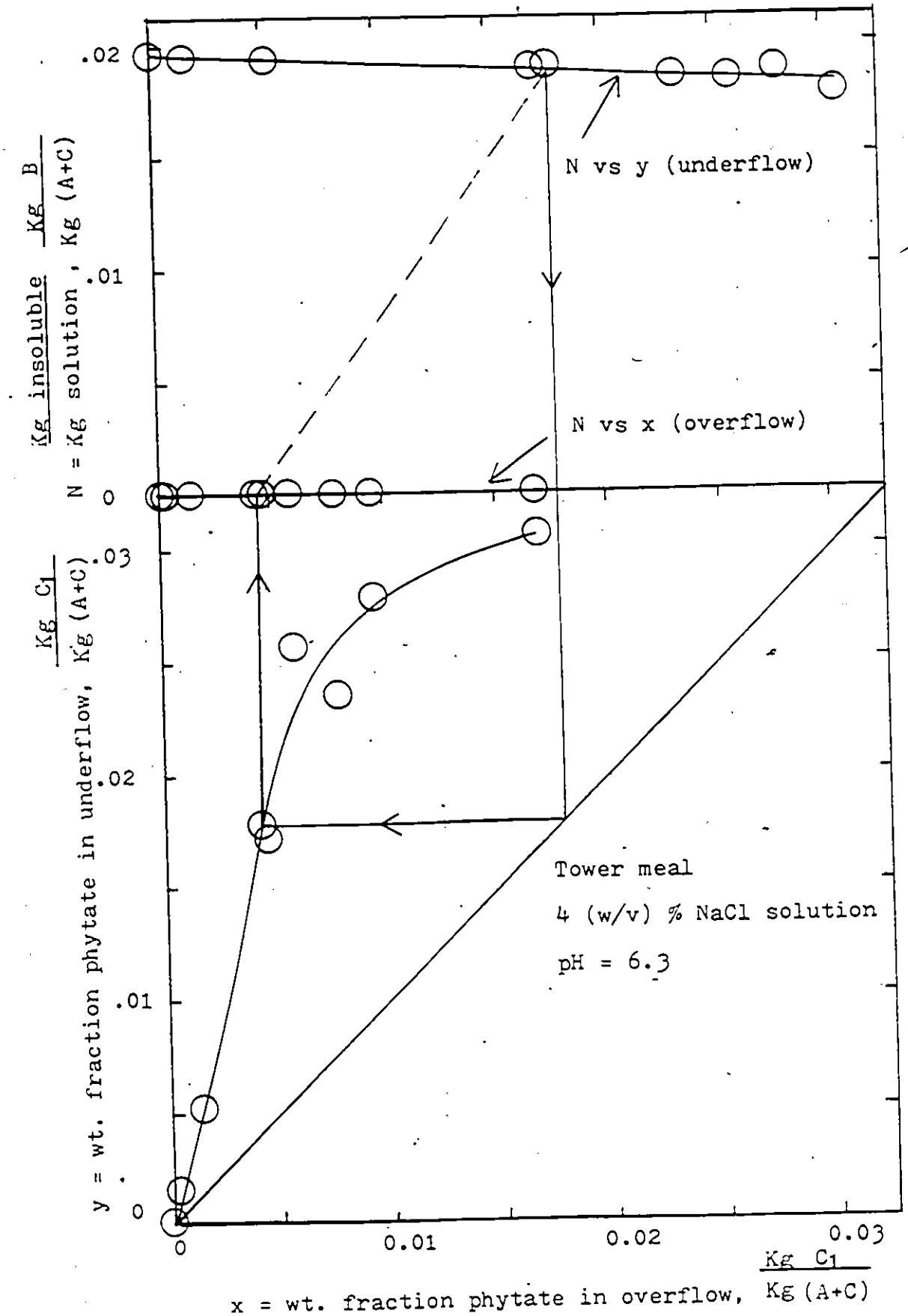


Figure 18 Phytate equilibrium diagram

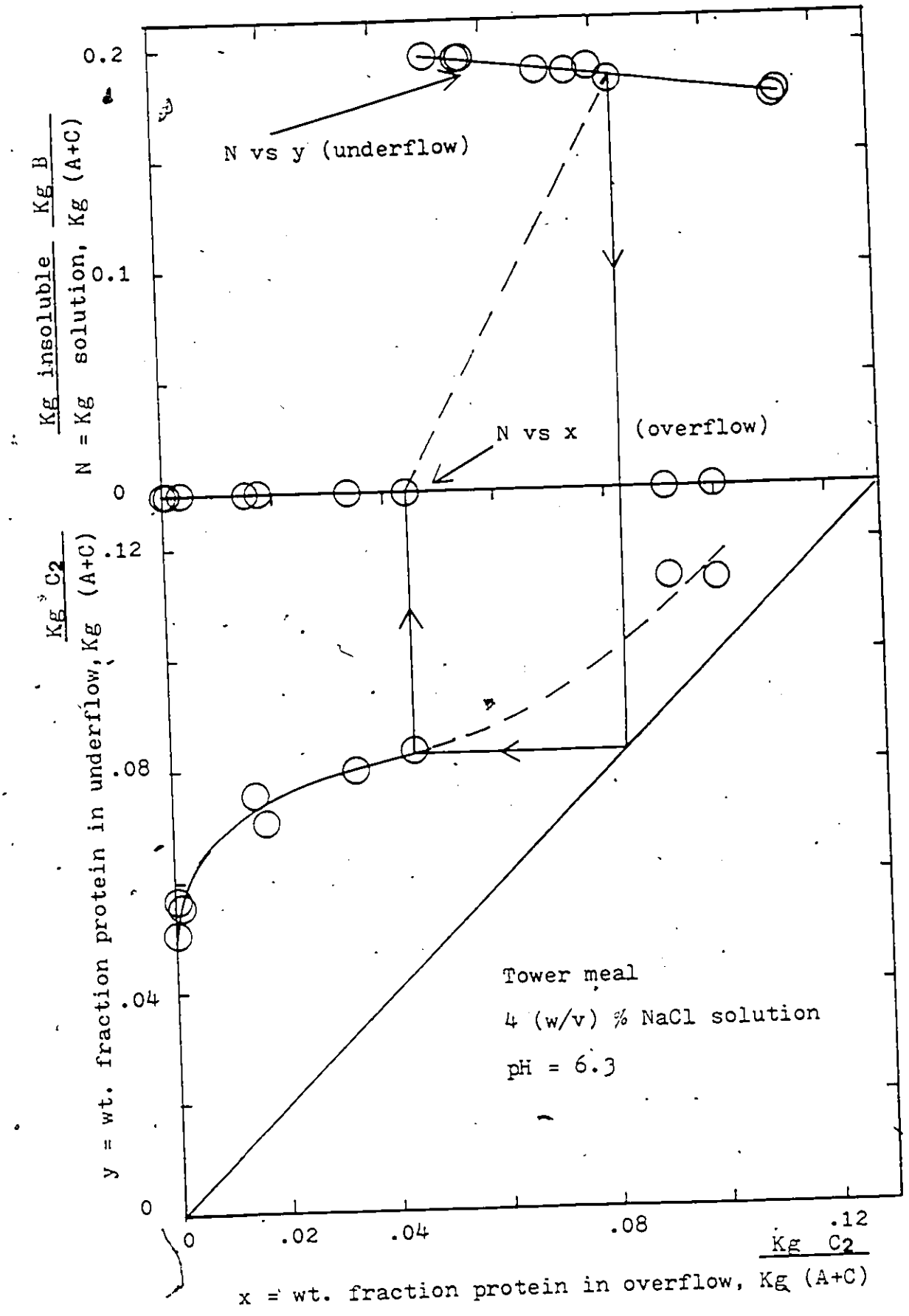


Figure 19 Protein (soluble) equilibrium diagram

in Appendix A10.

In Figure 18 and 19, the concentration of the insolubles (B) is expressed as N Kg of insolubles (B) per Kg of solvent (A) plus solubles ($C = C_1 + C_2$), i.e., N Kg B/Kg (A + B). Soluble solutes (C_1 or C_2) composition is expressed as weight fractions on an insoluble (B) - free basis. For the overflow, x_i is the weight fraction of soluble solute (C_1 or C_2) in the effluent solution. For the underflow, y_i is the weight fraction of the soluble solute (C_1 or C_2) in the raffinate phase.

For a given extraction procedure and conditions, it is possible to calculate graphically the extent of phytate and protein extraction from the Tower meal using a 4 (w/v) % sodium chloride solution. As an illustration, it is desired to estimate the percentage of the original phytate and protein left in the Tower meal sample after each extraction stage using a 4 (w/v) % sodium chloride solution and a v/n ratio of 10 ml/g for each of the extraction stages using a multistage crosscurrent extraction. The data obtained from the graphical calculation are tabulated in Appendix A11, and the sample calculations are given in Appendix B2. The phytate and the protein extraction curves in Figure 20 were drawn from the graphically determined values and were compared against the data previously obtained from an actual

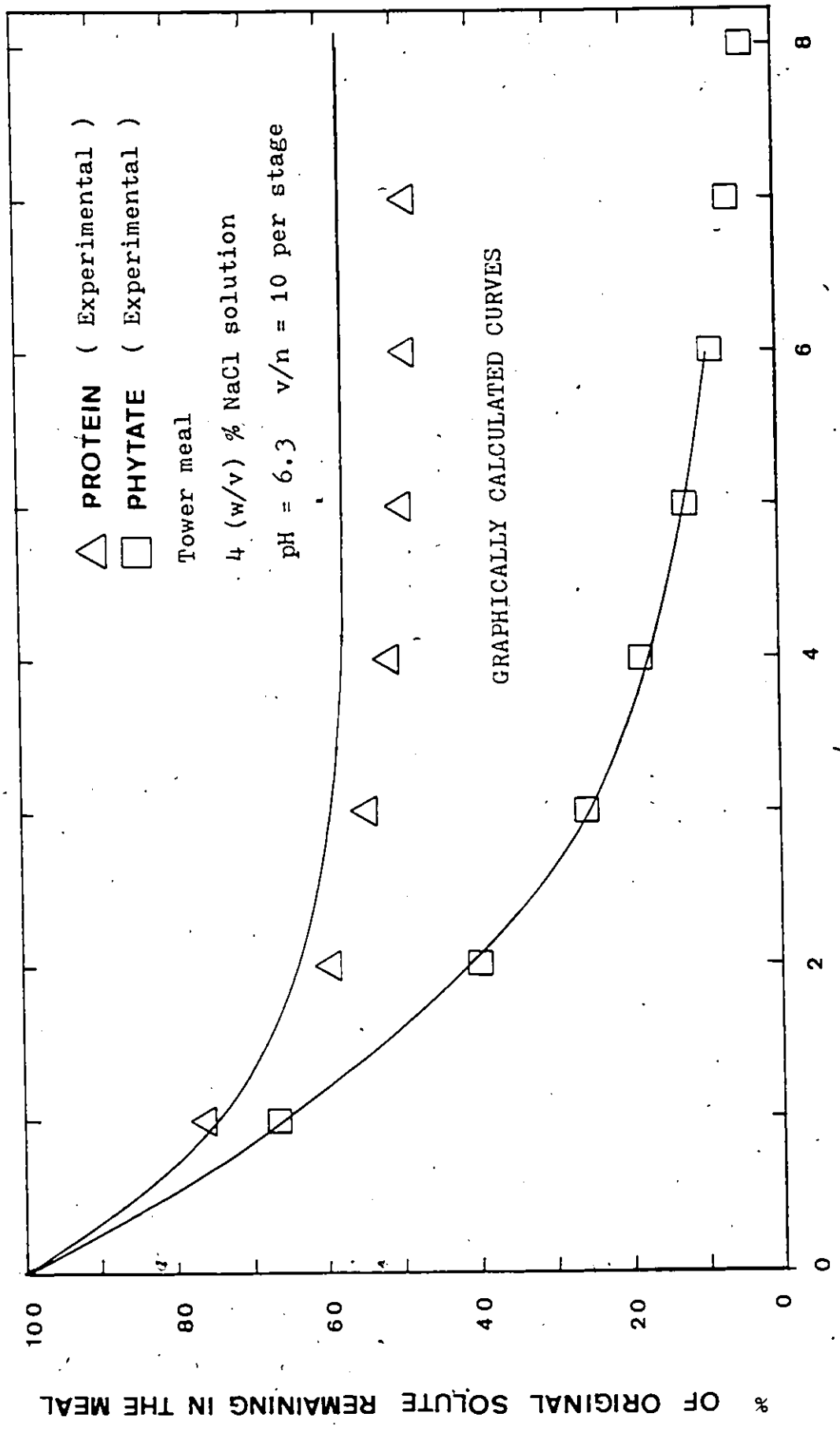


Figure 20 A comparison between graphically determined data and experimental data for multistage crosscurrent extraction of phytate

multistage crosscurrent extraction experiment. There is satisfactory agreement for the phytate extraction curve, but the results were less in agreement for the protein extraction curve as shown in Figure 20.

Membrane Selection and Development

During the extration of phytate with aqueous sodium chloride solution, it was observed that along with phytate and other phosphorus compounds, substantial amounts of proteins were also being extracted. It was decided to evaluate the feasibility of using ultrafiltration to selectively remove the phytate from the rapeseed extract.

Since the development of the first asymmetric cellulose acetate membrane by Loeb and Sourirajan (170), ultrafiltration (UF) and reverse osmosis (RO) membrane systems have been used in industrial concentration and fractionation applications. Among the few membrane materials developed, cellulose acetate has been the most commonly used. It still remains the standard to which the performance of the other membrane materials is compared.

The development and characteristics of cellulose acetate membranes have been extensively studied. Considerable advancement has been made in developing high flux cellulose acetate membranes. The performance characteristics of cellulose acetate membrane are fairly stable under properly controlled operating condition. Moreover, cellulose acetate membrane is easy and economical to make. Hence, cellulose acetate membrane was chosen for our study.

The use of cellulose acetate as membrane material has some inherent drawbacks. Cellulose acetate material is subject to hydrolysis when in contact with aqueous solution which leads to an increase in both the solvent and the solute fluxes. Chemically speaking, cellulose acetate is an ester and will react with water to form an alcohol and an acid. This hydrolysis reaction is highly base- and acid-catalyzed. Vos et al. (171) conducted a kinetic study for the hydrolysis of 39.8 weight per cent acetyl content cellulose acetate as a function of pH and temperature. Their results showed that the hydrolysis rate of the cellulose acetate material used was minimum in the pH region of 4 to 6, and that the hydrolysis rate was lower at lower temperature. Vos et al. (172) further calculated that the hydrolysis of the cellulose acetate materials could change the chloride permeation constant by a factor of 2 in about 4.3 years when the membrane is used in conjunction with aqueous sodium chloride solution at 23°C and a pH of 4.8. Hence it is important to operate the ultrafiltration under practical conditions where the hydrolysis rate is minimum in order to obtain long membrane life and steady performance.

Cellulose acetate, being a natural polymer, is also subjected to hydrolytic attack due to microbial effects. Furthermore, the cellulose acetate membrane is subjected to shrinkage at high temperature and to compaction at high pressure.

However, Cooper et al. (173) have observed that the use of cellulose acetate material of higher acetyl content will improve membrane resistance to biological attack.

The prime consideration in membrane selection and development is to obtain a match between the properties of the solutes to be separated and the separation characteristics of the membrane. Nevertheless, the initial criterion that determines the subsequent performance of a membrane is the composition of the membrane casting solution. Kutowy et al. (174) have shown that the casting solution composition used for this work, as presented previously in Table 5, was particularly suitable for making membrane for ultrafiltration purposes. In our case, we employed a low casting solution temperature of 4°C in order to reduce the rate of acetone evaporation during film casting. This in effect, decreased the sensitivity of membrane performance characteristics to any slight fluctuation in film casting conditions. Hence, a better reproducibility was obtained in the performance characteristics of the membrane produced. We further observed that a solvent evaporation time of 1 minute was necessary to obtain higher protein retention membrane.

The Effect of Gelation Bath Composition on Membrane Performance Characteristics

Previous investigators have demonstrated that the presence of aliphatic alcohols (such as methanol and ethanol) in the gelation bath, during the membrane gelation period, promotes the development of higher flux cellulose acetate membranes (174 - 176). In view of this, it was decide to find the "critical" ethanol composition of the gelation bath that will produce membrane capable of providing a high phytate-protein separation with a minimal protein loss. The results obtained from this investigation are given in Figure 21. The pure water permeation fluxes of the membranes were measured at 137.9 KPa and are included with the experimental data in Appendix A12.

The feed solution used for ultrafiltration was the extract of Candle flour prepared from 4 liters of 4 (w/v) % sodium chloride extraction solution at a v/n ratio of 500 ml/g. The feed flow rate was 2.5 L/min and the operating pressure was 137.9 KPa. The pH of the feed solution was unadjusted and was 6.3.

Our investigations showed that use of ethanol solution in the gelation bath instead of pure water resulted in membranes with much higher permeate flux and a corresponding much lower solute retention. This indicates that the effect of

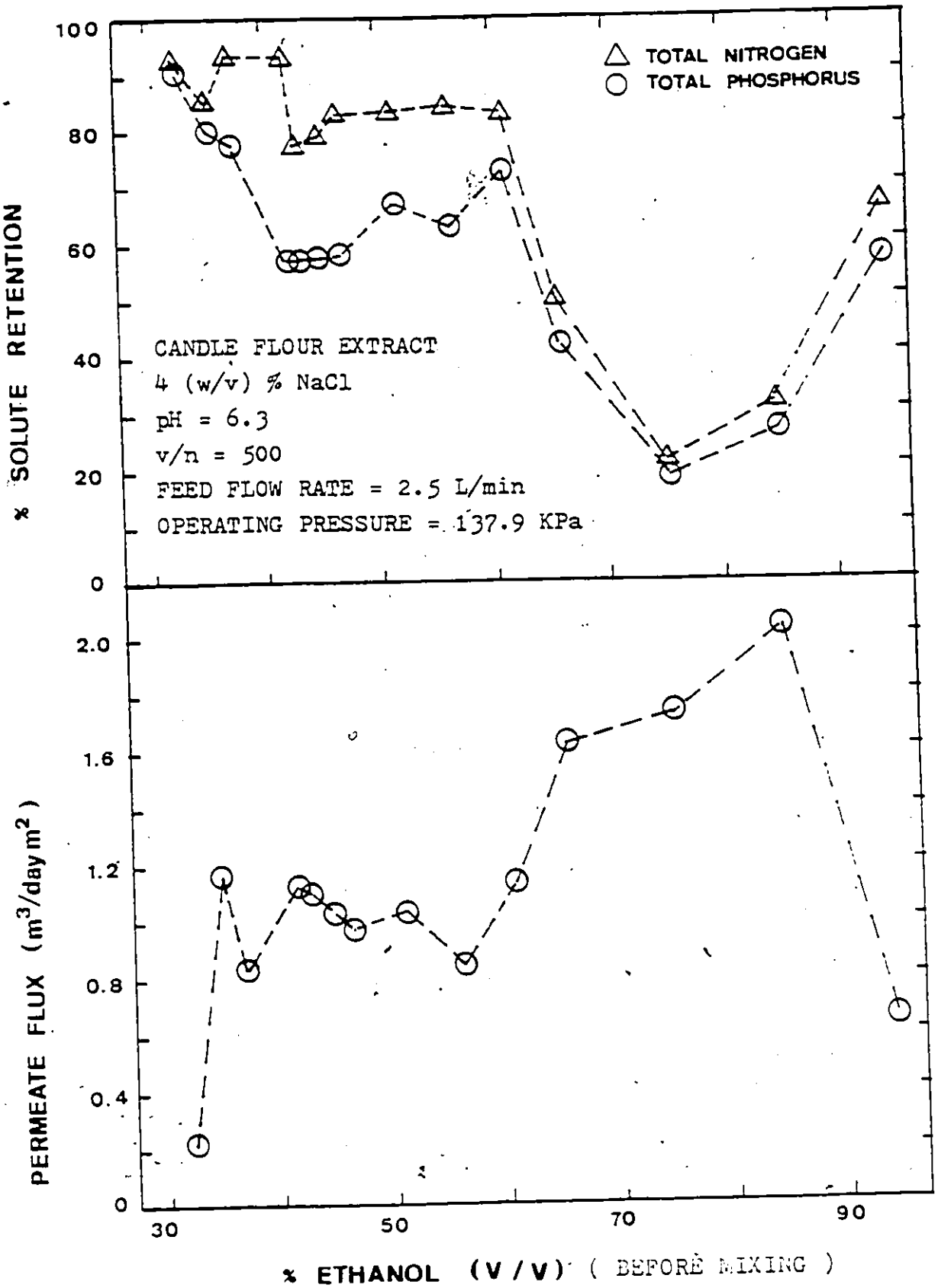


Figure 21 Effect of gelation bath composition on membrane performance characteristics

ethanol on the porous structure of the membrane was that of a non-solvent. A non-solvent will tend to decrease membrane homogeneity and to increase void and pore size (176). Consequently, a non-solvent will tend to increase membrane permeability and to decrease its structural strength. Hence, a more swollen membrane is produced when the cast membrane is immersed in an ethanol solution than when it is immersed in a pure water bath.

Membrane fabricated using a gelation bath whose ethanol composition was less than 28.00 (v/v) % (before mixing) gave extremely low permeate flux and were rejected from the study. Membrane Et-32.58, which was made from a gelation bath whose ethanol composition was 32.58 (v/v) % , showed very high retention for nitrogen and phosphorus compounds. Its percentage retention for nitrogen and phosphorus compounds was 92.44 and 90.50, respectively. The high solute retention suggests that the effective pore size of the membrane was probably too small to permit any significant permeation of either the phytate or the protein molecules. Therefore, the permeated solutes would most likely be non-phytate and non-protein micro-species.

From Figure 21, as the ethanol concentration in the gelation bath increased from 32.58 to 55.65 (v/v) %, there was

initially a dramatic increase in product permeate flux of the resulting membrane, and a corresponding decrease in its phytate retention. In contrast, there was no significant decrease in the nitrogen retention curve initially. However, as the ethanol concentration of the gelation bath increased, the effective pore size of the resulting membrane also increased and ultimately became large enough to permit the permeation of some of the smaller macromolecular proteins. The ethanol concentration of the gelation bath just prior to this occurence was called the "critical" ethanol concentration. Membrane fabricated at the "critical" ethanol concentration will provide the highest possible phytate-protein separation at a minimal loss of protein under the given conditions.

In Figure 21, the "critical" ethanol concentration is located within the region of 37.14 to 46.40 (v/v) %. Membranes fabricated within this region were classified as the "chosen" membranes since they were particularly suitable for this phytate-protein separation. These membranes were characterized by high protein retention and a corresponding low phytate retention. Their relatively high product permeate fluxes were also quite attractive.

Further increase in ethanol concentration beyond the 60.25 (v/v) % resulted in membrane whose effective pore size was too large to cause any significant separation between the phytate and the protein molecules as indicated by the total

phosphorus and the total nitrogen curves. A dramatic decrease in both phytate and protein retention and a sharp increase in product permeate flux were observed.

As the ethanol concentration of the gelation bath increased, the structural strength of the resulting membrane weakened since its degree of porosity and effective pore size increased. Membranes fabricated at high ethanol concentration are therefore more prone to structural compaction during the operation. Structural collapse or "cave in" could also be possible. The sudden decrease in pure water permeate flux (see Appendix A12) of the resulting membrane as observed at ethanol concentration beyond 65.25 (v/v) % can be attributed to the structural compaction effect. The sudden decrease in product permeate flux was observed in Figure 21 at 95.00 (v/v) % ethanol.

The compaction of the spongy bulk structure underneath the dense surface structure of the membrane could only affect its permeate flux. However, its separation characteristics could be affected if compaction occured at the dense surface layer of the membrane. This effect was exhibited by the increase of both phytate and protein retention curves at ethanol concentration beyond 74.71 (v/v) %.

Unsteady State Behavior of the Ultrafiltration of Rapeseed Extract

Figure 22 is presented to demonstrate the transient period encountered at the start-up of the ultrafiltration. The feed solution was the Tower meal extract prepared from 4 liters of 4 (w/v) % aqueous sodium chloride solution at a v/m ratio of 500 ml/g. The pH of the solution was unadjusted and was initially 6.3. The feed flow rate was 2.5 L/min and the operating pressure was 137.9 KPa. The tabulated data are given in Appendix A13.

The typical unsteady state behavior observed for the "chosen" membranes is exemplified by membrane ET-46.40. The code designates the gelation bath composition of the resulting membrane in volume per cent ethanol (before mixing). The "chosen" membranes generally have short transient period. Their steady state condition was usually reached within 40 minutes of operation. It was assumed that a steady state condition was attained when constant results were observed over a period of at least 30 minutes.

