CHEMICAL AND BIOCHEMICAL COMPONENTS OF BEACH PEA (*Lathyrus maritimus* L.)

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BY

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THIS WORK IS DEDICATED TO

MY PARENTS, LOVING WIFE AND DAUGHTER

ABSTRACT

Physico-chemical properties of Beach pea (*Lathyrus maritimus* L.) seeds were evaluated and their proximate composition determined. Results were also compared with those of green pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L.). Beach pea seeds had a very low grain weight, density, hydration capacity, hydration index, swelling capacity and swelling index as compared to green pea and grass pea. The contents of crude protein (% N x 6.25, 29.2%), crude fibre (12.0%), reducing sugars (0.2%), total phenolics (1.2%), ash (3.0%), and total free amino acids (0.6%) of beach pea were substantially higher than other peas examined. The contents of cysteine (1.6%), methionine (1.1%), and tryptophan (0.3%) in beach pea proteins were low, but still higher than those in green pea and Canadian and Indian grass peas. Beach pea lipids were dominated by linoleic acid (69.1%), similar dominance of linoleic acid was observed in green pea (45.1%) and Canadian grown grass pea (57.0%). The major macroelements of beach pea were potassium (475.8 mg/100g), phosphorus (413.2 mg/100g), magnesium (179.7 mg/100g), and calcium (144.2 mg/100g). The content of microelements, namely manganese, zinc, and iron in beach pea was 3.5, 3.0 and 9.4 mg/100g, respectively.

Samples of beach pea (*Lathyrus maritimus* L.) seeds and plant parts were analyzed in order to determine their chemical composition, total and free amino acids as well as minerals. The crude protein content of beach pea plant parts varied from 10.7 to 28.0%, soluble proteins 190.2 - 709.0 mg/100g, lipid 1.3 - 6.0%, ash 2.2 - 6.8%, crude fibre 10.7 - 35.5%, soluble sugars 0.1 - 12.2%, starch 0.8 - 26.5%, carbohydrate 55.8 - 81.5% and phenolic compounds 0.5 - 3.0%. The amino acid profile of seed proteins and other plant parts of beach pea showed that they were deficient in sulphur-containing amino acids.

Tryptophan was another limiting amino acid in plant parts, except in leaves (1.35 g/16g N). The content of free amino acids was highest in branches and stems (3147.9 mg/100g) and lowest in pod shells (150.7 mg/100g). Beach pea plant parts were a good source of minerals such as K, P, Ca, Mg, Na, Fe and Zn.

The biochemical composition of seeds and pod shells of beach pea was determined during growth and maturation. The content of crude and soluble protein, soluble sugars and phenolics was high in the fresh green seeds and pod shells, but these decreased rapidly during seed maturation. Meanwhile, the corresponding content of starch increased in seeds and decreased in pod shells. Glutamic acid was the predominant amino acid in seeds and aspartic acid was dominant in pod shells. Levels of arginine, alanine, and threonine were highest in fresh green seeds. Methionine and cysteine content increased during seed maturation, but declined in pod shells. Free amino acid content decreased rapidly during the latter stages of seed maturation. Potassium, calcium, sodium, phosphorus were the predominant minerals in fresh green seeds and pod shells, but iron content was highest in mature pod shells relative to that of other stages of maturation. Major changes in colour (pigment interchanges) also occurred during maturation.

Acetone-water (7:3, v/v) mixture containing 1% concentrated HCl served best for extraction of condensed tannins as compared with other solvents used in this study. Air classification of beach pea cotyledons and hulls concentrated protein content in cotyledons up to 35% and starch up to 37%. Meanwhile, the content of total phenolics and condensed tannins was reduced by up to 0.93 and 5.76%, respectively, in cotyledons of beach pea.

Beach pea hulls were also extracted with 70% (v/v) acetone containing 1% concentrated HCl in order to isolate, fractionate and partially characterize the compounds responsible for antioxidative activity of hulls. The UV absorption maxima showed that flavonoids were present in all three isolated fractions (I-III). β-Carotene-linoleate model system studies indicated that antioxidant activity of separated fractions and crude extract were in the order of fraction III > crude extract > fraction II > fraction I. Silica gel TLC plates sprayed with a solution of β-carotene and linoleic acid indicated that many of the individual compounds present were antioxidative in nature. Furthermore, separation of fraction III on a semi-preparative HPLC showed the presence of (+) catechin and (-) epicatechin as the main phenolic compounds present.

Methanol-ammonia-water extraction system efficiently removed β-N-oxalylamino-L-alanine (BOAA) and reduced the content of non-protein nitrogen, phenolics and condensed tannins from beach pea seeds with a concomitant increase in protein content of the processed meal.

The nitrogen solubility of beach pea seeds was lowest at pH 4.5. The content of glutelin in beach pea seeds, its cotyledons and hulls was higher than those of green and grass pea seeds. Meanwhile, the corresponding albumin and globulin were lower than those of green pea and Canadian grass pea. Albumin fraction contained the highest amount of sulphur-containing amino acids as compared to those of other protein fractions and the amount of these amino acids was higher in beach pea than those of other peas examined. Polyacrylamide gel electrophoresis (PAGE) results of protein isolates as well as protein fractions showed that major storage proteins of beach pea were in the range 22 -

40 kDa and 43 - 116 kDa.

Beach pea protein isolates prepared using sodium hydroxide had 87% protein, while those extracted with sodium hexametaphosphate had 85% protein. Functional properties, such as water binding, fat binding, foaming, foam stability, emulsion, emulsion stability and *in-vitro* digestibility (pepsin-trypsin; 80.6 - 82.6% and pepsin-pancreatin; 78.6 - 79.2%) of beach pea protein isolates were comparable to those of green pea and grass pea protein isolates.

Starch from beach pea was isolated and its physicochemical properties compared with those of green pea and grass pea starches. The yield of beach pea starch was 12.3% on a whole seed basis. The shape of the granules was round to elliptical, with granules 6-17 μm in diameter. Scanning electron micrographs (SEM) revealed the presence of smooth surfaces with many granules occurring in clusters. The total amylose content of beach pea starch was 29%, of which 5.9% was complexed by native lipids. The X-ray diffraction pattern of beach pea starch was of the "C" type and the X-ray intensities were much weaker than in other legume starches. The starch exhibited a restricted two stage swelling pattern and moderate amylose leaching. Native granules of beach pea were hydrolyzed readily by a 2.2 N HCl solution (49% in 20 d) and porcine pancreatic α-amylase (35% in 24 h). The gelatinization temperature range was 60-74.2 °C and the enthalpy of gelatinization was 1.6 Cal/g. The results showed that starch chain associations within the amorphous and crystalline domains of beach pea starch are much weaker than those in green pea and grass pea starches.

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LIST OF ABBREVIATIONS

AACC	=	American Association of Cereal Chemists'
ACS	=	American Chemical Society
AML	=	Amylose Leaching
ANOVA	=	Analysis of Variance
AOAC	=	Association of Official Analytical Chemists
BAPN	=	β-Aminopropionitrile
BHA	=	Butylated Hydroxyanisole
BOAA	=	β-N-Oxalylamino-L-alanine
BSA	=	Bovine Serum Albumin
BU	=	Brabender Units
BV	=	Biological Value
BVA	=	Brabender Viso Amylogram
CC	=	Column Chromatography
CM	=	Chloroform-Methanol
CPS	=	Counts Per Second
CRD	=	Complete Randomised Design
db	=	Dry Basis
DP	=	Degree of Polymerization
DTT	=	Dithiothreitol

DSC = Differential Scanning Calorimetry

EA = Emulsion Activity

EPA = Esterified Phenolic Acid

EPC = Epicatechin

ES = Emulsion Stability

FAME = Fatty Acid Methyl Ester

FAO/WHO = Food and Agriculture Organization/World Health Organization

FFA = Free Fatty Acid

FID = Flame Ionization Detector

FPA = Free Phenolic Acid

GC = Gas Chromatography

HPLC = High Performance Liquid Chromatography

HLB = Hydrophillic-Lipophilic Balance

IBD = Iodine Binding Capacity

IBPA = Insoluble Bound Phenolic Acid

IMP = Industrial Membrane Process

IR = Infra-Red

IVPD = In Vitro Protein Digestibility

K = Constant

kDa = Kilo Dalton

KI = Potassium Iodide

kV = Kilo Volt

LH = Low Hydroxyl Group

M = Molarity

MUFA = Monounsaturated Fatty Acid

MWM = Molecular Weight Marker

N = Normality

NA = Not Analyzed

NAS = National Academy of Sciences

ND = Not Detected

NPAGE = Non-Denaturing Polyacrylamide Gel Electrophoresis

NPN = Non-Protein Nitrogen

NR = Not Recorded

NSI = Nitrogen Solubility Index

P = Phosphorus

PA = Phytic Acid

PCA = Perchloric Acid

PDI = Protein Dispersibility Index

PER = Protein Efficiency Ratio

PI = Protein Isolate

PUFA = Polyunsaturated Fatty Acid

PW = Propanol-Water

R	=	Solvent-to-Seed Flour Ratio
r	=	Regression Coefficient
R_f	=	Response Factor
SAS	=	Statistical Analytical System
SEM	=	Scanning Electron Micrograph
SF	=	Swelling Factor
SD	=	Standard Deviation
SDS-PAGE	=	Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis
SHMP	=	Sodium Hexametaphosphate
TCA	=	Trichloro Acetic Acid
TFA	=	Trifluoroacetic Acid
TLC	=	Thin Layer Chromatography
T-RNA	=	Tetrahymena Relative Nutritive Value
UV	=	Ultra Violet
v/v	=	Volume/Volume
w/v	=	Weight/Volume

CHAPTER 1

INTRODUCTION

Beach pea (*Lathyrus maritimus* L.) is a shoreline legume which has recently attracted attention as a potential cold-climate crop. The word "legume" is derived from the latin "legumen" which means seeds harvested in pods. The term food legume is used to cover both the immature pods and seeds as well as mature dry seeds used for human food. Food legumes offer a relatively inexpensive source of valuable protein. The seeds of these plants which are most commonly consumed as food may be easily and economically stored for relatively long periods.

The grain legumes including groundnut and soybean collectively are ranked fifth after wheat, rice, corn and barley in terms of annual world production. World pea production was about 11.7 million metric tons in 1997 (Bi-weekly bulletin of Agriculture and Agri-Food Canada, 1998). Leguminosae (16,000-19,000 species in approximately 750 genera) is the third largest family of flowering plants. However, only about 12 species are widely used in the food industry in the form of unripe pods, immature seeds or mature dry seeds. These include common beans, field peas, chickpeas, cowpeas, green gram, black gram, lentils, and pigeonpeas as well as soybeans and groundnut (Deshpande and Damodaran, 1990). Pea production in Canada is 2.29 million metric tons in 1998 (Bi-weekly bulletin of Agriculture and Agri-Food Canada, 1998).

