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STUDIES OF AQUEOUS PROCESSING OF RAPESEED INTO OIL AND MEAL

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled STUDIES OF AQUEOUS PROCESSING OF RAPESEED INTO OIL AND MEAL submitted by Mamat @ Shafie Bin Embong in partial fulfilment of the requirement for the degree of Doctor of Philosophy.

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ABSTRACT

Conventional methods of rapeseed oil extraction; expeller processing, prepress solvent extraction, and solvent extraction, although efficient in extracting the oil, are causing damage to meal protein. In addition, the last two processes are prone to a constant danger of explosion and fire hazard. Canada has become the world's largest exporter of rapeseed. It is now known that rapeseed protein is one of the most balanced vegetable proteins, comparable to or even better than soybean. However, its utilization even for animal feeds is limited due mainly to the presence of toxic substances. The purpose of the aqueous rapeseed oil extraction is hopefully to overcome these problems.

The general objective of this study was to examine the feasibility of aqueous extraction of rapeseed oil with particular emphasis on the yield and the quality of the oil extracted. The rapeseed was ground, boiled and reground to a fine slurry which was then blended and stirred. The slurry was centrifuged into solid, liquid and oil fractions. The oil fraction was partly in the form of an emulsion and partly in the form of free oil. The emulsion was recentrifuged, frozen and thawed to obtain the free oil. The solid residue was dried to a constant weight and analysed.

The yield of oil was determined from the amount of residual oil in the dried meal.

Fine grinding was essential to get a maximum oil yield. Boiling for 5 min was necessary to inactivate the myrosinase and hence to lower the sulfur levels in oil. Blending for 15 min was needed to break down the cells further and increase oil yield. The optimum stirring conditions were: temperature, 70 ± 2 C; solid-to-water ratio, ca. 1:3, pH, 6.6 ± 0.1; and stirring time, 1 h.

By these laboratory batch conditions more than 90% of rapeseed oil could be extracted. Compared with industrial crude oil, the aqueous extracted crude oil contained lower amounts of sulfur (4-6 ppm), phospholipids (0.03-0.05%), free fatty acids (0.2%) and peroxide value (1.1 to 1.4 mequiv/kg of oil). The meals from the aqueous process contained about the same amounts of protein, fiber, and ash as soxhlet extracted meals. However, the aqueous extracted meals contained 4 to 7% residual oil under a single extraction cycle. However, multiple aqueous extraction that should reduce the amount of the residual oil in the meal was not employed. The protein in the meal was less soluble in water and salt solution, but was more soluble in alkaline solution and aqueous ethanol. The minimum solubility of the protein was at pH 5.0. The amino acid analyses did not show any significant effect of processing

on the quality of protein. During the aqueous extraction process, 58 to 79% of glucosinolates was removed from the meal. This process also yielded a whey containing ca. 0.5% true proteins of which ca. 65% could be precipitated by adjusting to pH 4-4.5 and heating at 85°C for 10 min.

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I. REVIEW OF LITERATURE

A. Aqueous Extraction of Vegetable Oils

1. Introduction

Conventional extraction technology, which employs either hydraulic press, screw press (expeller), solvent extraction, or prepress solvent extraction, is primilarly directed towards the production of edible oil. As a result little attention is given to the quality of the protein residue. In recent years, however, heightened awareness of the importance of proteins in human nutrition has provided motivation for possible changes in existing oil extraction processes and practices. These changes fall into two broad categories with the first arising from the fact that the quality requirements for food-grade products are different from those for feed-grade. This is basically a matter of sanitation and concern for the presence of end by-products, such as fiber and other indigestible matter. The second category is concerned with more fully exploiting the potential nutritive value of oil seed proteins. Of importance here is the avoiding of damage to the proteins and the inactivation or removal of the antinutritional factors present in some oil seeds which can reduce the nutritive value of the protein or can be toxic to humans (Cater et al., 1974).

Although a number of earlier studies have been reported on aqueous extraction of oil from oleaginous materials (e.g. Van Deurs, 1928; Hocker, 1930; Ludecke, 1938; Nyrop, 1938; and Robledano and Luzuriaga, 1948), it was only in the last two decades that the importance of extracting a good quality protein from the oil seed by the aqueous method has been realized (e.g. Sugarman, 1956; Rhee et al., 1972a and 1973; Hagenmaier et al., 1972 and 1973; Cater et al., 1974; Hagenmaier, 1974 and Mieth et al., 1975a,b). This aqueous extraction method has been used to extract oils from many vegetable materials (Table 1). Two processes, CFTRI (Bhatia et al., 1966) and CHAYEN (Chayen, 1960), have been utilized for commercial production, and even these are of limited use today (Cater et al., 1974). This could very well be due to a lower recovery of the oil by these aqueous processes when compared with conventional methods, since oil yield is important from the point of view of the economics of the total process.

Some advantages of aqueous extraction over conventional solvent extraction are: less initial capital investment, safer operation due to the fact that no flammable solvent is being used, less operational danger and no air pollution from solvent losses. Furthermore, simultaneous separation of oil and protein during the aqueous extraction process results in fewer steps being needed for protein

Table 1. Aqueous Extraction of Vegetable Oil

Oleaginous Material	Reference
Cocoa bean	Hocker (1930)
Palm fruit	Couche (1967)
Olive	Couche (1967), Vallee (1971), Montedoro and Petruccioli (1973)
Soybean	Sugarman (1956)
Peanut	Sugarman (1956), Dangoumau (1958), Subrahmanyan et al. (1959), Eapen et al. (1966), Rhee et al. (1972a and 1973a,b, and c), Cater et al. (1974)
Sunflower seed	Spinove and Gekker (1968), Haganmaier (1974), Mieth et al. (1975a)
Coconut	Beckman (1930), Moore (1948), Robledano and Luzuriaga (1948), Bhati (1963), Bousser and Bermier (1969), Pedro and Carlos (1969), Puertollano et al. (1970), Hagenmaier et al. (1972 and 1973), Cater et al. (1974)
Oleaginous material in general	Van Deurs (1928), Hocker (1930), Ludecke (1938), Chayen and Ashworth (1953), Kao (1956), Sugarman (1956), Georgia Technical Research Institute (1960)

isolate production. Another advantage of aqueous processing is the possibility of utilizing certain chemicals to remove or inactivate undesirable substances (Rhee et al., 1973a; and Cater et al., 1974). For example, hydrogen peroxide and sodium hypochlorite are effective for the destruction of aflatoxins in peanut (Rhee et al., 1972b).

The disadvantages of aqueous extraction include; lower efficiency of oil extraction and recovery, with the process yielding only about 95% as much oil as conventional methods; the necessity of demulsification to recover the oil if an emulsion should be formed; and an increased potential for microbial contamination resulting from wetting of the materials during processing. Also, the resulting higher oil content of the meal may cause off-flavor and hence storage stability problems (Cater et al., 1974).

2. Unit Operations

Removal of oil and protein from oil seeds can be achieved by aqueous extraction of comminuted seed followed by centrifugal separation of the slurry into oil and/or an oil emulsion, and solid and aqueous phases (Cater et al., 1974). This process consists of grinding, solid-liquid separation, centrifugation, demulsification and drying of products.

a) Grinding

Grinding is the first critical step in aqueous extraction of oil and protein from oil bearing materials. The seed cells must be ruptured to release their contents and to increase the efficiency of extraction (Cater et al., 1974). Insufficient grinding results in losses of oil in the residue. This was observed during the aqueous extraction of peanut (Subrahmanyan et al., 1959; Rhee et al., 1972a), sunflower (Hagenmaier, 1974; Mieth et al., 1975a) and coconut (Puertollano et al., 1970; Hagenmaier et al., 1972 and 1973; Dendy and Timmins, 1974). Also, there is an accumulation of fat containing cell particles in the emulsion during centrifugation (Mieth et al., 1975a). Excessive grinding can produce finer oil globules which are difficult to demulsify (Hagenmaier et al., 1972; Cater et al., 1974; Mieth et al., 1975a). In addition, there would be more nonprotein compounds such as polysaccharides and lipids in the protein isolates (Mieth et al., 1975a). These compounds lower the quality of the isolate by reducing the protein content and solubility, and storage stability. Within certain limits Rhee et al. (1972a) observed that the degree of grinding of peanut has little effect on protein extraction.

Grinding can be done either wet or dry, depending mainly upon the initial moisture content, and the chemical composition and physical structure of the oil seeds.

Coconut, with an initial moisture content near 50%, is ground wet to avoid a costly drying step (Cater et al., 1974). Wet grinding is generally considered superior to dry grinding in rupturing cells, because the moisture softens the cell walls (Subrahmanyan et al., 1959, and Cater et al., 1974). However, wet grinding of peanut produces a more stable emulsion (Rhee et al., 1973a; Cater et al., 1974). Since peanut and sunflower have low moisture contents they are best ground dry (Rhee et al., 1972a; Cater et al., 1974; and Hagenmaier, 1974). The amount of protein recovered as a concentrate after dry grinding is higher than that after wet grinding (Rhee et al., 1973a). By wet grinding more protein remains in the whey.

The method of grinding has marked effect on the subsequent recovery of free oil. When grinding was done vigorously in a Waring Blendor in the presence of aqueous media prior to extraction, a very stable emulsion, rather than a free oil, was obtained. However, when an Urschel mill was used, there was a maximum recovery of free oil (Rhee et al., 1972a).

During grinding of coconut, variation of the water to coconut ratio from 1:1 to 20:1, temperature from 30-85°C, and pH from 5.8 to 7.0 did not prove to have any significant effect on the overall yields of oil and protein (Dendy and Timmins, 1973). However, alkaline grinding conditions

could result in some saponification of the oil. Sison et al. (1968) observed that maximum extraction of protein occurred when grinding of coconut was done at pH 6.6.

b) Extraction

Basically, extraction is achieved by dispersing the comminuted oil bearing material in water and then agitating the dispersion to facilitate the extraction of the constituents. For maximum extraction of oil and other constituents this process can be varied to take into account the ease of oil release from the cells. This depends on the physical structure of the oil bearing materials. Rac (1967) showed that the size of the cells and the thickness of cell walls influences the extraction of oil with hexane. Due to the softness of the coconut's internal physical structure, its oil is effectively extracted from the comminuted meat by washing and pressing the slurry (Hagenmaier et al., 1972; Hagenmaier et al., 1973; Cater et al., 1974). However, in the case of peanut and sunflower, maximum extraction could only be obtained by stirring the dispersion (Rhee et al., 1972a; Hagenmaier, 1974). Chayen and Ashworth (1953) developed a shock wave process for extraction of oil from oleaginous materials, with the aim of producing a good quality protein. This wet rendering process was

employed by Eapen et al. (1966) for the extraction of peanut oil. However, their recovery of oil was low.

c) Solid-liquid Separation and Centrifugation

Removal of the major portion of the undissolved solids, which are mainly fibrous material; undissolved carbohydrates and proteins, has been found to be necessary with coconut and peanuts in order to obtain efficient recovery of oil by centrifugation. This separation could be achieved by hand by squeezing the ground coconut with water through cheese cloth (Hagenmaier et al., 1972; Gunetileke and Laurentius, 1974), by filtering through vibration or pressing-type screens (Hagenmaier et al., 1973; Cater et al., 1974; Rhee et al., 1973a,c; Dendy and Timmins, 1973; Subrahmanyan et al., 1959; Eapen et al., 1966), or by clarifying or basket centrifuges (Cater et al., 1974; Hagenmaier, 1974; Dendy and Timmins, 1973; Eapen et al., 1966). The selection of a particular method depends on the physical nature of the material in the dispersion, and also on the operating costs (Cater et al., 1974).

During the extraction of peanut oil, Eapen et al. (1966) and Rhee et al. (1973c) observed that removal of suspended solids by a 3-way centrifuge could not be used efficiently because of the large volume of solids in peanut slurry. The self-opening type 3-phase centrifuge had to be

opened at very short intervals to discharge the solids. This reduces the oil separation efficiency. Centrifugal sedimentation of the suspended solids caused emulsification and resulted in inefficient separation of the oil (Eapen et al., 1966). Removal by filtration was too slow and incomplete, since fine particles tended to block the screen. The efficiency of the separation of solids and oil depended on the careful conditioning of the seed. When the moisture content of the seeds was between 8.4-14.3%, the rate of filtration was fast, but when the moisture content of the seed was below 8%, the filtration rate was slow. The filtration rate also depends on roasting temperature. With roasting temperatures above 140°C, the filtration rate was slow. Rhee et al. (1973c) observed that a continuous clarifying centrifugation of peanut dispersion through a Westfalia disc-type centrifuge was as effective as a combination of prefiltration and pressing steps for removing the majority of the solids. This method was less laborious and minimized the loss of material.

In the solid-liquid separation of sunflower slurry, Hagenmaier (1974) found that basket centrifugation gave a far superior separation of oil and residue than batch centrifugation, although there was no difference in protein extraction between the two separation techniques. However, Dendy and Timmins (1973) obtained higher yields of coconut oil with a sieve-press operation than with a basket

centrifuge separation.

In the aqueous process, three phase centrifugation is an important step in which the liquid or slurry containing both dissolved and undissolved matter is separated into oil or an emulsion and solid and aqueous phases (Cater et al., 1974). In this centrifugation the oil can be recovered as free oil (e.g. Eapen et al., 1966; Rhee et al., 1972a) or as an oil-in-water emulsion (e.g. Subrahmanyan et al., 1959. Hagenmaier et al., 1972 and 1973; Sugarman, 1956), depending on the conditions of the process. When centrifugation was done at the isoelectric pH of peanut protein, a greater amount of free oil could be obtained than under alkaline conditions, which gave stable emulsions (Rhee et al., 1972a). Free oil could be separated from peanut by centrifugation when the seed was conditioned to prevent the breakdown of its fiber (Eapen et al., 1966). Heating before centrifugation could also yield free oil during the centrifugation (Pedro and Carlos, 1969; Vallee, 1971).

Centrifugation brings about concentration and packing of the oil-in-water emulsion in the continuous phase (Gunetileke and Laurentius, 1974), and it is a factor in demulsification of oil by cooling (to be discussed later).

Centrifugation in the isolectric pH range of

coconut protein (i.e. near pH 4.0); binds more oil to protein than centrifugation at pH 8.0 (Hagenmaier et al., 1972). A higher yield of oil after laboratory centrifugation at 4,000 x g for 30 min than after pilot plant centrifugation at 7,800 rpm for 45 sec was due to insufficient centrifugal force and retention time in the latter case for the separation of oil from other constituehts (Rhee et al., 1973c).

d) Demulsification

Various methods of demulsification have been used in aqueous extraction of oil from oleaginous materials. The nature of the protein forming the envelope around oil droplets and, hence, the nature and strehgth of the bonds between the oil ahd protein is the most important factor in determining the stability of the emulsion. Covalent bonding between lipids and proteins is rare, and hydrogen bonding is of minor significance, although it is indirectly important only when the interacting groups are near. Hydrophobic bonding is the most important factor in a lipid-protein complex. Other factors in the seed, such as phospholfpids, also affect the stability of the emulsion (Karel, 1973; Dendy and Timmins, 1974). Karel (1973) established an equation for the rate of coalescence of droplets in the demulsification process:

$$\frac{d\bar{v}}{dt} = K \frac{\phi}{\eta_w} e^{-E_1/RT}$$

with $\bar{v}$, average droplet volume; ϕ, volume fraction of oil; η_w, viscosity of aqueous phase; E, energy barrier; t, time; T, absolute temperature; and K, R. constants.

i) Elevated Temperature

In aqueous processes, heat treatment is used by many workers in the extraction step. During this extraction, demulsification takes place. In the traditional method, as discussed by Andaya et al. (1961), coconut cream was boiled in order to get the oil. Rajasekhran and Sreenivasan (1967) separated coconut oil and protein from an aqueous extraction of kernel by heating and centrifugation. Recently, during the demulsification of coconut cream, the emulsion was heated to 80-90°C before it was subjected to shear force (Hagenmaier et al., 1972, 1973).

ii) Low Temperature

Demulsification at low temperatures was employed in the aqueous extraction of oil from coconut (Robledano and Luzuriaga, 1948; Peters, 1960; Roxas, 1963; Gunetileke and Laurentius, 1974) and peanut (Subrahmanyan et al., 1959).

In their work, Robledano and Luzuriaga (1948) subjected the cream obtained by centrifugation to enzyme action, freezing, and thawing to break the emulsion. Peters (1960) reported breaking the coconut milk emulsion by freezing and thawing. Roxas (1963) was able to demulsify coconut milk by freezing it to -4°C, followed by thawing. Recently, it was shown that cooling at or below 17°C was sufficient to break coconut milk emulsion (Gunetileke and Laurentius, 1974). In this process, adequate packing of the oil globules by centrifugation was a prerequisite for breaking the emulsion under chilled conditions. Furthermore, it was observed that the lower the temperature of the cream, the shorter the time required for complete release of oil. Oil was not released if the temperature of the cream was above 17°C. Subrahmanyan et al., (1959) obtained peanut oil by freezing and thawing the emulsion.

When the water phase of an emulsion freezes into ice crystals, the oil droplets are forced into narrow channels of unfrozen fluid between ice crystals (Young, 1934). The increasing concentration of electrolytes in this unfrozen water results in supercooling and reduction in electrical charge (Borosikhino et al., 1961) The oil droplets are compressed and distorted by the growing ice crystals (Pospelova et al., 1962). Only those globules of a size bigger than the voids between ice crystals are

subjected to this pressure. Coalescence occurs at the part of the globule surface which is not protected by emulsifier or proteins. In practice, both freezing and thawing rates affect the stability of the emulsion (Singleton et al., 1960). Rapid freezing and a moderate rate of thawing causes greater instability.

Freezing could rupture the protein envelope (Robledano and Luzuriaga, 1948; Roxas, 1963) and could destroy or reduce the emulsifying power of proteins due to their irreversible aggregation (Borosikhino et al., 1961; Roxas, 1963). As observed under the microscope, cooling of coconut cream to 17°C or below resulted in crystallization of oil (Gunetileke and Laurentius, 1974). On thawing, larger droplets were formed due to the coalescence of these crystals. Crystalization of oil may rupture the protein envelope.

iii) Shear Force

Demulsification by shear force was employed by Sugarman (1956) to break the emulsion of oleaginous materials (e.g. peanut and soybean). This technique requires control of the emulsion pH at 4, and reduction of the moisture content below a critical level (i.e. 28%) above which the emulsion cannot be broken. This method was later used at the Georgia Technical Research Institute (1960)

for simultaneous extraction of oil and protein from oleaginous material and, recently, the group working at Food Protein Research and Development Center, Texas A & M University used it for the extraction of oil and protein from peanut, and sunflower (Hagenmaier et al., 1972, 1973; Hagenmaier, 1974; Cater et al., 1974). Shear force could be achieved by stirring (Rhee et al., 1972a, 1973), colloid mill (Georgia Technical Research Institute, 1960) and high velocity centrifugal pump (Hagenmaier et al., 1972, 1973; Cater et al., 1974; Hagenmaier, 1974).

When shear force is applied to the emulsion, there will be some denaturation of proteins. With the air trapped in the emulsion, the surface tension created around the trapped air tends to stretch the proteins and so cause denaturation. The protein envelope will be disrupted by the shear force thus releasing the oil droplet. Stirring and pumping causes forced convections and a greater rate of coalescence. During stirring, small and large droplets move at different rates, resulting in more coalescence. This process is called "Orthokinetic flocculation". However, at a high rate of shear, larger droplets are elongated and sub-divided (Kitchener and Mussellwhite, 1968). A disadvantage of this method was shown by Hagenmaier et al. (1972) when the protein precipitated from aqueous extract of coconut proved to be quite oily and the oil content of

this protein could only be reduced to 40% even with quite severe agitation. However, the protein recovered was not irreversibly denatured.

iv) Centrifugation

Centrifugation is used for solid-liquid separation, phase separation, concentration of emulsion into cream, and demulsification. In comparing the demulsification of peanut oil emulsion by heating, acidification, or centrifugation, Dangoumau (1958) observed that only centrifugation was satisfactory. Dendy and Timmins (1974) and Hagenmaier et al., (1972) reported that coconut oil emulsions could be completely broken by centrifugation. During centrifugation, oil droplets are assembled and compressed against each other, thus resulting in coalescence. Kitchener and Mussellwhite (1968) suggested that compressed droplets take the form of a polyhedron which has a larger surface area than that of spherical droplets of equal volume. This increase in surface area stretches the protein envelope, and, so causes it to rupture. The smaller oil globules, because of their relatively high surface tension, will require more work to be done on them to rupture the enclosing protein membrane than will the larger globules (Dendy and Timmins, 1974). They need a

longer period of centrifugation, approximately 30 minutes, in order to appear in the oil phase.

v) Hydrogen Ion Concentration

It was observed that most oil-in-water emulsions are least stable at isoelectric points of the proteins in the system (Rhee et al., 1972a). Van Deurs (1928) brought the pH of the emulsion below 5 by addition of acid or lactic acid bacteria to obtain a continuous phase of the oil or fat. Lava et al. (1941) published a semicommercial method of extracting coconut oil in which the emulsion was broken by adjusting the pH in the range of 3 to 5.6. During the demulsification of coconut oil emulsion by cooling, the pH of the cream was 6.1-6.4 (Gunetileke and Laurentius, 1974). Hagenmaier et al. (1972) adjusted the pH of coconut cream to 4.0 before it was demulsified by shear force. The same pH value was used by Rhee et al. (1972a) in the demulsification of peanut oil emulsion by stirring.

vi) Other Methods of Demulsification

Addition of surfactants to olive oil emulsion was reported in a series of publications by Martinez et al. (1961, 1962, 1966). Sodium diisobutyl naphtalene sulfonate and sodium isooctylsulfo-succinate were used to break the

emulsion. Several workers (Alexander, 1921; Beckman, 1930; Horovitz - Vlasova and Novetelnov, 1935; Soliven and Leon, 1938) observed that, if the emulsion was allowed to ferment, the oil and protein fractions separated. Recently, Puertollano et al. (1970) patented a method for separation of oil and protein from coconut milk by fermentation using Lactobacillus plantarum and L. delbrueckii . The optimum conditions for rapid fermentatation were: 1:1 to 1:2 (w/v) of coconut meat/water; a microaerophilic condition; temperature of 40° to 50°C; pH between 4.7 to 5.5. Nevertheless, even under controlled conditions only 60% of the milk produced from individual coconuts was separated into oil and protein. At least two factors were considered to be responsible for the demulsification of coconut milk during fermentation. The first was the removal of fermented sugars which may serve as emulsifiers, while the second was the precipitation of soluble protein due to acid being produced during fermentation.

e) Drying

Removal of water from the solid residues from aqueous oil extraction processes can be a problem. In drying of the wet carbohydrate meal and protein using tray drier, Subrahmanyan et al. (1959) observed that the

outer surface of the lumps became discolored during the process. The inside of the lumps of carbohydrate meal remained white, in contrast to the inside of protein lumps which became darker than the outside. To reduce the discoloration of protein meal it was necessary to spread wet protein more finely than was the case for wet carbohydrate meal. According to Cater et al. (1974), spray drying of high protein products, such as peanut concentrates and isolates, presents no major technical problems, since the proteins are relatively heat stable and the products are not hygroscopic. However, spray-drying of the whey-type coconut products has been difficult because of the lower protein content and the hygroscopic nature of the sugars and salts present.

3. Factors Affecting the Yield of Extraction

Factors which influence the efficiency of extraction include solid-to-water ratio, degree of grinding (already discussed), kinds of salts and their concentrations, pH of the dispersion, extraction time, and temperature and degree of agitation (Cater et al., 1974; Mieth et al., 1975b). Some other factors are the morphological structure and composition of seed, especially the amount of seed coat, size and rigidity of the cells, the storage conditions and

physiological properties of seed material, the temperature of pretreatment of the seed, and the number of extraction cycles (Mieth et al., 1975b).

a) Solid-to-water Ratio

This factor is not important when aqueous extraction is carried out by washing and pressing, as in the extraction of coconut (Hagenmaier et al., 1972 and 1974; Cater et al., 1974). However, it is critical when the oil is extracted by stirring or agitation, as in the case of peanut and sunflower (Rhee et al., 1972a; Sugarman, 1974; Subrahmanyan et al., 1959, Bhatia et al., 1966; Hagenmaier, 1974). Optimum extraction of oil and protein from oil bearing material depend on the solid-to-water ratio. The optimum solid-to-water ratio recommended by different workers are shown in Table 2.

b) Temperature and Time

Temperature is an important factor for maximum extraction of oil and protein. Various workers employing aqueous extraction process have used temperatures ranging from room temperature to 90°C (Table 3). The choice of extraction temperature depends on the emphasis placed on the two main objectives of the process. They are: firstly,

Table 2. The Reported Optimum Solid-to-water Ratios in Aqueous Oil Extraction

Solid-to-water Ratio	Oleaginous Material	Reference
1:10	Sunflower seed	Hagenmaier (1974)
1:5 to 1:12	Peanut	Sugarman (1956)
1:7	Peanut	Subrahmanyan et al. (1959), Bhatia et al. (1966)
1:6	Peanut	Rhee et al. (1972a, 1973 a,b and c)

Table 3. The Recommended Temperature For Aqueous Oil Extraction

Temperature	Oleaginous Material	Reference
Room temperature	Coconut	Pedro and Carlos (1969) Bousser and Bermier (1969)
80°C	Coconut	Roxas (1963), Hagenmaier et al. 1972 and 1973
90°C	Coconut	Bhati (1963)
Room temperature	Peanut	Dangoumau (1958)
60-65°C	Peanut	Subrahmanyan et al. (1959), Rhee et al. (1972a and 1973a, b and c)
Room temperature	Sunflower seed	Hagenmaier (1974)

to prevent irreversible denaturation of protein and to recover a good quality oil, and, secondly, to recover at least 95% of the oil (Hagenmaier et al., 1972). The first objective requires the use of lower temperatures, while the second is best achieved with the use of higher temperatures.

Rhee et al. (1972a) observed that the extraction of oil and protein increased with temperature. However, a temperature of 40°-44°C was found to be satisfactory for protein extraction, while a temperature of 60°-64°C was needed for optimum oil extraction. In an experiment with aqueous processing of full-fat sunflower seeds, Hagenmaier (1974) observed that there was no change in free fatty acid during extraction over the range of 5° to 65°C.

Time of extraction is only critical when stirring is required during the process. The extraction of oil and protein from peanut increased with the time, with a maximum after 30 min (Rhee et al., 1972a). In other studies by Rhee et al. (1973a,b,c), extraction was done for 1 hour. In the case of sunflower, the extraction time was 45 min (Hagenmaier, 1974).

c) pH of Extraction

Different pH values for aqueous extraction of oleaginous materials were recommended by different workers (Table 4). Rhee et al. (1972a) obtained the maximum extraction of peanut oil when protein solubility was at a minimum. As the pH of the dispersion departed from the point of minimum protein solubility, i.e. pH 4.00 ± 0.25, the amount of oil extracted decreased while the amount of protein released increased. Maximum protein extraction was obtained at pH 8. However, Rhee et al. (1973b) later found that maximum recoveries of both protein and oil from peanut were obtained at pH 8.0. Extraction at pH values higher than 8.0 considerably reduced the solubility of protein in the isolates, with no significant increase in the recovery of the protein, and increased the loss of oil through saponification. The protein content of the isolates increased, while their oil content decreased, as the extraction pH was raised from 7 to 10. In the case of sunflower, the optimum extraction of oil was achieved at pH 10, although minimum solubility of protein occurred near pH 5.0 (Hagenmaier, 1974).

Table 4. The Recommended pH of Aqueous Oil Extraction

pH of Extraction	Oleaginous Material	Reference
Neutral pH (water)	Coconut	Bousser and Bermier (1969)
	Peanut	Dangoumau (1958), Subrahmanyan et al. (1959)
4.00 ± 0.25	Peanut	Rhee et al. (1972a and 1973 a and c)
8.0	Peanut	Rhee et al. (1973b)
10.0	Peanut	Eapen et al. (1966)
10.0	Sunflower seed	Hagenmaier (1974)

d) Additives

Addition f chemicals other than acids and alkalies were employed by some workers during the extraction process. The use of surfactants to demulsify the olive oil from olive pastes resulted in a greater yield of oil (Martinez et al., 1966). Montedoro and Petruccioli (1973) obtained an increase in the yield of olive oil when six different hydrolases were added, either individually or in combination, during the extraction process. In another study Lanzani et al. (1975), using four enzymes, obtained an increase in oil yield of sunflower from 30 to 52%, and peanut from 72 to 78%. In the case of rapeseed, oil recoveries were 44, 49 and 75% using polygalacturonase, cellulase and pectinase, respectively. However, the maximum recovery of rapeseed oil was 78% using 1.5% pectinase and 1.5% polygalacturonase. The addition of NaCl to water was found to increase the extraction of protein and of good quality oil (Linow et al., 1971; Mieth et al., 1975a). Rhee et al. (1972a) observed that, with each of four salts used (KCl, NaCl, $CaCl_2$, and $MgCl_2$), the peanut protein extractability at all the concentrations tested was suppressed under neutral and alkaline conditions, but was enhanced in the range of pH 3.5 - 5.5. Sodium sulfite was added during the aqueous extraction of sunflower in order to prevent discoloration of the protein residue (Hagenmaier, 1974).

e) Extraction Cycle

The amount of oil extracted increases with the number of extraction cycles. Mieth et al. (1975b) suggested that the residue of the first extraction cycle should be reextracted in order to obtain more oil. Hagenmaier et al. (1972) found that two washes were adequate to remove all the oil globules clinging to the coconut residue. Similarly, washing of the residue retained in the perforated bowl of a basket centrifuge was carried out during the extraction of sunflower oil (Hagenmaier, 1974). In another study, Hagenmaier et al. (1973) extracted ground coconut three times with hot water prior to pressing. In order to reduce the oil content of the peanut protein concentrate and to increase the recovery of free oil, the solids were washed several times (Rhee et al., 1973c). The washing step reduced the oil content of the concentrate from ca. 30% for the unwashed product to ca. 10% for the three-fold washed product and increased the amount of free oil from 83% without washing to about 89% with three washings. Washing more than three times did not result in any significant increase in oil or protein recovery. In their experiment on aqueous extraction of sunflower, Mieth et al. (1975a) reextracted the residue from the emulsion, and the washings from the residue of the first centrifugation in order to obtain a 95% extraction of oil.

4. Yield and Quality of the Extracted Oils

In general, aqueous extraction methods lead to lower recovery of the oil than do the standard methods of oil extraction. The variation in the results reported by various workers for the yield of oil and protein was, as was already discussed, attributed to the degree of grinding (Mieth et al., 1975b). The amount of oil remaining in the residue of peanut as reported by Chayen and Ashworth (1953), Sugarman (1956), Subrahmanyan et al. (1959), and Eapen et al. (1966), was 9 to 10%. Dangoumau (1958) reported a 98% recovery of peanut oil after aqueous extraction. While preparing peanut protein isolate Bhatia et al. (1966) produced protein isolates of relatively low fat content (4%), and obtained an oil recovery in the order of 91 - 94%. Recently, Rhee et al. (1972a) reported a high recovery of peanut oil (approximately 96%) and the amount of residual oil present in protein concentrates and isolates was ca. 2.5% and 3.0%, respectively. At the maximum pH (i.e. pH 8) for extraction of protein and oil, Rhee et al. (1973b) obtained a protein isolate which contained ca. 6% oil. In summing up the recent work of Rhee et al. on peanut , Cater et al. (1974) reported the oil extracted were 89% and 86%, respectively, for preparation of protein concentrate and isolate. The protein concentrate contained 8% oil, while the protein isolate contained 9% oil.

In the aqueous extraction of coconut oil, Robledano and Luzuriaga (1948) obtained a yield 1% lower than that of the copra method, however, because refining was not necessary, the overall oil recovery rate, in terms of fresh meat, was ca. 10.2% higher than that of the copra method. Using the Krauss-Maffei process, Samson et al. (1971) reported about 14% of the coconut oil remained with the residue, and 7% with the protein. The data obtained by Timmins (1972, unpublished) at the Tropical Products Institute in London indicate that their residue and protein contain ca. 8% and 5 - 10% of the oil, respectively (Hagenmaier et al., 1972). Recently, at the Food Protein Research and Development Center, Texas A & M University, Hagenmaier et al. (1973) developed a process in which the oil remaining was 5.8% in the residue. In their report on aqueous extraction of coconut, Cater et al. (1974) obtained a 93% recovery of the initial oil content. The soluble fraction, skim milk, contained 5.1% oil.

Aqueous processing of full-fat sunflower seeds, yielded 86% of the oil (Hagenmaier, 1974). The dried residue contained 14.2% of the total oil. A higher yield (95%) was reported by Mieth et al. (1975a).

The quality of oils processed by aqueous methods of extraction is higher than that obtained by standard extraction procedures. The oil is a water-washed product which requires little, if any, further treatment, except,

possibly, removal of water, either by recentrifugation or vacuum drying (Cater et al., 1974). A good quality coconut oil is obtained by aqueous extraction methods (Robledano and Luzuriaga, 1948; Thieme, 1968; Hagenmaier et al., 1973; Cater et al., 1974). The coconut oil obtained by this process does not have to undergo a further refining process (Robledano and Luzuriaga, 1948). According to Hagenmaier et al. (1973 and 1975), coconut oil obtained by this process contained 0.2 to 0.4 free fatty acids with an iodine number of 7.5. Hence, it is superior to the crude coconut oil obtained by crushing copra which had ca. 5% free fatty acids.

In their studies on integrated processing of peanut for the separation of major constituents, Subrahamanyan et al. (1959) obtained two types of oil with different quality. Skipin oil, which forms ca. 70% of the total oil, has a free fatty acid value of 0.17%. Its moisture content of 0.04% gives a slight turbidity appearance. Separator oil, which comprises less than 30% of the oil yield, is low in free fatty acids (0.04%) and moisture (0.01%). Rhee et al. (1973b) conducted a study on the effect of processing pH on the properties of peanut protein isolates and oil, and observed that the free fatty acid value increased slightly from 1.2 ± 0.5 to 1.5 ± 0.2

while increasing the extraction pH from 7 to 10. Under the same conditions, a similar increase was observed for saponification number (191.0 ± 1.2 to 194.0 ± 1.0) and unsaponificable matter (0.2 ± 0.3 to 0.5 ± 0.2%). The refining loss increased substantially with extraction pH, i.e., from 1.8% at pH 7.0 to ca. 4% at pH 10.0.

B. Rapeseed

1. Introduction

Among the edible vegetable oils, rapeseed ranks fifth in the world production and is exceeded only by soybean, peanut, cottonseed and sunflower seed oils (Downey, 1965; Ohlson, 1972). Canada has become the world's largest exporter of rapeseed. Rapeseed oil has been shown to be an excellent edible oil for various uses (Tape et al. 1970). Rapeseed meal is used extensively in livestock and poultry feedings.

There is a general world wide shortage and increasing demand for protein. Rapeseed protein, the best vegetable protein known so far (Sosulski and Sarwar, 1973; Ohlson and Tear, 1974) is an ideal potential source of protein. However, its utilization in human foods is limited due mainly to the presence of toxic substances, glucosinolates and high fiber content. The present technology of rapeseed oil extraction does not include a suitable procedure to leave the protein in meal in a digestable form, free from those deleterious matter (Radwan and Lu, 1976).

2. Oil Extraction

The oil extraction of rapeseed is similar to that employed for other high oil content seeds. Currently, three methods of extraction are being employed in Canada; expeller processing, prepress solvent extraction and straight solvent extraction. The prepress solvent extraction is the most widely used (Youngs et al. 1972).

a) Expeller Pressing

In this process, the oil is mechanically squeezed from the seed. The major steps of expeller pressing include crushing, cooking for approximately 30-60 min at temperature up to 105-110°C followed by screw-press oil extraction. The objective of crushing is the reduction of the particle size which breaks the seed structure to allow oil separation and which allows a uniform cooking and drying and facilitates the rate of extraction. The cooking of the crushed seed completes the breakdown of oil cells, coagulates the cell wall proteins making it permeable to flow of oil, increases the fluidity of the oil due to the coalesce of small droplets into larger ones, causes insolubilization of phosphatidic material in order to reduce refining loss, and decreases the affinity of the oil to the solid surfaces of the meal. Cooking also deactivates

lipases resulting in lower free fatty acid value and hence lower refining loss, gives a proper plasticity for efficient pressing, increases the quality of oil cake by destruction of myrosinase and lipoxidase; dries the material to a suitable moisture content since there is less affinity of oil to the meal when the moisture is low, and destroys molds and bacteria. For an efficient extraction the moisture of the material entering the press should be 3% or less. During the pressing, extremely high pressure (15,000-20,000 lb/in^2) and temperatures (ca. 150°C) are developed. Although the oil content of the cake can be reduced to 4% by this process, it may be necessary to leave 6% to 7% to avoid damage to the cake. The cake coming out from the expeller is both hot and dry and water may be added to reduce the temperature and increase the moisture. The cake is then ground into meal. (Youngs et al. 1972; Norris, 1964; Youngs, 1965).

b) Prepress Solvent Extraction

In this process, only 70% to 80% of the oil is removed in the expeller; less pressure is required and consequently less heat is generated and hence, less heat damage is imparted to the protein. The cake containing 15% to 20% oil is reground and extracted with hexane in a continuous countercurrent extraction to remove as much oil as

possible. The solvent in the meal is stripped in desolventizers. The meal obtained contains ca. 2% fat (Youngs et al., 1972; Clandinin and Tajcnar, 1961).

c) Direct Solvent Extraction

Normally, high oil content seeds, such as rapeseed are not directly solvent extracted because the crushed seed tends to disintegrate into fine particles when placed in the solvent. This causes the counter-current movement of the meal and miscella to become impractical. A process known as "Filtration-Extraction" has been developed to overcome this problem and has been employed in rapeseed extraction (D'Aquin et al., 1953). The crushed, cooked seed is fed continuously into a horizontal cylinder counter-current to the stream of solvent, with slow agitation to give maximum extraction of oil and minimum disintegration of the meal. The slurry is deposited on a filtration bed and washed with a decreasing strength of miscella and finally with pure solvent. As in prepress solvent extraction, the solvent is removed in desolventizers and the resulting meal contains only ca. 1% oil (Youngs et al., 1972).