In Figure 22, there was a rapid decline in the product permeate flux during the initial period of ultrafiltration, after which the permeate flux remained fairly constant. The decline in the permeate flux was due to the formation of fouling deposits on the membrane surface. The fouling depos-

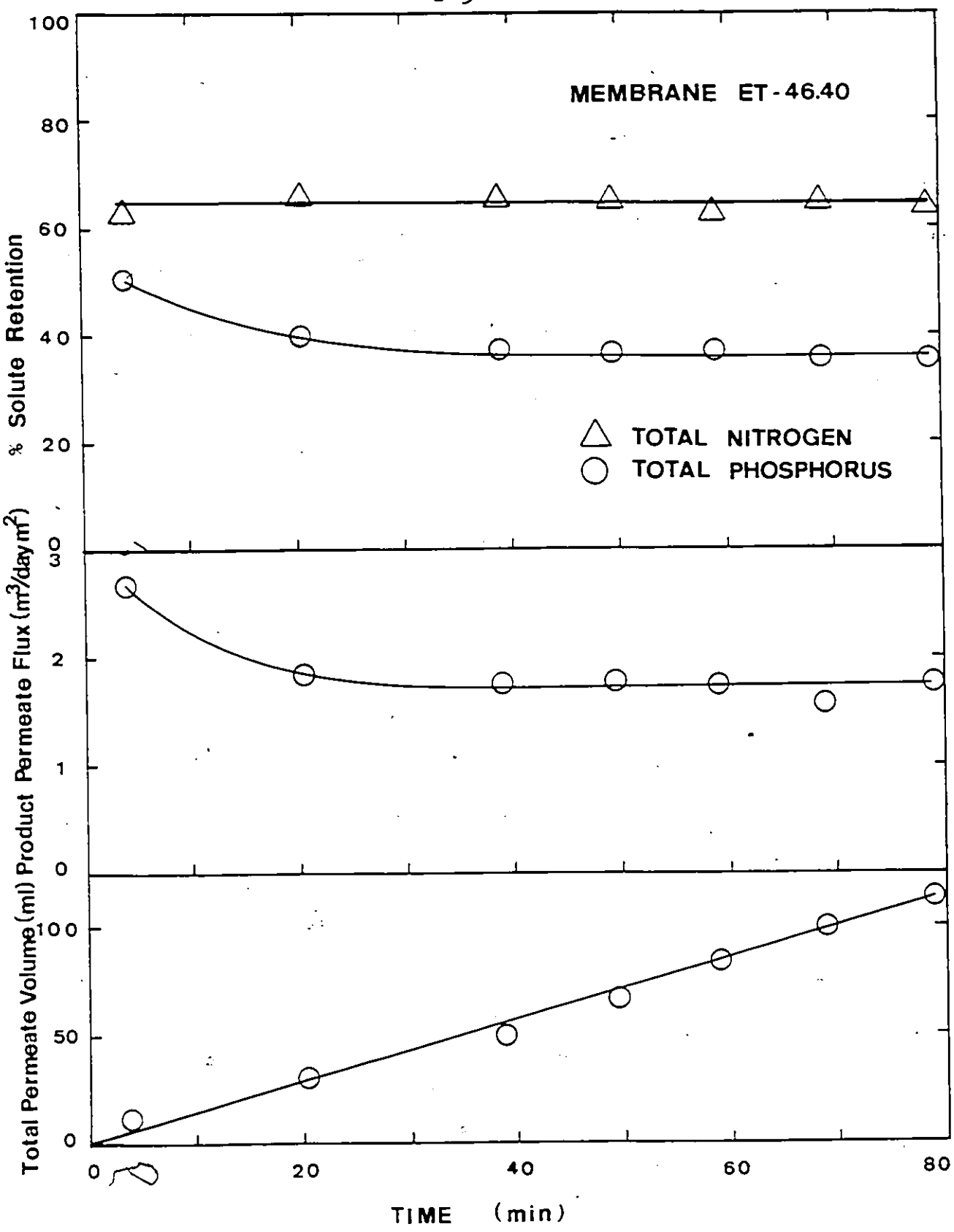


Figure 22 Unsteady state behavior of the ultrafiltration of Rapeseed extract

its were visibly observed on the membrane surface and were associated with the concentration polarization of proteins and other organic macromolecules. Membrane fouling effects are generally more severe for higher flux UF membrane.

Figure 22 shows that the total nitrogen retention curve approached its steady state value sooner than the total phosphorus retention curve, indicating a longer transient period for phytate- membrane interaction. Lower retention for total nitrogen was observed with the Tower meal extract than with the Candle flour extract. This was probably due to higher content of non-protein nitrogeneous micro-species in the Tower meal extract. The non-protein nitrogeneous content of the rapeseed extracts was estimated by measuring the percentage of total nitrogen retention of UF membranes that were found to be impermeable to the protein molecules. Membranes ET-28.02 and ET-32.58 estimated that the non-protein nitrogeneous species of the Tower meal extract constituted 25 to 30 per cent of its total nitrogen content. On the other hand, the non-protein nitrogeneous species of the Candle flour was estimated by membrane ET-32.58 to be 7.5 per cent of its total nitrogen content.

The Removal of Phytate by Continuous Diafiltration

Prior to the study of the effect of some of the operating parameters on the separation and flow characteristics of the "chosen" UF membranes, it was important to investigate beforehand whether or not a complete removal of the phytate from the dissolved proteins was attainable by the membrane processing technique. This was carried out by the continuous diafiltration experiments using the previously described UF system.

During the course of continuous diafiltration, the total bulk feed solution volume was maintained constant by periodically adding 4 (w/v) % sodium chloride solution to the retentate. The feed solution used was the Candle flour extract prepared from 4 (w/v) % sodium chloride extraction solution at a v/n ratio of 500 ml/g. The pH of the feed solution was unadjusted and was 6.3. The feed flow rate was 2.5 L/min and the applied operating pressure was 137.9 KPa. The results obtained with six "aged" membranes ET-38.99 in series are plotted in Figures 23 and 24, and are tabulated in Appendix A14.

Figure 23 shows the changes in bulk feed solution composition with the volume of dilution (V_D). The volume of dilution is defined as the ratio of the total volume of liquid permeated to the initial feed solution volume (V_o). The

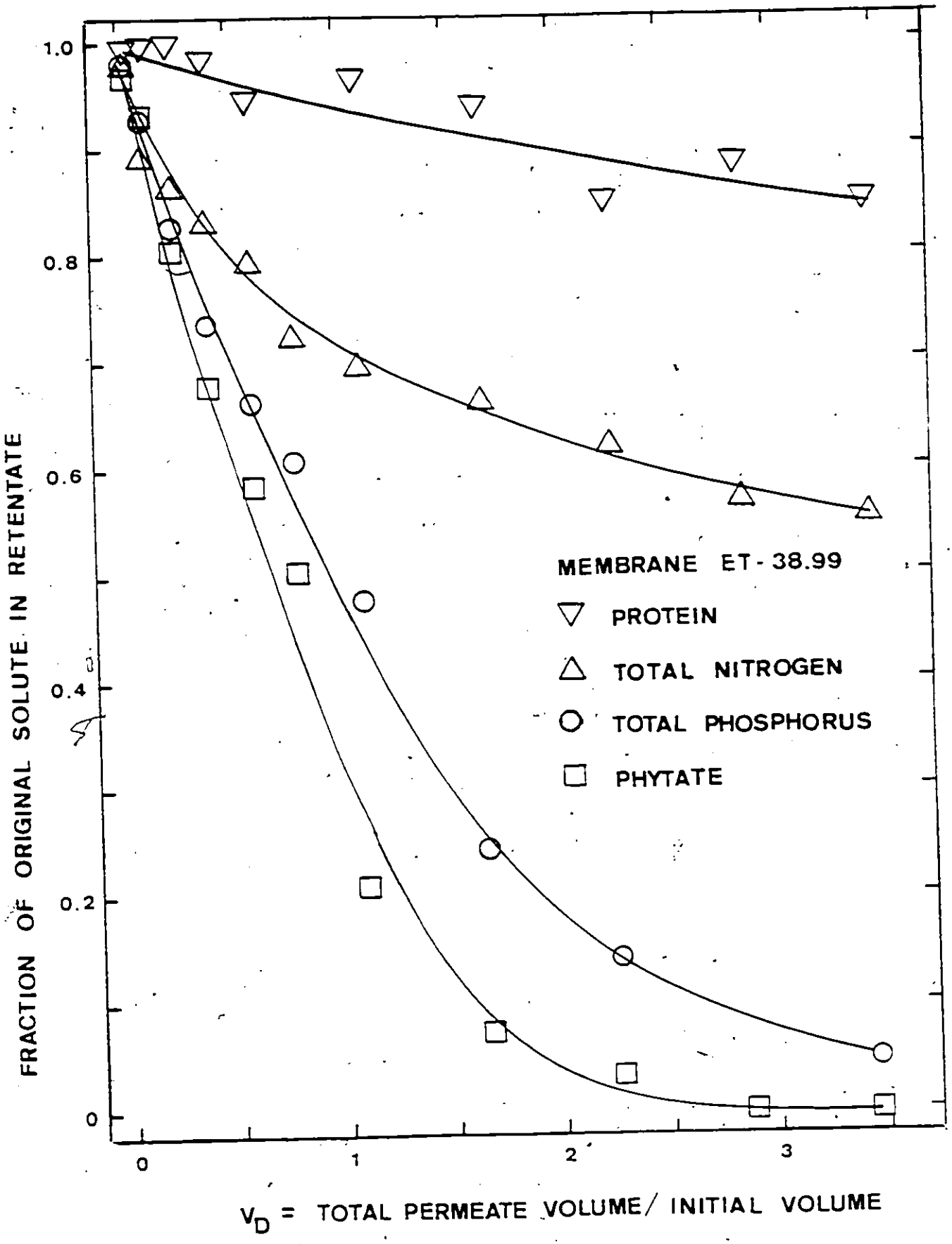


Figure 23 The changes in the bulk feed solution composition during the course of continuous diafiltration

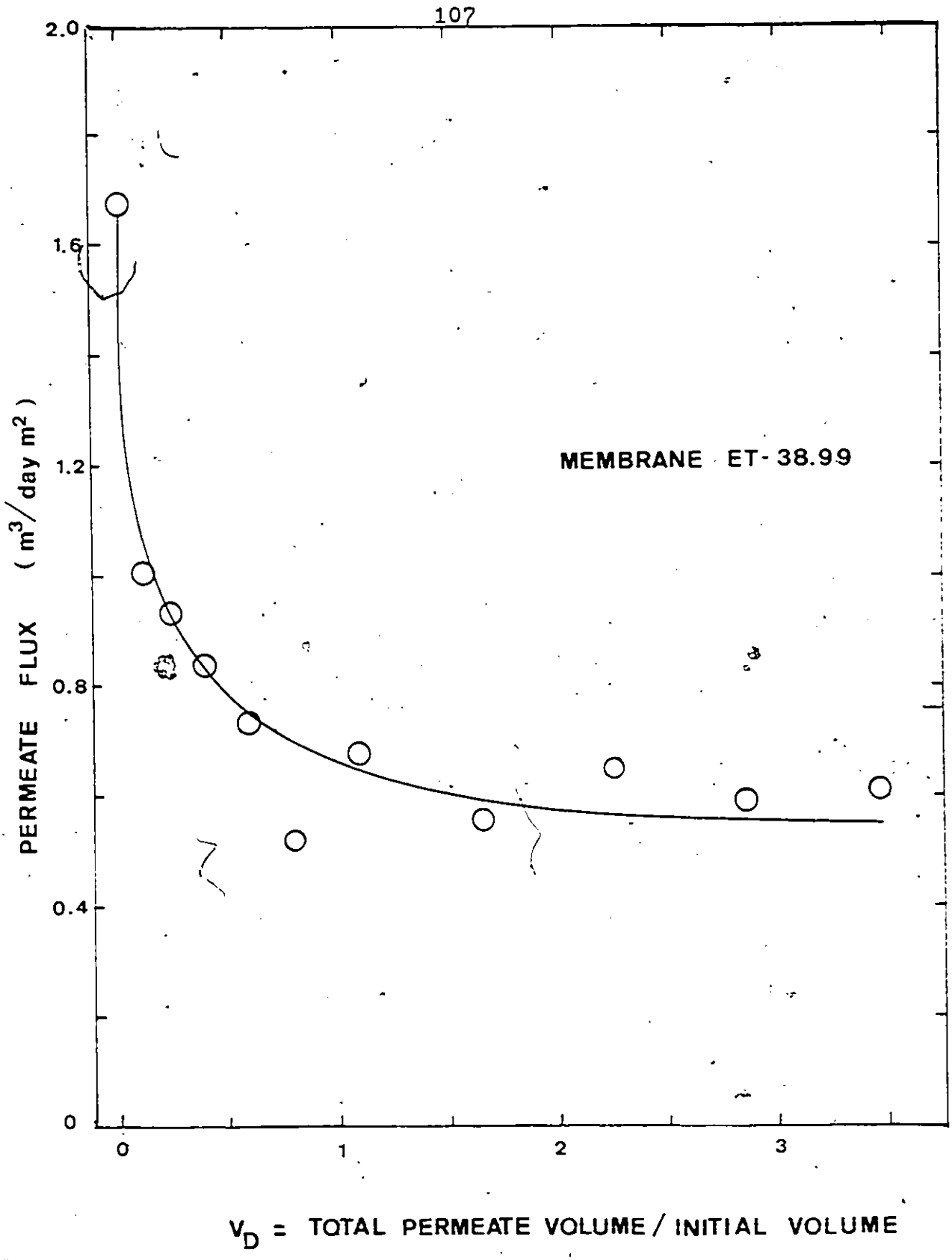


Figure 24 The changes in product permeate flux during the course of continuous diafiltration

initial feed solution volume was 2.5 liters. In Figure 23, after a V_D = 1.5, the total nitrogen curve is almost in parallel with the protein curve. Prior to this, the greater slope of the initial portion of the total nitrogen curve can be attributed to the removal of the smaller non-protein nitrogeneous species. At V_D = 2.75, a complete removal of phytate was achieved. The corresponding loss of protein was relatively low at 13.5 %. The significant loss of protein indicates that at least some of the "aged" UF membranes ET-38.99 were permeable to the low molecular weight proteins of the rapeseed extract. Rapeseed meals are known to contain proteins of widely different isoelectric points and molecular weights (177, 148). According to Lönnerdal and Janson (177), the low molecular weight proteins accounted for 20-40 % of the rapeseed meal proteins.

The "aged" UF membranes ET-38.99 had been used for almost three months prior to this continuous diafiltration experiments. These membranes had been subjected to extreme operating condition, including high and low pH solutions. Operating at extreme pH will certainly accelerate the rate of hydrolysis of the cellulose acetate and thereby, will impair the membrane performance characteristics. Unfortunately, the solute retention of these "aged" UF membranes ET-38.99 were not measured during the continuous diafiltration experiments.

A comparison between the "new" and "aged" UF "chosen" membranes is given in Table 8. Generally, "aged" membranes have lower solute retention and lower permeate flux than their initial values when they were first used. The degree of deterioration in membrane performance characteristics is greatly depended on the extensiveness of its use and the severeness of the operating conditions.

For a membrane separation process to be an economical viable unit operation, the membrane must maintain a high trans-membrane flux over long period of operating time. In Figure 24, the product permeate flux decreased sharply and leveled off with increasing volume of dilution (V_D). The initial decrease in permeate flux was associated with membrane compaction and membrane fouling effects. The permeation curve leveled off at V_D = 1.5 and the corresponding operating time was 289 minutes (see Appendix A14). The permeate flux remained fairly constant thereafter for a period of about 2000 minutes. The unusual long transient period was also observed with the other "aged" membranes.

The results show that phytate can be completely removed from the rapeseed extract by ultrafiltration and that a fairly high trans-membrane flux was maintained over a long period of operating time. A lower protein loss and a higher trans-membrane flux could be obtained if "newer" "chosen" UF membranes were used.

Table 8 A Comparison Between "New" and "Aged" Membranes

Membrane		ET-37.14	ET-38.99	ET-40.83	ET-42.68	ET-44.52	ET-46.40
Pure Water Permeate Flux (m^3/day m^2)	1st Run	1.5018	0.8606	2.6788	2.5381	2.5788	2.3626
	17th Run	1.3930	0.8187	2.1014	2.4389	2.2703	1.9242
Product Permeate Flux (m^3/day m^2)	1st Run	0.8393	0.6316	1.1233	1.1074	1.0253	0.9219
	17th Run	0.5661	0.5209	0.5817	0.7363	0.7398	0.6547
% N Retention	1st Run	93.20	85.84	71.67	77.34	78.47	83.00
	17th Run	82.06	78.80	73.91	75.54	72.83	76.63
% P Retention	1st Run	77.15	81.19	58.08	56.06	57.07	53.41
	17th Run	59.18	51.25	43.53	30.39	44.43	36.81

Effect of Sodium Chloride on the Removal of Phytate by Ultrafiltration

During the removal of phytate from the rapeseed extract by continuous diafiltration, 4 (w/v) % sodium chloride solution was periodically added back into the feed tank instead of pure water. The sodium chloride in the processing solution must be maintained at a certain concentration in order to retain the low phytate retention of the membrane. This was observed when UF experiments using the water extract of of the rapeseed sample failed to produce a reasonable phytate-protein separation.

Phytic acid and its known salts have molecular weights of less than 1,000 and are therefore expected to freely permeate through the UF membrane. However, our observations on UF experiments made with the water extract of the rapeseed sample and with only sodium phytate in water have shown that phytate barely passed through the "chosen" UF membranes. Similar observations were reported by previous investigators on the removal of phytate from the soybean extract by dialysis and by ultrafiltration (139, 146, 147).

In addition, we observed that the permeation of phytate was greatly influenced by the presence of added sodium chloride. This is illustrated in Figure 25 with membrane BT-42.68. The feed solution used for ultrafiltration was 4 li-

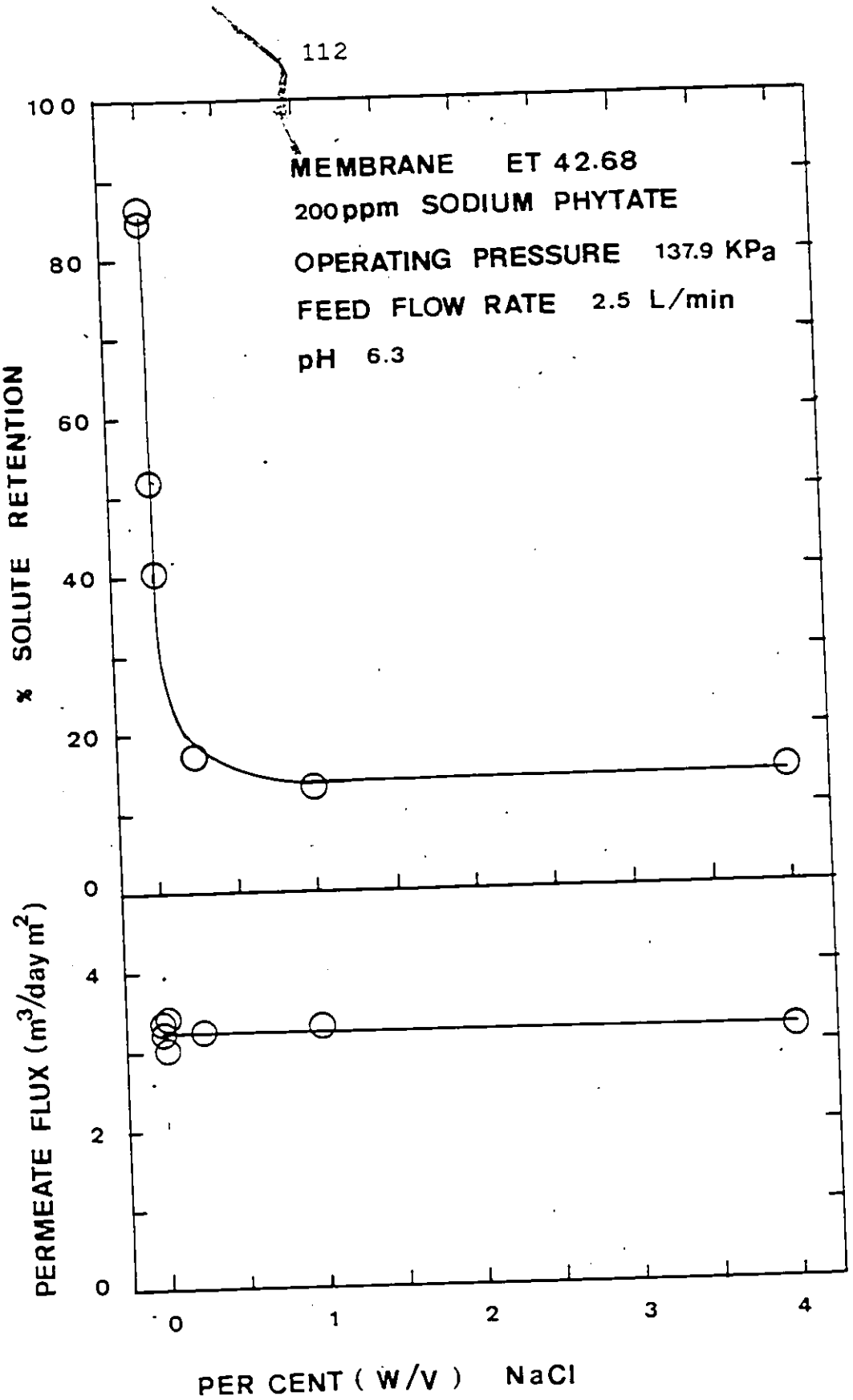


Figure 25 Effect of sodium chloride on the removal of sodium phytate by ultrafiltration

ters of 200 ppm of sodium phytate sample. The pH of the feed solution was adjusted to 6.3 with hydrochloric acid. The feed flow rate was 2.5 L/min and the applied operating pressure was 137.9 KPa. The data are tabulated in Appendix A15. Similar results obtained for three other "chosen" UF membranes are included in Appendix A15.

In Figure 25, the retention of phytate for membrane ET-42.68 was measured to be 86.38 % in the absence of added sodium chloride, and 17.13 % in 0.25 (w/v) % of sodium chloride. The dramatic decrease in the phytate retention with added sodium chloride indicated the effectiveness of sodium chloride in enhancing the membrane permeation of phytate. The product permeate flux remained constant with increasing sodium chloride concentration indicating no significant increase in effective osmosis pressure difference across the membrane. This was expected since sodium chloride was observed to be freely permeating the "chosen" UF membranes. These observations are presented in Table 9.

Omosaiye and Cheryan (147) reported that phytate did not readily permeate the UF membrane with a 50,000 molecular weight exclusion limit. Since earlier reports indicated that phytate exists as soluble, unionized salts, except at high solution pH (129, 178 - 182), they deducted that the solubility characteristics of phytic acid were not the reason for

Table 9 The Ultrafiltration of 4 (w/v) % Sodium Chloride Solution.

(Feed Flow Rate = 2.5 L/min, Operating Pressure = 137.9 KPa)

Membrane	Pure Water Permeate Flux ($m^3/day\ m^2$)	Product Permeate Flux ($m^3/day\ m^2$)	% Solute Retention
ET-39.91	2.6176	2.4464	0.00
ET-41.76	3.2052	3.0223	0.00
ET-41.76	2.8570	2.7148	0.00
ET-43.60	2.3102	2.2274	0.00

its non-removal during ultrafiltration. Hence, they concluded that interactions occurred between the phytate and the protein which impeded the passage of phytate through the membrane. Our ultrafiltration studies, however, have shown limited permeation of phytate even in the absence of protein in the system as demonstrated by the sodium phytate solution without the added sodium chloride. This shows that the conclusion made by Omosaiye and Cheryan is insufficient to explain our observed phenomenon.