Food legumes constitute an important part of the human diet in many countries throughout the world, particularly in the tropical and subtropical regions (Koehler *et al.*, 1987) providing an important source of protein, vitamins and minerals (Meiners *et al.*,

1976; Barampama and Simard, 1993). The protein content of legumes is generally about twice that of most cereals. They also serve as a good source of carbohydrates and dietary fibre (Koehler *et al.*, 1987). Legume proteins, considered in isolation, have a somewhat lower nutritional value than most other classes of protein, but they contribute substantially in fulfilling the protein requirements when combined with other proteins in a mixed diet. Current nutritional recommendations suggest that the intake of cereals, legumes, fruits and vegetables be increased for better overall health and management of chronic ailments such as cardiovascular diseases, diabetes and cancer (Scientific Review Committee, 1990). Food legumes are also known to contain several undesirable attributes such as beany, bitter or grassy flavours, and components such as enzyme inhibitors, lectins, phytates, polyphenolics (tannins), flatulence causing sugars, cyanogenic compounds, lathyrogens, esterogens, saponins, antivitamins, and allergens (FAO, 1977; Salunkhe, 1982). While some of these constituents are heat-labile (e.g., trypsin inhibitors, haemagglutinins, saponins and cyanogenic glycosides), others are heat-stable (e.g., polyphenolic tannins and lathyrogens). Of these factors, tannins and lathyrogens are the most important constituents in *Lathyrus* species (Padmanaban, 1980; Roy, 1981).

Tannins complex strongly with proteins, essential amino acids, enzymes, metal ions and cause astringency, depression of food/feed intake, increased excretion of endogenous protein, disturbances of the digestive tract, and toxicity by themselves or by their metabolites (Singleton, 1981). Tannins may also form complexes with carbohydrates and make them unavailable for absorption. Recently, antioxidative, anticarcinogenic,

antimutagenic and antitumorigenic activities of certain plant tannins have also been reported (Thompson, 1993).

Lathyrogens are another important class of antinutritional/toxic constituent of *Lathyrus* species. It has long been known that consumption of seeds of *Lathyrus* species causes "lathyrism" in animals as well as humans (Murti *et al.*, 1964; Haimanot *et al.*, 1990; Spencer *et al.*, 1991; Dwivedi, 1994). Between the 18th and the 20th centuries, outbreaks of lathyrism occurred in certain regions of Europe (e.g. France, Spain), Africa (e.g. Ethiopia) and Asia (e.g. Afghanistan, Bangladesh, India, and Russia) where consumption of seeds of *Lathyrus* species was evidenced. Lathyrism is generally associated with the consumption of seeds of *Lathyrus sativus, Lathyrus cicera, Lathyrus clymenum* and *Lathyrus latifolius.* Two distinct types of lathyrism are recognized: neurolathyrism and osteolathyrism. Neurolathyrism is characterized by nervous disorders such as hyperirritability, weakness, paralysis of leg muscles and convulsions, while osteolathyrism (odoratism) brings pathological changes and deformation of bone structures, particularly those of the spine, ribs, and legs (Murti *et al.*, 1964).

The nutritional quality of a protein depends primarily on its essential amino acid content. In addition to the 20 common amino acids in all living organisms, there are also "uncommon" amino acids which are usually found in both lower and higher plants. Over 600 non-protein amino acids have been classified as secondary metabolites in plant species (Addis and Narayan, 1994). Generally, legumes and other plants synthesize, concentrate and store uncommon amino acids in their seeds and leaves. The site and onset of

biosynthesis, storage, utilization and disappearance of these amino acids varies from species to species (Cheeke, 1985). However, presence of certain uncommon amino acids in some plants is responsible for their survival in competition with seemingly more normal plants in the same environment (Roy and Spencer, 1989). Uncommon amino acids may be responsible for protecting seeds and plant parts from attack by animals, insects, fungi or microorganisms (Janzen, 1969).

Lathyrus seeds contain approximately 27-30% crude protein; this level is somewhat higher than that of most other legumes. Even though the seed proteins may be of high quality, antinutrients/toxins present can render them unsuitable for consumption. Roy (1981) reported the presence of some toxic amino acids in the storage tissues of *Lathyrus* legumes. Spencer *et al.* (1986) confirmed that β-N-oxalylamino-L-alanine (BOAA) was the compound responsible for neurolathyrism in humans. Furthermore, Padmanaban (1980) has shown that β-aminopropionitrile (BAPN) and β-(γ-glutamyl)aminopropionitrile of *Lathyrus* species are responsible for osteolathyrism in experimental animals.

As noted earlier, many unusual amino acids exist in nature. A number of these have chemical structures similar to amino acids regularly found in proteins. These free amino acids are important precursors or intermediates in metabolism, but they can express their toxicity by inhibiting the activities of some proteolytic enzymes in biological systems (Roy, 1981). For foods containing these amino acids, their consumption by animals, including humans, poses a potential health risk.

Lathyrus sativus is a well-known *Lathyrus* species, sometimes referred to as grass pea or chickling vetch. Grass pea is a relatively productive crop compared to other pulses in the regions characterized by poor soil and adverse climatic conditions. Its yield normally ranges from 1000 to 2000 kg/hectare in the United States while it is approximately 5200 kg/hectare in Canada; its crude protein content ranges from 27 to 30% of the seed weight (National Academy of Sciences (NAS), 1972; Duke *et al.*, 1981). Both the yield per unit area and crude protein content of grass pea are somewhat higher than those of most other legumes. Recently, grass pea has been introduced to the Canadian Prairies since it was considered a good feed source for animals. A selection and breeding program has been initiated at the Agriculture and Agri-Food Canada Research Station in Manitoba in order to improve the quality of fodder. Research carried out at this research unit revealed that grass pea contains 0.22 to 7.20 g BOAA/kg, and condensed tannins of up to 4.38 g/kg seed (Deshpande and Campbell, 1992a). Therefore, grass pea seeds may be used as a small portion of the feed or be detoxified before use as a human food ingredient.

In contrast to grass pea, beach pea (*Lathyrus maritimus* L.) is relatively unknown to plant breeders, farmers, and consumers. Beach pea grows along the shorelines of Arctic and Subarctic regions from Greenland to Siberia and Japan (Fernald, 1950) and is also found along the shores of Newfoundland, Nova Scotia, Quebec as well as Ontario (Scoggan, 1950; Hitchcock, 1952; Lamourex and Grandtner, 1977). It is a relatively productive pulse crop in regions characterized by poor soil and adverse climatic conditions. Preliminary trials have shown promise in both green house and field conditions at the

Atlantic Cool Climate Crop Research Centre of Agriculture and Agri-Food Canada, St. John's Newfoundland. Therefore, beach pea is an excellent candidate as a potential cold-climate crop, already under trial at the above research centre as well as at the University of Vermont, USA. The vegetative parts of beach pea are sometimes used as a fodder for cattle (Bal and Barimah-Asare, 1992). However, to the best of our knowledge beach pea seeds have not been used for feed or food purposes except for occasional use as an additive to regular peas during shortage of food by stranded sailors (Fernald and Kinsey, 1958; Erichson-Brown, 1979). The high amounts of tannins in beach pea are located in the seed coats and lathyrogens are present in their cotyledons. However, no information is available on the utilization of beach pea seeds, their nutritional quality and content of antinutrients such as tannins and lathyrogens. Therefore, the major nutrients and antinutritional factors of beach pea seeds need to be studied in order to evaluate their potential use in feed and food formulations.

Development of processed legumes, using traditional methods, in the developing countries may have a positive impact on reducing many of their endogenous antinutritional factors. These traditional methods include milling, dehulling, soaking, germination, fermentation and cooking (Mosse and Pernollet, 1982). However, these techniques have not been tried for processing of beach pea.

1.1 Hypothesis and objectives of the present study

Hypothesis for the present study was that there will not be any difference in the content of nutritional parameters and antinutritional and/or toxic components in beach pea and grass pea as both of them belong to the same genus *Lathyrus*. Another hypothesis was that there will be a difference in the presence of nutritional and antinutritional constituents of beach pea and green pea since these two legumes differ in their genus as well as species. For testing these hypothesis the following objectives were defined.

The main objectives of this study were: (1) To identify and quantify the chemical and biochemical components of beach pea. (2) To evaluate nutritional quality of beach pea in comparison with those of green pea and grass pea. (3) To use different detoxification methods (dehulling, and methanol-ammonia-water extraction) for the removal of antinutritional and/or toxic compounds from beach pea, green pea and grass pea. (4) To develop a simple method to reduce the content of seed coat polyphenolics (tannins) in order to obtain a higher protein recovery from the seeds/meals. (5) To extract maximum amounts of polyphenolics using different solvent systems from beach pea seeds and hulls and study the antioxidant activity of polyphenolic fractions. (6) To evaluate the distribution of protein fractions in anatomical parts of beach pea, green pea and grass pea seeds and study their surface characteristics. (7) To prepare protein isolates using sodium hydroxide or sodium hexametaphosphate and study their effects on the functional properties of protein isolates. (8) To characterize starch from beach pea and compare it with the starches isolated from green pea and grass pea.

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of *Lathyrus* species

A geographical study of the North American Pacific strand and dune flora has shown that beach pea occurs at various places southward from Alaska to California (Cooper, 1936). Beach pea is physiognomically classified under beach meadow group (Talbot and Talbot, 1994), and is a relatively unknown *Lathyrus* species.

The biological classification of the beach pea plant in comparison with green pea and grass pea is given below (Table 2.1). Beach pea and grass pea are classified under the genus *Lathyrus* and green pea comes under the genus *Pisum*. Beach pea belongs to herbs, perennial, often climbing by means of tendrils, having several stems, with procumbent, striate, 2.5 to 3.5 mm in diameter; from 0.2 to 1 m or more in length. Leaves of beach pea are slightly fleshy, pinnate, ending in a simple or branched tendril. Flowers of beach pea are papilionate, 2-2.5 cm long and purple in colour (Figure 2.1A). Pods of beach pea are compressed, oblong, continuous within, 2-valved, dehiscent, more or less spherical, 4-4.5 mm in diameter, smooth, seeds 6-7 numbers, dark green in colour (Figure 2.1B premature pods; 2.1C mature pods). *Lathyrus* genus is widespread in the temperate regions of both hemispheres and is grown wild or cultivated (e.g. grass pea). *Lathyrus* species are cultivated mainly in India and, to a limited extent, in the Mediterranean and South American regions. *Lathyrus sativus* legume is known by a variety of names (Chichkling vetch, Khesari, Kesari, Grass pea, *Lathyrus* pea, Indian vetch, Teori, Teora, Lak, Lakh, Lakhori, Lang, Lanka, Chattrimattri, Latri matar, etc.) in

Table 2.1 Biological classification of beach pea, green pea and grass pea[1]

Classification	Beach pea	Green pea	Grass pea
Division	Magnoliophyta	Magnoliophyta	Magnoliophyta
Class	Spermatophyta	Spermatophyta	Spermatophyta
Subclass	Dicotyledons	Dicotyledons	Dicotyledons
Order	Rosales	Rosales	Rosales
Super order	Calyciflorae	Calyciflorae	Calyciflorae
Family	Fabaceae	Fabaceae	Fabaceae
Subfamily	Papilionoideae	Papilionoideae	Papilionoideae
Genus	*Lathyrus*	*Pisum*	*Lathyrus*
Species	*Lathyrus maritimus* L.	*Pisum sativum* L.	*Lathyrus sativus* L.

[1]Adapted from Allen and Allen (1981).

