CHARACTERISTICS OF SOLVENT EXTRACTED FLAXSEED (Linum usitatissimum L.) MEALS

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CHARACTERISTICS OF SOLVENT EXTRACTED FLAXSEED (*Linum usitatissimum L.*) MEALS

BY

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ABSTRACT

Flaxseed meal was prepared by a two-phase solvent extraction system consisting of alkanol, ammonia, water and hexane. Methanol, ethanol and isopropanol were used as the alkanol and the prepared meals were evaluated with respect to their effect on nutrients, antinutrients and functional characteristics. Commercially available flaxseed meal was also studied.

Approximately 46 to 49% meal and 46 to 50% oil were recovered from dry seeds depending on the extraction system employed. The removal of 4.2 to 5.7% polar substances from the seeds resulted in an increase of 13 and 10% in the content of crude protein and ash, respectively. The presence of ammonia in the polar phase had little effect on the non-protein nitrogen content.

A method was developed to isolate and quantify individual cyanogenic glycosides of linseed using chromatographic techniques. The cultivar used for this study was free of linamarin and contained 4.42±0.08 mg/g of linustatin and 1.90±0.03 mg/g of neolinustatin in the defatted meal on a dry basis. The extraction system, consisting of 10% (w/w) ammonia in 95% (v/v) methanol, removed 57% of linustatin and neolinustatin present in the original samples. A higher content of water, up to 15% in the methanol-ammonia-water phase, removed 67 to 68% of cyanogenic glycosides but resulted in a sticky, dark-coloured meal. Increased contact time (30 min) and solvent-to-seed ratio (R, 13.3) were more effective as 78% to 81% of cyanogenic glycosides were removed by this process. A two stage extraction with

methanol-ammonia-water/hexane gave similar results, but, a three stage extraction removed approximately 92.5% of cyanogenic glycosides present in flaxseed.

The content of total phenolic acids (220±13 mg/100g), condensed tannins (136±13 mg/100g) and soluble sugars (7.69±0.16%) of defatted meals were reduced by 10-48%, 26-74% and 5-46%, respectively. Defatted flaxseed meals contained 2.4±0.13 to 2.8±0.37% of phytic acid and solvent extraction resulted in a slight increase in its content in the products. Flaxseed meal was low in methionine, lysine and tryptophan compared to the FAO/WHO reference values. Methanol-ammonia-water/hexane extraction had little effect on the content of amino acids but resulted in lowering of the content of some of the fatty acids possibly due to the removal of some phopholipids by the polar phase.

Flaxseed meal had a very high water absorption (9.7g H_2O/g) and water hydration capacity (5.2 g H_2O/g) and they were not altered by extraction with the two-phase solvent processing. The presence of ammonia in the extraction system enhanced the fat absorption of the meals 2.6-3.2 fold and increased the pH by almost one unit. Nitrogen solubility of the meals was fairly high (46-65%) and extraction with methanol-ammonia improved the nitrogen solubility of the products at their natural pH. Minimum value of the nitrogen solubility in the meals was observed at pH between 3.0-3.5. Emulsifying capacities of the meals were 64.5% to 80.6% and they were fairly stable to heat and retained 95 to 100% of the emulsifying activity. Whippability of the meals was between 55 to 70% and the foams were stable.

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LIST OF ABBREVIATIONS

IV	- Iodine value
LEAR	- Low erucic acid rapeseed
LHF	- Linolenic acid hardening flavour
LDL	- Low density lipoproteins
FAO/WHO	- Food and agriculture organization/ World health organization
R	- Solvent-to-seed ratio
ACS	- American chemical society
AOAC	- Association of official analytical chemists
AACC	- American association of cereal chemists
TCA	- Trichloroacetic acid
HPTLC	- High performance thin layer chromatography
TLC	- Thin layer chromatography
NMR	- Nuclear magnetic resonance
HPLC	- High pressure liquid chromatography
FPA	- Free phenolic acid
EPA	- Esterified phenolic acid
IBPA	- Insoluble bound phenolic acid
k	- Constant
r	- Regression coeffcient

NSI - Nitrogen solubility index

SAS - Statistical analysis system

THIS WORK IS DEDICATED

TO

MY LOVING PARENTS

CHAPTER 1

INTRODUCTION

Oilseed meals have been widely used as feedstuffs for a long time. With rapid expansion in usage of plant proteins as food ingredients, several non-conventional sources including oilseed meals have gained importance. Food utilization of soybean, rapeseed/canola, sunflower and groundnut has been extensively studied for this purpose.

Oilseeds, like other plant seeds, store nutrients for use during the germination process. Seed contains food materials required by the embryo for its initial development; proteins, carbohydrates, lipids, growth factors, enzymes, minerals. They also contain several chemical compounds which protect the seed. Components of oilseeds include both nutrients and factors without any nutritive value or even antinutritional properties. The latter compounds may be present in minute quantities but could potentially limit the use of protein meals unless they undergo prior treatment and/or extraction.

In general, most of the antinutrients are derived as secondary metabolites of the plant and include chemical compounds such as cyanogens, phenolic compounds, enzyme inhibitors, phytates, allergens, alkaloides, goitrogens, lathrogens, etc. The ability of these compounds to bind to major nutrients such as proteins, carbohydrates, minerals and vitamins reduces their bioavailability and may also cause toxic effects.

Plant-derived protein ingredients are commonly used in comminuted meat products, imitation foods and texturized products (Dabrowski *et al.*, 1991). It is acknowledged that oilseed proteins in food products are not intended for use based on their nutritive value but are used mainly for the functional properties they impart. These non-conventional protein sources bring about appropriate interactions with other major food ingredients (eg. oil, water) to facilitate their incorporation in food formulations. Therefore, the diverse functional properties of oilseed protein ingredients influence the utilization of such products (Smith, 1971).

The final nutrient content and functional properties of oilseed meals are determined by the processing conditions they have been subjected to. Oils from the seeds are extracted by pre-pressing or dissolution in organic solvents (Johnson and Lusas, 1983). Seeds or press cakes are usually prepared by cracking, heating and flaking prior to direct solvent extraction or are expeller-pressed, ground and flaked before extraction. These physical treatments greatly affect the nutritional and functional properties of the seed meals.

Solvent extraction is a process for transporting materials from one phase to another for the purpose of separating one or more components from the mixtures (Johnson and Lusas, 1983). In oil-bearing seeds, crude oil is separated by pressing or solvent extraction of seeds which contain proteins, carbohydrates and other components. Various solvents and extraction methods have been used commercially and others have been proposed based on laboratory results, mainly emphasizing

maximum yield of oil. Processes have been developed for simultaneous extraction of oil and removal of antinutritive components from oilseeds (Johnson and Lusas, 1983). Meanwhile, obtaining a good quality meal with unaltered nutritive value and functional properties is of practical importance.

Flax or linseed which is the third largest oilseed crop in Canada (Anonymous, 1989) is primarily grown for its oil content. It is used as a drying oil because of its high content of α-linolenic acid. However, little attention has been paid to the utilization of protein meals in food formulations. Presence of antinutritive components (eg. cyanogens, antivitamins, etc.) limits the use of meals particularly for monogastric animals, but the effective removal of these components may enhance the use of linseed meal in food and feed formulations.

The objectives of this study were to upgrade linseed meal by a two-phase solvent extraction system consisting of different alcohols (absolute or otherwise) with or without ammonia and to evaluate nutritional and antinutritional components of the resultant meals as well as the functional properties of the meals.

CHAPTER 2

LITERATURE REVIEW

2.1 Linseed - General considerations

2.1.1 Production and cultivation of linseed in Canada

According to global production data on oilseeds, linseed is the sixth major oilseed crop in succession to soybean, cottonseed, rapeseed, groundnut and sunflower (Shahidi, 1990). The major oilseeds produced in Canada were canola (3.057 million tonnes), soybean (1.176 million tonnes), linseed (0.531 million tonnes), mustard (0.165 million tonnes) and sunflower (0.069 million tonnes) in the 1989-90 crop year (Anonymous, 1989). Canada has become the biggest producer of linseed in the world by contributing 27.7% of the total global production in the year 1989-90 (Anonymous, 1989).

Linseed plant is mainly grown in Western Canada, from latitude 49° to 57° north, from longitude 96° to 121° west, and 225 to 1000 m above sea level (Dorrel, 1975). Linseed cultivars may be of spring or winter type depending on the planting season. They are also classified as those for fibre or seed production and sometimes for both. In agronomic terms, linseed is preferred as a rotation crop because it is not usually affected by the diseases that attack cereals and other oilseed crops. Furthermore, the clean straws are used in industrial application and no fibrous residues are left in the soil (Dorrel, 1975).

2.1.2 Botany of the plant

The flax or linseed (*Linum usitatissimum L.*) plant belongs to the family *Linaceae*, which includes about 290 species (Gray, 1981) and it is the only member of the *Linum* genera that is cultivated as a field crop (Dillaman, 1946; Dorrel, 1972). This dicotyledonous plant is known to have been cultivated in ancient Egypt and perhaps even by prehistoric man (Dybing and Lay, 1981). The name "usitatissimum" meaning most useful is most appropriate for it as both stem and seeds have been used as economic products since antiquity.

The mature linseed plant is 0.2-1.2 m high with a compound inflorescence and is profusely branched especially in winter varieties which are mainly grown for seeds (Dybing and Lay, 1981). Typical main-stem leaves are about 5 mm wide and 25-50 mm long, arranged in alternate pairs. Flowers are white or varied shades of blue. The mature fruit or ball is a dry capsule that can be indehiscent, semidehiscent or dehiscent (Dybing and Lay, 1981). Linseed plant requires cool weather at the time of blooming and ample moisture and moderate temperature until ripening of the fruit (Easterman, 1968).

Economically-important tissues of linseed include vascular bundles of the stem and oil-containing tissues of the seed. Fibres obtained from stems consist of elongated, thick walled sclerenchyma cells from the phloem regions of vascular bundles (Easu, 1977; Dybing and Lay, 1981). The seeds are yellow to brown in colour, flattened or lens-shaped and 2-6 mm in diameter. In the seed, both embryo and endosperm are oil-

storing tissues. However, the embryo is the major tissue in both size and oil content. It contains 75-85% oil in the mature seed (Dybing, 1968 and 1971).

2.1.3 Uses of plant

2.1.3.1 Fibre

Historically, linseed plant may have been used mainly for its fibre. Flax fibre is used for making linen. Nowadays, there are more uses for flax fibre in industries such as manufacturing of rugs, fine writing papers, currency paper, fine carbon papers, cigarette papers, etc. (Easterman, 1968).

2.1.3.2 Oil

Linseed contains up to 48% crude oil in the seed, on a dry weight basis (Patterson, 1989). The fatty acid composition of linseed oil (Table 2.1) shows that linolenic acid content can vary from 22 to 72% of the total fatty acids (Anderson, 1971; Dorrel, 1970; Patterson, 1989). This feature distinguishes linseed from other vegetable oils and explains the principal use of it as a drying oil in the industry. Linseed oil has been extensively used as a "drying oil vehicle" in paints, varnishes, lacquers, enamels, oil cloth, linoleum, patent leather, textiles, printing ink, soap, shoe polish, and numerous other speciality products (Easterman, 1968; Dybing and Lay, 1981). The drying property of the oil is measured in terms of its "Iodine Number" or "Iodine Value (IV)" and expressed as the ability of the oil to absorb oxygen when it

Table 2.1. Oil content and fatty acid composition of some oilseeds[1].

Component	Canola (LEAR)	Corn germ	Linseed	Soybean
Oil content (%)	44.0-48.0	3.5-5.0	32.0-48.0	18.0-23.0
Fatty acid (w/w %)				
C14:0	-	<0.3	-	<0.1
C16:0	2.5-60.0	9.0-14.0	7.0-9.3	7.0-14.0
C16:1	<0.6	<0.5	-	<0.5
C18:0	0.9-2.1	0.5-4.0	2.1-3.7	3.0-5.5
C18:1	50.0-66.0	24.0-42.0	17.2-19.5	18.0-26.0
C18:2	11.0-23.0	32.0-62.0	15.5-19.1	50.0-57.0
C18:3	8.3-13.0	<2.0	22.0-72.0	5.5-10.0
C20:0	0.1-1.2	<1.0	-	<0.6

[1] Adapted from Patterson (1989).

is exposed to air. Both genetic and seasonal variations (temperature, rainfall) lead to a wide range of IV, from 130 to 205, but values of 180-185 may be taken as typical (Easterman, 1968; Patterson, 1983).

Use of linseed oil to enrich the polyunsaturated fatty acid content in animal tissue lipids, including man is currently being investigated (Holub, 1990; Jiang *et al.*, 1991). Linseed oil can be hydrogenated to prepare edible products. Hydrogenation can also minimize the risk of linolenic hardening flavour, LHF (Patterson,1983). It has been shown that nickel-catalysed hydrogenation is not suitable for linseed oil and would tend to produce a hard material with a high melting point. The copper-catalysed hydrogenation has produced an original flavour product that is suitable for margarine blends (Anderson, 1971). Recent developments in biotechnology would allow the development of low linolenic linseed varieties (Dorrel, 1972). However, the ever widening cultivation of canola, soybean and sunflower may overshadow the use of hydrogenated linseed oil for edible products (Patterson, 1983, 1989).

2.1.3.3 Seed and seed meal

Whole flaxseed has found a variety of uses in speciality food products. These include hot or dry cereals and bread flour ingredients or flaxseed tea as well as pharmaceuticals (Dybing and Lay, 1981). The small seeds are usually pre-pressed and solvent extracted to obtain oil. The "linseed meal" (with hulls) remaining after oil extraction affords a high protein component which is traditionally used as an animal

feed ingredient. It has been reported that use of linseed meal for livestock resulted in improved health, superior milk production and improved carcass grade as well as finish and sleek appearance (Peterson, 1958). However, inclusion of linseed meal in amounts greater than 14% in the feed of dairy animals resulted in inferior quality of butter, cheese and milk (Singh, 1979). Possible use of linseed meal as a protein supplement for swine has indicated a 25% limit for inclusion of linseed protein meal in the diet (Peterson, 1958; McDonald *et al.*, 1966). In general, linseed meal is not considered as a satisfactory ingredient for poultry feed because of retardation of growth. Diets containing 5% linseed meal have resulted in a 10% death rate in turkey poults (McDonald *et al.*, 1966). However, flaxseed has shown no adverse effects on growth of rats even at a 40% level in the diet (Ratnayake *et al.*, 1992). A major fraction of dietary fibre of flax was found to be fermentable. Higher levels (40%) of flax in rat diets were able to lower the content of serum low density lipoproteins (LDL) but elevated the oxidative stress on tissues (Ratnayake *et al.*, 1992).

2.2. Chemical composition of linseed meal

2.2.1 Proximate composition

Only a few reports on compositional characteristics of linseed meal are available in the literature. The proximate composition reported for linseed meal indicates that it has a similar protein content when compared with canola and soybean (Table 2.2). The more recent results reported by Bhatty and Cherdkiatgumachai (1990)

Table 2.2. Proximate composition of some oilseed meals (%).

Component	Linseed[1]	Rapeseed[2]	Soybean[2]
Crude protein (Nx6.25)	43.9	43.1	50.4
Ash	6.4	7.0	6.8
Crude fat	-	2.3	0.5
Crude fibre	-	10.7	6.9
Total dietary fibre	15.6	-	-
Total carbohydrates	30.6	-	-
Nitrogen free extract	-	36.9	35.4

[1] Bhatty and Cherdkiatgumachai (1990).
[2] Josefson (1972).

and by Madhusudhan and Singh (1983) confirmed the previous reports by Peterson (1958) and Mandhokhot and Singh (1979). The protein content of linseed meal varies from 36% to 45%, on a dry weight basis, depending on genetic and environmental variations (Singh, 1979; Madhusudhan and Singh, 1983; Bhatty and Cherdkiatgumachai, 1990). There are few reports available on the amino acid composition of linseed proteins and these show that lysine and methionine are the limiting amino acids and the meal needs to be supplemented when used in monogastric feed formulations (Singh, 1979; Bhatty and Cherdkiatgumachai, 1990).

The mineral composition of linseed meal as reported by Bhatty and Cherdkiatgumachai (1990) showed the presence of macro elements in the decreasing order of K, P, Mg, Ca, S and Na and micro elements as Fe, Zn, Mn and Cu. Vitamin A was found at 18.8 IU/100g and vitamin E was present at about 0.6 IU/100 g. Vitamin B_3 (niacin) was the predominant B vitamin (9.1 mg/100g). High total dietary fibre content of linseed meal compares well with other oilseed meals and this is largely due to its high content of mucilage (Bhatty and Cherdkiatgumachai, 1990).

2.2.2 Antinutritional components

Cyanogenic glycosides and anti-pyridoxin factor or linatine, are the major antinutrients reported in linseed meal. Even though the presence of phytic acid (Madhusudhan and Singh, 1983; Bhatty and Cherdkiatgumachai, 1990), phenolic acids (Dabrowski and Sosulski, 1984) and enzyme inhibitors (Madhusudan and Singh, 1983)

Table 2.3. Amino acid composition of some oilseed meals and FAO/WHO reference values (g/16 g N).

Amino acid	Rapeseed[1]	Soybean[1]	FAO/WHO Reference[2]
Isoleucine	3.7	4.8	4.0
Leucine	6.3	7.6	7.0
Lysine	5.8	6.1	5.5
Methionine	1.8	1.5	3.5 (+cystine)
Phenylalanine	3.5	5.0	6.0 (+tyrosine)
Tyrosine	2.6	2.9	-
Threonine	3.8	4.0	4.0
Tryptophan	1.3	1.0	1.0
Valine	4.8	5.0	5.0
Alanine	3.9	4.5	-
Arginine	5.6	6.9	-
Aspartic acid	6.2	11.9	-
Cystine	2.4	1.7	-
Glycine	4.3	4.4	-
Glutamic acid	16.6	20.6	-
Histidine	2.7	2.6	-
Proline	6.4	-	-
Serine	3.7	5.2	-

[1] Josefson (1972).
[2] FAO/WHO (1973).

in linseed meal has been reported, their deleterious effects have not been considered to be as important as those of cyanogenic glycosides and linatine.

2.2.2.1 Cyanogenic glycosides

Both vegetative parts and seeds of flax contain cyanogenic glycosides. The presence of monosaccharide cyanogenic glycosides in linseed (linamarin and lotaustralin, Figure 2.1) has been reported (Butler, 1965; Conn, 1981). Smith *et al.* (1980) have reported that two disaccharide cyanogenic glycosides (Figure 2.1.) may also be isolated from linseed meal. The proposed trival names are linustatin (2-[(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-2-methylpropanenitrile) and neolinustatin ([(2R)-[(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-2-methylbutanenitrile]). Both these compounds were shown to protect farm animals against selenium toxicity (Smith *et al.*, 1980).