Based on the analysis of their defatted soy flour which indicated that calcium and magnesium are 2.6 and 5.9 moles per mole of phytate respectively, de Rham and Jost (146) claimed that phytate exists as insoluble calcium phytate in the soy extract. They concluded that phytate can be dialyzed with a concentrated sodium chloride solution because of the shift of the equilibrium from the insoluble calcium phytate towards the soluble sodium phytate, i.e.

$$\text{sodium phytate} + Ca^{++} \rightleftharpoons \underline{\text{calcium phytate}}\downarrow + Na^{+}$$

On the ultrafiltration of sodium phytate - water system, we observed low phytate permeation in the absence of added sodium chloride. However, the semi-quantitative analysis of the sodium phytate sample used in our UF experiments, as presented in Table 10, showed that the molar ratio of the

Table 10 Semi-Quantitative Analysis of the Sodium Phytate Sample

SEMI-QUANTITATIVE ANALYSIS

No: 11-394

Sample No. Lot 4249-A

Method: XRF

No. of Elements: 32

From: Ottawa University
P.O. 81-04-01-933
Date: April 13, 19 81

Analyst:

MAJOR ELEMENTS (%)	<.003	.003-.01	.01-.03	.03-0.1	0.1-0.3	0.3-1.0	1.0-3.0	3.0-10.0	> 10.0	REMARKS
SiO_2	X									
Al_2O_3	X									
Total Fe (Fe_2O_3)				X						
MgO	X									
CaO	X									
Na_2O									X	
K_2O	X									
TiO_2	X									
TRACE ELEMENTS (%)										
V	X									
Cr	X									
Mn			X							
Co	X									
Ni	X									
Cu	X									
Zn		X								
As	X									
Sr	X									
Y	X									
Zr			X							
Nb	X									
Mo	X									
Ag	X									
Sn		X								
Sb	X									
Ba	X									
La	X									
Ce	X									
W	X									
Pb	X									
Bi	X									
Th	X									
U	X									

total metal impurities to phytate was only 1 : 12.7. Theoretically, the required stoicheometric molar ratio for divalent metal ion to phytate is 6 : 1 and for trivalent metal ion to phytate is 4 : 1. Hence, the low permeation of phytate can not be solely attributed to the formation of insoluble metal-phytate complexes, and the effectiveness of the sodium chloride in enhancing the phytate permeation can not be simply explained by the displacement of the equilibrium. It must also be pointed out that de Rham and Jost (146) based their metals - phytate molar ratios on the composition of the soy flour and not of the soy extract.

In order to explain the observed phenomena of the ultrafiltration of phytic acid, a postulate is made based on the structural configuration of the phytic acid molecule. The structure of the phytic acid (Figure 1) indicates that the association among the phytate molecules is highly favorable. When the six phosphate groups of the phytate molecule are not saturated with the positively charged mineral ions, these phosphate groups can form hydrogen bonds with the surrounding water molecules and with the phosphate groups of the other phytate molecules. The formation of the intermolecular hydrogen bonds among the phytate molecules most probably involve the hydrogen of the hydroxyl group of one molecule and the oxygen of the O = P group of another molecule. The phytate molecule therefore does not exist as a single isolated

molecule, but rather as a huge hydrated molecular aggregate with the other phytate molecules. This may be the primary reason for the limited permeation of phytate in the absence of added sodium chloride, despite its low molecular weight.

In order for the phytate molecules to be readily permeable through the UF membrane, they should exist in the soluble form and not be strongly associated with each other and/or with protein molecules. The hydrogen bondings among the phytate molecules and between the phytate and the water molecules could be precluded by saturating the phytate molecules with positively charged ions. Furthermore, previous experimental evidence (33, 129, 139, 146, 178, 184), showed that sodium phytate as being the most water soluble salt of phytic acid. Hence, sodium ion is probably the logical choice of cation to saturate the phytate molecules to increase their membrane permeation. This, together with the displacement of the equilibrium by the excess sodium ions (146), could probably be sufficient to account for the effectiveness of added sodium chloride in enhancing the permeation of phytate by ultrafiltration.

Unsteady State Behaviour of the Ultrafiltration of Sodium Phytate Solution

Experiments were conducted to estimate the approximate time required for approaching steady state condition during the ultrafiltration of sodium phytate feed solution. The feed solution used was 4 liters of 200 ppm sodium phytate solution with 4 (w/v) % sodium chloride. The feed flow rate was 2.5 L/min and the operating pressure was 137.9 KPa. The pH of the feed solution was adjusted to 6.3 with hydrochloric acid. The results obtained with UF membranes ET-37.14, ET-46.40, ET-65.25, and ET-74.71 are plotted in Figures 26 to 29 and are tabulated in Appendix A16.

From Figures 26 to 29, the phytate permeation curves for membranes cast in lower ethanol concentration baths (UF membranes ET-37.14 and ET-46.40) approached their steady state value much sooner than that for membranes casted in higher ethanol concentration baths (UF membranes ET-65.25 and ET-74.71). The reverse was true for their product permeation curves. Similar observations were made with the Tower meal extract. Whether this behaviour was related to the structural strength of the membrane, its porosity and its pore size, are not certain. No further work was done to elucidate an explanation to these observations. For the "chosen" membranes ET-37.14 and ET-46.40, it was observed that 45

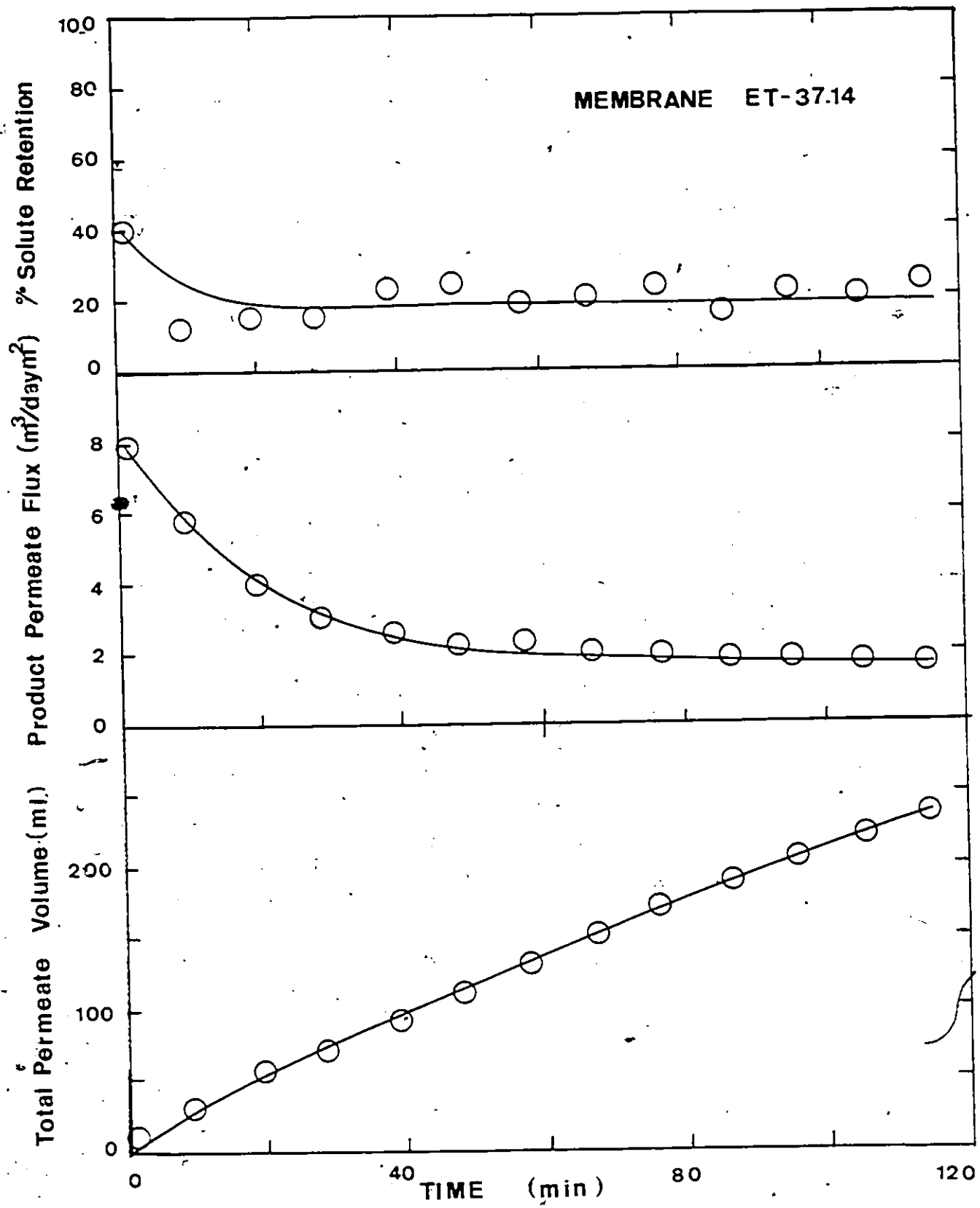


Figure 26 Unsteady state behaviour of the ultrafiltration of sodium phytate solution

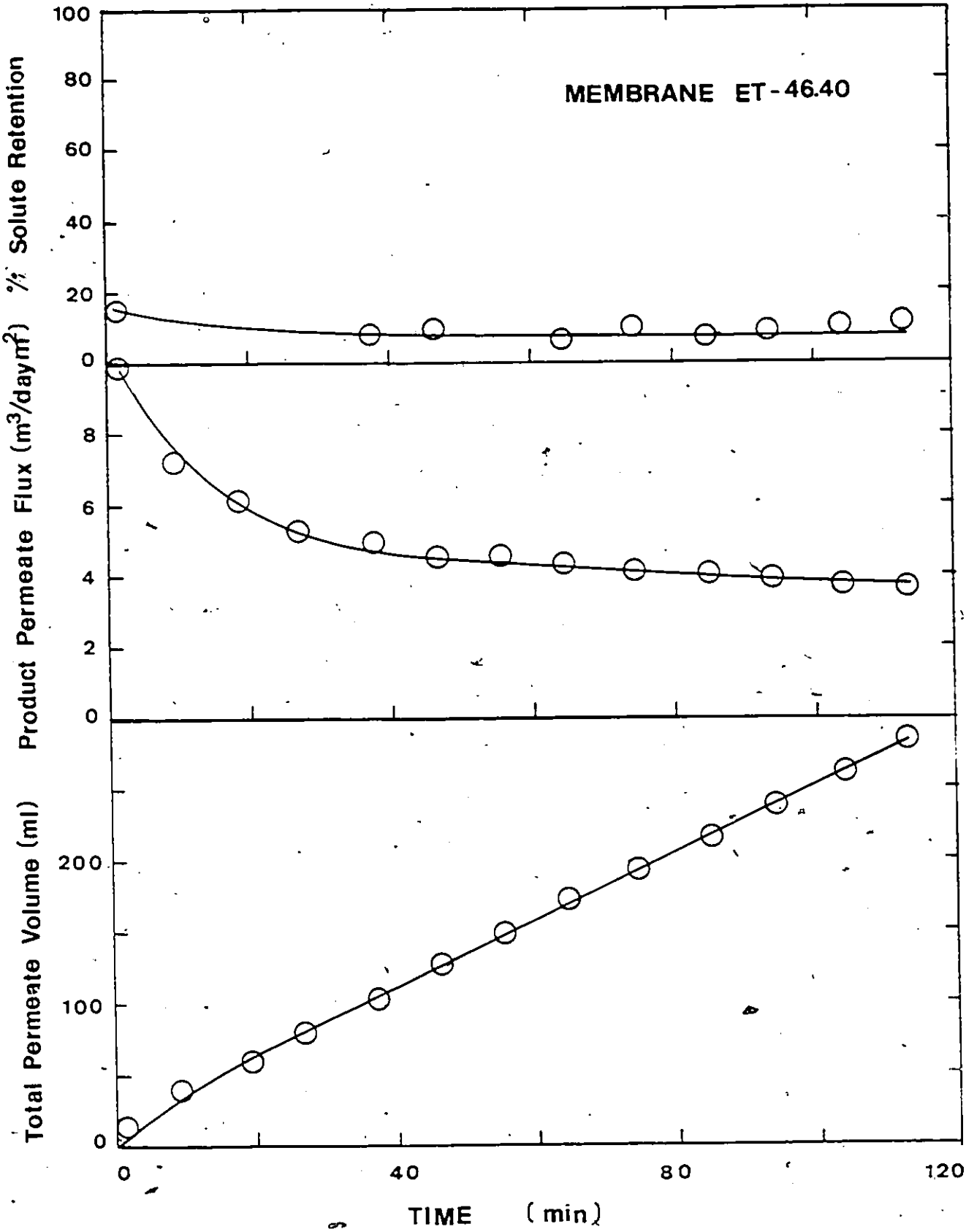


Figure 27 Unsteady state behaviour of the ultrafiltration of sodium phytate solution

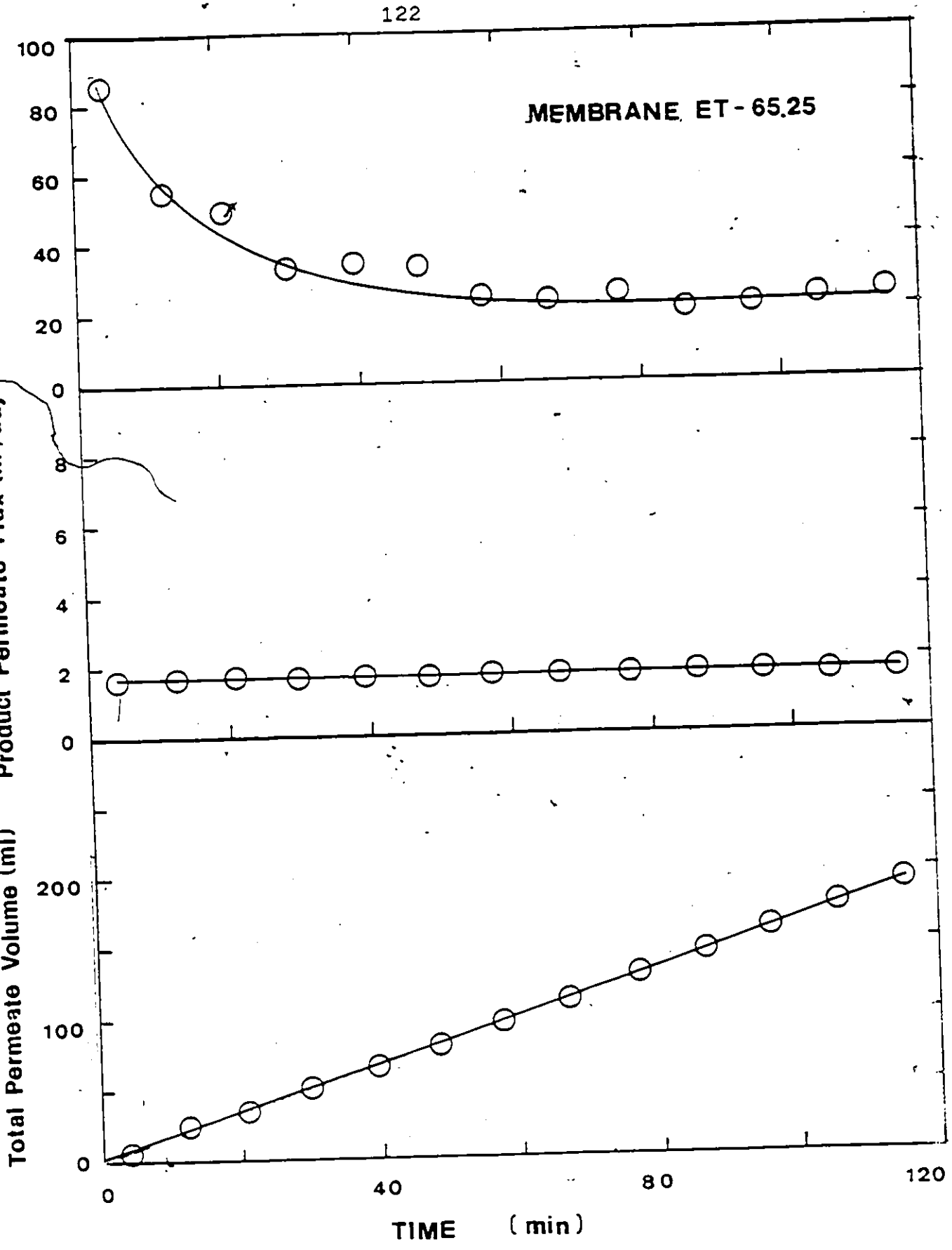


Figure 28 Unsteady state behaviour of the ultrafiltration of sodium phytate solution

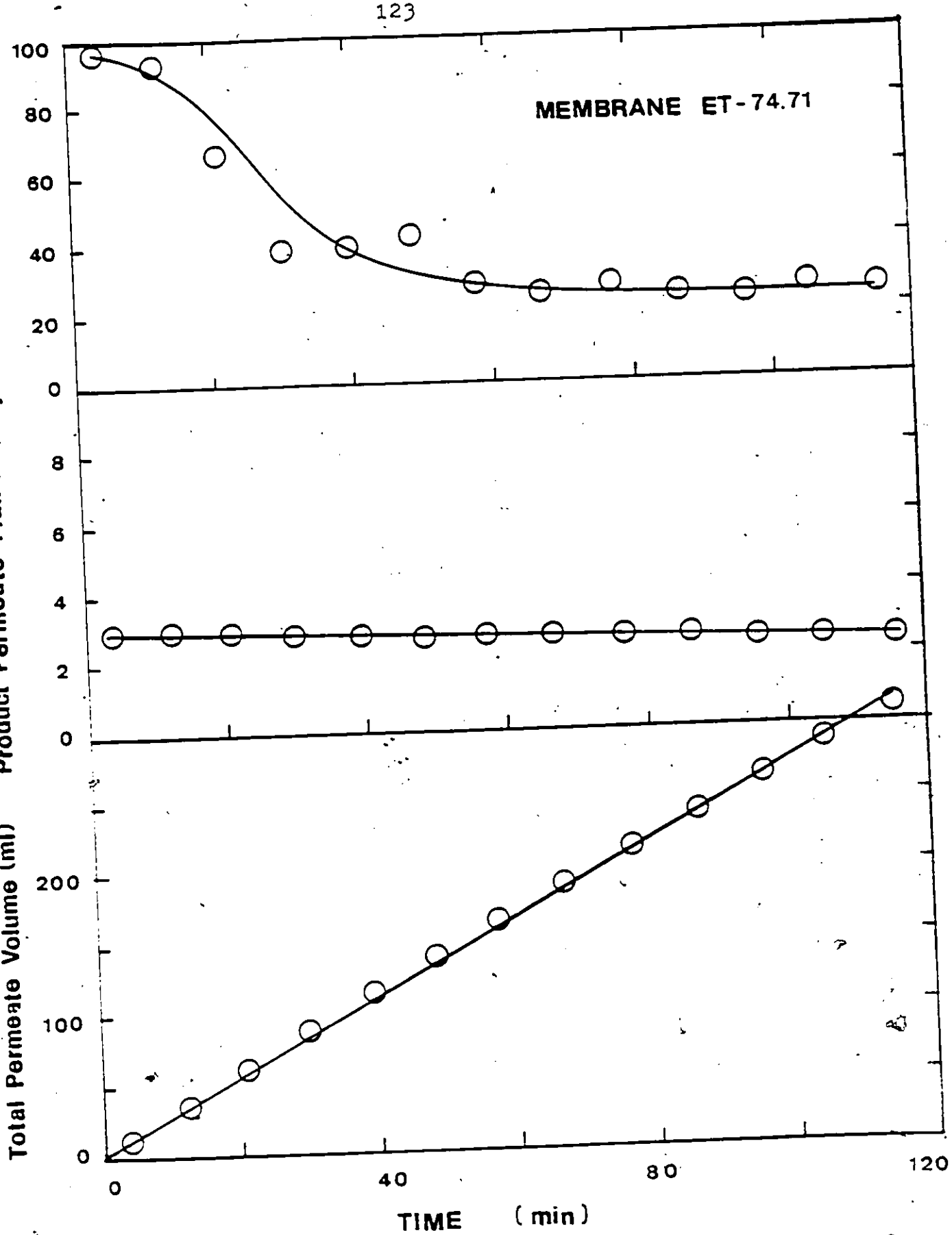


Figure 29 Unsteady state behaviour of the ultrafiltration of sodium phytate solution

minutes of ultrafiltration was sufficient to obtain the steady state values for phytate retention and product permeate flux.

Effect of Feed Solution pH on the Removal of Phytate from Rapeseed Extract by Ultrafiltration

Our earlier works showed that the presence of sufficient amount of sodium chloride in the processing solution was essential for obtaining high permeation of phytate during ultrafiltration. However, the presence of protein and cationic impurities in the processing solution may affect the extent of phytate permeation even with added sodium chloride. The protein and the cationic impurities may interact with phytate to form various phytate complexes. The nature of interactions between phytic acid, protein, and cationic impurities are, in turn, influence by the concentration, pH, and ionic strength of the solution. Hence depending on the chemical environment of the system, phytate may exist as free phytic acid, cation-phytate compounds, protein-phytate complexes, or protein-cation-phytate complexes. The postulated mechanisms for phytic acid in solution are therefore as follows:

$$\text{Cation} + \text{Phytic acid} \rightleftharpoons (\text{Cation-phytic acid}) \qquad \text{(I)}$$

$$\text{Protein} + \text{Phytic acid} \rightleftharpoons (\text{Protein-Phytic acid}) \qquad \text{(II)}$$

$$\text{Protein} + \text{Cation} + \text{Phytic acid} \rightleftharpoons$$

$$(\text{Protein-Cation-Phytic acid}) \qquad \text{(III)}$$

Since only the free phytic acid and the soluble nonionized cation-phytate compounds are readily permeating the "chosen" UF membrane in the presence of sufficient amount of sodium chloride, the extent of phytate removal from the system by ultrafiltration will therefore depend on the relative magnitudes of the association constants governing the above equilibrium equations.

The performance of the membranes on phytate - protein separation was affected by the pH of the feed solution as demonstrated by Figures 30 and 31 for UF membranes ET-38.99 and ET-37.14. The tabulated data are given in Appendix A17. The feed solution used was the extract of the Candle flour prepared from 4 (w/v) % sodium chloride extraction solution at a v/m ratio of 500 ml/g. The feed flow rate was 2.5 L/min and the operating pressure was 137.9 KPa. The pH of the feed solution was adjusted with hydrochloric acid and sodium hydroxide solution.