Figure 2.1 **Beach pea** [A: flowering stage; B: premature seed and pod development stage; C: mature seed and pod development stage]

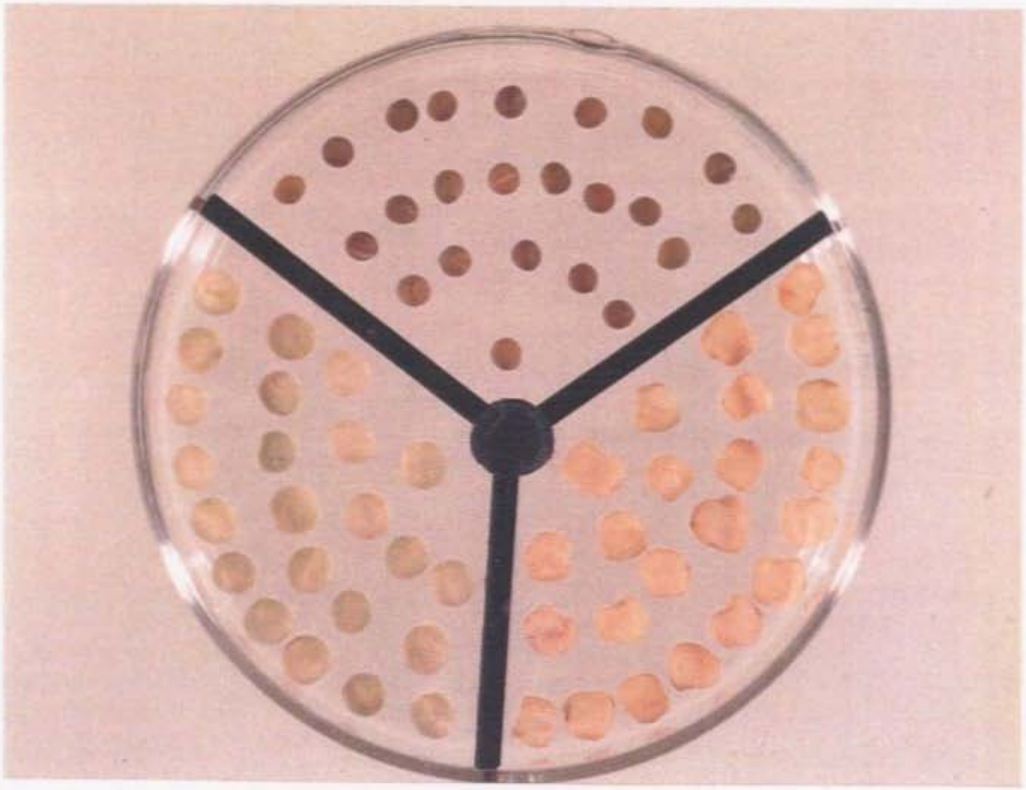
different parts of the world (Bhat *et al.*, 1984). *Lathyrus* is thought to have originated from West Asia and Southern Europe.

Lathyrus sativus belongs to the Fabaceae family. The flowers of *Lathyrus* species are dull white, blue, pink or purple and are classified under papilionoideae. Pods are flat, dorsally broader with two ridges and short, 3-5 cm in length. Each pod contains 6-7 seeds. Seeds are rhomboid or triangular, dull white, grey brown and variously mottled (Figure 2.2). They are well adapted to rather dry areas, yet tolerate water logging, grow well on poor land, and are resistant to cool weather. *Lathyrus* occur in meadows, along seashores, lake and stream banks, roadsides, and in thickets, fields, and waste areas. Historical evidence has shown that *Lathyrus sativus* was considered one of the most economical pulses for fodder and green manure in rice fields during the cold winters in India and Java (Fernald, 1950).

2.2 Importance of *Lathyrus* species

Biological nitrogen fixation, particularly of the symbiotic type, plays a crucial ecological role in maintaining adequate nitrogen resources in the plant world. Quite distinctive in this respect are the numerous members of the giant family Leguminosae which can thrive without any fixed nitrogen or with a minimal supply of nutrients from the soil. Specific bacteria (*Rhizobium* species) which invade the root hairs and establish a mutually beneficial association inside their cortical root swellings or nodules convert the free air nitrogen into fixed nitrogen for eventual plant protein assimilation and storage.

Figure 2.2 Variation in size, shape and colour of legume seeds: (A) Beach pea (top), (B) Grass pea (bottom right), and (C) Green pea (bottom left).



Leguminous plants develop an efficient means for meeting their nitrogen requirements and thereby have an evolutionary advantage over most other living organisms.

The roots of beach pea plant are nodulated by *Rhizobia* under natural growth conditions. The nodules of *Lathyrus* are of the indeterminate type resembling *Pisum*, *Vicia* and *Lens* species, and their symbionts are known to be related (Wilson, 1939). Some species have horizontal root systems with potential in erosion control (Allen and Allen, 1981).

Lathyrus sativus is an essential staple food crop in North-Central India, Bangladesh, China and Ethiopia (Spencer *et al.*, 1986). Unlike other legumes, grass pea thrives very well under adverse climatic conditions, and requires very little, if any, management input and attention during its growth cycle. In the western world, renewed interest in the cultivation of grass pea is evident mainly due to its desirable agronomical characteristics, especially drought resistance, development of very low neurotoxin (β-N-oxalylamino-L-alanine,)-containing genotypes, and as an animal feed (Low *et al.*, 1990). Grass pea is also an excellent source of fodder. In addition, its deep tap root system and nitrogen-fixing ability makes it an ideal choice in sustainable agriculture.

As compared to grass pea, beach pea has a very low BOAA content, but a higher concentration of crude protein and other nutrients. Beach pea is not known to plant breeders, farmers, and consumers. If beach pea cultivation starts on agricultural land, then it may be in a position to compete with other *Lathyrus* legumes in terms of yield, nutritive value and nitrogen fixation for improving soil fertility.

Legume seeds, like other plant seeds, store nutrients for use during the germination process. The seeds contain materials such as proteins, carbohydrates, starch, lipids, growth factors (hormones), enzymes, and minerals required by the embryo for its initial development. They also contain several chemical compounds which are essential to protect the seed during adverse conditions. *Lathyrus* seeds, similar to other legume seeds, contain both nutrients and antinutrients. The latter compounds (Table 2.2) may be present in minute quantities, but could potentially limit the use of beach pea protein meals unless they undergo prior treatment and/or removal.

2.3 Antinutrients

2.3.1 Lathyrogenic compounds

Plants belonging to the *Lathyrus* species are known to be sometimes toxic to man and animal due to the presence of large amounts of lathyrogenic compounds, especially under severe drought conditions. Both the vegetative parts and seeds of *Lathyrus* species contain unusual ninhydrin-reactive toxic amino acids; these are nitrogenous secondary metabolites responsible for osteolathyrism and neurolathyrism. The toxic γ-glutamyl derivative of β-aminopropionitrile (BAPN) and β-N-oxalylamino-L-alanine (BOAA) are present in *Lathyrus* species (Bell, 1962; Murti *et al.*, 1964; Rao *et al.*, 1964). It has been shown that β-(γ-glutamyl)aminopropionitrile is mostly responsible for the inhibition of synthesis of desmosine and isodesmosine which results in failure of cross-linking between the polypeptide chains in elastin and presumably in collagen, and ultimately osteolathyrism (O'Dell *et al.*, 1966). Bell and O'Donovan (1966) and Roy and Narasinga

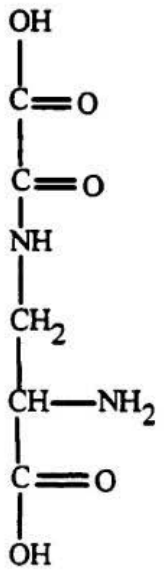
Table 2.2 Antinutritional components of some selected pea seeds[1]

Parameter	Grass pea	Green pea	Chickpea
α-Amylase inhibitor (Units/g)	3.6-91.4	14-80	4-6
Trypsin inhibitor (Units/mg)	133.3-173.9	NR	NR
Tannins (mg/100g)	0-438	500-1050	78-272
Phytic acid (%)	NR	0.89	0.28
Raffinose (%)	0.80[2]	0.29[3]	1.0
Stachyose (%)	1.20[2]	0.70[3]	2.5
Verbascose (%)	2.35[2]	0.82[3]	4.2
BOAA[4] (mg/100g)	120-1100	NR	NR

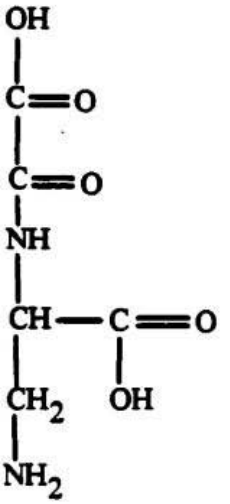
[1]Adapted from Salunkhe and Kadam (1989), [2]Naczk *et al.* (1992a), [3]Phillips and Abbey (1989).

[4]BOAA, β-N-oxalylamino-L-alanine; NR, Not reported.

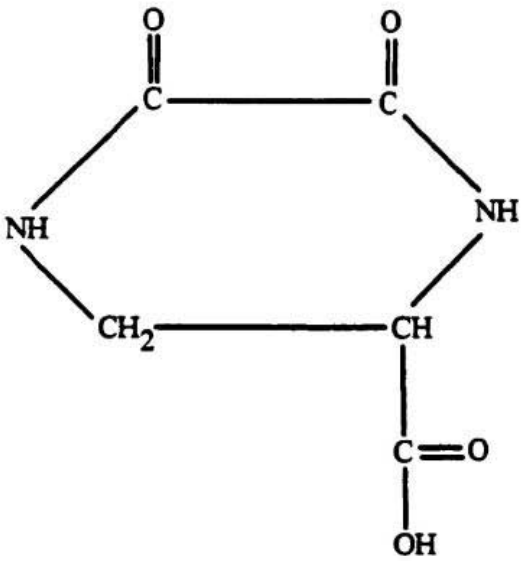
Rao (1968) have shown that BOAA (β-isomer) exists naturally in an isomeric mixture with the α-isomer. This formation could be due to the migration of oxalyl moiety from the β-amino to the α-amino group of BOAA (Figure 2.3). β-isomer is the main neurotoxic component of *Lathyrus sativus* seed, while α-isomer has been shown to be less toxic to experimental animals (Padmajaprasad *et al.*, 1997). The proportion of α- to β-isomers in *Lathyrus sativus* seeds is approximately 5:95 (Roy, 1981). There are different nomenclatures suggested for the β-isomer of the neurotoxin from *Lathyrus sativus*. These are β-N-oxalylamino-L-alanine (BOAA) (Nagarajan *et al.*, 1965), β-N-oxalylamino-α,β-diaminopropionic acid (Ox-dapro or ODAP) (Adiga *et al.*, 1963), and L-3-oxalylamino-2-aminopropionic acid (OAP) (Mehta *et al.*, 1976). The α-isomer of the neurotoxin has been referred to as the α-isomer of N-oxalylamino-α, β-diaminopropionic acid (Bell and O'Donovan, 1966; Roy and Narasinga Rao, 1968), and as L-2-oxalylamino-3-aminopropionic acid (Wu *et al.*, 1976). The α-isomer of the neurotoxin has been shown to be produced in the plant by a non-toxic rearrangement (Wu *et al.*, 1976). Rao *et al.* (1964) have reported that β-form of N-oxalylamino-L-alanine (BOAA) is responsible for the neurotoxicity (Neurolathyrism) in humans. Neurolathyrism is characterized by such symptoms as muscular rigidity, weakness and paralysis of the leg muscles and death in extreme cases upon excessive consumption of *Lathyrus* seeds especially those grown under drought conditions (Shourie, 1945). In most recorded cases, the onset of the disease is sudden. It has generally been concluded from the nature of the sysmptoms that the disease primarily affects the central nervous system (Rao *et al.*,



β-N-oxalylamino-L-alanine
(β-isomer)



α-N-oxalylamino-L-alanine
(α-isomer)



Possible intermediate

Figure 2.3 Structure of α- and β-isomers of N-oxalylamino-L-alanine and the hypothetical cyclic intermediate.