Cyanogenic glycosides are β-glycosidic derivatives of α-hydroxynitriles. They are mainly derived by multi-step biosynthetic sequences from the amino acids namely L-phenylalanine, L-tyrosine, L-leucine, L-isoleucine and L-valine. It has been shown that synthesis of linamarin and lotaustralin (Butler, 1965) and linustatin and neolinustatin (Conn, 1981) in linseed is closely associated with valine and isoleucine metabolism, respectively. Linamarin and lotaustralin are cyanohydrins of acetone and 2-butanone, respectively. The unstable cyanohydrin moiety is stabilized by a

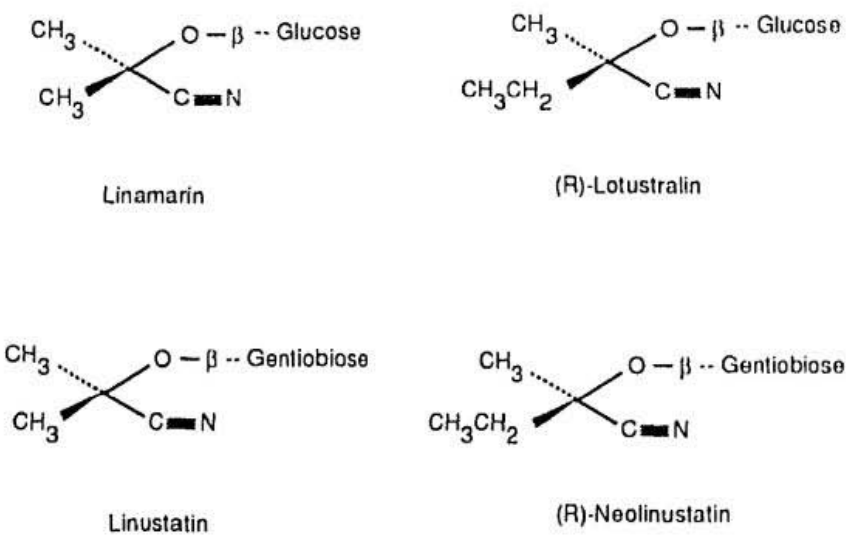


Figure 2.1. Structures of possible cyanogenic glycosides in linseed.

glycosidic linkage to a single sugar residue (D-glucose) in linamarin and lotaustralin while a disaccharide (D-gentiobiose) stabilizes linustatin and neolinustatin. The aliphatic cyanogenic glycosides are referred to as bound cyanide. The molecule HCN is regarded as free non-glycosidic cyanide. It is well documented that cyanogenic glycosides are present in fodder crops like white clover, root crops like cassava, nuts like macadamia, bitter almonds and also plants like flax, sorghum, bamboo and lima beans (Montgomery, 1980). Even though the legume family is recognized as a cyanogenic genera, soybean meal contains a very little amount (0.07-0.3 μg/g of meal) of cyanide (Honig *et al.*, 1983).

Under normal physiological conditions, tissues of cyanophoric plants contain little or no detectable HCN. When plant tissues are disrupted, HCN may be rapidly released from cyanogenic glycosides upon hydrolysis. The catabolism of cyanogenic glycosides is initiated by cleavage of their carbohydrate moiety by one or more β-glucosidases, thus yielding the corresponding α-hydroxynitriles (Figure 2.2). This intermediate may decompose either spontaneously or enzymatically in the presence of α-hydroxynitrile lyase to yield HCN and an aldehyde or ketone (Poulton, 1989).

Potential toxic levels of cyanogenic glycosides for animals consuming plant materials containing cyanogenic glycosides depend on factors such as species and size of animal, the level of β-glucosidases in the plant, the length of time between tissue disruption and ingestion, the presence and nature of other components in meal and the rate of detoxification of HCN by the animal (Poulton, 1989). For acute toxicity to

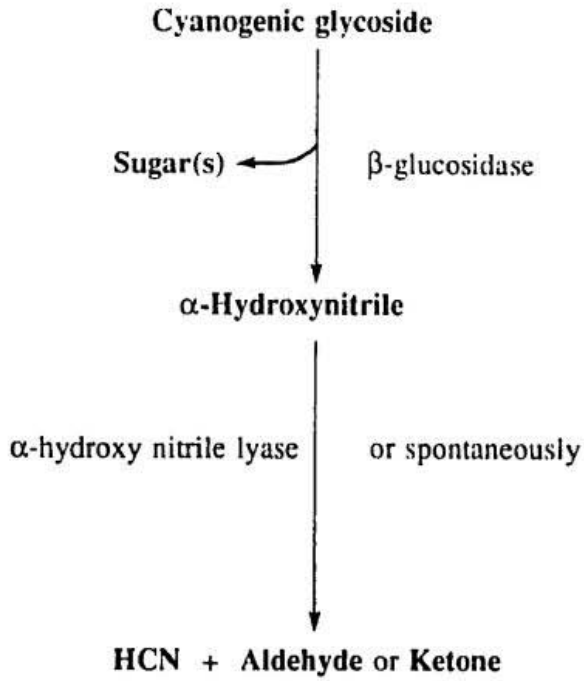


Figure 2.2 Breakdown of cyanogenic glycosides

occur, enough plant material must be ingested in a sufficiently short period by the animal. In human, the minimum lethal dose of HCN taken orally is approximately 0.5-3.5 mg/kg body weight (Poulton, 1989).

Cyanide exerts an acute toxic effect by combining with metalloporphyrin-containing enzyme systems (Conn, 1981). The most important one is its affinity to cytochrome oxidase. Cyanide concentration of approximately 33 μM can completely inhibit the mitochondrial electron transport system, thus swiftly preventing utilization of oxygen by the cell (Conn, 1981).

Several detoxification methods for cyanogenic glycosides of cassava have been reported. However, few effective means are available for detoxification of cyanogenic glycosides. In general these glycosides are heat stable, thus cooking has little or no effect on the content of cyanogens but tends to inactivate the endogenous β-glucosidases present (Poulton, 1989).

Isolation and quantification of cyanogenic glycosides using endogenous β-glucosidase in cassava is well documented (Wood, 1966; Cooke, 1978; Cooke *et al.*, 1978; Ikediobi *et al.*, 1980). Only very few reports are available on separation (Smith *et al.*, 1980; Brimer *et al.*, 1983) and quantification (Brimer *et al.*, 1983) of cyanogenic glycosides of linseed.

2.2.2.2 Anti-pyridoxin factor

Incorporation of high levels of linseed meal in chicks diets has shown typical

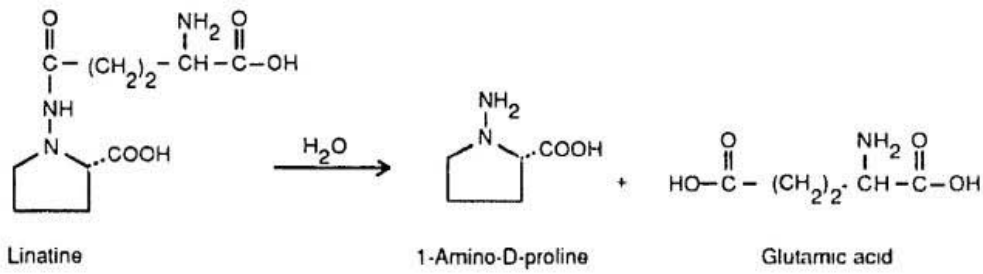


Figure 2.3. Linatine and its hydrolytic products.

symptoms of a B vitamin deficiency and this has been alleviated by addition of pyridoxin to the ration (Kratzer *et al.*, 1946) which also improved the growth rate of the birds (Klostermann *et al.*, 1960). It was suggested that linseed meal contains a substance which acts as a vitamin B_6 antagonist and counteracts the vitamin B_6 naturally present in the feed. Klostermann *et al.* (1967) were able to isolate and characterise this vitamin B_6 antagonist as well as to synthesize it. The trival name "linatine" was suggested for this compound because of its association with plant, *Linum usitatissimum*. Although linatine was first observed in seeds, its presence in immature linseed plants was recognized later (Nugent, 1971).

Klostermann *et al.* (1967) identified linatine as 1-amino-D-proline, combined via a peptide linkage with γ-glutamic acid (Figure 2.3). Linatine was found to be very soluble in water but insoluble in anhydrous organic solvents. Hydrolysis of linatine produced 1-amino-D-proline (DAP), an asymmetrically substituted secondary hydrazine. DAP readily condenses with the carbonyl groups of pyridoxal (PL) and pyridoxal phosphate (PLP) to form a stable hydrazone (Klostermann, 1974). Linatine, DAP, and other α-hydrazino acids have also been shown to possess bactericidal properties (Klostermann, 1974).

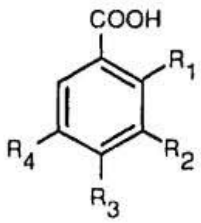
2.2.2.3 Phenolic compounds

Oilseed flours are reported to contain a considerable amount of phenolic acids as well as other phenolic compounds. Natural phenolic compounds are hydroxylated

derivatives of benzoic and cinnamic acids, coumarins, flavonoids, condensed tannins and lignin (Ribereau-Gayon, 1972; Sosulski, 1979a). Phenolic acids generally belong to the benzoic acid (C6+C1) and the cinnamic acid (C6+C3) families (Figure 2.4). Benzoic acids are widely distributed in nature and these include *p*-hydroxybenzoic, protocatechuic, vanillic, gallic, syringic, *o*-hydroxysalicylic and gentisic acids (Figure 2.4). Cinnamic, *p*-coumaric, caffeic, ferulic and sinapic acids are found in most oilseeds and occur frequently in the form of esters with quinic acid or with sugars (Sosulski, 1979a). Coumarins have C6+C3 structure of the cinnamic acids but the C3 chain is formed into an oxygen containing heterocycle. Presence of coumarins in oilseeds is less pronounced.

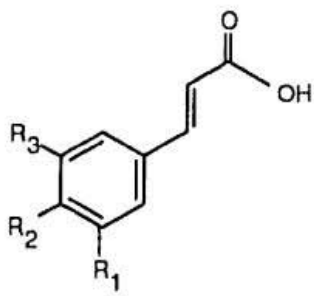
Flavonoids are a major group of plant phenolics and have a C6+C3+C6 structure in common; flavones, flavonols, flavanones, anthocyanidins, chalcones, aurones, etc. (Figure 2.5). Their structures differ from one another by the nature of the C3 group. The flavonols which are commonly found in oilseeds are present as glycosides. Flavanols do not occur as glycosides but have a great tendency to polymerise and form condensed tannins (eg. flavan-3-ol).

The polymeric phenols, principally hydrolysable tannins, yield gallic and ellagic acids as well as glucose upon acid hydrolysis. Condensed tannins yield only flavanols and a brown residue. Lignin releases a variety of benzoic and cinnamic acid derivatives as well as other unrelated compounds (Sosulski, 1979a).



Benzoic acids

R_1	R_2	R_3	R_4	
H	H	OH	H	-- p-Hydroxybenzoic acid
H	OH	OH	OH	-- Gallic acid
OH	H	H	OH	-- Gentisic acid
OH	H	H	H	-- Salicylic acid
H	H	OH	OH	-- Protocatechuic acid
H	OCH_3	OH	OCH_3	-- Syringic acid
H	H	OH	OCH_3	-- Vanillic acid



Cinnamic acids

R_1	R_2	R_3	
H	OH	OH	-- Caffeic acid
H	OH	H	-- p-Coumaric acid
H	OH	OCH_3	-- Ferulic acid
OCH_3	OH	OCH_3	-- Sinapic acid

Figure 2.4. Phenolic acids of oilseed meals.

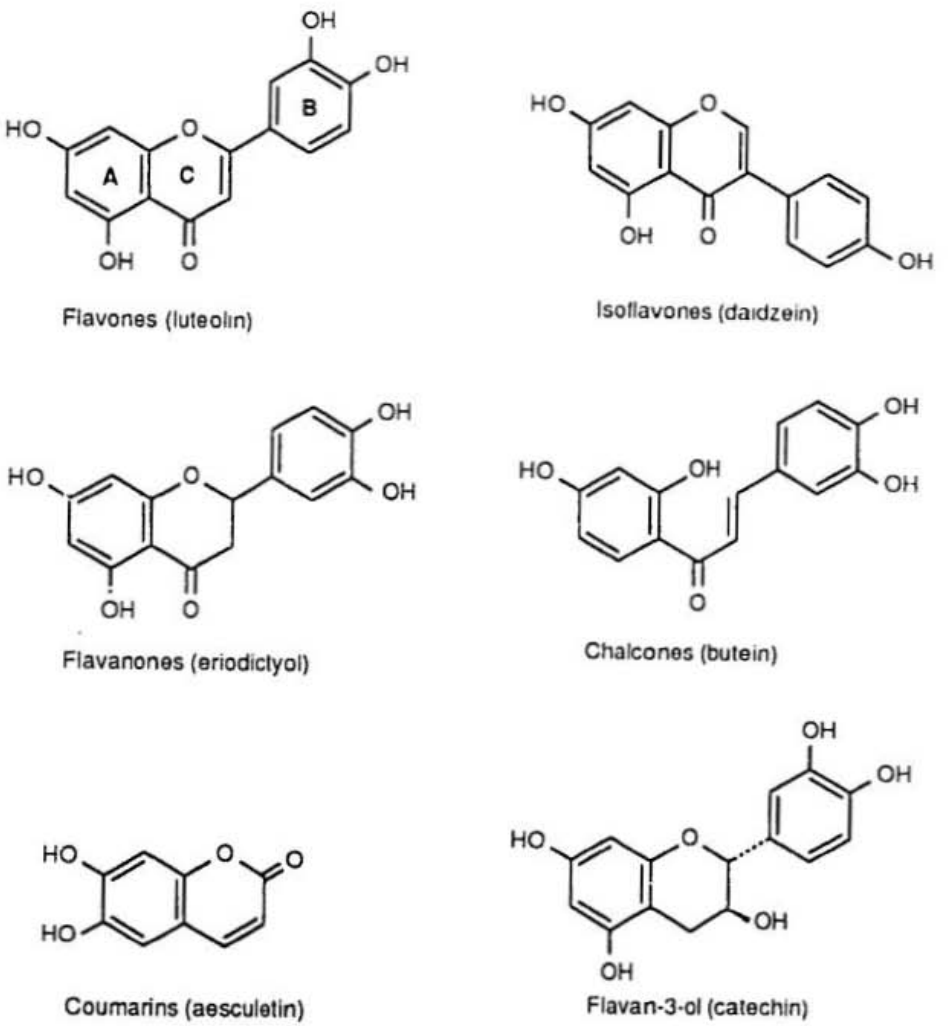


Figure 2.5. Polyphenols of oilseed meals.

Phenolic compounds contribute to the dark colour, bitter taste and astringency of oilseed meals. In canola, phenolic acids in the free form (13-14% of total phenolic acids) are recognized to contribute to the taste of products (Kozlowska *et al.*, 1990) and predominantly contain sinapic acid (Kozlowska and Zadernowski, 1988). Esterified phenolic acids constitute up to 80% of the total phenolic acids of canola; mainly as choline ester of sinapic acid referred to as sinapine (Krygier *et al.*, 1982). Sinapine is identified as being responsible for the bitter taste of *Brassica* oilseeds (Clandinin, 1961; Sosulski, 1979a). Phenolic acids released from insoluble residues also contain a large amount of sinapic acid (Koslowska *et al.*, 1983a,b).

The green to brown colour of sunflower protein isolates has been ascribed to the presence of chlorogenic acid (Carter *et al.*, 1972; Sosulski, 1979a). It has also been found that development of a specific off-flavour during heating of soybean flour is caused by the formation of 4-vinylguaiacol and 4-vinylphenol from *p*-coumaric and ferulic acid precursors (Olson and Alder-Nissen, 1979).

In addition to the sensory effects, phenolic acids of oilseed meals interact with other seed nutrients. Atmospheric or enzyme-catalysed oxidation of phenolics of oilseeds results in production of quinoidal compounds and hydrogen peroxide. Both of these products are destructive to labile amino acids, denature proteins and inhibit enzyme activity (Sosulski, 1979a). In oilseeds, cinnamic acid and its esters are the preferred substrates for phenolases and polyphenol oxidases. The *o*-diphenols,

especially caffeic and chlorogenic acids are oxidized to form *o*-quinoaes by the action of enzyme phenol oxidase. The *o*-quinones so produced react non-enzymatically to polymerize or bind covalently to amino, thiol and methylene groups. The ε-amino group of lysine and SH group of methionine are usually attacked and are thus rendered nutritionally unavailable to the digestive systems of monogastrics (Sosulski, 1979a). This has been reported to be a serious problem in sunflower, where chlorogenic and caffeic acids contribute about 70% to the total phenolic acids present in the meal (Sabir *et al.*, 1974a,b). In cottonseed meal, the aldehydic groups of gossypol react readily with ε-amino group of lysine present in cottonseed globulin proteins, especially at high temperatures of oil extraction (Beradi and Goldblatt, 1980). The other important property of many phenolics possessing *o*-dihydroxy grouping (catechol) is their ability to chelate metal ions (Harbone, 1980). There is evidence that tannins bind proteins and polyvalent cations such as those of iron.

It has been established that oilseed meals possess antioxidant properties mainly because of the presence of phenolic compounds. Flavonoids and cinnamic acid derivatives are known as primary antioxidants which act as free radical acceptors and chain breakers (Pratt and Hudson, 1990). The metal-chelating activity of the flavonoids (Hudson and Lewis, 1983) and their ability to form resonance-stabilized free radicals (Dziedzic and Hudson, 1983) facilitates their action as strong antioxidants. The antioxidant activity of phenolic acids and their esters depends on the

number of hydroxy groups, and their effectiveness would be strengthened by the presence of steric hinderance in the molecule (Dziedzic and Hudson, 1983). Phenolic compounds in soybean flour have been identified as isoflavone glycosides (Rakis, 1972; Naim *et al.*, 1973), caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, syringic and vanillic acids (Arai *et al.*, 1966). In cottonseed meal, quercetin and rutin (Whittern *et al.*, 1984) and in peanut meal, dihydroquercetin and taxifolin (Pratt and Miller, 1984) have been identified as antioxidative flavonoids. Methanolic extracts of canola and mustard flour were shown to possess antioxidant activity in meat model systems and their activity was proportional to the total content of phenolics present (Shahidi *et al.*, 1992).

2.2.2.4 Phytic acid

Phytic acid or *myo*-inositol hexaphosphate; 1,2,3,4,5,6-hexakis-dihydrogen phosphate (Figure 2.6) has been found in cereals and legumes up to a level of approximately 5% by weight (de Bolland *et al.*, 1975). Phytic acid is the major storage form of phosphorus in the seeds and it is produced as a secondary product of carbohydrate metabolism (Loewus and Loewus, 1980). Phosphorus from phytic acid comprises 50-60% of the total organic phosphorus present in soybean (Erdman, 1979) and 78-79% of that in canola (Upperstorm and Sevensson, 1980; Naczk *et al.*, 1986a).

Phytic acid molecule has 12 replaceable protons (Figure 2.6) and is negatively

charged at pH values encountered in food and feedstuff. Therefore, it is highly reactive towards positively charged groups such as metal ions and proteins (Erdman, 1979; Thompson, 1990). In general, one or two phosphate groups of phytic acid may bind with cations (Figure 2.6.c; Gosselin and Coughlan, 1953). The mixed salt of phytic acid is formed when several cations complex within the same phytic acid molecule. The binding of phytic acid with minerals is pH dependent, and complexes of varying solubilities are formed (Cheryan, 1980). Most polyvalent metal ions, especially calcium (Reinhold *et al.*, 1973), magnesium (Nolan *et al.*, 1987), zinc (Erdman, 1979; Nosworthy and Cladwell, 1988; Champagne and Phillipy, 1989) and iron (Davis and Nightingale, 1975) bind to phytic acid and form insoluble complexes which makes them unavailable for metabolism.