The following steps are suggested by Youngs et al. (1972) in order to produce high quality rapeseed meal:

1) When the seed is crushed, the enzyme is brought into contact with the glucosinolates. The moisture content of

the seed at this point should be between 6-10%. Above 10% moisture, hydrolysis of glucosinolates will proceed rapidly. On the other hand, below 6% moisture, the enzyme is only slowly inactivated by heat. Moisture either in the form of water or steam, should not be added during cooking since it tends to result in local high moisture areas favoring hydrolysis of the glucosinolates. 2) The temperature of the seed as soon as it enters the cooker, should be raised to 80-90°C as rapidly as possible. Slow rate of heating favors hydrolysis since the rate of the hydrolysis increases with increasing temperature until inactivation occurs. 3) The temperature in the cooker and the desolventizers should not exceed 105°C, as there is a danger of damaging protein quality at high temperature. Expeller temperatures are difficult to determine but these should be as low as practicable.

3. Rapeseed Oil

A few years ago the utilization of rapeseed oil in human foods was questionable due to the high content of erucic acid in the oil. However, with the production of low erucic acid rapeseed oil, this oil is now being used in the food industry for products such as salad oils and dressing, mayonnaise, cooking oils, margarine, and shortenings. Numerous reviews on the uses and nutritional

aspects of rapeseed oil have been reported (e.g. Rocquelin et al., 1971; Runer and Ohlson, 1971; Downey et al., 1974; Beare, 1975; Vles, 1974; Runer and Honkanen, 1972; Jorgensen, 1972).

a) Sulfur Content of Rapeseed Oil

Rapeseed oil is unique in that it contains high amounts of sulfur in comparison with other vegetable oils. During the extraction of the oil, a small amount of glucosinolates is hydrolyzed into isothiocyanates, oxazolidinethione and other sulfur compounds which are soluble in oil and can poison the catalyst during hydrogenation process (Persmark, 1972a; Sosulski, 1974). The presence of 5 ppm of sulfur reduced the catalytic activity by ca. 50% (Babuchowski and Rutkowski, 1969). Sulfur content of 12 ppm was associated with marked decrease in the hydrogenation value for rapeseed oil (Bhatty and Sosulski, 1972). Oils containing over 20 ppm of sulfur are difficult to hydrogenate (Sosulski, 1974).

Seven sulfur compounds were found in industrial extracted rapeseed oils (Daun, 1975). Four of these were identified as phenylethyl isothiocyanate, 3 - butenyl isothiocyanate, 4 - pentenyl isothiocyanate and 5 - vinyl 2 - oxazolidinethione. The main constituents are 3 - butenyl isothiocyanate and 4 - pentenyl isothiocyante (Franzke et al.,

1975. The ratio of 3 - butenyl isothiocyanate to 4 - pentenyl isothiocyanate in oils was reported to be approximately the same ratio present in the meal (Daun, 1975). Daun (1975) observed that approximately the same amounts of 3 - butenyl isothiocyanate were in expelled, solvent extracted and prepress-solvent extracted crude oils and there was no substantial difference in oils from B. napus L. and B. campestris L.. Franzke et al. (1975) reported that isothiocyanate content was higher in solvent extracted oils than in pressed oils. According to Daun (1975) the oxazolidinethione content in expelled, solvent extracted, and crude oils ranged from 2.0 to 26.1 ppm of sulfur. However, Franzke et al. (1975) observed that oxazolidinethione was only found in solvent extracted oils. The highest contents of oxazolidinethione were found in oils which contained large amounts of gum (Daun, 1975). The unidentified compounds were present in much larger quantities in oils from B. campestris L. than in oils from B. napus L. and in expelled oils than in solvent extracted oils.

Various amounts of sulfur in crude oils were reported by different workers; 500 ppm (Kucera and Hejtmanek, 1957), 9 - 45 ppm (Franzke et al., 1972), 12 - 43 ppm (Kurucz and Peredi, 1974), 5 - 15 ppm (Persmark, 1972a) and 16 - 57 ppm (Teasdale, 1975). The amount of sulfur is higher in solvent extracted oil than in expelled oil (Von Fellenberg, 1945; Zeman and Zemanova, 1967; Daun and Hougen, 1976).

This is due to an association of sulfur compounds with gum and colored material which are preferentially extracted with the solvent (Daun and Hougen, 1976). Sulfur content in oil is also affected by the glucosinolate content in the seed. A lower level of sulfur was obtained from low-glucosinolate seed than high-glucosinolate seed. Crude oil extracted from B. napus L. cultivar Bronowski contained traces of each sulfur compound. Sosulski (1974) suggested that a high quality rapeseed oil should contain 2 to 7 ppm of sulfur.

The amounts of sulfur in rapeseed oil are affected by subsequent processing of oil. Industrial degumming, refining, bleaching and especially deodorizing removed almost all the sulfur compounds (Kucera and Hejtmanek, 1957; Franzke et al., 1972; Kurucz and Peredi, 1974; Drozdowski et al., 1975; Daun and Hougen, 1976). Hydrogenation of a refined oil also reduced its sulfur content from 8 ppm to 1 ppm (Daun and Hougen, 1976).

The effects of seed conditions such as moisture content, heat treatment, and the quality of seed were also studied. There was a gradual increase in sulfur content of the oil with increasing moisture content of the seed (Daun and Hougen, 1976). However, the rate of increase was higher for high-glucosinolate seed than low-glucosinolate seed. Dry heating of high-glucosinolate seed resulted in increasing amounts of sulfur in the oil. However, with

low-glucosinolate seed, dry heating did not produce any sulfur in the oil. Prolonged heating of high-glucosinolate seed further increased the sulfur content of the oil which could be due to partial pyrolysis of glucosinolates yielding oil - soluble compounds. The sulfur levels of oils from green, frost - damaged, and bin - heated seed were higher than that of sound seed. The highest sulfur content was observed in oil extracted from 75% green seeds.

The sulfur content of laboratory extracted oil from dry ground seed ranged 2 to 5 ppm (Sosulski et al., 1972; Bhatty and Sosulski, 1972). Boiling of rapeseed before diffusion extraction of glucosinolates reduced the sulfur content to less than 10 ppm (Sosulski et al., 1972; Bhatty and Sosulski, 1972; Kozlowska et al., 1972a). In diffusion extraction of glucosinolates, alkaline solutions were more effective in reducing the sulfur content of oil than acidic solutions (Sosulski, et al., 1972). The amount of sulfur in the oil decreased with increasing temperature of the NaOH solutions (Kozlowska et al., 1974). The sulfur level increased during 1 h of extraction and then decreased with further extraction. With the alkaline solutions and boiling, sulfur level in oils ranged between 12 and 38 ppm (Bhatty and Sosulski, 1972; Kozlowska et al., 1972a). By using 50% ethanol in NaOH solution, no sulfur in the oil was detected by Kozlowska et al. (1972a) however, 2 to 5 ppm was obtained by Bhatty and Sosulski (1972).

4. Effect of Processing on Quality of Rapeseed Meal

Industrial processing conditions can cause positive or negative changes in the natural properties of the seeds (Rutkowski, 1970). During the processing of rapeseed, extensive denaturation of protein occurs. However, this is generally considered desirable for feeding purposes since it makes the protein more readily assimilable by the animals. On the other hand excessive heat can result in losses of certain amino acids e.g. lysine (Youngs et al., 1972).

Protein damage can occur in various processing steps: in the cooker and the expeller in the expeller process; in the cooker, expeller and desolventizer in prepress solvent extraction; and in the cooker and desolventizer in straight solvent extraction. The extent of the damage in these operations will depend on temperature, time, moisture content, mechanical factors (Rutkowski, 1970), reducing sugar content and possibly on the content of other constituents in the seed (Youngs et al. 1972). Thermal processes are the most important factors influencing the quality of the products, while mechanical factors, humidity and solvent are the less important factors (Rutkowski, 1970).

The protein damage due to heat was reported in the processing of many oil seeds, including cotton seed (Conkerton et al., 1957), sesame (Carter et al., 1961),

mustard seed (Mustakas et al., 1962; McGhee et al., 1964), sunflower (Renner et al., 1953a), peanut (Bensabat et al., 1958), soybean (Evans and Butts, 1948; Liener, 1958), and rapeseed (Clandinin et al., 1959; Clandinin and Tajcnar, 1961). These findings revealed that the amino acids affected include basic amino acids (lysine, arginine, and histidine) as well as cystine, serine and trytophan. Lysine is reported to be the most sensitive. The solubility and digestibility of rapeseed proteins are also affected by heat during the processing (Rutkowski, 1970).

The protein damage is generally proportional to the extent of heat i.e. time and temperature beyond a certain critical temperature (Bender, 1972). Extensive spontaneous bin-heating of clean rapeseed (B. campestris L.) caused substantial losses in tryptophan, lysine, histidine, arginine and threonine (Bell and Giovannetti, 1973). Heating the rapeseed (B. napus L. cultivar Bronowski) at 110, 115 and 120°C for 1 or 2 h resulted in a reduced content of available lysine which was correlated with a decrease in total lysine content (Josefsson, 1975a). Autoclaving rapeseed meal at 121°C for 2, 4 and 6 h reduced the liberation of lysine during hydrolysis by ca. 20, 50 and 60% respectively, while autoclaving at 4 and 6 h in addition destroyed arginine, phenylalaine and tyrosine (Sarwar et al.,

1975). The loss of arginine (45%) for 6 h autoclaving was almost double that observed for 4 h treatment. Autoclaving for 2, 4 and 6 h increased feacal excretion of lysine by 10, 50 and 70% respectively. Autoclaving for 2 h had little or no effect on the availability of N and amino acids except lysine which was slightly reduced. However, autoclaving 4 and 6 h reduced the availability of N and most of amino acids by ca. 20 and 50% and lysine by 50 and 70% respectively.

The moisture content of the protein containing material is a critical factor in determining the extent of damage during heating. Lea and Hannan (1949) established that protein damage is most extensive at 10-14% moisture. Carpenter et al. (1962) observed that the loss of available lysine is greatest at 4-14% moisture. However, the dry materials are relatively resistant to heat (Miller, 1956) and boiling in excess water usually gives no damage (Bender, 1972; Renner et al., 1953b; Taira et al., 1965). In case of rapeseed, the losses of amino acids due to extensive spontaneous bin heating was observed when the rapeseed was stored at 9 or 10% moisture content. In his experiment, Josefsson (1975a) observed that the damage of lysine occurred when low - glucosinolate rapeseed meal was heated at 12% moisture content.

Some few years ago, most of the rapeseed meal

was produced in Canada by the expeller method. Currently the meals are produced mainly by the prepress - solvent or straight solvent process (Youngs, 1965). The present meals are subjected to less heat during processing; as a result they are nutritionally superior (Clandinin, 1967). In the expeller processing, Clandinin and Tajcnar (1961) demonstrated that a decrease in the temperature of both the cooker and conditioner resulted in an increase in lysine content in the meal; the lysine content of the meal correlated directly with the final oil content of the meal (Clandinin et al., 1959; Clandinin and Tajcnar, 1961). A marked reduction in the lysine content was observed in the meal with less than 6% oil. When the meal contained 6% or more residual oil, the average lysine content was 4.8% of the protein. When the oil was removed by a petroleum ether solvent (Clandinin and Bayly, 1963) and the resulting meal had not been heated, the average lysine content of six varieties of rapeseed tested was 5.3% of the protein (Clandinin and Tajcnar, 1961).

The protein solubilities of expeller processed meal were lower than those of prepress-solvent and solvent processed rapeseed meals (Anwar and Clandinin, 1971; Donefer, 1974). Sosulski and Bakal (1969) and Girault (1973) also noted that the solubility of nitrogenous material in rapeseed is lower for the industrial processed meal than

laboratory defatted meal.

A cooperative study carried out by Giovannetti and Bell (1971) indicated that there is no undue destruction of lysine in the three processing methods which yield meals of similar lysine content corresponding to those in the raw seed.

Besides proteins, other constituents of the meal may undergo changes during the processing. It was observed by Zeman et al. (1964) that the mineral matter in the meal is unaffected by the production process. Similarly all vitamins, except for tocopherol undergo little changes in the process (Rutkowski, 1970). The contents of crude fiber, starch and pentosans in rapeseed meal remain unchanged while the monosaccharides content changes considerably due to the non-enzymatic browning reaction (Hrdlicka et al., 1964, 1965a). Greater losses were observed with the more drastic processing conditions. The darker color of the meal is also the result of the complete decomposition of pigments, such as carotenoids and chlorophylls at 115°C (Rutkowski, 1970). Glucose liberated from glucosinolates by enzymatic hydrolysis can react with amino acids producing dark compounds (Rutkowski and Kozlowska, 1969).

During the processing glucosinolate content may be altered in two ways; first by enzymatic hydrolysis and second by chemical modification on heating in the presence

of moisture and other constituents of the seed (Youngs, 1965). Reynolds and Youngs (1964) found that if no water was added during cooking, virtually all glucosinolates remained in the meal, whereas cooking with the addition of moisture resulted in a substantial drop of these compounds in the meal. Josefsson (1975a) observed that there was generally a reduced glucosinolate content in the meals when the seed had been heated for 2 h at 105, 110, 115 and 120°C, as well as when the seed had been treated for 60 min at 115 or 120°C.

5. Rapeseed Meal

a) Components

i) Protein

Protein is the most important constituent of the rapeseed meal; its amount and amino acid pattern determine the value of the meal. The protein content of rapeseed meal varies from 30 - 48% depending on such factors as variety (Clandinin and Bayly, 1963), environmental conditions (Wetter et al., 1970) and to a limited extent on processing technology (Rutkowski, 1970). Of the total nitrogen about 9% was reported to be nonprotein nitrogen (Quinn and Jones, 1976). Based on amino acid analysis, Sosulski and Sarwar (1973) suggested a factor of 5.50 instead of 6.25 for calculating the rapeseed protein content.

Varietal difference was observed by Clandinin et al. (1959) and Clandinin and Bayly (1963) who found that the protein content of B. napus L. is higher than that of B. campestris L.. Higher protein content in B. napus L. meals compared to B. campestris L. meals is due to the fact that the seeds of B. campestris L. are smaller than those of B. napus L. resulting in higher fiber and lower protein contents (Josefsson, 1972; Sarwar and Sosulski, 1973).

The protein content of rapeseed meal could be

increased through plant breeding. It was reported that there was a negative correlation between the percentage of oil and protein (Stolle, 1954; Sosulski, 1973). The introduction of yellow - seeded rapeseed with thinner seed coats, has resulted in an increase in oil as well as protein (Stringam and Harvey, 1973; Stringam et al., 1974). Numerous workers have reported the increase in protein content in rapeseed concentrates prepared by removing the hull and in rapeseed isolates prepared by dissolving and precipitating the proteins (e.g. Ohlson, 1973; Anjou and Fecske, 1974; Thompson et al., 1976).

The nitrogen content of rapeseed usually increased in response to the addition of fertilizers (Wetter et al., 1970; Josefsson, 1970; Josefsson and Appelqvist, 1968).

Numerous workers have reported the extraction, isolation and characterization of rapeseed protein. A number of solvents were being used by various workers for the extraction and isolation; these include, water, dilute NaOH and HCl, aqueous NaCl and aqueous ethanol. In their work Shaikh et al. (1968) extracted defatted rapeseed meal with aqueous NaOH at pH 8 to 11 and precipitated the protein at pH from 4 to 6 and observed that the extraction at pH 10 and the precipitation at pH 4 gave the most satisfactory result.

Studies on the isolation of proteins from rapeseed, soybean, flax and sunflower successively by water and aqueous NaCl, ethanol and NaOH revealed that the proteins in rape and turnip rape were less water-soluble than that of soybean but correspondingly more soluble in salt solutions (Sosulski and Bakal, 1969). Gheyasuddin and Ali (1975) observed that water soluble and salt soluble proteins represented ca. 32 and 44% respectively of the total proteins.

In 1971 Korolczuk and Rutkowski obtained the highest extractability of nitrogenous compound (more than 80% of total N) at pH 9.5-10.0 and 30-45°C and the lowest extractability (20-25% of total N) at pH 6.5-8.0 and 80-100°C. Using NaOH solution, Girault (1973) was able to extract 89% of the total rapeseed flour N. With aqueous NaCl, 67-70% (Finlayson et al., 1969), less than 44% (Owen et al., 1971), 75% (Lo and Hill, 1971), and 70% (Girault, 1973) of the total N of the rapeseed meal were extracted. Kodagoda et al. (1973a) extracted proteins from rapeseed meal sequentially by water, HCl solution at pH 2 and NaOH solution at pH 10 and also by direct 0.01N NaOH solution. After the maximum precipitation, they obtained 11, 13 and 42; and 61% protein respectively.

During isolation of rapeseed protein using sodium hexametaphosphate Thompson et al. (1976) obtained highest yield of nitrogen (97%) when rapeseed flour was double

extracted with 2% sodium hexametaphosphate at pH 7.0 first with a rapeseed flour - solvent ratio 1:10 and second with a ratio of 1:6 at 25°C for 30 min. Chamnanwej (1971) observed that NaOH solution was the most effective solvent for the extraction of rapeseed protein but it appears to cause an alteration of the proteins. Two salt solutions (NaCl and sodium pyrophosphate) yielded a comparable amount of protein, however, they have different efficiency in their extracting power on the various classes of proteins.

The studies on characteristics of rapeseed meal protein showed two major protein fractions from salt extraction, a neutral protein (12S protein) and a basic protein (1.7S protein) (Bhatty et al., 1968; Finlayson et al., 1969). The 12S protein acts as a single molecule at pH values of 7.5 to 9.0 but dissociates into 7S components at pH 3.6 and 3S components at pH 2.2 (Finlayson et al., 1969).

Rapeseed was reported to contain over 30 protein species and the majority of these proteins have isoelectric points in the neutral pH region (Quinn and Jones, 1976). In another study Janson et al. (1971) observed that the rapeseed proteins were composed of ca. 20 weakly acidic proteins, ca. 20 neutral proteins and 4-6 basic ones. The water extract contains mainly acidic and neutral proteins whereas the HCl solution (pH 2) extract is made up of mainly basic proteins (Kodagoda et al., 1973a). The basic proteins

account for ca. 20% of the total soluble protein and have molecular weights in the range of 15,000-20,000 (Janson et al., 1971) and ca. 5% of the soluble protein has M.W. of 50,000-75,000 and the bulk of the protein has M.W. from ca. 120,000 to 150,000 (Janson, 1971; Janson et al., 1971). Different molecular weights of protein species were reported by other workers (Chamnanwej, 1971; Kodagoda et al., 1973a; Ohlson and Tear, 1974; Quinn and [redacted] 1976).

Realizing the importance of ami[redacted]or human nutrition, extensive studies on the a[redacted]composition of rapeseed protein have been carried out by many workers. Several workers reported differing amino acid compositions of rapeseed protein. Rapeseed meal has been reported to be nutritionally inferior due to its low lysine content (e.g. K[redacted]tzer et al., 1954; Klain et al., 1956). However, it was established that high temperature of processing was responsible for the low lysine content in the meal (Clandinin et al., 1959; Clandinin and Tajcnar, 1961; Clandinin, 1967).

The result obtained by Tape et al. (1970) indicated that essential amino acids of rapeseed meal and flour, with the exception of arginine, methionine and cystine, were uniformly between 40 to 60% of that in casein, suggesting a balanced characteristic of the amino acid pattern. Rapeseed meal protein was reported to be deficient in valine

and isoleucine (Tape et al., 1970), in methionine (Ballester et al., 1970a), in lysine and methionine (Hrdlicka et al., 1965b), in valine, isoleucine and histidine (Gheyasuddin and Ali, 1975), and in methionine and cysteine (Thompson et al., 1976).

In 1972, Clandinin et al.(a) mentioned that rapeseed meal contained ca. 92% of lysine found in soybean meal. Work by Ballester et al. (1970a) and Ohlson (1972 and 1973) showed that rapeseed protein contained a high amount of lysine, comparable to that of soybean. Ohlson (1972 and 1973) also reported that rapeseed protein contained a considerably higher level of methionine than soybean protein. In their study, Sarwar et al. (1975) observed that compared to the soybean meal, the rapeseed meal contained higher amounts of histidine, threonine, valine, methionine, cystine, glycine and proline but lower amount of arginine, isoleucine, leucine, phenylalanine, aspartic acid and glutamic acid.

Work by Sosulski and Sarwar (1973) showed that soybean and rapeseed proteins contained high proportion of essential amino acids as compared with flax, sunflower and safflower proteins. Rapeseed proteins also contained high level of methionine + cystine and proline while soybean proteins had a higher level of phenylalanine. High ratings were given to soybean and rapeseed meal and isolates

however, soybean was deficient in S-containing amino acids while the rapeseed meals were low in isoleucine. Since isoleucine deficiency is uncommon in normal diet, they concluded that the rapeseed products appeared to have the best protein for the supplementation of human diet.

ii) Crude Fiber

The crude fiber of a meal is mainly composed of lignin and insoluble carbohydrates, cellulose and hemicellulose. In all cases, the contents of crude fiber of rapeseed meal are reported to be high; e.g. 12.6 - 16.7% (Sosulski and Bakal, 1969) and 13.2 - 14.7% (Ballester et al., 1970a). The crude fiber content of rapeseed meal is considerably higher than soybean (e.g. Ohlson, 1972) and peanut meals (Rosen, 1958) but is similar to that of sunflower (Klain et al., 1956; Morrison et al., 1953) and cottonseed meals (Altschul et al., 1958). The high crude fiber content in rapeseed meal is due to the small size of the seed and large proportion of seed coat, rich in fiber (Wetter, 1965; Ohlson, 1972).

The utilization of commercial rapeseed meal would be enhanced if the crude fiber content could be reduced. This is done by dehulling the seed and by plant breeding. The hull can be removed by dry dehulling before oil extraction (Ohlson, 1973), wet dehulling after diffusion

extraction (Sosulski, 1974) and air-classification of the meal (Tape et al., 1970). Significant reduction of crude fiber could be achieved through the introduction of yellow-seeded cultivars (Stringam and Harvey, 1973; Stringam et al., 1974).

iii) Lipid

The amount of residual oil in rapeseed meals depends on the method of oil extraction. The meals from prepress-solvent and straight solvent extraction may contain 1 to 4% of oil (e.g. Clandinin et al., 1959; Bayley, 1974). With expeller oil extraction, 6 to 7% of oil is left in the meals. The amount of ether extract of rapeseed meal tends to be higher than that of soybean meal due to the add-back of gums to the rapeseed meal (Clandinin et al., 1972b; Anjou, 1972).

The fatty acid composition of residual oil shows similar pattern to rapeseed oil provided that the meal contains more than 1% oil (Lehman et al., 1964). The fatty acid of the residual oil contains less erucic, eicosenoic and linolenic acids and more of the other acids than are found in typical rapeseed oil. Presumably due to its low content, the residual oil does not play any essential role as an energetic component of the meal (Rutkowski, 1971). However, its presence increases the digestible energy,

metabolizable energy and total digestible nutrient values of the meal (Clandinin et al., 1972).

iv) Carbohydrates

In their study Matet et al. (1949) observed that the carbohydrate content of European rapeseed meal was similar to that of linseed meal but was slightly higher than that of sunflower meal, and it varied from 20 to 25%. Different values were reported by other workers; ca. 38% (Hrdlicka et al. 1964) and less than 20% (Theander and Aman, 1974). This carbohydrate consisted of mainly polysaccharides, with small amount of mono-, di- and tri-saccharides. Different workers reported different constituents and amounts of oligosaccharide fractions of the carbohydrates. The constituents reported were; sucrose, stachyose, raffinose, glucose, fructose, galactinol, digalactosyl glycerol, myo-inositol and traces of arabinose, galactose, ribose, xylose, rhamnose, galacturonic acid and digalactosyl inositol (Mizuno, 1958; Hrdlicka et al., 1964; Siddiqui et al., 1973; Theander and Aman, 1974). The predominant components were reported to be sucrose and fructose by Mizune (1958), sucrose and glucose by Hrdlicka et al. (1964), sucrose and stachyose by Siddiqui et al. (1973) and Theander and Aman (1974).

v) Ash, Minerals and Vitamins

The ash content of the rapeseed meals was reported to range from: 6 to 7% for the meals from Western Canada (Klain et al., 1956), 5.8 to 6.6% for the meals from Chile (Ballester et al., 1970a), 7 to 8% for the meals from Sweden (Ohlson, 1972), and 7.0 to 7.3% for meals from Poland (Rutkowski, 1971). The variation among samples appears to be rather small (Ohlson, 1972).

The mineral content of rapeseed meal is relatively higher than that of peanut but is comparable with cottonseed and soybean meals. The analysis of the ash shows that there is considerable variation in the content of Ca, P, Cu, Fe, Mg, Mn, Zn, and Se among the rapeseed meals (Bragg, 1974). Published data indicate that rapeseed meal is comparatively richer in Ca, P, Mn, Se, Zn and Mg than other oil meals (Bell et al., 1967; Ballester et al., 1970a; Clandinin et al., 1972). Recently, Bragg (1974) showed that with the exception of Cu, the contents of other minerals were higher in rapeseed meal than corresponding values normally cited for soybean meal and the author concluded that the rapeseed meal appeared to be a better source of Ca, P, Zn, Fe, Mg, Mn, and Se. Unpublished data by Motzok showed that 70% of phosphorus in rapeseed meal was in the form of inorganic compounds as compared to only

32% in soybean meal. Since phosphorus is an expensive ingredient in feed rations, the rapeseed meal is considered to have this advantage over soybean meal (Clandinin et al., 1972).

Like other oil seed meals, rapeseed meal contains a high proportion of B-group vitamins. There are no significant differences in vitamin contents between rape and turnip rape varieties (Klain et al., 1956). In comparison with other oil seed meals, rapeseed meal contains higher amounts of choline and niacin, similar in riboflavin and lower in thiamine and pantothenic acid. The tocopherol content of rapeseed meal is about 45mg/kg including 12mg of the alpha form (Ames, 1956; Hjarde et al., 1962).

vi) Other Constituents

Other minor but also important constituents of rapeseed meal are tannin and phytic acid. Both the prepress - solvent and solvent processed rapeseed meals contained 2.6% (Fenvick and Hoggan, 1976) to 3.0% tannin (Clandinin and Heard, 1968). The feeding of tannic acid causes a reduction in growth (Vohra et al., 1966; Glick and Joslyn, 1970a; Peaslee and Eintellig, 1973) and an increase in the level of excreted nitrogen (Vohra et al., 1966; Glick and Joslyn, 1970b) in rats, mice and chickens.

It also resulted in the formation of yellow-green mottling egg yolks (Potter et al., 1967; Fry et al., 1972) and in lowering the metabolizable energy content of the diets (Vohra et al., 1966; Yapar and Clandinin, 1972). However, there would be little metabolic disorders affecting the chicken if the rapeseed meal is kept below the 10% level (Fenwick and Hoggan, 1976).

The rapeseed protein concentrate contained ca. 5% phytic acid (Hermansson et al., (1974). Inspite of the phytic acid content, there was no reduction in the intestinal absorption of Ca, Zn, and Fe in rats fed on a diet containing rapeseed protein concentrate (Eklund and Agren, 1974).

b) Biological Value

The nutritive value of rapeseed meal is dependent on protein content, amino acid composition and contents of crude fiber, minerals and vitamins, and the presence of toxic substances (Josefsson, 1972). The biological value of rapeseed meal was inferior to that of soybean meal and casein due to the goitrogenic factor, high fiber content, and unfavorable amino acid balance (Drouliscos and Bowland, 1969). The voluntary intakes of diets containing rapeseed meal were depressed by the presence of glucosinolates especially at high levels (Bell et al., 1971; Sarwar et al., 1973; McDonald, 1974; Josefsson, 1975b). This palatability problem is due to isothiocyanates, which have pungent taste and odour and, in high concentrations, can damage skin, the mucous membrane and the surface of the alimentary canal (Josefsson, 1972).

A number of workers reported that the digestibility of rapeseed meal and protein was less favorable than soybean and sunflower meals, casein, heat-damaged casein, soybean protein isolate, and zein (e.g. Rutkowski, 1971; Bayley, 1973; McDonald and Gregoire, 1973). In a more detailed study, Sarwar et al. (1973) reported that the apparent digestibility of rapeseed meal was lower than casein, soybean meal, and turnip rapeseed meals; however, it was

comparable to sunflower, and was higher than flax and safflower meals. In the case of rapeseed protein isolate, the apparent digestibility was similar to that of soybean meal, but lower than flax, sunflower, safflower and turnip rapeseed meals, and casein. The digestibility of the meal increased when its glucosinolate content was low (Ballester et al., 1970b; Bell, 1975).

The apparent digestibility coefficient of the individual amino acids was low for rapeseed protein (McDonald and Gregoire, 1973; Bayley, 1973; Sarwar et al., 1975). However, there were two notable exceptions, with lysine in rapeseed meal being comparable to that of soybean meal, while methionine was superior to that in the soybean meal.

The protein efficiency ratio (PER) values of rapeseed meal and turnip rapeseed meal were similar to those of sunflower, safflower and flax meal, but lower than soybean meal and casein (Sarwar et al., 1973). The PER value of rapeseed meal increased substantially after heat inactivation of myrosinase (Josefsson, 1975b) and removal of glucosinolates (e.g. Bock, 1967; Tape et al., 1970; Ballester et al., 1973; Eklund and Agren, 1974). The PER values of rapeseed flour (Tape et al., 1970) rapeseed protein concentrates (Gorrell et al., 1974; Ohlson, 1973) or rapeseed protein isolates (Sarwar et al., 1973; Thompson

et al., 1976) were higher than that of casein. Furthermore, the rapeseed isolate prepared by extraction with sodium hexametaphosphate and precipitation at pH 2.5 was reported to be equal in PER to cheese whey protein concentrate (Thompson et al., 1976). Ohlson and Tear (1974) stated that the nutritive value of rapeseed protein concentrate was higher than that of any other vegetable protein known.

c) Functional Properties and Uses in Foods

Research in the area of the functional properties of rapeseed protein is a relatively recent development. In order for it to be an acceptable food ingredient, rapeseed protein must have good functional properties. The water absorption capacity of rapeseed protein concentrate was 500 - 700%, compared to 400% for texturized soybean flour, and its water binding capacity was 300 - 400%, compared to 200% for texturized soybean flour (Ohlson, 1973).

The emulsifying capacity and emulsion stability of rapeseed protein concentrate were comparable to those of soybean products (Ohlson, 1973). When protein concentrates and isolates were prepared by a sequential extraction with water and acidic and alkaline solutions, it was observed that the water extracted isolate had a higher emulsifying capacity than the other isolates (Kodagoda et al., 1973b).

The emulsifying capacity of concentrates was lower than that of corresponding isolates. The emulsions containing isolate and concentrate extracted by water were the most stable. Foaming properties of rapeseed protein concentrate were also comparable to those of soybean products (Ohlson, 1973). The specific volume of whipped egg-albumin increased when acidic extracted isolate was added, but decreased upon addition of other isolates (Kodagoda et al. 1973b), however, all concentrates decreased the specific volumes. The water extracted isolate showed the greatest foaming stability. All modified products obtained from solubilization of rapeseed protein concentrate with alkali, acid, or the enzymes, pepsin and papain had higher solubility, emulsifying, and foaming properties, and three of seven modified preparations had a better swelling ability than did the original rapeseed protein concentrate (Hermansson et al., 1974).

During baking, replacement of 5% of the wheat flour with rapeseed protein isolates or concentrates decreased the loaf volume by 10 - 15%, and 20%, respectively. However, the addition of 0.5% emulsifier restored the loaf volume in most breads (Kodagoda et al., 1973b). It has been shown that rapeseed protein concentrate can be used in different foods, e.g., meat balls and hamburgers, at a 2-3 times higher concentration than soybean protein

without any detectable off-flavor development (Ohlson, 1973; Ohlson and Tear, 1974). Rapeseed flour has been incorporated into meat and bakery products where it was found to be organoleptically acceptable (Sims, 1971). The weight-gain of calves fed with rapeseed concentrate as a substitute for cow's milk was the same as that of calves given fish protein concentrate, and was even greater than that of those given skimmed milk (Ohlson and Tear, 1974). There was no off-flavor or odor development, or deterioration in protein quality when rapeseed protein concentrate was stored in a moisture-vapor-proof packaging material for six months at 40°C (Ohlson, 1973).

Work on spinnability of alkaline solutions of rapeseed globulin and casein, and of rapeseed albumin starch sulfate and casein has shown that the spinnability depends on such factors as protein concentration, NaOH concentration of the spinning solution, and the HCl content and temperature coagulating bath (Schmandke et al., 1976).

d) Toxicity of Rapeseed Meal

The main factor which limits the use of rapeseed meal and protein for human consumption and animal feed is the presence of glucosinolates. Numerous reports have demonstrated that feeding rapeseed meals caused a

depression in growth and in many cases enlargements of the thyroid in poultry (e.g. Clandinin et al., 1959; Jackson, 1969; Clandinin and Bayly, 1960) rats (e.g. Bell, 1957a; Bell and Baker, 1957; Belzile et al., 1963; Manns et al., 1963), and swine (e.g. Manns and Bowland, 1963a,b; Manns et al., 1963; Bayley et al., 1969; Bell, 1965). Feeding experiments have also shown that the goitrogenic substances in rapeseed meal have negative effects on the egg production of mature hens (e.g. Clandinin et al., 1959; Clandinin and Bayly, 1960; Clandinin and Tajcnar, 1961; Marangos et al., 1974; Olmu et al., 1975), as well as on the mortality of hens (e.g. Marangos et al., 1974; Olmu et al., 1975), and on the rate of maturation, reproductivity and lactation of swine (e.g. Robblee, 1965; Rutkowski, 1971). Glucosinolates also affect the adrenal cortex, pituitary kidneys and liver, especially in non-ruminant animals (Van Etten, 1969).

Ruminants have the highest resistance to the glucosinolates of rapeseed meal and generally show no marked goitrogenic effect and no adverse effects on the rates of weight gain or reproduction (e.g. Bunger et al., 1964; Virtanen et al., 1963; Bell and Devlin, 1972). Robblee (1965) indicated that both the yield and flavor of milk are unaffected by the inclusion of solvent-extracted rapeseed meal in the ration. However, in another experiment,

Ingalls et al. (1970) observed that high levels of rapeseed meal in grain rations resulted in somewhat lower milk yield and fat content. Feeding of sheep with a ration containing rapeseed meal had no detrimental effect on the wool (Rutkowski, 1971). It was suggested that glucosinolates are effectively destroyed or altered during ruminant digestion (Virtanen et al., 1963; Bell and Devlin, 1972). Palatability of rapeseed meal has been indicated as a factor limiting the amount of rapeseed meal used in rations for ruminants (Bell and Devlin, 1972; Ingalls and Walden, 1972).

Despite the unfavorable effects of glucosinolates, rapeseed meal has been recommended for use in feeding rations of poultry, swine and ruminants (e.g. Robblee, 1965; Clandinin et al., 1972a; Bowland and Bell, 1972; Bell and Devlin, 1972; Meyer, 1975). Depending on the type, stage or condition of the animals, 3 to 20% of rapeseed meal were used in the feeding rations.

e) Glucosinolates

The chemical nature of glucosinolates has long been the subject of the investigations of many workers, but significant progress only began subsequent to the studies by Kjaer et al. in 1953. Using paper chromatography, Kjaer and

Jensen (1956) detected six glucosinolates in B. napus L.: progoitrin (major), gluconapin (major), glucobrassiconapin (major), sinalbin (minor), and both tentatively identified glucocoiberin (minor), and gluconasturitium (minor). These constituents, when hydrolysed by myrosinase (thioglucosidase), gave rise, respectively, to (L)-5-2-vinyl-oxazolidinethione (non volatile), 3-butenyl isothiocyanate (volatile), 4-pentenyl isothiocyanate (volatile), p-OH-benzyl isothiocyanate (volatile), 3-methyl sulfinyl propyl isothiocyanate (non volatile), and 2-phenyl ethyl isothiocyanate (volatile).

The isothiocyanates have a considerably lower goitrogenic activity than oxazolidinethione (Fertman and Curtis, 1951; Gmelin and Virtanen, 1960), and they can be transformed into thiocyanates (Ettlinger and Lunden, 1957), which are also characterized by weaker goitrogenic activity (Fertman and Curtis, 1951; Gmelin and Virtanen, 1960). Other researchers (Bell, 1957a; Bell and Baker, 1957; Belzile et al., 1963) reported that both isothiocyanates and oxazolidinethione have roughly equal effects on growth rate, however, males were affected more severely than females. Bell and Belzile (1965) suggested that the only isothiocyanates which have significant antithyroid activity are those capable of cyclizing to form oxazolidinethione.

The action of isothiocyanates and thiocyanates is similar. They prevent the uptake of iodine by the thyroid gland and release its accumulated iodine. In this manner they block the first phase of the biosyntheses of thyroid hormones, however, they do not affect the thyroid gland mechanisms. The goitrogenic effect of these compounds can be overcome by the addition of iodine to the ration (Rutkowski, 1971).

Oxazolidinethione and nitriles are formed from glucosinolates, progoitrin or epi-progoitrin, which, on hydrolysis by myrosinase (thioglucosidase) gives rise to 2-hydroxy-3-butenyl-isothiocyanate. This in turn cyclized to L-5-vinyl-2-oxazolidinethione, a compound of high antithyroidal activity (Kjaer et al., 1959; Astwood et al., 1949). Recently, Van Etten et al. (1966, 1969a) showed that, when rapeseed meals were allowed to autolyze, the hydrolysis products included nitriles. Earlier studies have shown that nitriles have toxic effect on mammals (Virtanen, 1965; Tookey et al., 1965). This toxic effect was greater than that of the oxazolidinethione (Van Etten et al., 1969b).

The mechanism of goitrin influence on the thyroid gland is not fully understood, however, it is known that it blocks the irreversible mechanism connected with the binding of iodine in the thyroid, and, thus,

induces a partial suppression of tiroksine synthesis (Rutkowski, 1971). The decrease of tiroksine in blood, which results from this partial suppression, stimulates the hypophysis to secrete excessive amount of thyrotropine which affects the thyroid gland by stimulating its growth (Clandinin and Tajcnar, 1961). The goiter caused by these compounds cannot be alleviated by increasing the iodine intake, so damage can occur when rapeseed meal is used in feeding of non-ruminants (Rutkowski, 1971).