1969).

The content of these lathyrogenic compounds in *Lathyrus* species depends on cultivar, geographical area and climatic conditions, with cultivar having the most important effect. The content of β-N-oxalylamino-L-alanine in *Lathyrus sativus* seeds ranges from 0.22 to 11.00 g/kg (Radha Ayyagari *et al.*, 1989; Deshpande and Campbell, 1992b).

2.3.1.1 Biosynthesis

Naturally-occurring neurotoxic amino acids (Neurolathyrogens) such as β-cyanoalanine, β-N-oxalylamino-L-alanine and osteolathyrogens, β-aminopropionitrile, and β-(γ-glutamyl)aminopropionitrile are biosynthetically inter-related (Figure 2.4). The biosynthetic pathways of some toxic amino acids and nitriles in *Lathyrus* plants have been postulated by Murti *et al.* (1964) and Sarma and Padmanaban (1969). Nigam and Ressler (1964) have shown that labelled serine incorporation into the dipeptide [β-(γ-glutamyl)aminopropionitrile] in *Vicia sativa* and *Lathyrus* species is possible only in the presence of cyanide. The incorporation of cyanide into serine is catalyzed by an enzyme prepared by *Vicia sativa* and also from *Lathyrus* species (Floss *et al.*, 1965). It has been suggested that β-cyanoalanine may be formed through non-specific enzyme reactions catalyzed by cysteine sulphydrase. Ressler *et al.* (1961) proposed that β-cyanoalanine itself may be derived from asparagine by a hypothetical enzyme, "amide dehyrase", although the evidence obtained so far has confirmed only the reverse pathway (Figure

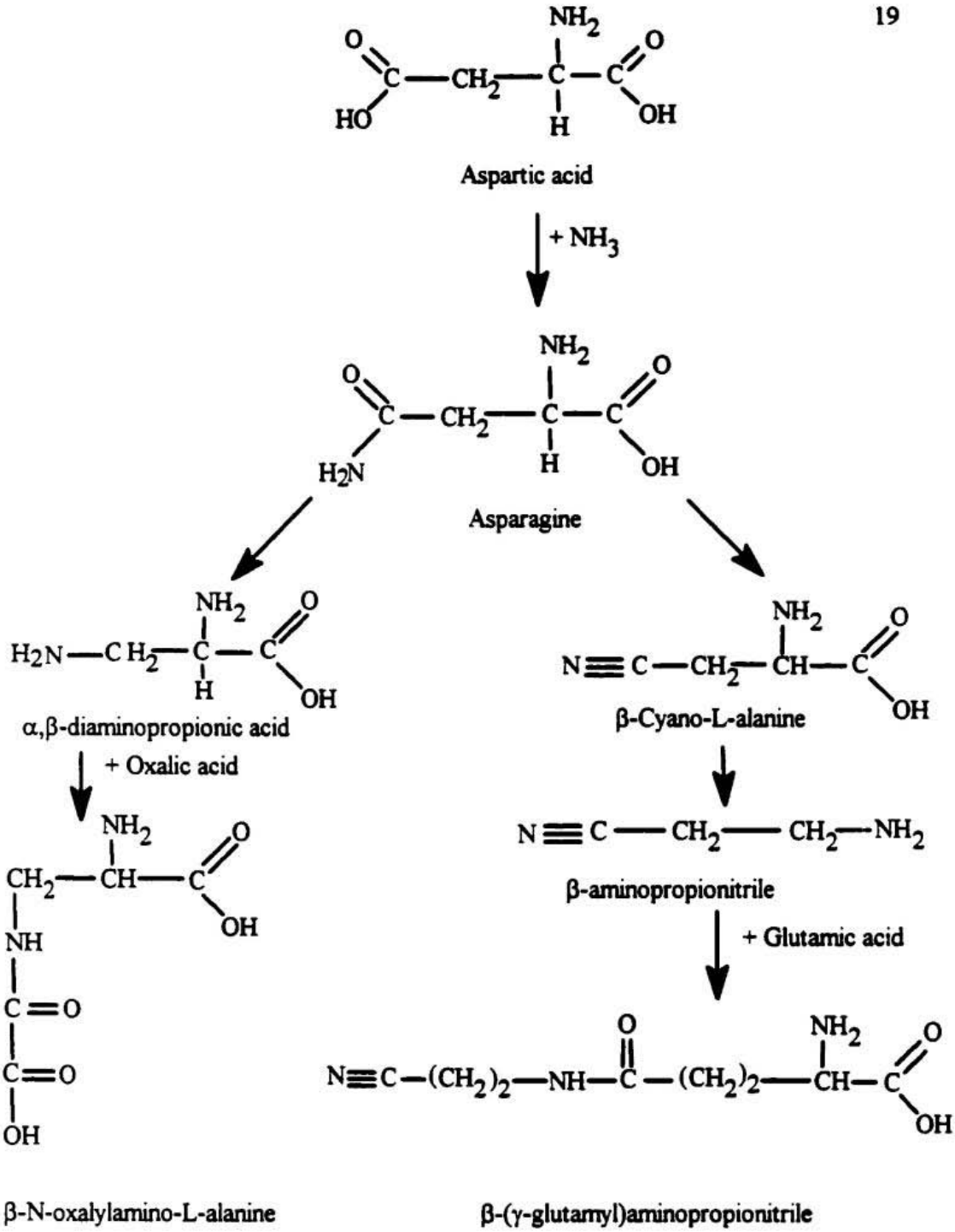
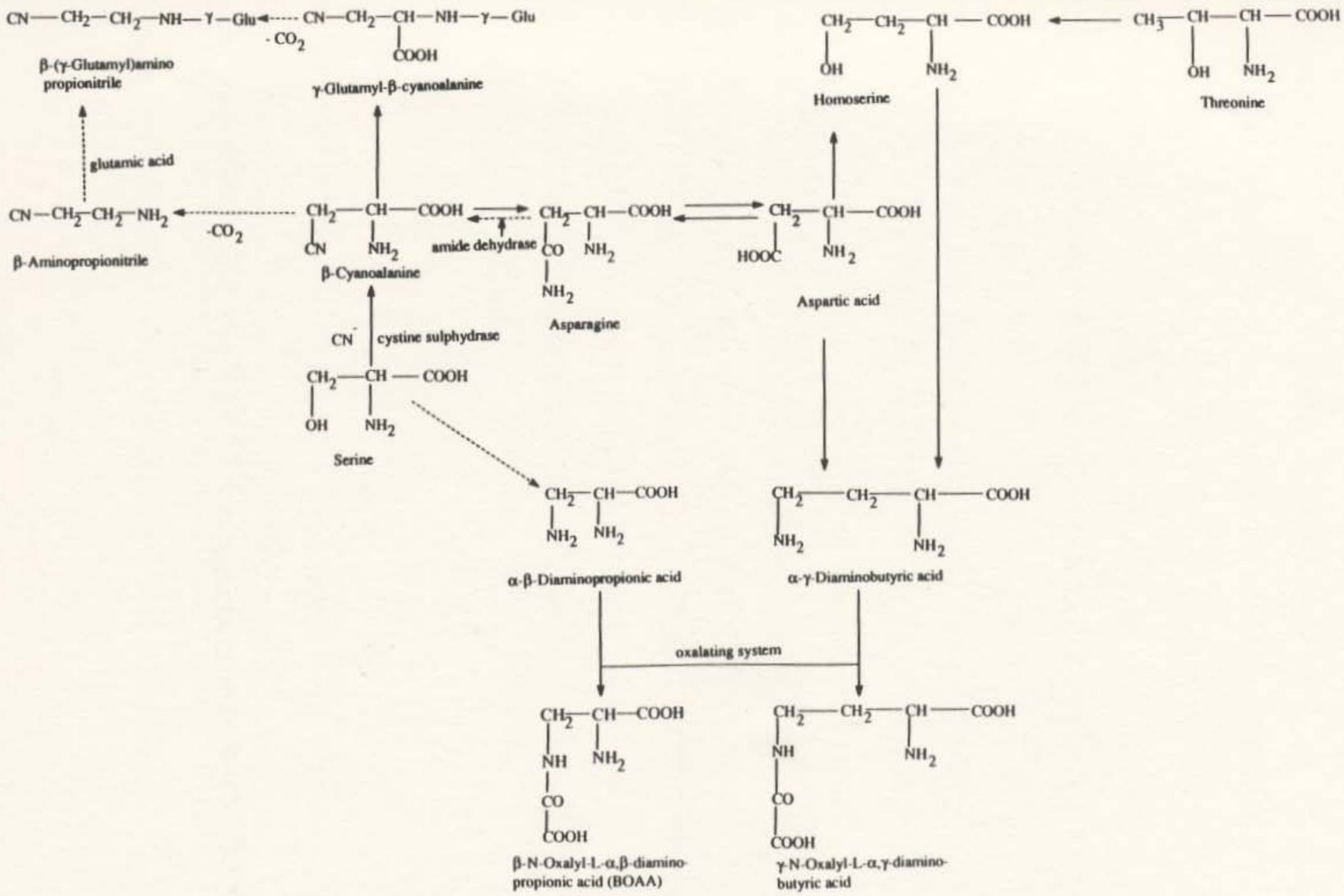


Figure 2.4 Possible pathways for biosynthesis of β-N-oxalylamino-L-alanine and β-(γ-glutamyl)aminopropionitrile.

2.5). Ressler *et al.* (1961) suggested that α, β-diaminobutyric acid may be derived from β-cyanoalanine by another hypothetical enzyme, "nitrile reductase". The pathways from asparagine to β-cyano-L-alanine and the formation of α,γ-diaminobutyric acid from β-cyano-L-alanine were suggested by Ressler *et al.* (1961); subsequently this was supported by autoradiographic studies (Tschiersch, 1964). It is resonable to assume that α, β-diaminopropionic acid may also be formed from the same source, the $-CONH_2$ group being replaced by an $-NH_2$ by analogy with the well-known Hofmann reaction. The subsequent step of acylation with oxalic acid is an acceptable biochemical process (Figure 2.5; Murti *et al.*, 1964). Kuo *et al.* (1994) reported that the incorporation of [^{14}C]-label from the precursor β-(isoxazolin-5-one-2-yl)-L-alanine into β-BOAA occurs in intact fruits as well as in separated pericarp and immature seeds, in both high and low toxic varieties of *Lathyrus sativus*. This incorporation gradually decreases in the pericarp while increasing in the maturing seeds. It was observed that L-(^{3}H) homoserine and DL-(1-^{14}C) aspartic acid are efficiently incorporated into the α, γ-diaminobutyric acid from aspartic acid (Nigam and Ressler, 1966). Ikegami *et al.* (1993) reported that cysteine synthase which is present in *Lathyrus sativus* catalyses the formation of β-(isoxazolin-5-one-2-yl)-L-alanine (BIA), the biosynthetic precursor of the neurotoxin BOAA and some other heterocyclic β-substituted alanines from *O*-acetyl-L-serine (OAS) as an additional catalytic activity.