Phytic acid can also react with proteins, depending on the pH of the medium. At pH values below isoelectric point of proteins, phytic acid binds directly with the positively charged proteins as a result of electrostatic attraction (Figure 2.6.a). At intermediate pH values above the isoelectric point of the protein both phytic acid and protein molecules are negatively charged and phytic acid binds primarily with proteins mediated by polyvalent cations such as calcium or magnesium (Figure 2.6.b). However, direct binding of proteins with phytic acid does not take place to any considerable extent (Cheryan, 1980). Complexing of proteins with phytic acid, directly or through mediation by mineral ions, may alter the protein structure and this in turn

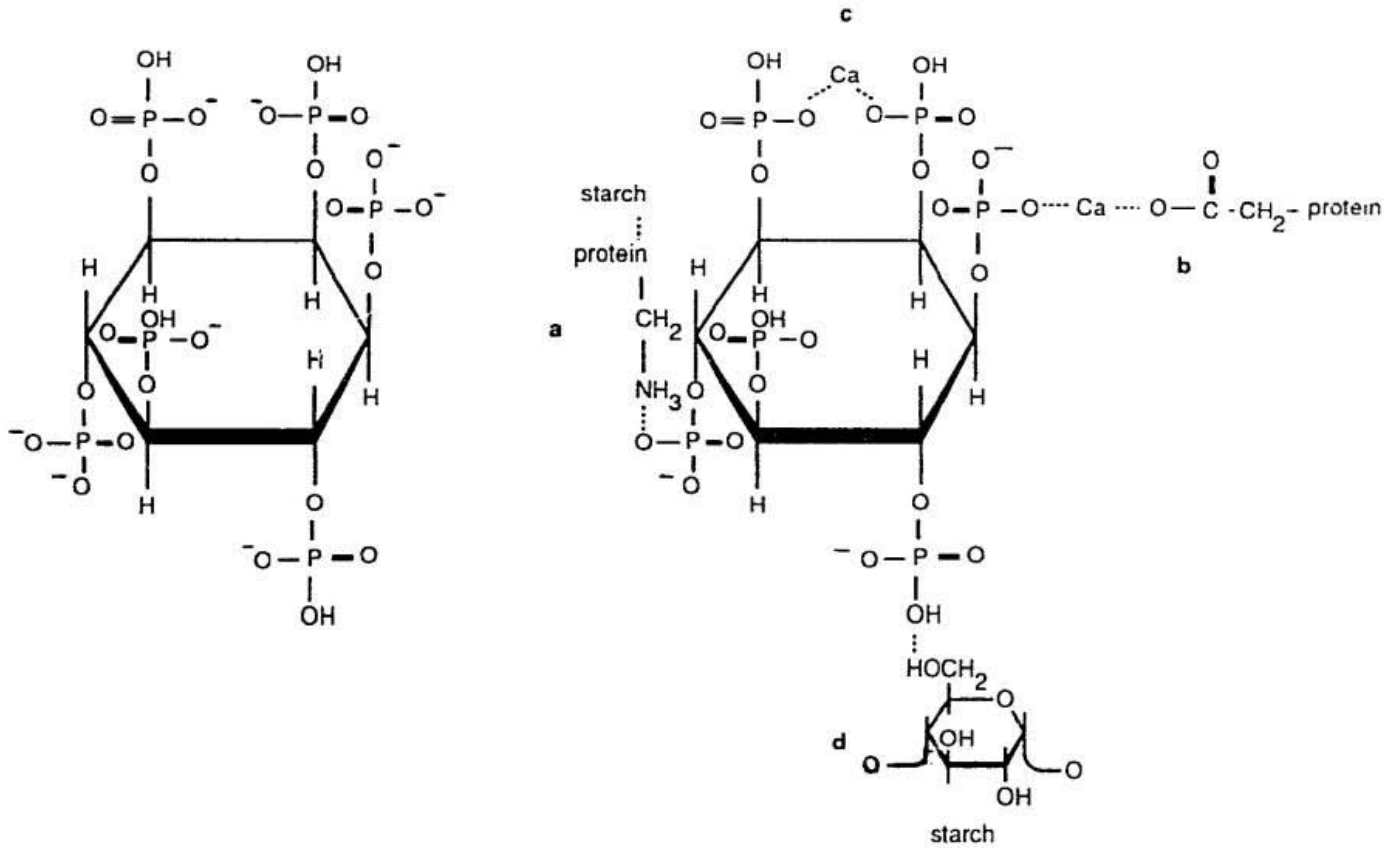


Figure 2.6. Proposed structure of phytic acid and its possible interactions with food nutrients.

may decrease solubility as well as changes in functionality and digestibility (Cosgrove, 1980). Binding of starch molecules with phytic acid is theoretically possible but this has not yet been demonstrated. This type of binding can occur readily through the formation of phosphate link or indirectly through its association with proteins (Figure 2.6.d; Thompson, 1986, 1989)

Phytic acid exists typically as salts of calcium, magnesium or potassium (Mills and Chong, 1977; Yiu *et al.*, 1983). In rapeseed (Yiu *et al.*, 1983), peanut, cottonseed (Saio *et al.*, 1977) and soybean (Tombs 1967; Maga, 1982) phytic acid is found in globloids inside the protein bodies of cotyledon cells. However, in cereals phytic acid is largely found in the protein bodies of the aleurone layer (Thompson, 1990).

Phytates are not easily removed by traditional processing of oilseed meals. The intact phytic acid remains embedded with proteins. The location and the strong association of phytic acid with proteins tend to concentrate it together with proteins during preparation of protein concentrates and isolates from oilseed meals.

2.3 Functional properties of oilseed meals

The functional properties of oilseed meals play an important role in formulation of different food products. Acceptability of such products depends on their sensory quality, nutritional value and functional properties, all of which are affected by the method of meal preparation. Functional properties which determine the usefulness of plant protein materials in food are governed by the physicochemical

properties that affect the behaviour of the colloidally suspended proteins in the food products. The structure, texture, flavour and colour of the formulated products are affected by the functionality of their ingredients.

2.3.1. Solubility

Solubility of a protein meal is affected by the pH as well as the presence of salts in the medium and it is generally reported as the percentage solubility of nitrogen or protein in a water dispersion. Water dispersible nitrogen compounds are proteins, peptides and free amino acids present in the meals. Solubility of a protein meal or product greatly influences its functional properties (Kinsella, 1982).

2.3.2 Water absorption

Water absorption of a protein is affected by several factors; amino acid composition, protein conformation, surface hydrophobicity, and the presence of other compounds that absorb water are amongst these factors (Kinsella, 1982). The extent of hydration is directly proportional to the content of hydrophilic side chains of proteins. The side chain groups involved are carboxyl, amino, imidazole, carbonyl, sulphydryl, hydroxyl and guanidino groups (Briskey, 1970). Water binding is diminished as polar groups are blocked by amidation of carboxyl groups and these in turn influence the solubility of proteins. Temperature and pH are important factors affecting water binding because carboxyl and amino groups are ionisable and the

polarity can be controlled by varying the pH and the temperature (Sosulski *et al.*, 1977). Other factors that influence the water absorption of oilseed meals are the gelation of carbohydrates and swelling of crude fibres (Narayana and Narasinga Rao, 1982). Sensory characteristics associated with the degree of hydration include juiciness, dryness and mouthfeel of the product. The properties that influence water absorption may also control water retention during cooking, processing and storage (Sosulski *et al.*, 1977).

2.3.3. Fat absorption

Fat absorption of protein meals measures the lipophilicity which is dictated primarily by the apolar amino acids in proteins (Kinsella, 1982). Fat absorption may vary depending on the source of protein, extent of processing, particle size, temperature, etc. (Lin *et al.*, 1974). Denaturation of proteins may mask their apolar groups and this can reduce fat absorption. Oil or water retention properties of protein meals may also be affected by food processing conditions where water or oil is incorporated as ingredients along with the meal. Overall qualities of food products such as shrinkage during processing, mouthfeel and storage stability are affected by oil or water retention properties of their constituent ingredients (Beuchat *et al.*, 1975).

2.3.4 Foaming and emulsifying properties

A foam may be defined as a colloidal system consisting of gasses suspended

in a very viscous liquid (Aurand and Woods, 1973). Proteins can serve as good foaming agents by preventing the coalescence of gas bubbles dispersed in the liquid. Exposure of hydrophobic regions of proteins keeps them in solution as a foam. The pH and ionic strength of the medium affect the foam stability indirectly by influencing the nitrogen solubility. Foam stability is closely related with protein solubility (Sosulski, 1979b); therefore, a good foam stability is observed at a higher nitrogen solubility. Non-protein nitrogen compounds, carbohydrates and minerals also affect foam stability (Cherry and McWaters, 1981). It has been reported that extensively heat-denatured proteins show poor foam stability (Bickermann, 1953; Narayana and Narasingha Rao, 1982; Tasneem *et al.*, 1982).

The formation of an emulsion by a protein is mainly due to solubilized protein molecules, and enhanced by the surface activity strength and the ability of the protein to stabilize oil-water emulsion (Sosulski, 1977). The emulsifying capacity of soluble proteins is based on the hydrophobic-lipophilic balance in the molecule which determines their affinity for oil and water. The amino acid composition, protein configuration in the solution, pH and the ionic strength of the aqueous phase influence the emulsifying properties of the proteins (Sosulski, 1979b). Lin *et al.* (1974) have shown that oil emulsification capacities of sunflower and soybean meals were not related to their liquid absorption properties but were governed by the high protein solubilities which were associated with their emulsifying properties.

2.4 Oilseed processing

The conventional extraction process of oilseeds of small size with high oil content is similar. Seeds are first crushed to fracture the seed coat and rupture the cells. This increases the surface-to-volume ratio and enhances oil extraction. In canola oil processing, crushed seeds are cooked at 90-120°C for 15-20 min to inactivate the enzymes present (Anjou, 1972). The crushed and cooked seeds are then pre-pressed to reduce the oil content and compress the material to large cake fragments. The cakes are then flaked and solvent extracted with hexanes using percolating-type extractors. Soybean seeds are fragmented, then flaked, and solvent extracted (Brecker, 1971; Anjou, 1972). The seeds are extracted to give a uniform and low residual oil in the resultant meal (0.5-1.0% of flakes) and to enhance functional properties of the meals. Linseed may be pre-pressed by hydraulic means, by expulsion or by solvent extraction of fresh seeds or cake that has already been partially deoiled by pre-pressing (Delvaux and Bertrand, 1964).

2.5. Two-phase solvent extraction process

Schllingman and Praeve (1978) and Schllingman and Vertery (1978) have reported the use of methanol-ammonia to rupture microbial cell walls followed by extraction of lipids from single cell proteins. Later, Schllingman and von Rymon-lipinski (1982) extended this work to extract lipids from oilseeds. A two-phase extraction process was developed for canola. The process uses the advantage of

alkanol-ammonia-water/hexane to extract the oil into hexane while simultaneously removing undesirable components out of the oilseed meal. It differs in almost every essential step from the conventional process as summarised by Shahidi *et al.* (1988).

Use of different alcohols, water contents in alcohols for two-phase solvent extraction of canola was described by Diosady *et al.* (1985), Rubin *et al.* (1986) and Shahidi *et al.* (1988). The effectiveness of removal of glucosinolates by alkanol and alkanol-ammonia solutions can be ranked as methanol >> ethanol > isopropanol > t-butanol. Isopropanol and t-butanol without water dissolved little ammonia; however, when more than 5% (v/v) water was added in the alkanol phase, sticky, dark-coloured meal was produced. Therefore, use of 95% alkanols was recommended. t-Butanol did not give a phase separation of the polar phase and hexane, causing difficulties in removing the gum fractions. Only ethanol and methanol gave two separate phases in the extraction system, thus allowing simultaneous extraction of oil and polar materials from canola seeds (Shahidi *et al.*, 1988).

The two-phase solvent extraction system described above has been shown to be a useful medium for extraction of undesirable components from rapeseed and canola meals. The effectiveness of glucosinolate removal from different canola/rapeseed cultivars depended on the alkanol used, ammonia concentration, solvent to seed ratio, and the contact time of ground seed or meal with the solvent (Naczk *et al.*, 1986b; Shahidi *et al.*, 1988). Over 97% of sinigrin (92.9% of total glucosinolates) originally present in the seeds of mustard (*Brassica juncea*) was removed by this two-

phase solvent extraction system consisting of methanol, ammonia and water (Shahidi and Gabon, 1988,1989 and 1990). The two-phase solvent extraction system was also effective in partially removing phenolic compounds (phenolic acids and condensed tannins) of canola and rapeseed (Naczk *et al.*, 1986a; Shahidi and Naczk, 1988; Naczk and Shahidi, 1989; Shahidi and Naczk, 1989).

Removal of some undesirable plant constituents by ammonia treatment is well documented. Ammoniation has been found to inactivate any aflatoxin present, as contaminant in cottonseed and peanut meals (Mann *et al.*, 1971; Gardner *et al.*, 1971) and shelled corn (Brekke *et al.*, 1978). The tannin content of high-tannin sorghum grains was reduced after treatment with ammonia and the treated products supported growth in chicks similar to that shown by low-tannin sorghum (Price *et al.*, 1979). Kirk *et al.* (1966) used ammoniation treatment to reduce concentration of glucosinolates as well as sinapine which resulted in improved palatability and nutritional quality of crambe seed meals. Coxworth and McGregor (1980) reported that the strong flavour of mustard can be completely removed by ammoniation, thus indicating that glucosinolates present can be extracted, left intact, altered or destroyed. Keith and Bell (1982) reported that ammoniation in combination with heating reduced the content of glucosinolates in the meals. Goh *et al.* (1982) showed that ammoniated ethanol or a combination of ammonia and steam were effective in hydrolysing sinapine to sinapic acid and choline, and overcame the problem of fishy odour in eggs which occurred in some strains of chicken fed sinapine-containing feeds (Goh *et al.*, 1979).

2.6 Laboratory preparation of linseed meal

There has been a considerable interest in detoxifying and upgrading linseed meal (Madhusudhan and Singh, 1985 a,b; Dev and Quensel, 1986, 1989). Preparation of a detoxified linseed meal by boiling in water (Madhusudhan and Singh, 1985a) and also preparation of a protein isolate has been reported (Madhusudhan and Singh, 1983). The resultant protein isolate was low in hemagglutinating activity but their proteolytic or trypsin inhibitor activity remained unchanged. The amino acid content was not very different from that of the original meal; however, there was a reduction in the content of available lysine. Recent work published by Dev and Quensel (1986 and 1989) has shown the importance of high swelling properties of mucilage in linseed coat. Most of the works reported on linseed meal has not referred to removal of cyanogenic glycosides even though it is the main limiting factor for usage.

Investigations by different research groups have so far shown that linseed is a potential ingredient for food product formulations. However, effective methods of detoxification and meal or protein preparations and their industrial application have not been thoroughly studied. Linseed meal needs to be upgraded so that it can be used as a food ingredient.

CHAPTER 3

MATERIALS AND METHODS

3.1 Meal preparation

Seeds of flax/linseed and commercial linseed meals were obtained from Omega Nutrition, Vancouver, British Columbia or through the Flax Council of Canada, Winnipeg, Mannitoba. Laboratory preparation of meals from seed or commercial meals by the two-phase solvent extraction was carried out as shown in Figure 3.1.

Seeds were first ground using a Moulinex coffee grinder before solvent extraction. Ground seeds or commercial meal (75 g) were blended for 2 min at low speed (approximately 15 000 rpm) in a 4 L commercial Waring blender with 500 ml of hexane or an absolute or 95% (v/v) alkanol, (R=6.7; volume of solvent in ml/weight of seed in g) with or without ammonia (10% w/w). Ammonia was bubbled into the absolute or 95% (v/v) alkanol at 0°C. The final concentration of ammonia in the solution was adjusted by mixing of the resultant solution with enough alkanol to obtain a 10% (w/w) ammonia concentration in the final mixture. After a quiescent period of 15 min at room temperature, 500 ml hexane was added and the slurry was blended again for 2 min. The meal was separated by vacuum filtration using Whatman No. 41 filter paper, rinsed 3 times with 125 ml of alkanol and dried at 40°C under vacuum. The meal was further defatted with hexane using a Soxhlet apparatus and air dried overnight. The two liquid phases were separated and the hexane layer was evaporated to recover the oil. The alkanol (polar) phase was re-extracted three times

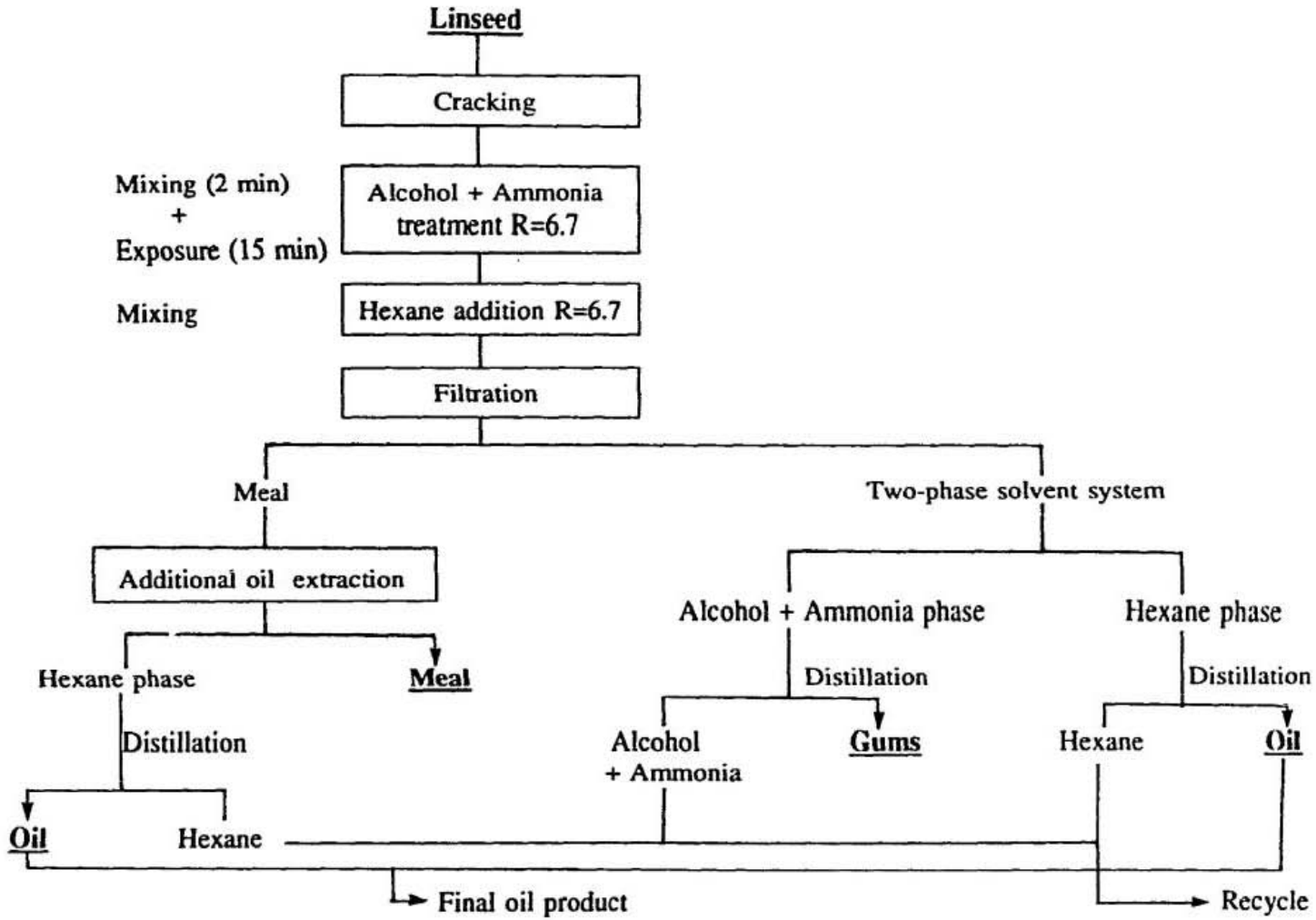


Figure 3.1. Flow diagram for two-phase solvent extraction system (adapted from Shahidi *et. al.*, 1988).

Table 3.1. Composition of the polar phase in various solvent extraction systems.