Figures 30 and 31 show that the phytate - protein separation of the "chosen" membrane was greatly improved by shifting the processing solution pH from the normal 6.3 to 5.5. A much lower phytate retention was observed at pH of 5.5 indicating that phytate was less associated with the protein at this condition. It was therefore suspected that pH 5.5 might be the mean isoelectric pH for the bulk rapeseed proteins in the Candle flour extract

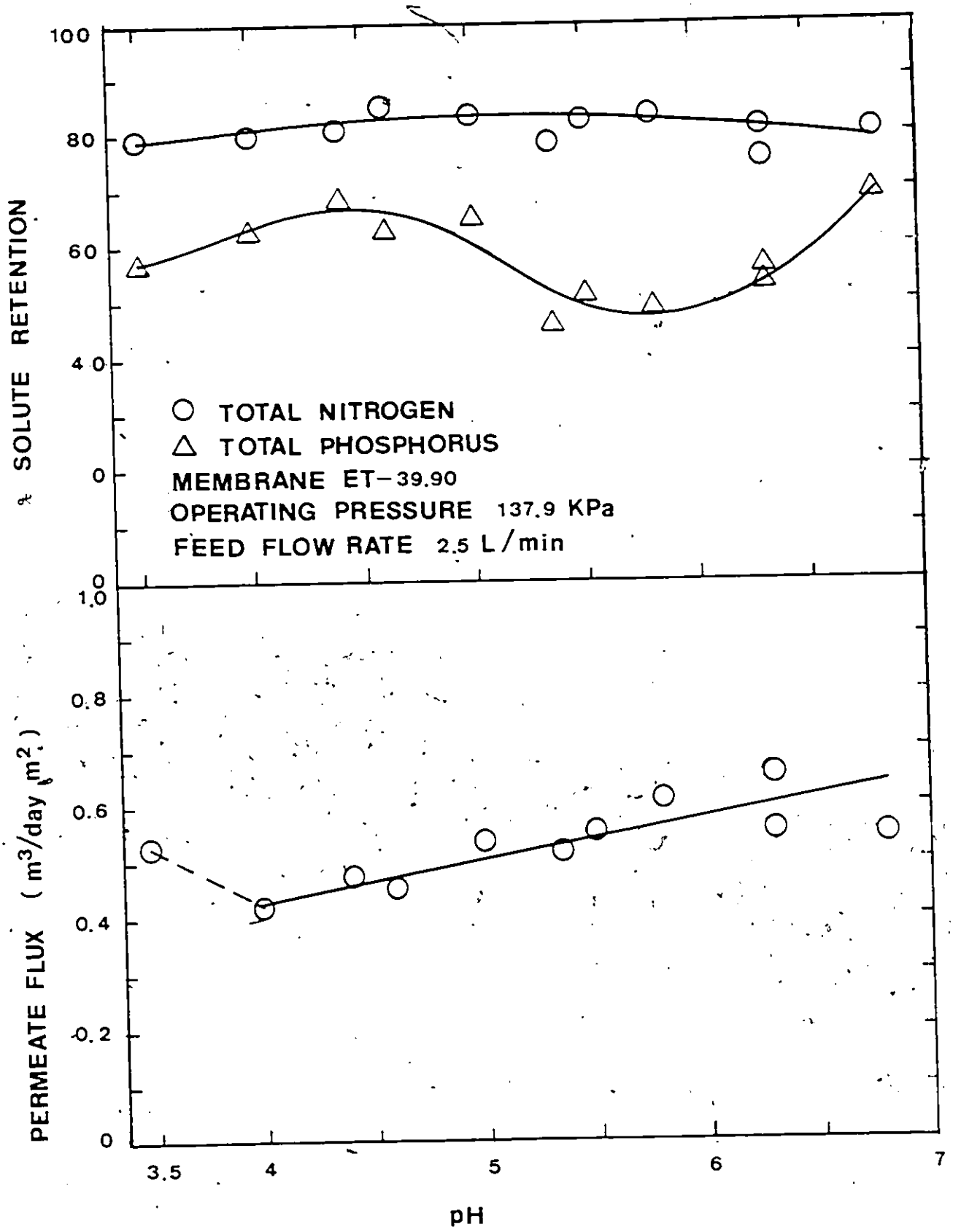


Figure 30 Effect of feed solution pH on the removal of phytate from rapeseed extract by ultrafiltration

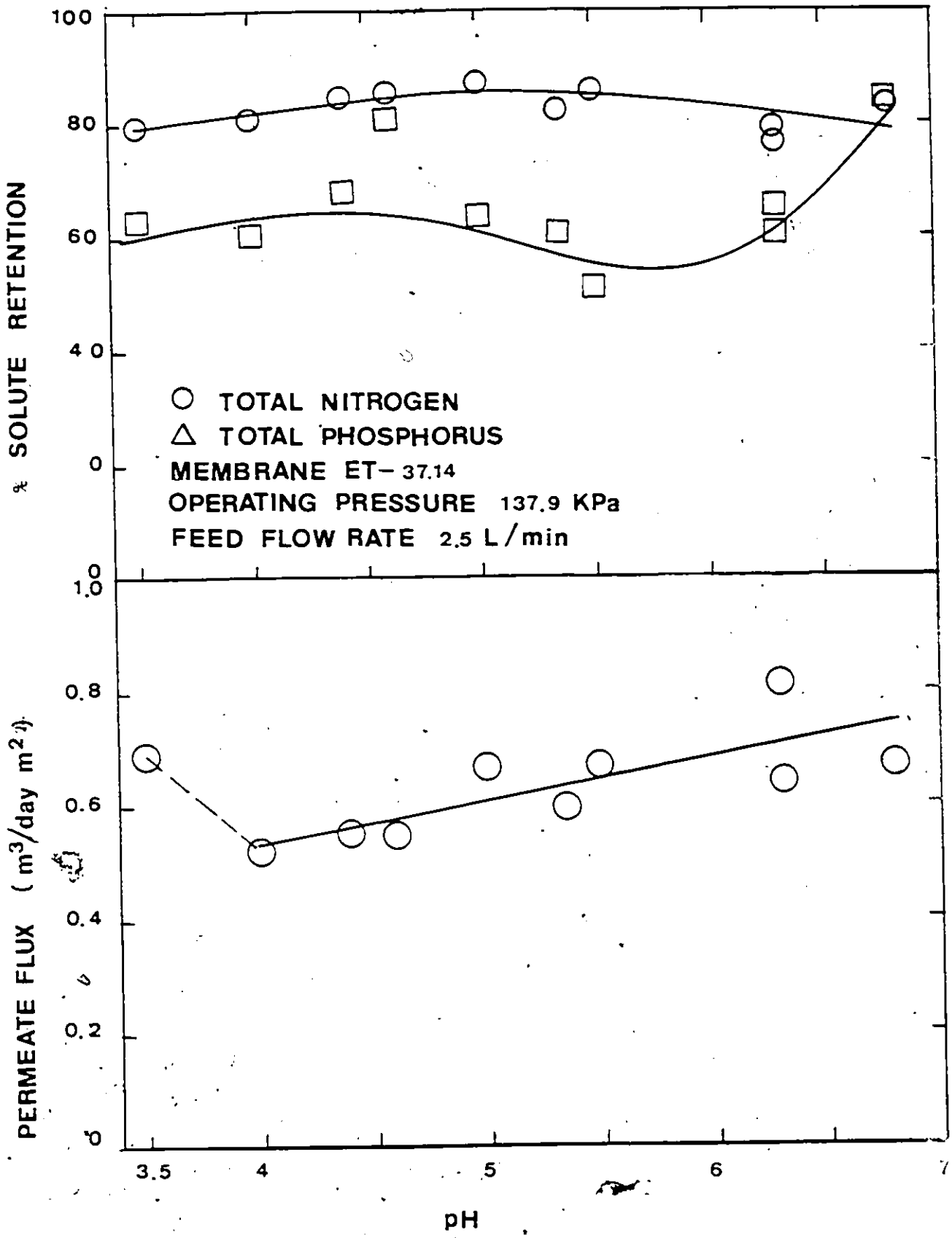


Figure 31 Effect of feed solution pH on the removal of phytate from rapeseed extract by ultrafiltration

solution. However, attempts made to verify this point by potentiometric titration of the extract solution failed to identify the isoelectric points of the extracted proteins.

Below the pH of 5.5, the phytate retention of the "chosen" UF membranes increased with decreasing pH. This effect was associated with the increase in electrostatic attraction between the positively charged protein and the negatively charged phytate molecule (Equilibrium II). In all of our experimental conditions, the phytate molecule will be strongly negatively charged since six of its twelve replaceable protons are strongly dissociated at pH of 1.8 (178). Protein, on the other hand, is positively charged at pH below its "true" isoelectric point. Hence, a protein-phytate complex could be formed as a result of strong electrostatic attraction (144, 179, 180, 185). This "true" isoelectric pH may varies from one protein to another. Since the extracted rapeseed proteins are almost totally rejected by the "chosen" UF membrane, the phytate that is bound to the protein will also not pass through the membrane.

Above the pH of 5.5, the amount of phytate removed decreased with increasing pH. This effect was attributed to the increasing strength of the multivalent cation linkage with increasing pH (Equilibrium III) and / or

decreasing solubility of the cation-phytate salts at high pH (Equilibrium I). Inspite the fact that the electrostatic effects are minor at high pH, there was a fairly strong association between the aqueous extracted protein of the oilseeds and the phytate at alkaline pH as shown by the electrophoritic results reported by O'Dell and de Boland (186). Experimental evidence by other investigators (144, 179, 185, 187) indicated that the multivalent cations, particularly the alkaline-earth metals, mediate the binding between phytate and protein. Furthermore, higher pH favors the formation of calcium and magnesium salts of phytic acid, compounds with limited solubility (129, 146, 185). Above pH 10.3, the phytate is almost completely insoluble (188). Some other divalent cationic salts of phytate are also insoluble at alkaline pH (49 - 52).

The observed behaviour of the product permeation flux was quite interesting. It decreased linearly with decreasing pH as illustrated in Figures 30 and 31. Whether this was due to the change in membrane characteristics or solution characteristics is not known. Further decrease in solution pH caused precipitation. Precipitation was observed at pH 3.50. The increase in product permeate flux at that pH was therefore associated with the change in solution system. The effect of precipita-

tion on phytate - protein separation was not very noticeable.

Effect of Variation in Feed Flow Rate on the Ultrafiltration of Albumin Bovine Fraction V Solution

To prevent the solute concentration build up in the solution at the vicinity of the membrane surface, it is necessary to introduce a high degree of turbulent flow within the UF cell unit. A high feed flow rate combined with proper geometry of the flow system can accomplish this purpose.

The effect of feed flow rate on the separation and flow characteristics of the "chosen" membranes was examined using the albumin bovine fraction V solution. The "chosen" membranes used were ET-39.91, ET-41.76, and ET-43.60. The feed solution was 8 liters of 60 ppm of albumin bovine fraction V in water. The feed solution pH was unadjusted and was measured to be 6.75. The UF experiments were performed at a fixed operating pressure of 137.9 KPa. The feed flow rate was varied from 0.5 to 2.5 L/min. The results of these UF experiments are tabulated in Appendix A18. The data obtained from a "chosen" UF membrane ET-41.76 are plotted in Figure 32.

As illustrated in Figure 32, the product permeate flux increased linearly with feed flow rate. The protein retention also increased with the feed flow rate. The protein retention was close to 100 % at the feed flow

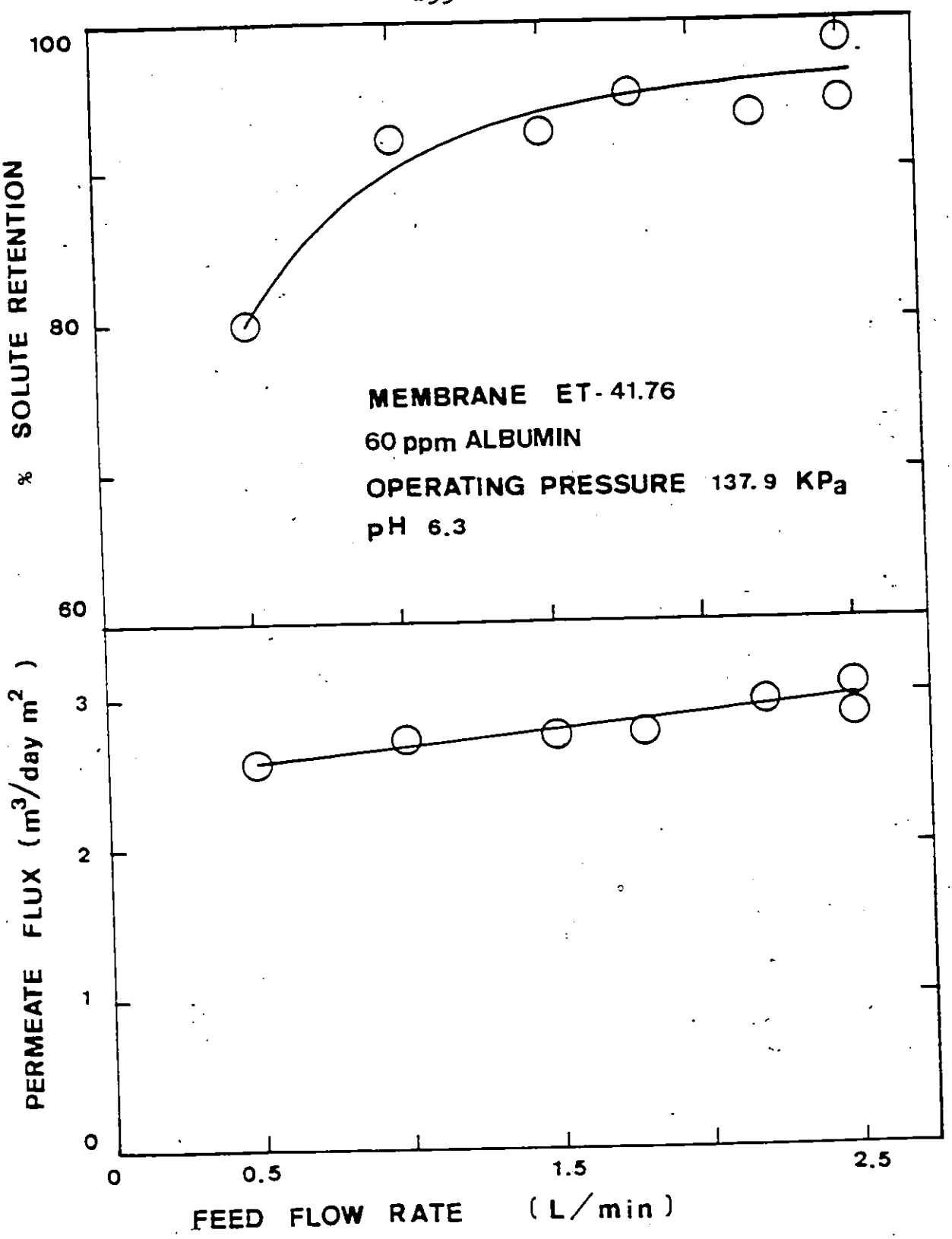


Figure 32 Effect of feed flow rate on the ultrafiltration of albumin bovine fraction V solution

rate of 2.5 L/min. However, the protein retention of the "chosen" UF membranes ET-41.76, as exemplified by Figure 32, was observed to be much lower at the lower feed flow rate in comparison to that observed with the other "chosen" UF membranes (.see Appendix A18).

An increase in feed flow rate increases turbulence inside the cell unit. This in turn increases the solute mass transfer coefficient and thereby decreases the concentration polarization , i.e., the amount of solute (protein) arriving at the membrane surface decreases as cross flow increases. Consequently, the effective solute (protein) concentration at the membrane surface is reduced, along with the concentration driving force for trans-membrane solute transport. This effect will be more pronounced with the larger effective pore size and greater porosity UF membrane since the cross flow will be contending with a greater degree of concentration polarization. An increase in turbulence also decreases the thickness of the boundary layer and thereby decreases its effective resistance to mass transfer, particularly the transport of solvent. Hence, an increase in feed flow rate resulted in an increase in protein retention and product permeate flux.

Effect of Variation in Feed Flow Rate on the Ultrafiltration of Sodium Phytate Solution

The effect of feed flow rate on the separation of sodium phytate was demonstrated in the absence of added sodium chloride and in the presence of 1 (w/v) % sodium chloride. The "chosen" UF membranes used were ET-39.91, ET-41.76, and ET-43.60. The feed solution was 8 liters of 200 ppm of sodium phytate in water. The feed solution pH was adjusted to 6.3 with hydrochloric acid. The applied operating pressure was 137.9 KPa and the feed flow rate was varied from 0.5 to 2.5 L/min. The results were very similar to that obtained for the albumin bovine fraction V . The data are tabulated in Appendix A19. The data obtained from a "chosen" UF membrane ET-41.76 are plotted in Figure 33.

Figure 33 shows that the product permeate flux increased linearly with the feed flow rate. The same permeation flux curve was obtained for both with and without the added sodium chloride indicates that the free permeation of sodium chloride was not affected by the changes in the feed flow rate. The increase in product permeate flux with increasing cross flow was due to the decrease in the effective resistance of the boundary layer to solvent transport with increasing turbulence. The

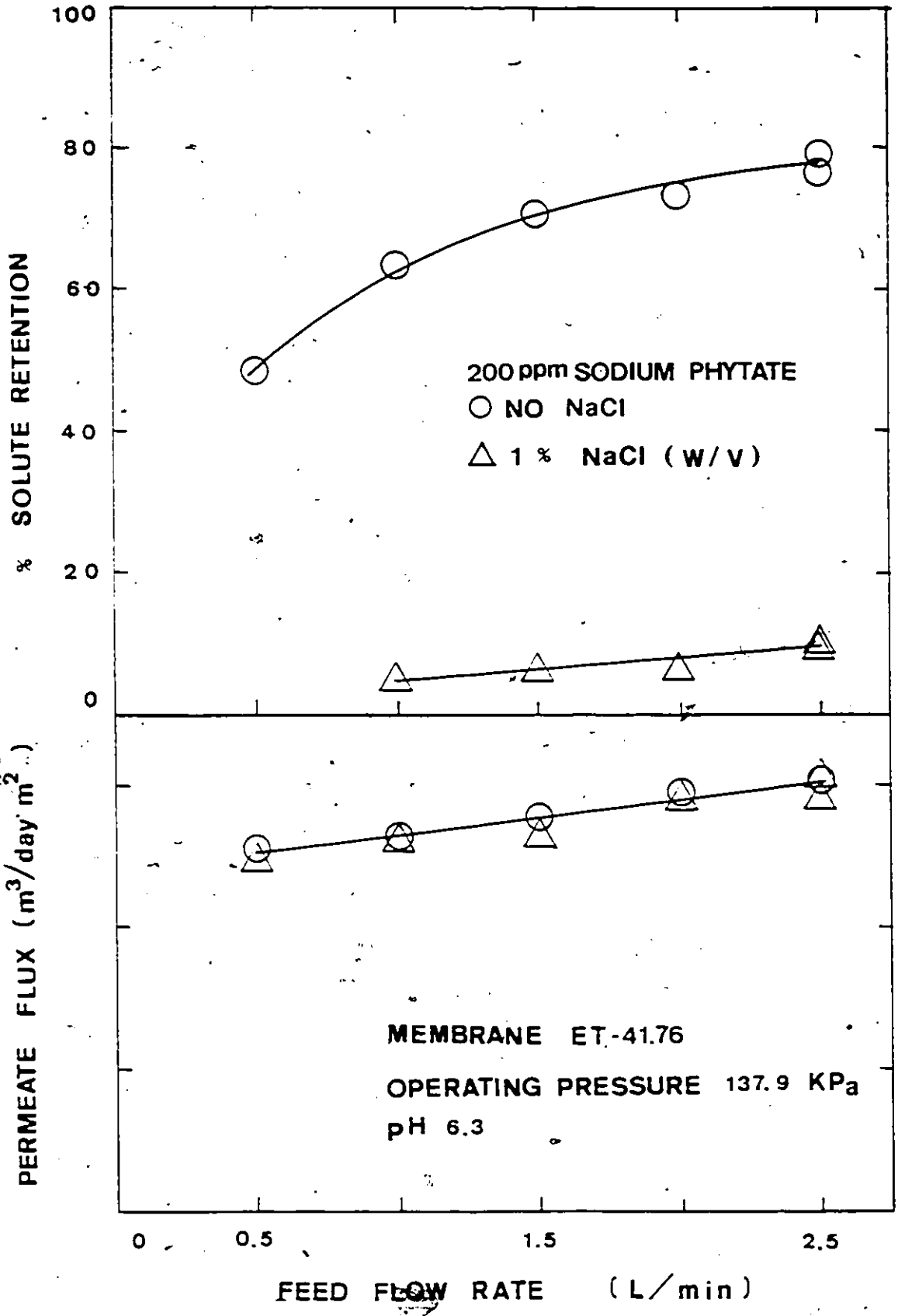


Figure 33 Effect of feed flow rate on the ultrafiltration of sodium phytate solution

permeation of phytate was lower at the higher feed flow rate because of decreasing concentration polarization with increasing cross flow. It was observed that the variation of feed flow rate had a greater effect on phytate removal when sodium chloride was not added in the feed solution, particularly at the lower feed flow rate. However, the addition of sodium chloride in the feed solution greatly enhanced the removal of phytate at all tested feed flow rate.

The effect of feed flow rate on the membrane permeation of phytate was also investigated with the 75 ppm of sodium phytate solution in the presence of 1 (w/v) % sodium chloride. The UF experiments were also performed at the same operating conditions using the same set of "chosen" UF membranes. The data obtained are included in Appendix A19. The results were compared to the data obtained previously for the 200 ppm of sodium phytate solution with added 1 (w/v) % sodium chloride. The comparison made for a "chosen" UF membrane ET-41.76, at the two different feed concentrations, is plotted in Figure 34. It was observed that at both feed concentrations, the product permeate flux and the phytate retention increased linearly with increasing feed flow rate.

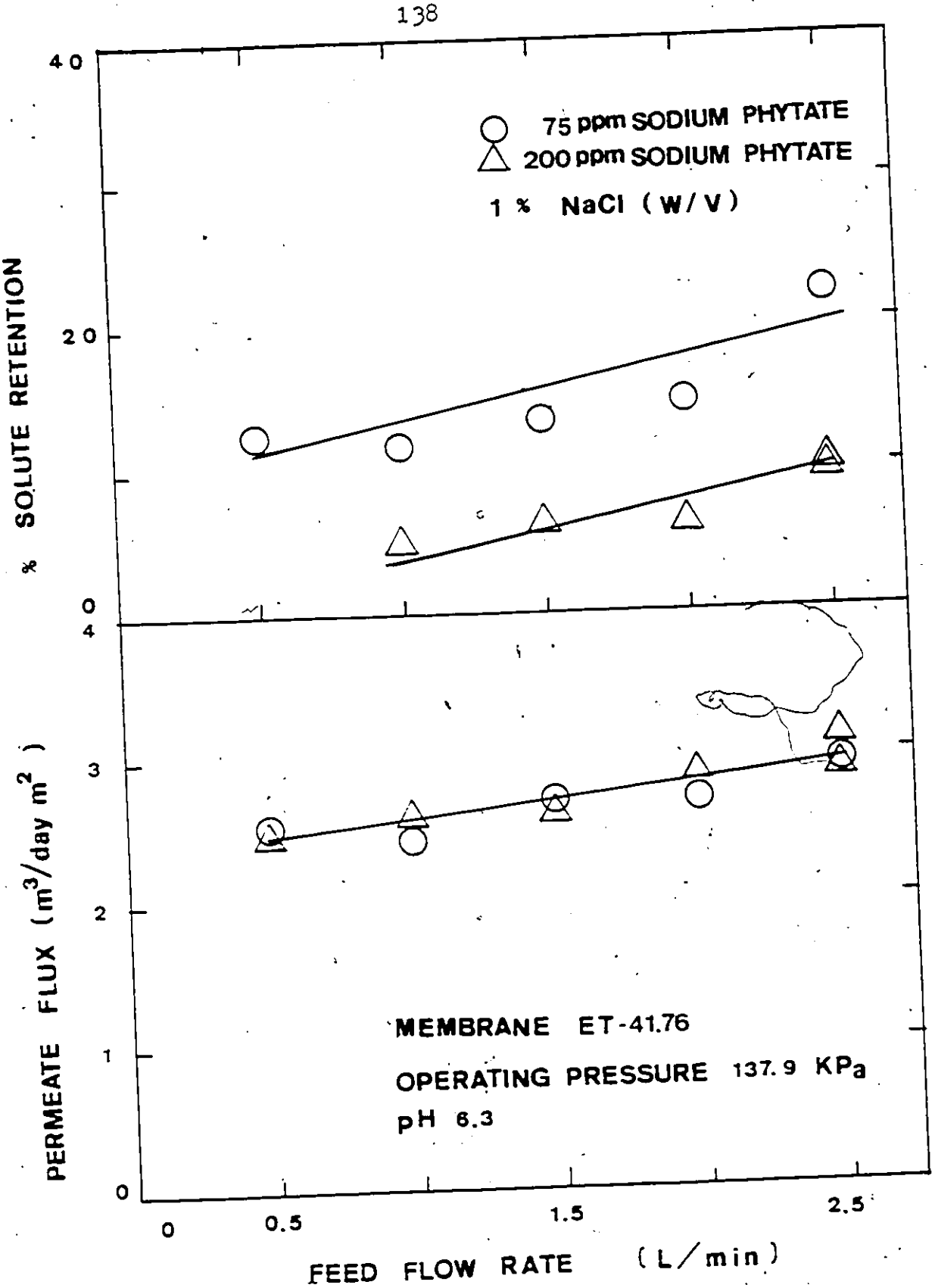


Figure 34 Effect of feed flow rate on the ultrafiltration of sodium phytate solution

Generally, at a given operating pressure and feed flow rate, both the solute retention and the product permeation flux decrease with increasing feed concentration. The latter effect is simply due to the increase in osmotic pressure, and hence a decrease in effective driving pressure for fluid flow. The effective driving pressure for fluid flow through the membrane is the applied operating pressure minus the difference between the osmotic pressure of the membrane boundary layer solution and of the membrane permeated product solution. In addition, there is a greater probability of membrane fouling at higher concentration. However, there was no observable difference in the product permeate fluxes at the two different feed concentrations, as shown in Figure 34. Their magnitude of concentration difference could be insufficient to cause any significant difference between their effective osmotic pressure driving forces. It can be seen from Figure 34 that the permeate of the more concentrated feed solution (200 ppm) was higher in solute content and accordingly, was higher in osmotic pressure. Hence, the higher osmotic pressure of the more concentrated feed solution could be offset by the higher osmotic pressure of its permeation product. The net effect could, therfore, be no difference in their effective driving pressure for fluid flow. Furthermore, the membrane fouling effect will be less influential with the sodium

phytate - water system. The lower retention of phytate at higher feed concentration can be attributed to the greater degree of concentration polarization and consequently, a greater concentration driving force for the trans-membrane solute transfer.

Effect of Variation in Feed Flow Rate on the Ultrafiltration of Rapeseed Extract

The effect of feed flow rate on the removal of phytate from the rapeseed extract by ultrafiltration was demonstrated with the "chosen" UF membranes ET-39.91, ET-41.76, and ET-43.60. The feed solution was 6 liters of Tower meal extract prepared from 2 (w/v) % sodium chloride extraction solution at a v/n ratio of 200 ml/g. The pH of the feed solution was unadjusted and was 6.3. The applied operating pressure was 137.9 KPa and the feed flow rate was varied from 0.5 to 2.5 L/min. The data are tabulated in Appendix A20. The results obtained from a "chosen" UF membrane ET-41.76 are plotted in Figure 35.