Figure 2.5 Biosynthesis of lathyrogens [solid arrows indicate experimentally confirmed pathways; dashed arrows indicate lack of experimental proof]. (adapted from Roy and Spencer, 1989).


β-(γ-Glutamyl)amino propionitrile
γ-Glutamyl-β-cyanoalanine
Homoserine
Threonine
glutamic acid
β-Aminopropionitrile
β-Cyanoalanine
amide dehydrase
Asparagine
Aspartic acid
cystine sulphydrase
Serine
α-β-Diaminopropionic acid
α-γ-Diaminobutyric acid
oxalating system
β-N-Oxalyl-L-α,β-diamino-propionic acid (BOAA)
γ-N-Oxalyl-L-α,γ-diamino-butyric acid

2.3.1.2. Chemistry

β-N-Oxalyl-L-α,β-diaminopropionic acid is highly acidic in character and forms oxalic acid and diaminopropionic acid upon acid hydrolysis. This compound has a specific rotation of -36.9^0 and has apparent pK values of 1.95, 2.95 and 9.25, corresponding to its two carboxyl groups and one amino group, respectively. This compound also has a melting point of 236-237 ^{0}C (Rao *et al.*, 1964). Rao *et al.* (1964) reported that the crystallized compound hydrolized with 4 N HCl and then recrystallized in ether, the crystalline pellet was L-α,β-diaminopropionic acid and the ether-soluble portion was decolourized with acidified permanganate, but did not reduce Tollen's reagent, formed an insoluble calcium salt, and produced a red colour on heating with indole and sulphuric acid. A small quantity of this compound, when melted with diphenylamine over a free flame, cooled, and dissolved in alcohol, gave a blue colour, which is a specific test for the presence of oxalic acid (Huntress and Mulliken, 1941). The isolated L-α,β-diaminopropionic acid and oxalic acid, when compared with authentic compounds showed the same R_f values.

2.3.1.3 Biological detection

There are several biological methods that can indirectly determine the content of lathyrogens in *Lathyrus* species. These methods involve feeding or administration of lathyrogens to experimental animals followed by measuring the time required for manifestation of neurological disorders. Neurotoxic amino acids, such as β-cyanoalanine,

and α,γ-diaminobutyric acid, were administered to male weaning rats at different concentrations. The rats showed typical neurological symptoms, such as convulsions, tremors, weakness of the hind legs, among others (Ressler *et al.*, 1961; Ressler, 1962). Similar results were obtained when these neurotoxic amino acids were administered to chicks (Adiga *et al.*, 1963; Rao and Sarma, 1966; Padmanaban, 1980). Moslehuddin *et al.* (1987) and Rotter *et al.* (1991) reported that highly toxic diets from *Lathyrus* seeds when fed to chicks caused a decrease in weight, and sometimes death due to the high concentration of lathyrogens.

2.3.1.4 Chemical analyses

Extraction of lathyrogenic compounds from defatted pea seeds may be achived using 60 or 70% ethanol with shaking for 6 h (Rao *et al.*, 1964; Rao, 1978; Briggs *et al.*, 1983; Deshpande and Campbell, 1992b). Lathyrogenic compounds may also be extracted by mechanical shaking in water for 12 h (Geda *et al.*, 1993) or in 6% ice-cold perchloric acid (Capony and Demaille, 1983). Various chemical procedures have been used for quantification of total osteolathyrogens as well as neurolathyrogens of plants. For naturally-occuring lathyrogens, which are non-protein amino acids or their derivatives in *Lathyrus* species, ninhydrin reaction can be used to detect and quantitate them. Ninhydrin reacts with the primary or secondary amino groups, and the absorbance of the complex so formed is measured spectrophotometrically (Mathews and van Holde, 1990). β-aminopropionitrile (osteolathyrogen) gives a characteristic green colour with

ninhydrin, (Garbutt and Strong, 1957). The neurotoxic amino acids, β-cyanoalanine, also gives a green colour with ninhydrin (Padmanaban, 1980) while BOAA and its α-isomer produce a violet and greyish violet complex (Roy and Spencer, 1989); the adducts formed can be subjected to a quantitative assay for lathyrogens. Neurotoxic lathyrogens may also be quantified spectrophotometrically using *o*-phthalaldehyde as an assay reagent (Rao, 1978; Briggs *et al.*, 1983; Tekle-Haimanot *et al.*, 1993).

Chromatographic separation of lathyrogenic compounds by column chromatography (Rao *et al.*, 1964), TLC (Addis and Narayan, 1994), and high pressure liquid chromatography (HPLC) with derivatization (Geda *et al.*, 1993; Khan *et al.*, 1993, 1994) has been established. Lathyrogenic compounds from different species of *Lathyrus* may also be separated, identified and quantified by high voltage electrophoresis (Addis and Narayan, 1994).

2.3.1.5 Mechanisms of toxicity

Lathyrogenic compounds have various actions in different biological systems. Early theories on the mode of action of osteolathyrogens were based on monoamine oxidase inhibition, chelation, and antinicotinamide effect (Levene, 1963). For example, L-α,γ-diaminobutyric acid causes chronic ammonia toxicity in adult rats by inhibiting ornithine trans-carbambamylase activity in liver which results in reduced urea synthesis (Roy and Spencer, 1989). β-N-Oxalylamino-L-alanine has been reported to significantly increase the ammonia concentration of blood and brain in young rats, resulting in accumulation of glutamine in the brain. Ammonia production may be a consequence of increased

catabolism of proteins by the neurotoxin (Cheema *et al.*, 1971). Oseteolathyrogens such as β-(γ-glutamyl)aminopropionitrile or β-aminopropionitrile (BAPN) interfere with the cross-linking of collagen and elastin by irreversible inhibition of the enzyme necessary for the development of connective tissues, thereby loosing their structural integrity (Cheeke, 1985). It was also proposed that the collagen effect could be due to alterations in the desmosine and isodesmosine. However, it was found that concentrations greater than those required to affect the collagen were necessary to induce changes in mucopolysaccharides of connective tissues (Rosmus *et al.*, 1966).

β-N-Oxalylamino-L-alanine, β-cyanoalanine, and α,γ-diaminobutyric acid are neurotoxic amino acids in *Lathyrus sativus* species and are involved in neurolathyrism. Spencer *et al.* (1986) compared clinical manifestations of neurolathyrism in humans to those of animals fed a nutritionally balanced diet containing seed, a seed extract and purified neurolathyrogen such as BOAA. Human symptoms included weakness and stiffness in legs, muscle cramp, heaviness, numbness, itching of the back, frequent urination with hesitation, abdominal cramp, diarrhoea, excessive thirst, sleepiness, short-term memory loss and excessive dreaming. Bridges *et al.* (1991) reported that neurolathyrogens can cross the blood-brain barrier, accumulate in the central nervous system following intravenous administration, induce severe convulsions and cause neurological damage to the retina and spinal cord. Monkeys which were fed the same diet demonstrated signs of tremor, periodic jerks, a mild-to-moderate increase in the tone of leg muscles, and striking hind limb extensor posturing. These results show that

primates fed lathyrogens appear to acquire corticospinal disfunction similar to that appearing in other animals after consuming *Lathyrus sativus* (Spencer *et al.*, 1986). β-Cyano-L-alanine, a potent inhibitor of rat liver cystathionase, may possibly be linked to cystathionurea in rats after its ingestion (Pfeffer and Ressler, 1967). This compound inhibits aspartate decarboxylase (Tate and Meister, 1969), asparaginase, and glutaminase of certain prokaryotes. Vivanco *et al.* (1966) reported that administration of α, γ-diaminobutyric acid to rats, orally, intraperitoneally, or by stomach tube, produces characteristic convulsive disorders. Appropriate amounts of the toxin and increased levels of γ-aminobutyric acid and glutamate were detected in the brain at the time neurological signs were observed (Vivanco *et al.*, 1966; O' Neal *et al.*, 1968).

2.3.1.6 Biological significance

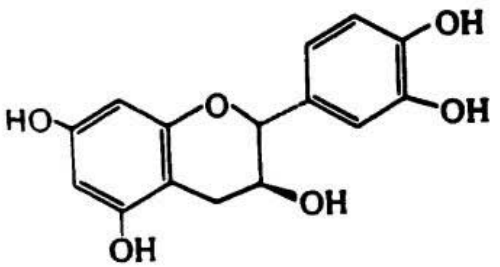
Non-protein amino acids (such as neurotoxic and osteotoxic amino acids) can be potent toxicants able to benefit the plant by affording protection against predation and disease and by improving its competition for habitat resources with other plants (Bell, 1971). Simola (1967) reported that the presence of an uncommon amino acid in the genus *Lathyrus* may prevent hybridization.

2.3.2 Tannins and phenolic acids

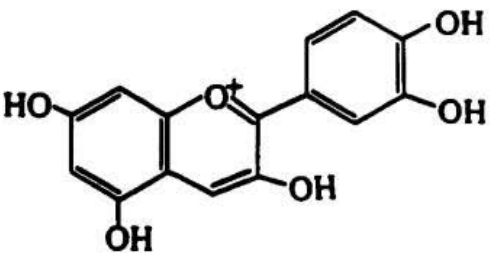
Tannins are complex polyphenolic compounds present in a wide variety of plant materials. Polyphenolic compounds are classified as phenolic acids and derivatives, tannins, and flavonoids. The flavonoids are subclassified into anthocyanins, flavones,

flavonols, and related substances. Anthocyanin pigments are known to be responsible for nearly all the pink, scarlet, red, mauve, violet, and blue colours of flowers, leaves, fruits, fruit juices, and wines, but nonetheless flavones and flavonols do make a significant contribution, either as yellow pigments or as copigments of anthocyanins (Harborne, 1965). According to Horowitz (1964) the taste of flavanones has a definite relation to their chemical structures. Astringency is closely connected to the tanning reactions of condensed tannins (Rossi and Singleton, 1966a, b) and their ability to inhibit enzymes. Based on their structural features, tannins are classified either as hydrolyzable tannins, which generally have gallic acid as their monomers, or condensed tannins such as catechin, gallocatechin, leucocyanidin, leucodelphinidin, and dimeric procyanidins (Naczk *et al.*, 1992b) (Figure 2.6). The condensed tannins are dimers, oligomers and polymers of polyhydroxyflavan-3-ol monomer units linked by 4 → 6 or 4 → 8 bonds. During the maturation of seeds/fruits, increased condensation of phenolics (condensed tannins) (Goldstein and Swain, 1963) is accompanied by a decrease in the total phenolics and astringencey (Craft, 1961). This decrease in the content of phenolics during maturation may be due to polymerization of existing polyphenolic compounds to high molecular weight insoluble polymers such as lignins (Kadam *et al.*, 1982).