Extraction	Alkanol	Water % (v/v) in alkanol	Ammonia % (w/w) in alkanol/water
1	None	0	0
2	Methanol	0	0
3	Methanol	5	0
4	Methanol	0	10
5	Methanol	5	10
6	Ethanol	5	10
7	Isopropanol	5	10

at an alkanol to hexane ratio of 2:1 (v/v) to recover additional oil. The alkanol phase was evaporated under vacuum to recover the dissolved solids. Different solvent combinations employed for extraction of linseed are summarized in Table 3.1. Mass balance of the materials due to the process was evaluated. The recovered meals were stored in "Nasco" whirl pack plastic bags (Polycello, Amherst, Nova Scotia) and kept at room temperature until use.

3.2 Chemical composition

All chemicals used were ACS grade and results of the experiments were calculated on the dry weight bases of the meals.

3.2.1 Moisture

Samples of meals (1.000-2.000 g) were weighed into pre-weighed aluminium dishes and placed in a forced-air oven (Fisher Isotemp 300, USA) preheated to 105±1°C. Samples were held at this temperature until a constant weight was attained, then transferred to a desiccator, cooled and weighed immediately. The moisture content was calculated as the percentage of weight loss of the sample due to drying (AOAC, 1990).

3.2.2 Ash

Samples weighing 2.000-3.000 g were transferred into clean porcelain crucibles

charred using a flame and then placed in a temperature-controlled furnace (Lab Heat, Blue M, Illinois) which was preheated to 550°C. Samples were held at this temperature until grey ash remained and then transferred to a desiccator, cooled and weighed immediately. Ash content was calculated as percentage weight of the remaining matter (AOAC, 1990).

3.2.3 Crude protein

Samples (100-200 mg) were weighed on nitrogen-free papers and placed in the digestion tubes of a Büchi 430 (Büchi Laboratoriums-Technik AG, Flawil/Schweiz) digester. The samples were digested with two Kjeltabs catalyst pellets (Profamo, Quebec) and 20 ml of concentrated H_2SO_4 in the Kjeldahl digester (Büchi 430) until a clear solution was obtained. Digested samples were diluted with distilled water (50ml); alkali (150 ml of 25% NaOH) was added and the released ammonia was steam distilled (Büchi 321) into 4% H_3BO_3 (50 ml) containing twelve drops of an end point indicator (N-point indicator, EM Science, New Jersey) until 200 ml distillate was collected. The content of ammonia in the distillate was determined by titrating it against 0.1N H_2SO_4 (AOAC, 1990). The content of crude protein in samples was calculated by multiplying the percentage of nitrogen by a factor of 6.25.

3.2.4 Non protein nitrogen

The content of non-protein nitrogen was determined by the method of Bhatty

and Finlayson (1973) as modified by Naczk *et al.* (1985). One gram of meal was shaken with 40 ml of 10% trichloroacetic acid (TCA) solution at 20°C for 1 h using a wrist-action shaker (Burrel, Pittsburgh, Pennsylvania). The insoluble residue was removed by centrifugation at 5000xg for 10 min and the residue was treated three times with 15 ml of 10% (w/v) TCA solution. The supernatant was collected as before and was made up to 100 ml with distilled water and an aliquot of it was taken for determination of soluble nitrogen using the Kjeldahl procedure (AOAC, 1990).

3.2.5 Cyanogenic glycosides

3.2.5.1 Preparation of standards

3.2.5.1.1 Linamarin

Pure linamarin (phaseolunatin) was obtained from Sigma Chemical Company (St. Louis, Missouri).

3.2.5.1.2 Linustatin and neolinustatin

Defatted linseed meal (100 g) was extracted with 80% ethanol (1:10, w/v) at 70°C for 1 h. The solid material recovered by evaporating the aqueous ethanol solution (70°C, ambient pressure) was eluted on a silicic acid column (100 mesh, Malinckrodt, 1.15 cm i.d. x 18 cm). Solvent systems, namely chloroform (400 ml), chloroform/methanol (4:1, v/v, 800 ml) and chloroform/methanol (2:1, v/v, 1200 ml), were employed in sequence. The eluate of the last solvent system was evaporated

under vacuum to dryness. The dried residue was dissolved in methanol and loaded on thin layer chromatography (TLC) plates (Silica gel, 60 Å mean pore diameter, 2-25 µm mean particle size, 250 µm thickness, Sigma Chem. Co., St. Louis, Missouri) and the chromatogram was developed using chloroform/methanol/water (65:35:10, v/v/v) as the mobile phase in a glass chamber 22 x 22 x 10 cm (Fisher Scientific Ltd. Toronto, Ontario). Glycoside fractions were identified by spraying of plates with 10% (v/v) sulphuric acid and heating at 120°C for 10 min. Linamarin, raffinose (Sigma Chem. Co., St. Louis, Missouri), and sucrose (Bio-rad, Richmond, California) were chromatographed on the same plate to identify sugars.

The dried residue recovered from chloroform/methanol (2:1, v/v) was separated on two different columns. First, the sample on a prepacked liquid chromatography column (Lichroprep RP-8, Merck, Germany) was eluted with methanol/water (15%, v/v). Fractions of 5 ml were collected on a fraction collector (LKB Bromna 2112, Sweden) and the activity of the fractions was monitored by TLC using solvent system chloroform/methanol/water (65:35:10, v/v/v). The eluates corresponding to the spots C; linustatin and D; neolinustatin (Figure 4.1) which came together on this column were further separated by eluting on a silica gel column (Silica gel 60, 70-230 mesh, BDH, Ontario, 1.25 cm i.d. x 40 cm) with chloroform/methanol/water (65:35:10, v/v/v). Fractions were collected as before and the fractions containing the glycosides were identified using TLC. Two types of TLC plates; pre-coated with silica gel (60 Å mean pore diameter, 2-25 µm mean particle size, 250 µm thickness, Sigma Chem.

Table 3.2. Composition of mobile phases (by volume) used for separation of linustatin and neolinustatin on TLC and HPTLC.

Mobile phase	Composition	Reference
1: Chloroform-methanol-17% NH_4OH	2:2:1	Smith *et al.* (1980)
2: Ethyl acetate-acetone-chloroform-methanol-water	40:30:12:10:8	Brimer *et al.* (1983)
3: Chloroform-methanol-water	65:35:10	Amarowicz *et al.* (1992)
4: n-Propanol-water	85:15	Gasparic and Churacek (1978)
5: n-Butanol-acetone-water	75:75:25	Dini *et al.* (1989)
6: Ethyl acetate-acetic acid-methanol-water	60:15:15:10	Gasparic and Churacek (1978)

Co., St. Louis, Missouri) and high performance TLC or HPTLC (60 Å mean pore diameter, 5-10 µm mean particle size, 200 µm thickness, Sigma Chem. Co., St. Louis, Missouri) were also used with six different solvent systems for this separation as summarized in Table 3.2. The compounds C and D separated on TLC by solvent system chloroform-methanol-water (65:35:10, v/v/v) were scraped off, isolated and chemical structures were confirmed by Mass Spectroscopy and NMR (^{1}H and ^{13}C).

3.2.5.2 Quantification by high pressure liquid chromatography (HPLC)

Cyanogenic glycosides in linseed meal (4-5 g) were extracted into 50 ml of boiling 80% ethanol (v/v) over a 10 min period (Brimer *et al.*, 1983). After 1 min homogenization, using a Polytron (Brinkmann, Westmount, New York), the homogenate was centrifuged at 5000xg for 10 min and the supernatant was collected. The residue was re-extracted two more times. Combined supernatants were evaporated at 40°C under vacuum and the residue was dissolved in 10 ml of methanol. To this solution 20 ml of chloroform was added and mixed well. Precipitating polar compounds were removed by centrifugation. The supernatant was evaporated to dryness under pressure and redissolved in 4 ml of 15% (v/v) HPLC-grade methanol in water. For HPLC analysis samples were filtered through a 0.45µ nylon filter (Cameo 11, MSI, Westboro, Massachusetts) into the HPLC sample vials and then directly used for analysis. Linamarin (Sigma Chem. Co., St. Louis, Missouri), linustatin and neolinustatin, isolated as described previously, were used as stan-

dards.

A Shimadzu (Kyoto, Japan) HPLC system consisting of two model LC-6A pumps with a mixing chamber, a model SIL-6B auto injector, a model SCL-6B system controller and a Model CR501 Chromatopac data processor were used. The detector used was a differential refractometer (Waters Associates, Miliford, Massachusettes). A 10μ particle size CSL-spherisorb-ODS2 reversed phase analytical column (4.5mm x 24mm) from Chromatographic Specialties Inc. (Brockville, Ontario) and a guard column (4.5 mm x 5 cm) coupled to the analytical column were used. Solvent used was 15% (v/v) methanol. Flow rate of 0.8 ml/min, injecting volume of 20 μl and analysis time of 14 min were employed.

The effect of water content (5, 10 and 15%, v/v) in the polar phase, solvent-to-seed ratio (6.7 and 13.3) and quiescent period after blending (15 and 30 min) on the content of cyanogenic glycoside of the meal was studied for methanol-ammonia-water/hexane extraction. The effect of multi-stage (two and three) extraction was also examined for the same solvent system. Meals were prepared as mentioned previously and by changing the parameters of interest.

3.2.6 Phenolic acids

The free, esterified and insoluble bound phenolic acids of the meals were isolated using the procedure of Krygier *et al.* (1982). Meals (2 g) were extracted six times with a 40 ml of methanol/acetone/water (7:7:6, v/v/v) at room temperature using

a Polytron homogenizer for 60 sec at 10 000 rpm. After each extraction, samples were centrifuged for 15 min at 5000xg and supernatants were collected. Combined supernatants were evaporated at 30°C under vacuum to 40 ml and the pH of the mixture was adjusted to 2 using 6N HCl. The samples were centrifuged at 5000xg and supernatants were extracted 6 times with diethyl ether/ethyl acetate mixture (1:1, v/v) at a supernatant to solvent ratio of 1:1 (v/v). The ether extracts were combined and evaporated to dryness at 30°C under vacuum. The extracted phenolic acids contained free phenolic acids (FPA).

The aqueous layer remaining after extraction of free phenolic acids and the precipitate after centrifugation were then treated with 30 ml 4N NaOH under nitrogen for 4 h at room temperature to release esterified phenolic acids. The resultant hydrolysate was acidified to pH 2 using 6N HCl and extracted into an ether mixture and dried, as before, to yield esterified phenolic acids (EPA).

The remaining meal after extraction of free and esterified phenolic acids was treated with 20 ml of 4N NaOH under nitrogen at room temperature, then acidified with 6N HCl to pH 2 and centrifuged at 5000xg for 15 min. The supernatants were extracted 6 times with diethyl ether/ethyl acetate mixture as before, and extracts were evaporated to dryness under vacuum to obtain insoluble bound phenolic acids (IBPA).

The dried phenolic acids (FPA, EPA and IBPA) were dissolved in methanol separately and contents of phenolic acids were determined colorimetrically using Folin Denis reagent as described by Swain and Hillis (1959). To a 0.5 ml of methanolic

solution of phenolic extracts, 0.5 ml of Folin Denis reagent, 1 ml of saturated Na_2CO_3 and 8 ml of distilled water were added and mixed well. After 30 min standing at room temperature samples were centrifuged and absorbance was read at 725 nm. The free, esterified and insoluble bound phenolic acids were expressed as trans-ferulic acid equivalents using the equation, $C = k(0.150A_{725}-0.0017)$, $r = 0.9940$ (For details see Figure A.1. in the Appendix). The total phenolic acid content was calculated as the sum of free, esterified and insoluble-bound fractions and expressed as mg per 100g of meal.

3.2.7 Condensed tannins

The condensed tannins of linseed meals were isolated by adopting the method described for rapeseed meals by Shahidi and Naczk (1989). One gram of sample was extracted at room temperature twice with 10 ml of 70% (v/v) aqueous acetone using a Polytron homogenizer for 1 min, at 10 000 rpm. After centrifugation (10 min, 5000xg), the supernatants were collected, combined and evaporated to dryness at 30°C under vacuum. The extracted tannins were then dissolved in 10 ml of methanol and the content of condensed tannins in the methanoic solutions were determined colorimetrically by the method of Price *et al.* (1978). To 1.0 ml of methanolic solution of condensed tannins, 5 ml of 0.5% vanillin reagent or 5.0 ml of 4% HCl was added. The absorbance of the samples and blank was measured at 500 nm after a 20 min standing at room temperature. (+)-Catechin (3.5 mole of water per mole of catechin,

Sigma Chem. Co., St.Louis, Missouri) was used as a standard and the content of condensed tannins in the samples was expressed as catechin equivalents, using the equation $C = k(2.2467A_{500}-0.0561)$, $r = 0.9963$ (For details see Figure A.2. in the Appendix). The content of condensed tannins of the meals was calculted and expressed as mg per 100g of meal.

3.2.8 Phytic acid

Phytic acid in the prepared meals was extracted according to the method of Tongkonchitr *et al.* (1981) as modified by Naczk *et al.* (1986a). Two grams of meal were extracted with 40 ml of 1.2% HCl containing 10% Na_2SO_4 for 2 h using a wrist-action shaker. The slurry was centrifuged for 20 min at 5000xg. Five millilitres of the supernatant was mixed with 5 ml of distilled water and 6 ml of 0.4% $FeCl_3.6H_2O$ in 0.07N HCl solution. The mixture was heated in a boiling water bath for 45 min and then cooled to room temperature. The resulting ferric phytate precipitate was collected by centrifugation at 5000xg for 15 min and the supernatant was discarded. The precipitate was mixed thoroughly with 5 ml of 4% Na_2SO_4 in 0.07N HCl and the mixture was centrifuged again. The recovered ferric phytate was digested using 6 ml 1:1 (v/v) mixture of concentrated H_2SO_4 and concentrated HNO_3. The digestion was terminated when white fumes hung over the liquid. A 10 ml portion of distilled water was added to the warm digest and the solution was heated in a boiling water bath for 30 min to destroy pyrophosphate and the mixture was then

diluted with distilled water to 100 ml.

The phytate phosphorus was determined according to the method described by Nahapetian and Bassiri (1979). To 1 ml of diluted digest, 4 ml distilled water, 3 ml 1.5 N H_2SO_4, 0.4 ml 10% $(NH_4)_6Mo_7O_{24}.4H_2O$ and 0.4 ml 2% ascorbic acid were added and mixed. The solution was allowed to stand for 20 min and absorbance was measured at 660 nm. The content of phosphorus in the mixtures was calculated from the equation $C = k(77.54A_{660}-0.39)$, $r = 0.9900$, (For details see Figure A.3. in the Appendix) using KH_2PO_4 as standard. The phytic acid content (%) was calculated by multiplying the phytate phosphorus content of the meal by 3.55 which is derived from the empirical formula $C_6P_6H_{18}O_{24}$.

3.2.9 Total soluble sugars

To one gram of sample, 50 ml of 80% ethanol was added in a 100 ml volumetric flask which was then gently brought to a boil and maintained for 15 min. The flask was cooled and the contents diluted to the volume with 80% ethanol followed by filtration (Finley and Fellers, 1973) using S&S 576 filter papers. Sugar content of the diluted samples was measured, as sucrose equivalents by the anthrone method (Carrol *et al.*, 1955). A 1 ml sample was mixed with 10 ml of cold anthrone and kept for 10 min in a boiling water bath. The flask was then cooled to room temperature and absorbance was read at 620 nm. The content of total soluble sugars was calculated using the equation $C = k(0.3060A_{620}+0.004)$, $r = 0.9975$ (For details

see Figure A.4. in the Appendix) and expressed as percent sucrose equivalents in the dry meal.

3.2.10 Analysis of amino acids

Samples (10-15 mg) were digested in 6N HCl at 110°C under a stream of nitrogen (Blackburn, 1978). The amino acid composition of the hydrolysates was determined using a Beckman 121 MB Amino Acid Analyser (Beckman Instruments, Polo Alto, California). Cysteine and methionine were determined by performic acid oxidation prior to their digestion in 6N HCl. Cysteine and methionine were measured as cysteic acid and methionine sulphone, respectively (Blackburn, 1978). Analysis of tryptophan was performed by uv absorption after hydrolysis of the sample with 3N mercaptoethane sulphonic acid at 110°C and under vacuum as described by Penke *et al.* (1974).

3.2.11 Analysis of fatty acids

Fatty acid composition of the oil recovered from hexane layer was determined. Fatty acid methyl esters (FAME) were prepared by transmethylation of the lipids in 6% H_2SO_4 in 99.9 mole% of methanol at 65-70°C for 15 h (Keough and Kariel, 1987). After extraction of the methyl esters into hexane, they were analysed using a Perkin-Elmer 8310 GC equipped with a 30 m x 0.25 mm column (SP 2330, Supelco, Oakville, Ontario). Oven temperature was 180°C, the injection port and flame

ionisation detector temperatures were 230°C and 250°C, respectively. The flow rate of the helium carrier gas was 25 ml/min. Identification of FAME was based on the comparison of their retention times with standards (Supelco Inc. Oakville, Ontario). Quantification was performed by the computer control using area normalization.

3.3. Functional properties

3.3.1 Water absorption

Water absorption of the meals was determined by a combination of the AACC (1976) method and that of Sosulski (1962) as described by Naczk *et al.* (1986a). A 2 g sample was dispersed in 20 ml of distilled water. The contents were mixed further over 60 min at every 10 min for 30 sec using a glass rod. The contents were centrifuged at 2000xg for 15 min. The supernatant was carefully decanted and tubes were inverted and drained for 15 min before weighing. The absorbed water was expressed as percentage increase in the sample weight.

3.3.2 Water hydration capacity

Water hydration capacity (WHC) of the meals was measured by the methods of AACC (1976) and Quinn and Paton (1979). Five grams of meal were weighed into a pre-weighed 50 ml centrifuge tube and distilled water was added in small unmeasured volume increments while stirring with a glass rod after each addition until the mixture was thoroughly wet. The stirring rod was wiped on the sides of the tube

which was then centrifuged at 2000xg for 10 min. The resulting supernatant was discarded and the contents were weighed. If no supernatant appeared, more distilled water was added and the procedure was repeated. The approximate water hydration capacity (WHC_{approx}) was calculated as:

$$WHC_{approx}\ (gH_2O/g\ meal) = \frac{(\text{weight of tube + sediments}) - (\text{weight of tube + 5.0})}{5}$$

To determine water hydration capacity accurately, enough meal was added to four pre-weighed tubes (amount of meal = $15/WHC_{approx} + 1$, where 15 is the desired total weight of sample and water). Volume of water to be added was calculated as, 15-weight of material (g). To the four tubes were added volumes of water equal to 1.5 and 0.5 ml more and 1.5 and 0.5 ml less than that of the calculated value. The contents in each tube were vigorously mixed with a stirring rod for 2 min and then centrifuged, as before. The two tubes, one with and one without supernatant represented the limits which the WHC values occurred. The WHC values were presented as the mid point between these two volumes divide by the weight of the material in grams.

3.3.3 Fat absorption

Fat absorption of the meals was determined using the methods described by Lin *et al.* (1974) and Sosulski *et al.* (1976). A 2 g sample in 12 ml of pure soybean

oil was placed into a 50 ml centrifuge tube and the contents were stirred for 30 sec every 5 min. After 30 min, the tubes were centrifuged at 1600xg for 25 min. The free oil was decanted and the absorbed oil was determined as the weight percent difference.

3.3.4 pH

The pH of 10% (w/v) dispersion of meals in CO_2-free distilled water was determined using a Fisher Accumet 810 pH meter.