As observed in Figure 35, the phosphorus (phytate) retention decreased with increasing feed flow rate, while the nitrogen (protein) retention was not significantly affected by the feed flow rate variation. The product permeate flux increased rapidly with feed flow rate. This indicates that an increase in cross flow was highly effective in reducing the concentration polarization of proteins, thereby reducing the membrane fouling effect. An increase in crossflow also increases turbulence, hence decreasing the hydrodynamic boundary layer resistance to fluid flow. It was observed earlier that in the absence of protein, the

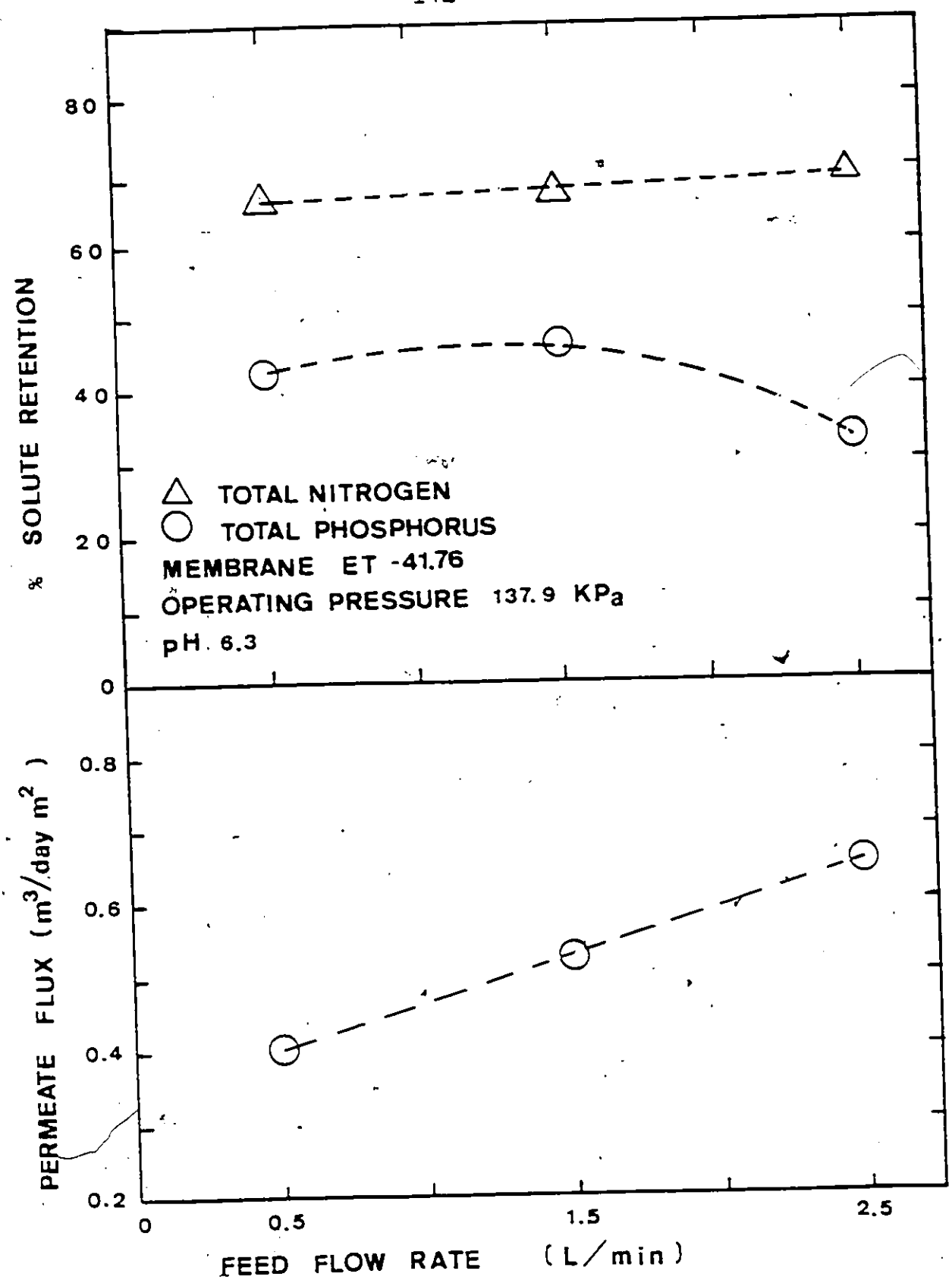


Figure 35 Effect of feed flow rate on the ultrafiltration of rapeseed extract

phytate retention increased with feed flow rate. However, the presence of protein molecules in the solution will strongly hinder the permeation of phytate due to strong phytate - protein interaction, particularly at the vicinity of the membrane surface, where the concentration polarization effect is greater. An increase in feed flow rate will reduce the concentration polarization of protein which in effect will increase the permeation of phytate. This explains the decrease in phytate retention with increasing feed flow rate in the presence of protein, as obtained with the Tower meal extract.

Effect of Variation in Applied Operating Pressure on the Ultrafiltration of Rapeseed Extract

The effect of applied operating pressure on the removal of phytate from the rapeseed extract by ultrafiltration was demonstrated with the "chosen" UF membrane ET-39.91, ET-41.76, and ET-43,60. The feed solution was 6 liters of Tower meal extract prepared from 2 (w/v) % sodium chloride extraction solution at a v/n ratio of 200 ml/g. The pH of the feed solution was unadjusted and was 6.3. The feed flow rate was 2.5 L/min and the applied operating pressure was varied from 68.95 to 179.26 KPa. The experimental results are tabulated in Appendix A21. The data obtained for the "chosen" UF membrane ET-43.60 are plotted in Figure 36.

As illustrated in Figure 36, an increase in operating pressure increased both the solute retention and the product permeate flux. The increase in protein and phytate retention with increasing operating pressure could be due to a decrease in the average effective pore size on the membrane surface because of membrane compaction effect, and / or an increase in the preferential sorption of the membrane for pure water at higher pressure. The increase in product permeate flux with applied operating pressure was mainly due to an increase in pressure driving force

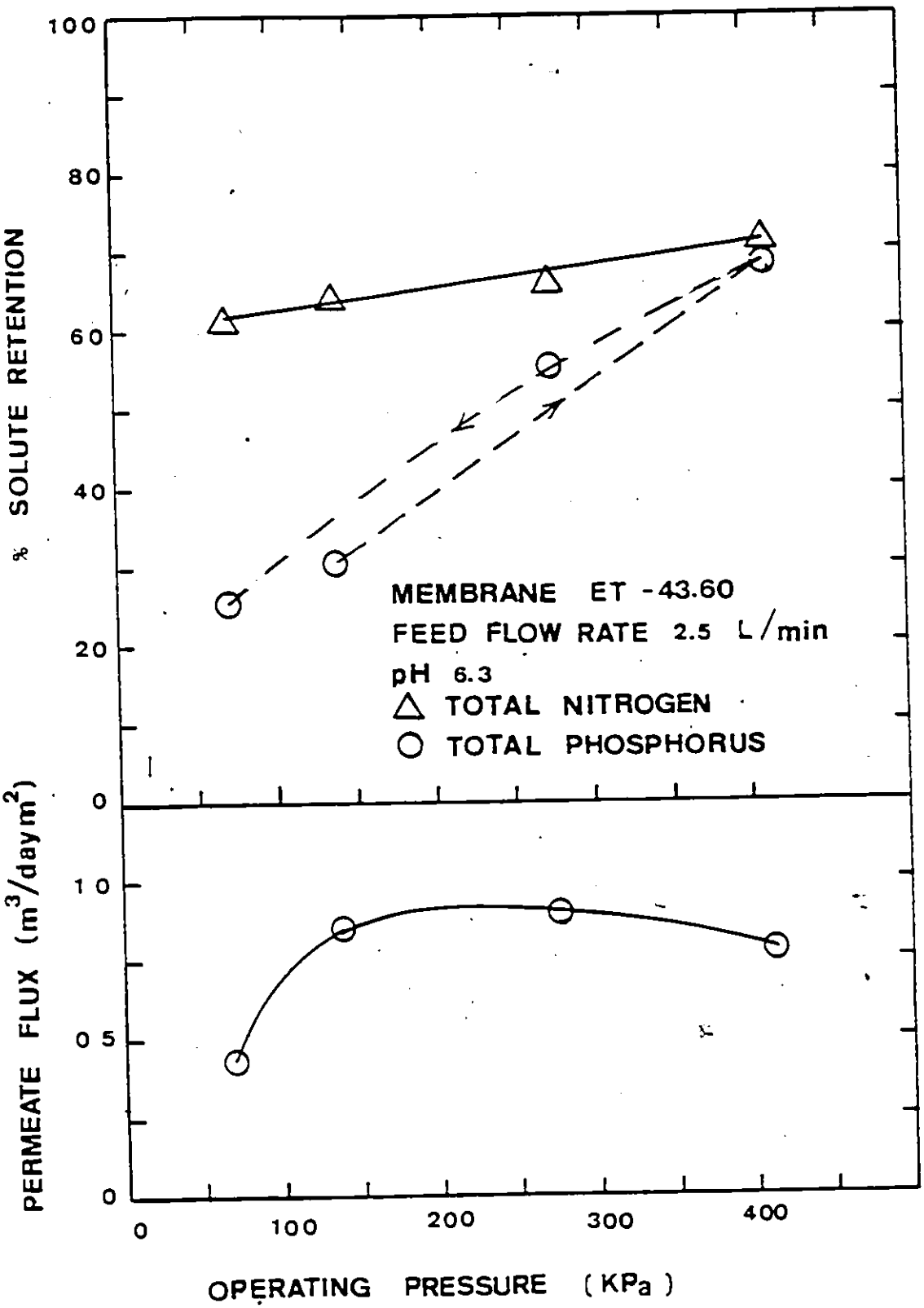


Figure 36 Effect of operating pressure on the ultrafiltration of rapeseed extract

for fluid flow. However, at higher values of operating pressure, the product permeate flux ceased to increase further, while the protein and phytate retention continued to increase markedly with increasing operating pressure. The increase retention of protein and phytate will result in an increase in effective osmotic pressure difference across the membrane. Nevertheless, this change in effective osmotic pressure difference would not be sufficient by itself to account for the increase in the overall resistance observed at the higher operating pressure. The increase in the pressure driving force, due to the increase in operating pressure, will increase the build up of proteins in the concentration polarization region. This will eventually increase the thickness of the polarization gel layer. Hence, an increase in the pressure driving force for fluid flow is counterbalanced by an increase in the overall resistance to fluid flow arising from the increase in the thickness of the gel layer.

The slight decrease in product permeate flux beyond the operating pressure of 275 KPa, as observed in Figure 36, could possibly due to membrane compaction and / or membrane fouling effects. The observed hysteresis effect could also be attributed to membrane compaction and / or membrane fouling effects. The pressure dependence of phytate retention indicated in Figure 36 is important. It

shows that in order to remove the largest possible amount of phytate from the rapeseed extract by ultrafiltration, a low operating pressure must be used.

An Economic Evaluation of the Proposed Process for the Production of Low - Phytate Rapeseed Protein Products.

A schematic flow diagram of the proposed process for the production of low - phytate rapeseed protein products is given in Figure 37. However, the actual operation process will depend on the type and quality of the rapeseed feed and on the processing objectives.

In Figure 37, phytate is removed from the rapeseed meal by a multistage crosscurrent extraction process using aqueous sodium chloride extraction solution. The insoluble meal is separated and washed (to remove the salt) by centrifugation and is dried to obtain a low - phytate reduced protein rapeseed meal product. The liquid extract phase is filtered and pasturized to reduce its microbiological load prior to the recovery of the dissolved protein by membrane processing. This pretreatment of the extract is expected to improve the membrane performance. A sequence of ultrafiltration - continuous diafiltration - ultrafiltration is employed in that order, so as to optimize the extent of phytate removal versus processing time. The purpose of the continuous diafiltration is two - fold, namely (1) for ensuring complete removal of phytate, and (2) for desalination. During the course of continuous diafiltration, sodium chloride solution is added back to the retentate for the phytate removal

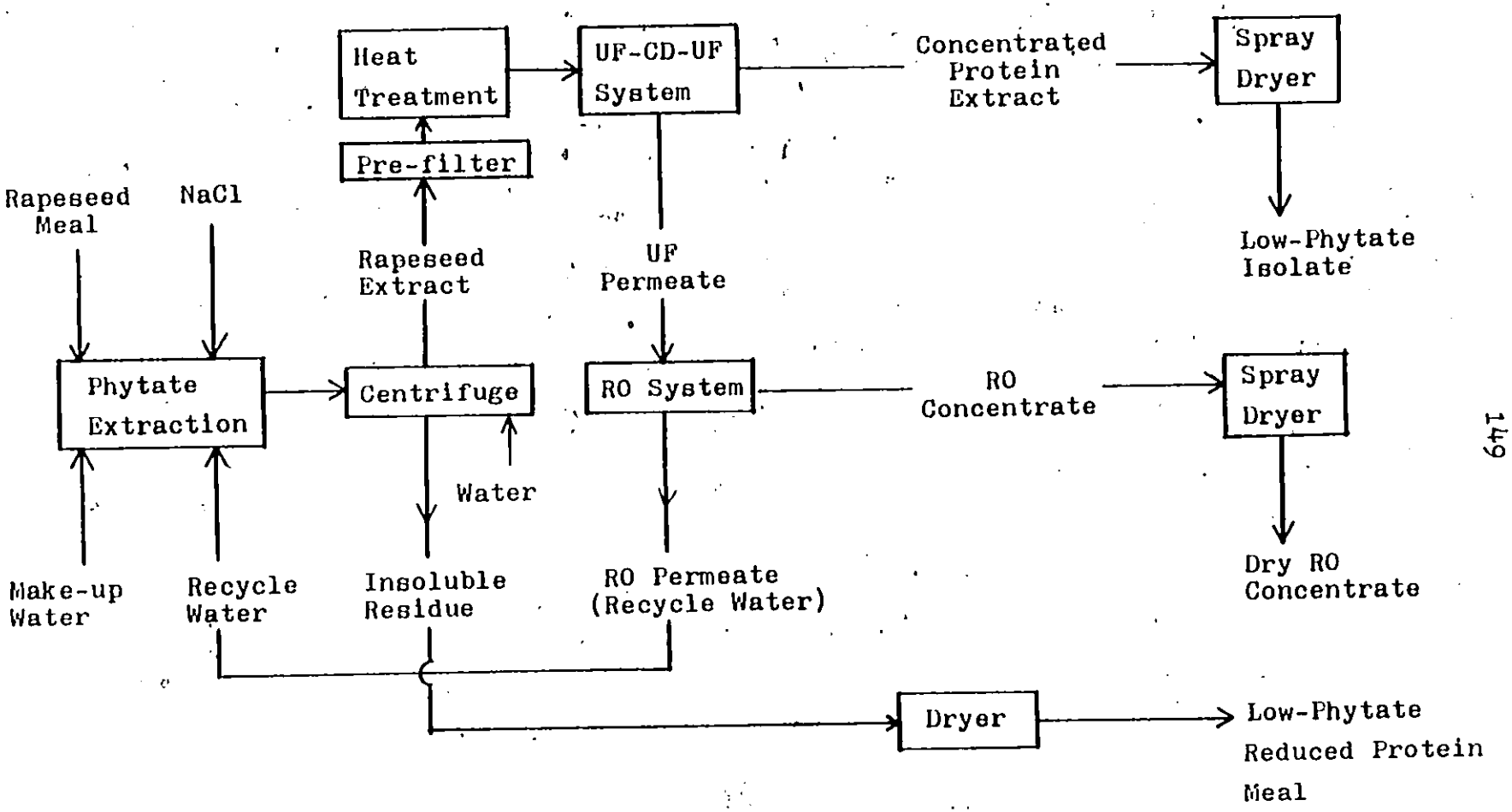


Figure 37 A schematic flow diagram of the proposed process for the production of low-phytate rapeseed protein products (UF = Ultrafiltration; CD = Continuous Diafiltration; RO = Reverse Osmosis).

stage, and water is added back to the retentate for the desalination stage. The continuous diafiltration will also help to overcome the problem of concentration polarization. The final concentrated protein solution from the UF unit is spray dried to obtain a low - phytate rapeseed protein isolate. For economical and environmental considerations, the permeate from the UF operation is processed by the reverse osmosis unit to reclaim the water and the sodium chloride to be use by the phytate extraction process. The phytate concentrate from the RO operation could possibly be processed to obtain purified phytate for pharmaceutical use.

An economic analysis was performed on the proposed process given in Figure 37. The predesign cost estimation was based on an average size plant processing 30 million pounds of dehulled deoiled rapeseed meal annually, operating 300 days per year and 24 hours per day. It was estimated that 1 pound of dehulled deoiled rapeseed meal would yield about 1/2 pound of low - phytate rapeseed meal, 2/5 pound of low - phytate rapeseed isolate, and 9/100 pound of reverse osmosis concentrate. The material loss was estimated to be 1/100 pound. The actual yield data for the products will depend on the type and the quality of the rapeseed meal being processed. An overall material balance for the raw material and products is given in Table 11.

Table 11 . An Overall Material Balance for a Low-Phytate Rapeseed Protein Production Plant (Dry Basis)

Dehulled Deoiled Rapeseed Meal Consumption Rate (1,000 lb / yr)	Product Production Rate (1,000 lb / yr)		
	Low-Phytate Rapeseed Meal	Low-Phytate Rapeseed Isolate	Reverse Osmosis Concentrate
30,000	15,000	12,000	2,700

The total material loss was 300,000 lb/yr.

The estimated capital costs for the proposed plant is given in Table 12. The cost estimation was based on the cost figures obtained by Hensley and Lawhon (189). The cost data were updated to suit the present processing plant. The cost figures given in Table 12 are based on Marshall and Stevens all industry index of 696.9 for the first quarter of 1981.

The operation processes of Figure 37 were divided into four major unit operations (Table 12). The major and auxiliary equipments were included in each of the equipment divisions. The "Building and Miscellaneous" division include all buildings, process piping and wiring, distribution and yard materials, a capital cost contingency factor, field labor, and engineering costs. Equipment installation and freight costs were estimated as 20 % and 1.5 % of the f.o.b. equipment cost, respectively. Process building and warehouse requirements were determined by the production capacity of the proposed plant. Process buiding and warehouse costs were taken as $ 46 / ft^2 and $ 24 / ft^2, respectively. Service building costs were considered at 4 % of the total f.o.b. equipment costs. Process piping and wiring materials, distribution material, and yard materials costs were calculated at 10 %, 15 %, and 5 % of the f.o.b. equipment costs, respectively. A capital-cost contingency factor was included as 5 % of the total capital

Table 12 Capital Costs (including shipping and installation)

Equipment Division		Capital Costs ($ 1,000)
I	Material Transfer and Handling	524
II	Phytate Extraction	2,002
III	Membrane Processing	2,808
IV	Drying Equipments	1,785
V	Buildings and Miscellaneous	5,652
	Total	12,771

costs from the equipment divisions I - IV plus buildings and plant materials. Field labor and engineering costs were calculated at 20 % and 10 %, respectively, of the total capital costs of the equipment divisions I - IV.

The annual production costs, excluding the raw material cost, is given in Table 13. The production costs for equipment divisions I - IV included depreciation, utilities, operating labor, membrane replacement, maintenance, and miscellaneous expenses. Equipment depreciation was considered at 10 % annually. Electricity and thermal energy costs were calculated at $ 0.035/Kw-hr and $ 2.50/10^6BTU (based on natural gas), respectively. Operating labor was considered at $ 16/hr (included benefits). The operating costs for "Building and Miscellaneous" division consisted of fixed and variable costs. Building depreciation (based on 15 year life), supervision, indirect payroll costs, and general work expenses were included in the fixed costs. Operating supplies, laboratory and service costs, and contingencies were included in the fixed costs. The energy and labor requirements for the proposed plant is given in Table 14.

The estimated capital costs for the proposed plant, as obtained from Table 12, was $ 0.43/lb of rapeseed meal processed. The production costs, as shown in Table 13, was $ 0.19/ lb of rapeseed meal processed producing 1/2 pound

Table 13 Production Costs (excluding raw material cost)

Equipment Division		Production Costs ($ 1,000 / yr)
I	Material Transfer and Handling	598.8
II	Phytate Extraction	1,266.9
III	Membrane Processing	935.4
IV	Drying Equipments	1,315.6
V	Buildings and Miscellaneous	1,505.9
	Total	5,612.6

Table 14 Energy and Labor Requirements

Equipment Division	Energy Consumption (1,000 kw-hr/yr)	Energy Consumption (10^{10} BTU/yr)	Operating Labor (man-hr/yr)
I Material Transfer and Handling	263	---	22,200
II Phytate Extraction	1,297	12.1	36,000
III Membrane Processing	6,249	---	12,500
IV Drying Equipments	---	31.4	14,400

of low - phytate rapeseed meal, 2/5 pound of low - phytate rapeseed isolate, and 9/100 pound of dried RO phytate concentrate. The current price quotation (May 29, 1981) for deoiled rapeseed meal with hull is $ 203.25 - $ 225.50 per short ton. Presently, there is no market price for the dehulled deoiled rapeseed meal and no company makes it.

The profitability of the low - phytate rapeseed protein production plant was evaluated at the 20 per cent level of return after the taxes. The raw material cost was taken as $ 225.50 per short ton. The sale of the low-phytate meal was at 60 % feed cost. The calculation is summarized in Table 15. The selling price of the low - phytate rapeseed isolate, as shown in Table 15, was $ 0.97 per pound. The calculated selling price of the isolate does not include the selling price of the RO phytate concentrate. In mid-1978, conventional soybean isolates were selling for U.S. $ 0.80-0.95 per pound in carload lots (189). In comparison, the selling price of the low - phytate rapeseed isolate would be competitive with the commercial soy isolate. This preliminary cost estimation suggests that the proposed plant would be economically feasible.

Table 15 Profitability of the Low - Phytate Rapeseed Protein Production Plant.

1) Raw Material Cost	$ 3,382,500 / yr
2) Production Costs	$ 5,612,600 / yr
3) Total Costs (1 + 2)	$ 8,995,100 / yr
4) Net Profit (20% level of return)	$ 1,799,020 / yr
5) Taxes	$ 1,799,020 / yr
6) Gross Profit (4 + 5)	$ 3,598,040 / yr
7) Total Sales (3 + 6)	$ 12,593,140 / yr
8) Sale of Low-Phytate Meal (at 60 % feed cost)	$ 1,014,750 / yr
9) Sale of Low-Phytate Isolate	$ 11,578,390 / yr
10) Low-Phytate Isolate Selling Price	$ 0.97 / lb

CONCLUSIONS

This study has presented a potential commercial method for the manufacture of low-phytate or phytate-free rapeseed protein products. Such products, in the form of meal or flour, can be produced by removing the phytate with dilute aqueous sodium chloride solution using established extraction techniques. Those extracts high in dissolved protein can yield further product by first removing the phytate using continuous diafiltration and then concentrating the resulting solution by ultrafiltration. Consequently, a low-phytate or phytate-free rapeseed isolate is obtained from the extract.

The extraction experiments showed that phytate is virtually completely removed from deoiled rapeseed using dilute sodium chloride solution. Important factors governing the extent of phytate dissolution and protein losses were believed to be: sodium chloride concentration; type of rapeseed; v/n ratio; and the initial pH of the extraction medium (limited to the range of 3 to 10 to avoid protein denaturation). Testing of these variables led to the following findings.

Extent of phytate dissolution:

- increased dramatically and approached completion rapidly with increasing sodium chloride concentration and with increasing v/n ratio

- was independent of the type of rapeseed and of the initial pH of the extraction medium.

Extent of protein dissolution:

- increased and approached a limiting value rapidly with increasing sodium chloride concentration. This limiting value of protein dissolution was found to be dependent on the type of rapeseed and its processing history
- was independent of the v/n ratio and of the initial pH of the extraction medium.

It was further demonstrated that the degree of phytate removal and protein loss from a deoiled rapeseed can be estimated graphically using equilibrium diagrams constructed from experimental values. The results obtained for a multistage crosscurrent extraction experiment showed excellent agreement between the estimated values and the actual experimental data in the case of phytate. Less satisfactory agreement was obtained for protein.

Membranes for the ultrafiltration were successfully prepared by proper choice of casting solution (for optimum permeate flux), and controlled alcohol content of the gelation bath (to give appropriate pore size and porosity). The membranes were characterised by high protein - low phytate retention. Fabrication was simple and inexpensive. It was demonstrated by a continous diafiltration experiment

that a complete removal of phytate from a rapeseed extract was attainable with a relatively low protein loss.

The phytate-protein separation by ultrafiltration was shown to be greatly affected by the solution conditions and operating parameters. The following conclusions can be drawn from the ultrafiltration experiments:

- the presence of sodium chloride in the process solution was essential in order to obtain a high degree of phytate permeation
- the optimum pH for phytate-protein separation was around 5.5
- it was necessary to operate at a high feed flow rate and at a low operating pressure in order to obtain the highest possible phytate-protein separation.