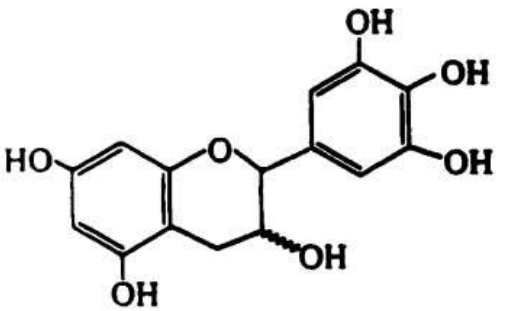
Sosulski and Dabrowski (1984) fractionated the phenolic constituents of defatted flours and hulls of ten legume species and classified them into free acids, soluble esters, and insoluble-bound residues. They reported that the total content of free phenolic acids (trans-ferulic, trans-*p*-coumaric, and syringic acids) in legume flours varied from 1.8 to



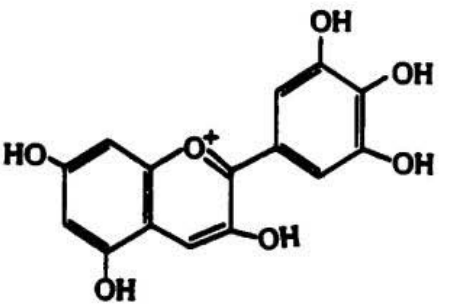
Catechin



Cyanidin



Gallocatechin



Delphinidin

Figure 2.6 Structures of monomers of polyphenols of pea seed meals.

16.3 mg/100g of sample (Figure 2.7).

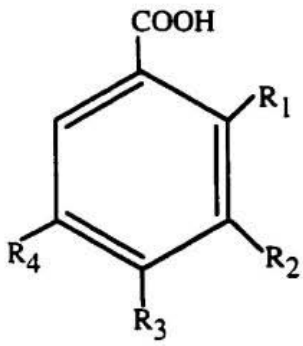
Polyphenolic compounds in legumes have received considerable attention largely as a result of their possible influence on the nutritional and aesthetic qualities of foods, biochemical and physiological functions, and their pharmacological and toxicological implications (Jadhav *et al.*, 1989).

2.3.2.1 Chemistry and biogenesis

The term "tannin" was historically used to describe a chemically heterogeneous group of compounds which precipitated proteins. Molecular weights of tannins ranged between 500 and 3000 Da and, besides giving the usual phenolic reactions, had special properties such as the ability to precipitate alkaloids, gelatin and other proteins (Swain and Bate-Smith, 1962). The condensed tannins, or proanthocyanidins (Hagerman and Butler, 1994), are flavanol-based compounds. Condensed tannins react in alcoholic solutions of strong mineral acids to release the corresponding anthocyanidin, with a characteristic colour. Structural diversity of the proanthocyanidins is a consequence of the substitution patterns and stereochemistry of the flavanol subunits. Structural complexity also results from the diversity of positions for interflavan bond formation, and from the stereochemical variation in the interflavan bond.

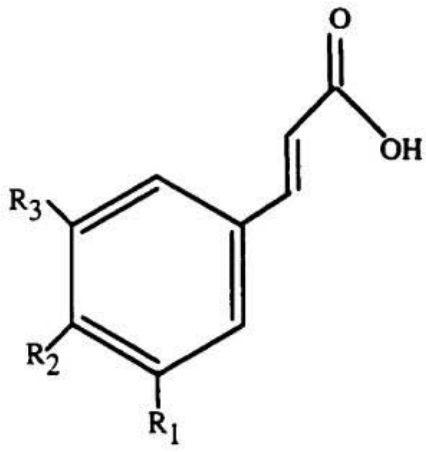
The hydrolyzable tannins may be hydrolyzed to a carbohydrate, usually glucose, and a phenolic, either gallic acid or its dimer ellagic acid. On the other hand, condensed tannins mainly ellagitannins, yields several derivatives of gallic acid besides gallic acid

Benzoic acid derivatives



R_1	R_2	R_3	R_4	
H	H	OH	H	*p*-Hyroxybenzoic acid
H	OH	OH	OH	Gallic acid
OH	H	H	H	Salicylic acid
H	H	OH	OH	Protocatechuic acid
H	OCH_3	OH	OCH_3	Syringic acid

Cinnamic acid derivatives



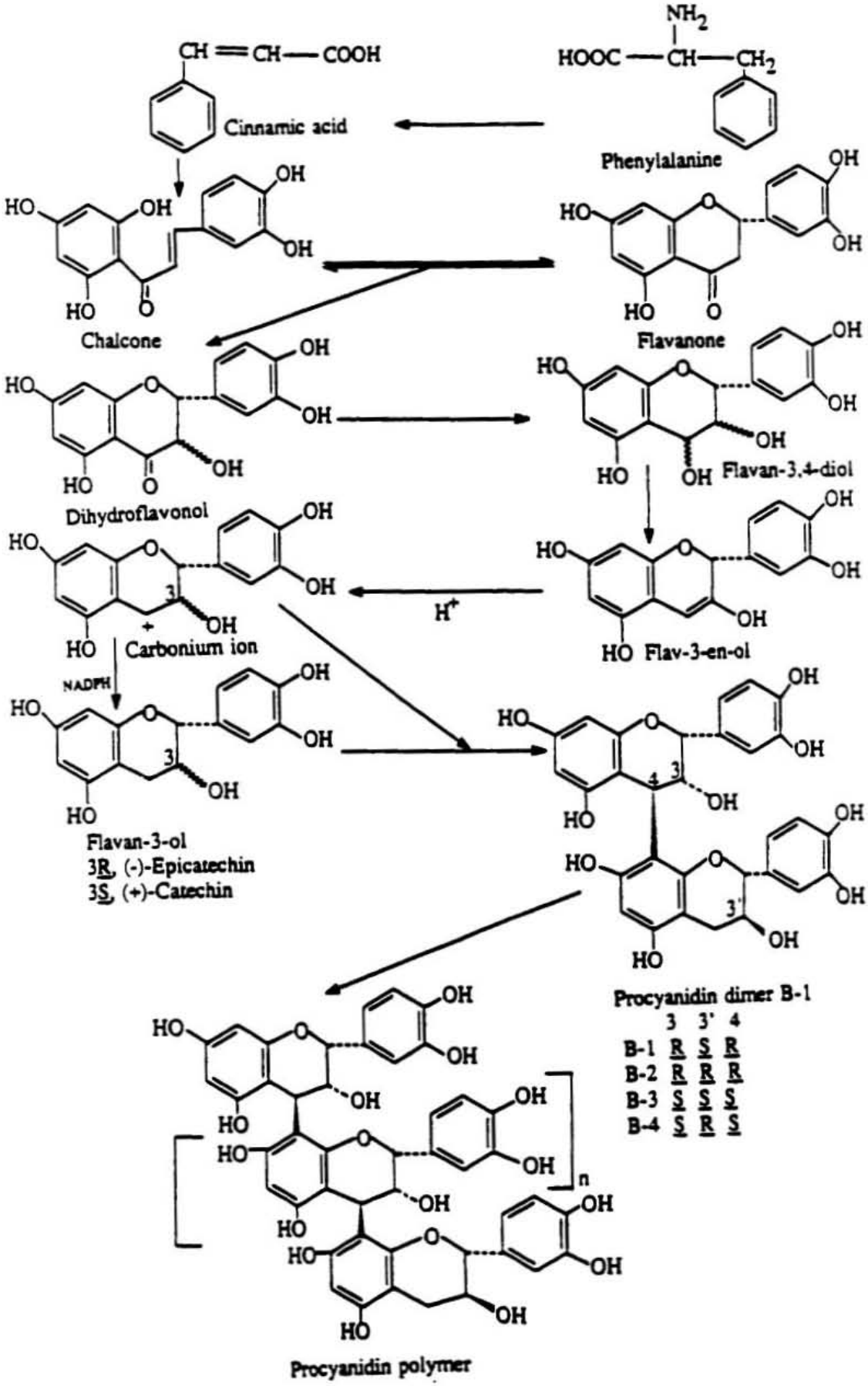
R_1	R_2	R_3	
H	OH	H	*p*-Coumaric acid
H	OH	OCH_3	Ferulic acid
OCH_3	OH	OCH_3	Sinapic acid

Figure 2.7 Phenolic acids of pea meals.

itself. The resistance of the condensed tannins towards hydrolysis arises from the condensation of two or more molecules of flavonoids such as flavan-3-ols (catechins) or flavan-3,4-diols or a mixture of the two. The chemical term "proanthocyanidins" has been used for those colourless natural products which are converted to anthocyanidins on heating with acids (Freudenberg and Weinges, 1962). Weinges *et al.* (1969) implicated the term "lecuoanthocyanidin" to represent monomeric proanthocyanidins such as flavan-3,4-diols and condensed proanthocyanidin or procyanidin for flavan-3-ol dimers and oligomers.

Freudenberg and Weinges (1962) proposed that flavan-3,4-diol is capable of producing a carbonium ion at the reactive benzylic C_4 -OH which could react with the nucleophilic centres of another flavan to give a $C_4 \rightarrow C_6$ or $C_4 \rightarrow C_8$ linkage. According to Hathway (1958), the condensed tannins are formed either by autoxidation or enzymatic reaction involving polyphenoloxidase. Polyphenoloxidase combines an oxidation product of a flavan, namely an *O*-quinone, with the A or B ring of another oxidized flavan. As a result, catechin condenses to give a head to tail polymer, while gallocatechin polymerizes in a tail to tail manner (Hathway and Seakins, 1957). The degree of polymerization of the procyanidins appears to be characteristic of specific plants controlled by metabolic activities during maturity (Haslam *et al.*, 1977). The structure, stereochemistry, distribution, metabolism, and biosynthetic pathways of plant procyanidins (condensed tannins) and associated flavan-3-ols are well explained by Haslam (1977) (Figure 2.8).

Figure 2.8 Biosynthesis of polyphenols [Tannins]. (adapted from Haslam, 1977).



	3	3'	4
B-1	R	S	R
B-2	R	R	R
B-3	S	S	S
B-4	S	R	S

In addition to tannins, plants contain a wide variety of nontannin phenolics (Hagerman *et al.*, 1997). These phenolics, which are usually relatively low molecular weight (<500Da), are distinguished from tannins by their inability to precipitate proteins. Dietary nontannin phenolics have different metabolic fates from tannins because of their different reactivity.

2.3.2.2 Chemical analyses

Chemical methods for assessing tannins require their extraction from tissues, separation, and isolation from other compounds and detection or quantification by spectrophotometric methods. The solvents widely used for extraction of tannins are methanol (Naczk *et al.*, 1992b), ethanol (Shahidi *et al.*, 1994), and acetone (Amarowicz *et al.*, 1996a). These solvent systems destroy the cell membrane and simultaneously dissolve the phenolic compounds. Sephadex LH-20 or G-50 chromatography has proven to be a more successful and widely used technique for fractionation of tannins (Davis and Hoseney, 1979). Compounds that are more sensitive but do not allow recovery of the intact tannins, involve the formation of coloured products with vanillin in concentrated HCl or *p*-toluenesulphonic acid and precipitation with gelatin, neutral lead acetate, or ferric chloride. Thin-layer and paper chromatographic techniques have also been employed to check the purity of the isolated condensed tannins (Jones *et al.*, 1976; Leung *et al.*, 1979). In general, the methods used for tannin analysis are based on either general phenolic reactions, protein precipitation, functional group reactions, or HPLC. A large

proportion of the current assays involve spectrophotometry of tannins or their chromogen. UV spectrophotometry appears to be a simple, fast, and convenient procedure (Sharp *et al.*, 1978) as it excludes partial purification of tannins encountered by Haslam and Gupta (1978). The Prussian blue method (Price and Butler, 1977) or the Folin-Denis method (Ribereau-Gayon, 1972) may be used to measure both tannin and nontannin phenolics in plant extracts. Methods based on the formation of coloured phenolic-metal ion complexes are also useful for measuring total phenolics (Hagerman and Butler, 1978). The property of tannins to form insoluble complexes with proteins has been adopted for quantification of tannins from plant materials (Hagerman and Butler, 1978). The insoluble complexes can be dissolved in SDS-triethanolamine solution and treated with ferric chloride to form a violet-coloured complex with condensed tannins. The biological activity of condensed tannins could be reported as percentage inhibition of porcine pancreatic α-amylase (Davis and Hoseney, 1979).