3.3.5 Nitrogen solubility index (NSI)

The nitrogen solubility index (NSI) for each sample was determined according to the method of AACC (1976). Five grams of sample were weighed into a 250 ml Erlenmeyer flask and 200 ml of distilled water was added in small portions while stirring thoroughly to obtain a uniform dispersion. The contents of the flasks were mixed at room temperature for 2 h using a wrist action shaker. The mixture was then transferred carefully into a 250 ml volumetric flask. Two drops of antifoam A were added and the mixture was then diluted to the mark with water while mixing thoroughly. Forty millilitre aliquots were centrifuged for 10 min at 1500xg. The supernatant was passed through a funnel equipped with a glasswool plug. Twenty five millilitres of the clear liquid were transferred into Kjeldahl tubes for subsequent determination of nitrogen. The amount of water-soluble nitrogen in the sample was

determined and NSI was expressed as percentage of the ratio of the content of water soluble nitrogen to that of total nitrogen in the meal.

The effect of pH on the NSI of hexane-extracted and methanol-ammonia-water/hexane treated meals was monitored. The pH was adjusted (from 2 to 11.8) by addition of 1% HCl or 1% NaOH solution (Naczk *et al.* 1985).

3.3.6 Oil emulsification properties

The emulsifying activity or capacity of each sample was determined by the methods of Yasumatsu *et al.* (1972) and Naczk *et al.* (1985). A 1.25 g of meal was homogenized with 50 ml of water for 30 sec using a Polytron homogenizer at 10 000 rpm. Pure soybean oil (25 ml) was added to each and the mixture was homogenized for 90 sec. The emulsion so obtained was divided evenly into four tubes which were then centrifuged at 1100xg for 5 min. Emulsifying activity was calculated as percentage of the volume of the emulsified layer over the volume of emulsion before centrifugation.

Emulsion stability was determined using the material prepared for the measurement of emulsifying activity. The mixture was heated at 85°C for 15 min and cooled as described by Inklarr and Fortuin (1969) and Naczk *et al.* (1985). After cooling to room temperature, the mixture was evenly divided into four portions and transferred into 50 ml centrifuge tubes and centrifuged at 1100xg for 5 min. The emulsion stability was expressed as the percent emulsified activity remaining after

heating.

3.3.7 Whippability and Foam stability

Whippability and foam stability were determined by the methods of Lin *et al.* (1974) and Naczk *et al.* (1985). One hundred millilitre of 3% (w/v) dispersion of meal in distilled water was homogenized for 60 sec using a Polytron homogenizer at 10 000 rpm. The mixture was then transferred immediately into a 250 ml measuring cylinder and the foam volume was noted. The percentage volume increase was calculated as given by Lawhon and Cater (1971) and expressed as whippability. Foam stability was expressed (on the basis of 100 ml of a 3% w/v dispersion) as the volume of the foam remaining after 0.5, 10, 20, 40, 60, 120 min of quiescent period.

3.4 Statistical analysis

All experiments and/or measurements were replicated 3 to 6 times. Mean ± standard deviation was calculated in each case. Analyses of variance and Tukey's studentized range test (Snedecor and Cochran, 1980) for difference between means were performed on Statistical Analysis System (SAS Inc. 1990, North Carolina, USA). Relationships of parameters were assessed by a linear regression method.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Mass balance of extractions

Results for mass balance of laboratory prepared linseed meal and commercial linseed meal following solvent extraction with hexane, methanol, ethanol and isopropanol combined with water and/or ammonia are summarized in Table 4.1. Both 5% (v/v) water and 10% (w/w) ammonia in alcohol were used since these combinations were found to afford optimum recovery of oil and best quality meal for canola (Rubin *et al.*, 1986, Shahidi *et al.*, 1988). Recovery of the meal and oil from the hexane phase and solids from the polar phase added up to 95-99%, of initial weight of the seed or meals on a dry weight basis. Losses during extractions varied from 1.0 to 4.9%, which is considered reasonable since it was difficult to quantitatively transfer the slurries, cakes or solvents from one unit operation to another in the laboratory preparations.

Different solvent extractions of seeds yielded 46.4 to 50.0% of dried meal. The presence of water in methanol slightly lowered the recovery of the meal (46.0-47.5%), but resulted in the recovery of 4.8 to 5.7% of solids from the polar phase. Isopropanol at this proportion of water and ammonia did not give two separate phases with hexane. The recovered solids, based on the analyses of canola meals, may include phospholipids, phenolic compounds and soluble sugars (Shahidi *et al.*, 1988) as well as breakdown products of other polar matters. Johnson and Lusas (1983) have

reported that alcohol/water mixtures have the most commercial potential as alternative solvents for oil extraction. As the water content in alkanol increases the solvent becomes more polar and its capacity to extract non-lipid matters (pigments, sugars, etc.) as well as phosphatides also increases. However, a practical problem due to the high water content is deposition of phosphatides in the meals which results in the production of a dark-coloured and sticky meal (see Chapter 2). The amount of oil extracted into hexane was not influenced, to any great extent, by the nature of the alkanol used and in all cases 45.9 to 49.2% oil was recovered from the seeds. Commercial, cold-pressed linseed meals contained about 15% oil.

In canola, a major advantage of the two phase solvent extraction was considered to be the simultaneous removal of glucosinolates and extraction of oil from crushed seeds as reviewed by Shahidi *et al.* (1988). In a laboratory scale, the recovered oil accounted for 72 to 82% of the total oil present in linseed. In canola seeds it was reported that 78.5 to 88.8% of total oil was extracted (Rubin *et al.*, 1986; Shahidi *et al.*, 1988). The solvents, after recovery of solids and oils from the alkanol and hexane phases, respectively, may potentially be recycled. Johnson and Lusas (1983) have reported the recycling of alkanols used for extraction of oils from oilseeds.

Table 4.1. Mass balance of different extractions.

Solvent	Yield on a dry basis (%)			Loss (%)
	Meal	Oil	Solids	
Laboratory prepared meal: Hexane only	48.9±1.0	49.2±0.6	0.0	1.9
Methanol-hexane	46.7±1.0	45.9±1.9	5.2±0.1	2.2
Methanol-water/hexane	46.7±2.0	47.0±0.5	4.8±0.1	1.5
Methanol-ammonia/hexane	47.6±2.0	46.6±0.8	4.8±0.7	1.0
Methanol-ammonia-water/hexane	46.4±2.0	47.1±0.8	5.6±0.1	0.9
Ethanol-ammonia-water/hexane	48.1±1.0	46.8±1.8	4.2±0.1	0.9
Isopropanol-ammonia-water/hexane	50.0±3.0	48.0±0.5	no phase separation	1.2
Commercial meal: Hexane only	82.5±5.0	15.1±3.0	0.0	2.4
Methanol-ammonia-water/hexane	74.2±2.0	14.3±1.0	6.9±0.2	4.6

4.2 Effect of solvent extraction on chemical composition

4.2.1 Effect solvent extraction on content of crude protein, ash and non-protein nitrogen of linseed meal

The content of crude protein in the extracted linseed meals was in the range of 42.9 to 48.6% (Table 4.2). The solvents containing water and ammonia gave a crude protein content of 46.0 to 48.6%. Extraction with methanol/ammonia/water-hexane enhanced the crude protein content of the products by about 13% as compared to the hexane extracted meal. The non-protein nitrogen content did not show a considerable change when the extraction medium contained ammonia (Table 4.2). The increase in crude protein content of the meals was mainly due to the concentration effect caused by the removal of polar matters (4-6%) into the alkanol phase. Commercial linseed meal had a slightly lower content of crude protein than that in the defatted linseed. The two-phase solvent extracted canola meal was reported to contain a crude protein content of 25% higher than its hexane extracted counterpart (Shahidi *et al.*, 1988). It was also reported that the non-protein nitrogen content of the extracted meal was reduced by about 50%.

The ash content of the meals was also increased by about 8% after solvent extraction, again due to the dissolution of 4.8 to 5.7% of polar matters of the meal into the alkanol phase. Presence of water in the polar phase did not influence the ash content to any great extent. The ash content of the meal was not affected by isopropanol-ammonia-water/hexane extraction.

Table 4.2. Effect of solvent extraction on contents of crude protein, ash and non-protein nitrogen compounds of linseed meal[1].

Solvent	Crude protein (%N X 6.25)	Ash (%)	Non-protein nitrogen (% of total nitrogen)
Laboratory prepared meal: Hexane only	42.9±0.3^{c}	5.57±0.15^{c}	11.0±0.5^{ab}
Methanol/hexane	43.5±0.7^{c}	5.93±0.13^{ab}	9.77±0.75^{c}
Methanol-water/hexane	46.8±0.3^{b}	5.80±0.11^{bc}	10.0±0.9^{bc}
Methanol-ammonia/hexane	46.0±0.1^{b}	6.02±0.10^{a}	10.3±0.6^{bc}
Methanol-ammonia-water/hexane	48.6±0.3^{a}	6.03±0.07^{a}	11.7±0.3^{a}
Ethanol-ammonia-water/hexane	46.3±0.2^{b}	5.97±0.17^{ab}	11.8±0.2^{a}
Isopropanol-ammonia-water/hexane	46.0±0.4^{b}	5.62±0.02^{c}	9.45±0.20^{c}
Commercial meal: Hexane only	41.2±0.5	6.17±0.02	9.24±0.08
Methanol-ammonia-water/hexane	47.6±0.2	6.70±0.30	9.60±0.20

[1] Values in the same column bearing different superscripts are significantly ($P<0.05$) different.

4.2.3 Separation of cyanogenic glycosides of linseed meal

Separation of glycoside extracts of defatted linseed meal (iv) on Silica gel plates is shown in Figure 4.1. The eluted glycosides by methanol/chloroform/water (65:35:10, v/v/v) were tentatively identified and five separate spots (A, B, C, D and E) were apparent. Spots A, B and E had R_f values similar to those of pure raffinose (i), sucrose (ii) and linamarin (iii), respectively. Spots C and D were thought to be disaccharide glycosides of linseed. The spot below that of linamarin (E) is suspected to be a sugar.

The chromatograms obtained for separation of linustatin (C), neolinustatin (D) and linamarin (E) on TLC and HPTLC plates are presented in Figures 4.2 and 4.3, respectively. The observed R_f values are given in Table 4.3. The mobile phases 1 and 2 (Table 3.2) were reported as classical solvent systems for separation of cyanogenic glycosides (Smith *et al.*, 1980; Brimer *et al.*, 1983). System 3 (Table 3.2) has been used to separate glycosides from rapeseed (Amarowicz *et al.*, 1992). Systems 4, 5 and 6 (Table 3.2) were used to separate oligosaccharides (Gasparic and Churacek, 1978; Dini *et al.*, 1989). The mobile phases used were able to secure a good distance between linamarin and neolinustatin. Solvent systems 1 and 3 were effective in separating linustatin and neolinustatin on TLC. It was confirmed that solvent system 2 was good for total glycoside determination but not for separation of individual glycosides (Brimer *et al.*, 1983). HPTLC resulted in a much better separation of glycosides using all solvent systems, except 5.

Figure 4.1. Chromatogram (TLC) of glycoside extracts of linseed meal. (i,A), raffinose; (ii,B), sucrose; (iii,E), linamarin; and (iv), glycoside extract of linseed.

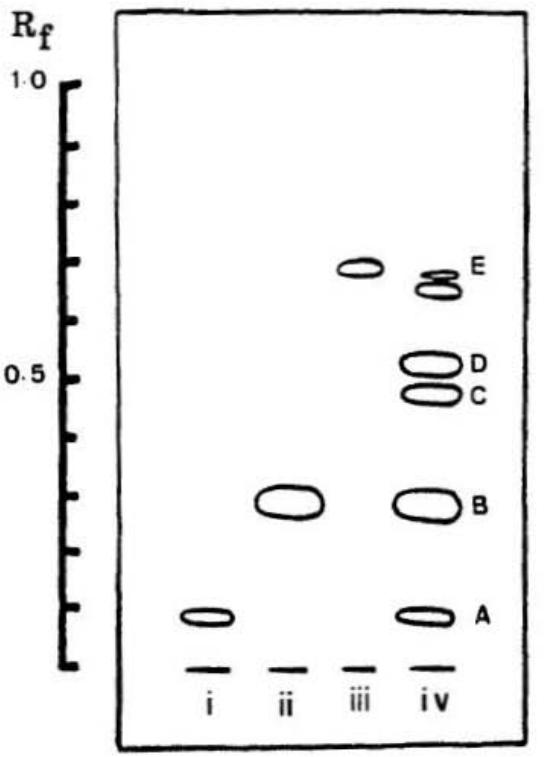

R_f
1·0
0·5
E
D
C
B
A
i
ii
iii
iv

Figure 4.2. Chromatograms (TLC) of cyanogenic glycosides of linseed meal. C, neolinustatin; D, linustatin; and E, linamarin (1 to 6 refers to mobile phases in Table 3.2).

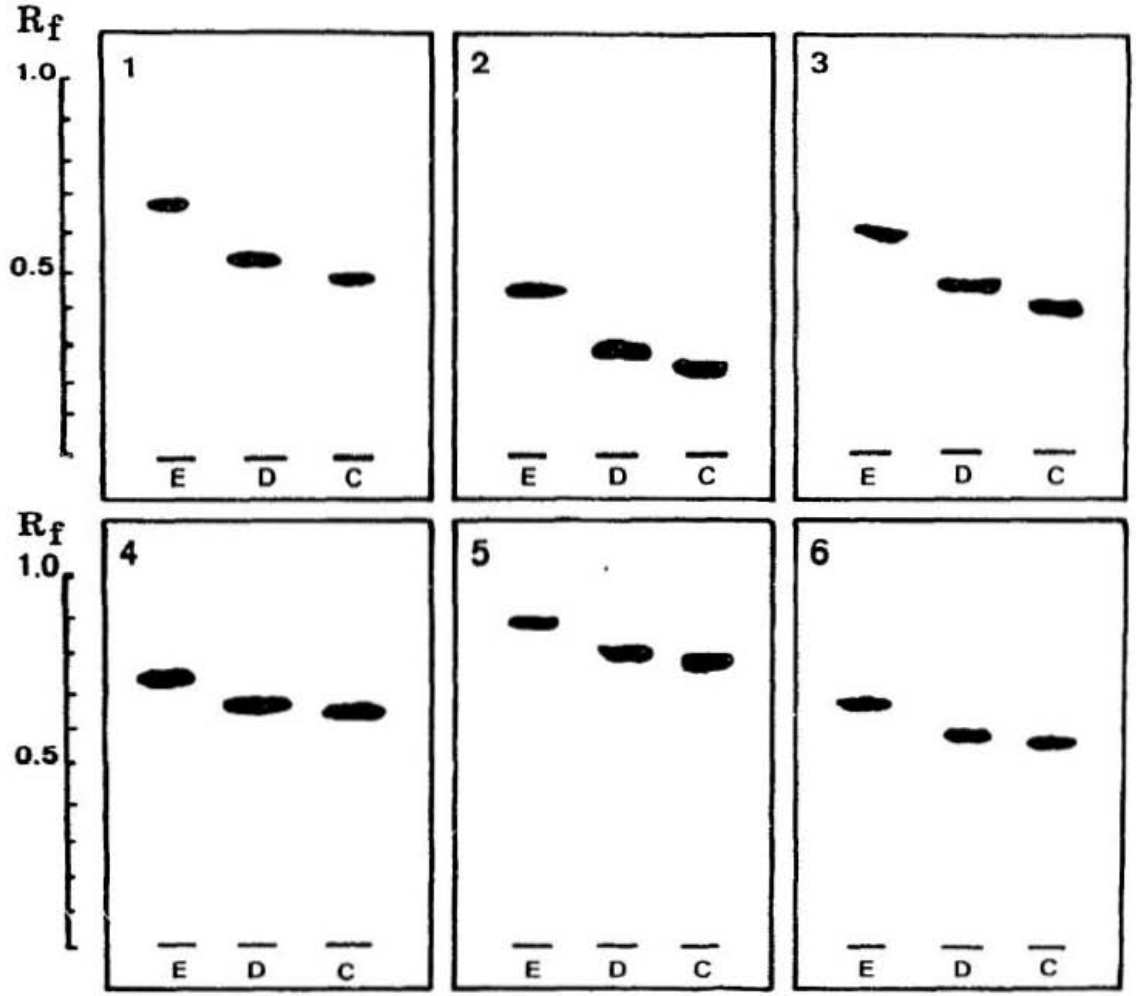

Rf
1.0
0.5
1
2
3
E
D
C
Rf
1.0
0.5
4
5
6
E
D
C

Figure 4.3. Chromatograms (HPTLC) of cyanogenic glycosides of linseed meal. C, neolinustatin; D, linustatin; and E, linamarin (1 to 6 refers to mobile phases in Table 3.2).

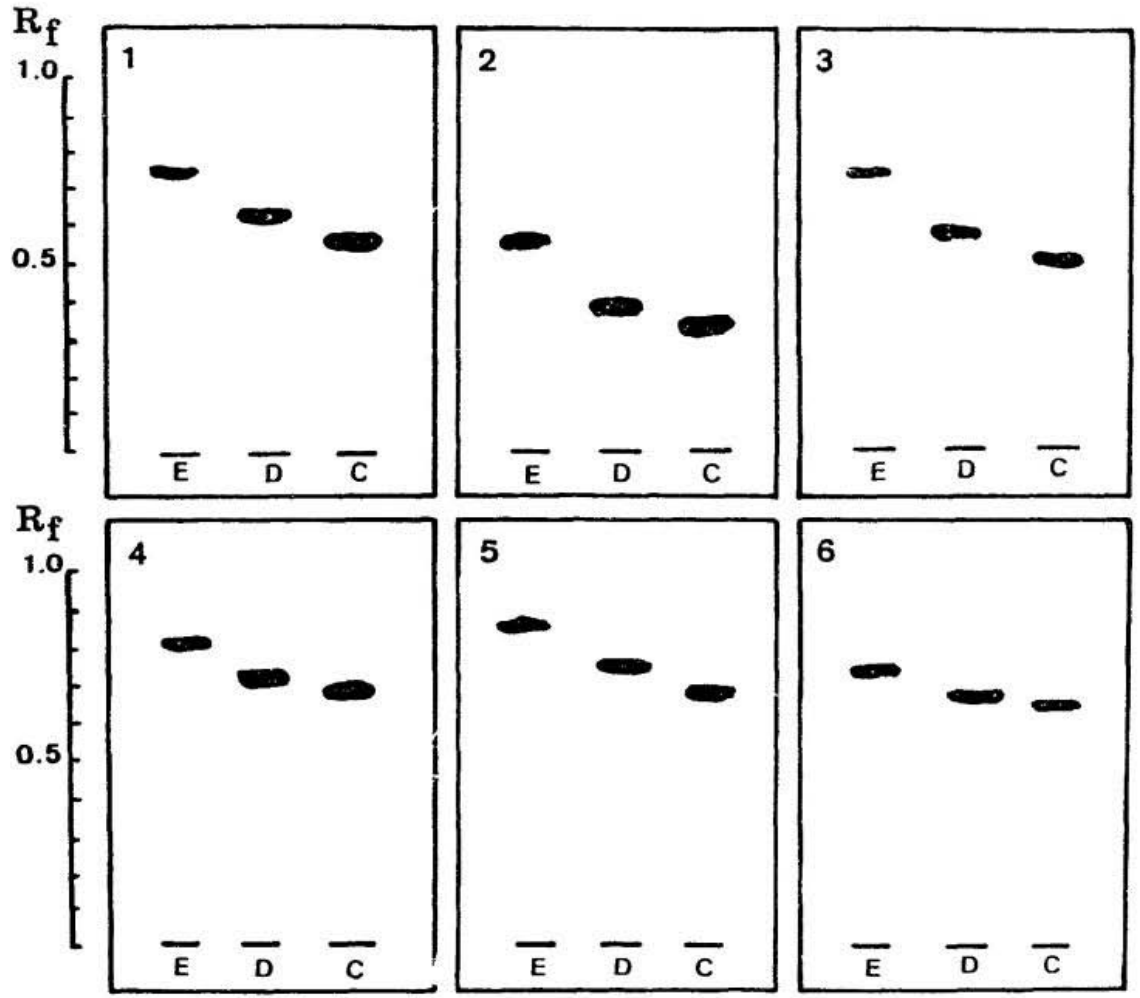

R_f
1.0
0.5
1
2
3
4
5
6
E
D
C

Table 4.3. R_f values for linamarin, linustatin and neolinustatin.