Glucosinolates are diffusely distributed in parenchymal tissues of the seed (Guignard, 1890). Myrosinase, or thioglucosidase glycohydrase (EC 3. 2. 3. 1), is located in special cells called idioblasts (Guignard, 1890; Kjaer, 1960). The destruction of the cellular structure allows the glucosinolates to come into contact with myrosinase. With sufficient moisture, this enzyme hydrolyzes the glucosinolates, liberating glucose, bisulphate and biologically active compounds ('mustard oil') which are divided into three groups: goitrin and related nitriles; organic isothiocyanates; inorganic thiocyanate (Rutkowski, 1971; Josefsson, 1972).

After the establishment of the general structure of the glucosinolates, and particularly sinigrin, Ettlinger and Lunden (1957) revised the proposed mechanism of the hydrolysis of glucosinolates (Fig. 1). As found in

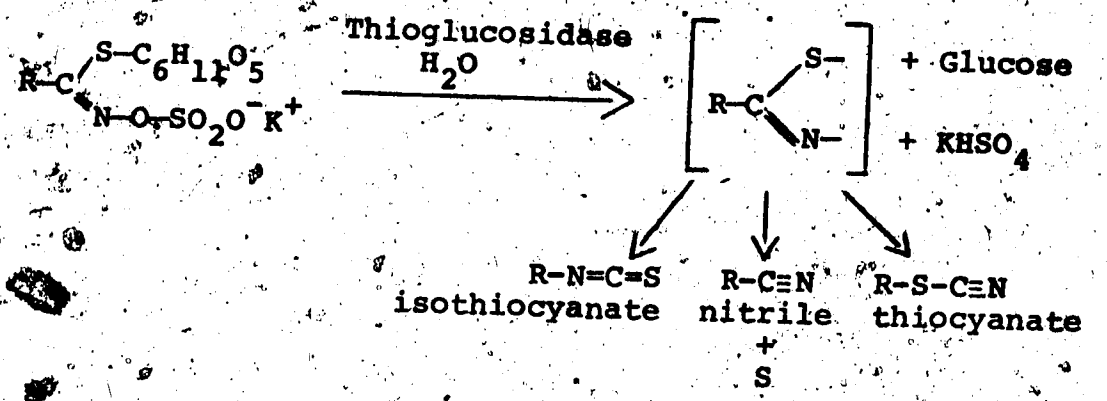

Fig. 1. Reaction Mechanism of Myrosinase on Glucosinolates (Ettlinger and Lundeen, 1957).

crushed mustard seed, moisture, temperature and time have a marked effect on the enzymatic hydrolysis of glucosinolates. Hydrolysis proceeds rapidly above a moisture content of 13%, and at temperatures from 40 - 72°C. At a moisture level of 15.5% and temperature of 55°C, hydrolysis was over 90% complete within 1 min and 99% complete after 15 min (Mustakas et al., 1962).

The content of glucosinolates in rapeseed meal primarily varies with variety, and environmental and processing conditions. The difference in glucosinolate content with variety has been reported by many workers (e.g. Wetter and Craig, 1959; Appelqvist, 1962; Youngs et al., 1972). In a more extensive study on the glucosinolate content of rapeseed meals derived from some varieties of rape and turnip rape grown at various localities in Europe, Appleqvist and Josefsson (1967) and Josefsson and Appleqvist (1968) obtained the results shown in Table 5. Considerable differences were observed in the amount of glucosinolate between species, and between winter and summer types of the same species; with summer types containing less glucosinolates.

The isothiocyanate content was similar for both B. napus L. and B. campestris L., but the oxazolidinethione content was higher in B. napus L. (Wetter and Craig, 1959; Clandinin et al., 1959; Josefsson and Appelqvist, 1968;

Table 5. The Glucosinolate Content in Seed Meals of Rape and Turnip Rape Grown in Various Localities in Europe*

Species	Glucosinolate Content (% of dry matter)		
	gluconapin (isothiocyanate)	progoitrin (oxazolidinethione)	gluconapin + progoitrin
B. napus L.			
Winter type	0.83 - 2.30	2.4[illegible] - 5.[illegible]	3.39 - 7.77
Summer type	0.05 - 1.80	0.12 - 4.26	0.18 - 5.75
B. campestris L.			
Winter type	2.84 - 3.84	0.1[illegible] - 0.65	3.09 - 4.30
Summer type	1.[illegible]1 - 2.47	1.6[illegible] - 1.30	3.01 - 3.59

* From Appelqvist and Josefsson (1967) and Josefsson and Appelqvist (1968).

Youngs et al., 1972). Within the B. napus L. species, plants of cultivar Bronowski contained the lowest amounts of glucosinolates, (0.18 - 0.64% of dry matter) (Josefsson and Appelqvist, 1968; Downey et al., 1969; Josefsson and Munck, 1973). However, this variety is not suitable for commercial production in Canada due to its long growing season and its lower yields of seed and oil (Finlayson et al., 1973). A new variety of B. napus L., called "Tower", which contains ca. 0.15% glucosinolates, is satisfactory for the production in some areas of the prairies which have a long growing season (Radwan and Lu, 1976; Clandinin and Robblee, 1976). Selection within varieties Echo and Arlo of B. campestris L. resulted in isolation of plants with seeds free of OH-pentenyl isothiocyanate, or oxazolidinethione, or both (Downey et al., 1969).

The environmental conditions under which the plants are grown can influence the glucosinolate contents of the seed. The addition of sulfur fertilizers caused an increase in the amounts of glucosinolates in the seed (Downey and Wetter, 1964; Josefsson and Appelqvist, 1968). However, the addition of large amounts of nitrogenous fertilizers caused a decrease in the glucosinolate content.

f) Detoxification of Rapeseed Meal

After the extraction of rapeseed oil, the bulk of the glucosinolates remain in the meal (Youngs, 1965). Though glucosinolates are harmless (Belzile et al., 1963), they can be hydrolized to toxic products in the digestive tract by myrosinase or other enzymes which are in the food or are produced by intestinal bacteria, especially E. coli and A. aerogenes (Greer, 1962; Bell and Belzile, 1965) or Paracolobactrum Spp. (Oginsky et al., 1965). Thus, it is important that these glucosinolates and their hydrolytic products be removed. Studies on the removal of glucosinolates were carried out on both the rapeseed as well as the crambe seed (e.g. [illegible]as et al., 1976; Sosulski, et al., 1972).

i) Heat Treatments and Reactions with Salts and Other Compounds

Different methods have been tried with varying degree of success, to remove the glucosinolates from the meal. These include: dry heating or toasting, autolysis and distillation, autoclaving and steam stripping, and treatments with chemical compounds plus heating. Glucosinolates and their hydrolytic products are removed during heat treatments due both to their volatility and to their thermal destruction. Dry heating or toasting removed most

of glucosinolates (Rutkowski et al., 1966; Rutkowski and Kozlowska, 1969), but reduced the protein solubility (Rutkowski and Kozlowska, 1969), and available lysine (Josefsson, 1974).

Autolysis and distillation improved the quality of rapeseed meal, especially of B. campestris L. (Goering et al., 1960). Different amounts of glucosinolates were reported to have been removed by autoclaving and steam-stripping (Belzile and Bell, 1963; Belzile et al., 1963; Ballester et al., 1970b; Bock et al., 1974). This procedure caused a gradual deterioration of protein quality with the time of treatment. In addition, the nitrile contents of crambe seed meal was increased (Mustakas et al., 1976).

Treatment of crambe seed meal with ammonia and heat, or sodium carbonate under pressure effectively destroyed glucosinolates, however, the resulting meal caused palatability problems (Kirk et al., 1965; Mustakas et al., 1968). Certain metallic salts, especially ferrous sulfate, when added to rapeseed and crambe seed meals, caused decomposition of glucosinolates to nitriles, which could then be removed by steam stripping (Youngs, 1967; Sallans et al., 1967; Youngs and Perlin, 1967; Austin et al., 1968). However, a decomposition product of oxazolidine-thione, a hydroxynitrile (1-cyano-2-hydroxy-3-butene), which

is toxic to animals, was only slightly steam volatile and it remained in the meals.

Glucosinolates in rapeseed flour can be eliminated by heating in the presence of sodium metabisulfite as a catalyst (Janzani et al., 1974). Treatment with H_2O_2 lowered the glucosinolate content of the flour but was not as effective as water extraction (Anderson et al., 1975). Also, the H_2O_2 treatment caused the oxidation of methionine to its sulfoxide and sulfone, and cysteine to cysteic acid.

ii) Extraction of glucosinolates

Toxic constituents can be extracted either from ground or intact rapeseed before oil extraction, or from the meals. The extraction procedure is based on the principle that glucosinolates and their hydrolytic products, isothiocyanates and oxazolidinethione, are soluble in polar solvents (Tookey et al., 1965; Sosulski et al., 1972). However, glucosinolates are more soluble in water than isothiocyanates or oxazolidinethione (Belzile and Bell, 1966).

Various extracting solvents have been used, such as isopropanol, ethanol, or acetone, or their aqueous mixtures, ether, buffer solutions, or water. Using organic

[illegible] and their aqueous mixtures, different amounts of glucosinolates were extracted by various workers (Van Etten et al., 1965; Bell, 1957b; Tookey et al., 1965; Afzalpurkar et al., 1974; Mukherjee, 1975). Aqueous acetone was the most effective for removal of glucosinolates (Afzalpurkar et al., 1974). Increasing the water content enhanced the extraction of glucosinolates. When buffer solutions were used most of the glucosinolates were extracted from enzyme-deactivated meal, however, there was considerable lysine destruction (Szewczuk et al., 1969).

The extraction of glucosinolates from the meal by water also occurred with varying degrees of success (Bell, 1957b; Grussendorf, 1953; Allen and Dow, 1953; Belzile et al., 1963; Ballester et al., 1970b and [illegible] Rozlowska et al., 1972a; Eklund and Agren, 1974; Mustakas et al., 1976). The most recent work showed that practically all glucosinolates could be removed.

Extraction of the glucosinolates from the meal results in high losses of dry matter, soluble carbohydrates and meal nitrogen. The loss of dry matter can be reduced by adjusting the pH to 3.5-4.5 or 8-9 during aqueous methanol or aqueous acetone extraction (Van Etten et al., 1965), or by lowering the moisture content of acetone (Tookey et al., 1974). Other disadvantages of meal extraction methods are the long extraction period (15 h)

(Ballester et al., 1970b; Kozlowska et al., 1972) slow drying of the meal slurry, and the dark appearance of the product (Kozlowska et al., 1972a). However, the time of extraction could be shortened to 2 h for aqueous NaCl extraction (Owen et al., 1971) and continuous water extraction (Ballester et al., 1973).

Rapeseed protein isolates, which were prepared by extracting the proteins from the meals with aqueous NaOH or salt solutions and precipitating them at isoelectric points, contained at most traces of glucosinolates (e.g. Shaikh et al., 1968; Sarwar et al., 1973; Thompson et al., 1976).

Glucosinolates can be extracted from ground or intact rapeseed before the extraction of oil. Extractions by water of glucosinolates from enzyme-deactivated ground rapeseed were carried out by Eapen et al., (1969), Tape et al. (1970), Kozlowska et al. (1972a), Ohlson (1973), Anjou and Fecske (1974) and Mukherjee (1975). Practically all the glucosinolates could be removed from the ground rapeseed. In general, the extraction of glucosinolates from ground rapeseed also resulted in high losses of dry matter, carbohydrates, nitrogen and oil. In addition, drying of the wet slurry and recovery of extracted solids would increase the costs of the process.

Work on extraction of glucosinolates from intact rapeseed was carried out to overcome the high loss of material and the difficulty of drying the wet slurry (Sosulski et al., Kozlowska et al., 1972a and 1974; Sosulski, 1974). Boiling the seed before diffusion extraction, or the use of high temperatures or highly alkaline solvents were effective in controlling the myrosinase activity and sulfur levels in oils. The extraction of glucosinolates was enhanced by frequent use of fresh solvent, high temperature, high water-to-seed ratio, a neutral to alkaline aqueous medium, and by dehulling of the seed. Practically all glucosinolates could be extracted from the seed using this method. The disadvantages of the diffusion extraction method were the long extraction times and the large volume of water utilized for the process. The use of ethanol in the solvent, in addition to increasing cost, reduced the protein solubility.

iii) Biological Detoxification and Removal of Glucosinolates by Plant Breeding.

Biological methods of detoxification of rapeseed meal by Geotrichum candidum (Staron, 1970 and 1974) and lactic acid bacteria (Kozlowska et al., 1972b) have been reported. Glucosinolate contents were effectively reduced during fermentation. The removal of glucosinolates by plant breeding was previously mentioned in Section B5e.

C. Statement of Objectives and Definition of Terms

From the literature survey, it is apparent that the present conventional methods of rapeseed oil extraction have a number of disadvantages, such as the danger of explosion and fire hazard, loss of solvent, and causing damage to the quality of rapeseed protein which is one of the best vegetable proteins. Further, there is no provision in the processing steps for the removal of glucosinolates and their hydrolytic products, isothiocyanates and oxazolidinethione. In addition, the rapeseed oil extracted by these methods contained a high level of sulfur which can poison the nickel catalyst during the hydrogenation process, causing an increase in the cost of this process. Most of these problems could be solved if the rapeseed oil is extracted by aqueous method.

The general objective of this study is to examine the feasibility of aqueous extraction of rapeseed oil under laboratory conditions. In particular the objectives of this study are

1) to establish the steps and the parameters of the process for the optimum oil yield
2) to investigate the quality of the oil extracted by the aqueous process
3) to investigate the quality of the meal, especially the protein. The solubility and the amino acid

composition of the protein are to be studied. It is also important to know the amounts of glucosinolates extracted simultaneously during the process

4) to minimize the problems of whey (aqueous fraction) which are associated with this process.

A number of terms will be used in the text. In order to avoid confusion and to have a better understanding of these terms, it is necessary to define them here.

Industrial crude oil is the crude oil obtained from Canbra Food Co. Lethbridge, Alberta. The oil was extracted by prepress-solvent method from B. campestris L. var. Span.

Aqueous extracted crude oil refers to the oil which was extracted at optimum conditions by the aqueous extraction process established in this study.

Soxhlet extracted crude oil is the oil obtained by soxhlet extraction method using petroleum ether (B.p. 30-60°C).

Residual oil refers to the oil which was left in the meal and extracted by the soxhlet method.

Oil fraction is the oil obtained after the first centrifugation step of aqueous oil extraction. This oil is comprised of oil in the form of an emulsion and free oil.

Free oil refers to the oil obtained after the first centrifugation step of aqueous oil extraction. This oil is not in the form of an emulsion.

Liquid fraction (whey) refers to the supernatant water after the first centrifugation step of aqueous oil extraction.

Solid fraction is the rapeseed particles which settled at the bottom of the tube after the centrifugation steps of aqueous oil extraction.

Aqueous extracted meal refers to the solid fraction obtained after the first centrifugation step of aqueous oil extraction. This solid fraction was dried either in an oven at 105°C or by freeze-drying.

Industrial-extracted meal is the meal obtained from Canbra Food Co. Lethbridge, Alberta. It is the solid residue left after prepress solvent extraction of oil from B. campestris L. var. Span.

Soxhlet extracted refers to solid residue left after oil extraction by the soxhlet method.

II. MATERIALS AND METHODS

1. Materials

The rapeseed, Brassica campestris L. var. Echo, was obtained from the Alberta Wheat Pool and was stored in a cold room at 5°C for 5 years. The seed contained 5.6% moisture, 20.0% protein, and 42.6% oil. The viabiltiy of the seed determined by a germination test was 45%. This rapeseed was the major source of material for this study. It was used largely in the study of the process parameters for aqueous extraction of rapeseed oil, and effect of the process on the quality of the aqueous extracted crude oil and the quality of the protein in the aqueous extracted meal. Although this rapeseed sample had been stored for sometime, it was used for this study because it was readily available. The long storage conditions may not affect the aqueous extraction process, however, they did influence the quality of the oil.

Although most of the study was done with the rapeseed variety Echo, it was considered appropriate to employ a fresh rapeseed variety which was currently used in the industrial process. Two rapeseed varieties, Span and Torch, as well as industrial crude oil and industrial extracted meal were obtained from Canbra Food Co. Lethbridge, Alberta. Span seed contained 9.5% moisture, 18.6%

protein, and 46.2% oil. Torch seed contained 6.4% moisture, 18.9% protein, and 46.3% oil. The viabilities of both Span and Torch seeds were ca. 99%. The aqueous extraction process steps established with variety Echo were applied to the two fresh rapeseed samples. These two rapeseed varieties were also used to study the effect of dry heating of rapeseed before the oil extraction, on the quality of the aqueous extracted crude oil. The quality of the meals from these two varieties, was also studied. The industrial crude oil and industrial extracted meal were used for quality comparison with the crude oils and meals obtained by the aqueous extraction method.

It is necessary to explain the situation relating to the supply of the fresh rapeseed samples. The first shipment of rapeseed from Canbra Food Co. was the variety Span. However, when a second shipment of the same rapeseed variety was requested from the company, the rapeseed from this shipment was the variety Torch. Hence, the two varieties were used in conjunction with each other in the study on the quality of the oil.

2. Aqueous Extraction Procedure

The general laboratory procedure for the aqueous extraction of rapeseed oil is schematically shown in Fig. 2. This procedure was the result of preliminary studies using B. campestris L. var. Echo on various process parameters with the aim of maximizing of the oil yield.

a) Grinding

The rapeseed was first ground dry in a rotating-disc type Quaker City Mill (Model 4 - 5, Straub Co., Philadelphia, Pa.). Grinding was the first step in breaking up the cells for the release of oil. It was observed that the finer this initial grinding, the better the oil yield. However, the rapeseed became more sticky, more heat was generated in the grinder and the overall operation was slow. It was also found that the grinding operation was faster and the rapeseed particles were less sticky, if the moisture content of the rapeseed was low.

b) Boiling

The finely ground rapeseed was added slowly with stirring to boiling water and boiled for 5 min. Unlike in the aqueous extraction of coconut, peanut and sunflower

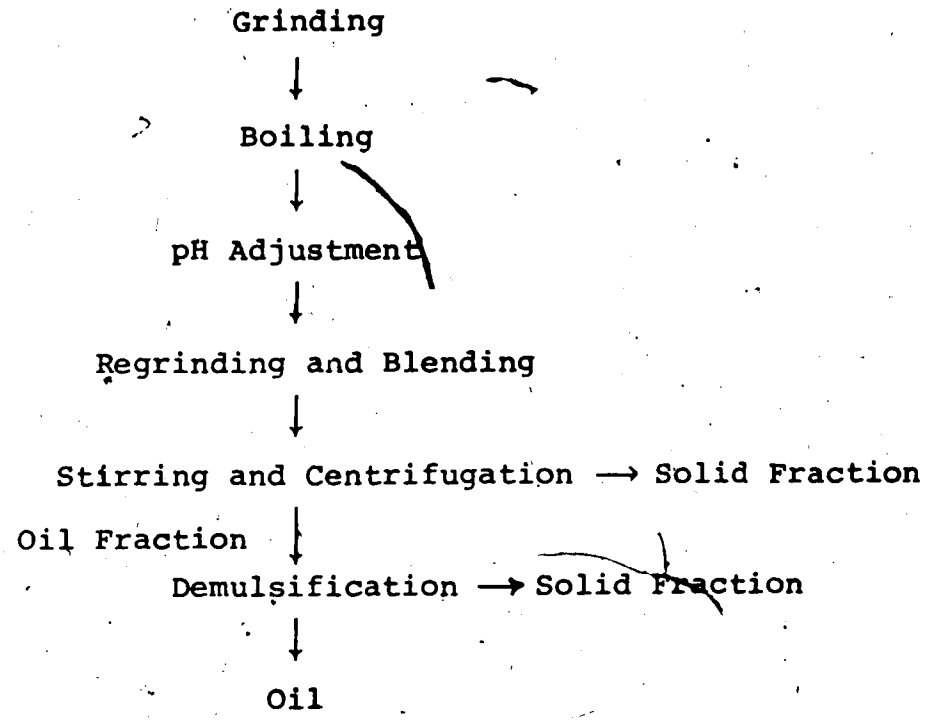

Fig. 2. General Flow Diagram of the Aqueous Rapeseed Oil Extraction

seed oils recently carried out at Texas A & M University (e.g. Hagenmaier et al., 1972; Rhee et al., 1972; Hagenmaier, 1974), boiling was a necessary step in aqueous extraction of rapeseed oil for deactivation of myrosinase (to be discussed). Furthermore, boiling helps the breaking down of the cells (to be discussed). The ground rapeseed was added slowly with stirring in order to avoid localized spots of low temperature which could cause a high activity of myrosinase and so result in a high sulfur content in the oil. It is important that the amount of water used in boiling be measured so that the solid-to-water (s/w) ratio is optimum. During these processing steps, especially boiling and blending, some water was lost via evaporation. When possible, the container was covered with aluminum foil to minimize this loss. Under these conditions, 160 - 180 ml of water was lost before the material was stirred. Thus, that amount of water had to be added in addition to the 1:3 s/w ratio (for Echo variety) in order to obtain the optimum s/w ratio during stirring (to be discussed).

c) pH Adjustment

After cooling, the slurry was adjusted with NaOH up to pH 7.3 ± 0.1 from its original pH of 5.2 ± 0.1. The

adjustment of pH was made at this stage in order to counteract the buffering capacity of rapeseed slurry, thus minimizing the time for final pH adjustment, and giving a more stable pH, a critical factor in obtaining maximum yield (to be discussed). Furthermore, it gave a better pH environment for the extraction of oil during the second grinding and blending operations. The pH after blending was normally 6.5 ± 0.3.

d) Regrinding and Blending

The slurry was reground four times into a very fine slurry in the mill mentioned above, and then blended in a Waring blender at very high speed. These two steps were necessary to cause further breakdown of the cells. This second grinding operation was not required if the rapeseed was initially ground to very fine particles.

e) Stirring and Centrifugation

After blending, 100 ml of the slurry was stirred in a 500 ml conical flask fitted with a condensor, and held in a water bath. The condensor was needed to maintain the s/w ratio during stirring. The stirred slurry was centrifuged for 10 min in a Servall Superspeed Centrifuge (Ivan Servall, Inc. Conn.).

f) Demulsification

The oil fraction obtained from centrifugation was recentrifuged at 9,000 rpm for 10 min. The free oil was pipetted out and the compact mass of emulsion was frozen, then thawed in an oven at 90°C, and centrifuged into oil, liquid and solid fractions. This freeze - thaw step, essentially according to Gunetileke and Laurentius (1974), was included as a convenient way of breaking the emulsion under our conditions. Alternative emulsion breaking steps such as the use of a high velocity pump for phase inversion by shear (Hagenmaier et al., 1972) were not investigated.

g) The Yield

The solid fraction from the first centrifugation step was dried in the oven at 105°C, ground and redried to a constant weight. The oil content of the aqueous extracted meal was determined by the Soxhlet extraction method and used in calculating the oil yield by a simple mass balance.

Calculation:

$$\text{Total oil in meal} = X \times Y/100-Y = A\% \text{ of oil}$$

$$\text{Percent oil extracted} = \frac{(Z-A) \times 100}{Z}$$

where; Z = percent oil in rapeseed (on a dry basis)

X = 100 - Z, percent non fat solid

Y = percent residual oil in the meal

The yield obtained from this laboratory process was not equal to the overall theoretical yield because some of the rapeseed material left on the grinder, blender, boiling container and flask, and some lipid (0.2%) in the liquid fraction were not taken into account. Also, the small amount of solid fraction after the demulsification step was not added to the aqueous extracted meal for soxhlet extraction. However, all of these losses might be recovered, if desired.

3. Analyses of Crude Oils

a) Determination of Sulfur

The sulfur content of the oil was determined using the method of Sosulski (1975) modified as follows: 0.5 to 5 ml (depending on sulfur content) of rapeseed oil was reacted with 25 ml glacial acetic acid and 1.2 g magnesium turnings in a 250 ml flat-bottom flask fitted with a condenser. A dry lead acetate paper was placed on the top of the condenser with the help of a rubber ring and steel rim before the reaction took place. The flask was fitted

with a special tube to bubble nitrogen through the reacting mixture. The reaction was stopped after 30 min. After rinsing with distilled water the lead acetate paper was dried in a dark room. The sulfur content was determined by comparing the intensity of the dark color with standards (0 to 12 ppm) prepared by identical procedure using 5 ml of standard solutions of Na_2S in 0.1 N NaOH The lead acetate paper was made from Whatman student grade filter paper dipped in saturated lead acetate solution and dried.

b) Determination of Phospholipids

The phospholipids were obtained by microdetermination of phosphorus (Morrison, 1964) and multiplying by a factor of 25 (Persmark, 1972b). A drop of rapeseed oil was weighed (0.010 - 0.015 g) and heated with 0.3 ml of conc. H_2SO_4 in a graduated tube containing a glass bead for 30 min. Three drops of hydrogen peroxide were added to the tube which was then reheated until the solution turned clear. The tube was boiled for another 1 min and allowed to cool. The wall of the tube was washed and the solution was diluted to ca. 4 ml with distilled water. 0.1 ml of 33% (w/v sodium sulfite, 1.0 ml of 2% (w/v) ammonium molybdate solution and 0.01 g ascorbic acid were

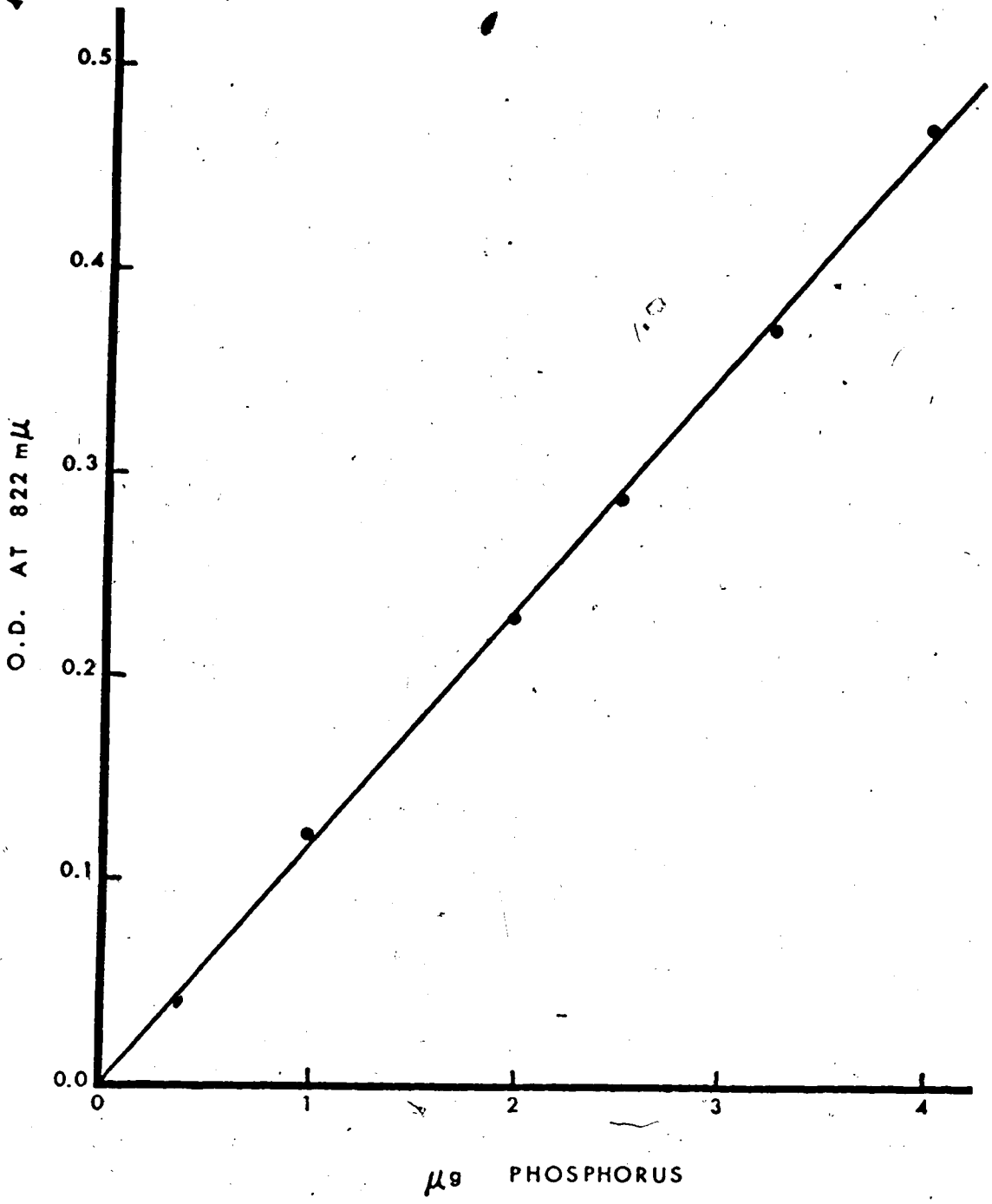

Fig. 3. Standard Curve for Determination of Phosphorus

added sequentially to the tube. After each addition, the tube was shaken to facilitate mixing. The tube was heated in boiling water for 10 min and cooled. The volume of the solution was adjusted to 8.3 ml with distilled water and the optical density was read at 822 mμ against water. The average optical density of several blanks was subtracted from that of the solution and the amount of phosphorus was determined from a standard curve prepared from standard phosphorus solution using $NaH_2PO_4 \cdot 2H_2O$.

c) Determination of Free Fatty Acids

The free fatty acid contents were determined as described in A.O.A.C. (1975). The oil (7.05 g) in neutralized alcohol (50 ml) was titrated with 0.25 N NaOH while vigorously shaking the flask until a permanent faint pink color of phenolphthalein indicator persisted for 1 min. The volume of 0.25 NaOH used corresponds to percent free fatty acids expressed as oleic acid.

d) Determination of Peroxide Values

The peroxide values of the oils were determined according to A.O.A.C. (1975). The I_2 liberated from the reaction of saturated KI solution (0.5 ml) and the oil (5.00 ± 0.05 g) in 3:2 v/v acetic acid - chloroform

solution was titrated with 0.01 N $Na_2S_2O_3$ solution while vigorously shaking the flask, until the blue color of starch indicator just disappeared.

Peroxide value (milliequivalent peroxide/kg sample)

= S x N x 100/g sample.

Where, S = ml of $Na_2S_2O_3$ (blank corrected)

N = normality of $Na_2S_2O_3$.

e) Determination of Unsaponifiable Matter

The unsaponifiable matter was determined according to A.O.C.S. (1974). The unsaponifiable compounds remaining after the saponification of oil (2.0g) by 50% aqueous KOH solution (1.5 ml) in alcohol (25 ml), were extracted with ethyl ether. After washing, drying and weighing, the residue was titrated with 0.02 N NaOH.

Unsaponifiable matter (%)

$$= \frac{100(\text{wt. of residue} - \text{wt. of fatty acid} - \text{wt. of blank})}{\text{wt. of sample}}$$

f) Determination of Moisture and Volatile Matter

The amounts of moisture and volatile matter of the oil were determined according to A.O.A.C. (1975). The oil (5 ± 0.2 g) was dried to a constant weight at room temperature and a pressure of less than 100 mm Hg in a vacuum oven. The loss in weight was reported as weight of moisture and volatile matter.

4. Analyses of Meal

a) Determination of Nitrogen

The N contents of the meals were determined by two methods, the microkjeldahl, and the ammonium electrode. The ammonium electrode method was used to determine the N in the solution obtained from the experiments which were conducted on the extractability of meal protein in relation to pH and temperature. Since there were many samples from these experiments, the ammonium electrode method was used as it is faster than the microkjeldahl. Analyses of the two procedures showed that the results were comparable. All other N determinations were conducted by the microkjeldahl procedure. The percentage of crude protein was calculated by using a factor of 6.25.

The microkjeldahl method was according to the A.O.A.C. (1975). The sample was digested to a clear solution by concentrated H_2SO_4 (2.0 ml), with potassium sulfate and mercuric oxide as catalysts. A modified Parnas-Wagner distillation apparatus was used for steam generation and distillation. The titration of distillate was made with 0.02 N HCl until the first appearance of the violet color of the methyl red - methylene blue indicator.

In the ammonium electrode method the digestion of the sample was conducted in a similar manner to the microkjeldahl method. After the digestion step, the cooled content of the flask and 5 - 6 washings with distilled water were transfered to a 250 ml volumetric flask. A solution of 10 M NaOH and 2 M NaI (6.3 ml) was added, both to bring the pH to the range of 11 to 13, and to complex mercury. The solution was brought up to volume with distilled water and allowed to cool to room temperature. 100 ml of the solution was transferred to a beaker, stirred gently with a magnetic stirrer to avoid vortexing and the ammonium electrode tip was immersed in the solution. When equilibrium had been reached (ca. 3 min), the reading was taken on the expended scale of the millivolt range of a Fisher Accumet pH Meter (Research Model 320). Then 10 ml of standard ammonium chloride

solution (1 to 10 ppm depending on ammonia concentration of the solution) was added to the solution. After equilibrium was reached the new reading was noted. Using the difference in the two readings the concentration of N can be calculated using tables provided by Orion Research Inc.

b) Determination of Crude Fiber

The determination of crude fiber of the meal was conducted according to the A.O.C.S. (1974). The meal sample (2g) together with ca. 1g of acid washed asbestos was digested with boiling 1.25% H_2SO_4 (200 ml) and then boiling 1.25% NaOH. The difference in weight of the dried residue before and after incineration at 600 ± 15°C for 30 min gave the amount of crude fiber

$$\% \text{ crude fiber in meal} = \frac{(A - B)\ 100}{W}$$

where, A = the loss in weight from incineration

B = the loss in weight from asbestos blank

W = weight of sample

c) Determination of Total Ash

The amount of total ash in the meal was determined according to the A.O.C.S. (1974). The sample (5g) was incinerated at 600 ± 15°C to a constant weight.

d) Determination of Oil Content

The oil content of the meal was determined by extracting the meal in a soxhlet apparatus using petroleum ether (b.p. 30° - 60°C) for 8 h or overnight.

e) The Determination of Solubility Characteristics of Meal Protein

The solubility characteristics of the meal protein were determined according to Lund and Sandstrom (1943). The meal (2.0g) was successively extracted with distilled water, 5% sodium chloride, 70% ethanol and 0.2% sodium hydroxide. All the extractions were carried out at room temperature except the ethanol extraction which was conducted in a water bath at 65°C. The sample was extracted in 25 ml of the solvent for 20 min, centrifuged at 1,200 x g for 15 min, and the supernatant was decanted. Three successive extractions were made with each solvent, then the supernatant was combined and weighed. The residue was dried and weighed. Nitrogen determinations were conducted on the supernatants and the dried residue. The proportion of nitrogen extracted by each solvent was then calculated on the basis of the total nitrogen recovered.

f) The extraction of Meal Protein in Relation to pH and Temperature

The extraction of N compounds of the meal in aqueous solutions was determined according to Korolczuk and Rutkowski (1971). The freeze-dried meal (5.0g) was mixed with 100 ml of distilled water and the mixture was adjusted to various pH with concentrated NaOH or HCl. It was then transferred to a flat bottom flask, which was fitted with a reflux condenser and placed in a water bath. The N of the meal was extracted with continuous stirring for 30 min at 24, 60, 70 and 80°C. After cooling under tap water the mixture was centrifuged at 2,000 rpm (480 x g) for 20 min. The N in the supernatant was determined.

g) Determination of Amino Acids

The chromatographic analysis of amino acids was carried out in the Department of Biochemistry on a Durrum 500 Amino Acid Analyser (Durrum Instrument Corporation, Palo Alto, Ca.) using a column 50 cm long and 1.75 mm in diameter packed with 8 ± 2 micron Durrum type 6A resin which was 8% cross-linked. The analytical procedure was conducted as follows: The column was operated under a back pressure of 2,500 p.s.i., with a buffer flow rate of 10 ml/h. The sodium acetate - sodium chloride buffer

systems were at pH 3.25, 4.25 and 7.90 and were run for 51.37, 16.39 and 45.44 min respectively. Ninhydrin reagent for the color development with amino acids was prepared by dissolving 40.0g ninhydrin and 1.25g hydrindantin in 2 liters of a solvent consisting of 75% dimethyl sulfoxide and 25% 4M sodium acetate (pH 5.2).

The samples for chromatographic analysis of amino acids were prepared in the following manner: The rapeseed meal (1.5 to 3.0 mg), or the liquid fraction (0.1 ml) was hydrolyzed with 1 ml of 6N HCl at 105°C for 22 h in an evacuated sealed glass tube. The hydrolysate was evaporated to dryness in a vacuum dessicator in the presence of NaOH pellets. The dried hydrolysate was dissolved in 0.5 to 2.5 ml of sodium citrate buffer of pH 2.2, and 0.04 ml of this solution was used for the determination.

h) Determination of Isothiocyanates and Oxazolidinethione

The determination of isothiocyanates and oxazolidinethione was carried out at the Department of Plant Science using a procedure which was essentially that described by Youngs and Wetter (1967). Myrosinase solution (100 µl) was added to a vial containing 10 to 15 mg dried rapeseed meal which became thoroughly wetted after

1 - 5 min. Myrosinase solution was made up of 3 mg myrosinase/ml of distilled water at pH 7.0 or citrate buffer at pH 7.0. One glass bead and 2.0 ml of internal standard solution were added into the vial which was then capped and shaked for 1 h. The internal standard solution was made up of 4 µl n-butyl isothiocyanate and 4 µl -hyl myristate/100 ml methylene chloride.