2.3.2.3 Mechanisms of antinutritive action of tannins

Tannins in peas have been determined by various methods and expressed as catechin- or tannic acid-equivalents. The tannin contents of several pea varieties have been reported to range from 0 to 4% (Reddy *et al.*, 1985). The antinutritional effects of certain types of tannins include high toxicity to some animal and avian species (Singleton, 1981). However, varying effects have been observed in different biological systems. Since polyphenols (tannins) form insoluble complexes with proteins, carbohydrates and other food components, their presence in foods interferes with the utilization of dietary proteins

in nonruminants (Martin-Tanguy *et al.*, 1977) and ruminants (Kumar and Singh, 1984).

The tannin-protein interaction may depend on the size, conformation, and charge of the protein molecule. Tannins may reversibly complex with proteins via hydrogen bonding of their hydroxyl groups with carbonyl functionality of peptide bonds in proteins, or irreversibly by oxidation to quinones which may in turn combine with reactive groups (e.g. $-NH_2$) of protein molecules. The phenol-protein complex may also be stabilized by ionic bonds between the phenolate anion and the cationic site of the protein molecules and/or hydrophobic interactions between aromatic ring structure of tannins and hydrophobic regions of proteins (Kumar and Singh, 1984). Tannins are also considered potent inhibitors of digestive enzymes due to their capacity to bind with enzyme proteins as well as other substrates. The phenolic compounds can affect the enzymes by either reducing their solubility due to the formation of an insoluble protein-phenolic complex or by inhibiting the enzyme-inhibitor complex. For example, tainting of eggs laid by hens fed on tannin-containing seed meals (tainting effect) may be a manifestation of the formation of a tannin-trimethylamine oxidase complex. The formation of the enzyme-inhibitor complex prevents the conversion of trimethylamine to the odourless and water-soluble trimethylamine oxide (Fenwick *et al.*, 1984).

The other important property of condensed tannins is related to their metal-precipitating ability due to the presence of *ortho*-dihydroxyl groups in the flavonoid ring. The precipitation of metal ions inhibits the absorption of dietary minerals. The inhibition may be due to the formation of insoluble metal-tannin complexes in the gastrointestinal

tract, thus making the metal ions unavailable for absorption.

The affinity of tannins for polysaccharides is strongly dependent on the molecular size, conformational mobility and shape, as well as water solubility of polyphenols. Thus, an increase in molecular size and conformational flexibility of tannins enhances their affinity for carbohydrates. Davis and Hoseney (1979) reported that 40-60% tannins may bind starch depending on the source of tannins as well as the type of starch.

2.3.2.4 Biological significance

Bate-Smith (1958) apparently made the first connection between phenolic substances and textural quality of plant products. It was shown, microscopically, that lignin is not the only material that takes part in the encrustation and toughening of plant cell walls. Leucoanthocyanins, polymerized to form condensed tannins, are apparent in the toughness. The biochemical function of tannins has long been debated. The importance of tannins to the plant is believed to be due to their effectiveness as repellents to predators, whether animal or microbial. This function depends on the combined properties of astringency which renders the plant tissue unpalatable, and protein precipitation, which denatures salivary proteins of predators and inactivates microbial extracellular enzymes (Gupta and Haslam, 1980; Rhodes, 1985). Zucker (1983) explained that, due to their large molecular size and structure, condensed tannins are tightly complexed to proteins, pectins and cellulose of the cell wall in such a way to deny microbial extracellular enzymes binding sites to these substrates, thus providing protection against microbial attack. The function of tannins may be related to pea seed

hardness since this defect is thought to be a physiological response to environmental stress (Hincks and Stanley, 1986). Lignin-like materials are deposited around pea/bean cotyledon cells and promote hardening, both as a result of their own mechanical strength as well as their action in preventing water imbibition and swelling (Hincks and Stanley, 1986, 1987). Ma and Bliss (1978) and Deshpande *et al.* (1982) examined whole and dehulled seeds from nearly 39 cultivars of common legumes for tannins and reported that the ratio of tannin content of testa to cotyledon was about 4:1. High concentrations of tannins in grains during the milk stage of development resist bird attack, while tannins in mature grains are responsible for decreased preharvest seed sprouting (Salunkhe *et al.*, 1982b).

2.3.3 Phytic acid

Phytic acid (myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate) is one of the typical antinutritent in legumes (Belavady and Banerjee, 1953). It comprises approximately 5% by weight of edible legumes, cereals, oilseeds, pollens and nuts (Cheryan, 1980). Phytic acid is the major storage form of phosphorus; nearly 60 to 90% of the total phosphorus in seeds, and is produced as a secondary product of carbohydrate metabolism (Loewus and Loewus, 1980). The proportion of phytic acid reaches up to 60 to 80% of the dry weight of globoids of dicotyledons (Lui and Altschul, 1967). Phytic acid exists as salts of calcium, magnesium or potassium (Mills and Chong, 1977; Yiu *et al.*, 1983). Phytic acid, which is a strong acid, forms a variety of salts with several heavy

metals, such as zirconium, thorium, titanium and uranium in 6 N HCl (Ryabchikor *et al.*, 1956; Alimarin and Tozel, 1958). Phytic acid has 12 replaceable protons (Figure 2.9A) and is negatively charged at pH conditions generally 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 2.9Ba; 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 Phillippy, 1989), and iron (Davis and Nightingale, 1975) bind to phytic acid and form insoluble complexes which makes them unavailable for metabolism.

The ability of phytic acid to complex with proteins depends on pH of the medium. At pH below isoelectric point of proteins, phytic acid binds directly with the positively charged proteins as a result of electrostatic attraction (Figure 2.9Bb). At intermediate pH 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 2.9Bc). 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 structure of proteins which may decrease their solubility, functionality and

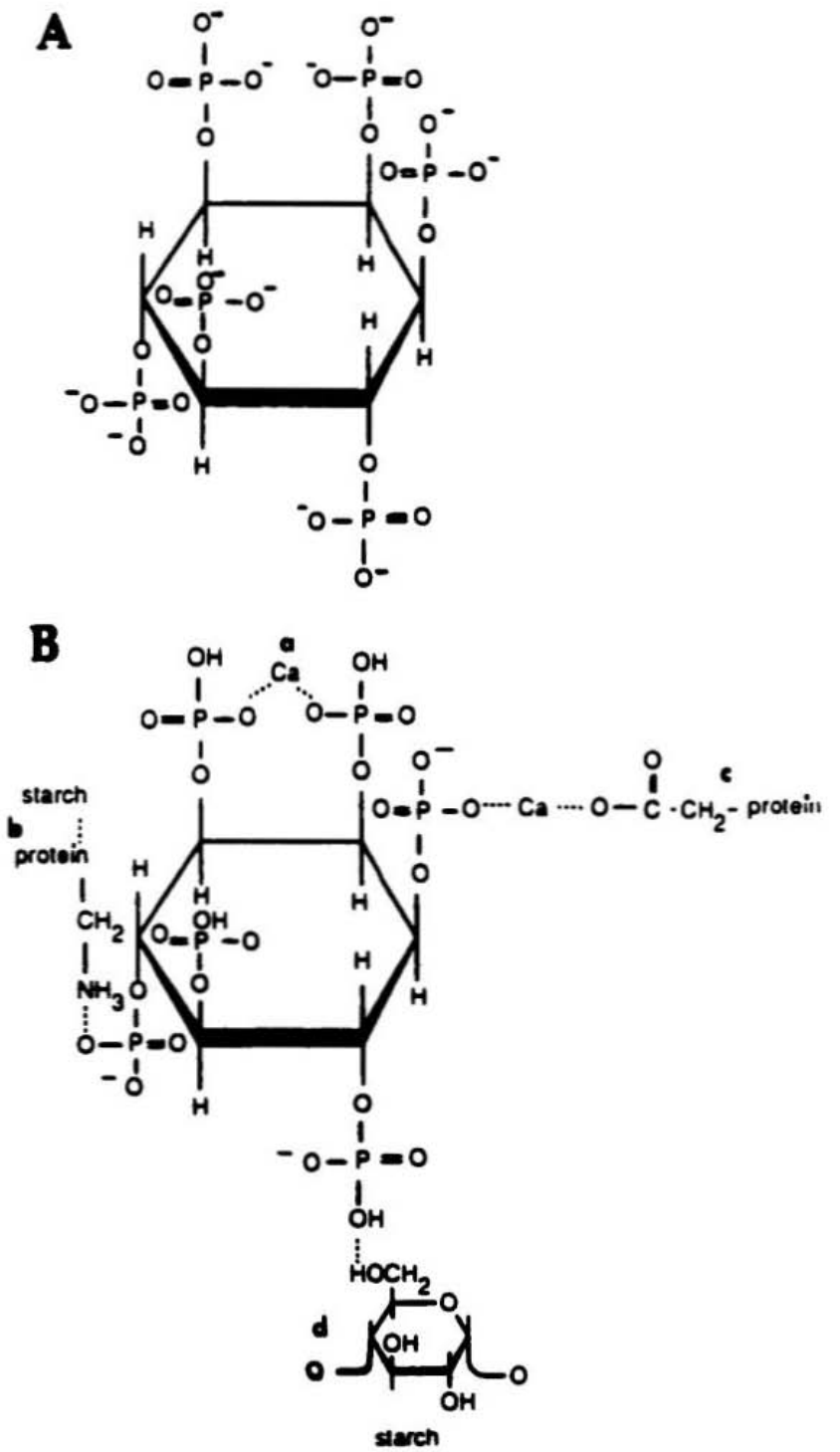


Figure 2.9 **Charged structure of phytic acid (A) and its intreaction with food nutrients (B).**

digestibility (Cosgrove, 1980).

Phytic acid appears to be structurally capable of binding with starch through phosphate linkages or indirectly through its association with proteins (Figure 2.9Bd; Thompson, 1986, 1989). The nutrient digestibility may also be affected by binding of phytic acid with digestive enzymes. These, in turn, may be responsible for both the adverse and beneficial health effects of phytic acid in foods passing through the digestive system (Thompson, 1986, 1989). Phytates are not easily removed by traditional processing of pea seeds. 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 pea seed meals.

2.3.4 Oligosaccharides

Mature seeds of legumes contain oligosaccharides of the raffinose family, namely raffinose, stachyose and verbascose in which galactose is attached to sucrose through α-linkages (Calloway *et al.*, 1971; Shallenberger and Moyer, 1961; Olson *et al.*, 1994; Figure 2.10). These oligosaccharides have been shown to be responsible for flatulence manifested by rectal gas expulsion, abdominal rumbling, cramp, diarrhoea, and nausea following consumption of legume seeds (Steggerda, 1968; Abdel-Gawad, 1993).