Mobile phase[1]	TLC plate			HPTLC plate		
	Linamarin	Neolinustatin	Linustatin	Linamarin	Neolinustatin	Linustatin
1	0.68	0.54	0.48	0.74	0.63	0.56
2	0.44	0.28	0.24	0.55	0.38	0.33
3	0.59	0.45	0.39	0.73	0.58	0.50
4	0.73	0.67	0.66	0.80	0.71	0.68
5	0.89	0.80	0.78	0.85	0.74	0.69
6	0.69	0.59	0.57	0.73	0.66	0.64

[1] Refers to mobile phases in Table 3.2.

4.2.3 Effect of solvent extraction on the content of cyanogenic glycosides of linseed meal

The HPLC chromatogram of standard linustatin (C), neolinustatin (D) and linamarin (E) is shown in Figure 4.4 (1). Linustatin had a retention time of 6.4 m and linamarin and neolinustatin had retention times of 6.8 min and 11.8 min, respectively. A typical HPLC chromatogram of the glycoside extracts of linseed meal is presented in Figure 4.4(2). All the samples gave chromatograms having two distinct peaks identical to the retention times of linustatin and neolinustatin. It was clear that linamarin was not present in the samples analysed. It has been reported that linseed meal contains both mono and disaccharide cyanogenic glycosides (Butler, 1965; Conn, 1981). However, Frehner *et al.* (1990) have observed that during development of linseed fruits, disaccharide cyanogenic glycosides predominate over monosaccharide cyanogenic glycosides. These observations were supported by a study done by Oomah *et al.* (1992) using Canadian flaxseed cultivars which showed that linamarin was present either in very low levels or absent in several cultivars. The cultivar used in this study was devoid of linamarin.

The content of linustatin and neolinustatin in solvent extracted linseed meals is summarized in Table 4.4. The linustatin content of the hexane extracted meal was higher than its neolinustatin content. The degree of removal of both glycosides by a particular solvent extraction system was similar. A 4 to 57% reduction in the contents of cyanogenic glycosides was observed, the greatest reduction was due to meals which

Figure 4.4. HPLC profiles of cyanogenic glycoside standards and extracts of linseed meal. (1), standards of linustatin, C; neolinustatin, D; and linamarin, E; (2), glycoside extracts of linseed meal and (3), (2) + linamarin E.

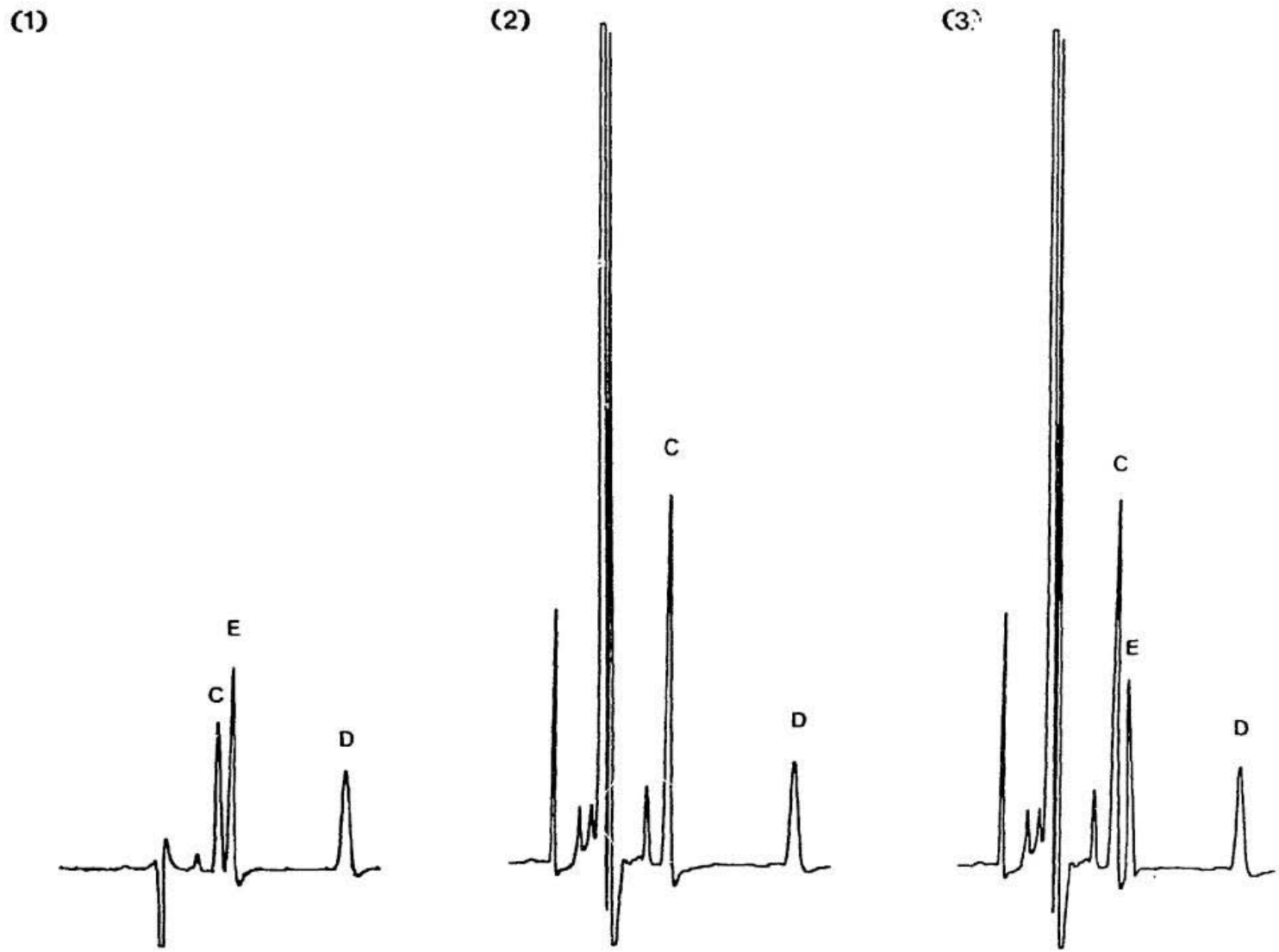

(1)
E
C
D
(2)
C
D
(3)
C
E
D

Table 4.4. Effect of solvent extraction on contents of linustatin and neolinustatin in linseed meal as quantified by HPLC[1].

Solvent	Linustatin (mg/g)	Removal (%)	Neolinustatin (mg/g)	Removal (%)
Laboratory prepared meal: Hexane only	4.42 ± 0.08[a]	0.0	1.90 ± 0.03[a]	0.0
Methanol/hexane	4.26 ± 0.09[a]	3.6	1.81 ± 0.23[a]	5.3
Methanol-water/hexane	2.69 ± 0.17[d]	39.1	1.19 ± 0.09[b]	37.3
Methanol-ammonia/hexane	3.26 ± 0.38[c]	26.2	1.39 ± 0.17[b]	26.8
Methanol-ammonia-water/hexane	1.92 ± 0.17[e]	57.0	0.81 ± 0.04[c]	57.3
Ethanol-ammonia-water/hexane	3.99 ± 0.17[b]	9.7	1.79 ± 0.09[a]	5.8
Isopropanol-ammonia-water/hexane	3.36 ± 0.15[c]	23.9	1.31 ± 0.06[b]	31.0
Commercial meal Hexane only	1.41 ± 0.32	0.0	1.97 ± 0.14	0.0
Methanol-ammonia-water/hexane	1.72 ± 0.22	60.8	0.69 ± 0.05	64.9

[1] Values bearing different superscripts are significantly (P<0.05) different.

were extracted with methanol-ammonia-water/hexane. Ethanol-ammonia-water/hexane and methanol/hexane were the least effective solvent combinations for removal of cyanogenic glycosides. Reduction of cyanogenic glycosides is a very important factor when considering utilization of linseed meal for feed or food formulation.

Modification of the extraction conditions in methanol-ammonia-water/hexane system resulted in a much better removal of the two disaccharide cyanogenic glycosides in flaxseed meal. An increase in the water content in the alkanol phase up to 15% (v/v), increased the removal of cyanogenic glycosides to 77-79%. This may be due to the increased polarity of the polar phase. However, the latter combination gave a very dark, sticky meal that formed hard clumps upon drying. Extending the contact time of the meal with solvent, up to 30 min showed a slight improvement in the removal of cyanogenic glycosides. Increase of solvent-to-seed ratio to R=13.3 for both the 15 and 30 min contact time increased the removal of linustatin and neolinustatin by 20%. None of these combinations had an apparent adverse effect on the meal quality.

A two-stage extraction with methanol-ammonia-water/hexane (10%, w/w ammonia; 5%, v/v water; and R=6.7) resulted a similar effect in enhancing the removal of cyanogenic glycosides as that of increasing the solvent-to-seed ratio to 13.3. A three-stage extraction was very effective in reducing the contents of both linustatin and neolinustatin, by 92.5%, from linseed meal. However, it is not clear that whether the cyanogenic glycosides are broken down into soluble compounds which

Table 4.5. Effect of water content in methanol-ammonia-water phase on the concentration of linustatin and neolinustatin in linseed[1].

Water content (% v/v)	Linustatin (mg/g)	Removal (%)	Neolinustatin (mg/g)	Removal (%)
5	1.92 ± 0.17	57.0	0.81 ± 0.04	57.3
10	1.59 ± 0.04	64.0	0.67 ± 0.04	65.0
15	1.41 ± 0.04	68.2	0.59 ± 0.08	69.0

[1] R=6.7; ammonia (10%, w/w); quiescent period 15 min.

Table 4.6. Effect of polar phase-to-seed ratio (R, v/w) and quiescent period on the content of linustatin and neolinustatin in linseed[1].

Quiescent period (min)	R	Linustatin (mg/g)	Removal (%)	Neolinustatin (mg/g)	Removal (%)
15	6.7	1.92 ± 0.17	57.0	0.81 ± 0.04	57.3
15	13.3	0.99 ± 0.04	77.6	0.41 ± 0.01	78.4
30	6.7	1.73 ± 0.15	60.8	0.73 ± 0.05	61.6
30	13.3	0.85 ± 0.04	80.5	0.36 ± 0.01	81.0

[1] Ammonia (10%, w/w); water (5%, v/v) in methanol.

Table 4.7. Effect of number of extraction stages on the content of linustatin and neolinustatin of methanol-ammonia-water/hexane extracted linseed meal[1].

Number of extractions	Linustatin (mg/g)	Removal (%)	Neolinustatin (mg/g)	Removal (%)
1	1.92 ± 0.17	57.0	0.81 ± 0.04	57.3
2	0.88 ± 0.05	80.0	0.40 ± 0.03	78.9
3	0.33 ± 0.04	92.5	0.15 ± 0.03	92.1

[1] R=6.7; ammonia (10%, w/w); water (5%, v/v); quiescent period 15 min.

are then extracted or are made chemically bound to other components, thus rendering them unavailable for detection. Degradation of cyanogenic glycosides in methanol-ammonia-water/hexane needs further studies.

Ammonia treatment (2.5M ammonium hydroxide) of cassava leaves for reducing the content of cyanogenic glycoside content has been reported by Padmaja (1989). The above treatment was unsuccessful since the increased alkalinity of the samples resulted in the deactivation of the enzymes which were used in subsequent determination of cyanogens. Apart from their contribution to the chemical defence mechanism of the plant (Conn, 1979, 1981), cyanogenic glycosides (linamarin and linustatin) were found to serve as a storage form of nitrogen which can be converted to asparagine that is required in germination and growth of the seedlings of *Hevea brasilliensis* or rubber plant (Selmar *et al.* 1988).

4.2.4 Effect of solvent extraction on contents of total phenolic acids and condensed tannins of linseed meal

The total content of phenolic acids of linseed meals obtained as the sum of free, esterified and insoluble-bound fractions is presented in Table 4.8. Individual values of each phenolic acid fraction are given in Table A.4 (Appendix). Hexane-extracted linseed meal contained 0.22% (220 mg/100g) of phenolic acids on a dry weight basis. Other studies on linseed meal have indicated the presence of 60.4 mg/100g (Kozlowska *et al.*, 1983) and 81.2 mg/100g (Dabrowski and Sosulski, 1984)

Table 4.8. Effect of solvent extraction on the content of total phenolic acids and condensed tannins of linseed meals[1].

Solvent	Total phenolic acids[2] (mg/100g)	Condensed tannin[3] (mg/100g)
Laboratory prepared meal:		
Hexane only	220 ± 13[a]	136 ± 13[a]
Methanol-hexane	190 ± 11[b]	100 ± 8[b]
Methanol-water/hexane	192 ± 20[ab]	73.4 ± 5.1[c]
Methanol-ammonia/hexane	176 ± 12[b]	65.3 ± 6.2[d]
Methanol-ammonia-water/hexane	132 ± 7[c]	35.6 ± 5.3[f]
Ethanol-ammonia-water/hexane	187 ± 13[b]	53.4 ± 6.1[e]
Isopropanol-ammonia-water/hexane	188 ± 19[a]	42.5 ± 3.5[f]
Commercial meal:		
Hexane only	214 ± 12	125 ± 7
Methanol-ammonia-water/hexane	141 ± 9	70.0 ± 8.1

[1] Values bearing different superscripts are significantly ($P<0.05$) different.
[2] expressed as ferulic acid equivalents.
[3] expressed as (+)-catechin equivalents.

phenolic acids in thesamples. Ferulic acid was found to be the predominant phenolic acid in linseed meal (Dabrowski and Sosulski, 1984). Both of these studies used hull-free linseed meal/flour.

Bhatty and Cherdkiatgumachai (1990) reported that hulls of linseed comprised about 37.5% of the oil-free weight of the dried seed meal. Phenolic compounds of linseed hulls have not been reported. However, studies on other oilseed meals have shown that hulls contain a considerable amount of phenolic compounds (Durkee, 1971). The higher content of phenolic acids in linseed meals in this study may be attributed to the presence of hulls.

The alkanol-water, with or without added ammonia, resulted in the removal of about 10 to 48% of phenolic acids present in the original meal. The reduction of phenolic acids was much higher when ammonia was present in the solvent extraction system. Methanol in combination with ammonia and water resulted the highest (48%) removal of total phenolic acids. It has been reported that treatment of oilseeds with ammonia (gaseous or alkanol solution) reduces the content of phenolic acids (Kirk *et al.*, 1966; McGregor *et al.*, 1983; Goh *et al.*, 1982; Naczk and Shahidi, 1989).

The present results for linseed meal lend further support to the previous findings although the degree of reduction of phenolics in linseed was not as high as those reported for canola, crambe or mustard. This variation in the removal efficiency of linseed phenolics may arise from the existing differences in seed microstructures which may govern the extraction of phenolic acids. Therefore, different extraction

efficiencies may be observed for different botanical families/genera.

Ibrahim and Shaw (1970) reported the phenolic compounds in linseed cotyledon and young shoots are esters and glycosides of *p*-coumaric, caffeic, ferulic and sinapic acids. Presence of C-glycosides and O-glycoflavones was also noted. These authors suggested that phenolic compounds may be responsible for the greater resistance of young flax plants to "flax rust" disease.

The content of condensed tannins of flax meal as affected by different solvent extraction systems and expressed as (+)-catechin equivalents, is presented in Table 4.8. About 0.14% (136 mg/100g) condensed tannins were present in linseed meal, on a dry weight basis. Tannins of linseed may originate from polymeric phenols of seed coat materials or hulls. The two-phase solvent extraction system reduced the tannin content of the meals by 26 to 74%. Presence of ammonia gave rise to a higher reduction of tannins, thus methanol-ammonia-water/hexane and isopropanol-ammonia-water/hexane treatments reduced the tannin content of the meals by 74% and 68%, respectively. Shahidi and Naczk (1989) reported a similar reduction of condensed tannins in canola meals upon methanol-ammonia-water/hexane extraction.

Ammoniation is considered as an effective chemical treatment for the removal of tannins from cassava leaves (Padmaja, 1989), sal seed meal (Gandhi *et al.*, 1985) and sorghum seeds (Price *et al.*, 1979). The mechanism of removal of tannins and phenolic acids by ammoniation treatment may arise from extraction of these polar compounds into a polar solvent. Furthermore, in basic solutions, phenolic compounds

may form phenolic anions and phenolates which are more soluble in the extraction solutions. However, the contents of bound phenolics remained unchanged. The removal of condensed tannins and phenolic acids from oilseed meals is considered important since they may influence in the formation of off-flavours, dark colours could also bind minerals and vitamins, thus limiting the use of the meals as protein ingredients in food formulations (Sosulski, 1979a).

4.2.5 Effect of solvent extraction on the content of phytic acid of linseed meal

The content of phytic acids in the extracted meals is presented in Table 4.9. Meals contained 2.4-3.2% of phytic acid; Bhatty and Cherdkiatgumachai (1990) reported the presence of 2.4% phytic acid in linseed meal. However, Madhusudhan and Singh (1985a) reported 4.2% phytic acid in linseed meal. The phytic acid content of hexane extracted linseed meal was in the range of those reported for soybean meal, 1.0-1.5% (de Boland *et al.*, 1975; Lolas *et al.*, 1976) and canola meal, 3.0-5.0 (Shah *et al.*, 1976; Nwokola and Bragg, 1977; Uppstrom and Sevensson, 1980; Naczk *et al.*, 1986a).

None of the two-phase solvent combinations were able to reduce the content of phytic acid in linseed meal and indeed an increase in phytic acid content in the resultant meals was noted which may be due to a concentration effect. However, it has been established that phytic acid in oilseeds such as canola, soybean and sun-flower is present in association with storage protein bodies (Thompson, 1990).

Therefore, in the preparation of protein concentrates from canola, soybean and sunflower a parallel increase in the content of phytic acid may be noted. Removal of polar matters from the flaxseed meals resulted in the concentration of proteins and a similar enhancement in the content of phytic acid in this study.

Omosiye and Cheryan (1979) reported that hydrolysis of phytate facilitated separation of phytic acid from soy protein by employing an ultrafiltration process. The control of pH during the extraction and subsequent purification steps combined with ultrafiltration and diafiltration has effectively reduced the content of phytic acid in canola protein isolates (Tzeng *et al.*, 1988; 1990). However, Ratnyake *et al.* (1992) reported that phytate in flaxseed has no effect on zinc status of liver and tibia of rats fed with diets containing flaxseed of up to 40%.

4.2.6 Effect of solvent extraction on the content of soluble sugars of linseed meal

The soluble sugars determined by the anthrone method showed a reduction due to the extraction of linseed with alkanols (Table 4.9). Methanol-ammonia-water/hexane extraction reduced the content of soluble sugars by 46%. Other extractions, except isopropanol-ammonia-water/hexane, removed 26 to 39% of the soluble sugars.

Extraction of Cruciferae oilseeds with methanol-ammonia-water/hexane removed 50.0 to 77.5% of the total soluble sugars originally present in rapeseed and mustard meals (Naczk and Shahidi, 1990). In canola, sucrose was the dominant sugar

Table 4.9. Effect of solvent extraction on the content of total soluble sugars and phytic acid in linseed meal[1].