In determining isothiocyanates 3 µl portions of solution were injected into two gas-liquid chromatographs, one at lower temperature and the other at higher temperature. The low-temperature chromatography conducted was for determining butenyl isothiocyanate and pentenyl isothiocyanate while the high-temperature chromatograph run was for methylthiobutyl isothiocyanate, phenylethyl isothiocyanate, and methythiopentyl isothiocyanate. The lower-temperature run was performed on a Varian Aerograph Series 1200, at these conditions: injection port temperature 140°C; oven temperature 110°C; detector temperature 160°C; column, 4% Carbowax 400 on chromasorb G 60/70 in 3/16 in. x 4 ft. stainless steel tubing; gas flow rates: air 100 ml/min, H_2 60 ml/min and N_2 60 ml/min. The high-temperature run was carried out on a Hewlett Packard, 5750 Research Chromatograph at these conditions: injection port temperature 230°C; oven temperature 210°C; detector temperature 260°C; column, 10% FFAP on chromasorb

W 60/70 in 3/16 in. x 4 ft. stainless steel tubing; and gas flow rates were as above. The run was completed in 3 min.

To determine oxazolidinethione, 50 ml of the methylene chloride solution from the vial was added to a test tube containing 3 ml of 95% alcohol. The tube was stoppered with a "Nalgene" stopper and allowed to stand overnight (or a minimum of 4 h). The optical densities at 235, 245 and 255 mμ were obtained on a Unicam SP 1800, Ultraviolet Spectrophotometer. The blank consisted of 3 ml of 95% alcohol and 50 μl of methylene chloride.

Calculation

Low Temperature Isothiocyanates

The peak areas of internal standard (Ma), butenyl isothiocyanate (Ba) and pentenyl isothiocyanate (Pa) were determined. The areas were corrected by molecular weight correction factors:

$$Ma \times 1.13 = Mac$$

$$Ba \times 1.11 = Bac$$

$$Pa \times 1.00 = Pac$$

The amount of standard used was 80.0 μg. Then

$$\frac{80}{Mac} = F \text{ (μg isothiocyanate/unit area)}$$

e.g. F x Bac = µg butenyl isothiocyanate.

Then: $\frac{\text{µg butenyl isothiocyanate}}{\text{sample wt in mg}}$ = mg butenyl isothiocyanate/g of meal

Similar steps were used to calculate high temperature isothiocyanates, however, the molecular weight correction factors were as follows: internal standard, 1.00; methylthiobutyl isothiocyanate, 1.86; phenylethyl isothiocyanate, 1.19; and methylthiopentyl isothiocyanate, 1.69.

Oxazolidinethione

$$O.D._{245} - \left(\frac{O.D._{235} + O.D._{255}}{2}\right) = C.O.D. \text{ (Corrected O.D.)}$$

$$\frac{C.O.D. \times 1764.0}{\text{wt. of sample in mg}} = \text{mg OZT./g of meal.}$$

This was corrected for the interference of isothiocyanates

mg OZT./g of meal - (total isothiocyanates in mg/g of meal x 0.1) = Actual mg OZT./g of meal.

Extraction of myrosinase.

The preparation of myrosinase solution was made with yellow mustard (B. hirta) seed because this seed contains a high concentration of myrosinase. The mustard

seed (100g) was defatted with acetone, dried, and extracted twice with 300 ml of water. The mixture was centrifuged and the supernatant was made up to a 30% acetone solution which was then centrifuged. The supernatant was brought up further to a 70% acetone solution, which was recentrifuged. The precipitate (myrosinase) was dissolved in acetone and freeze dried.

i) Determination of Total Lipid in the Aqueous Fraction

The total lipid content in the aqueous fraction was determined according to Bligh and Dyer (1959). The sample (40g) was shaken with a chloroform:methanol (47 : 114 v/v) mixture in a separatory funnel. The bottom layer (chloroform) was transferred to a weighed flat bottom flask. When all chloroform was evaporated on a hot plate, the flask was heated in an oven at 105°C to a constant weight. The difference in weight was taken as the amount of total lipid in the aqueous fraction.

III. RESULTS AND DISCUSSION

1. Preliminary Studies

In the initial stages of this study, the work primarily involved the establishing of general processing steps for the aqueous extraction of rapeseed oil (shown in Fig. 2). Some of the results of the preliminary studies have already been mentioned in "Aqueous Extraction Procedure", while the rest will be discussed here.

The effect of boiling time was examined, and it was found that there was no difference in the amount of oil extracted when the ground rapeseed was boiled for either 5 or 60 min. Studies on the effect of blending and stirring showed that they do influence the oil yield. An experiment was carried out on the effect of the volume of material stirred in the 500 ml flask upon the oil extraction. The results showed that volumes from 50 to 250 ml had no significant effect on the amount of oil extracted.

In one experiment, the temperature of the slurry was adjusted to various temperatures from 12 to 77°C before it was centrifuged and it was found that there was no difference in the amount of oil extracted. However, in another experiment, there was slightly more oil remaining in the solid fraction when the initial

temperature of the slurry was at 87 ± 1°C than there was at 5 ± 1°C. This was probably due to coagulation of protein at high temperature, thus causing greater adsorption of oil on the solid particles.

It was also observed that the volume of material centrifuged had no effect on the yield of oil. However, if the slurry was diluted before centrifugation, slightly less oil was extracted, and the greater the dilution, the lower the oil yeild. In one study, instead of the initial steps of grinding, boiling and regrinding before blending, whole rapeseed was boiled and then ground to a fine slurry. Only ca. 65 to 80% of oil could be extracted with this procedure. This indicated that boiling of ground rapeseed was necessary for further break down of the cells. Other process parameters, that were found to affect the oil yield were the subject of more detailed study.

2. Optimization of Process Parameters

After establishing the general processing steps for the aqueous extraction method, the next step was to study the optimization of the process. The investigation included the effect of blending, stirring, and centrifugation. The effect of the degree of grinding was not studied because there was no provision in the grinder for

the control of the particle size. Initially a series of experiments were performed to obtain the approximate optimum condition of each parameter. During each experiment, one parameter was varied, while the remaining parameters were held at constant arbitrarily selected values. Consequently, the yields were generally low but these experiments did provide the basis for further optimization studies.

a) The Effect of Blending

The effect of blending time upon the extraction of oil and free oil formation is shown in Fig. 4. In this experiment, the rapeseed material from a second grinding was blended for, 0, 3, 5, 10, 15, 20 and 35 min in a Waring blendor at a very high speed. The material was subsequently stirred and centrifuged at 9,000 rpm. The conditions of stirring were: temperature, 70 ± °C; time, 1 h; solid-to-water ratio (s/w), 1:3, and pH, 6.6 ± 0.1. The results indicate that the oil yield improved from ca. 65% without blending to ca. 90% at 35 min of blending. A significant increase in oil extracted was obtained from 0 (ca. 65%) to 5 min (ca. 82%) of blending. However, the optimum time of blending seemed to be about 15 min when ca. 88% of the oil was extracted. Further blending from 15 to 35 min only increased the amount of oil extracted by ca. 2%. In this experiment, the blending step caused an increase of ca. 25% in the oil yield compared to that without blending.

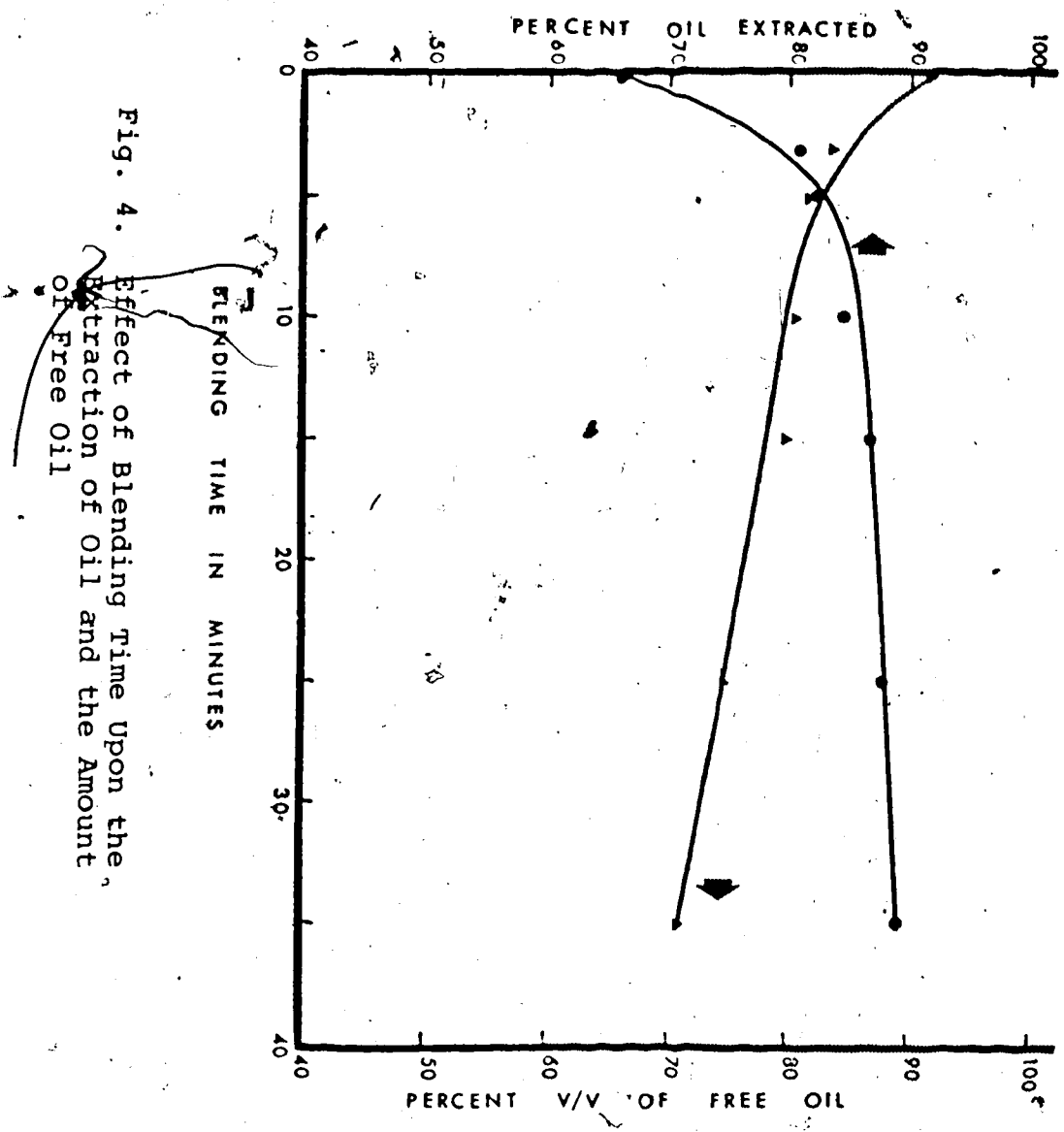

Fig. 4. Effect of Blending Time Upon the Extraction of Oil and the Amount of Free Oil

The amount of free oil obtained after the subsequent stirring and centrifugation decreased from ca. 91% without blending to ca. 71% after 35 min of blending (Fig. 4). The results also indicate that the decrease in the amount of free oil was greater within 10 min of blending. The decrease was due to the fact that blending causes small oil droplets to form hence stabilizing the emulsion. Similarly, Rhee et al. (1972a) observed that blending peanut in the presence of aqueous media prior to extraction resulted in a very stable emulsion. Blending was not used in aqueous extractions of coconut, peanut and sunflower seed oils (Hagenmaier et al., 1972; Rhee et al., 1972a; Hagenmaier, 1974). Although blending did give a more stable emulsion, it was a necessary processing step in this laboratory procedure. It is probable that blending caused a further disintegration of the individual cells. The necessity of blending in aqueous extraction of rapeseed oil could be due to the high amount of fiber in the seed and the rigidity of the cells, or to inadequate grinding process.

b) The Effect of Stirring

Stirring is an important processing step for improving the oil yield. During stirring, three forces, centrifugal, shear and frictional, are probably involved in both the extraction and the demulsification of the oil. A number of parameters of the stirring procedure were investigated. These were the effects of solid-to-water

ratio (s/w), time and vigorousness of stirring, temperature, and pH of rapeseed material. Experiments in these studies were conducted at the approximate optimum conditions determined in the preliminary studies, except for the parameter under investigation which was varied.

i) The Effect of the Solid-to-Water Ratio (s/w)

The effect, during stirring, of the solid-to-water ratio (s/w) of the slurry on the amount of oil extracted is shown in Fig. 5. With B. campestris L. var. Echo, a broad optimum was obtained with an apparent maximum of oil extracted at an s/w ratio of ca. 1:3.35. With two other rapeseed varieties, Span and Torch, sharper optima were observed with both having an apparent maximum of oil extraction at an s/w of 1:2.5. In this experiment, the maximum amounts of oil extracted were ca. 95, 93, and 93% for Echo, Span and Torch varieties, respectively. The difference in maximum s/w ratios between varieties Span and Torch on one hand and Echo on the other might be due to the oil contents of the seed which were 46.2 and 46.3% (wet basis) for Span and Torch, respectively, and 42.6% for Echo. The difference might also be due to the prolonged storage of the Echo seed. The s/w ratios obtained in this

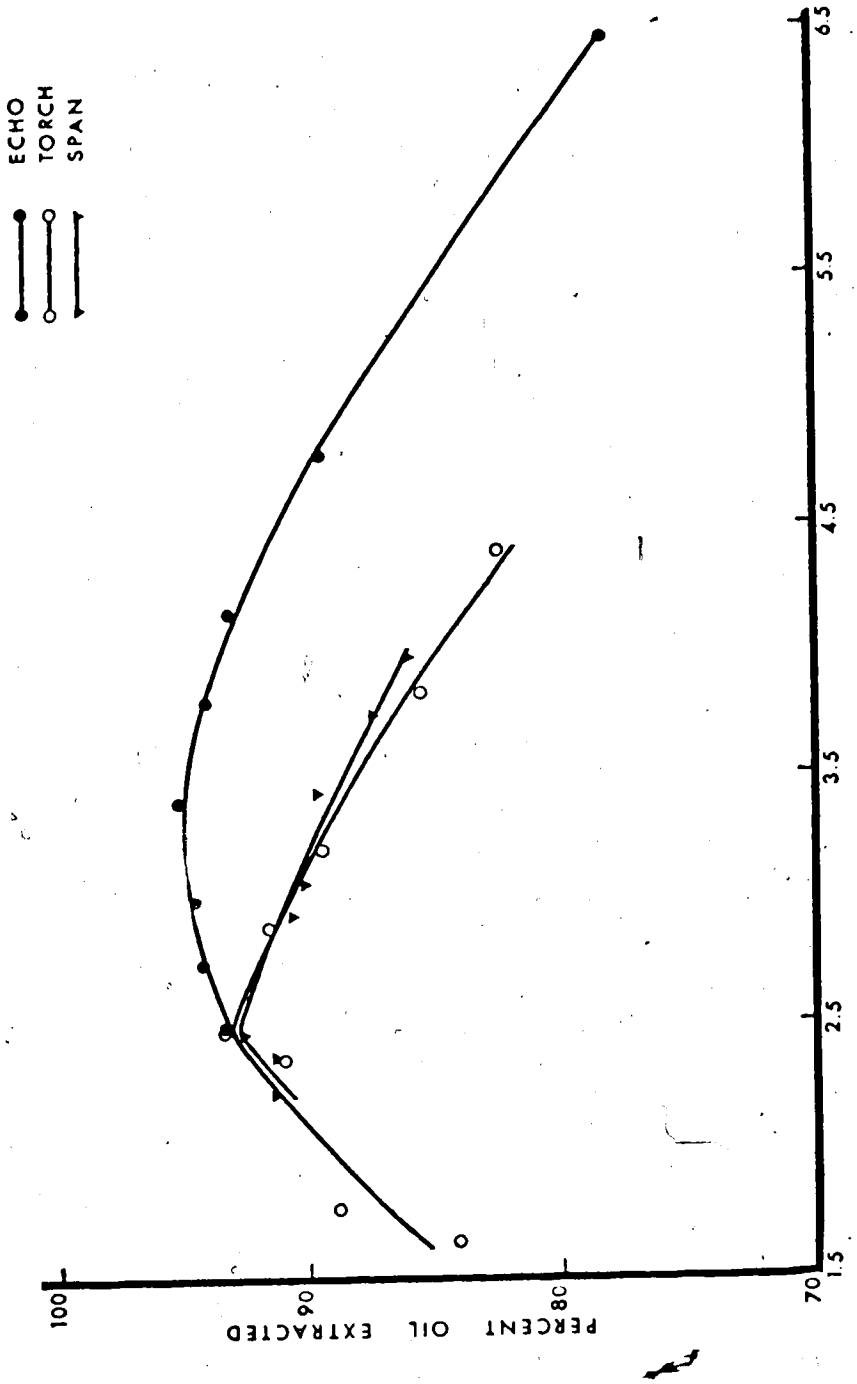

Fig. 5. Effect of Solid-to-water Ratio During Stirring on the Amount of Oil Extracted

study were higher than the s/w ratios recommended in the literature. Hagenmaier (1974) used an s/w of 1:10 for sunflower seed oil extraction. Ratios varying from 1:5 to 1:12 were reported for aqueous extraction of peanut oil (Sugarman, 1956; Subrahmanyan et al., 1959; Bhatia et al., 1966; Rhee et al., 1972a). A high s/w ratio is desirable for further processing of the solid and liquid fractions, and for minimizing protein loss from the solid fraction. However, with a high s/w ratio, lower amounts of glucosinolates could be extracted from the solid fraction.

The amount of oil extracted in relation to s/w ratio seems to correlate with the degree of demulsification during stirring. With the three varieties studied, the amounts of free oil formation in relation to s/w ratios agreed very closely with the amounts of oil extracted (Fig. 6). The maximum amounts of free oil formation, ca. 69, 69, and 65%, were obtained at s/w ratios of about 1:3.35, 1:2.75, and 1:2.5, respectively, for rapeseed of variety Echo, Span and Torch. Oil droplets coalesce with each other during stirring. With a low s/w ratio, the volume fraction of oil is also low, thus the rate of coalescence is low (Karel, 1973), leaving small oil droplets which could be easily adsorbed by the solid residue during centrifugation. However, with a greater s/w ratio,

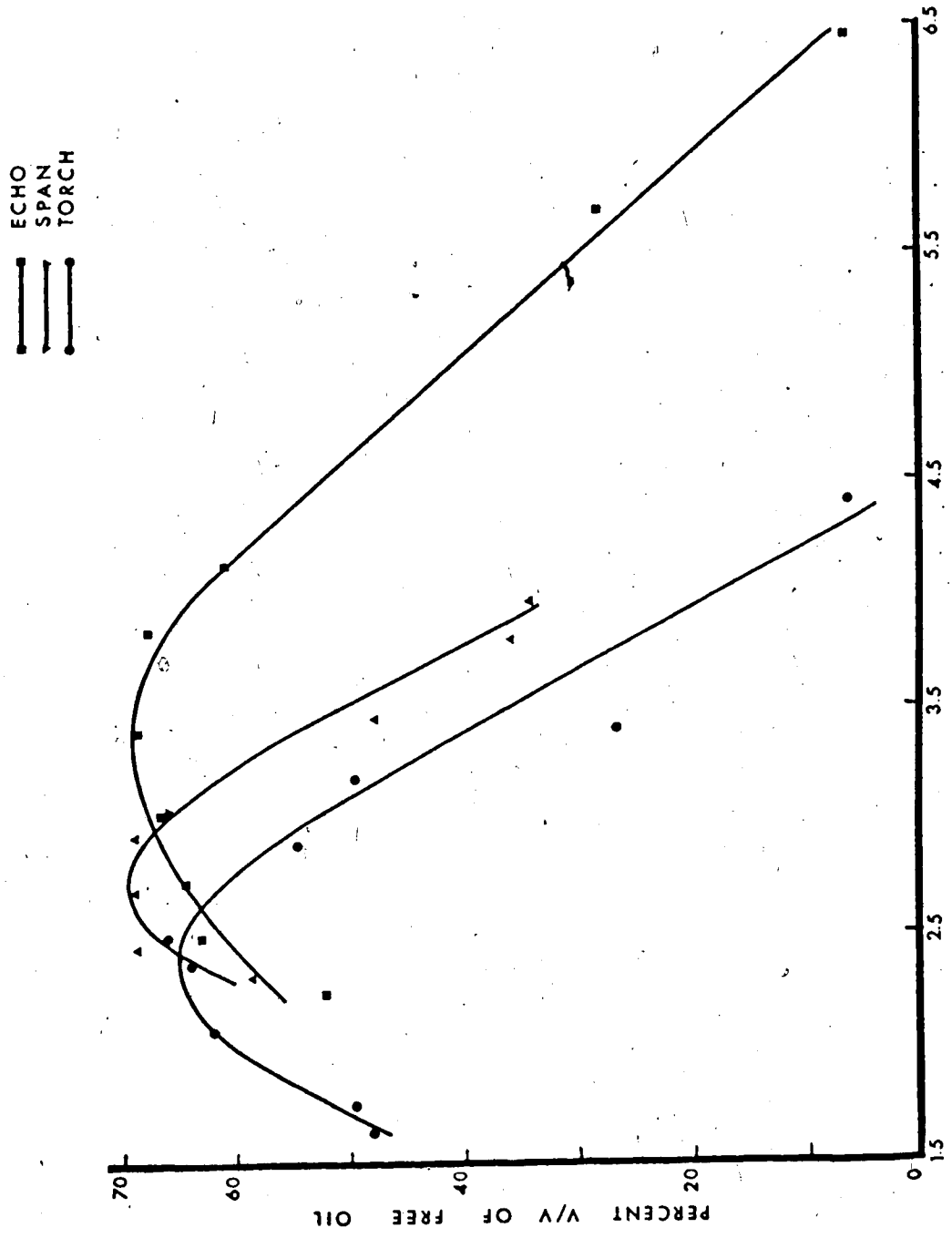

Fig. 6. Effect of Solid-to-water Ratio During Stirring on the Amount of Free Oil

although the volume fraction of oil is high, the volume fraction of solid is also high, thus reducing the coalescence of the oil droplets.

ii) The Effect of Time

The effects of stirring time upon the extraction of oil, and on the amount of free oil are shown in Fig. 7. In this experiment the rapeseed slurry was stirred at 70 ± 2°C for 0, 15, and 30 min, and 1, 2, 3, 4, and 5 h, and then centrifuged. The results indicated that the amount of oil extracted increased with the time of stirring, from near 75% with no stirring to about 92% after 5 h of stirring. However, the increase was significant only within 1 h of stirring, i.e., from ca. 75% to ca. 91%. After 1 h, the additional increase was only about 1%. Hence, the optimum stirring time for rapeseed oil extraction was taken as 1 h. This was comparable to data reported in the literature. The maximum amount of peanut oil was extracted between 30 min (Rhee et al., 1972a) and 1 h (Rhee et al., 1973a, b,c) , while the maximum amount of sunflower oil was obtained at 45 min (Hagenmaier, 1974).

In this study the amount of free oil was 0% when the rapeseed slurry was not stirred, but this was increased to ca. 63% after 15 min of stirring. Continued stirring increased the amount of free oil to ca. 78%

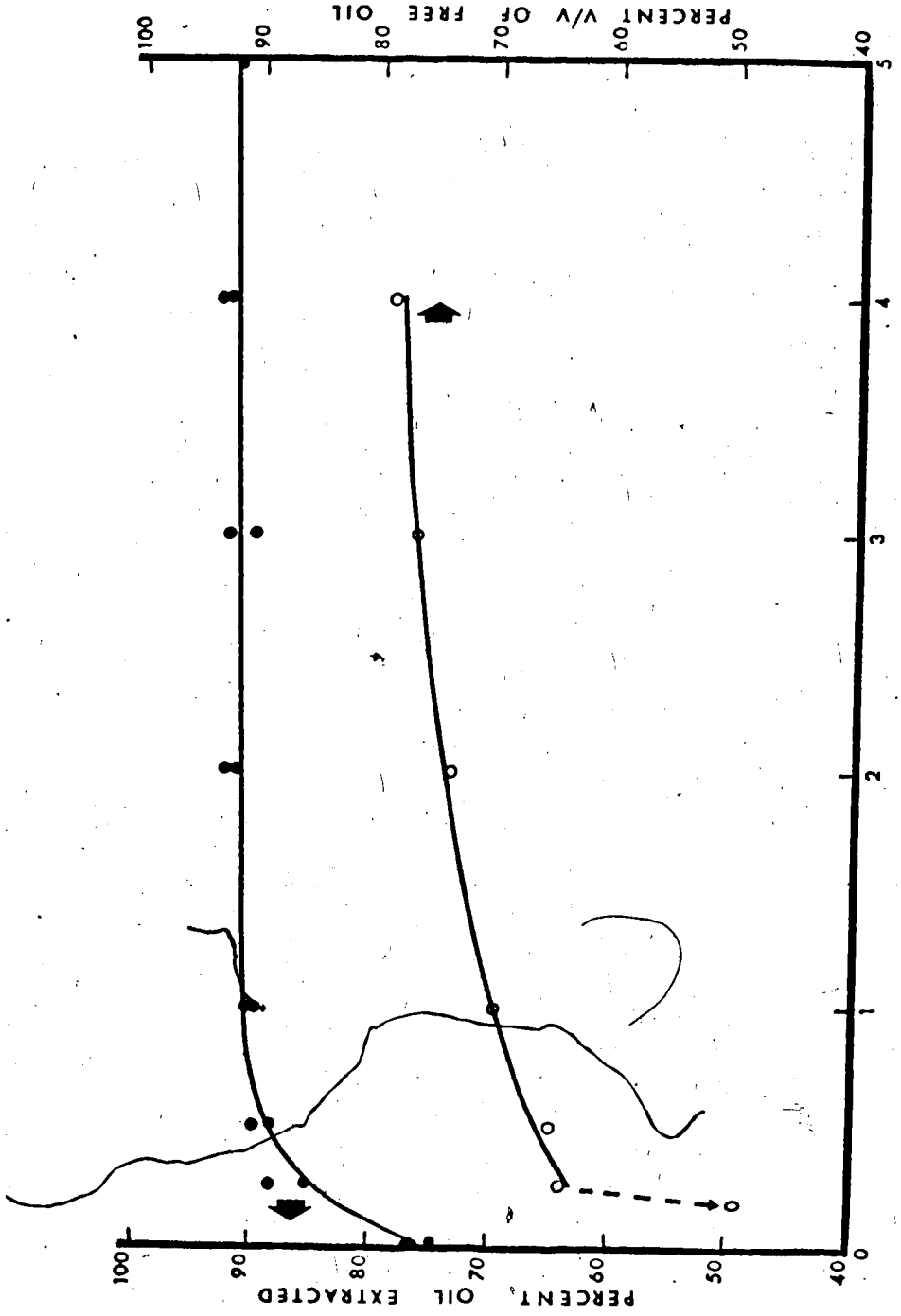

Fig. 7. Effect of Stirring Time Upon the Extraction of Oil and the Amount of Free Oil

after 4 h, indicating that the rate of coalescence of oil droplets was greatest during the first 15 min of stirring. This was probably due to the size distribution of the oil droplets. Bigger oil droplets were easily demulsified within 15 min of stirring, while very small oil droplets were stable and could not be demulsified easily by stirring. Hence, the stirring step helped both to extract and to demulsify the emulsion.

The vigorousness of stirring also influenced the amounts of oil extracted. In a preliminary study, it was found that ca. 71% of the oil was extracted at slow speed (stirred at speed 3 of the heavy duty Bell stirrer) and 84% at high speed (stirred at speed 5). This could be due to the fact that, at the high speed, a greater energy was imparted to the rapeseed material causing a greater release of oil from the cells. It was also found that higher speed of stirring resulted in a higher amount of free oil (ca. 80%) as compared to slow speed (ca. 13%). These results indicate that a greater rate of collision and coalescence occured during stirring at higher speed that at the lower speed. Furthermore, it is possible that with a greater force of stirring the protein envelope around the oil droplets could be more easily disrupted.

iii) The Effect of Temperature

The effect of temperature during stirring upon the extraction of oil is shown in Fig. 8. In this experiment the temperature of the water bath was adjusted to 24, 41, 68 and 80°C during stirring. It can be seen that the extraction of oil improved with the increase in temperature. The amount of oil extracted increased from ca. 78% at 24 ± 1°C to ca. 92% (average of three readings) at 80 ± 1°C. The results also indicate that the rate of increase in oil yield became less at high temperatures. The increase in oil extracted at high temperatures could be due to an increase in the permeability of the cell walls and to increased fluidity of the oil globules within the cells. The optimum temperature of stirring seemed to be 70°C, since, with a further increase of temperature to 80°C, only an additional 1% of oil could be extracted.

It was observed that the amount of free oil also increased with the increase in temperature. Hence, at high stirring temperatures, a greater degree of coalescence among the small oil droplets occurred, giving rise to a higher amount of free oil. This greater degree of coalescence at high temperature could be due to the loosening of the protein envelope and other surfactants, e.g.

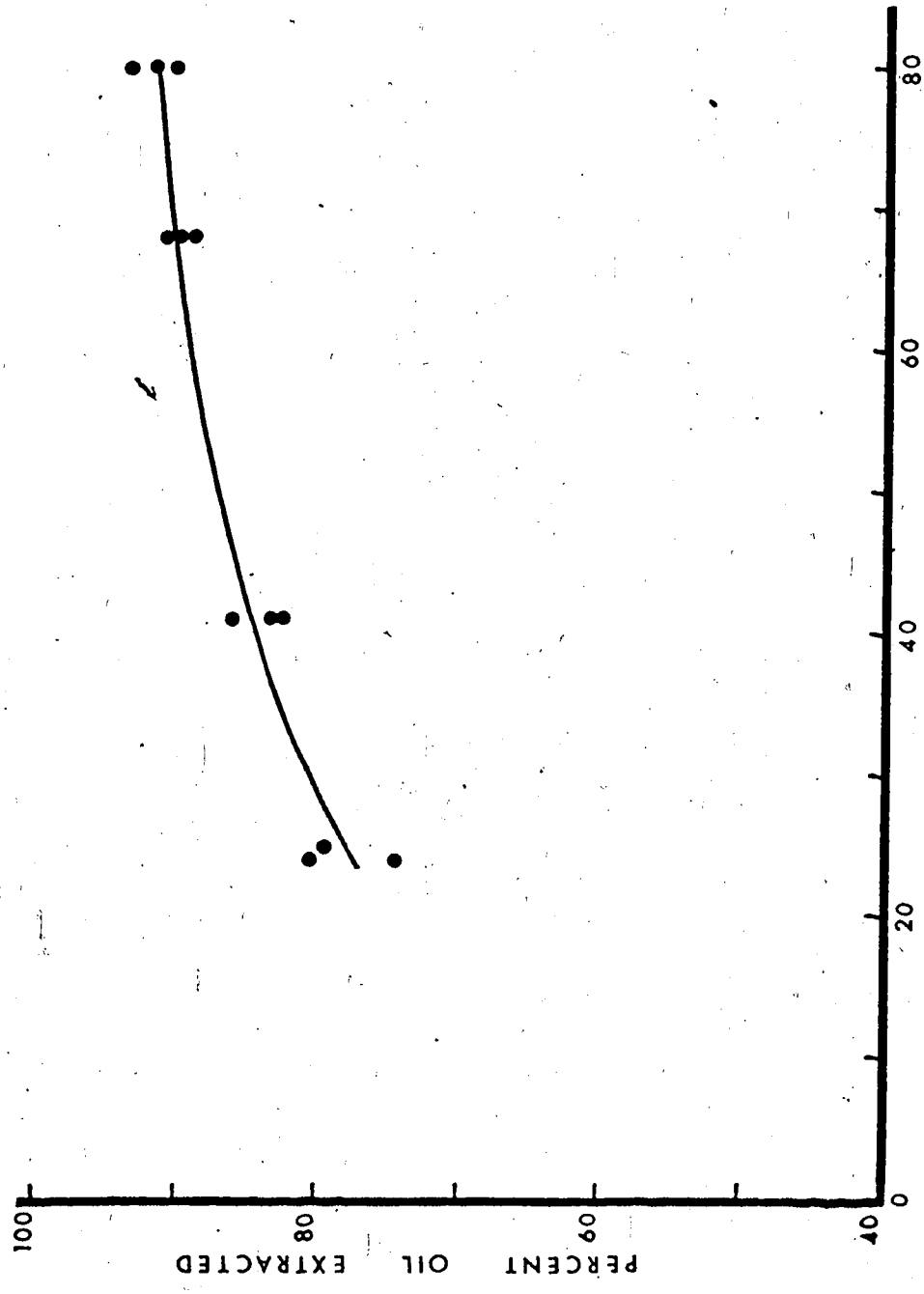

Fig. 8. Effect of Temperature of Rapeseed Slurry During Stirring Upon the Extraction of Oil

phospholipids, around the small oil droplets. Similar observations were mentioned by Karel (1973) and Lissant (1974), who suggested that at high temperatures the surface film material becomes more soluble in the continuous phase and tends to migrate away from the interface, thus allowing the droplets to coalesce.

iv) The Effect of pH

The effects during stirring of pH of the rapeseed slurry on the extraction of oil and on the amount of free oil are shown in Fig. 9. In this experiment the pH of the rapeseed slurry was adjusted to 2.9, 3.9, 5.0, 5.4, 5.9 , 6.1, 6.2, 6.5, 7.3, 8.1, and 8.8 with concentrated HCl or NaOH, as necessary, before it was stirred and centrifuged. The results indicate that the optimum pH for the oil extraction was 6.6 ± 0.1, while the lowest yield of oil extracted was in the region of pH 4 to 5. There was a slight increase in oil yield at pH values lower than 4.0. At the optimum pH of 6.6 ± 0.1, about 92% of oil was extracted, whereas only about 82% was extracted at pH 5. The results also indicate that more oil could be extracted at pH values above 6.6 ± 0.1 than below. Hence, during the rapeseed oil extraction; it is imperative that the pH of the slurry should not be much lower than

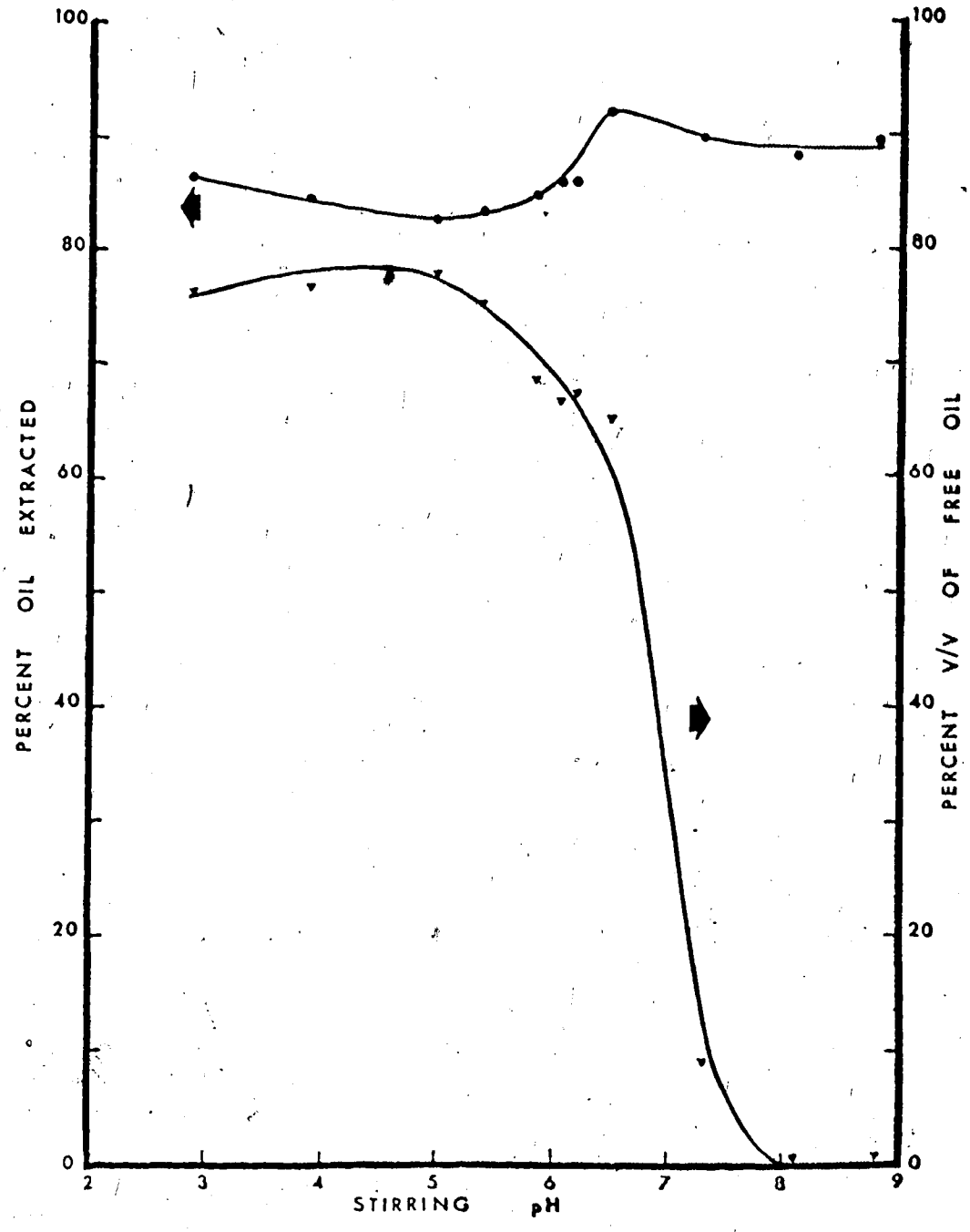

Fig. 9. Effect of pH of the Rapeseed Slurry During Stirring on the Extraction of Oil and the Amount of Free Oil

6.6. Other pH optima reported in literature varied from 4 to 10 for peanut oil extraction (Rhee et al., 1972a and 1973 a,b,c; Subrahmanyan et al., 1959; Eapen et al., 1966) and 10.0 for sunflower oil extraction (Hagenmaier, 1974).

The rapeseed proteins, like other seed proteins, are comprised of structural, catalytic and storage proteins, of which the last two seem to play a major role in the aqueous extraction of the oil. In general, the lowest solubilities of catalytic and storage proteins are in the region of pH 4.5 and 7.0, respectively. Two minimum solubilities of rapeseed proteins were reported between pH 3.0 to 4.0 and between 5.7 and 8.0 (Korolczuk and Rutkowski, 1971; Kodagoda et al., 1973; ElNockrashy et al., 1975; Quinn and Jones, 1976). In this study, the maximum and minimum yield of oil extracted correspond closely to those reported minimum solubilites of storage and catalytic proteins respectively.