A postulate based on the molecular structure of phytic acid was developed to explain the observed phenomena of the ultrafiltration of phytate as affected by the presence of sodium chloride.

Finally, a pre-design cost evaluation of the proposed process for the production of low-phytate rapeseed protein products showed that the production plant would be economically feasible. The calculated selling price of the low-phytate rapeseed isolate was competitive with commercial soy isolates.

RECOMMENDATIONS

Further studies should include the followings:

- the optimum phytate extraction temperature.
- the feasibility of preparing a low - phytate rapeseed isolate by effectively extracting the protein using aqueous ethanol - sodium chloride solution while retaining the phytate in the meal.
- the extraction and ultrafiltration of glucosinolates.
- the feasibility of using a dispersed mixture of deoiled rapeseed in aqueous sodium chloride solution as feed for direct removal of these antinutrients by ultrafiltration.
- the pretreatment of the rapeseed extract, such as pasteurization, for bacteriological control and for improvement in membrane performance.
- the sanitation of the ultrafiltration equipment and operation process with a view to formulate a practical cleaning procedure in order to control the microbial growth and to extend the useful lifetime of the membrane.
- the nutritional quality of the low - phytate rapeseed meal and isolate (including the effect of their sodium content).
- if they are nutritionally desirable products, then a pilot plant operation should be undertaken to study its feasibility.

REFERENCES

1) Adolphe, D., "Canola, Canada's Rapeseed Crop", Canola Council of Canada, No. 56, 1980.

2) Bengtsson, L., Von Hofsten, A., and Loof, B., in "Rapeseed; Cultivation, Composition, Processing and Utilization", Appelqvist, L. A., and Ohlson, R., ed., Elsevier, Amsterdam, 1972.

3) Nagy, J.C., and Furtran, W.H., "The Socio-Economic Costs and Returns from Rapeseed Breeding in Canada", University of Saskatchewan, 1977.

4) Abdellatif, A.M.M., and Vles, R.O., in "Proc. Internat. Conf. on Sci. Technol. and Marketing of Rapeseed and Rapeseed Products", Rapeseed Association of Canada, 1970.

5) Beare-Rogers, J.L.; Ibid.

5a) Consolidated Regulation of Canada, 1978.

6) Vaisey-Genser, M., and Eskin, N.A.M., "Canola Oil; Properties, Processes and Food Quality", Rapeseed Association of Canada, No.55, 1979.

7) Beare-Rogers, J.L., in "Prog. Chem. Fats and Other Lipids", 15, 29, 1977.

8) McCutcheon, T., Umemura, T., Bhatnagar, M.K., and Walker, B.L., Lipids, 7, 454, 1976.

9) Beare-Rogers, J.L., in "Proc. Symp. on Rapeseed Oil, Meal and By-Product Utilization", Rapeseed Association of Canada, No. 45, 1977.

10) Kramer, J.K.G., Hulan, H.W., Mahadevan , S., Sa er, F.D., and Corner, A.H., Ibid.

11) Friend, D.W., Kramer, J.K.G., and Corner, A.H., Can. J. An. Sci., 56, 361, 1976.

12) Appelqvist, L.A., in "Rapeseed; Cultivation, Composition, Processing and Utilization", Appelqvist, L.A., and Ohlson, R., ed., Elsevier, Amsterdam, 1972.

13) Oliver, S.L., McDonald, B.E., and Opuszynska, T., Can. J. Physiol. Pharmacol., 49, 448, 1971.

14) Lo, M.T., and Hill, D.C., Federation Proc., 30, 641,1971.

15) Lo, M.T., and Hill, D.C., Can. J. Physiol., 50, 373, 1972.

16) Bell, J.M., Benjamin, B.R., and Giovanetti, P.M., Can. J. An. Sci., 52, 407, 1972.

17) Ballester, D., Rodrigo, R., Nakouzi, J., Chichester, C.O., Yanez, E., and Mönckeberg, F., J. Sci. Food Agric., 21, 143, 1970.

18) Tape, N.W., Sabry, Z.I., and Bapen, K.E., J. Inst. Can. Technol. Aliment. 3, 78, 1970.

19) Lo, M.T., and Hill, D.C., J. Sci. Food Agric., 22, 128, 1971.

20) Owen, D.F., and Chichester, C.O., Cereal Chem., 48, 91, 1971.

21) Eklund, A., Ågren, G., and Langler, T., J. Sci. Food Agric., 22, 650, 1971.

22) Eklund, A., Ågren, G., Langler, T., Stenram, U., and Nordgren, H., Ibid, 22, 653, 1971.

23) Ågren, G., and Eklung, A., Ibid, 23, 1457, 1972.

24) Campbell, L.D., and Eggum, B.O., in "Research on Canola Seed, Oil, Meal and Meal Fractions", 6 th Progress Report, Canola Council of Canada No. 57, 1980.

25) Eklund, A., Ågren, G., Nordgren, H., and Stenram, U., J. Sci. Food Agric., 25, 343, 1974.

26) Eklund, A., Nutr. Rept. Intern., 7, 647, 1973.

27) McLaughlan, J.M., Jones, J.D., Shah, B.G., and Beare-Rogers, J.L., Ibid, 11, 327, 1975.

28) Shah, B.G., Jones, J.D., McLaughlan, J.M., and Beare-Rogers, J.L., Ibid, 13, 1, 1976.

29) Posternak, S., Compt. Rend. Soc. Biol., 55, 1190, 1903.

30) Anderson, R.J., J. Biol. Chem., 17, 171, 1914.

31) Neuberg, C., Biochem. Z., 2, 557, 1908.

32) Courtois, J.E., Scikagaku (J. Japan Biochem. Soc.), 26, 85, 1954.

33) Johnson, L.F., and Tate, M.E., Can. J. Chem., 47, 63, 1969.

34) Fennessey, J.P., and Nowacki, W., Helv. Chim. Acta, 51, (6), 1475, 1968.

35) Truter, M.R., and Tate, M.E., J. Chem. Soc. (Phys. Org.), 70, 1970.

36) McCance, R.A., and Widdowson, E.M., Biochem. J., 29, 2694, 1935.

37) Oke, O.L., Indian J. Med. Res., 53, 417, 1965.

38) de Boland, A.R., Garner, G.B., and O'Dell, B.L., J. Agr. Food Chem., 23, 1186, 1975.

39) O'Dell, B.L., de Boland, A.R., and Koirtyohann, S.R., J. Agr. Food Chem., 20, 718, 1972.

40) Hamilton, T.S., Hamilton, B.C., Johnson, B.C., and Mitchell, H.H., Cereal Chem., 28, 163, 1951.

41) Martinez, W.H., in "Evaluation of Proteins for Humans", Bowell, C.E., ed., Avi Pub. Co., Westport, Conn., 1975.

42) de Turk, E.E., Holbert, J.R., and Hawk, B.W., J. Agr. Res., 46, 121, 1933.

43) Earley, E.B., and de Turk, E.E., J. Am. Soc. Agron., 36, 803, 1944.

44) Yatsu, L.Y., J. Cell Biol., 25, 193, 1965.

45) Lui, N.S.T., and Altschul, A.M., Arch. Biochem. Biophys., 121, 678, 1967.

46) Dieckert, J.W., and Dieckert, M.C., in "Symposium: Seed Proteins", Inglette, G.E., ed., Avi Pub. Co., Westport, Conn., 1972.

47) Pomeranz, Y., Cereal Chem., 50, 504, 1973.

48) Oberleas, D., Ph.D. Dissertation, University of Missouri, 1964.

49) Oberleas, D., Muhrer, M.E., and O'Dell, B.L., in "Zinc Metabolism", Prasad, A.S., ed., Charles C. Thomas, Springfield, Ill., 1966.

50) Oberleas, D., Muhrer, M.E., and O'Dell, B.L., J. Nutr., 90, 56, 1966.

51) Vohra, P., Gray, G.A., and Kratzer, F.H., Proc. Soc. Exp. Biol. Med., 120, 447, 1965.

52) Maddaiah, V.T., Kurnick, A.A., and Reid, B.L., Ibid, 115, 391, 1964.

53) Alimarin, I.P., and Zozel, L.Z., Inst. Abschei. Neorg. Khim., 3, 114, 1957; Chem. Abstr., 51, 2659b, 1958.

54) Ryabchikov, D.I., Belyaeva, V.K., and Ermakov, A.N., Zh. Anal. Khim., 11, 658, 1956; Chem. Abstr., 51, 7940e, 1957.

55) Beck, G., Mikrochem. Mikrochem. Acta, 34, 62, 1948; Chem. Abstr., 42, 8703, 1948.

56) Mellanby, E., Spec. Dept. Ser. Med. Res. Council, London, No. 93, 1925.

57) Mellanby, E., J. Physiol., 109, 488, 1949.

58) Bruce, H.M., and Callow, R.K., Biochem. J., 28, 517, 1934.

59) Harris, R.S., and Bunker, J.W.M., J. Nutr., 9, 301, 1935.

60) McCance, R.A., and Widdowson, E.M., J. Physiol., 101, 44, 1942.

61) Harrison, D.C., and Mellanby, E., Biochem. J., 33, 1660, 1939.

62) Mellanby, E., "A Story of Nutritional Research", Williams and Wilkins, Baltimore, 1950.

63) Todd, W.R., Elvehjem, C.A., and Hart, E.B., Am. J. Physiol., 107, 146, 1934.

64) Tucker, H.F., and Salmon, W.D., Proc. Soc. Exp. Biol. Med., 88, 613, 1955.

65) Prasad, A.S., Miale, A., Jr., Farid, Z., Sandstead, H.H., Schulert, A.R., and Darby, W.J., Arch. Internal Med., 111, 407, 1963.

66) O'Dell, B.L., and Savage, J.E., Federation Proc., 16, 394, 1957.

67) Smith, W.H., Plumlee, M.P., and Beeson, W.M., J. An. Sci., 21, 399, 1962.

68) Oberleas, D., Muhrer, M.E., and O'Dell, B.L., Ibid, 21, 57, 1962.

69) Forbes, R.M., and Yohe, M., J. Nutr., 70, 53, 1960.

70) Savage, J.E., Yohe, J.M., Pickett, E.E., and O'Dell, B.L., Poultry Sci., 43, 420, 1964.

71) Edwards, H.M., Jr., Ibid, 45, 412, 1966.

72) O'Dell, B.L., and Savage, J.E., Proc. Soc. Exp. Biol. Med., 103, 304, 1960.

73) Likuski, H.J.A., and Forbes, R.M., J. Nutr., 84, 145, 1964.

74) O'Dell, B.L., Yohe, J.M., and Savage, J.E., Poultry Sci., 43, 415, 1964.

75) Likuski, H.J.A., and Forbes, R.M., J. Nutr., 85, 230, 1965.

76) Oberleas, D., in "Toxicant Occurrancy Naturally in Foods", 2nd ed., NRC Nat. Academy of Sci., Washington, D.C., 1973.

77) Forbes, R.M., Federation Proc., 19, 643, 1960.

78) O'Dell, B.L., Newberne, P.M., and Savage, J.E., J. Nutr., 65, 503, 1958.

79) Byrd, C.A., and Matrone, G., Proc. Soc. Exp. Biol. Med., 119, 347, 1965.

80) Moore, C.V., Am. J. Clin. Nutr., 3, 3, 1955.

81) Anonymous, Nutr. Rev., 25, 218, 1967.

82) Klavins, J.V., and Kaufman, N., Brit. J. Exp. Path., 43, 172, 1962.

83) McCance, R.A., Edgecombe, C.N., and Widdowson, E.M., Lancet, 2, 126, 1943.

84) Sharpe, L.M., Peacock, W.C., Cooke, R., and Harris, R.S., J. Nutr., 41, 433, 1950.

85) Sathe, V., and Krishnamurthy, K., Ind. J. Med. Res., 41, 453, 1953.

86) Liebman, M., and Driskell, J., Nutr. Rep. Int., 19 (3), 281, 1979.

87) Cowan, J.W., Esfahani, M., Salvi, J.P., and Azzam, S.A., J. Nutr., 90, 423, 1966.

88) Cosgrove, D.J., Rev. Pure and Appl. Chem., 16, 209, 1966.

89) Foy, H., Konde, A., and Austin, W.H., Nature, 183, 691, 1959.

90) Welch, R.M., and Campen, D.R., J. Nutr., 105, 253, 1975.

91) Davies, N.T., and Nightingale, R., Br. J. Nutr., 34, 243, 1975.

92) Koepke, J.A., and Steward, W.B., Proc. Soc. Exp. Biol. Med., 115, 927, 1964.

93) Murray, J., and Stein, N., Ibid, 133, 86, 1970.

94) Murray, J., and Stein, N., in "Trace Element Metabolism in Animals", Mills, C.F., ed., Livinstone, Edinburgh, 1970.

95) Multani, J.S., Cepurneek, C.P., Davis, and Saltman, P., Biochem., 9, 3970, 1970.

96) Hussain, R., Walker, R.B., Layrisse, M., Clark, P., and Finch, C.A., Am. J. Clin. Nutr., 16, 464, 1965.

97) Layrisse, M., Martinez-Torres, C., and Roche, M., Ibid, 21, 1175, 1968.

98) Elwood, P.C., Newton, D., Eakins, J.D., and Brown, D.A., Ibid, 21, 1162, 1968.

99) Kent-Jones, D.W., and Amos, A., J. Modern Cereal Chem., 412, 284, 1957.

100) Widdowson, E.M., and McCance, R.A., Lancet 1, 588, 1942.

101) Wozenski, J., and Woodburn, M., Cereal Chem., 52, 665, 1975.

102) Davidson, S., and Passmore, R., "Human Nutrition and Dietetics", 3rd ed., Williams and Wilkins, Baltimore, 1966.

103) McCance, R.A., and Widdowson, E.M., Nature, 153, 650, 1944.

104) Hussain, R., and Patwardhan, V.N., Ind. J. Med. Res., 47, 676, 1959.

105) Apte, S.V., and Venkatachalam, P.S., Ibid, 50, 516, 1962.

106) Apte, S.V., and Venkatachalam, P.S., Ibid, 52, 213, 1964.

107) Walker, A.R.P., Fox, F.W., and Irving, J.T., Biochem. J., 42, 452, 1948.

108) McCance, R.A., and Widdowson, E.M., J. Physiol., 101, 304, 1942.

109) Hathaway, M.L., "Magnesium in Human Nutrition", Home Economics Research Report No. 19, Agricultural Research Service, U.S. Dept. of Agriculture, Washington, D.C., 1962.

110) Cullumbine, H., Basnayake, V., Lemottee, J., and Wickramanayake, T.W., Brit. J. Nutr., 4, 101, 1950.

111) Guenther, W., and Sell, J.L., J. Nutr., 104, 1446, 1974.

112) Seelig, M.S., Am. J. Clin. Nutr., 14, 342, 1964.

113) Davis, P.N., Norris, L.C., and Kratzer, F.H., J. Nutr., 77, 217, 1961.

114) Rackis, J.J., and Anderson, R.L., Food Product Development, 11 (10), 38, 1977.

115) Reinhold, J.G., Farodji, B., Abadi, P., and Ismail-Beigi, F., J. Nutr., 106, 493, 1976.

116) Reinhold, J.G., Nasi, K., Lahimgarzadeh, A., and Hedayai, H., Lancet 1, 283, 1974.

117) Reinhold, J.G., Ismail-Beigi, F., and Faradji, B., Nutr. Rep. Int., 12, 75, 1975.

118) Davies, N.T., Hristic, V., and Flett, A.A., Ibid, 15 (2), 207, 1977.

119) Kratzer, F.H., Allred, J.B., Davies, P.N., Marshall, B.J., and Vohra, P., J. Nutr., 68, 313, 1959.

120) Forces, R.M., Ibid, 74, 194, 1961.

121) Vohra, P., and Kratzer, F.H., Ibid, 82, 249, 1964.

122) Nielsen, F.H., Sunde, M.L., and Hoekstra, Ibid, 89, 35, 1966.

123) Lease, J.G., Ibid, 96, 126, 1968.

124) Ferrel, R.E., Wheeler, E.L., and Pence, J.W., Cereal Sci., 14, 110, 1969.

125) Kennedy, B.M., and Schelstraete, M., Cereal Chem., 52, 173, 1975.

126) Vix, H.L.E., Gardner, H.K., Jr., Lambou, M.G., and Rollins, M.L., in "Symposium: Seed Proteins", Inglett, G.E., ed., Avi Pub. Co., Westport, Conn., 1972.

127) Wolf, W.J., and Briggs, D.R., Arch. Biochem. Biophys., 186, 85, 1959.

128) Nakayama, O., Fukamachi, C., Kubo, S., Suruga, S., Miyamaga, S., Horike, S., and Watanabe, T., Report of the Food Research Institute, 14B, 52, 1960.

129) Smith, A.K., and Rackis, J.J., J. Am. Chem. Soc., 79, 633, 1957.

130) Fontaine, T.D., Pons, W.A., and Irving, W.G., J. Biol. Chem., 164, 487, 1946.

131) Bourdillon, J., J. Biol. Chem., 189, 65, 1951.

132) Carlson, C.W., U.S. Dept. Agr. ARS-71-35, 81, 1967.

133) Lease, J.G., Barnett, B.D., Lease, E.J., and Turk, D.E., J. Nutr., 72, 66, 1960.

134) Lease, J.G., Poultry Sci., 45, 237, 1966.

135) O'Dell, B.L., Feedstuffs, 32, 35, 1962.

136) Nelson, T.S., Shieh, T.R., Wodzinski, R.J., and Ware, J. H., Poultry Sci., 47, 1842, 1968.

137) Nelson, T.S., Shieh, T.R., Wodzinski, R.J., and Ware, J. H., J. Nutr., 101, 1289, 1971.

138) Chang, R., Schwimmer, S., and Burr, H.K., J. Food Sci., 42 (4), 1098, 1977.

139) McKinny, L.L., Solars, W.F., and Stezkorn, E.A., J. Biol. Chem., 178, 117, 1949.

140) Ford, J.R., Mustakas, G.C., and Schmutz, R.D., JAOCS, 55, 371, 1978.

141) Sagdullaev, Sh. Sh., Turakhozhaev, N.T., and Shakirov, T.T., Khim. Prir. Soedin., Inst. Khim. Rast. Veshchestv. Tashkent, U.S.S.R., 4, 530, 1976.

142) Rizaev, N.U., Uzb. Khim. Zh., 16 (2), 85, 1972.

143) Harland, B.F., and Oberleas, D., Cereal Chem., 54 (4), 827, 1977.

144) Okubo, K., Waldrop, A.B., Iacobucci, G.A., and Myers, D.V., Ibid, 52, 263, 1975.

145) Hartman, G.H., Jr., JAOCS, 56 (8), 731, 1979.

146) de Rham, O. and Jost, T., J. Food Sci., 44 (2), 596, 1979.

147) Omosaiye, O., and Cheryan, M., Cereal Chem., 56 (2), 58, 1979.

148) Gillberg, L., and Törnell, B., J. Food Sci., 41, 1063, 1976.

149) Radwan, M.N., and Lu, B.C.-Y., JAOCS, 53, 142, 1976.

150) Kutowy, O., Thayer, W.L., and Sourirajan, S., Canadian Patents and Development Ltd., Ottawa, Patent Application Case No. 6379, 1976.

151) Carter, J.W., Psaras, G., and Hoyland, G., Trans. Inst. Chem, Engrs., 46 (10), T265, 1968.

152) Kesting, R.E., "Synthetic Polymeric Membranes", McGraw-Hill, New York, 1971.

153) Merten, U., Lonsdale, H.K., Riley, R.L., and Vos, K.D., Reverse Osmosis Membrane Research, U.S. Dept. Interior, Office of Saline Water, R&D Progress Report No. 265, 1967.

154) Riley, R.L., Gardner, J.O., and Merten, U., Science, 143, 801, 1963.

155) Riley, R.L., Gardner, J.O., and Merten, U., Desalination, 1 (1), 30, 1966.

156) Michael , A.S., Bixler, H.J., and Hodges, R.M., Jr., J. Colloid Sci., 20, 1034, 1965.

157) Kesting, R.E., Barsh, M.K., and Vincent, A.L., J. Appl. Polymer Sci., 9, 1873, 1965.

158) Sourirajan, S., Ind. Eng. Chem. Fundamentals, 3, 206, 1964.

159) Lang, C.A., Analytical Chem., 30, 1692, 1958.

160) Bredford, M.M., Analytical Biochem., 72, 248, 1976.

161) Pons, W.A., Stansbury, M.F., and Hoffpauir, C.L., J. Assoc. Official Agric. Chem., 36, 492, 1953.

162) Sosulski, F.W., and Bakal, A., Can. Inst. Food Tech. J., 2, 28, 1969.

163) Finlayson, A.J., Bhatty, R.S., and Christ, C.M., Can. J. Botany, 47, 679, 1969.

164) Owen, D.E., and Chichester, C.O., Cereal Chem., 48, 91, 1971.

165) Lo, M.T., and Hill, D.C., J. Sci. Food Agric., 22, 128, 1971.

166) Kodagoda, L.P., Young, C.Y., Nakai, S., and Pourie, M.D., Can. Inst. Food Tech. J., 6 (3), 135, 1973.

167) Keshavarz, E., Cheung, R.K.M., Lui, R.C.M., and Nakai, S., Can. Inst. Food Sci. Technol. J., 10 (2), 13, 1977.

168) Becker, K.W., Baczewski, K.C., and Klein, D.J., U.S. Patent 3,966,982, June 29, 1976.

169) Jones, J.D., and Holme, J., U.S. Patent 4,158,656, June 19, 1979.

170) Loeb, S., and Sourirajan, S., Advan. Chem. Soc., 38, 117, 1962.

171) Vos, K.D., Burris, F.O., Jr., Riley, R.L., J. Applied Polymer Sci., 10, 825, 1966.

172) Vos, K.D., Hatcher, A.P., and Merten, U., Ind. Eng. Chem. Prod. Res. Develop., 5, 211, 1966.

173) Copper, R.C., and Richard, M.G., in "Proceedings of the Third Annual Conference of the National Water Supply Improvement Association, Key Largo, Florida, July 13-17, 1972.

174) Kutowy, O., Thayer, W.L., and Sourirajan, S., Can. Patents and Develop. Ltd., Ottawa, Patent Application Case No. 6379, 1976.

175) Carter, J.W., Psaras, G., and Hoyland, G., Trans. Inst. Chem. Engrs., 46(10), T265, 1968.

176) Kesting, R.E., "Synthetic Polymeric Membranes", McGraw-Hill, New York, 1971.

177) Lönnerdal, B., and Janson, J.-C., Biochim. Biophys. Acta, 278, 175, 1972.

178) Crean. D.E.C., and Haismann, D.R., J. Sci. Food Agric., 14, 824, 1963.

179) Okubo, K.,Myers, D.V., and Iacobucci, G.A., Cereal Chem., 53, 513, 1976.

180) Hill, R., and Tyler, C., J. Agric. Sci., 44, 324, 1954.

181) de Boland, A.R., Garner, G.B., and O'Dell, B.L., J. Agric. Food Chem., 23, 1186, 1975.

182) Lolas, G.M., and Markakis, P., Ibid, 23, 13, 1975.

183) Baker, E.C., Mustakas, G.C., Erdman, J.W., Jr., and Black, L.T., JAOCS, , 541, 1981.

184) Sandergren, E., J. Inst. Brew., 54, 200, 1948.

185) Saio, K., Koyama, E., and Watanabe, T., Agric. Biol. Chem., 31, 1195, 1967.

186) O'Dell, B.L., and de Boland, A., J. Agric. Food Chem., 24, 804, 1976.

187) Saio, K., Koyama, E., and Watanabe, T., Agric. Biol. Chem., 32, 448, 1968.

188) Goodnight, K.C., Hartman, G.H., and Marquardt, R.F., U.S. Patent 4,072,670, 1978.

189) Hensley, D.W., and Lawhon, J.T., Food Technology, May, 46, 1979.

190) Eklund, A., Upsala J. Med. Sci., 80, 5, 1975.

191) Lolas, G.M., Palamidas, N., and Markakis, P., Cereal Chem., 53 (6), 867, 1976.

APPENDIX A
****

EXPERIMENTAL DATA

APPENDIX A1 EFFECT OF HYDROCHLORIC ACID CONCENTRATION ON THE DISSOLUTION OF PHYTATE FOR THE COMMERCIAL TOWER MEAL.

Normality of HCl	% Phytate Dissolution
0.000	10.50
0.056	15.56
0.113	37.14
0.170	79.98
0.226	89.55
0.282	94.58
0.339	98.65
0.452	94.04
0.565	98.66
1.356	100.00
1.695	97.68

v/m = 200 ml/g

APPENDIX A2 EFFECT OF SULFURIC ACID CONCENTRATION ON THE DISSOLUTION OF PHYTATE FOR THE COMMERCIAL TOWER MEAL.