Solvent	Soluble sugars[2] (%)	Phytic acid (%)
Laboratory prepared meal:		
Hexane only	7.69 ± 0.16^{a}	2.40 ± 0.13^{bc}
Methanol-hexane	5.64 ± 0.24^{bc}	2.23 ± 0.10^{bc}
Methanol-water/hexane	4.71 ± 0.15^{d}	2.29 ± 0.03^{bc}
Methanol-ammonia/hexane	5.10 ± 0.12^{cd}	2.50 ± 0.30^{ab}
Methanol-ammonia-water/hexane	4.17 ± 0.18^{e}	3.05 ± 0.30^{a}
Ethanol-ammonia-water/hexane	5.67 ± 0.10^{b}	2.40 ± 0.10^{bc}
Isopropanol-ammonia-water/hexane	7.31 ± 0.47^{a}	2.94 ± 0.30^{a}
Commercial meal:		
Hexane only	7.93 ± 0.23	2.77 ± 0.37
Methanol-ammonia-water\hexane	3.97 ± 0.13	3.23 ± 0.25

[1] Values bearing different superscripts are significantly ($P<0.05$) different.
[2] expressed as sucrose equivalents.

present and its content was reduced by 78 to 89%. Higher molecular weight sugars (such as stachyose and raffinose) were removed less effectively, 19.6 to 53.4% (Naczk and Shahidi, 1990; Shahidi *et al.*, 1990). The application of a solvent system consisting of methanol-ammonia-water/hexane was effective in the removal of flatulence-causing sugars from commercial soybean meal (Myhara *et al.*, 1989). The difference in the results for canola and soybean meal was suggested to arise from differences in the seed microstructures and other treatments prior to solvent extraction (Naczk and Shahidi, 1990). Bhatty and Cherdkiatgumachai (1990) have reported only the composition of monosaccharides in linseed which indicated that glucose was predominantly present and pentosans accounted for 12% of the total sugars in linseed meal. Linseed hulls contained up to 56% monosaccharides (Bhatty and Cherdkiatgumachai, 1990). Further studies on composition of sugars in flaxseed are required, however, the presence of sucrose and raffinose was tentatively noted in this study.

4.2.7 Amino acid composition of meals

The amino acid composition of laboratory prepared linseed meals extracted with hexane and methanol-ammonia-water/hexane and also hexane extracted commercial meal are presented in the Table 4.10. The content of essential amino acids of the meals was slightly less than the FAO/WHO reference values. In particular, methionine, lysine and tryptophan levels were lower than the reference values (Table 2.3). The amino acid composition of linseed meal was similar to that of canola.

Table 4.10. Amino acid composition of hexane and methanol-ammonia-water/hexane extracted, and commercial linseed meals (g/16g N).

Amino acid	Hexane extracted	Methanol-ammonia-water/Hexane extracted	Hexane extracted commercial meal
Histidine	2.69 ± 0.24	2.46 ± 0.05	2.36 ± 0.03
Isoleucine	4.78 ± 0.54	4.54 ± 0.15	4.19 ± 0.03
Leucine	6.70 ± 0.62	6.39 ± 0.15	5.96 ± 0.04
Lysine	4.38 ± 0.37	4.14 ± 0.08	3.92 ± 0.02
Methionine	1.45 ± 0.09	1.41 ± 0.05	1.24 ± 0.05
Cysteine	3.29 ± 0.56	3.39 ± 0.21	3.16 ± 0.23
Phenylalanine	5.13 ± 0.05	4.91 ± 0.08	4.63 ± 0.00
Tyrosine	2.21 ± 0.19	2.12 ± 0.03	1.98 ± 0.02
Threonine	3.40 ± 0.30	3.33 ± 0.04	3.00 ± 0.00
Tryptophan	0.46 ± 0.10	0.46 ± 0.05	0.25 ± 0.09
Valine	5.75 ± 0.07	5.64 ± 0.17	5.02 ± 0.06
Alanine	4.81 ± 0.50	4.64 ± 0.10	4.61 ± 0.01
Aspartic acid	9.18 ± 0.60	9.16 ± 0.60	8.03 ± 0.19
Arginine	11.50 ± 0.33	11.20 ± 0.10	9.78 ± 0.06
Glycine	6.44 ± 0.35	6.26 ± 0.25	5.64 ± 0.17
Glutamic acid	16.70 ± 0.43	16.36 ± 0.33	14.45 ± 0.63
Proline	3.64 ± 0.24	3.65 ± 0.10	3.32 ± 0.03
Serine	4.93 ± 0.03	4.99 ± 0.24	4.48 ± 0.03

Commercial meals showed slightly lower values for all amino acidsas compared with laboratory-prepared meals. Methanol-ammonia-water/hexane extraction gave slightly lower amino acid values but these were not as low as those of the commercial meal.

4.2.8 Fatty acid composition of oils

The fatty acid composition of linseed oil recovered from the hexane phase is presented in Table 4.11. Fatty acid composition of hexane-extracted oil was similar to that reported in the literature. Methanol-ammonia-water treatment did not alter the relative fatty acid composition of the oil to any extent. However, somewhat lower values were observed for oil prepared by the latter extraction procedure. Removal of phospholipids by methanol-ammonia-water might have been responsible. Naczk *et al.* (1985) reported about 10% of the total phosphorus was removed by methanol/hexane extraction of canola. They suggested that most of the dissolved phosphorus was present in the methanol layer and probably contained phospholipids and the resultant oil was considered as degummed.

4.3 Effect of two-phase solvent extraction on functional properties of meals

4.3.1 Effect of solvent extraction on water absorption and water hydration capacity of linseed meal

Linseed extracted with hexane absorbed 9.7 times as much water as its original weight (Table 4.12). Other solvent extractions did not alter the water absorption,

Table 4.11. Fatty acid composition (w/w %) of linseed oil prepared by hexane and methanol-ammonia-water/hexane extractions.

Fatty acid	Hexane extracted	Methanol-ammonia-water/ hexane extracted
C14:0	0.20 ± 0.07	0.25 ± 0.05
C16:0	5.05 ± 0.26	4.83 ± 0.20
C18:0	4.54 ± 0.75	3.64 ± 0.07
C20:0	0.33 ± 0.01	0.33 ± 0.01
C22:0	0.15 ± 0.03	0.21 ± 0.01
C16:1	0.10 ± 0.00	0.13 ± 0.01
C18:1	14.8 ± 0.1	14.5 ± 0.1
C18:2	17.5 ± 0.1	17.1 ± 0.1
C18:3N3	55.6 ± 0.3	54.4 ± 0.9
C18:4N3	0.17 ± 0.00	0.58 ± 0.02
C20:2	0.16 ± 0.05	0.17 ± 0.06
C20:3	0.10 ± 0.00	0.09 ± 0.00

except for the isopropanol-ammonia-water/hexane system. The degree of water absorption in linseed meal was considerably higher than that of canola which was 3.7 (Diosady *et al.*, 1985; Naczk *et al.*, 1985). Bhatty and Cherdkiatgumachai (1990) found that linseed meal can absorb 8-fold and the hulls can absorb 13-fold their weights of water. It has been reported that linseed hulls contain 2.7% mucilage from the outer endosperm (Peterson, 1958) which absorbs water and swells (Dev and Quensel, 1986). Gelation of carbohydrates and swelling of crude fibres are two important factors that influence the water absorption of oilseed meals (Narayana and Narasingha Rao, 1982). The higher water absorption values in the solvent extracted meals may have resulted from the hull polysaccharides. The larger difference in water absorption of linseed meal as compared to other oilseed meals (canola or soybean) is presumably due to the presence of different constituents as well as existing differences in the conformational characteristics of its proteins.

The water hydration capacity values (Table 4.12) suggest that hexane-extracted meals require 5.2 times their weight of water to become hydrated. Extraction with alkanols resulted in an increase in the water hydration capacity values. However, all the meals extracted with solvents including ammonia did not get wet at the first contact of the meal with water and required a thorough mixing to become wet. The ability to physically hold water against gravity is related to the viscosity of food systems and is influenced by pH, ionic strength and temperature (Kinsella, 1979). Swelling or expansion of particles upon imbibition of water is an important functional

Table 4.12. Effect of solvent extraction on water and fat absorption of linseed meal[1].

Solvent	Water absorption (%)	Water hydration capacity (gH_2O/g)	Fat absorption (%)
Laboratory prepared meal:			
Hexane only	973 ± 3[a]	5.2 ± 0.2	266 ± 4[a]
Methanol/hexane	975 ± 7[ab]	5.9 ± 0.3	275 ± 3[b]
Methanol-water/hexane	973 ± 5[a]	5.8 ± 0.2	296 ± 2[d]
Methanol-ammonia/hexane	980 ± 4[ab]	6.3 ± 0.3	307 ± 4[e]
Methanol-ammonia-water/hexane	973 ± 11[ab]	5.8 ± 0.1	324 ± 3[f]
Ethanol-ammonia-water/hexane	977 ± 7[ab]	6.0 ± 0.2	286 ± 2[c]
Isopropanol-ammonia-water/hexane	981 ± 1[b]	6.5 ± 0.1	276 ± 1[b]
Commercial meal:			
Hexane only	977 ± 3	6.1 ± 0.2	135 ± 4
Methanol-ammonia-water/hexane	982 ± 6	6.3 ± 0.1	197 ± 3

[1] Values in the same column bearing different superscripts are significantly ($P<0.05$) different.

property in foods like processed meats, doughnuts and custards, where the proteins should mix with water without dissolution while concurrently imparting body, thickening power and viscosity of the food (Hermansson and Akesson, 1975; Kinsella, 1979).

4.3.2 Effect of solvent extraction on fat absorption of linseed meal

The fat absorption of the meals was strongly influenced by the presence of ammonia in the alkanol phase (Table 4.12). Hexane-extracted meals absorbed 2.6 times their original weight of oil. Extraction with methanol-ammonia-water/hexane enhanced the fat absorption by 3.2 fold. A similar trend was observed in methanol-ammonia-water/hexane extracted commercial linseed meal. Kinsella (1982) and Dench *et al.* (1981) have reported that fat absorption of a protein meal depends mainly on physical entrapment of oil by a capillary attraction process. Degree of water and fat absorption and their retention during cooking and processing greatly influences the juiciness, dryness, mouthfeel and even the storage ability of the product in which oilseed meal is incorporated (Sosulski *et al.*, 1977). The observed lower fat absorption value for commercial linseed meal may be due to changes in the protein conformation during commercial processing.

4.3.3 Effect of solvent extraction on pH of linseed meal

The presence of ammonia in the alkanol phase increased the pH of a 10% (w/v) dispersion of the meal in distilled water by about one unit (Table 4.13) as compared with alkanol and alkanol-water extracted meals. Diosady *et al.* (1985) and Naczk *et al.* (1985) have reported a similar pH increase in methanol-ammonia-water/hexane extracted canola meal. This increase in the pH value may be due to the presence of some residual ammonia in the meals. However, the content of non-protein nitrogen did not show any change by ammonia-containing solvent extraction systems. Kirk *et al.* (1966) have reported that ammonia-treated crambe meals retained some ammonia, both vacuum labile and chemically bound. However, all the labile ammonia was removed after 20 h of drying under vacuum.

4.3.4 Effect of solvent extraction on nitrogen solubility index (NSI) of linseed meal

Nitrogen solubility index (NSI) of the prepared meals at their natural pH is presented in Table 4.13. Hexane-extracted linseed meal at pH 5.98 had 55% of its total nitrogen in solution. Methanol alone and methanol-water extraction gave lower NSI values. Ethanol and isopropanol-ammonia-water/hexane extractions also gave a slightly lower NSI values, but methanol in combination with ammonia was able to

Table 4.13. Effect of solvent extraction on pH and nitrogen solubility index (NSI) of linseed meal[1].

Solvent	pH	NSI (% total nitrogen)
Laboratory prepared meal: Hexane only	5.98±0.03^{ab}	55.0±0.2^{c}
Methanol/hexane	5.97±0.02^{a}	46.2±0.5^{a}
Methanol-water/hexane	6.02±0.01^{ab}	47.5±1.2^{a}
Methanol-ammonia/hexane	6.67±0.01^{c}	65.0±0.9^{f}
Methanol-ammonia-water/hexane	6.82±0.03^{e}	60.7±0.2^{e}
Ethanol-ammonia-water/hexane	6.71±0.02^{d}	53.7±0.7^{b}
Isopropanol-ammonia-water/hexane	6.04±0.04^{b}	56.1±0.4^{d}
Commercial meal: Hexane only	6.09±0.03	42.4±0.6
Methanol-ammonia-water/hexane	6.84±0.02	53.9±1.0

[1] Values in the same column bearing different superscripts are significantly ($P<0.05$) different

improve the NSI values at natural pH of the meals. Naczk *et al.* (1985) reported that methanol-ammonia-water/hexane treatment lowers the NSI values of canola. Nitrogen solubility is considered as a reliable index of functional potential of protein-rich products.

Changes of NSI values as a function of pH of the solution was monitored for both hexane-extracted and methanol-ammonia-water/hexane extracted linseed meals (Figure 4.5). The solubility curves for both meals showed one minimum only. The minimum solubility of nitrogen (12% and 20%) occurred at a pH between 3 and 3.5 for both meals. The numerical values were however, slightly lower than those reported by Smith *et al.* (1946) and Dev and Quensel (1986) for linseed flour. Hexane-extracted linseed meals had a somewhat lower nitrogen solubility than their methanol-ammonia-water/hexane extracted counterparts. Both meals exhibited a higher NSI in the alkali pH range than in the acidic pH range indicating that at pH 11.0 more than 69-70% nitrogen was in solution for both meals.

4.3.5 Effect of solvent extraction on emulsifying properties of linseed meals

Emulsifying properties of laboratory prepared, solvent-extracted meals were evaluated by both the emulsifying activity and emulsion stability. Emulsifying activity of the meals ranged from 64.5 to 80.6% (Table 4.14) of the added soybean oil. Methanol-water extraction showed a slight decrease in emulsifying activity while other solvent extraction systems did not show much of an effect. Commercial linseed

Figure 4.5. pH dependance of nitrogen solubility index of linseed meal.
●——●, hexane extracted; ▼-----▼, methanol-ammonia-water/hexane extracted.

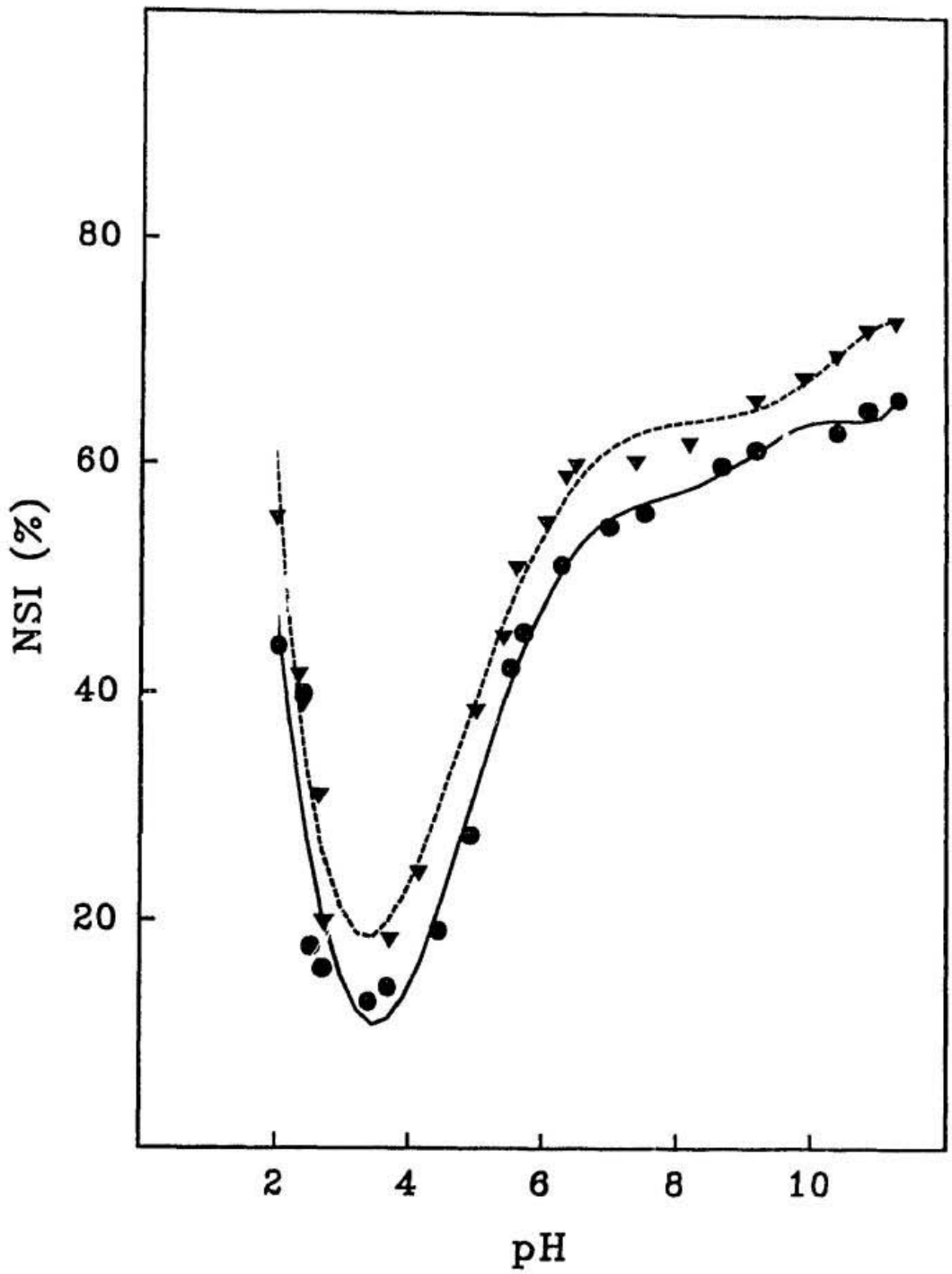

80
60
40
20
NSI (%)
2
4
6
8
10
pH

Table 4.14. Effect of solvent extraction on emulsifying properties and foaming ability of linseed meal[1].