The rapeseed oil emulsion was found to be least stable at minimum solubility of catalytic proteins (i.e. pH 4 to 5) as shown by the maximum formation of free oil after centrifugation (Fig. 9). In their work on aqueous extraction of peanut oil, Rhee et al (1972a) observed that the emulsion was least stable at isoelectric pH of the protein present in the system. In this study the maximum free oil (ca. 78%) was obtained at pH of minimum oil extraction (i.e. pH 4-5), whereas only ca. 65% of the total oil yield was

free oil at the pH of maximum oil extraction (pH 6.6 ± 0.1). The percentage of free oil dropped drastically from pH 6.5 to 8.0. Above pH 8, all oil extracted was in the form of an emulsion. This great reduction in the amount of free oil at pH values higher than 6.5 was probably due to the increase in the solubility of the proteins and to the changes in the emulsifying capacity of proteins and other emulsifying agents e.g. phospholipids, and mono- and diglycerides in the solution. An increase in amount of protein dissolved in the aqueous fractions at pH values lower or higher than 4 to 5 was observed during the experiment. With pH values of rapeseed slurry between 4 to 5, the aqueous fractions were clear, however, at pH values lower or higher than 4 to 5, the aqueous fractions were cloudy and turbid. This turbidity increased as the pH value departed from 4 to 5. The amount of free fatty acids (FFA) increased slightly with increasing pH during equeous extraction of peanut oil from 7 to 10 (Rhee et al., 1973b). The increase in FFA would likely result in an increase in the amounts of mono- and diglycerides.

At pH of minimum solubility of catalytic proteins (pH 4 to 5) less oil was released from rapeseed material giving the minimum yield at this pH range. This was also observed by Hagenmaier et al. (1972) in aqueous

extraction of coconut oil. At the isoelectric point of rapeseed storage proteins (pH 5.7 to 8.0) more oil was released during stirring and centrifugation. Hence, the catalytic proteins may be involved more with emulsification of oil droplets whereas the storage proteins may be involved with the adsorption of the oil.

c) The Effect of Centrifugation

The effects of centrifugation parameters on the oil yield were studied in considerable detail since centrifugation was found to be a major factor influencing the oil yield in this aqueous extraction process. The influence of centrifugation, as carried out with a Servall Superspeed Centrifuge, on the extraction of oil is shown in Fig. 10. In this experiment, the rapeseed material, after stirring, was centrifuged at 3,400 (1,397 x g), 4,125, 5,100, 6,100, 7,400, 8,500, 9,000 rpm (9,750 x g). Since the Servall Superspeed Centrifuge had only a speed control setting, the time required to reach the various final speeds (or accelerations) was variable. For instance, the times to reach final speeds of 3,400 and 9,000 rpm were 6 min and 20 sec, and 2 min and 13 sec, respectively. When centrifugation was started, the speed control was immediately set to the desired final speed

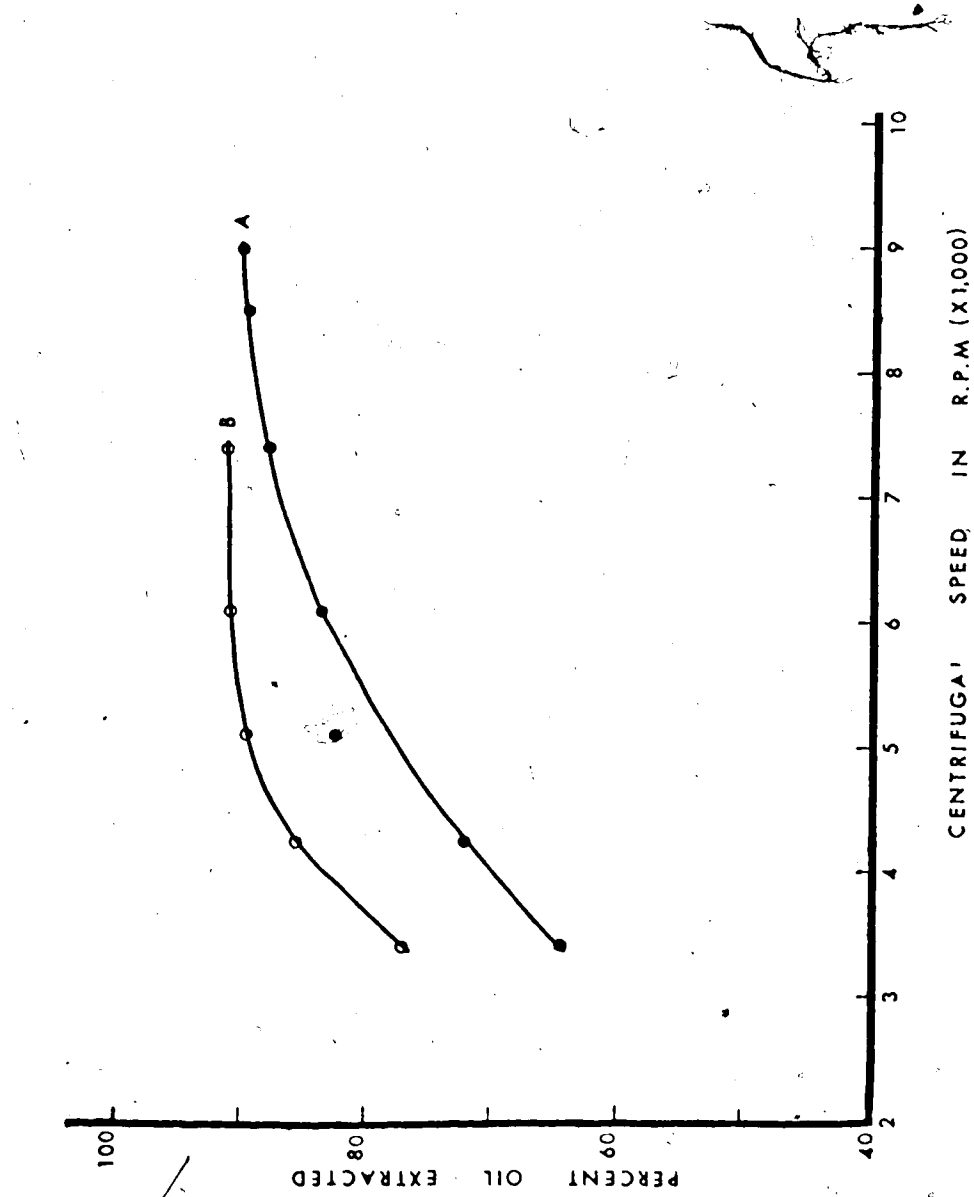

Fig. 10. Effect of Centrifugation on the Extraction of Oil (Using a Servall Superspeed Centrifuge)

NOTE: A: with various final speed settings
B: with final speed setting at 9,000 rpm

and the centrifugation was stopped as soon as it reached that speed. The results shown by the lower graph in Fig. 10 indicate that the oil yield improved with the final speed (or acceleration) from ca. 65% at 3,400 rpm (1,397 x g) to ca. 90% at 9,000 rpm (9,750 x g). A significant increase in oil yield was obtained when the final speeds were between 3,400 and 7,000 rpm. Only ca. 3% more oil was released when the final speed was increased from 7,000 to 9,000 rpm. In a preliminary experiment, it was found that no oil was extracted when the rapeseed material was centrifuged at final speed of 2,200 rpm. These results indicate that the maximum amount of oil extracted using this centrifuge was at a final speed of 9,000 rpm.

The plot of the final centrifugal accelerations against their required times (Fig. 11) shows that the rate of change of acceleration was variable. The rate of change is low when the final centrifugal acceleration is also low, and this rate of change increases with an increase in the final centrifugal acceleration.

In another experiment, the rapeseed material was centrifuged with the final speed control set at 9,000 rpm. The centrifugation was immediately stopped when the speed reached those speeds corresponding to the values in the previous experiment. The times to reach these speeds are

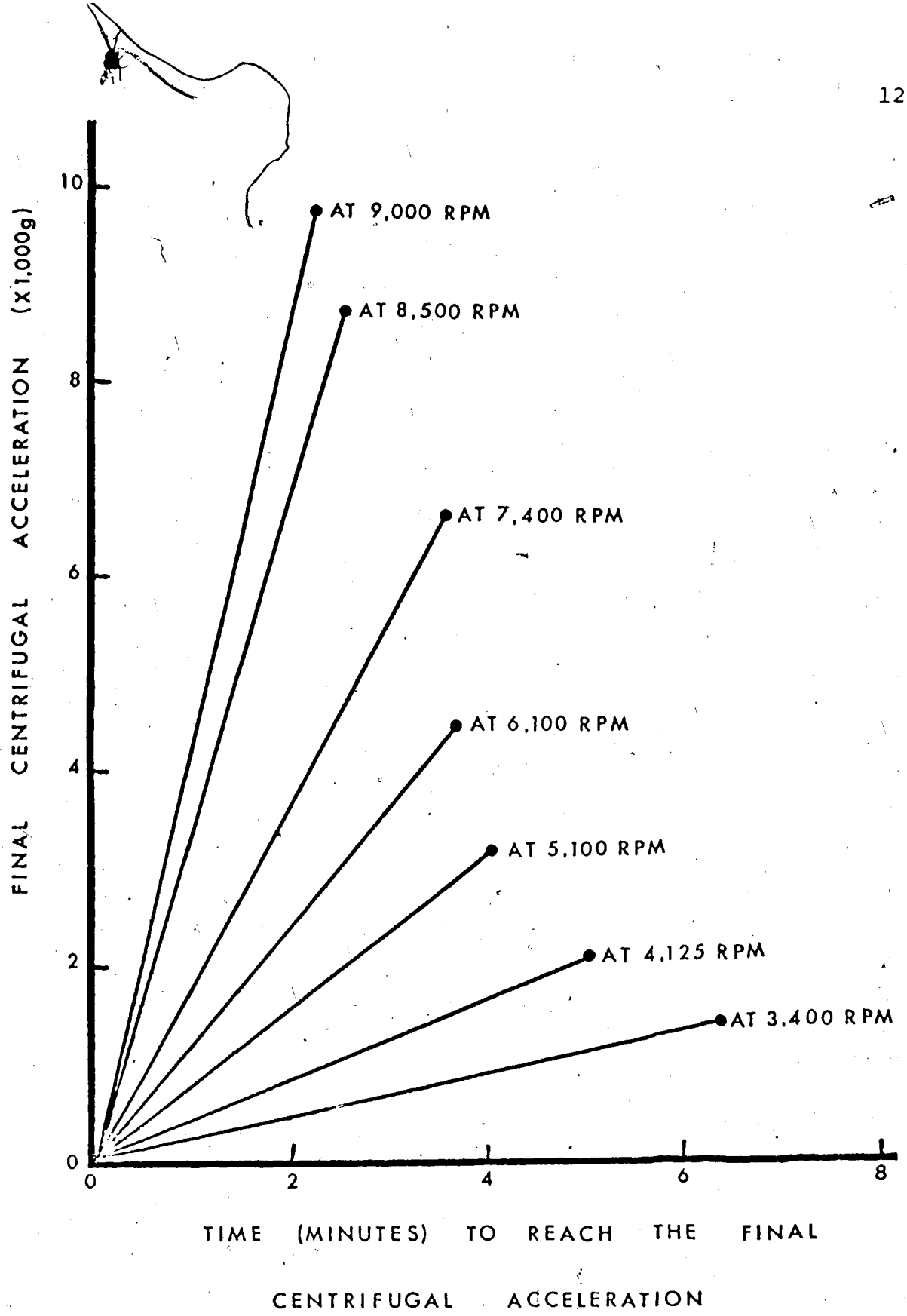

Fig. 11. The Rate of Change of Acceleration with Various Final Speed Settings (Servall Superspeed Centrifuge)

given in Table 6. The results of this experiment are given by the upper graph in Fig. 10.

Table 6. The Time to Reach Various Speeds with the Final Speed Control Set at 9,000 rpm

Speed in rpm	Time in Sec
3,400 (1,397xg)	16
4,125 (2,063xg)	24
5,100 (3,144xg)	31
6,100 (4,470xg)	40
7,400 (6,604xg)	62

These two graphs show that with the highest acceleration used (9,000 rpm setting) to reach the particular speed, the amount of oil released was greater even though the final speed (or acceleration) was the same. For instance, at a final speed of 3,400 rpm, ca. 65% of oil was released with the 3,400 rpm speed setting, whereas the amount of oil extracted was ca. 78% with the 9,000 rpm speed setting. With the 9,000 rpm speed setting, the oil yield was independent of the final speeds greater than ca. 5,000 rpm. The results show that under these conditions it took only

ca. 31 sec for the maximum release of oil droplets from the solid particles. The results of these two experiments indicate that the oil yield was more dependent on the come-up time (time required to reach the final speed or the rate of change of acceleration) than on the final speed or final acceleration.

The effect of centriguation time was also studied using the same centrifuge. It was observed that at the final centrifugal speed of 7,400 rpm the time of centrifugation between 0 and 60 min had little effect on the oil yield, indicating that the release of oil from solid particles took place before the final speed was reached.

The effect of centrifugation was also studied with a Beckman model J-21 Centrifuge (Beckman Inst., Inc. Palo Alto, Cal.). In one experiment, the rapeseed material was centrifuged at various final speeds ranging from 2,500 (ca. 840 x g) to 17,000 rpm (ca. 35,000 x g). With this centrifuge the rate of change of acceleration (or come-up conditions), was constant. Under this constant rate of change of acceleration (or come-up conditions), no significant difference in oil yield was observed between the final centrifugal speeds of 2,500 and 17,000 rpm. In another experiment, the time to reach the final speed of 7,500 rpm was varied from 22 sec to 17 min and 14 sec. The results show that the oil yield decreased with

increasing come-up time (Fig. 12). About 90% of oil was released when the time to reach the final speed of 7,500 was 22 sec, whereas the oil yield was only ca. 76% with the come-up time of 17 min and 14 sec. Hence, these two experiments further proved that the come-up time was the critical factor in aqueous extraction of rapeseed oil.

The effects of centrifugation in the aqueous oil extraction have not been previously studied. The effects observed in this study may be due to one or more of the following three factors.

A) Hindered Settling

In the rapeseed slurry the solid particles and oil droplets are close together. Thus during the centrifugation, the velocity gradient of each particle is affected by the presence of neighboring units. The oil droplets in the slurry either adhere to the solid particles by surface tension or are free in the water. The particles, in settling, displace the water (McCabe and Smith, 1956). The velocity of the displaced water is greater with respect to the particle than with respect to the apparatus. The displaced water, due to its velocity, will cause the separation of free oil droplets from the solid particles and the release of the oil droplets which are adhered to the solid particles. The shorter the come-up time or the greater

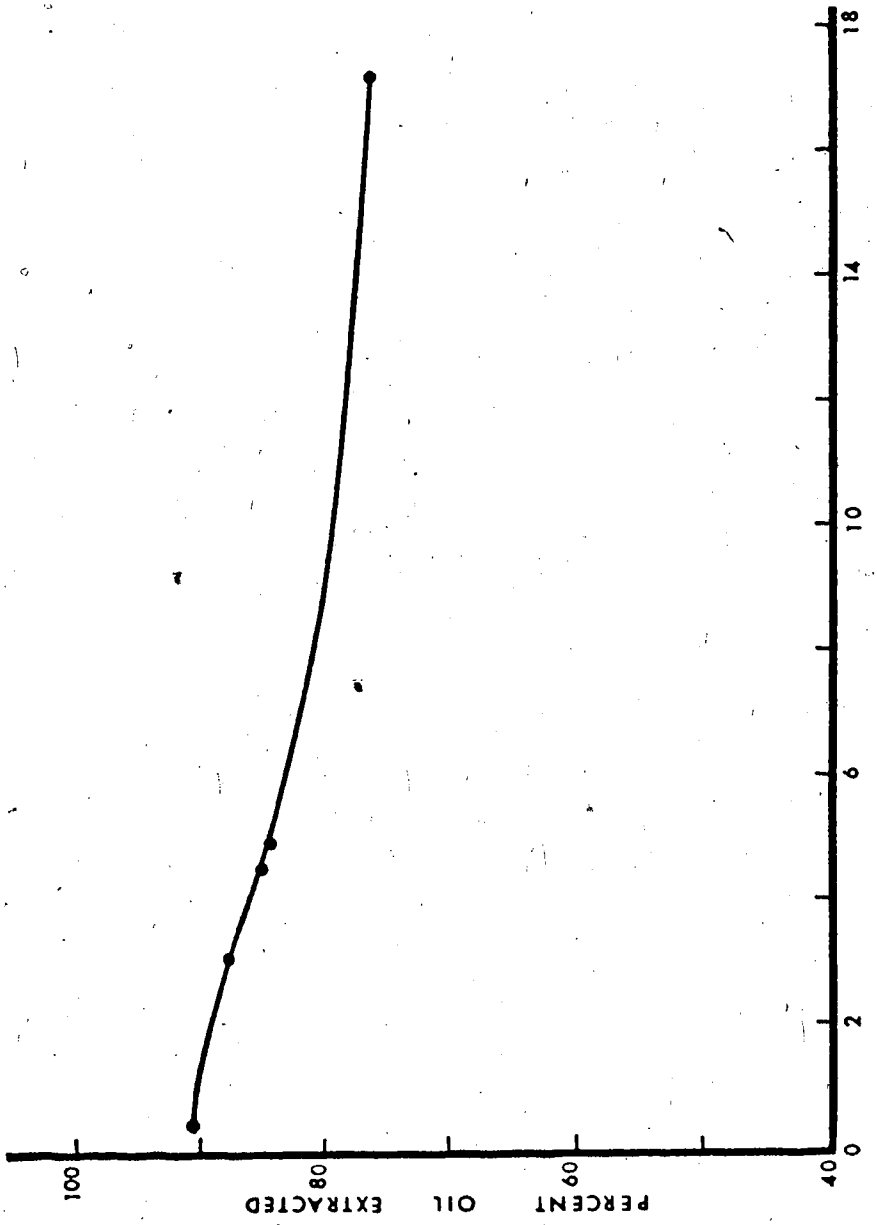

Fig. 12. Effect of Come-up Time on the Extraction of Oil (Using a Beckman Model J-21 Centrifuge)

the rate of change of acceleration, the greater will be the velocity of the displaced water, resulting in a greater release of oil droplets adhered to the solid particles.

B) Turbulent Flow

When the rapeseed slurry is centrifuged with a long come-up time or a low rate of change of acceleration, the solid particles will settle smoothly at low velocities. The drag force resisting the motion of particles which arises from the frictional force of the water is also low. Under these conditions the solid particles settle together with the oil droplets. Some of the free oil droplets will be trapped by the solid particles. With a shorter come-up time or a greater rate of acceleration, the solid particles settle at greater velocities and this may cause a turbulent flow pattern of water around each particle. The drag force acting on the particles is also greater. With a greater drag force and turbulent flow, a greater number of oil droplets, adhered to the solid particles, will be released and separated. In addition, fewer free oil droplets will be trapped by the solid particles.

C) Buoyant Force

The size of oil droplets and the strength of

interfacial tension between the oil droplets and the solid particles are variable. When the slurry is centrifuged there will be a downward force acting on the particles and an upward force acting on the oil droplets (buoyant force), since the density of oil is lower than that of water. The greater the size of the droplet, the greater will be the buoyant force acting on it. Under a condition of long come-up time or low rate of change of acceleration, only the larger oil droplets and those with a weak surface tension adhesion to solid particles will be released from the solid particles. With a shorter come-up time or a greater rate of change of acceleration, the buoyant force is greater, thus causing more oil droplets to be released from the solid particles.

If the density of the aqueous phase is increased, such as by the addition of salts, the frictional force acting on the particles, and the buoyant force acting on the oil droplets would be greater. These forces would increase the number of oil droplets released from the solid particles, thus increasing the oil yield as observed by Linow et al. (1971) and Mieth et al. (1975a).

Due to the shortness of the centrifuge tubes, (ca. 10 cm) the settling time of the solid particles is expected to be short. Thus the initial rate of change of acceleration is the most critical factor determining the

amount of oil separated from the solid particles. Once the solid particles settle to the bottom of the tube, there will be no further release of the oil droplets. Thus the final speed (or acceleration) and the time of centrifugation after reaching the final speed are not important factors in determining the release of oil droplets from the solid particles.

3. Results of Oil Analyses

After establishing the optimum process parameters for the aqueous extraction, it was essential to study the quality of the aqueous extracted crude oil. The quality of the aqueous extracted crude oil was compared with the quality of a soxhlet extracted crude oil and an industrial crude oil which was obtained from Canbra Food Co.. For this comparison, sulfur, phospholipids, free fatty acids, peroxide values, unsaponifiable matter, and moisture and volatiles were analysed. A more detailed study was carried out on the sulfur content of the aqueous extracted crude oil since this property is the most important factor in determining the quality of the crude rapeseed oil. The effects of dry heating the seed on the quality of the aqueous extracted crude oil were also studied.

a) Sulfur Content

i) Sulfur Content of the Aqueous Extracted Crude Oils

Sulfur content is an important factor in determining the quality of rapeseed oil due to the fact that sulfur in the oil would poison the catalyst during the hydrogenation process. The presence of 5 ppm of sulfur reduced the activity of the catalyst during the hydrogenation process by ca. 50% (Babuchowski and Rutkowski, 1969). It has been found that oils are difficult to hyrogenate if they contain over 20 ppm sulfur whereas oils containing 2 to 7 ppm sulfur are considered good quality oils (Sosulski, 1974).

One of the steps of the aqueous extraction of rapeseed oil was the boiling of ground rapeseed. This treatment inactivated enzymes such as myrosinase and lipase. The inactivation of myrosinase would prevent the hydrolysis of glucosinolates into sulfur containing compounds which are soluble in oil, thereby resulting in an oil with lower sulfur content.

The sulfur contents of aqueous, soxhlet, and industrial extracted crude oils are shown in Table 7. The sulfur content of the aqueous extracted crude oil from untreated seeds of the Echo variety was 4.5 ppm and that from untreated seeds of the Span variety was 6 ppm. The

Table 7. Sulfur Content of the Oils

Sample and Treatment	Sulfur Content (ppm)*
A. Industrial crude oil	10
B. B. campestris L. var. Echo	
1. Aqueous extracted crude oils	
a) Untreated seed	4.5
b) Seed boiled	3
c) Seed dried in oven at 105°C for 2 days	23
2. Soxhlet extracted crude oil (untreated seed)	<2
C. B. campestris L. var. Span	
1. Aqueous extracted crude oils	
a) Untreated seed	6
b) Seed dried at 55°C for ca. 17 h	15
c) Seed dried at 105°C for ca. 17 h	12
d) Seed boiled and dried at 105°C for ca. 17 h	4
D. B. campestris L. var. Torch	
1. Aqueous extracted crude oil (seed dried at 55°C for 3 days)	50
2. Soxhlet extracted crude oil (untreated seed)	<2

* average of two analyses. All samples under B, C and D were composite samples of at least five extractions each.

apparently higher amount of sulfur in oil from the Span variety could probably be due to the higher moisture content of the Span seed (9.5%) than the Echo seed (5.6%). The study by Daun and Hougen (1976) showed that there was a gradual increase in the sulfur content of the oil when increasing amounts of water were added to the seed. When the seed is ground, the glucosinolates come into contact with myrosinase and before the enzyme is inactivated by boiling, the enzyme hydrolyzes the glucosinolates. The extent of this hydrolysis depends on the moisture content of the seed. If the seed was boiled to inactivate the enzyme before grinding, the sulfur levels of oil were further reduced to 3 ppm for the Echo variety and to 4 ppm for the Span variety.

The sulfur contents of aqueous extracted crude oils were higher (4.5 - 6 ppm) than that of the soxhlet extracted crude oils (less than 2 ppm) but lower than that of the industrial crude oil (10 ppm) (Table 7). Higher levels of sulfur in Canadian crude oils were reported: 16 - 57 ppm (Teasdale, 1975) and 18 - 31 ppm (Daun and Hougen, 1976). Persmar (1972a) reported relatively small amounts of sulfur (5 - 15 ppm) present in rapeseed oil extracted in Sweden Lower levels of sulfur (1 - 5 ppm) were also obtained by other workers in laboratory extracted oils of untreated seeds (Bhatty and Sosulski, 1972; Sosulski et al., 1972;

Kozlowska et al., 1972a). In their study Daun and Hougen (1976) produced laboratory extracted oils free of sulfur from untreated seed, regardless of the glucosinolate content of the seed.

In the industrial process, the sulfur levels in expeller-extracted oils were lower than in the subsequently solvent extracted oil fractions (Von Fellenberg, 1945; Zeman and Zemanova, 1967; Daun and Hougen, 1976). This was attributed to the association of sulfur compounds with the gum and colored material of the seed which were preferentially extracted by the solvent (Daun and Hougen, 1976). In the aqueous extracted crude oils, no apparent relationship between the sulfur content and the gums or colored material was observed. The phospholipid levels in aqueous extracted crude oils ranged between 0.03 to 0.05% (to be discussed) whereas the sulfur contents ranged from 3 ppm when the seed was boiled, to 50 ppm when the seed was dried in an oven for 3 days at 55°C (to be discussed). Although aqueous extracted crude oils from the untreated Span seed and the heated Torch seed (55°C for 3 days) contained the same amount of phospholipids (0.04%), the two oils contained different amounts of sulfur; 6 ppm for Span oil and 50 ppm for Torch oil. During the study, it was observed that lighter colored oils were obtained if the material was stirred or boiled at higher pH values. Similar results were reported by Eapen et al., (1969) that comparatively

lighter colored oils were obtained when the seed was boiled in 0.5% alkali. However, our results did not show any correlation between the color and the sulfur content of the oils.

ii) The Effect of Dry Heating

Heating the seed before grinding influenced the sulfur level of the aqueous extracted oil. Dry heat treatment of rapeseed before grinding was carried out in order to reduce the moisture content of the seed so that, as mentioned earlier, the grinding operation would be faster and the rapeseed particles would be less sticky. However, the results show that heating the rapeseed before grinding increased the sulfur content of the oil (Table 7). When Span seed was heated at 55° and 105°C for ca. 17 h, the levels of sulfur in the oils were 15 and 12 ppm respectively. The sulfur content of oil from the Echo seed, heated at 60°C for 2 days, was 23 ppm. Higher sulfur content (50 ppm) was found in oil extracted from Torch seed which was heated at 55°C for 3 days. These results indicate that the level of sulfur in the oil increased with the heating time.

Heating rapeseed with a moisture content greater than 10% at 50°C to 70°C caused rapid hydrolysis of

glucosinolates; however, at a moisture content of 8% very little hydrolysis occured (Pritchard, 1974). A similar effect of heating on the amount of sulfur in a laboratory solvent extracted oil was observed by Daun and Hougen (1976). Heating a high-glucosinolate seed (B. napus L. var. Oro; 5% moisture) for 12 and 144 h increased the sulfur content of the resulting oils from 0 to 16 and 84 ppm respectively. However, heat treatment of a low-glucosinolate seed (B. napus L. var. 569 - 2895; 4% moisture) did not seem to produce any sulfur in the oil. In their study on the effect of bin heating of rapeseed, Bell et al. (1972) found 55 ppm sulfur in the oil. Heating rapeseed from 90 to 135°C caused an increase in the amounts of isothiocyanates and vinyl-oxazolidinethione (Clandinin et al., 1959; Clandinin, 1962; Appelqvist and Josefsson, 1967).

It was suggested that heating caused partial pyrolysis of the glucosinolates to yield oil-soluble sulfur-containing compounds (Bell et al., 1972; Daun and Hougen, 1976). In this study it was found that if the rapeseed was boiled before heating in an oven at 105°C overnight the sulfur content in the oil was only 4 ppm. The results of this study indicate that hydrolysis by myrosinase, and not the pyrolysis of glucosinolates was responsible for the increase in the sulfur levels of the oil. This hydrolysis occurred during the dry heating of

unboiled rapeseed. Unlike boiling, dry heating in an oven, even at 105°C, would not easily inactivate the enzymes as found by VanEtten et al. (1966).

iii) The Effect of pH

The effects of pH during boiling and stirring upon the sulfur content of the oil were also studied. In one experiment concentrated HCl or NaOH was added as necessary to the boiling water before ground rapeseed was added. The pH of the rapeseed slurry was measured after boiling and cooling. The results indicate that pH during boiling had a significant effect on the sulfur level in the oil (Fig. 13). The sulfur content of the oil increased with an increase in pH during boiling from ca. 2 ppm at pH 3.1 to ca. 10 ppm at pH 7.6. However, in the study on the diffusion extraction of glucosinolates from rapeseed Sosulski et al. (1972) found that alkaline solutions were more effective in reducing the sulfur level in the oil. The results from this study probably reflect the activity of myrosinase before its inactivation by boiling. Just before inactivation, the myrosinase with an excess amount of water, could hydrolyze the glucosinolates and the extent of this hydrolysis increases with pH of the boiling water. This is in accordance with myrosinase

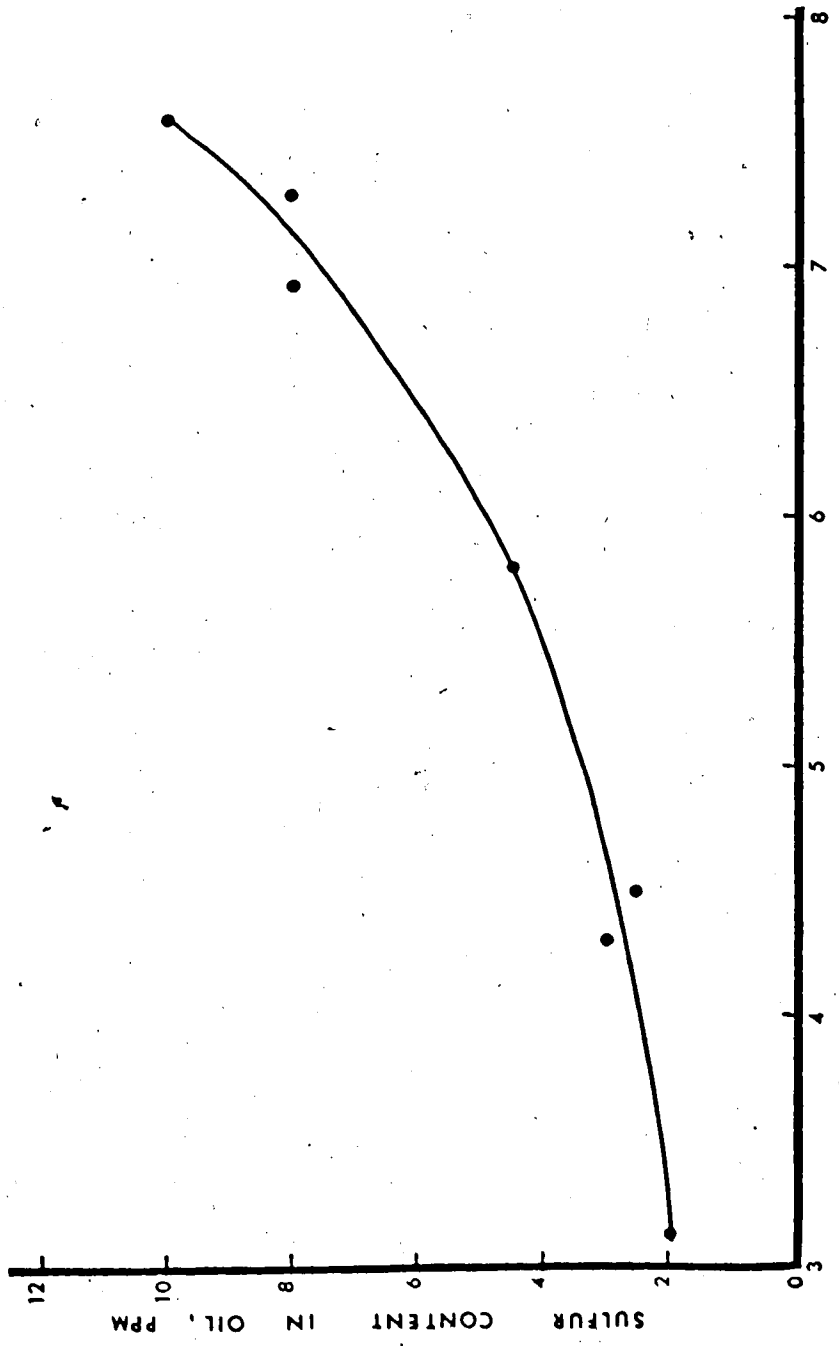

Fig. 13. Effect of pH of Rapeseed Slurry During Boiling on the Sulfur Content of the Oil

activity in crambe seed meal which was found to increase with the pH and had an optimum at pH ca. 9.0 (Tookey, 1973). Under acidic conditions, hydrolysis of the glucosinolates of both rapeseed and crambe seeds gave rise to nitriles (Youngs et al., 1972; VanEtten et al., 1966) In another experiment, the pH of the rapeseed slurry was adjusted from 3.9 to 9.7 before it was stirred. Under the conditions of pH examined, the sulfur contents of the oils ranged from 3 to 5 ppm (Table 8). Hence, the pH of

Table 8. The Effect of pH During Stirring on the Sulfur Content of the Oil

ph During Stirring	Sulfur in ppm
9.7	3.0
8.1	4.0
6.6	5.0
5.0	4.0
4.5	3.5
3.9	5.0

slurry during stirring had no significant effect on the sulfur content of the oil. This was due to the fact that myrosinase was already inactivated during the boiling step.

The overall results of the study on sulfur content reveal that the aqueous extracted crude oil contained low amounts of sulfur due to the boiling step. Boiling before grinding further reduced the sulfur level in the oil. Without enzyme inactivation dry heating would increase the sulfur content. Low pH conditions during the boiling of ground rapeseed lowered the sulfur level in the oil. However, boiling the rapeseed in acidic environment might reduce the quality of rapeseed protein in the meal and cause corrosion to the boiling container.

b) Phospholipid Content

The phospholipid contents of aqueous, soxhlet and industrial crude oils are compared in Table 9. The results show that phospholipid contents in aqueous extracted crude oils from untreated seed were much lower (0.05% for Echo variety and 0.04% for the Span variety), than in soxhlet extracted crude oils (0.32% for Echo variety and 0.36% for Torch variety), and the industrial extracted crude oil from Canbra Food Co. (0.8%). The same level of phospholipids (0.6 to 0.9%) in industrial rapeseed crude oil was reported by Klein and Crauer (1974). However, Teasdale (1975) reported the amount of phospholipids in Canadian rapeseed crude oils as 3.5% maximum, while

Table 9. Phospholipid Content of the Oils

Sample and Treatment	Percent Phospholipid*
A. Industrial crude oil	0.80
B. B. campestris L. var. Echo	
1. Aqueous extracted crude oil (untreated seed)	0.05
2. Soxhlet extracted crude oil (untreated seed)	0.32
C. B. campestris L. var. Span	
1. Aqueous extracted crude oils	
a) Untreated seed	0.04
b) Seed dried at 55°C for ca. 17 h	0.04
c) Seed dried at 105°C for ca. 17 h	0.03
d) Seed boiled and dried at 105°C for ca. 17 h	0.03
D. B. camprestris L. va. Torch	
1. Aqueous extracted crude oil (seed dried at 55°C for 3 days)	0.04
2. Soxhlet extracted crude oil (untreated seed)	0.36

* average of two analyses. All samples under B, C and D were composite samples of at least five extractions each.

degummed oils contained a maximum of 0.6% phospholipids. Higher amount of lecithin (ca. 2%) was also reported by Anjou (1972). In Swedish rapeseed oils the phospholipid contents were reported as 0.51 to 0.75%, and the degummed oils contained 0.013 to 0.4% phospholipids (Persmark, 1972b; Hoffmann, 1973).

Phospholipid content depends on the method of oil extraction; in pressed oil the amount of phospholipids was 0.5% whereas in the solvent extracted oil the phospholipid content was 1.2% (Persmark, 1972b). The smaller amount of phospholipids in the soxhlet extracted crude oils than in the industrial crude oil was probably due to the mild extraction conditions of soxhlet method. The phospholipid contents in aqueous extracted crude oils from the Span seed, dried overnight at 55 and 105°C, were 0.04 and 0.03% respectively. The aqueous extracted crude oils from Span seed, boiled and dried overnight at 105°C, and from Torch seed, dried for 3 days at 55°C, contained 0.03 and 0.04% phospholipids respectively. Hence, these results indicate that the amount of phospholipids did not vary significantly with the heat treatments of the seeds. The low amount of phospholipids in aqueous extracted crude oils is probably due to the fact that most of the phospholipids are hydrated and precipitated during the extraction process. This is clearly shown by the higher amount of phospholipids

(2.38%) in the residual oil in the aqueous extracted meal. Hence, the oil obtained by aqueous extraction could be considered equivalent to a degummed oil.

In industrial processing of rapeseed oil, phospholipids are often removed by degumming the oil with a small amount of water at the extraction plant followed by degumming with phosphoric acid at the refining plant (Anjou, 1972). The industrial crude lecithin produced from degumming process contains 30 to 40% oil (Persmark, 1972b). Rapeseed phospholipids (lecithin), inferior to soybean lecithin in color as well as in flavor, taste and general appearance, has not been used extensively in food industry (Persmark, 1972b). However, it is often added back to the meals (Anjou, 1972; Clandinin et al., 1972b). Since phospholipids are to be removed from the oil, a greater amount of phospholipids represents higher refining loss. Hence, due to a low phospholipid content, the refining loss of aqueous extracted crude oils would be comparatively less than the present industrial extracted crude oil.

c) Free Fatty Acid Content

The free fatty acid contents of aqueous, soxhlet and industrial crude oils are shown in Table 10. The results show that the amounts of free fatty acids (FFA)

Table 10. Free Fatty Acid Content of the Oils

Sample and Treatment	Percent Free Fatty Acid*
A. Industrial crude oil	0.55
B. *B. campestris* L. var. Echo	
1. Aqueous extracted crude oil (untreated seed)	0.70
2. Soxhlet extracted crude oil (untreated seed)	1.00
C. *B. campestris* L. var. Span	
1. Aqueous extracted crude oils	
a) Untreated seed	0.16
b) Seed dried at 55°C for ca. 17 h	0.14
c) Seed dried at 105°C for ca. 17 h	0.17
d) Seed boiled and dried at 105°C for ca. 17 h	0.16
D. *B. campestris* L. var. Torch	
1. Aqueous extracted crude oil (seed dried at 55°C for 3 days)	0.12
2. Soxhlet extracted crude oil (untreated seed)	0.16

* average of two analyses. All samples under B, C and D were composite samples of at least five extractions each.

in aqueous extracted crude oil from the untreated Span seed was lower (0.16%) and that from the untreated Echo seed was slightly higher (0.7%) than industrial crude oil (0.55%). The soxhlet extracted crude oil from Echo seed contained a slightly higher level of FFA (1.0%) than the aqueous extracted crude oil from the same variety. However, this was not clearly observed in the case of oils from Torch seed; the soxhlet extracted crude oil contained 0.16% FFA and the amount of FFA in aqueous extracted crude oil was 0.12%.