Normality of H_2SO_4	% Phytate Dissolution
0.000	10.50
0.036	39.02
0.072	57.68
0.108	70.44
0.144	82.61
0.216	91.39
0.288	94.18
0.360	97.42
0.720	100.00
1.080	99.98
1.440	94.31
3.600	95.27

v/n = 200 ml/g

APPENDIX A3 EFFECT OF SODIUM SULFATE CONCENTRATION ON THE DISSOLUTION OF PHYTATE FOR THE COMMERCIAL TOWER MEAL.

% Na_2SO_4 (w/v)	% Phytate Dissolution
0.00	10.07
0.50	71.80
1.00	81.07
2.00	83.91
4.00	90.43
6.00	95.17
10.00	100.00

v/n = 200 ml/g

APPENDIX A4 EFFECT OF SODIUM CHLORIDE CONCENTRATION ON THE DISSOLUTION OF PHYTATE AND NITROGEN-CONTAINING SUBSTANCES. (v/n = 200 ml/g, pH = 6.30)

% NaCl	Candle Flour		Tower Meal		Commercial Tower Meal	
(w/v)	Phytate	Total N	Phytate	Total N	Phytate	Total N
0.00	33.26	28.90	39.52	28.59	-----	10.94
0.20	-----	-----	-----	34.65	-----	17.97
0.50	72.28	65.42	70.87	38.14	73.47	22.75
1.00	85.93	68.43	80.01	36.39	82.44	24.38
2.00	-----	-----	-----	-----	92.68	-----
4.00	97.39	76.00	91.59	39.29	97.86	27.09
10.00	100.00	78.38	100.00	38.41	98.86	27.38

APPENDIX A5 EFFECT OF ETHANOL CONCENTRATION ON THE DISSOLUTION OF PHOSPHORUS- AND NITROGEN-CONTAINING SUBSTANCES. (v/v = 50 ml/g, pH = 6.30)

% ETOH	Candle Flour		Tower Meal		Commercial Tower Meal	
(v/v)*	Total P	Total N	Total P	Total N	Total P	Total N
0.00	43.95	32.82	52.05	23.45	44.30	12.72
4.72	25.66	29.46	40.59	21.45	28.91	10.41
46.40	4.94	8.48	5.08	6.98	5.85	4.64

* (v/v) % BEFORE MIXING.

APPENDIX A6 EFFECT OF ETHANOL CONCENTRATION ON THE DISSOLUTION OF PHOSPHORUS- AND NITROGEN-CONTAINING SUBSTANCES IN THE PRESENCE OF 4 (w/v) % SODIUM CHLORIDE.

(v/m = 50 ml/g, pH = 6.30)

% ETOH	Candle Flour		Tower Meal		Commercial Tower Meal	
(v/v)*	Total P	Total N	Total P	Total N	Total P	Total N
0.00	79.83	75.41	81.73	40.45	78.45	25.60
4.72	61.95	63.84	65.57	32.25	60.98	25.19
23.39	31.85	58.51	27.85	29.56	-----	-----
46.40	11.80	50.70	7.50	28.10	8.95	22.37

* (v/v) % BEFORE MIXING.

APPENDIX A7 EFFECT OF (v/n) RATIO ON THE DISSLUTION OF PHOSPHORUS- AND NITRGEN-CONTAINING SUBSTANCES. (4 (w/v) % NaCl, pH = 6.30)

v/n	Candle Flour		Tower Meal	
(ml/g)	Total P	Total N	Total P	Total N
5.0	51.19	-----	-----	-----
10.0	56.35	73.00	52.52	38.72
13.3	-----	74.44	-----	-----
20.0	64.67	74.43	62.78	-----
50.0	79.83	75.41	81.73	40.45
100.0	91.70	73.36	84.71	-----
200.0	100.00	73.73	94.30	38.47
500.0	99.17	72.63	94.04	42.22

APPENDIX A8 A Multistage Crosscurrent Extraction of Tower Meal Using Deionized Distilled Water. (v/m = 10 ml/g per extraction stage)

Number of Stages	% of Original Solute Remaining in the Meal	
	Phytate Phosphorus	Total Nitrogen
1	80.38	77.63
2	78.44	65.87
3	76.49	62.85
4	75.19	61.62
5	74.48	60.76
6	70.84	60.12
7	70.40	59.75
8	70.18	59.57

APPENDIX A9 A Multistage Crosscurrent Extraction of Tower Meal Using 4 (w/v) % Sodium Chloride Solution. (v/m = 10 ml/g per extraction stage, pH = 6.3)

Number of Stages	% of Original Solute Remaining in the Meal		
	Total Phosphorus	Phytate Phosphorus	Total Nitrogen
1	68.39	66.86	76.13
2	41.62	39.65	59.13
3	29.07	25.94	54.11
4	21.88	18.36	50.80
5	16.48	12.31	49.53
6	13.14	8.66	48.75
7	11.29	6.32	48.22
8	8.94	4.29	47.64

APPENDIX A10 Equilibrium Data for Phytate and Protein Extraction from the Tower Meal Using 4 (w/v) % Sodium Chloride Solution.

N (Overflow) $\frac{B}{A+C}$	X_1 $\frac{C_1}{A+C}$	X_2 $\frac{C_2}{A+C}$	N (Underflow) $\frac{B}{A+C}$	Y_1 $\frac{C_1}{A+C}$	Y_2 $\frac{C_2}{A+C}$
0	0.00017	0.00040	0.19676	0.00016	0.05038
0	0.00041	0.00090	0.19519	0.00163	0.05649
0	0.00150	0.00379	0.19456	0.00528	0.05586
0	0.00439	0.01546	0.18789	0.17863	0.07551
0	0.00473	0.01781	0.18915	0.01711	0.07016
0	0.00789	0.03398	0.18589	0.02338	0.07963
0	0.00597	0.04451	0.18465	0.02579	0.08318
0	0.00949	0.09157	0.17789	0.02791	0.11368
0	0.01682	0.10017	0.17748	0.03067	0.11291

APPENDIX A11 A Multistage Crosscurrent Extraction of Tower Meal Using 4 (w/v) % Sodium Chloride Solution as Obtained by Graphical Calculation. (v/n = 10 ml/g per extraction stage)

Number of Stages	% of Original Solute Remaining in the Meal	
	Phytate	Protein
1	66.35	74.43
2	41.70	64.56
3	25.27	59.92
4	17.16	57.90
5	12.34	57.90
6	9.16	57.90

APPENDIX A12 The Effect of Gelation Bath Composition on Membrane Performance Characteristics

UF Membrane*	% N Retention	% P Retention	Product Permeate Flux (m^3/day m^2)	Pure Water Permeate Flux (m^3/day m^2)
ET-32.58	92.44	90.50	0.2254	0.5011
ET- 35.32	85.02	79.64	1.1742	4.1765
ET-37.14	93.20	77.15	0.8393	1.5018
ET-41.76	93.00	56.87	1.1361	2.9146
ET-42.68	77.34	56.06	1.1074	2.5381
ET-44.52	78.47	57.07	1.0253	2.6788
ET-46.40	82.64	57.48	0.9763	1.9703
ET-51.01	82.86	66.60	1.0434	1.8220
ET-55.65	83.83	62.24	0.9676	1.6973

APPENDIX A12 (CONT'D)

UF Membrane*	% N Retention	% P Retention	Product Permeate Flux (m^3/day m^2)	Pure Water Permeate Flux (m^3/day m^2)
ET-60.25	82.56	72.60	1.1369	3.3000
ET-65.25	49.23	41.35	1.6280	8.7038
ET-74.71	21.02	17.71	1.7361	6.1250
ET-84.62	31.28	26.43	2.0345	3.0220
ET-95.00	66.67	57.28	0.6472	2.1274

Feed solution: Candle flour extract prepared from a 4 (w/v)% NaCl extraction solution at a v/h ratio of 500 ml/g; pH = 6.3.

Feed flow rate: 2.5 L/min.

Operating Pressure: 137.9 KPa.

*The code designates the gelation bath composition of the resulting membrane in volume per cent ethanol (before mixing).

APPENDIX A13 Unsteady State Behavior of the Ultrafiltration of Rapeseed Extract

Operation Time (min)	% N Retention	% P Retention	Product Permeate Flux (m^3/day m^2)	Total Permeate Volume (ml)
4.0	62.74	50.44	2.6873	10.68
20.5	66.67	39.71	1.8517	29.62
39.0	65.55	36.99	1.7538	48.35
49.5	65.60	36.66	1.7735	66.72
59.0	62.42	36.92	1.7421	83.30
69.0	65.11	35.66	1.5558	99.68
79.0	63.42	35.08	1.7471	116.08

"Chosen" UF membrane: ET-46.40

Feed solution: Tower meal extract prepared from a 4 (w/v) % NaCl extraction solution at a v/n ratio of 500 ml/g; pH = 6.3.

Feed flow rate: 2.5 L/min.

Operating pressure: 137.9 KPa.

APPENDIX A14 The Removal of Phytate by Continuous Diafiltration

Operating Time (min)	Volume of Dilution (V_D)	Product Permeate Flux ($m^3/day\ m^2$)	Fraction of Original Solute in Retentate			
			Protein	Total N	Phytate P	Total P
10	0.04	1.6783	.9949	.9783	.9705	.9770
41	0.12	1.0828	1.0000	.8913	.9311	.9264
95	0.24	0.9324	1.0000	.8641	.8066	.8276
175	0.40	0.8392	.9848	.8315	.6787	.7356
289	0.60	0.7361	.9444	.7935	.5836	.6621
450	0.80	0.5212	-----	.7228	.5049	.6069
635	1.10	0.6804	.9646	.6956	.2098	.4782
1055	1.66	0.5594	.9394	.6603	.0721	.2437
1440	2.26	0.6539	.8485	.6196	.0328	.1425
1865	2.86	0.5923	.8838	.5679	.0000	-----
2275	3.46	0.6140	.8485	.5516	.0000	.0460

APPENDIX A14 (CONT'D)

"Chosen" UF membrane: ET-38.99

Feed solution: Candle flour extract prepared from a 4 (w/v) % NaCl extraction solution at a v/n ratio of 500 ml/g; pH = 6.3.

Feed flow rate: 2.5 L/min.

Operating pressure: 137.9 KPa.

APPENDIX A15 Effect of Sodium Chloride on the Removal of Phytate by Ultrafiltration

	(w/v) % Sodium Chloride						
ET-42.68*	4.00	0.00	1.00	0.25	0.00	0.02	0.04
% Retention	14.48	86.38	13.21	17.13	84.63	51.91	40.35
PWP Flux	3.3978	3.2907	3.4709	3.3650	3.3726	3.5329	3.4187
PP Flux	3.1972	3.2036	3.3130	3.2591	3.3550	2.9997	3.4360
ET-42.68	4.00	0.00	1.00	0.25	0.00	0.02	0.04
% Retention	26.90	93.06	17.94	16.60	90.31	55.63	47.59
PWP Flux	3.2336	3.1015	3.2855	3.1888	3.2039	3.3482	3.2264
PP Flux	3.0797	3.1029	3.1586	3.1267	3.2232	2.9032	3.3325

* Data plotted in Figure 25.

Pure water permeate (PWP) flux and product permeat (PP) flux are in $m^3/day\ m^2$.

APPENDIX A15 (CONT'D)

	(w/v) % Sodium Chloride						
ET-42.68(2)**	4.00	0.00	1.00	0.25	0.00	0.02	0.04
% Retention	18.34	87.66	16.55	21.65	81.56	59.56	46.65
PWP Flux	3.0119	2.9262	3.2237	3.0881	-----	-----	3.7123
PP Flux	2.9958	2.9518	3.0812	3.0163	3.2056	2.9740	3.4446
ET-42.68(2)**	4.00	0.00	1.00	0.25	0.00	0.02	0.04
% Retention	31.31	94.60	29.62	34.40	95.27	71.58	73.59
PWP Flux	1.7646	1.6931	1.7819	1.7706	1.7600	1.8965	1.8044
PP Flux	1.7723	1.7664	1.8139	1.7819	1.8049	1.6220	1.7975

**UF membranes ET-42.68(2) were cast in a gelation bath of the same composition, i.e., 42.68 (v/v) % ethanol (before mixing), except that their gelation baths were used once previously for the casting of UF membrane ET-42.68.

APPENDIX A15 (CONT'D)

Feed solution: 200 ppm of Sodium Phytate solution (with and without added NaCl).

pH = 6.3

Feed flow rate: 2.5 L/min

Operating pressure: 137.9 KPa

APPENDIX A16 Unsteady State Behaviour of the Ultra-filtration of Sodium Phytate Solution

ET-37.14

Operating Time (min)	% Solute Retention	Total Permeate Volume (ml)	Product Permeate Flux (m^3/day m^2)
1.5	40.00	11.78	7.9049
9.5	12.23	32.20	5.8070
19.5	15.73	50.75	3.9877
28.5	15.12	71.28	3.0569
39.0	23.53	92.32	2.6245
48.0	24.78	111.70	2.2531
57.5	19.04	131.15	2.3496
67.0	20.25	151.80	2.0442
77.0	24.08	171.60	1.9435
86.5	18.11	189.30	1.8013
95.5	22.56	205.50	1.8238
105.5	21.04	221.40	1.7342
114.5	25.16	236.48	1.6392

APPENDIX A16 (CONT'D)

ET-46.40

Operating Time (min)	% Solute Retention	Total Permeate Volume (ml)	Product Permeate Flux (m^3/day m^2)
1.5	15.32	14.75	9.9021
9.5	-----	40.30	7.2503
18.5	-----	60.32	6.1930
27.0	-----	80.10	5.3119
37.5	7.35	103.00	4.9745
46.5	9.73	126.70	4.5717
55.5	-----	149.45	4.5919
64.5	6.54	171.72	4.3804
74.5	10.21	193.05	4.2092
85.0	7.14	215.65	4.0783
94.0	9.60	239.48	3.9189
104.0	10.44	262.35	3.7594
113.0	12.02	284.45	3.6587

APPENDIX A16 (CONT'D)

ET-65.25

Operating Time (min)	% Solute Retention	Total Permeate Volume (ml)	Product Permeate Flux (m^3/day m^2)
4.0	85.97	6.48	1.6301
12.5	54.62	20.65	1.7231
21.0	49.44	35.12	1.7307
30.3	33.14	50.15	1.6615
39.5	34.41	65.88	1.6728
48.5	33.63	80.80	1.6671
57.5	24.92	95.82	1.6951
67.0	23.05	111.90	1.7119
77.0	25.46	128.78	1.6867
86.5	20.18	144.75	1.7006
96.0	21.65	160.75	1.6917
105.5	23.89	176.38	1.6167
115.0	24.84	191.85	1.6615

APPENDIX A16 (CONT'D)

ET-74.71

Operating Time (min)	% Solute Retention	Total Permeate Volume (ml)	Product Permeate Flux (m^3/day m^2)
4.0	96.49	11.8	2.9706
12.5	93.21	37.1	3.0201
21.0	66.29	62.35	2.9580
30.0	38.52	88.15	2.8296
39.5	39.71	114.62	2.7804
48.5	42.62	139.08	2.6909
57.5	28.02	163.22	2.7133
67.0	25.54	188.70	2.6887
77.0	27.90	215.15	2.6383
86.5	24.78	240.02	2.6350
96.0	24.08	264.55	2.5678
105.5	26.90	288.48	2.5007
115.0	25.95	311.80	2.4470

Feed solution: 200 ppm of sodium phytate solution with 4 (w/v) % sodium chloride; pH = 6.3

Feed flow rate: 2.5 L/min

Operating Pressure: 137.9 KPa

APPENDIX A17 Effect of Feed Solution pH on the Removal of Phytate from Rapeseed Extract by Ultrafiltration

ET-38.99

Solution pH	% N Retention	% P Retention	PWP Flux (m^3/day m^2)	PP Flux (m^3/day m^2)
5.00	83.43	65.00	0.9098	0.5386
6.30	75.74	53.48	0.8548	0.6505
5.50	82.78	51.37	0.8684	0.5561
4.00	80.12	62.64	0.8560	0.4227
4.60	85.20	62.78	0.8600	0.4555
5.80	83.72	48.82	0.8350	0.6134
5.35	78.50	46.10	0.8606	0.5187
4.40	80.98	68.47	0.8895	0.47788
6.30	81.32	56.18	0.8173	0.5525
3.50	79.44	57.03	0.8497	0.5281
6.80	80.64	69.06	0.7638	0.5466

APPENDIX A17 (CONT'D)

ET-37.14

Solution pH	% N Retention	% P Retention	PWP Flux (m^3/day m^2)	PP Flux (m^3/day m^2)
5.00	87.57	64.25	1.6308	0.6709
6.30	76.73	60.43	1.5449	0.8146
5.50	86.11	51.07	1.5890	0.6702
4.00	80.12	60.99	1.5692	0.5226
4.60	85.75	-----	1.5363	0.5496
5.80	84.73	69.00	1.4529	0.7042
5.35	82.80	61.04	1.5227	0.5941
4.40	85.01	68.58	1.5602	0.5539
6.30	79.67	65.67	1.4282	0.6425
3.50	80.06	63.14	1.4591	0.6929
6.80	83.58	84.37	1.2703	0.6695

Feed solution : Candle flour extract prepared from a 4 (w/v) % sodium chloride extraction solution at a v/n ratio of 500 ml/g

Feed flow rate : 2.5 L/min

Operating pressure : 137.9 KPa

APPENDIX A18 Effect of Feed Flow Rate on the Ultrafiltration of Albumin Bovine Fraction V Solution

ET-39.91

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux (m^3/day m^2)
2.5	97.22	2.2825
1.5	95.39	2.0543
2.2	94.96	2.3312
0.5	91.75	1.8797
2.5	99.01	2.4772
1.0	94.78	2.0308
1.8	96.83	2.1281

ET-41.76* (* Data plotted in Figure 32)

2.5	94.60	2.8733
1.5	92.60	2.5754
2.2	93.78	2.9706
0.5	79.94	2.5846
2.5	98.85	3.0814
1.0	92.33	2.7289
1.8	95.25	2.7692

APPENDIX A18 (CONT'D)

ET-41.76

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux ($m^3/day\ m^2$)
2.5	93.39	2.5443
1.5	91.61	2.5141
2.2	93.28	2.6685
0.5	70.87	2.4235
2.5	96.05	2.7692
1.0	87.76	2.5175
1.8	93.83	2.5242

Et-43.60

2.5	100.0	2.0912
1.5	96.71	2.1096
2.2	97.14	2.3010
0.5	93.53	2.2892
2.5	98.11	2.3413
1.0	98.37	2.2221
1.8	98.02	2.2557

Feed solution: 60 ppm of albumin bovine fraction V in H_2O

Feed solution pH: 6.75 Operating Pressure: 137.9KPa

APPENDIX A19 Effect of Feed Flow Rate on the Ultra-filtration of Sodium Phytate Solution

ET-39.91 (Without added NaCl)

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux (m^3/day m^2)
2.5	86.31	2.4001
1.5	83.26	2.0720
0.5	64.45	1.8475
2.0	81.52	2.2533
1.0	76.02	1.9396
2.5	85.94	2.3898
(With 1 (w/v) % NaCl)		
2.5	17.22	2.5010
1.5	13.30	1.9893
0.5	7.78	1.8067
2.0	12.44	2.2466
2.5	13.76	2.3340

Feed solution : 200 ppm of sodium phytate solution

pH = 6.3

Operating pressure: 137.9 KPa

APPENDIX A19 (CONT'D)

ET-41.76* (Without added NaCl)

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux (m^3/day m^2)
2.5	76.82	3.0475
1.5	70.73	2.7799
0.5	48.72	2.5620
2.0	73.40	2.9617
1.0	63.87	2.6501
2.5	79.17	3.0470
* (With 1 (w/v) % NaCl)		
2.5	10.15	3.1346
1.5	6.43	2.6150
2.0	6.11	2.8967
1.0	5.01	2.6199
2.5	9.86	2.8857

* Data plotted in Figure 33

Feed solution : 200 ppm of sodium phytate solution

pH = 6.3

Operating pressure : 137.9 KPa

APPENDIX A19 (CONT'D)

ET-41.76 (Without added NaCl)

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux (m^3/day m^2)
2.5	72.74	2.6936
1.5	67.06	2.5209
0.5	45.18	2.3753
2.0	74.57	2.6800
1.0	62.15	2.4453
2.5	77.08	2.7142
(With 1 (w/v) % NaCl)		
2.5	8.28	2.8178
0.5	4.67	2.3287
2.0	6.79	2.6650
1.0	5.92	2.4654
2.5	9.40	2.6226

Feed solution : 200 ppm of sodium phytate solution

pH = 6.3

Operating pressure : 137.9 KPa

APPENDIX A19 (CONT'D)

ET-43.60 (Without added NaCl)

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux (m^3/day m^2)
2.5	89.29	2.2274
1.5	83.26	2.2792
0.5	56.21	2.2208
2.0	82.91	2.3130
1.0	76.02	2.2533
2.5	86.77	2.2447
(With 1 (w/v) % NaCl)		
2.5	15.89	2.3527
0.5	7.78	2.1620
2.0	12.22	2.3045
1.0	9.34	2.2659
2.5	11.47	2.1833

Feed solution : 200 ppm of sodium phytate solution

pH = 6.3

Operating pressure : 137.9 KPa

APPENDIX A19 (CONT'D)

ET-39.91 (With 1 (w/v) % NaCl)

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux (m^3/day m^2)
1.5	25.62	2.0140
0.5	22.95	1.8386
2.0	23.71	2.0798
1.0	25.62	1.7889
2.5	30.96	2.2405

ET-41.76* (With 1 (w/v) % NaCl)

1.5	13.45	2.7063
0.5	12.55	2.5299
2.0	14.51	2.6905
1.0	11.57	2.4264
2.5	22.12	2.8478

* Data plotted in Figure 34

Feed solution : 75 ppm of sodium phytate solution

pH = 6.3

Operating pressure : 137.9 KPa

APPENDIX A19 (CONT'D)

ET-41,76 (With 1 (w/v) % NaCl)

Feed Flow Rate (L/min)	% Solute Retention	Product Permeate Flux ($m^3/day\ m^2$)
1.5	7.27	2.4986
0.5	6.08	2.3796
2.0	10.98	2.4759
1.0	9.09	2.2717
2.5	13.19	2.5934
ET-43.60 (With 1 (w/v) % NaCl)		
2.5	32.48	2.2789
1.5	23.33	2.2317
0.5	17.99	2.1980
2.0	24.86	2.1169
1.0	22.57	2.0736
2.5	31.72	2.1092

Feed solution : 75 ppm of sodium phytate solution

pH $\simeq$ 6.3

Operating pressure : 137.9 KPa

APPENDIX A20 Effect of Feed Flow Rate on the Ultrafiltration of Rapeseed Extract

Membrane	Feed Flow Rate (L/min)	% Solute Retention		Product Permeate Flux ($m^3/day\ m^2$)
		Total N	Total P	
ET-39.91	2.5	73.79	48.31	0.6244
	1.5	69.71	54.10	0.4978
	0.5	69.71	48.31	0.3744
ET-41.76*	2.5	69.71	33.24	0.6531
	1.5	66.80	46.22	0.5281
	0.5	66.21	42.86	0.4068
ET-41.76	2.5	65.63	28.26	0.7393
	1.5	67.38	45.41	0.5988
	0.5	65.05	40.78	0.4369
ET-43.60	2.5	64.67	30.46	0.8577
	1.5	65.63	45.99	0.7154
	0.5	65.63	45.88	0.5019

Feed solution : Tower meal extract prepared from a 2(w/v)% NaCl extraction solution at a v/n ratio of 200 ml/g; solution pH = 6.3

Operating pressure : 137.9 KPa

* Data plotted in Figure 35

APPENDIX A21 Effect of Operating Pressure on the Ultrafiltration of Rapeseed Extract

Membrane	Operating Pressure (KPa)	% Solute Retention		Product Permeate Flux (m^3/day m^2)
		Total N	Total P	
ET-39.91	179.3	73.79	48.31	0.6244
	455.0	74.37	71.61	0.5773
	317.2	72.04	62.22	0.5854
	68.9	67.38	35.44	0.3873
ET-41.76	154.4	65.63	28.26	0.7393
	430.2	66.80	62.22	0.6869
	292.34	65.63	51.55	0.8328
	68.9	60.33	24.09	0.5141
ET-43.60*	137.9	64.67	30.46	0.8577
	413.7	71.46	68.59	0.7829
	275.8	65.63	55.26	0.9034
	68.9	61.55	25.36	0.4406

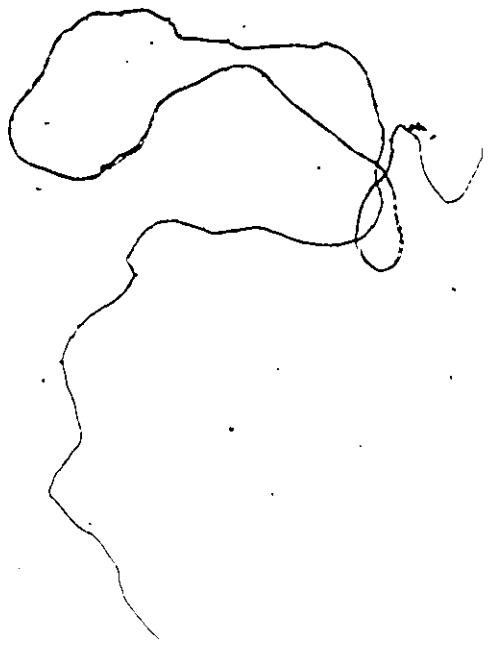
Feed solution : Tower meal extract prepared from a 2(w/v)% NaCl extraction solution at a v/n ratio of 200 ml/g; solution pH = 6.3

Feed flow rate : 2.5 L/min

* Data plotted in Figure 36

APPENDIX B
****

SAMPLE CALCULATIONS



APPENDIX B1 Sample Calculation of the Equilibrium Data for Phytate and Protein Extraction from the Tower Meal Using 4 (w/v) % Sodium Chloride Solution.