Solvent	Emulsifying activity (%)	Emulsion stability (%)	Whippability (% volume increase)
Laboratory prepared meal:			
Hexane only	77.5±3.1^{bc}	107±8^{b}	64±3^{b}
Methanol/hexane	74.9±4.2^{bc}	95±2^{a}	55±4^{a}
Methanol-water/hexane	64.5±3.4^{a}	107±8^{b}	58±4^{a}
Methanol-ammonia/hexane	73.5±2.4^{b}	101±3^{bc}	50±4^{a}
Methanol-ammonia-water/hexane	76.2±2.2^{b}	110±3^{b}	70±2^{c}
Ethanol-ammonia-water/hexane	78.8±1.4^{bc}	100±2^{c}	54±4^{a}
Isopropanol-ammonia-water/Hexane	80.6±1.5^{c}	102±3^{bc}	60±5^{b}
Commercial meal:			
Hexane only	78.4±1.4	100±2	37±1
Methanol-ammonia-water/Hexane	81.0±1.0	104±1	42±1

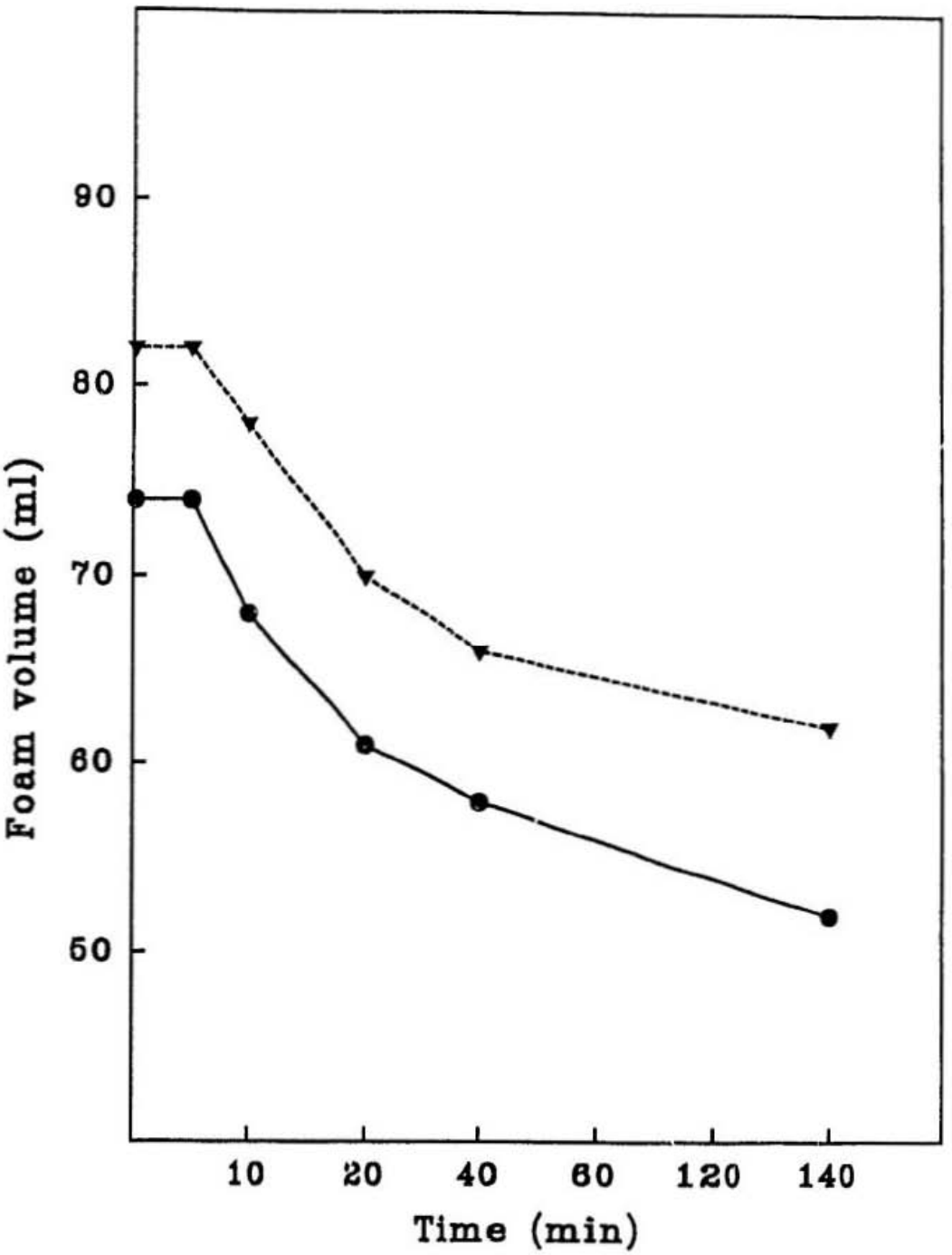
[1] Values in the same column bearing different superscripts are significanlty ($P<0.05$) different.

meal had a similar emulsifying activity as the hexane extracted meal. All the emulsions formed were fairly stable as 95-100% emulsifying activity was retained after heating of the emulsion at 80°C for 30 min (Table 4.14). Thus, heat treatment did not cause the emulsions to collapse. A similar situation has been observed in alpha-alpha proteins (Wang and Kinsella, 1976, Dench *et al.*, 1981). Wolf and Cowan (1975) suggested that fat absorption of protein meals is closely related to the formation and stabilization of emulsions. High protein solubilities ensured good oil emulsification activity in sunflower and soybean meals (Inklaar and Fourtin, 1969; Lin *et al.*, 1974; Kinsella, 1979). Therefore, nitrogen solubility is not the primary determinant of emulsifying properties of the meals. Presence of polysaccharides can also contribute to enhance the emulsifying activity and emulsion stability (Dev and Quensel, 1986). It has been shown that the presence of mucilage in prepared protein products from linseed meal enhances the emulsion stability of canned fish sauces and ice cream and also reduces the cooking loss of meat emulsions (Dev and Quensel, 1989).

4.3.6 Effect of solvent extractions on foaming properties of linseed meal

Foaming properties of meals were monitored as whippability or percentage volume increase and foam stability (Table 4.14). Whippability of the meals was between 55.0 and 70.0%. Methanol/hexane extraction lowered the foaming ability of the meals. Commercial linseed meal had the lowest whippability. Dev and Quensel

Figure 4.6. Time dependance of foam stability of linseed meal.
●——●, hexane extracted; ▼------▼, methanol-water-ammonia/hexane extracted meal.


90
80
70
60
50
Foam volume (ml)
10
20
40
60
120
140
Time (min)

(1986) observed that the foaming capacity of linseed meal protein isolate and flour at different pH values was proportional to the content of soluble proteins at the particular pH.

The foam produced by all meals had a fine stable "bubble" structure which may be unique for and characteristic of linseed meal. The change of foam volume with time for hexane and methanol-ammonia-water/hexane-extracted meals is presented in Figure 4.6. The good foam stability of linseed meal foam may partly be attributed to the presence of polysaccharides in the meal. Both non-protein nitrogen compounds and carbohydrates can stabilize the foams (Cherry and McWaters, 1981). It has been reported that finely divided solid particles are also able to stabilize foams (Dev and Quensel, 1988). Sosulski (1977) reported that a good nitrogen solubility also ensures good foam stability in the protein meal under investigation. It increases film thickness and viscosity of foam bubbles reduces air leakage and enhances stability (Kinsella, 1979). Linseed flour has been shown to increase in foam stability with decreasing pH of the medium (Dev and Quensel, 1986).

CONCLUSIONS

The two-phase solvent extraction of flaxseed with alkanol-ammonia-water/hexane resulted in simultaneous recovery of oil and production of a high quality meal. The meals so obtained had a high content of proteins (up to 49%) and had reduced levels of cyanogenic glycosides. While the starting material contained linustatin and neolinustatin, no linamarin was detected in the samples. A methanol-ammonia-water/hexane solvent system was the most effective in removing cyanogenic glycosides. Increasing water content in the polar phase as well as contact time or solvent-to-seed ratio or use of a multi-stage extraction process improved the efficiency of the removal of cyanogenic glycosides. A three-stage extraction process resulted in the highest reduction (92.5%) of cyanogenic glycosides. The content of total phenolic acids and condensed tannins was comparatively lower in flaxseed than those in the other oilseeds; solvent extraction reduced these even further. The soluble sugar content was also reduced but phytic acid was concentrated in solvent extracted meals. Methanol-ammonia-water/hexane extraction did not have any adverse effect on amino acid composition of the meal or fatty acid composition of the oil. Flaxseed meal had a very high water absorption and water hydration capacity. The fat absorption properties and pH of the meals were influenced by the presence of ammonia in the extraction system. While the original flaxseed meal had 55% soluble nitrogen compounds, the two-phase solvent extraction improved this property. Emulsifying ability and whippability of the products were not influenced by the solvent extraction process employed. All meals gave fairly stable emulsions and foams.

Based on the results shown, unrestricted utilisation of flaxseed meal in animal feed and human food formulations, as far as the contents of cyanogenic glycoside and phenolic acids are concerned, may be possible. However, further studies are needed to assess the fate of cyanogenic glycosides in alkanol-ammonia-water extraction and the effect of processing on the anti-pyridoxine factor (linatine).

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APPENDIX

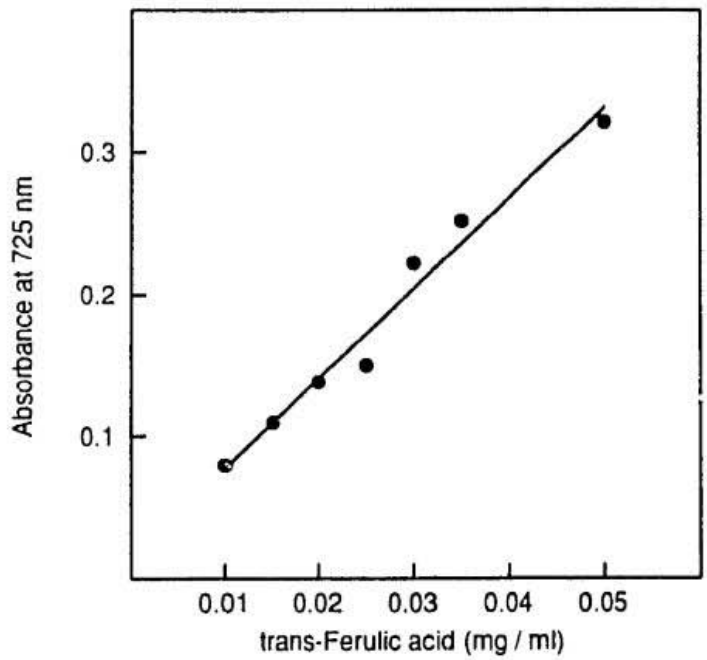

Figure A.1. Standard line of concentration dependance of phenolic acids as reflected in the absorbance of the ferulic acid-Folin Denis complex.

Regression coefficient (r) = 0.9940
Equation of the line (Y=aX+b) where,

Y = absorbance at 725 nm (A_{725})
X = concentration of ferulic acid in solution, mg/ml (C)
a = 6.6667
b = 0.0113

$A_{725} = 6.6667*C + 0.0113$

Therefore:

$C = 0.15*A_{725} - 0.0017$

Since extracted solutions have to be diluted, then:

$C = k(0.15*A_{725} - 0.0017)$

where k = dilution factor.

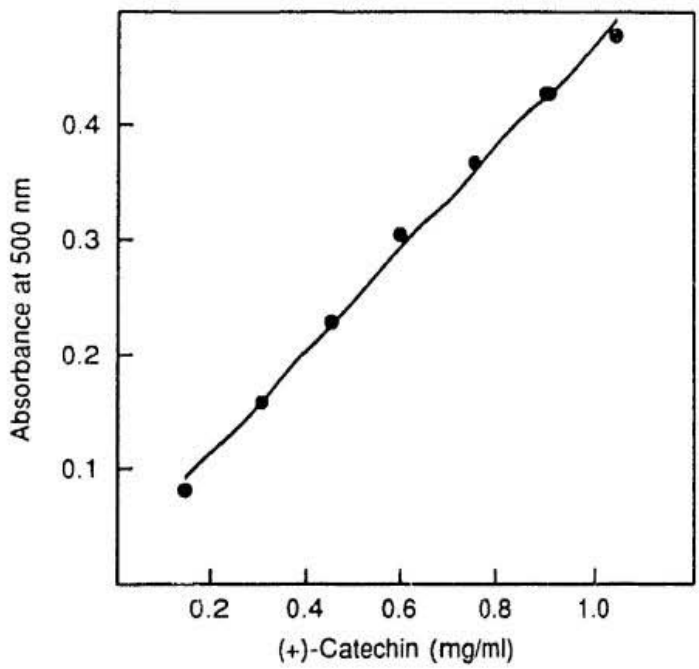

Figure A.2. Standard line of concentration dependance of condensed tannins as reflected in the absorbance of the (+)-catechin-vanillin complex.

Regression coefficient (r) = 0.9963
Equation of the line (Y=aX+b) where,

Y = absorbance at 500 nm (A_{500})
X = concentration of (+)-catechin in solution, mg/ml (C)
a = 0.4451
b = 0.025

$A_{500} = 0.4451*C + 0.025$

Therefore:

$C = 2.2467*A_{500} - 0.0561$

Since extracted solutions have to be diluted, then:

$C = k(2.2467*A_{500} - 0.0561)$

where k = dilution factor.

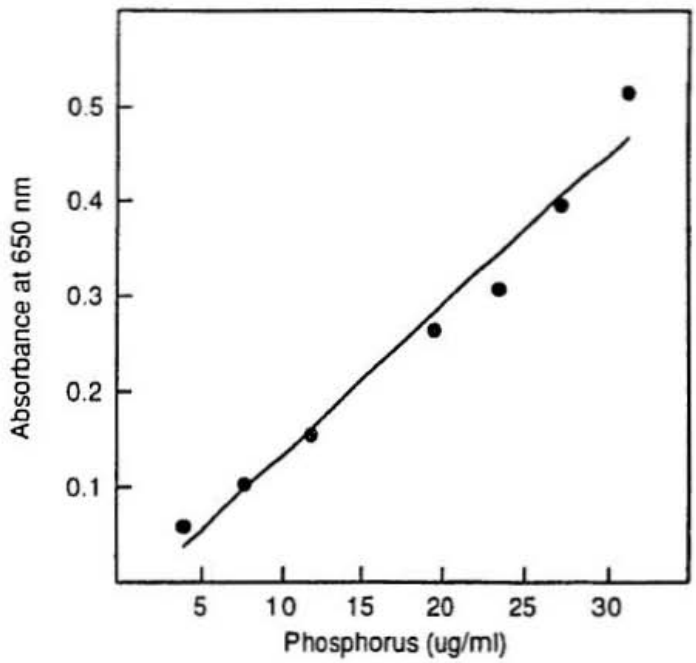

Figure A.3. Standard line of concentration dependance of phytic acid as reflected in the absorbance of the phosphorus-ammonium molybdate complex.

Regression coefficient (r) = 0.9900
Equation of the line (Y=aX+b) where,

Y = absorbance at 660 nm (A_{660})
X = concentration of phosphorus in solution, µg/ml (C)
a = 0.01289
b = 0.005

$A_{660} = 0.01289*C + 0.005$

Therefore:

$C = 77.54*A_{660} - 0.39$

Since extracted solutions have to be diluted, then:

$C = k(77.54*A_{660} - 0.39)$

where k = dilution factor.

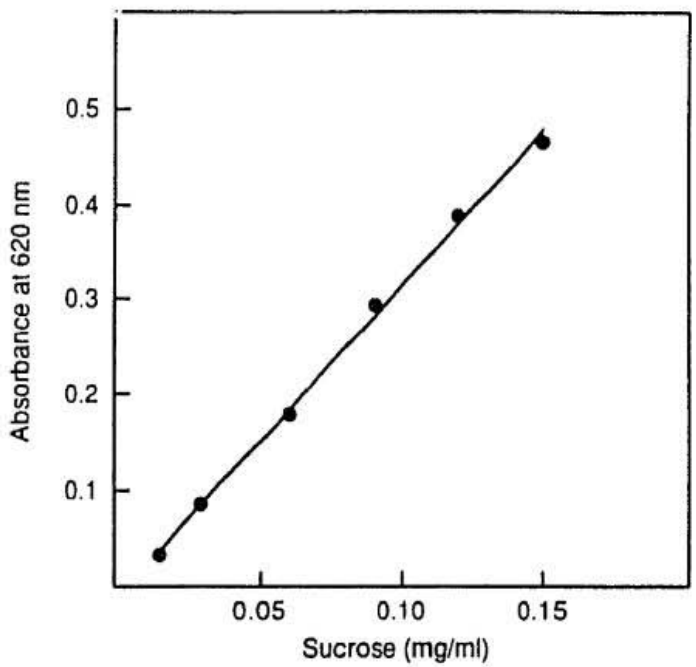

Figure A.4. Standard line of concentration dependance of total soluble sugars as reflected in the absorbance of sucrose-anthrone complex.

Regression coefficient (r) = 0.9975
Equation of the line (Y=aX+b) where,

Y = absorbance at 620 nm (A_{620})
X = concentration of sucrose in solution, mg/ml (C)
a = 3.2679
b = 0.0147

$A_{620} = 3.2679*C + 0.0147$

Therefore:

$C = 0.3060*A_{620} - 0.0045$

Since extracted solutions have to be diluted, then:

$C = k(0.3060*A_{620} - 0.0045)$

where k = dilution factor.

Table A.1. Mass balance of multi-stage extraction of linseed with methanol-ammonia-water/hexane; 5% (v/v) water, 10% (w/w) ammonia, R=6.7, 15 min quiescent period.

Extraction	Yield on dry basis (%)			Loss(%)
	Meal	Oil	Solids	
1 stage	46.2±2.0	47.1±0.8	5.7±0.1	0.8
2 stage	42.0±1.0	44.6±1.0	6.5±0.9	6.9
3 stage	41.5±2.0	45.7±1.0	6.9±1.0	5.9

Table A.2. Mass balance of extraction of linseed with methanol-ammonia-water/hexane containing different amounts of water; 10% (w/w) ammonia, R=6.7, 15 min quiescent period.

Water content (%, v/v)	Yield on dry basis (%)			Loss(%)
	Meal	Oil	Solids	
5	46.2±2.0	47.1±0.8	5.7±0.1	0.8
10	44.2±2.0	44.9±2.0	5.9±1.0	5.0
15	42.5±2.0	42.3±1.0	6.1±1.0	9.1

Table A.3. Mass balance of extraction of linseed with methanol-ammonia-water/hexane using different solvent-to-seed ratio (R) and quiescent period; water content 5% (v/v), ammonia content 10% (w/w).

Quiescent period (min)	R	Yield on dry basis (%)			Loss(%)
		Meal	Oil	Solids	
15	6.7	46.2±2.0	47.1±0.8	5.7±0.1	0.8
15	13.3	42.3±1.0	47.0±2.0	6.1±0.9	4.6
30	6.7	44.2±1.0	46.9±1.0	5.7±0.8	3.2
30	13.3	42.0±0.9	46.1±0.9	6.8±0.7	5.1

Table A.4. The contents of free, esterified and insoluble bound phenolic acids of two-phase solvent extracted linseed meal (mg/100g as ferulic acid equivalents).

Solvent	Free	Esterified	Insoluble bound
Laboratory prepared meal:			
Hexane only	34.8±1.4	130±7	55.3±4.4
Methanol/hexane	32.3±2.7	107±4	50.8±3.7
Methanol-water/hexane	29.1±1.7	108±9	55.2±4.1
Methanol-ammonia/hexane	32.9±2.5	99.8±6.1	43.5±4.0
Methanol-ammonia-water/hexane	26.1±1.5	66.4±4.2	39.5±4.3
Ethanol-ammonia-water/hexane	30.7±3.5	108±9	48.3±5.4
Isopropanol-ammonia-water/hexane	32.3±3.5	114±13	42.1±5.2
Commercial meal:			
Hexane only	32.9±1.6	123±5	58.1±3.0
Methanol-ammonia-water/hexane	26.2±2.6	68.1±6	46.8±4.6

Table A.5. Foam stability of two-phase solvent extracted linseed meal.

Solvent	Volume of foam remain (ml)[1]					
	0.5 min	10 min	20 min	40 min	60 min	120 min
Laboratory prepared meal: Hexane only	74.0	74.0	68.0	61.0	58.0	52.0
Methanol/hexane	60.0	60.0	58.0	56.0	56.0	43.0
Methanol-water/hexane	66.0	60.0	58.0	44.0	42.0	32.0
Methanol-ammonia/hexane	58.0	56.0	54.0	51.0	45.0	44.0
Methanol-ammonia-water/hexane	82.0	82.0	78.0	76.0	68.0	52.0
Ethanol-ammonia-water/hexane	62.0	60.0	51.0	51.0	52.0	48.0
Isopropanol-ammonia-water/hexane	72.0	72.0	68.0	68.0	60.0	54.0
Commercial meal: Hexane only	78.0	78.0	74.0	67.0	64.0	52.0
Methanol-ammonia-water/hexane	80.0	76.0	72.0	70.0	66.0	65.0

[1] 100 ml of 3% (w/v) solution in distilled water.