Varying amounts of FFA in industrial rapeseed crude oils have been reported in literature; 0.97% (Hoffmann, 1973); 0.7 to 0.8% (Klein and Crauer, 1974); 0.5% (Pritchard, 1974); 0.3 to 0.5% (Appelqvist, 1961); 0.4 to 1.5% (Teasdale, 1975). The amount of FFA in crude oil of good quality should not exceed 1.5% in order to avoid high refining losses (Olin, 1957).

High FFA contents of Echo rapeseed oils from both soxhlet and aqueous extraction methods could be due to the long storage time (5 years) and/or to the viability of the seed, which was found to be only ca. 45% compared to ca. 99% for Span and Torch seeds. Appelqvist and Johansson (1972) reported the values ranging from 1.3 to 2.8% for winter rape and 1.7 to 2.5% for winter turnip rape stored for 5 to 33 months. Higher percentages of FFA were reported for oil from germinated, mold infested or cracked

seeds. Furthermore, there is a parallel increase in FFA content with the decrease in viability during storage of rapeseed (Appelqvist and Loof, 1972; Ruhowicz, 1972).

The FFA contents of aqueous extracted crude oils from Span seeds dried overnight at 55 and 105°C were 0.14 and 0.17% respectively. The [illegible] extracted crude oils from Span seed boiled and dried [illegible] at 105°C and from Torch seed dried for [illegible] 55°C contained 0.16 and 0.12% FFA respectively. These results indicate that the FFA level in aqueous extracted crude oils was not affected by the heat treatments of the seed. The low levels of FFA in aqueous extracted crude oils is probably due to the quick inactivation of lipase by boiling. Inactivation of lipase is rapid at 90 to 100°C in an aqueous medium (Anjou, 1972).

During refining of the oil, FFA is normally removed by alkali or steam refining and is also removed in the deodorization process. The removal of FFA is essential to prevent any off-flavor development in the oil. Hence, the level of FAA in the oil represents a direct loss in the processing (Moysey and Norum, 1975). The total refining loss of industrial extracted crude oils from good quality seed, due to FFA and phospholipids, is ca. 8% or less (Downey et al., 1974). Thus, because of low levels of FFA and phospholipids, the refining loss of aqueous extracted crude oils would be comparatively low.

d) Peroxide Value

The peroxide values of aqueous, soxhlet and industrial extracted crude oils are shown in Table 11. The results show that the peroxide values of aqueous extracted crude oils of untreated seed were slightly higher (1.10 mequiv/kg of oil for the Span variety and 1.39 mequiv/kg of oil for the Echo variety) than soxhlet extracted crude oils (0.62 mequiv/kg of oil for the Echo variety and 0.74 mequiv/kg of oil for the Torch variety) but lower than industrial crude oil (5.45 mequiv/kg of oil). Appelqvist (1967) has reported that if the oil was extracted with all possible precautions, it would contain no detectable peroxide, however, if no precautions were taken, the peroxide value ranged from 4.54 ± 0.21 to 5.85 ± 0.48 mequiv/kg of oil. Higher values were reported for oils from heat-damaged, non-viable seeds (Appelqvist, 1967) and mechanically damaged seeds (Rutkowski and Makus, 1959).

Low peroxide values of the soxhlet extracted crude oils obtained in this study could be due to the minimal heat treatments of the rapeseed material and shorter time of exposure to light during the extraction (the soxhlet extraction was normally carried out overnight). Slightly higher values of peroxide in the aqueous extracted crude oils were probably due to the heat treatment during

Table 11. Peroxide Value of the Oils

Sample and Treatment	Peroxide Values in mequiv/kg of Oil*
A. Industrial crude oil	5.45
B. *B. campestris* L.var. Echo	
1. Aqueous extracted crude oil (untreated seed)	1.39
2. Soxhlet extracted crude oil (untreated seed)	0.62
C. *B. campestris* L. var. Span	
1. Aqueous extracted crude oils	
a) Untreated seed	1.10
b) Seed dried at 55°C for ca. 17 h	1.61
c) Seed dried at 105°C for ca. 17 h	1.32
d) Seed boiled and dried at 105°C for ca. 17 h	1.85
D. *B. campestris* L. var. Torch	
1. Aqueous extracted crude oil (seed dried at 55°C for 3 days)	2.03
2. Soxhlet extracted crude oil (untreated seed)	0.74

* average of two analyses. All samples under B, C and D were composite samples of at least five extractions each.

boiling, blending, stirring and thawing the frozen emulsion and longer exposure of the oil to light. Heat treatment of the seeds and exposure of oil to light and heat was found to result in higher peroxide values (Appelqvist, 1967).

In this study, dry heating of the seed in an oven caused an increase in the peroxide value of the oils. Compared to that of untreated seed, there was a slight increase in peroxide value when the seed of Span variety was dry heated in the oven overnight at 55°C (1.61 mequiv/kg of oil) and 105°C (1.32 mequiv/kg of oil). Higher peroxide value (2.03 mequiv/kg of oil) was obtained when the Torch seed was heated at 55°C for 3 days. These results indicate that the peroxide value increased with the amount of heat used in drying rapeseed in an oven. A higher peroxide value (1.85 mequiv/kg of oil) was also obtained when Span seed was boiled and dried in an oven overnight at 105°C. Since boiling of rapeseed would inactivate the enzyme lipoxidase, the increase in peroxide value during drying in an oven and during the aqueous extraction process would be due to non-enzymatic reactions. The results also indicate that storing of Echo seed for 5 years at 5°C did not seem to affect the peroxide value. In their study, Janicek and Pokorny (1963) observed that peroxide values changed irregularly during the storage of the seed.

e) Unsaponifiable Matter

The amounts of unsaponifiable matter in the oils are given in Table 12. The amounts of unsaponifiable matter were; 1.05 and 1.13% in aqueous extracted crude oils from untreated Echo and Span seeds respectively, 1.14 and 0.74% in soxhlet extracted crude oils from untreated Echo and Torch seed respectively, and 1.03% in industrial crude oil. The results from varieties Echo and Torch indicate that there was no significant difference in the amounts of unsaponifiable matter in oils obtained by aqueous and soxhlet extractions. Comparing the aqueous and industrial extracted crude oils from Span seed, there seems to be no significant effect of the two oil extraction methods on the amounts of unsaponifiable matter in the oils. With the exception of the value from the Torch variety, the amounts of unsaponifiable matter in the aqueous and soxhlet extracted crude oils were higher than those of industrial extracted crude oils (0.3 to 0.8%) reported by Teasdale (1975).

Varietal difference in the amount of unsaponifiable matter was observed in this study. Lower amounts were found in oils from Torch seed than Span and Echo seeds. Similarly, this varietal difference was also observed by Sietz (1967) and Appelqvist (1968). Sietz (1967) reported that the unsaponifiable matter of German cultivars

Table 12. Unsaponifiable Matter in the Oils

Sample and Treatments	Percent Unsaponifiable Matter*
A. Industrial crude oil	1.03
B. *B. campestris* L. var. Echo	
1. Aqueous extracted crude oil (untreated)	1.05
2. Soxhlet extracted crude oil (untreated)	1.14
C. *B. campestris* L. var. Span	
1. Aqueous extracted crude oils	
a) Untreated seed	1.13
b) Seed dried at 55°C for ca. 17 h	1.19
c) Seed dried at 105°C for ca. 17 h	1.25
d) Seed boiled and dried at 105°C for ca. 17 h	1.32
D. *B. campestris* L. var. Torch	
1. Aqueous extracted crude oil (seed dried at 55°C for 3 days)	0.75
2. Soxhlet extracted crude oil (untreated seed)	0.74

* average of two analyses. All samples under B, C and D were composite samples of at least five extractions each.

of rapeseed as; 0.3% for winter rape 0.6% for summer rape and winter turnip rape, and 0.8% for summer turnip rape. Higher values were obtained by Appelqvist (1968) for Swedish cultivars; 0.9% for winter rape, 1.0% for summer rape, 0.8% for winter turnip rape and 1.1% for summer turnip rape. The amounts of unsaponifiable matter in aqueous extracted crude oils from Span seed dried overnight at 55 and 105°C were 1.19 and 1.25% respectively. The aqueous extracted crude oil from Span seed boiled and dried overnight at 105°C contained 1.32% unsaponifiable matter. These results seem to indicate a small increase in the amount of unsaponifiable matter with the heat treatment of the seed.

f) Moisture and Volatiles Content

The moisture and volatile contents of the oils are given in Table 13. The amounts of moisture and volatiles were; 0.034 and 0.045% in aqueous extracted crude oils from untreated Echo and Span seeds respectively, 0.060% in industrial crude oil, and 0.069 and 0.075% in soxhlet extracted crude oils from untreated Echo and Torch seeds respectively. The amounts of moisture and volatiles in the aqueous extracted crude oils were slightly lower than in the industrial and soxhlet extracted oils. Slightly higher values of moisture and volatiles

Table 13. Moisture and Volatiles Content of the Oils

Sample and Treatment	Percent Moisture and Volatiles *
A. Industrial crude oil	0.060
B. B. campestris L. var. Echo	
1. Aqueous extracted crude oil (untreated seed)	0.034
2. Soxhlet extracted crude oil (untreated)	0.069
C. B. campestris L. var. Span	
1. Aqueous extracted crude oil	
a) Untreated seed	0.045
b) Seed dried at 55°C for ca. 17 h	0.054
c) Seed dried at 105°C for ca. 17 h	0.034
d) Seed boiled and dried at 105°C for ca. 17 h	0.040
D. B. campestris L. var. Torch	
1. Aqueous extracted crude oil (seed dried at 55°C for 3 days)	0.032
2. Soxhlet extracted crude oil (untreated seed)	0.075

* average of two analyses. All samples under B, C and D were composite samples of at least five extractions each.

in the industrial crude oil could probably be due to higher peroxide value and FFA, and in the soxhlet extracted crude oils to higher FFA and the solvent (petroleum ether) left in the oils.

The moisture and volatile contents of aqueous extracted crude oils from Span seed dried overnight at 55 and 105°C were 0.054 and 0.034 respectively. The aqueous extracted crude oils from Span seed boiled and dried overnight at 105°C and from Torch seed dried for 3 days at 55°C contained 0.040 and 0.032% respectively. Hence, there seemed to be no significant effect of heat treatment of the seed on the amount of moisture and volatiles in aqueous extracted crude oils. There was also no varietal difference.

4. Results of Meal Analyses

Meal is the by-product of rapeseed oil extraction. It is used within limits for animal feedings. It is also used as a fertilizer. The economic aspects of rapeseed oil extraction depend not only on the amount and the quality of oil extracted, but also on the quality of the meal. Thus, it was important that some research be done on the effects of aqueous oil extraction on the quality of the meal. Investigations in this study were carried out to determine the proximate composition of

the meal, solubility characteristics of the protein, extractability of protein in relation to pH and temperature, amino acid composition, and the glucosinolate content of the meal.

a) Proximate Composition

The proximate analyses of various meals are given in Table 14. The amounts of protein, fiber and ash were calculated on a moisture and oil free basis. The protein contents in percent of varieties Echo, Span and Torch meals obtained by aqueous and soxhlet extractions were 38.5 and 38.2, 40.5 and 41.1, and 39.9 and 39.5 respectively. The protein content of the industrial extracted meal of variety Span, obtained from Canbra Food Co., was 39.9%. These results show that there was no difference between the protein contents of aqueous and soxhlet extracted meals. However, there was a varietal difference. The protein contents of meals from varieties Span and Torch were higher than those of variety Echo. The industrial extracted meal of variety Span contained about the same amount of protein as aqueous and soxhlet extracted meals of the same variety which were prepared in our laboratory.

Table 14. Proximate Composition of Various Meals

Sample	Percent†			
	Protein*	Fiber*	Ash*	Residual Oil
B. campestris L. var.				
1. Echo				
a) Aqueous extracted meal	38.5+	16.5	7.1	5.5
b) Soxhlet extracted meal	38.2	16.0	7.6	0.0
2. Span				
a) Aqueous extracted meal	40.5	15.9	5.8	7.0
b) Soxhlet extracted meal	41.5	16.5	6.8	0.0
c) Industrially extracted meal	39.9	13.1	7.1	0.5
3. Torch				
a) Aqueous extracted meal	39.9	15.4	5.9	7.2
b) Soxhlet extracted meal	39.5	16.0	6.3	0.0

* on a moisture and oil free basis
† average of two analyses

The fiber contents in percent of aqueous and soxhlet extracted meals for the three varieties, Echo, Span and Torch were 16.5 and 16.0, 15.9 and 16.5, and 15.4 and 16.0, respectively. The fiber content of the industrial extracted meal, 13.0%, was lower than those of other meals. These results indicate that there was no significant difference between the fiber contents of aqueous and soxhlet extracted meals.

The ash contents in percent of variety Echo, Span and Torch meals obtained by aqueous and soxhlet extractions were 7.1 and 7.6, 5.8 and 6.8, and 5.9 and 6.3, respectively, while the ash content of industrial extracted meal was 7.1%. These results show that the ash contents of the aqueous extracted meals of these three varieties were slightly less than those of soxhlet extracted meals, and that the ash contents of variety Echo meals were slightly higher than those of varieties Span and Torch.

The amount of residual oil in soxhlet extracted meals was assumed to be 0.0%. The aqueous extracted meals of varieties Echo, Span and Torch contained 5.5, 7.0 and 7.2% residual oil, respectively. The amount of residual oil in industrial extracted meal was only 0.5%. The higher amounts of residual oil in aqueous extracted meals, due to a lower oil extraction efficiency during this process, will be discussed later. A higher amount

of residual oil would increase the metabolizable energy of the meals. On the other hand, it would also tend to cause off-flavor, and, hence, storage stability problems (Cater et al., 1974).

During the aqueous extraction process, simple sugars, some proteins, inorganic compounds and some non-protein nitrogen compounds including glucosinolates would likely be extracted in the liquid fraction. Phenolic constituents such as tannins (Schanderl, 1970), and caffeic and chlorogenic acids (Lo and Hill, 1972) would also be extracted in the liquid fraction. Due to the extraction of these compounds from the seed, the fiber and protein contents of the aqueous extracted meals would be expected to be higher than those of soxhlet extracted meals. However, in the case of crude fiber content, the results did not clearly indicate this to be so. The extraction of the non-nitrogen compounds in the liquid fraction would increase the protein contents of the aqueous extracted meals, however, the loss of some proteins and non-protein nitrogen (to be discussed) in the liquid fraction seemed to maintain the protein content in the aqueous extracted meals at the level comparable to that of solvent extracted meals. The ash contents of aqueous extracted meals were slightly lower than those of soxhlet extracted meals, indicating that comparatively more ash material was

extracted in the liquid fraction during the aqueous extraction process.

b) Solubility Characteristics of Meal Protein

The solubility characterists of the proteins in aqueous and soxhlet extracted meals from B. campestris L. var. Echo are shown in Table 15. The results from the same variety of rapeseed obtained by Sosulski and Bakal (1969) are included in the table for comparison. The aqueous extracted meal contained 17.7% water soluble proteins, 16.4% salt soluble proteins, 28.9% alkali soluble proteins, and 20.4% ethanol soluble proteins. The soxhlet extracted meal contained 33.6% water soluble proteins, 36.7% salt soluble proteins, 18.9% alkali soluble proteins, and 2.2% ethanol soluble proteins. These results show that aqueous extracted meal contained lower amounts of water and salt soluble proteins but higher amounts of alkali and ethanol soluble proteins than either the soxhlet extracted meal or the meals analyzed by Sosulski and Bakal (1969).

The amount of proteins in the residue left after the protein extractions was higher in aqueous extracted meal (16.6%) than in soxhlet extracted meal (8.5%) but slightly lower than the 19.5% reported by Sosulski and Bakal (1969). These changes in solubility characteristics were due to the moist heating during the aqueous

Table 15. Solubility Characteristics of Protein in the Meals as Determined by the Osborne Series of Four Solvents

Sample	Percent of total meal nitrogen soluble in				Percent nitrogen in residue
	H_2O	5%NaCl	70%EtOH	0.2%NaOH	
Brassica campestris var. Echo					
1. Soxhlet extracted meal	33.6*	36.7	2.2	18.9	8.5
2. Aqueous extracted meal	17.7	16.4	20.9	28.9	16.6
3. Aqueous extracted meal (without blending)	17.2	16.7	25.7	27.0	13.5
4. Results from Sosulski and Bakal (1969)	44.5	25.0	4.4	6.6	19.5

* average of two analyses

extraction process. In this aqueous extraction process, boiling for 5 min, blending for 15 min (temperature could go up to ca. 90°C), and stirring at 70°C ± 2°C for 1 h would affect the solubility characteristics of the proteins. Hence, the moist heat in the aqueous extraction process reduced the water and salt soluble proteins but increased the alkali and, to a greater extent, the ethanol soluble proteins. It also increased the amount of protein in the residue. Longer time and increased temperature of moist heat led to a progressive insolubilization of rapeseed protein in water and salt solution, whereas dry heat has a negligible effect on protein solubility (Girault, 1973). Similarly, due to heating, the solubility of protein in industrial processed meal was lower than laboratory defatted meals (Sosulski and Bakal, 1969; Girault, 1973).

These results also show that about the same amount of soxhlet extracted meal protein dissolved in water and in salt solution. The water soluble proteins in soxhlet extracted meal were lower, and salt and alkali soluble proteins were higher than those reported by Sosulski and Bakal (1969), however, the sum of these two protein fractions was similar to that reported by Sosulski and Bakal (1969). The difference in the protein solubility characteristics of soxhlet extracted meal

obtained in this experiment and those of Sosulski and Bakal (1969) could be due to the method of meal preparation or to the storage conditions for the seed used in this experiment. Sosulski and Bakal (1969) prepared their meal by extracting the oil from ground seed twice with hexane (solid-to-solvent ratio, 1:10) in a Waring blender for 1 h at room temperature and then desolventized the meal in a vacuum chamber at 45°C for 24 h. In this experiment, the soxhlet extracted meal was prepared by heating the seed in an oven for 1 h at 110°C, extracting the oil with petroleum ether in a soxhlet apparatus, and allowing the solvent to evaporate from the meal at room temperature.

One experiment was carried out to find the effect of blending on the solubility characteristics of the protein in the meal. In this experiment, the blending step was omitted from the aqueous extraction procedure. The resulting meal contained 17.2% water soluble proteins, 16.7% salt soluble proteins, 27.0% alkali soluble proteins and 25.7% ethanol soluble proteins. The amount of protein in the residue left after the protein extractions was 13.5%. Compared with the protein solubility characteristics of the meal obtained from the complete aqueous extraction, it seemed that blending caused a slight reduction in ethanol soluble fraction and a slight increase in the alkali soluble fraction. Blending also resulted in an increase in the

amount of protein left in the residue after the protein extractions. These results point to the fact that the boiling step was probably the major contributor to the changes in the protein solubility characteristics since it was the first heat treatment in the aqueous extraction process. It is likely that the subsequent heat treatments during blending and stirring would cause only minor change in the protein solubility characteristics. However, further work is necessary to investigate the effect of each step of the aqueous extraction process upon the protein solubility characteristics of the meal.

c) Extractability of Meal Protein in Relation to pH and Temperature

The extractability of rapeseed protein of aqueous extracted meal in relation to pH and temperature is shown in Fig. 14. At all pH values, except 2 and 10.7, the amount of protein extracted increased with the increase in the extraction temperature from 24 to 80°C. At pH 2, the amounts of protein extracted at 70 and 80°C were about the same, but both were lower than that extracted at 60°C. At a pH of 10.7, the amount of protein extracted at 60°C was ca. 54%, which was higher than that extracted at 70 and 80°C. Reports in the literature also reveal that the amount of protein extracted from

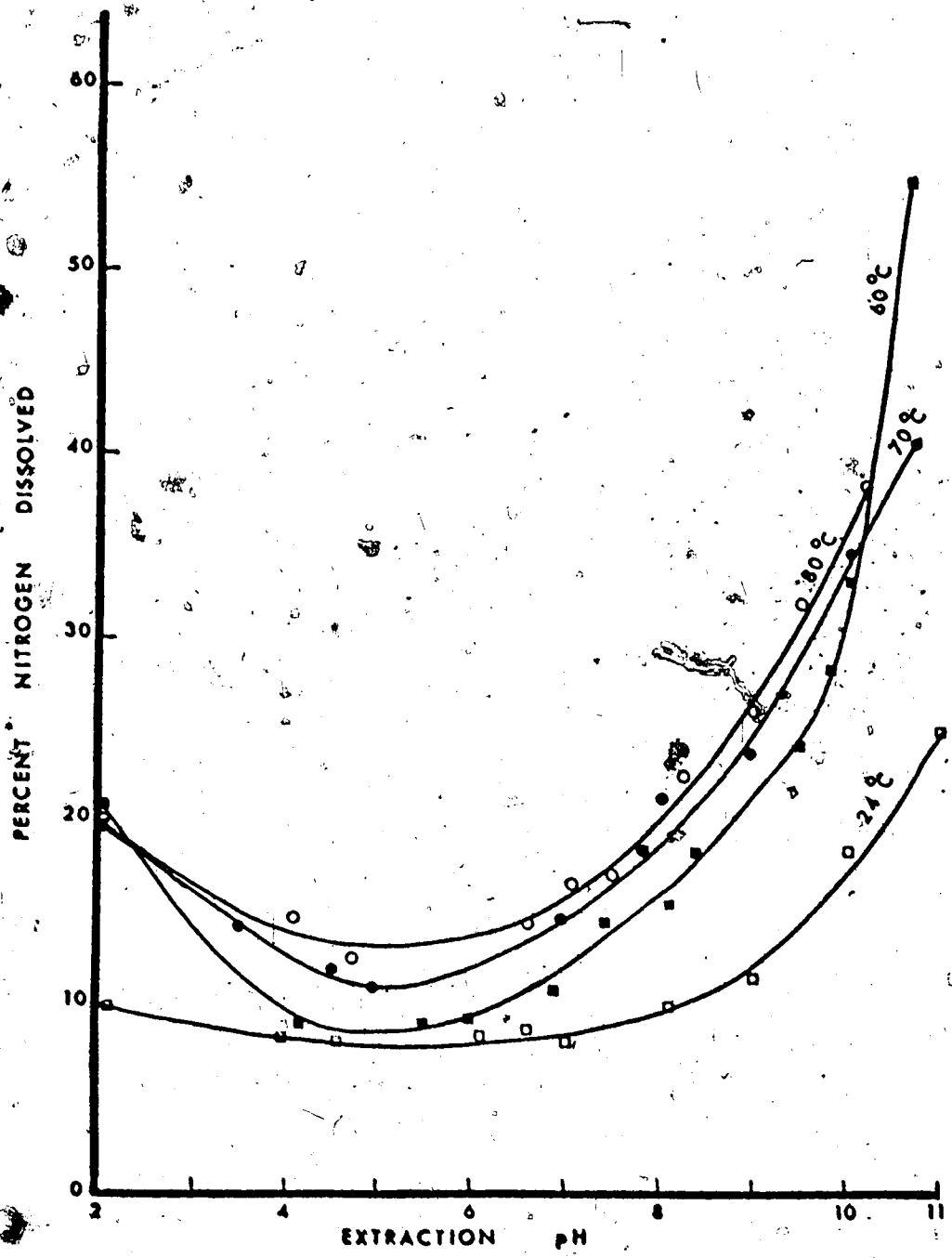

Fig. 14. Extractibility of Meal Protein in Relation to pH and Temperature

rapeseed meal at various pH values increased with the increase in extraction temperature from 25 to 90°C (Radwan and Lu, 1976; Hermansson et al., 1974). In comparison in this study, the amounts of protein extracted from aqueous extracted meal at various pH values and temperatures were lower than those extracted from different meals as reported by Korolczuk and Rutkowski (1971); Hermansson et al. (1974); Quinn and Jones (1976); Radwan and Lu (1976). This may be due to the moist heat treatments during the process (already discussed).

The results indicate that, with aqueous extracted meal, the minimum point of protein extraction at all temperatures studied was at about pH 5.0. The graphs also indicate that the minima were broader at lower than at high temperatures. This was especially true for the protein extractability at 24°C. At this extraction temperature a significant increase of the extracted protein above the minimum point was only observed at about pH 2 in the lower pH range and at about pH 8 in the higher pH range. Various minimum points of extractability for soxhlet extracted meals were reported in the literature (Korolczuk and Rutkowski, 1971; Radwan and Lu, 1976). Two minimum points of extractability reported by Korolczuk and Rutkowski (1971) were not observed in the present study. The results show that the minimum

extractability point increased with temperature, giving about 8, 9, 11 and 13% of the protein at 24, 60, 70 and 80°C, respectively. Similar results were reported by Radwan and Lu (1976).

These results also indicate that, with the temperatures and pH values studied, a better extraction of protein from aqueous extracted meal was obtained at pH values higher than 10. However, at high pH values the protein extracting solutions were dark in color, which could result in dark protein precipitates. This dark coloration may be due to the solubilization of dark pigments of the hull and to browning reactions, especially at high pH and temperature.

d) Amino Acid Composition of Original Rapeseed Sample, Soxhlet and Aqueous Extracted Meals and Liquid Fraction (B. campestris L. var. Echo)

The amino acid contents of four rapeseed protein samples are shown in Table 16. The four samples analyzed were the original rapeseed sample, soxhlet and aqueous extracted meals, and the liquid fraction (whey) from the aqueous extraction procedure. The original rapeseed sample was prepared by dry grinding the rapeseed before it was analyzed. Comparison of the results shows very little difference in the amino acid composition of the

Table 16. Amino Acid Composition of Four Samples from B. campestris L. var. Echo (g amino acid/16g meal N)

Amino Acid	Original Rapeseed Sample	Soxhlet Extracted Meal	Aqueous Extracted Meal	Liquid Fraction (whey)
Aspartic acid	6.63*	6.60	6.37	7.85
Threonine	4.76	4.65	4.51	3.64
Serine	4.88	4.77	4.85	4.41
Glutamic acid	15.15	15.28	14.81	16.96
Proline	7.54	7.86	8.47	4.15
Glycine	8.40	8.43	8.28	9.97
Alanine	6.37	6.28	6.14	7.39
½ Cystine	0.75	0.47	0.62	Trace
Valine	5.79	5.79	5.87	4.09
Methionine	1.54	1.77	2.02	1.78
Isoleucine	3.96	3.98	4.15	2.55
Leucine	6.92	6.88	7.19	4.66
Tyrosine	1.86	1.93	1.99	1.91
Phenylalanine	2.89	2.87	3.01	1.43
Histidine	2.25	2.22	2.18	1.64
Lysine	5.31	5.20	4.85	5.12
Ammonia	10.81	10.80	10.39	17.23
Arginine	4.17	4.20	4.27	3.20

* average of two analyses

soxhlet extracted meal and the original rapeseed sample. However, the original rapeseed sample contained slightly more ½ cystine and less proline than the soxhlet extracted meal.

The amino acid composition of aqueous extracted meal varied slightly from that of the original rapeseed sample and the soxhlet extracted meal. The aqueous extracted meal contained slightly less lysine and glutamic acid and slightly more methionine, leucine and proline than both the soxhlet extracted meal and the original rapeseed sample. This slight difference in amino acid composition could not be due to the aqueous oil extraction process, since boiling in excess water usually gives no protein damage (Bender, 1972; Renner et al., 1953b; Taira et al., 1965). Furthermore, it has been shown that the amino acid composition of rapeseed protein varied according to protein fractions and preparations (Kodagoda et al., 1973; Girault, 1973; Sosulski and Sarwar, 1973; Sarwar et al., 1975; Thompson et al., 1976). Hence, this difference is likely due to the fact that some proteins and free amino acids were extracted in the liquid fraction.

Proteins in the liquid fraction had a different pattern of amino acid distribution. They contained higher amount of threonine and aspartic acid, glutamic acid,

glycine, alanine, and lower amounts of ½ cystine, valine, isoleucine, leucine, phenylalanine, histidine, arginine, and proline than those of soxhlet and aqueous extracted meals and the original rapeseed sample. The amino acid composition in the liquid fraction was quite similar to the amino acid distribution in a water extracted protein concentrate as reported by Kodagoda et al. (1973).

e) Glucosinolate Contents

One of the advantages of the aqueous extraction of rapeseed oil is the simultaneous extraction of glucosinolates (Table 17). The glucosinolate content of soxhlet extracted meals from B. sampestris L. var. Echo, Span and Torch were 8.43, 9.18 and 6.52 mg/g of sample, respectively, and were reduced to 2.83, 1.92 and 2.78 mg/g of sample in aqueous extracted meals. Hence, 58 to 79% of the glucosinolates were removed from the meal during the aqueous oil extraction process. Extraction of individual glucosinolate was 46 to 79% for butenyl isothiocyanate, 41 to 71% for pentenyl isothiocyanate, 63 to 75% for methylsulphinylbutyl isothiocyanate, 67 to

Table 17. Glucosinolate Content of the Meals

Sample	Butenyl -	Pentenyl -	Methylsulplinyl-butyl -	[illegible]	oxazolidine-thione	Total Glucosindlates
	Isothiocyanate (mg/g)				mg/g	mg/g
1. B. campestris var. Span						
a) Soxhlet extracted meal	4.00*	2.88	0.20	0.47	1.63	9.18
b) Aqueous extracted meal	0.83	0.84	0.05	0.06	0.14	1.92
2. B. campestris var. Torch						
a) Soxhlet extracted meal	2.70	2.00	0.08	0.18	1.56	6.52
b) Aqueous extracted meal	1.46	1.19	0.03	0.06	0.04	2.78
3. B. campestris var. Echo						
a) Soxhlet extracted meal	3.01	3.00	0.63	0.0	1.79	8.43
b) Aqueous extracted meal	1.34	1.00	0.22	0.0	0.27	2.83
c) Aqueous extracted meal (after second extraction)	0.0	0.0	0.0	0.0	0.09	0.09

* average of two analyses

87% for phenylethyl isothiocyanate and 85 to 97% for vinyl-oxazolidinethione. The results indicate that vinyl-oxazolidinethione was more easily extracted than the isothiocyanates during aqueous oil extraction process. These results were contrary to some findings that vinyl-oxazolidinethione is more difficult to extract than isothiocyanates (Ballester et al., 1970 and 1973; Kozlowska et al., 1972a).

Glucosinolates were extracted into aqueous phase during the boiling, blending and stirring steps. It is apparent that grinding the rapeseed material to fine particles enhanced the extraction of glucosinolates in the subsequent steps. The diffusion rates of glucosinolates are enhanced by frequent change of solvent, high temperature, a high water-to-seed ratio, a neutral to alkaline pH of the aqueous medium and dehulling of the seed (Sosulski et al., 1972; Kozlowska et al., 1974)

An experiment was carried out in which the glucosinolates left in the aqueous extracted meal of Echo variety were further extracted by stirring for 1 h at 70°C and pH 5.0 with an s/w ratio of 1:15. A pH of 5.0 was used during this extraction because it was the minimum point of meal protein solubility (already discussed). Only a trace amount of vinyl-oxazolidinethione

(0.09 mg/g) was left in the meal after this second extraction. About 99% of the glucosinolates were extracted after this second extraction.

5. Protein in the Liquid Fraction

Liquid fraction (whey) from the aqueous oil extraction process contained ca. 1% crude protein of which about 50% remained after precipitation with 15% TCA (Table 18). Hence, the true protein amounted to ca. 50% of the crude protein in the liquid fraction. A study was carried out on the precipitation of these proteins by adjusting the liquid fraction to different pH values and by heating in boiling water for 10 to 15 min. After heating, the precipitated protein was centrifuged at 9,000 rpm (9,750 x g) for 15 min and the crude protein in solution after the centrifugation was determined. The results show that, after heating the liquid fraction at pH 4.0 and 4.5, 0.68% of crude protein remained in the solutions. At lower and higher pH values more crude protein remained in the solutions after the heat treament. These results indicate that pH values between 4.0 to 4.5 were the region of maximum protein precipitation. At the point of maximum protein precipitation, ca. 65% of the true protein, which is equivalent to ca. 32% of the total N in the

Table 18. Precipitation of Proteins From the Liquid Fraction

Sample and Treatment	Percent N Left	Percent Crude Protein (N x 6.25) Left
Original Liquid Fraction (Whey)	0.162	1.01
Treatment		
1. Boiling in H_2O for 10-15 min at pH		
2.0	0.134	0.8
3.0	0.117	0.73
3.5	0.112	0.70
4.0	0.109	0.68
4.5	0.109	0.68
5.0	0.110	0.70
6.0	0.133	0.83
7.0	0.139	0.87
2. Heating at 85°C for 10 min at pH		
4.0	0.107	0.67
3. Precipitation with 15 percent TCA	0.081	0.50

aqueous fraction, was precipitated. It was observed that heating accelerated the precipitation of the protein.

One experiment was carried out in which the liquid fraction was adjusted to pH 4.0 and heated at 85°C for 10 min. It was found that about the same amount as above of crude protein (0.67%) remained in the solution after these treatments (Table 18). Hence, heating at 85°C for 10 min was sufficient to precipitate the proteins. The fact that the aqueous medium resulting from this procedure was lighter in color than that after heating in boiling water for 15 min was probably due to less brow action. Furthermore, a finer precipitate was obtained during heating at 85°C for 10 min than during heating in boiling water for 10 to 15 min.

During the determination of residual lipid in the liquid fraction by extracting with a mixture of methanol and chloroform, it was observed that proteins precipitated out. However, this was not studied further.

6. Material Balance

An experiment was carried out in duplicate to determine the overall material balance of the aqueous extraction process. In this experiment, a great care was taken to recover all the material adhering to the

equipment used during each extraction step. The rapeseed (Echo variety) was ground and weighed (208.8 g). The material was boiled in 500 ml of water. Additional 150 ml of water was used for washing the equipment. The results of this experiment are summerized in Table 19. These results show that of the total rapeseed solid approximately 41.5% was recovered as oil and 50.1% as meal; 7.2% of the rapeseed solid was lost in the whey.

Of the original 88.7g oil in the seed, 81.8 g (i.e. ca. 92%) was recovered in the oil fraction. This yield was in agreement with the previously calculated yield values obtained throughout this study.

The amounts of total solids in the solid and liquid fractions were 98.8 g and 14.2 g respectively. Assuming that the meal contained ca. 7% oil (based on previous analyses), the amount of non-fat solid recovered in the meal would be ca. 92 g. Since the amount of non-fat solid of the starting material was 108.5 g, the recovery of the non-fat solid in the meal was ca. 85%. Together with 14.2 g of solid lost in whey, the total recovery of non-fat solid was ca. 98%.

Although ca. 650 g of water was used during the experiment only ca. 260 g was obtained in the liquid fraction. Hence, only ca. 40% of water used was recovered as liquid fraction. Some of the remaining water was lost

Table 19. Material Balance of the Aqueous Processing of Rapeseed

Starting materials.

1) rapeseed, 208.8 g (containing 88.7 g oil and 108.5 g non-fat solid)

2) water, 500 g for boiling and 150 g for washing.

	Oil	Meal	Whey
1. Material recovered (g)	81.8	≈ 330*	274
2. Dry matter (g)	81.8	98.8	14.2
3. [illegible] recovery [illegible] total dry [illegible] of seed)	41.5	50.1	7.2
4. Non-fat solid (g)		≈ 91.9*	N.D.
5. Percent recovery			
(a) oil	92.2	≈ 7.8	N.D
(b) non-fat solid	-	84.7	13.1

* estimated values

in drying the solid fraction which was previously analyzed to contain ca. 70% moisture. The remaining water was lost during the experiment especially during boiling and blending.

IV. CONCLUSIONS

1. Process Parameters

The results of this study led to the finalized laboratory aqueous rapeseed oil extraction processing steps shown in Fig. 15.

Grinding is a very important step in aqueous extraction of rapeseed oil. It should be emphasized here that the finer the material, the better the extraction. The second grinding was conducted to ensure that the rapeseed material was sufficiently ground. Unlike the aqueous extraction of coconut, peanut, and sunflower seed oils, boiling was a necessary step in aqueous extraction of rapeseed oil to inactivate myrosinase in order to control the sulfur level in the oil. It also inactivates lipase, thus resulting in a low free fatty acid content in the oil. In addition, boiling the ground rapeseed caused a further breakdown of the cells. However, it also caused denaturation of the rapeseed proteins resulting in low solubilities in water and salt solution.