Approximations made:

1) Phytate exists as $Ca_5Mg(C_6H_{12}O_{24}P_6 \cdot 3H_2O)_2$ in the rapeseed meal.
2) The total phosphorus in the rapeseed meal are phytate phosphorus.
3) The total nitrogen in the rapeseed meal are protein nitrogen.
4) The total soluble solutes in the rapeseed meal consist only of the soluble protein and the soluble phytate.
5) Constant (solvent / insolubles) underflow.
6) Composition of the adherent solution is the same as that of the bulk solution.

The following relationships were therefore obtained:

Phytate = Phytate P x 4.545

% Phytate P = 2.028 (w/w) % Tower meal sample

% Phytate = 9.217 (w/w) % Tower meal sample

% Soluble Phytate = 8.664 (w/w) % Tower meal sample

(Since only 94.0% of the total P of the Tower meal is extractable with the 4 (w/v) % NaCl solution.)

% Protein N = 7.528 (w/w) % Tower meal sample

% Protein = % Protein N x 6.25

= 47.050 (w/w) % Tower meal sample

APPENDIX B1 (CONT'D)

% Soluble Protein = 34.346 (w/w) % Tower meal sample

(Since only 73.0 % of the total N of the Tower meal is soluble in the 4 (w/v) % NaCl solution.)

% Total Soluble Solutes = 43.010 (w/w) % Tower meal sample

% Insolubles = 56.990 (w/w) % Tower meal sample

The constant (solvent / insolubles) underflow was calculated from the following experimental data:

(v/n), ml/g	10.00	10.00	20.00
(w/v) % NaCl	4.00	4.00	0.00
Weight of the meal, g	19.9997	9.9980	4.9999
Volume of the solvent, ml	200.00	100.00	100.00
Volume of the separated liquid phase obtained after extraction, ml	145.00	72.53	86.30
Volume of solvent adhered per weight of meal, ml/g	2.75	2.75	2.74

Constant (solvent/insolubles) underflow =

2.75 ml solvent/g meal x 1 g meal/0.5699 g insoluble

or 4.825 ml solvent per g insoluble.

APPENDIX B1 (CONT'D)

Experimental data # 1

(v/n) = 500.50

Weight of Tower meal = 0.1998 g

Weight of 4 (w/v) % NaCl solution =100 g

Weight of N extracted = 6.35 mg

Weight of P extracted = 3.81 mg

Experimental data # 2

(v/n) = 201.13

Weight of Tower meal = 0.4972 g

Weight of 4 (w/v) % NaCl solution =100 g

Weight of N extracted = 14.4 mg

Weight of P extracted = 9.08 mg

Experimental data #3

(v/n) = 49.92

Weight of Tower meal = 2.0033 g

Weight of 4 (w/v) % NaCl solution = 100 g

Weight of N extracted = 61.0 mg

Weight of P extracted = 33.2 mg

APPENDIX B1 (CONT'D)

Experimental data #4

(v/n) = 10.00 ml/g

Weight of Tower meal = 19.9997 g

Weight of 4 (w/v) % NaCl solution = 200 g

Weight of N extracted = 505.00 mg

Weight of P extracted = 197.00 mg

Experimental data #5

(v/n) = 10.00 ml/g

Weight of Tower meal = 10.0008 g

Weight of 4 (w/v) % NaCl solution = 100 g

Weight of N extracted = 291.5 mg

Weight of P extracted = 106.5 mg

Experimental data #6

(v/n) = 10.00 ml/g

Weight of Tower meal = 13.0005 g

Weight of 4 (w/v) % NaCl solution (used)* = 130 g

Weight of N in the initial extracted solution* = 328.25 mg

Weight of P in the initial extracted solution* = 128.05 mg

Weight of N in the final extracted solution = 1135.00 mg

Weight of P in the final extracted solution = 363.00 mg

* Tower meal extracted solution was used as the solvent instead of the fresh 4 (w/v) % NaCl solution.

APPENDIX B1 (CONT'D)

Experimental data #7

(v/n) = 10.00 ml/g

Weight of Tower meal used = 7.9994 g

Weight of 4 (v/n) % NaCl solution (used)* = 80 g

Weight of N in the initial extracted solution* = 324.00 mg

Weight of P in the initial extracted solution* = 67.80 mg

Weight of N in the final extracted solution = 600.00 mg

Weight of P in the final extracted solution = 110.60 mg

Experimental data #8

(v/n) = 10.00 ml/g

Weight of Tower meal = 1.9997 g

Weight of 4 (v/n) % NaCl solution (used)* = 20 g

Weight of N in the initial extracted solution* = 243.00 mg

Weight of P in the initial extracted solution* = 34.90 mg

Weight of N in the final extracted solution = 326.00 mg

Weight of P in the final extracted solution = 46.45 mg

* Tower meal extracted solution was used as the solvent instead of the fresh 4 (w/v) % NaCl solution.

APPENDIX B1 (CONT'D)

Experimental data #9

(v/n) = 10.02 ml/g

Weight of Tower meal = 2.9932 g

Weight of 4 (w/v) % NaCl solution (used)* = 30 g

Weight of N in the initial extracted solution* = 404.25 mg

Weight of P in the initial extracted solution* = 99.07 mg

Weight of N in the final extracted solution = 544.50 mg

Weight of P in the final extracted solution = 125.78 mg

* Tower meal extracted solution was used as solvent instead of the fresh 4 (w/v) % NaCl solution.

APPENDIX B1 (CONT'D)

Sample Calculation:

Experimental data # 5

(v/n) = 10.00 ml/g

Weight of Tower meal = 10.0008 g

Weight of 4 (w/v) % NaCl solution 100 g = 100,000 mg

Weight of N extracted = 291.5 mg

Weight of P extracted = 106.5 mg

Calculation:

Weight of protein extracted = 291.5 x 6.25 = 1821.88 mg

Weight of phytate extracted = 106.5 x 4.545 = 484.04 mg

Weight of insolubles = 10.0008 x 569.90 = 5699.46 mg

Total weight of soluble protein in the Tower meal =

10.0008 x 343.46 = 3434.87 mg

Total weight of soluble phytate in the Tower meal =

10.0008 x 86.64 = 866.47 mg

Total weight of solution after extraction =

100,000 + 1821.88 + 484.04 = 102305.92 mg

$$N\ (\text{Overflow}) = \frac{0}{102305.92} = 0.00 \ \frac{\text{mg insolubles}}{\text{mg extracted solution}}$$

$$X_1 = \frac{484.04}{102305.92} = 0.0047313 \ \frac{\text{mg phytate}}{\text{mg extracted solution}}$$

$$X_2 = \frac{1821.88}{102305.92} = 0.0178081 \ \frac{\text{mg protein}}{\text{mg extracted solution}}$$

APPENDIX B1 (CONT'D)

Weight of soluble phytate left unextracted =

866.47 - 484.04 = 382.43 mg

Weight of soluble protein left unextracted =

3434.87 - 1821.88 = 1612.99 mg

Weight of solvent adhered to the insolubles =

5699.46 x 4.825 = 27499.89 mg

Weight of adhered solution to the insolubles =

27499.89 x (102305.92/100000) = 28134.02 mg

Weight of solvent and soluble solutes adhered to the insolubles = 28134.02 + 384.43 + 1612.99 = 30131.44 mg

Weight of phytate in the underflow =

382.43 + (0.0047313 x 28134.02) = 515.54 mg

Weight of protein in the underflow =

1612.99 + (0.0178081 x 28134.02) = 2114.00 mg

$$N \text{ (Underflow)} = \frac{5699.46}{30131.44} = 0.1891532 \frac{\text{mg insolubles}}{\text{mg solvent \& solubles}}$$

$$Y_1 = \frac{515.54}{30131.44} = 0.0171097 \frac{\text{mg phytate}}{\text{mg solvent \& solubles}}$$

$$Y_2 = \frac{2114.00}{30131.44} = 0.0701592 \frac{\text{mg protein}}{\text{mg solvent \& solubles}}$$

APPENDIX B2 Graphical Calculation for Multistage Cross-current Extraction of Phytate from the ower Meal.

Notation:

A = solvent = 4 (w/v) % NaCl

B = insolubles

C = solubles = $C_1 + C_2$

C_1= soluble phytate

C_2= soluble protein

M = mixture

$N_M = B / (A+C)_M$

$z_i = C_i / (A+C)_M$

R = raffinate

$N_R = B / (A+C)_R$

$y_i = C_i / (A+C)_R$

Subscripts:

1 = phytate

2 = protein

ni = ith extraction stage

M = mixture

R = raffinate

APPENDIX B2 (CONT'D)

To calculate graphically the percentage of the original phytate and protein left in the Tower meal sample after each extraction stage using a 4 (w/v) % NaCl and a (v/n) ratio of 10 ml/g.

Basis : 10.00 g of meal

A = 100.00 g

B = 10.00 x .5699 = 5.699 g of insolubles

$(C_1)_S$ = 10.00 x 0.08664 = 0.8664 g of soluble phytate

$(C_2)_S$ = 10.00 x 0.34346 = 3.4346 g of soluble protein

$(C)_S$ = 0.8664 + 3.4346 = 4.3010 g of solubles

$(A+C)_M$ = 100.00 + 4.3010 = 104.3010 g

N_M = 5.699/104.3010 = 0.0546399

z_1 = 0.8664/104.3010 = 0.0083067

z_2 = 3.4346/104.3010 = 0.0329296

Graphical calculation for phytate:

From Figure 18, a tie line was drawn through the point M (N_M, z_1) to obtain $(R)_{n1}$, the raffinate from stage 1.

Stage 1:

$(N_R)_{n1}$ = 0.1870 = B / $(A+C)_{R,n1}$ = 5.699 / $(A+C)_{R,n1}$

$(A+C)_{R,n1}$ = 30.475935 g

$(y_1)_{n1}$ = 0.1825 = $(C_1)_{R,n1}$ / $(A+C)_{R,n1}$ = $(C_1)_{R,n1}$ / 30.475935

$(C_1)_{R,n1}$ = 0.5561858 g

% of soluble phytate left in the meal = $(C_1)_{R,n1}/(C_1)_S$ x 100

= 64.20

APPENDIX B2 (CONT'D)

Since only 94.0% of the total P of the Tower meal is soluble with the 4 (w/v) % NaCl solution, hence

% of total phytate left in the meal = (64.20 x 0.94) + 6.00

= 66.35

Stage 2:

$(A)_{n2}$ = 100.00 g

$(A+C)_{M,n2} = (A)_{n2} + (A+C)_{R,n1}$

= 130.475935 g

$(N_M)_{n2}$ = B / $(A+C)_{M,n2}$ = 5.699 / 130.475935

= 0.0436785

$(M)_{n2}$ was located at the line joining the points $(R)_{n1}$ and the origin (solute-free solvent).

A tie line was drawn through the point $(M)_{n2}$ to obtain $(R)_{n2}$.

$(N_R)_{n2}$ = 0.1905 = 5.699 / $(A+C)_{R,n2}$

$(A+C)_{R,n2}$ = 29.91601 g

$(y_1)_{n2}$ = 0.0110 = $(C_1)_{R,n2}$ / 29.91601

$(C_1)_{R,n2}$ = 0.3290761 g

% of soluble phytate left in the meal = (0.3290761/0.8664) x100

= 37.98

% of total phytate left in the meal = (37.98 x 0.94) + 6.00

= 41.70

APPENDIX B2 (CONT'D)

Similarly,

Stage 3:

$(A)_{n3}$ = 100.00g

$(A+C)_{M,n3}$ = 129.83769 g

$(N_M)_{n3}$ = 0.0438932

$(N_R)_{n3}$ = 0.1925

$(A+C)_{R,n3}$ = 29.605194 g

$(y_1)_{n3}$ = 0.0060

$(C_1)_{R,n3}$ = 0.1776311 g

% of soluble phytate left in the meal = 20.50

% of total phytate left in the meal = 25.27

Stage 4:

$(A)_{n4}$ = 100.00 g

$(A+C)_{M,n4}$ = 129.605194 g

$(N_M)_{n4}$ = 0.043972

$(N_R)_{n4}$ = 0.1940

$(A+C)_{R,n4}$ = 29.376288 g

$(y_1)_{n4}$ = 0.0035

$(C_1)_{R,n4}$ = 0.102817 g

% of soluble phytate left in the meal = 11.87

% of total phytate left in the meal = 17.16

APPENDIX B2 (CONT'D)

Stage 5:

$(A)_{n5}$ = 100.00 g,

$(A+C)_{M,n5}$ = 129.376288 g

$(N_M)_{n5}$ = 0.0440498

$(N_R)_{n5}$ = 0.1950

$(A+C)_{R,n5}$ = 29.225641 g

$(y_1)_{n5}$ = 0.0020

$(C_1)_{R,n5}$ = 0.0584512 g

% of soluble phytate left in the meal = 6.75

% of total phytate left in the meal = 12.34

Stage 6:

$(A)_{n6}$ = 100.00 g

$(A+C)_{M,n6}$ = 129.225641 g

$(N_M)_{n6}$ = 0.0441011

$(N_R)_{n6}$ = 0.1955

$(A+C)_{R,n6}$ = 29.150895

$(y_1)_{n6}$ = 0.0010

$(C_1)_{R,n6}$ = 0.0291508 g

% of soluble phytate left in the meal = 3.36

% of total phytate left in the meal = 9.16

APPENDIX B2 (CONT'D)

Graphical calculation for protein:

Similarly, from Figure 19, the following results were obtained.

Stage 1:

$(N_R)_{n1}$ = 0.1890

$(A+C)_{R,n1}$ = 30.153439 g

$(y_2)_{n1}$ = 0.074

$(C_2)_{R,n1}$ = 2.2313544 g

% of soluble protein left in the meal = 64.97

% of total protein left in the meal = (64.97 x 0.73) + 27.00

= 74.43

(Since only 73.0% of the total N is soluble.)

Stage 2:

$(A)_{n2}$ = 100.00 g

$(A+C)_{M,n2}$ = 130.153439 g

$(N_M)_{n2}$ = 0.0437867

$(N_R)_{n2}$ = 0.1935

$(A+C)_{R,n2}$ = 29.452196 g

$(y_2)_{n2}$ = 0.060

$(C_2)_{R,n2}$ = 1.7671317 g

% of soluble protein left in the meal = 51.45

% of total protein left in the meal = 64.56

APPENDIX B2 (CONT'D)

Stage 3:

$(A)_{n3}$ = 100.00 g

$(A+C)_{M,n3}$ = 129.605194 g

$(N_M)_{n3}$ = 0.043972

$(N_R)_{n3}$ = 0.1950

$(A+C)_{R,n3}$ = 29.225641 g

$(y_2)_{n3}$ = 0.053

$(C_2)_{R,n3}$ = 1.5489589 g

% of soluble protein left in the meal = 45.10

% of total protein left in the meal = 59.92

Stage 4:

$(A)_{n4}$ = 100.00 g

$(A+C)_{M,n4}$ = 129.225641 g

$(N_M)_{n4}$ = 0.0441011

$(N_R)_{n4}$ = 0.1960

$(A+C)_{R,n4}$ = 29.07653 g

$(y_2)_{n4}$ = 0.050

$(C_2)_{R,n4}$ = 1.4538265 g

% of soluble protein left in the meal = 42.33

% of total protein left in the meal = 57.90

* A pinch occured with the tie line after the 4th extraction stage.

APPENDIX C
****

HISTORICAL OUTLINE OF THE DEVELOPMENT OF IMPROVED CANADIAN RAPESEED VARIETIES. (3)

Prior to WWII	Seed stocks of the Polish (Brassica Campestris) species imported from Poland. Provided ancestryfor all the Polish varieties..
During WWII	Seed stocks of the Argentine (Brassica napus) species imported from Argentina. Provided ancestry for all the Argentine varieties.
1944	Mr. H.C. Neufeld of Nipawin made selections from Argentine stocks and turn these over to Dr. W.J. White at the Canada Agriculture Research Station at Saskatoon. Selections were made for uniformity, lodging resistance, high oil content in the seed, and low iodine value in the oil.
1954	Release of the first Canadian variety Golden.. A selection from Argentine with improved oil content and lodging resistance.
1958	The Swedish variety of Arlo was the first Polish rapeseed to be licenced in Canada.
1961	The licencing of Nugget made from selections of Golden. Improved oil content and a lower iodine content in the oil.
1963	The licencing of Tanka made from selections

of Golden. Improvement in yield and size of seed.

1964 The first Canadian variety of Polish rapeseed named Echo was licenced. This selection from Polish had an improved yield.

1966 Target was selected from Tanka and was a shorter plant which matured earlier and had a higher yield and a higher oil content.

1968 Oro was selected from crosses between a low erucic acid selection from Liho and Nugget. This was the first Canadian low erucic acid rapeseed variety.

1969 Polar selected from Polish and exhibited an improvement in oil and protein content of the seed.

1970 Turret was selected from Target with an improvement in yield and oil content.

1971 In response to the Canadian Governments decree in 1970 that rapeseed oil must have no more than 5 per cent erucic acid, two low erucic acid rapeseed varieties were licenced. The first was Zephyr developed from a similar genetic background as Oro and with the same characteristics of Oro. The second variety Span was developed and was the first Polish

LEAR variety. Span was developed from low erucic acid selections of Polish and Arlo.

1973 Two more LEAR varieties were licenced. Midas was developed from selections of crosses involving Target and another source involving a low erucic acid oil. The variety Torch was developed from a similar background as that of Span and exhibits an improvement in yield.

1974 Tower was the first variety to be licenced that exhibited both a low erucic acid and a low glycosinolate content (double-zero), which significantly improved the meal quality. This variety was developed from backcrosses to Turret of sources of both low erucic acid and low glucosinolate content.

1977 Two varieties released in 1977 exhibit low erucic and low glucosinolate qualities. An Argentine variety named Regent is now licenced and is similar to Tower but is a higher yielding variety with an increased oil content. Candle, Canada's first low glucosinolate Polish variety is also now licenced. It is lower yielding than Torch but higher in oil content. Candle is the first variety to partially exhibit a yellow seed coat which is

synonymous with low fiber content and thus an improvement in meal quality.

The Future

The following are the types of varietal improvements that the rapeseed breeders along with the rapeseed industry have set as goals for the breeding program.

- Higher yields.
- Earlier maturity.
- a lower fibre content in the meal (yellow seed coat)
- Higher oil and protein content in the seed.
- Disease resistance in particular to white rust.
- Greater lodging and shattering resistance.
- A higher linoleic and lower linolenic acid content in the oil giving a more polyunsaturated oil with better keeping qualities.
- High lysine content.
- A hybrid rape variety.

APPENDIX D
****

Content of phytate-P in protein concentrates prepared from some oilseeds. Analysis of pure sodium phytate is included as a methodolical control (190).

MATERIAL	PHYTATE - P (mg / g)*
Rapeseed, protein concentrate	6.89
Sunflower seed, lipid-protein concentrate	8.80
Nigerseed, lipid-protein concentrate	8.35
Poppy seed, press-cake flour	8.08
Sodium phytate	195.0

* Calculated on fat-free weight.

Content of phytate in some oilseeds and their protein products.

MATERIAL (REFERENCE)	% BY WEIGHT OF MATERIAL	PHYTATE - P (mg / g)
Sesame seed (161)	-----	5.47
Cottonseed kernels (161)	-----	6.20-10.69
Soybean (191)	1-1.5 % Phytic acid	
Sesame seed (181)	5.18 % Phytic acid	
Commercial soy protein isolate (145)	1.9-2.5 % Phytate	
Defatted soy flour (146)	1.5 % Phytate	
Soybean flour (138)	-----	3.06
Sesame meal (134)	1.00 % Phytate-P	
Full-fat soy flour (140)	0.40 % Phytate-P*	
Full-fat soy flour (183)	1.3 % Phytic acid	
Defatted rapeseed (148)	2.2-3.9 % Phytic acid	

* On a dry weight basis.

Content of phytate in the Brassica protein concentrates (27).

Protein Source	% Phytate*
Bronowski	5.27
Target	6.68
Tower	7.32
Mustard	6.41
Span	7.18
Karlshamn	7.50

* All values expressed on dry matter basis

* Phytate expressed as $Ca_5Mg(C_6H_{12}O_{24}P_6 \cdot 3H_2O)_2$ and = Phytate-P x 4.545.

APPENDIX E
****

APPENDIX E Experimental Equipment and Chemicals

Experimental Equipment :

1) Diaphragm metering pump, Pulsafeeder model 7120-S-E, Interpace Corp., Rochester, New York, U.S.A.

2) Pulsation dampener, Pulsafeeder model 38, Interpace Corp., Rochester, New York, U.S.A.

3) Spectrophotometer, Spectronic 710, Baush & Lomb, Rochester, New York, U.S.A.

4) Digital pH/mv meter, model 801, Orion Research Inc.,

5) Conductivity bridge, YSI model, Yellow Springs Instrument ., Yellow Springs, Ohio, U.S.A.

6) Micro Kjeldahl digestion rack unit, Labconco Corp., Kansas, Missouri, U.S.A.

7) Constant temperature shaker, Blue M, Blue M Electric Co., Blue Island, Illinois, U.S.A.

8) Centrifuge, IEC model HN-SII, International Equipment Co., Needham Heights, Mass.,U.S.A.

9) Water bath, Techne UB-1, Techne Cambridge Ltd., Cambridge, England.

10) Rotating jar mill, fabricated at the Dept. of Chemical Engineering, University of Ottawa, Ottawa, Ontario.

APPENDIX E (CONT'D)

Experimental Chemicals:

1) Cellulose Acetate, Acetyl 40.0%, ASTM visc. 25, Eastman Kodak Co., Lot No. A66.
2) Anhydrone (Magnesium perchlorate, anhydrous), 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.
3) Acetone, 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.
4) Ethyl Alcohol, 95 (v/v)%, Consolidated Alcohol (Toronto, Ontario).
5) Albumen Bovine Fraction V, Matheson Coleman & Bell.
6) Sodium Phytate, Anhydrous, ICN Pharmaceuticals Inc., Lot No. 4249-A.
7) Sodium Chloride, Crystal, 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.
8) Potassium Sulfate, Powder, 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.
9) Selenium Oxychloride, J.T. Baker Chemical Co., Lot No. 35,179.
10) Sulfuric Acid, Concentrated, 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.
11) Ammonium Sulfate, Certified Primary Standard, Fisher Scientific Co., Lot No. 735382.

APPENDIX E (CONT'D)

12) Brilliant Blue G (Coomassie Brilliant Blue G), Anhydrous, Sigma Chemical Co.

13) Phosphoric Acid, 85 %, Matheson Coleman & Bell.

14) Molybdic Anhydride, Certified A.C.S., Fisher Scientific Co., Lot No. 743708.

15) Molybdenum metal (-100 mesh-powder), 99.8% pure, Fisher Scientific Co., Lot No. 743441.

16) Potassium Phosphate Monobasic, 'fisher' Certified Primary Standard, Fisher Scientific Co., Lot No. 734559.

17) Potassium Permanganate Solution, 0.1N, 'Fisher' Certified, Fisher Scientific Co., Lot No. 762801-12.

18) Sodium Sulfate, Anhydrous (low in phosphate),'Fisher' Certified A.C.S., Fisher Scientific Co., Lot No. 711740.

19) Hydrochloric Acid, Concentrated, 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.

20) Sodium Hydroxide, 50 % Solution, 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.

21) Phenolpthalein, 'Baker Analyzed' Reagent, J.T. Baker Chemical Co.