Blending, although it gave a stable emulsion, was necessary for extraction of rapeseed oil. Without blending nearly all the oil obtained after centrifugation was free oil, however, the oil yield was low. Blending

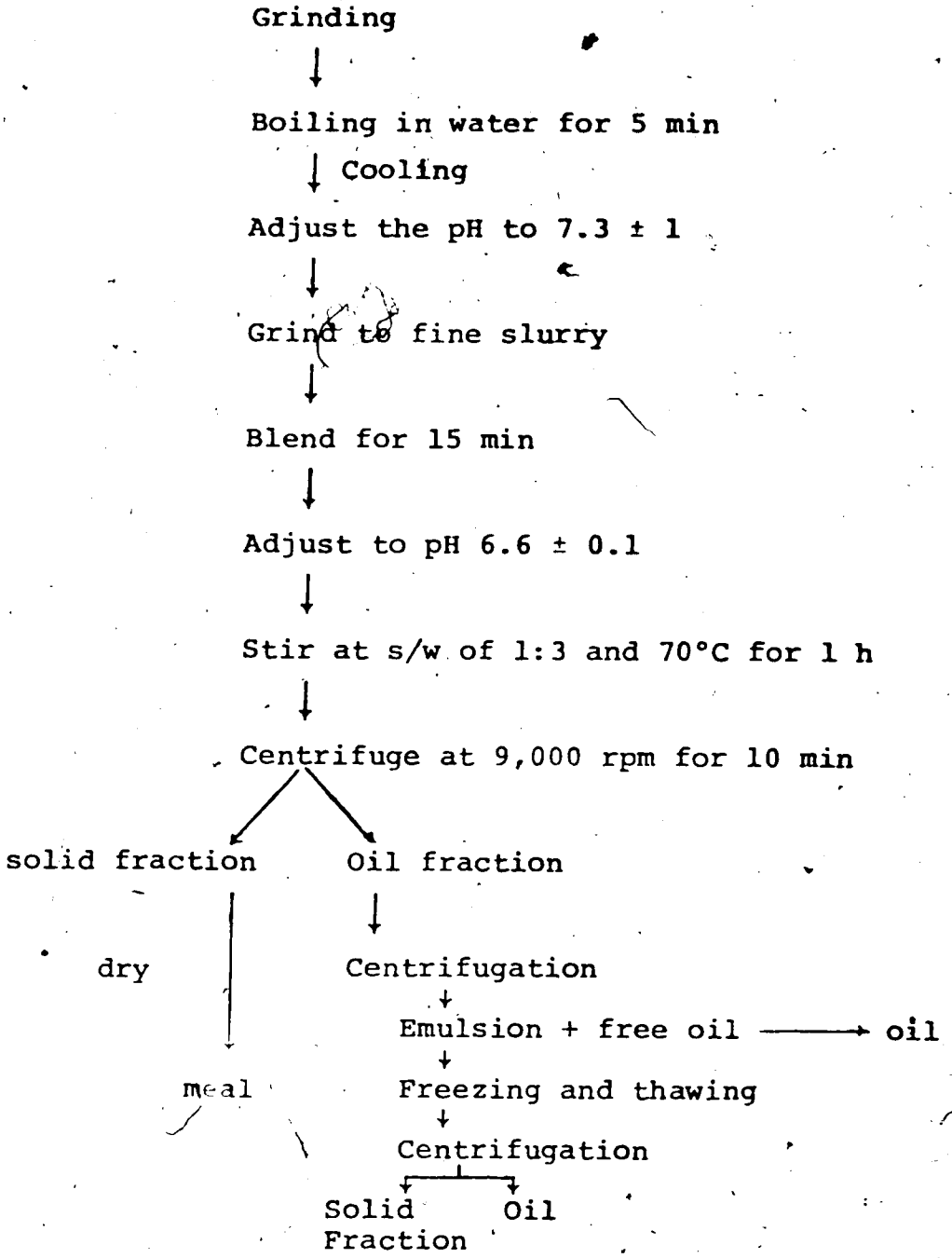

Fig. 15. Recommended Parameters for the Aqueous Rapeseed Oil Extraction

caused a further breakdown of the cells, resulting in an increase in oil yield. Stirring conditions, solid-to-water ratio, pH, temperature and time were studied. The results show there were optima for solid-to-water ratio, pH, temperature and time of stirring for the maximization of the yield. The importance of the centrifugation step was not realized in previous studies on aqueous extraction of oils. This study shows that the amount of oil extracted depended on the come-up time to reach the final speed or the rate of change of acceleration, rather than on the final speed or final acceleration of the centrifugation. Time of centrifugation after reaching the final speed did not affect the oil yield.

Some modifications of the process could be made for future studies on the aqueous extraction of rapeseed oil. In this study the centrifugation step was performed after the stirring step. However, a centrifugation step could be introduced after the second grinding step, so that some of the oil could be extracted prior to blending, stirring and centrifugation. The oil obtained after this proposed centrifugation step would be mainly in the form of free oil. Thus, less oil would be in the form of an emulsion after the subsequent blending, stirring and centrifugation steps. It follows that less emulsion would then need to be demulsified. The introduction of another centrifugation step might very likely

increase the oil yield.

A centrifugation step might also be introduced after the blending step. In this case a second blending would be introduced after this centrifugation. After the second blending step the material could be stirred and recentrifuged. The addition of these two steps would be expected to increase the oil yield.

A third modification of the aqueous oil extraction process could be a multiple extraction cycle. The liquid and solid fractions after the centrifugation step would again be blended, stirred and centrifuged. This multiple extraction cycle would obviously increase the oil yield.

2. The Yield of Aqueous Rapeseed Oil Extraction

In any vegetable oil extraction process, the yield of oil is the most important factor determining the economy of the extraction process. Other factors which affect the economy of an extraction process are the quality of oil and meal. Thus, the yield of oil by this aqueous extraction of rapeseed oil is critical in determining whether the process is feasible.

In this study the calculation of yield of oil was based on the amount of residual oil left in the dry meal. The maximum yields obtained were about 95, 95 and 93% for

B. campestris L. var. Echo, Span and Torch, respectively. The amounts of residual oil in aqueous extracted meals ranged from 4 to 7% for the three varieties. Comparable yields (86 to 96%) were reported in the literature for aqueous extractions of peanut, coconut and sunflower seed oils (Bhatia et al., 1966; Rhee et al., 1972; Hagenmaier et al., 1972; Hagenmaier, 1974; Mieth et al., 1975a). The yield of rapeseed oil in our laboratory aqueous extraction was lower than prepress solvent and straight solvent extractions (0.5 to 4.0% residual oil), but equivalent to expeller extraction (6.0 to 7.0% residual oil).

The above yield of aqueous oil extraction was obtained after only one extraction cycle. The yield should increase with the number of extraction cycles (Hagenmaier et al., 1972 and 1973; Rhee et al., 1973c; Mieth et al., 1975a, b). In one experiment the liquid fraction after centrifugation was replaced with water, which was then mixed with the solid fraction and recentrifuged. This treatment further increased the amount of oil extracted by 1.5 to 2%. The addition of salt was not included in this study, although it was found to increase the yield of sunflower seed oil (Mieth et al., 1975b). The use of salt in aqueous extraction of rapeseed would require an additional step of removing it

from the meal. Further investigation of the aqueous processing of rapeseed oil should be carried out to maximize the oil yield.

3. The Quality of the Oil and Meal

The quality of the aqueous extracted oil was better than industrial crude rapeseed oils. It contained lower amounts of phospholipids, sulfur, and free fatty acids, and a lower peroxide value than the industrial crude oil. Due to low phospholipid and free fatty acid contents, the refining loss during the processing of aqueous extracted rapeseed crude oil would be low. Water degumming of aqueous extracted crude oil would not be necessary. Low sulfur levels in aqueous extracted crude oil are desirable for the hydrogenation process of the oil. Also, the industrial crude oil was darker in color than aqueous extracted crude oil.

The meals obtained by the aqueous oil extraction contained about the same amounts of protein, fiber, and ash as the soxhlet extracted meals. The aqueous extracted meal contained low amounts of water and salt soluble proteins but high amounts of alkali and alcohol soluble proteins. The meal protein was more soluble at high pH and temperature. It would be more appropriate

to prepare protein concentrate rather than isolate from the aqueous extracted meal, since, due to low protein solubilities in water, salt and alkali solution, the recovery of protein in the isolate is expected to be low. However, more studies should be carried out on the protein solubilities of aqueous extracted meal, especially on solubility in relation to high pH and temperature.

The amino acid distribution of protein in aqueous extracted meal, although slightly different than those of soxhlet extracted meal and of the original rapeseed sample, seemed to indicate that the aqueous oil extraction process did not affect the protein quality of the meal. The aqueous extracted meals contained lower amounts of glucosinolates since 58 to 79% of glucosinolates were extracted during the process. If the oil is extracted by multiple extraction steps, more glucosinolates would be extracted. More studies are necessary to determine the factors affecting glucosinolate extraction during the aqueous oil extraction process. However, this glucosinolate problem could be best solved by plant breeding.

4. The Liquid Fraction (Whey)

The liquid fraction from aqueous oil extraction contained only a small amount of protein. However, it is

essential that this protein should be recovered. About 65% of the true protein could be precipitated by heating in boiling water or in water at 85°C at pH 4.0 to 4.5. Further studies on the precipitation of protein from the liquid fraction with salts and organic solvents should be carried out. The whey resulting from aqueous oil extraction process may not be a serious problem since comparatively small amount of water is used during the extraction. More studies concerning the liquid fraction should be carried out.

Reverse osmosis could be used to prepare the concentrate from the liquid fraction. However this could be an expensive undertaking. In addition the resulting concentrate might contain high amounts of simple sugars and glucosinolates. This whey problem could probably be best solved by ultra filtration. Bigger molecules such as proteins would be retained by the membrane, however, small molecules, such as simple sugars and glucosinolates, would be filtered through. The solution containing the small molecules might be used as a medium for single cell protein production.

Glucosinolates could probably also be removed from the liquid fraction by ion-exchange chromatography, however this could be an expensive technique.

5. The Importance of Aqueous Rapeseed Oil Extraction

The slightly lower yield of aqueous oil extraction could be partly balanced by the meal quality and partly by the quality of the oil. Aqueous oil extraction might preserve not only the quality of the proteins in the rapeseed, but also, some of the glucosinolates could be simultaneously extracted. With the introduction of low glucosinolates and low fiber rapeseed varieties, along with the good quality of rapeseed proteins, there will be a wider use of rapeseed meal for animal feedings and the possibility of uses in human foods. The importance of rapeseed meal should increase in the light of the current world situation in fats and oils production.

In recent years, the demand for meals has been relatively stronger than for vegetable oils. In some seasons, soybean oil production is mainly determined by the more rapidly increasing meal demand and hence the oil is almost considered as just a by-product. Thus, it is in surplus, especially at present. Furthermore, there are relatively large increases in supplies of palm and lauric oils (coconut and palm kernel oils). The availability of palm and lauric oils from Malaysia, Indonesia, the Philippines, Ivory Coast and other countries will continue to increase sharply for the next five years.

These palms have a productive life-span of at least 20 years for modern palm varieties, or 50 to 100 years for coconut palms. The marketing of soybean oil is becoming more difficult due to the supplies of low cost lauric oils, and even more so, of palm oil. However, the increase in meal demand could partly offset the costs of oil production. In addition to the increasing surplus of fats and oils, for the first time in about two decades there is a period of decline in consumption (Mielke, 1976). Thus, the future viability of the rapeseed industry may depend on expanding the utilization of meal in animal feeds as well as in developing products suitable for human use.

V. REFERENCES

Afzalpurkar, A.B., K.D. Mukherjee, and H.K. Mangold. 1974. Detoxification of rapeseed meal. International Rapeseed Conf., 4, Giessen, Germany, pp. 609-613.

Alexander, W. 1921. U.S. Patent 1,336,338. Cited by C.L. Puertollano, J. Banzon, and K.H. Steinkraus. 1970. J. Agric. Food Chem., 18: 579-584.

Allen, C.E. and D.S. Dow. 1952. The biological assessment of the nutritive value of rapeseed oilmeal as a dietary component. Sci. Agric., 32: 403-410.

Altschul, A.M., C.M. Lyman, and F.H. Thurber. 1958. Cottonseed meal. In: Processed Plant Protein Foodstuffs. Ed. A.M. Altschul. Academic Press Inc. Pub. New York, pp. 469 - 534.

American Oil Chemists' Society. 1974. Official and Tentative Method. A.O.C.S. Champaign, Ill.

Ames, S.R. 1956. Role of vitamin E (α-tocopherol) in poultry nutrition and disease. Poultry Sci., 35: 145-159.

Andaya, L.F., M.P. Santos, and A.L. Gonzales. 1961. Philipp. J. Sci. 90: 17-24. Cited by C.L. Puertollano, J. Banzon, and K.H. Steinkraus. 1970. J. Agric. Food Chem., 18: 579-584.

Anderson, G.H. G.S.K. Li, J.D. Jones, and F. Bender. 1975. Effect of hydrogen peroxide treatment on the nutritional quality of rapeseed flour fed to weanling rats. J. Nutr., 105: 317-325.

Anjou, K. 1972. Manufacture of rapeseed oil and meal. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 198-217.

Anjou, K. and A. Fecske. 1974. The production of rapeseed protein concentrate. International Rapeseed Conf., 4, Giessen, Germany, pp. 659-662.

Anwar, A. and D.R. Clandinin. 1971. Nitrogen solubility as a means of estimating the gross protein value of rapeseed meal. Poultry Sci., 50: 135-136.

Appelqvist, L.-A. 1961. Soeriges Utsadesforen. Tidskr., 71: 74-97. Cited by L.-A. Appelqvist. 1971. J. Amer. Oil Chem. Soc., 48: 851-859.

Appelqvist, L.-A. 1962. Hereditary variation in content of isothiocyanates and thiooxazolidones in seeds of rape and turnip rape. Acta Chem. Scand., 16: 1284-1285.

Appelqvist, L.-A. 1967. The effect of seed pretreatment and extraction conditions on the amount of minor compounds in seed oils. 1. Lipid peroxides. J. Amer. Oil Chem. Soc., 44: 206-208.

Appelqvist, L.-A. 1968. Lipids in Cruciferae. 1. Fatty acid composition in seeds of some Svalöf varieties and strains of rape, turnip rape, white mustard and false flax. Acta Agric. Scand., 18: 3-21.

Appelqvist, L.-A. 1971. Composition of seeds of Cruciferous oil crops. J. Amer. Oil Chem. Soc., 48: 851-859.

Appelqvist, L.-A. 1972. Chemical constituents of rapeseed. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 123-173.

Appelqvist, L.-A., and S.-A. Johansson. 1972. Unpublished data. Cited by L.-A. Appelqvist and B. Loof. 1972. In: Rapeseed. Cultivation, Composition, Processing, and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 60-100.

Appelqvist, L.-A. and E. Josefsson. 1967. Method for quantitative determination of isothiocyanates and oxazolidinethiones in digests of seed meals of rape and turnip rape. J. Sci. Food Agric., 18: 510-519.

Appelqvist, L.-A. and B. Loof. 1972. Postharvest handling and storage of rapeseed. In: Rapeseed. Cultivation, Composition, Processing, and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 60-100.

Association of Official Agricultural Chemists. 1975. Official Method of Analysis. 12th. ed. Washington, D.C.

Astwood, E.B., M.A. Greer, and M.G. Ettlinger. 1949. J. Biol. Chem. 181:121. Cited J.M. Bell, and R.J. Belzile. 1965. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, D.R. Clandinin, and L.R. Wetter, Can. Dept. Agric. Ottawa, Pub. No. 1257, p. 49.

Austin, F.L., C.A. Gent, and I.A. Wolff. 1968. Degradation of natural thioglucosides with ferrous salts. J. Agric. Food Chem., 16: 752-755.

Babuchowski, K. and Rutkowski, A. 1969. Seifen-Ole-Fette-Wachse, 95:727. Cited by R. Wettstrom. 1972. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, p. 233.

Ballester, D., R. Rodrigo, J. Nakouzi, C.O. Chichester, E. Yanez, and F. Monckeberg. 1970a. Rapeseed meal. II. - Chemical composition and biological quality of the protein. J. Sci. Food Agric. 21: 140-142.

Ballester, D., R. Rodrigo, J. Nakouzi, C.O. Chichester, E. Yanez, and F. Monckeberg. 1970b. Rapeseed meal. III. - A simple method for detoxification. J. Sci. Food Agric., 21: 143-144.

Ballester, D., B. Rodriguez, M. Rojas, O. Brunser, A. Reid, E. Yanez, and F. Monckeberg. 1973. Rapeseed meal. iv. Continuous water extraction and short-term feeding studies in rats with the detoxified product. J. Sci. Food Agric., 24: 127-138.

Bayley, H.S. 1973. Evaluation of rapeseed products as feed for growing pigs. In: Research on Rapeseed. Oil and Meal. Ed. P. Giovannetti and J.M. Bell. Pub. No. 32. Rapeseed Association of Canada, Ottawa, pp. 30-32.

Bayley, H.S. 1974. An examination of the source of variation between the findings of different investigations for the nutritional value of rapeseed meal. International Rapeseed Conf., 4, Giessen, Germany, pp. 471-477.

Bayley, H.S., C.Y. Cho, and J.D. Summers. 1969. Growth and digestibility studies to evaluate rapeseed meal as a protein supplement for swine. Can. J. Animal Sci., 49: 367-373.

Beare-Rogers, J.L. 1975. Vegetable oils and human nutrition. In: Oilseed and Pulse Crops in Western Canada - A symposium. Ed. J.T. Harapiak. Western Co-operative Fertilizers Ltd. pp. 588-603.

Beckman, J.W. 1930. Extracting oils etc. Brit. Patent 326,196. Chem. Abstr., 1930, 24: 4178.

Bell, J.M. 1957a. Growth depressing factors in rapeseed oilmeal. lV. Comparative feeding values of Argentine and Polish types and the effects of suplementary aureomycin, fishmeal, lysine, iodine and thyroid-active substances. Can. J. Animal Sci., 37: 43-49.

Bell, J.M. 1957b. Growth depressing factors in rapeseed oilmeal. III. Studies on counter-action by steam processing, extraction and dietary supplement. Can. J. Animal Sci., 37: 31-42.

Bell, J.M. 1965. Growth depressing factors in rapeseed oilmeal. VI. Feeding value for growing-finishing swine of myrosinase-free solvent-extracted meal. J. Animal Sci., 24: 1147-1151.

Bell, J.M. 1973. Improving rapeseed meal by air-classification. In: Research on Rapeseed. Oil and Meal. Ed. P. Grovannetti and J.M. Bell. Rapeseed Association of Canada, Pub. No. 32, pp. 90-92.

Bell, J.M. 1975. Nutritional value of low glucosinolate rapeseed meal for swine. Can. J. Animal Sci., 55: 61-70.

Bell, J.M. and E. Baker. 1957. Growth depressing factors in rapeseed oil-meal. 11. Studies on counteraction by dietary supplements. Can. J. Animal Sci., 37: 21-30.

Bell, J.M., and R.J. Belzile. 1965. Goitrogenic properties. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, D.R. Clandinin, and L.R. Wetter. Can. Dept. Agric. Ottawa, Pub. No. 1257, pp. 45-60.

Bell, J.M. and T.J. Devlin. 1972. Rapeseed meal for beef cattle and sheep. In: Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada. Pub. No. 16, pp. 21-23.

Bell, J.M., R.K. Downey, and L.R. Wetter. 1967. Oil and Meal from Canadian Rapeseed. Can. Dept. of Agric. Ottawa, Pub. No. 1183, p. 11.

Bell, J.M. and P.M. Giovannetti. 1973. The nutritive value of bin-heated rapeseed. In: Research on Rapeseed. Oil and Meal. Ed. P.M. Giovannetti and J.M. Bell. Rapeseed Association of Canada. Ottawa. Pub. No. 32, pp. 36-40.

Bell, J.M., P.M. Giovannetti, and C.G. Youngs. 1972. Effect of bin heating on the chemical composition and nutritional value of rapeseed fed to mice. Can. J. Animal Sci., 52: 517-524.

Bell, J.M., C.G. Youngs, and R.K. Downey. 1971. A nutritional comparison of various rapeseed and mustard seed solvent extracted meals of different glucosinolate composition. Can. J. Animal Sci., 51: 259-269.

Belzile, R.J., and J.M. Bell. 1963. Unpublished data. Cited by J.M. Bell, 1965. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, Dr.R. Clandinin, and L.R. Wetter. Can. Dept. Agric. Ottawa. Pub. No. 1257, p. 56.

Belzile, R.J., and J.M. Bell, 1966. Growth-depressing factors in rapeseed oil meal. VII. Effects of myrosinase activity on toxicity following treatment with buffered solution. Can. J. Animal Sci., 46: 165-169.

Belzile, R.J., J.M. Bell and L.R. Wetter. 1963. Growth-depressing factors in rapeseed oil meal. V. The effects of myrosinase activity on the toxicity of the meal. Can. J. Animal Sci., 43: 169-173.

Bender, A.E. 1972. Processing damage to protein foods. RAG. Bulletin, 2: 10-19.

Bensabat, L., V.L. Frampton, L.E. Allen, and R.A. Hill. 1958. Heat effect on peanut proteins. Effect of processing on the epsilon-amino groups of lysine in peanut protein. J. Agric. Food Chem., 6: 778.

Bhati, D.S. 1963. Modified Krauss-Maffei process for wet processing coconut. Coconut Bul. 17: 153-160. Cited by J.G. Woodroof, 1970. In: Coconuts: Production, Processing, Products. Ed. Woodroof, J.G. Avi Pub. Co. Inc. Westport, Connecticut, p. 77.

Bhatia, D.S., M.A.B. Parpia, and B.P. Baliga. 1966. Peanut protein isolate - production and properties. J. Food Sci. Technol. (India) 3: 2. Cited by K.C. Rhee, C.M. Cater and K.F. Mattil. 1972. J. Food Sci., 37: 90-93.

Bhatty, R.S., S.L. Mckenzie, and A.J. Finlayson. 1968. The proteins of rapeseed (B. napus L.) soluble in salt solution. Can. J. Biochem., 46: 1191-1197.

Bhatty, R.S., and F.W. Sosulski. 1972. Diffusion extraction of rapeseed glucosinolates with ethanolic sodium hydroxide. J. Amer. Oil Chem. Soc., 49: 346-350.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Bock, H.D. 1967. Wiss. Ztschr. Univ. Rostock, Mat. Naturwiss. Reiche No. 3, p. 153. Cited by A. Rutkowski. 1971. J. Amer. Oil Chem. Soc., 48: 863-868.

Bock, H.D., W. Kracht, T. Vocher, and M. Jaehnke. 1974. Improvement of the quality of rapeseed and extracted rapeseed meal. Tagungsber. Akad. Landwirtschaftswiss. D.D.R. 124: 175-183. Chem. Abstr., 1975, 83: 7933d.

Borosikhino, V.I., L.D. Skrylev, and S.G. Mokreshin. 1961. Colloid J. (U.S.S.R.) 23: 563. Cited by P. Sherman, 1968. In: Emulsion Science, Ed. L.P. Sherman, Academic Press, London, p. 132.

Bousser, R. and I. Bermier. 1969. Extraction of coconut oil from fresh pulp. Fr. Patent 1,564,857. Chem. Abstr. 72: 4197c (1970).

Bowland, J.P., and J.M. Bell. 1972. Rapeseed meal for pigs. In: Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada. Pub. No. 16, pp. 16-20.

Bragg, D.B. 1974. Mineral content and mineral availability of various rapeseed meals. In: Research on Rapeseed. Seed, Oil and Meal. Rapeseed Association of Canada, Ottawa. Pub. No. 35, pp. 106-107.

Bunger, H., E. Fimber, and F. Reising. 1964. Ztschr. Tierern. Futterm. 4: Cited by A. Rutkowski. 1971. J. Amer. Oil Che . Soc., 48: 863-868.

Carter, F.L., V.O. Cirino, and E.A. Layton. 1961. Effect of processing in the composition of sesame seed and meal. J. Amer. Oil Chem. Soc., 38: 148-150.

Cater, C.M., K.C. Rhee, R.D. Hagenmaier, and K.F. Mattil. 1974. Aqueous extraction - an alternative oilseed milling process. J. Amer. Oil Chem. Soc., 51: 137-141.

Carpenter, K.J., C.B. Morgan, C.H. Lea, and L.J. Parr. 1962. Chemical and nutritional changes in stored herring meal. 3. Effect of heating at controlled moisture contents on the binding of amino acids in freeze-dried herring press cake and in related model systems. Brit. J. Nutr., 16: 451-465.

Chamnanwej, N. 1971. Physical and chemical studies of the proteins of rapeseed (Brassica compestris var. Echo) M.Sc. thesis. Food Science Dept., University of Alberta.

Chayen, I.H. 1960. U.S. Patent 2,928,821. Cited by C.M. Cater, K.C. Rhee, R.D. Hagenmaier, and K.F. Mattil. 1974. J. Amer. Oil Chem. Soc., 51: 137-141.

Chayen, I.H., and D.R. Ashworth. 1953. Application of impulse rendering to the animal fat industry. J. Appl. Chem., 3: 529-537.

Christian, B.C. 1958. Rapeseed, mustard-seed, and poppy-seed meals. In: Processed Plant Protein Foodstuffs. Ed. A.M. Altschul. Academic Press Inc. Pub. New York, pp. 577-592.

Clandinin, D.R. 1962. The status of research on rapeseed oil meal as a feedstuff for poultry. Proc., Xllth. Worlds' Poultry Congr., pp. 259-263.

Clandinin, D.R. 1964. Unpublished data. Cited by C.G. Youngs. 1965. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, D.R. Clandinin and L.R. Wetter. Can. Dept. Agric. Ottawa. Pub. No. 1257, p. 30.

Clandinin, D.R. 1967. Nutrient composition of expeller, prepress-solvent, and solvent processed rapeseed meals. Poultry Sci., 46: 1596-1597.

Clandinin, D.R., and L. Bayly. 1960. Effects of feeding rapeseed oil meal on the structure of the thyroid glands in chickens. Poultry Sci., 39: 1239-1240.

Clandinin, D.R., and L. Bayly. 1963. Effect of variety and growing conditions on the protein and amino acid composition of rapeseed. Can. J. Animal Sci., 43: 65-67.

Clandinin, D.R., and J. Heard. 1968. Tannins in prepress-solvent and solvent processed rapeseed meal. Poultry Sci., 47: 688.

Clandinin, D.R., R. Renner, and A.R. Robblee. 1959. Rapeseed oil meal studies. 1. Effects of variety of rapeseed, growing environment and processing temperatures on the nutritive value and chemical composition of rapeseed oil meal. Poultry Sci., 38: 1367-1372.

Clandinin, D.R., and A.R. Robblee. 1976. Low glucosinolate rapeseed meal. In: Agriculture Bulletin. The University of Alberta, Winter 1976, pp. 7-10.

Clandinin, D.R., A.R. Robblee, and S.J. Slinger. 1972a. Rapeseed meal for poultry. In: Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada. Pub. No. 16, pp. 11-16.

Clandinin, D.R., S.J. Slinger, and J.M. Bell. 1972b. Composition of Canadian rapeseed meal. In: Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada. Pub. No. 16, pp. 8-10.

Clandinin, D.R., and E.W. Tajcnar. 1961. Rapeseed oil meal studies. III. Effects of variations in cooking and conditioning temperatures used during processing of rapeseed oil meals. Poultry Sci., 40: 291-298.

Conkerton, E.J., W.H. Martinez, G.E. Mann, and V.L. Frampton. 1957. Changes induced by autoclaving a solvent-extracted cottonseed meal. J. Agric. Food Chem., 5: 460-463.

Couche, R. A. 1967. Extraction of oil from palm fruit and olives. Neth. Patent 6,615,754. Chem. Abstr. 1967, 67: 99069x.

Cravens, W.W. and E. Sipos. 1958. Soybean oil meal. In: Processed Plant Protein Foodstuffs. Ed. A.M. Altschul. Academic Press Inc. Pub. New York, pp. 353-397.

D'Aquin, E.L., H.L.E. Vix, J.J. Spadars, A.V. Graci, P.H. Eaves, C.G. Reuther, K.J. Molaison, C.J. McCourtney, A.J. Crovetto and E.A. Gastrock. 1953. Filtration-extraction of cottonseed. Ind. Eng. Chem., 45: 247-254.

Dangoumau, A. 1958. Extraction of oil from oil seed with water. Rev. Franc. Crops Gras, 5: 245-251.

Daun, J.K. 1975. Sulfur compounds in rapeseed oil. Dissertion Abstr. Int. B, 36: 1979-1980.

Daun, J.K., and F.W. Hougen. 1976. Sulfur content of rapeseed oils. J. Amer. Oil Chem. Soc., 53: 169-171.

Dendy, D.A.V., and W.H. Timmins. 1973. Development of a process to extract protein and oil from fresh coconut. Oleagineux, 28: 589-594.

Dendy, D.A.V., and W.H. Timmins. 1974. Development of a process to extract protein and oil from fresh coconut. Oleagineux, 29: 37-43.

Donefer, E. 1974. Use of expeller rapeseed meal as a protein supplement for growing cattle. In: Research on Rapeseed. Seed, Oil and Meal. Rapeseed Association of Canada, Pub. No. 35, pp. 142-146.

Downey, R.K. 1965. Rapeseed botany, production and utilization. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, D.R. Clandinin and L.R. Wetter. Can. Dept. Agric. Ottawa. Pub. No. 1257, pp. 7-23.

Downey, R.K., B.M. Craig, and C.G. Youngs. 1969. Breeding rapeseed for oil and meal quality. J. Amer. Oil Chem. Soc., 46: 121-123.

Downey, R.K. A.J. Klassen, and J. McAnsh. 1974. Rapeseed. Canada's "Cindereila" Crop. 3rd ed. Rapeseed Association of Canada, Pub. No. 32.

Downey, R.K., and L.R. Wetter. 1964. Unpublished data. Cited by L.R. Wetter, 1965. In: Rapeseed Meal for Livestock and Poultry - A Review. Eds. Bowland, J.P., D.R. Clandinin, and L.R. Wetter. Can. Dept. Agr. Ottawa, Pub. 1257, p. 42.

Drouliscos, N.J. and J.P. Bowland. 1969. Biological evaluation of rapeseed meal, soyabean meal and casein fed to the weanling and the mature rat. Brit. J. Nutr., 23: 113-123.

Drozdowski, B. L.E. Goray, and H. Niewiadomski. 1975. Effect of industrial refining of rapeseed oil on the rate of hydrogenation. Tluszcze Jadalne 19: 73-87. Chem Albstr. 1975. 83: 26494C.

Eapen, K.E., S.S. Kalbag, and V. Subrahmanyan. 1966. Key operations in the wet-rendering of peanut for the isolation of protein, oil and starch. J. Amer. Oil Chem. Soc., 43: 585-589.

Eapen, K.E., N.W. Tape, and R.P.A. Sims. 1968. New process for the production of better-quality rapeseed oil and meal. I. Effect of heat treatment on enzyeme destruction and color of rapeseed oil. J. Amer. Oil Chem. Soc., 45: 194-196.

Eapen, K.E., N.W. Tape, and R.P.A. Sims. 1969. New process for the production of better-quality rapeseed oil and meal. II. Detoxification and dehulling of rapeseeds - feasibility study. J. Amer. Oil Chem. Soc., 46: 52-55.

Eklund, A., G. Agren, and T. Lang[illegible] 1971. Rapeseed protein fractions. 1. - Preparation of a detoxified lipid-protein concentrate from rapeseed (Brassica napus L.) by a water-ethanol extraction method. J. Sci. Food Agric., 22. 650-652.

Eklund, A., and G. Agren. 1974. Reproduction in rats fed on a glucosinolate - free rapeseed protein concentrate. International Rapeseed Conf., 4, Giessen, Germany, pp. 439-446.

ElNockrashy, A.S., K.D. Mukherjee, and H.K. Mangold. 1975. New varieties of rapeseed as a source of proteins. Fette, Seifen, Anstrichm., 77: 451-452.

Ettlinger, M.G., and A.J. Lunden. 1957. First synthesis of a mustard oil glucoside; the enzymatic Lossen arrangement. J. Amer. Chem. Soc., 79: 1764-1765.

Evans, R.J., and H.A. Butts. 1948. Studies on the heat inactivation of lysine in soybean oil meal. J. Biol. Chem., 175: 15-20.

Fenwick, R.G., and S.A. Hoggan. 1976. The Tannin content of Rapeseed meals. Br. Poultry Sci., 17: 59-62.

Fertman, M.B., and G.M. Curtis. 1951. Food and the genesis of goitre. J. Clin. Endocr., 11: 1361-1382.

Finlayson, A.J. 1965. Amino acid recoveries in the analyses of some feed samples. Can. J. Plant Sci., 45: 184-188.

Finlayson, A.J., R.S. Bhatty, and C.M. Christ. 1969. Species and varietal differences in the proteins of rapeseed. Can. J. Bot., 47: 679-685.

Finlayson, A.J., J. Krzymanski, and R.K. Downey. 1973. Comparison of chemical and agronomic characteristics of two Brassica napus L. cultivars Bronowski and Target. J. Amer. Oil Chem. Soc., 50: 407-410.

Franzke, C., R. Gobel, and E. Holstein. 1972. Studies on the behaviour of fat constituents during refining. Part II. Total sulfur content of colza oils differing in the degree of refining. Nahrung, 16: 867-871.

Franzke, C., R. Gobel, G. Noack and J. Seiffert. 1975. On the isothiocyanate and vinyl thio-oxazolidone contents of rapeseeds and rapeseed oil. Nahrung, 19: 583-593.

Fry, J.L., G.M. Herrick, G.M. Prine and R.H. Harms. 1972. Effect of bird resistant sorghums and tannic acid on yolk mottling. Poultry Sci., 51: 1540-1543.

Georgia Tech. Research Institute. 1960. Simultaneous extraction of oil and protein from oleaginous material. Brit. Patent 842,356. Chem. Abstr. 1961, 55: 6891i.

Gheyasuddin, S., and M. Ali. 1975. Protein and essential amino acid contents of mustard seed meal (Brassica napus L. and their distribution in various soluble fraction. Bangladesh J. Agric. Sci. 2: 61-66. Chem. Abstr. 1975, 83: 130273r.

Giovannetti, P. and J.M. Bell. 1971. Research on Rapeseed Meal. Rapeseed Association of Canada. Cited by C.G. Youngs. L.R. Wetter and G.S. Boulter. Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada. Pub. No. 16, pp. 4-7.

Girault, A. 1973. The study of some properties of rapeseed protein with a view to protein concentrate production. J. Sci. Food Agric., 24: 509-518.

Glick, Z., and M.A. Joslyn. 1970a. Food intake depression and other metabolic effect of tannic acid in the rat. J. Nutr., 100: 509-515.

Glick, Z., and M. A. Joslyn. 1970b. Effect of Tannic acid and related compounds on the absorption and utilization of protein in the rats. J. Nutr., 100: 516-520.

Gmelin, R., and A.I. Virtanen. 1960. The enzymic formation of thiocyanate (SCN^-) from a precursor (S) in Brassica species. Acta Chem. Scand., 14: 507-509.

Goering, K.J., O.O. Thomas, D.R. Beardsley, and W.A. Curran. 1960. Nutritional value of mustard and rapeseed meals as protein source for rats. J. Nutr., 72: 210-216.

Gorrell, A.D.L., D.M. Walker, and J.D. Jones. 1974. Rapeseed protein sources and amino acid supplementation of diets for weanling rats. Can. J. Animal Sci., 54: 659-667.

Greer, M.A. 1962. II. Thyroid hormones. The natural occurrence of goitrogenic agents. In: Recent Progress of Hormone Research. Ed. C. Pincus, Academic Press, New York. Vol. 18, pp. 187-212.

Grussendorf, W. 1953. Private communication. Cited by J.M. Bell, 1955. Can. J. Agric. Sci., 35: 242-251.

Guignard, L. 1890. J. Botanique, 4: 385. Cited by E. Josefsson. 1972. In: Rapeseed; Cultivation, Composition, Processing and Utilization. Ed. L.A. Appelqvist, and R. Ohlson. Elsevier Pub. Co. Amsterdam, p. 359.

Gunetileke, K.G., and S.F. Laurentius. 1974. Conditions for the separation of oil and protein from coconut milk emulsion. J. Food. Sci., 39: 230-233.

Hagenmaier, R.D. 1974. Aqueous processing of full-fat sunflower seeds: yield of oil and protein. J. Amer. Oil Chem. Soc., 51: 470-471.

Hagenmaier, R., C.M. Cater, and K.F. Mattil. 1972. Critical unit operations of the aqueous processing of fresh coconuts. J. Amer. Oil Chem. Soc., 49: 178-181.

Hagenmaier, R.D., C.M. Cater, and K.F. Mattil. 1973. Aqueous processing of fresh coconuts for recovery of oil and coconut skim milk. J. Food Sci., 38: 516-518.

Hagenmaier, R.D., C.M. Cater, and K.F. Mattil. 1975. Aqueous processing of coconuts: economic analysis. J. Amer. Oil Chem. Soc., 52: 5-9.

Hermansson, A.-M., D. Olsson, and B. Holmberg. 1974. Functional properties of proteins for foods - Modification studies on rapeseed protein concentrate. Lebens. - Wiss. - Technol., 7: 176-181.

Hjarde, W., H. Lieck, and H. Soendergaard. 1962. The contents of 5 members of the vitamin B complex and of vitamin E in feeding stuffs. Acta Agric. Scand., 12: 125-134.

Hocker, I.S. 1930. Extracting oil from cocoa beans or other vegetable materials. U.S. Patent 1,751,331. Chem. Abstr., 1930, 24: 2627.

Hoffmann, Y. 1973. Experiences in refining rapeseed oil by the Zenith process. J. Amer. Oil Chem. Soc., 50: 260A-267A.

Horodyski, A. 1962. Pamictnik Pulawski - Prace lung Zeszyt 8: 83. Cited by L.R. Wetter, H. Ukrainetz and R.K. Downey. 1970. In: Proceedings of the International Conference of the Science, Technology and Marketing of Rapeseed and Rapeseed Products. Rapeseed Association of Canada. Ste. Adele, Quebec, Canada, p. 102.

Horovitz-Vlasova, L.M., N.V. Novotelnov. 1935. Allg. Oel. Fett. Ztg. 32: 315. Cited by C.L. Puertollano, L. Banzon, and K.H. Steinkraus. 1970. J, Agric. Food Chem., 18: 579-584.

Hrdlicka, J., H. Kozlowska, J. Pokorný and A. Rutkowski. 1964. Über rapsschrote. 3. Mitt. Stickstoff-haltige substanzen der rapsschrote. Nahrung, 8: 537-543.

Hrdlicka, J., H. Kolowska, J., J. Pokorný and A. Rutkowski. 1965a. Über rapsschrote. 7. Mitt. Saccharide in extraktionsschroten. Nahrung, 9: 71-76.

Hrdlicka, J., J. Pokorný, J., A. Rutkowski, and M. Wojciak. 1965b. Über Rapsschrote. 8. Mitt. Aminosäuren. Nahrung, 9: 77-83.

Ingalls, J.R., and D.E. Waldern. 1972. Rapeseed meal in rations for dairy cattle. In: Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada. Pub. 16, pp. 24-27.

Ingalls, J.R., D.E. Waldern, and J.B. Stone. 1970. Rapeseed meal in ruminant rations and dairy cattle. In: Proceedings of the International Conference of the Science, Technology and Marketing of Rapeseed and Rapeseed Products. Rapeseed Association of Canada, Ste. Adele, Quebec, Canada, pp. 496-515.

Jackson, N. 1969. Toxicity of rapeseed meal and its use as a protein supplement in the diet of two hybrid strains of caged laying hens. J. Sci. Food Agric., 20: 734-740.

Janicek, G. and J. Pokorny. 1963. Scientific papers from Institute of Chemical Technology, Prage. Food Technol. 7: 173-179. Cited by L.-A. Appelqvist. 1967. J. Amer. Oil Chem. Soc., 44: 206-208.

Janson, J.C. 1971. Columns for large-scale gel filtration on porous gels fractionation of rapeseed proteins and insulin. J. Agric. Food Chem., 19: 581-598.

Janson, J.C., S.A. Liden, B. Lonnerdal, and J. Porath. 1971. Unpublished data. Cited by L.-A. Appelqvist. 1971. J. Amer. Oil Chem. Soc., 48: 851-859.

Jorgensen, E.A. 1972. Nutritional value of rapeseed oil. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 301-353.

Josefsson, E. 1970. Glucosinolate content and amino acid composition of rapeseed (Brassica napus L.) meal as affected by sulfur and nitrogen nutrition. J. Sci. Food Agric., 21: 98-103.

Josefsson, E. 1972. Nutritional value and use of rapeseed meal. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist, and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 354-377.

Josefsson, E. 1974. Effect of heat treatment on the content of glucosinolates and available lysine in rapeseed meal. Sver. utsaedesfoeren. Tidskr, 84: 199-204. Chem. Abstr. 1975, 82: 124086v.

Josefsson, E. 1975a. Effects of variation of heat treatment conditions on the nutritional value of low-glucosinolate rapeseed meal. J. Sci. Food Agric., 26: 157-164.

Josefsson, E. 1975b. Influence of glucosinolates and high molecular factors on the nutritional value of low-glucosinolate rapeseed meal. J. Sci. Food Agric., 26: 1299-1310.

Josefsson, E., and L.-A. Appelqvist. 1968. Glucosinolates in seed of rape and turnip rape as affected by variety and environment. J. Sci. Food Agric., 19: 564-570.

Josefsson, E., and L. Munck. 1973. Influence of genetically determined differences in glucosinolate content on the nutritional value of rapeseed meal. J. Sci. Food Agric., 24: 1265-1271.

Kao, W.S. 1956. Extraction of fatty oils and proteins from oleaginous materials. U.S. Patent. 2,773,889. Chem Abst. 1957, 51: 4740a.

Karel, M. 1973. Symposium: protein interaction in biosystem. Protein - lipid interactions. J. Food Sci., 38: 756-763.

Karvanek, M., J. Pokorny, H. Kozlowska, and A. Rutkowski. 1964. Uber rapsschrote. 5. Mitt. Mineralstoffe. Nahrung, 8: 675-680.

Kirk, L.D., G.C. Mustakas, and E.L. Griffin. 1965. Crambe seed processing: improved feed meal by ammoniation J. Amer. Oil Chem. Soc., 43: 550-555.

Kitchener, J.A., and P.R. Mussellwhite. 1968. The theory of stability of emulsion. In: Emulsion Science. Ed. P. Sherman, Academic Press, Longon, pp. 77-130.

Kjaer, A. 1960. Naturally derived isothiocyanates (mustard oils) and their parent glucosides. In: Progress in the Organic Chemistry of Natural Products. Vol. 18, Springer, Verlag. Vienna, pp.122-176.

Kjaer, A., B.W. Christensen, and S.E. Hansen. 1959. Isothiocyanates XXXIV. The absolute configuration of (-)-5-Vinyl-2-oxazolidinethione (Goitrin) and its glucoside progenitor (Progoitrin). Acta Chem. Scand., 13: 144-150.

Kjaer, A., J. Conti, and K.A. Jensen. 1953. iso-Thiocyanates. III. The volatile isothiocyanates in seeds of rape (Brassica napus. L.). Acta Chem. Scand., 7: 1271-1275.

Kjaer, A., and R.B. Jensen. 1956. iso-Thiocyanates XX. 4 - pentenyl isothiocyanate, a new mustard oil occurring as a glucoside (glucobrassicanapin) in nature. Acta Chem. Scand., 10: 1365-1371.

Klain, G.J., D.C. Hill, H.D. Branion, and J.A. Gray. 1956. The value of rapeseed oil meal and sunflower seed oil in thick starter rations. Poultry Sci., 35: 1315-1326.

Klein, K., and L.S. Crauer. 1974. Further developments in crude oil processing. J. Amer. Oil Chem. Soc., 51: 382A-385A.

Kodagoda, L.P., S. Nakai, and W.D. Powrie. 1973b. Some functional properties of rapeseed protein isolates and concentrates. Can. Inst. Food Sci. Technol. J., 6: 266-269.

Kodagoda, L.P., C.Y. Yeung, S. Nakai, and W.D. Powrie. 1973a. Preparation of protein isolates from rapeseed flour. Can. Inst. Food Sci. Technol. J., 6: 135-141.

Korolczuk, J., and A. Rutkowski. 1971. Extraction of N-Compounds of rapeseed meal in relation to pH and temperature. J. Amer. Oil Chem. Soc., 48: 398-399.

Kozlowska, H., J. Cwik, A. Rutkowski, and S. Weidner. 1972b. On rapeseed meals. Part XXII. Changes in the content of thioglucoside derivatives during the ensilaging of potatoes added with rapeseed meal. Nahrung, 16: 843-848.

Kozlowska, H., F.W. Sosulski, and B. Lossow. 1974. Rapeseed concentrate. International Rapeseed Conf., 4, Giessen, Germany, pp. 651-657.

Kozlowska, H., F.W. Sosulski, and C.G. Youngs. 1972a. Extraction of glucosinolates from rapeseed. Can. Inst. Food Technol. J., 5: 149-154.

Kratzer, F.H., P.N. Davis, D.E. Williams, and B.J. Marshall. 1954. Factors influencing the growth of chicks and poults fed rations containing rapeseed oil meal. J. Nutr., 53: 407-418.

Kucera, Z., and M. Hejtmanek. 1957. Colorimetric determination of sulfur in rape oil. Prumysl potravin, 8: 199-200. Chem. Abstr., 1957, 51: 12511c.

Kurucz, E., and J. Peredi. 1974. Uber den ITC und VTO-Gehalt der ungarischen rapsschrote und rapsole. International Rapeseed Conf., 4, Giessen, Germany, p. 363. Cited by J.K. Daun and F.W. Hougen. 1976. J. Amer. Oil Chem. Soc., 53: 169-171.

Lanzani, A., M.C. Petrini, O. Cozzoli, P. Gallovresi, C. Carola, and G. Jacini. 1975. Use of enzymes for vegetable oil extraction. Riv. Ital. Sostanze Grasse, 52: 226-229. Chem. Abstr. 1975, 83: 176919j.

Lanzani, A., M.C. Petrini, G. Jacini. 1974. Prepared proteins. Antithyroidal substances in rapeseed. Inf. Grasas Aceites, 11: 55-58. Chem. Abstr. 1975. 83: 191533x.

Lava, V.G., P.E. Torres, and S. Sanvictores. 1941. Philipp. J. Sci., 74: 247. Cited by C.L. Puertollano, J. Banzon, and K.H. Steinkraus. 1970. J. Agric. Food Chem., 18: 579-584.

Lea, C.H., and R.S. Hannan. 1949. Studies of the reaction between proteins and reducing sugars in the "dry" state. 1. The effect of activity of water, of pH and of temperature on the primary reaction between Casein and glucose. Biochem. Biophys. Acta, 3: 313-325.

Liener, I.E. 1958. Effect of heat on plant proteins. In: Processed Plant Protein Foodstuffs. Ed. A.M. Altschul. Academic Press, New York, pp. 79-129.

Linow, F., G. Mieth, L. Prahl, J. Pohl, J. Kroll, D. Raue, and R. Elspass. 1971. Simultaneous isolation of fats and proteins from oil-containing plants. Ger. Patent 2,056,896. Chem. Abstr. 1971, 75: 139593n.

L nt, K.J. 1974. Emulsion and Emulsion Technology. Part I. Marcel Dekker, Inc., New York.

Lo, M.T. and D.C. Hill. 1971. Evaluation of protein concentrates prepared from rapeseed meal. J. Sci. Food Agric., 22: 128-130.

Lo, M.T., and D.C. Hill. 1972. Composition of the aqueous extracts of rapeseed meals. J. Sci. Food Agric., 23: 823-830.

Ludecke, K. 1938. Oils and fats. Ger. Patent 662,794. Chem. Abstr. 1938, 32: 95386.

Lund, A.P. and W.M. Sandstrom. 1943. The protein of various tree seeds. J. Agric. Res., 66: 349-355.

Manns, J.G., and J.B. Bowland. 1963a. Solvent-extracted rapeseed oil meal as a protein source for pigs and rats. I. Growth, carcass characteristics and production. Can. J. Animal Sci., 43: 252-263.

Manns, J.G., and J.P. Bowland. 1963b. Solvent-extracted rapeseed oil meal as a protein source for pigs and rats. II. Nitrogen digestibility and retention; and energy and dry matter digestibility. Can. J. Animal Sci., 43: 264-270.

Manns, J.G., J.P. Bowland, V.E. Mendel, and S. Zivkovic. 1963. Solvent-extracted rapeseed oil meal as a protein source for pigs and rats. III. Blood serum protein bound iodine standard metabolic rates thyroid activity, adrenal and gonad weights. Can. J. Animal Sci., 43: 271-278.

Marangos , A., R. Hill, B.M. Laws, and D. Muschamp. 1974. Influence of three rapeseed meals and a mustard seed meal on egg and broiler production. Br. Poultry Sci., 15: 405-514.

Martinez, J.M.M., M.F. Catalina, C.G. Herrera, and C.J. del Valle. 1962. Emulsion problems in olive oil extraction III. Anales Real Soc., Espan. Fis. Quim. Ser. B., 58: 81-86. Chem. Abstr. 1962, 57: 8676c.

Martinez, J.M.M., C.G. Herrera, C. Janer, J. Pereda, and V. Flores. 1966. Effect of surfactive agents on olive oil extraction. Afinidad 23 (246): 527-530. Chem. Abst. 1967, 66: 114715e.

Martinez, J.M.M., C.G. Herrera, C.J. del Valle, and R.M. Delgado. 1961. Interfacial problems in the extraction of olive oil II. Anales Real Soc. Espan. Fis. Quim. 57: 453-464. Chem. Abstr. 1962, 56: 10647a.

Matet, J., R. Montagne, and A. Buchy. 1949. Nutritive value of rapeseed oil meal. Oleagineux, 4: 145-154.

McCabe, W.L., and J.C. Smith. 1956. Unit Operations of Chemical Engineering. McGraw-Hill Book Co. Inc. New York, p. 362.

McDonald. 1974. A study of the effect of level and source of rapeseed meal on feed intake by the rat and pig. In: Research on rapeseed. Seed, Oil and Meal. Rapeseed Association of Canada, Ottawa, Pub. No. 35, pp. 124-129.

McDonald, B.E. and R.J. Gregoire. 1973. A study of the digestion of rapeseed meal by the growing pig. In: Research on Rapeseed. Oil and Meal. Ed. P. Giovannetti and J. M. Bell. Rapeseed Association of Canada, Ottawa, Pub. No. 32, pp. 44-56.

McGhee, J.E., L.D. Kirk, and G.C. Mustakas. 1964. Mustard seed processing; simple methods for following heat damage to protein meals. J. Amer. Oil Chem. Soc., 41: 359-362.

McKillcan, M.E., and J.A.G. Lacrose. 1970. Residual lipids of hexane-extracted rapeseed meal. J. Amer. Oil Chem. Soc., 47: 256-258.

Meyer, W.K. 1975. Utilization of rapeseed meal by Canadian feed manufactures. Canadian Rapeseed Annual Meeting, R.A.C. Mexico. Cited by H.T. Armstrong. In: Oilseed and Pulse Crops in Western Canada - A Symposium. Ed. J.J. Harapiak. Western Co-operative Fertilizers Ltd., p. 105.

Mielke, S. 1976. World supply and demand situation for oilseeds, oils, and meals. J. Amer. Oil Chem. Soc., 53: 224-231.

Mieth, G., J. Kroll, J. Pohl, and J. Bruckner. 1975a. Trends in the processing of oil seeds and fruits. The simultaneous extraction of oils and proteins. Nahrung, 19: 687-696.

Mieth, G., J. Kroll, J. Pohl, F. Linow, and L. Prahl. 1975b. Studies on the simultaneous isolation of proteins and fats from oil seeds. Part 1. The principle of the procedure. Nahrung, 19: 955-960.

Miller, D.S. 1956. The nutritive value of fish proteins. J. Sci. Food Agric., 7: 337-343.

Miller, R.W., C.H. Van Etten, C. McGrew, and I.A. Wolff. 1962. Amino acid composition of seed meals from forty-one species of Cruciferae. J. Agric. Food Chem., 10: 426-430.

Mizuno, T. 1958. Carbohydrates of the rapeseed. Nippon Nogei Kagaku Kaishu. 32: 340-343. Chem. Abstr. 1960, 54: 19874h.

Montedoro, G., G. Petruccioli. 1973. Enzyme treatments in the extraction of olive oil by mechanical means. Riv. Ital. Sostanze Grasse 50: 331-343. Chem. Abstr. 1974, 80: 58575j.

Moore, O.K. 1948. The coconut palm - Mankinds' greatest provider. Econ. Bot. 2: 3-14. Cited by J.G. Woodroof, 1970. In: Coconuts: Production, Processing, Products. Ed. J.G. Woodroof Avi. Pub. Co. Inc. Westport, Connecticut, pp. 75-76.

Morrison, W.R. 1964. A fast, simple and reliable method for the microdetermination of phosphorus in biological materials. Ana. Biochem., 7: 218-224.

Morrison, A.B., D.R. Clandinin, and A.R. Robblee. 1953. The effect of processing variables on the nutritive value of sunflower seed oil meal. Poultry Sci., 32: 492-496.

Motzoke, I. Unpublished data. Cited by D.R. Clandinin, A.R. Robblee and S.J. Slinger. In: Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada, Pub. No. 16, p. 11.

Moysey, E.B., and Norum, E.R. 1975. Storage, Drying and Handling of Oilseeds and Pulse Crops. In: Oilseed and Pulse Crops in Western Canada. Ed. J.T. Harapiak, Western Co-operative Fertilizers Ltd., pp. 508-540.

Mukherjee, K.D. 1975. Extraktion des glucosinolate aus qualitatsraps zur herstellung von proteinkonzentraten. Fette, Seifen, Anstrichm., 77: 433.

Mustakas, G.C., L.D. Kirk, and E.L. Griffin. 1962. Mustard seed processing: bland protein meal, bland oil, and allyl isothiocyanate as a by-product. J. Amer. Oil Chem. Soc., 39: 372-377.

Mustakas, G.C., L.D. Kirk, E.L. Griffin, and A.N. Booth. 1976. Crambe seed processing: removal of glucosinolates by water extraction. J. Amer. Oil Chem. Soc., 53: 12-16.

Mustakas, G.C., L.D. Kirk, E.L. Griffin, and D.C. Clanton. 1968. Crambe seed processing. Improved feed meal by soda ash treatment. J. Amer. Oil Chem. Soc., 45: 53-57.

Mustakas, G.C., L.D. Kirk, V.E. Sohns, and E.L. Griffin. 1965. Mustard seed processing: Improved method for isolating the pungent factor and controlling protein quality. J. Amer. Oil Chem. Soc., 42: 33-37.

Norris, F.A. 1964. Extraction of fats and oils. In: Bailey's Industrial Oil and Fat Products. Ed. D. Swern, Interscience Pub. New York, pp. 637-718.

Nyrop, A. 1938. Extracting oils. Brit. Patent 497,547. Chem. Abstr., 1939, 33: 4070.

Oginsky, E.L., A.E. Stein, and M.A. Greer. 1965. Myrosinase activity in bacteria as demonstrated by the conversion of progoitrin to goitrin. Proc. Soc. Exp. Bio. Med., 119: 360-364.

Ohlson, R. 1972. Projection and prospects for rapeseed and mustard seed. J. Amer. Oil Chem. Soc., 49: 522A-526A.

Ohlson, R. 1973. Rapeseed protein concentrate for human consumption. PAG Bulletin, III: 21-23.

Ohlson, R., and J. Tear. 1974. Properties and use of rapeseed protein concentrate. International Rapeseed Conf., 4, Giessen, Germany, pp. 663-667.

Olin, E. 1957. Sveriges Utsadesforening Tidskr., 67: 91. Cited by K. Anjou. 1972. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 198-217.

Olmu, J.M., A.R. Robblee, D.R. Clandinin, R.T. Hardin. 1975. Effects of Span rapeseed meal on productive performance, egg quality, composition of liver and hearts, and incidence of fatty livers in laying hens. Can. J. Animal Sci., 55: 71-76.

Owen, D.F., C.O. Chichester, J. Granadino, and F. Möncke-berg. 1971. A process for producing nontoxic rapeseed protein isolate and an acceptable feed by-product. Cereal Chem., 48: 91-96.

Peaslee, M.H., and F.A. Eintellig. 1973. Reduced fedundity in mice on tannic acid diet. Comparative Gen. Pharm., 4: 393-397. Cited by R.G. Fenwick and S.A. Hoggan. 1976. Br. Poultry Sci., 17: 59-62.

Pedro, S.C., and R.A. Carlos. 1969. Extraction of oil from fresh coconut. Oleagineux 24: 419-421. Chem. Abstr. 1970, 73: 86722d.

Persmark, U. 1972a. Analysis of rapeseed oil. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 174-197.

Persmark, V. 1972b. Rapeseed lecithin. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 288-300.

Peters, F.E. 1960. Ph.D. thesis, Purdue University. Cited by K.G. Gunetileke and S.F. Laurentius. 1974. J. Food Sci., 39: 230-233.

Pospelova, K.A., T.A. Vorobeva, and P.I. Zubov. 1962. Colloid J. (U.S.S.R) 24: 511. Cited by P. Sherman, 1968. In: Emulsion Science. Ed. P. Sherman, Academic Press, London, p. 132.

Potter, D.K., H.L. Fuller, and C.D. Blackshear. 1967. Effect of tannic acid on egg production and egg yolk mottling. Poultry Sci., 46: 1508-1512.

Pritchard, J.L.R. 1974. Processing of rapeseed - oil extraction. Chem. Ind. (London), 17: 697-700.

Puertollano, C.L., J. Banzón, and K.H. Steinkraus. 1970. Separation of the oil and protein fractions in coconut (Cocos nucifera Linn.) by Fermentation. J. Agric. Food Chem., 18: 579-584.

Quinn, J.R. and J.D. Jones. 1976. Rapeseed Protein. pH solubility and electrophoretic characteristics. Can. Inst. Food Sci. Technol. J., 9: 47-50.

Rac. M. 1967. Influence of cellular structure on some technological properties of certain oleaginous raw materials. Rev. Fr. Crops Gras., 14: 223-228.

Radwan, M.N., and B.C.Y. Lu. 1976. Solubility of rapeseed protein in aqueous solution. J. Amer. Oil Chem. Soc., 53: 142-144.

Rajasekharan, N., and A. Sreenivasan. 1967. The use of coconut preparations as a protein supplement in child feeding. J. Food Sci. Technol. (India), 4: 59. Cited by R. Hagenmaier, C.M. Cater, and K.F. Mattil. 1972. J. Amer. Oil Chem. Soc., 49: 178-181.

Renner, R., D.R. Clandinin, A.B. Merison, and A.R. Robblee. 1953a. The effect of processing temperatures on the amino acid content of sunflower seed oil meal. J. Nutr., 50: 487-490.

Renner, R., D.R. Clandinin, and A.R. Robblee, 1953b. Action of moisture on damage done during overheating of soybean oil meal. Poultry Sci., 32: 583-585.

Reynolds, J.R., and C.G. Youngs. 1964. Effect of seed preparation on efficiency and quality in Filtration of rapeseed. J. Amer. Oil Chem. Soc., 41: 63-65.

Rhee, K.C., C.M. Cater, and K.F. Mattil. 1972a. Simultaneous recovery of protein and oil from raw peanuts in aqueous system. J. Food Sci., 37: 90-93.

Rhee, K.C., K.R. Natarajan, C.M. Cater, and K.F. Mattil. 1972b. J. Amer. Peanut Res. Education Assoc., 4 215. Cited by C.M. Cater, K.C. Rhee, R.D. Hagenmaier, 1974. J. Amer. Oil Chem. Soc., 51: 137-141.

Rhee, K.C., K.F. Mattil, and C.M. Cater. 1973a. Recovers protein from peanuts. Food Eng., 45: 82-86.

Rhee, K.C., C.M. Cater, and K.F. Mattil. 1973b. Effect of processing pH in the properties of peanut protein isolates and oil. Cereal Chem., 50: 395-404.

Rhee, K.C., C.M. Cater, and K.F. Mattil. 1973c. Aqueous process for pilot plant-scale production of peanut protein concentrate. J. Food Sci., 38: 126-128.

Robblee, A.R. 1965. Status of rapeseed meal as a protein supplement. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, D.R. Clandinin and L.R. Wetter. Can. Dept. Agric. Ottawa, Pub. No. 1257, pp. 93-96.

Robledano, R., and E.R. de Luzuriaga. 1948. Philippine Patent 1. Cited by J.G. Woodroof. In: Coconut: Production, Processing, Products. Ed. J.G. Woodroof. 1970. Avi Pub. Co. Inc., Westport, Connecticut, pp. 76-77.

Rocquelin, G., J.P. Sergiel, B. Martin, J. Lecilerc, and R. Cluzan. 1971. The nutritive value of refined rapeseed oils. A review. J. Amer. Oil Chem. Soc. 48: 728-732.

Rosen, G.D. 1958. Groundnuts (peanuts) and groundnut meal. In: Processed Plant Protein Foodstuffs. Ed. A.M. Altschul. Academic Press Inc. Pub. New York, pp. 419-468.

Roxas, F.G. 1963. Recovery oil from oleaginous meats of nuts, beans and seeds. U.S. Patent 3,083,365. Chem. Abstr. 1963, 59: 2110a.

Ruhowicz, K. 1972. Cited by L.-A. Appelqvist and B. Loof. 1972. In: Rapeseed. Cultivation, Composition, Processing, and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 60-100.

Runer, V., and E. Honkanen. 1972. Edible products from rapeseed oil. In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam. pp. 249-273.

Runer, V., and R. Ohlson. 1971. A review of rapeseed oil uses for edible purposes. J. Amer. Oil Chem. Soc., 48: 860-862.

Rutkowski, A. 1970. Effect of processing on the chemical composition of rapeseed meal. In: Proceedings of the International Conference of the Science, Technology and Marketing of Rapeseed and Rapeseed Products. Rapeseed Association of Canada. Ste. Adele, Quebec, Canada, pp. 496-515.

Rutkowski, A. 1971. The feed value of rapeseed meal. J. Amer. Oil Chem. Soc., 48: 863-868.

Rutkowski, A., and H. Kozlowska. 1969. Rapeseed presscake. XV. Degradation of the sulfurated compounds during processing of the presscake. Oleagineux, 24: 687-690. Chem. Abstr. 1970, 73: 28817c.

Rutkowski, A., H. Kozlowska, and I. Pokorny. 1966. Investigation on rape groats. X. Influence of thermal processing on quality of rape groats. Roczniki Technol. Chem. Zywnosei. 12: 275-293. Chem. Abstr. 1966, 65: 11248c.

Rutkowski, A., and D.-I.Z. Makus. 1959. The influence of the quality of rapeseed on the stability of oil. Fette, Seifen, Anstrichm., 61: 532-535.

Saben, H.S., J.P. Bowland, and R.T. Hardin. 1971a. Digestibility and metabolizable energy values for rapeseed meals and for soybean meal fed to growing pigs. Can. J. Animal Sci., 51: 419-425.

Saben, H.S., J.P. Bowland, and R.T. Hardin. 1971b. Effect of method of determination of digestible energy and nitrogen and on metabolizable energy values of rapeseed meal and soybean meals fed to growing pigs. Can. J. Animal Sci., 51: 427-432.

Sallans, H.R. 1964. Factors affecting the composition of Canadian oilseeds. J. Amer. Oil Chem. Soc., 41: 215-218.

Sallans, H.R., K.L. Phillips, J.F.T. Spencer, C.G. Youngs, P.S.S. Dowson, and G.D. Sinclair. 1967. Oil seeds. Prairie Regional Laboratory, NRC Review. pp. 57-58.

Samson, A.S., C.M. Carter, and K.F. Mattil. 1971. Preparation and characterization of coconut protein isolates. Cereal Chem., 48: 182-190.

Sarwar, G., D.W.F. Shannon, and J.P. Bowland. 1975. Effect of processing condition on the availability of amino acids in soybean and rapeseed proteins when fed to rats. Can. Inst. Food Sci. Technol. J., 8: 137-141.

Sarwar, G., F.W. Sosulski, and J.M. Bell. 1973. Nutritional evaluation of oilseed meals and protein isolates by mice. Can. Inst. Food Sci. Technol. J., 6: 17-21.

Schanderl, S.H. 1970. Tannins and related phenolics. In: Methods in Food Analysis. Ed. M.A. Joslyn, 2nd. ed. Acad. Press, New York. pp. 701-725.

Schmandke, H., R. Maune, R. Friebel, V. Webers, H. Anger, and I. Kormann. 1976. Experiments on spinning of rapeseed proteins to fibers. Nahrung, 20: 25-32.

Schwenke, K.D., B. Raab, J. Uhlig, H. Tkocz, J. Behlke, M. Böttger, and U. Freimuth. 1973. Seed protein. Part III. The isolation and characterization of albumen from sunflower seed and rapeseed. Nahrung, 17: 791-809.

Shaikh, I.A., M. Arshad, M.Y.I. Hague, and S.M. Ali. 1968. Nutritional properties of rapeseed cake. J. Sci. Agric. Res., 11: 263-267.

Siddiqui, I.R., P.J. Wood, and G. Khanzada. 1973. Low molecular weight carbohydrates from rapeseed (Brassica compestris L.) meal. J. Sci. Food Agric., 24: 1427-1435.

Sietz, F.G. 1967. Über das samenöl verschiedener Brassica-Arten. Fette, Seifen, Amstrichm., 69: 325-328.

Sims, R.P.A. 1971. Edible protein products from Cruciferae seed meals. J. Amer. Oil Chem. Soc., 48: 733-736.

Singleton, W.S., R.R. Benerito, and J.L. White. 1960. Factors affecting oil particle size in the freezing and thawing of fat emulsion. J. Amer. Oil Chem. Soc., 37: 88-92.

Sison, B.C. et al. 1968. Coconut protein studies: studies on the optimum conditions of extraction and preparation of protein from coconut meat. Tech. Bull. Natn. Inst. Sci. Technol., Philipp., 2. Cited by D.A.V. Dendy and W.H. Timmins. 1973. Oleagineux, 28: 589-594.

Soliven, F.A., and A.I. de Leon. 1938. Phillip. Agr. 27: 200. Cited by C.L. Puertollano, J. Banzon, and K.H. Steinkraus. 1970. J. Agr. Food Chem., 18: 579-584.

Sosulski, F.W. 1973. Extraction and nutritive value of rapeseed proteins. In: Research on Rapeseed. Oil and Meal. Ed. P.M. Giovannetti and J.M. Bell. Rapeseed Association of Canada, Ottawa, Pub. No. 32, pp. 103-107.

Sosulski, F.W. 1974. Extraction and nutritive value of rapeseed proteins. In: Research on Rapeseed. Seed, Oil and Meal. Rapeseed Association of Canada, Ottawa, Pub. No. 35, pp. 168-173.

Sosulski, F.W. 1975. Personal communication.

Sosulski, F.W., and A. Bakal. 1969. Isolated proteins from rapeseed, flax and sunflower meals. Can. Inst. Food Sci. Technol. J., 2: 28-32.

Sosulski, F.W., and G. Sarwar. 1973. Amino acid composition of oilseed meals and protein isolates. Can. Inst. Sci. Technol. J., 6: 1-5.

Sosulski, F.W., F.S. Soliman, and R.S. Bhatty. 1972. Diffusion extraction of glucosinolates from rapeseed. Can. Inst. Food Sci. Technol. J., 5: 101-104.

Spinov, R.I., and I.I. Gekker. 1968. Extracting oil from vegetable materials. U.S.S.R. Patent 214,001. Chem. Abstr. 1968, 69: 2877e.

Staron, T. 1970. A method of bilogical detoxifying rapeseed meal. In: Proceedings of the International Conference of the Science, Technology and Marketing of Rapeseed and Rapeseed Products. Ste. Adele, Quebec, Canada. Rapeseed Association of Canada, Ottawa, pp. 321-327.

Staron, T. 1974. Detoxification of rapeseed cakes by a biological method. Aliment. Vie, 62: 165-179. Chem. Abstr. 1975, 83: 7534z.

Stolle, G. 1954. Zuchter, 14: 202. Cited by L.-A. Appelqvist. 1972: In: Rapeseed. Cultivation, Composition, Processing and Utilization. Ed. L.-A. Appelqvist and R. Ohlson. Elsevier Pub. Co. Amsterdam, pp. 123-173.

Stringam, G.R., and B.L. Harvey. 1973. Morphological characteristics associated with seed coat color in rapeseed. In: Research on Rapeseed. Oil and Meal. Ed. P. Giovannetti and J.M. Bell. Rapeseed Association of Canada, Ottawa, Pub. No. 32, pp. 2-3.

Stringam, G.R., D.I. McGregor and S.H. Powlowski. 1974. Chemical and morphological characteristics associated with seedcoat color in rapeseed. International Rapeseed Conf., 4, Giessen, Germany, pp. 99-108.

Subrahmanyan, V., D.S. Bhatia, S.S. Kalbag, and N. Subramanian, 1959. Integrated processing of peanut for the separation of major constituents. J. Amer. Oil Chem. Soc., 36: 66-70.

Sugarman, N. 1956. Process for extraction of oil and protein simultaneously from oil bearing materials. U.S. Patent 2,762,820. Chem. Abstr. 1957. 51: 4031e.

Szewczuk, A., P. Mastalerz, and W. Nadwyczawski. 1969. Acid hydrolysis of rapeseed-meal feeds. Przem. Ferment. Rolny. 13: 15-20. Chem. Abstr. 1970, 72: 131155p.

Taira, H., H. Taira, K. Sugimura, and Y. Sakurai. 19 . Studies on amino acid contents of processed soybean. VII. The influence of added water on the heat destruction of amino acids in defatted soybean flour. Agric. Biol. Chem. (Toyko), 29: 1080-1083.

Tape, N.W., Z.I. Sabry, K.E. Eapen. 1970. Production of rapeseed flour for human consumption. Can. Inst. Food Technol. J., 3: 78-81.

Teasdale, B.F. 1975. Processing of vegetable oils. In: Oilseed and Pulse Crops in Western Canada - A Symposium. Ed. J.T. Harapiak, Western Co-operative Fertilizers Ltd., pp. 551-585.

Theander, O. and P. Aman. 1974. Carbohydrates in rapeseed and turnip rapeseed meals. Internationl Rapeseed Conf., 4, Giessen, Germany, pp. 429-438.

Thieme, J.G. 1968. Coconut Oil Processing. Food and Agriculture Organization of the United Nation. Rome, pp. 9-32.

Thompson, L.V., P. Allum-Poon and C. Procope. 1976. Isolation of rapeseed protein using sodium hexametaphosphate. Can. Inst. Food Sci. Technol. J., 9: 15-19.

Timmins (Unpublished). Cited by D.R. Hagenmaier, C.M. Cater, and K.L. Mattil. 1972. J. Amer. Oil Chem. Soc., 49: 178-181.

Tookey, H.L. 1973. Solubilization and selected properties of crambe seed Thioglucosidase (Thioglucosidase Glucohydrolase, EC3.2.3.1). Can. J. Biochem., 51: 1305-1310.

Tookey, H.L., C.H. VanEtten, J.E. Peters, and I.A. Wolff. 1965. Evaluation of enzyme-modified, solvent extracted crambe seed meal by chemical analysis and rat feeding. Cereal Chem., 42: 507-514.

Tristram, G.R., and R.H. Smith. 1963. The amino acid analysis of some purified proteins. In: Advances in Protein Chemistry. Ed. C.B. Anfinsen, and M.L. Anson. Academic Press, New York, Vol. 18. pp. 227-315.

Trzebny, W. 1964. Effect of nitrogenous fertilizers on the content of volatile isothiocyanates and thiooxazolidone in the seeds of four winter rape varieties. Pamietnik Pulawski. 15: 91-100. Chem. Anstr. 1965, 63: 4900g.

Vallee, F. 1971. Continuous extraction of olive oil. Fr. Patent 2,048,251.

Van Deurs, J.A.S. 1928. Extraction of fat from animal or vegetable materials. Brit. Patent 303,413. Chem. Abstr. 1929, 23: 4586.

VanEtten, C.H. 1969. In: Toxic Constituents of Plant Food Stuffs. Ed. I.E. Liener. Academic Press, New York, pp. 120-128. Cited by M.N. Radwan and B.C.-Y. Lu. 1976. J. Amer. Oil Chem. Soc., 53: 142-144.

VanEtten, C.H., M.E. Daxenbichler, J.E. Peters, and H.L. Tookey. 1966. Variation in enzymatic degradation products from the major thioglucosides in Crambe abyssinica L. and Brassica napus L. seed meals. J. Agric. Food Chem., 14: 426-430.

VanEtten, C.H., M.E. Daxenbichler, J.E. Petters, and I.A. Wolff. 1965. Seed meal detoxification. Seed meal from Crambe abyssinica. J. Agric. Food Chem., 13: 24-27.

VanEtten, C.H., M.E. Daxenbichler, and I.A. Wolff. 1969a. Natural glucosinolates (thioglucosides) in foods and feeds. J. Agric. Food Chem., 17: 483-491.

VanEtten, C.H., W.E. Gagne, D.R. Robbins, A.N. Booth, M.E. Daxenbichler and I.A. Wolff. 1969b. Biological evaluation of crambe seed meals and derived products by rat feeding. Cereal Chem., 46: 145-155.

Virtanen, A.I. 1965. Studies on organic sulphur compounds and other labile substances in plants. Phytochemistry, 4: 207-228.

Virtanen, A.I., R. Gmelin, M. Kiesvaara, M. Kreula, E. Puronen, M. Saarivirta, and P. Vilkki. 1963. Biochemical Inst. Helsinki. Cited by F. Whiting. 1965. In: Rapeseed Meal for Livestock and Poultry. - A Review. Ed. J.P. Bowland, D.R. Clandinin, and L.R. Wetter. Can. Dept. Agric. Ottawa, Pub. No. 1257, pp. 61-68.

Vles, R.O. 1974. Nutritional aspects of rapeseed oils. International Rapeseed Conf., 4, Giessen, Germany, pp. 17-30.

Vohra, P., F.H. Kratzer, and M.A. Joslyn. 1966. The growth depressing and toxic effects of Tannins to chicks. Poultry Sci., 45: 135-142.

VonFellenberg, T. 1945. Comparative studies of raw and refined rapeseed oil. Mitt. Lebensm. Hyg., 36: 355-359. Chem. Abstr., 1946, 40: 3624$_8$.

Wetter, L.R. 1965. The chemical composition of rapeseed meal. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, D.R. Clandinin, and L.R. Wetter. Can. Dept. Agr., Ottawa, Pub. No. 1257, pp. 32-44.

Wetter, L.R., and B.M. Craig. 1959. Varietal and environmental effects on rapeseed. 1. Isothiocyanate and thiooxazolidone content. Can. J. Plant Sci., 39: 395-399.

Wetter, L.R., H. Ukrainetz, and R.K. Downey. 1970. The effect of chemical fertilizers on the content of oil, protein and glucosinolates in Brassica including rapeseed. In: Proceedings of the International Conference of the Science, Technology and Marketing of Rapeseed and Rapeseed Products. Rapeseed Association of Canada. Ste. Adele, Quebec, Canada, pp. 92-112.

Yapar, Z., and Clandinin, D.R. 1972. Effect of tannins in rapeseed meal on its nutritional value for chicks. Poultry Sci., 51: 222-228.

Young, P.A. 1934. Freezing phenomena in cresoap emulsion of petroleum oils. Plant Physio., 9: 795-804.

Youngs, C.G. 1965. Processing of rapeseed meal. In: Rapeseed Meal for Livestock and Poultry - A Review. Ed. J.P. Bowland, D.R. Claninin, and L.R. Wetter. Can. Dept. Agric. Ottawa. Pub. No. 1257, pp. 24-32.

Youngs, C.G. 1967. Fat and oils in Canada. Vol. 2, Dept. of Industry, Trade and Commerce, Ottawa, Ont., p. 39.

Youngs, C.G., and A.S. Perlin. 1967. Fe(11) -catalyzed decomposition of sinigrin and related thioglycosides. Can. J. Chem., 45: 1801-1804.

Youngs, C.G., and L.R. Wetter. 1967. Micro-determination of the major individual isothiocyanates and oxazolidinethione in rapeseed. J. Amer. Oil Chem. Soc., 44: 551-554.

Youngs, C.G., L.R. Wetter, and G.S. Boulter. 1972. Processing of Canadian rapeseed for high quality meal. In: Canadian Rapeseed Meal in Poultry and Animal Feeding. Rapeseed Association of Canada. Pub. No. 16, pp. 4-7.

Zeman, I., J. Pokorny, H. Kozlowska, and A. Rutkowski. 1964. Uber rapsschrote. 6. Mitt. Rest-felt in extraktionschroten. Nahrung, 8: 681-690.

Zeman, I. and D. Zemanova. 1967. Tluszcze Jadalne, 11:111. Cited by J.K. Daun and F.W. Hougen. 1976. J. Amer. Oil Chem. Soc., 53: 169-171.