The formation of flatus during digestion occurs when intestinal microflora respond to some substances entering the large intestine by producing carbon dioxide, hydrogen, and sometimes methane. Abdel-Gawad (1993) reported that raffinose family of

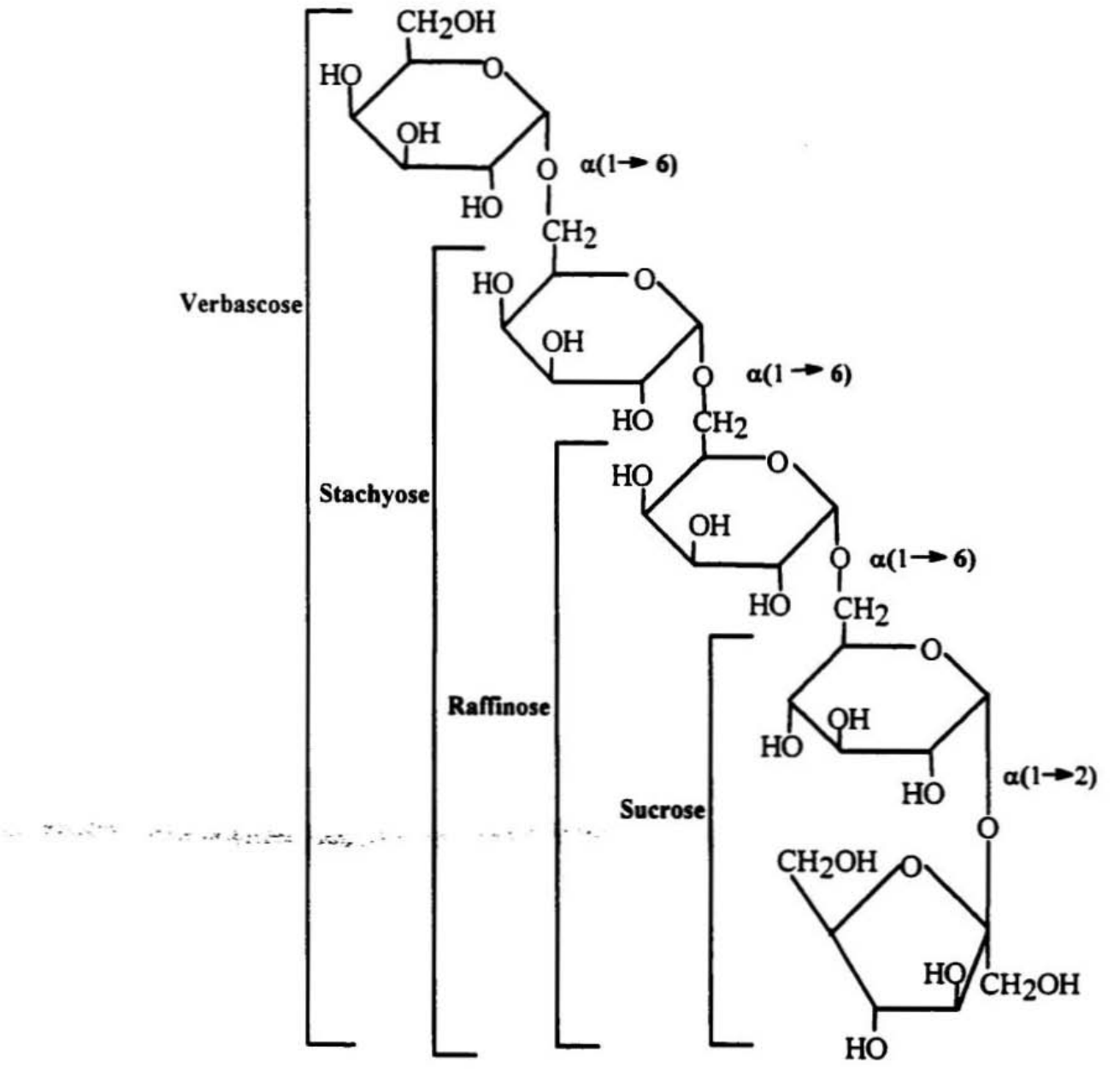


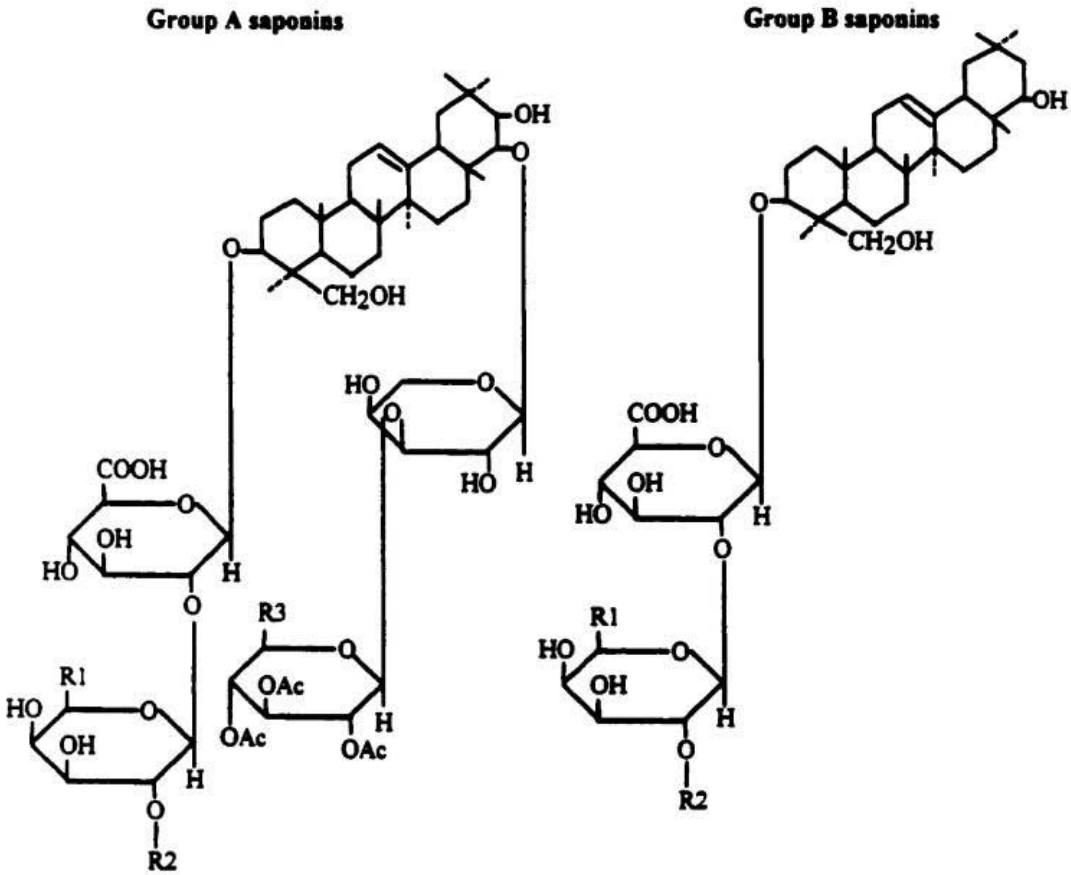
Figure 2.10 Representative structures of oligosaccharides.

oligosaccharides account for 67.3, 63.2, 53.0 and 51.0% of the total soluble sugars in cowpea, faba bean, lentil and common bean, respectively.

2.3.5 Saponins

Saponins are a chemically complex group of compounds commonly found in legumes such as soybean, peas, beans, lentils, peanuts, and alfalfa sprouts. Saponins are also found in some plants used as flavourings, herbs or spices such as fenugreek, sage, ginseng, quillaja bark, thyme, nutmeg and sarsaparilla (Oakenful and Sidhu, 1990). Burrows *et al.* (1987) reported six kinds of "group A" and five kinds of "group B" saponins that occur in soybean as shown in Figure 2.11. The structures of saponins are charactrized by the presence of a steroid or triterpene group, referred to as the aglycone, linked to one or more sugar molecules. Presence of both polar (sugar) and non-polar (steroid or triterpene) groups provides saponins with strong surface-active properties which are responsible for many of the adverse and beneficial biological effects of saponins. Saponins, being amphiphilic compounds, act as natural surfactants and interact readily with cell membranes.

Due to their high biological activity, saponins have been studied by many researchers for their physiological activities, including haemolytic, goitrogenic, antioxidative and hypolipidemic properties (Price *et al.*, 1987). Beneficial activities of saponins are related to their effects on lowering plasma cholesterol levels and antiviral activity against human immunodeficiency virus (HIV), *in-vitro* (Nakashima *et al.*, 1989). Saponins are believed to have hypocholesterolemic, immunostimulatory, antioxidative, antitumorigenic,



	R1	R2	R3
acetyl-soyasaponin A 1	CH_2OH	β-D-glu	CH_2OAc
acetyl-soyasaponin A 2	CH_2OH	H	CH_2OAc
acetyl-soyasaponin A 3	H	H	CH_2OAc
acetyl-soyasaponin A 4	CH_2OH	β-D-glu	H
acetyl-soyasaponin A 5	CH_2OH	H	H
acetyl-soyasaponin A 6	H	H	H

	R1	R2
soyasaponin I	CH_2OH	α-L-rham
soyasaponin II	H	α-L-rham
soyasaponin III	CH_2OH	H
soyasaponin IV	H	H
soyasaponin V	CH_2OH	β-D-glu

Figure 2.11 Chemical structures of saponin group A and group B.

antiviral, antifungal, antibacterial and antidiabetic properties (Thompson, 1993).

Saponins can cause local inflammation, if provided intravenously to mammals, and in large doses can result in death due to massive release of erythrocyte debris and reduction in the oxygen-carrying capacity of the blood (Scott *et al.*, 1985). Saponins can decrease absorption and utilization of nutrients caused either by the inhibition of metabolic and digestive enzymes (Cheeke, 1971) or binding with nutrients (West and Greger, 1978). However, there is little evidence of significant mammalian toxicity after ingestion (Price *et al.*, 1987), although incidences of livestock poisoning due to the presence of saponins have been documented (Williams *et al.*, 1984).

2.4 Removal of antinutritional and/or toxic compounds from pea seeds

Recently, removal of pea polyphenolics and other antinutritional factors by appropriate processing methods has received considerable attention, largely due to their possible influence on the nutritional and aesthetic qualities of foods (Reddy *et al.*, 1985; Table 2.3). Several methods may be considered to remove undesirable components from pea seeds. Breeding pea varieties devoid of or with little undesirable componetnt(s) is one approach. Such an approach clearly requires long-term efforts to consider agronomical consequences of genetic manipulation. Another approach is to remove these unwanted components by physical, chemical, or biological means. These techniques may include dehulling, soaking, heat processing, germination, fermentation, selective extraction, membrane filtration, irradiation, and enzymatic treatments or any of their combinations.

Table 2.3 Effects of some selected antinutrients in foods

Component	Effect(s) in food or food products	Reference
Polyphenols	Reduction in protein digestibility, inhibition of several enzymes, astringency taste	Goldstein and Swain, 1965, Marquardt and Ward, 1979, Bressani and Elias, 1980
Phytate	Reduced mineral bioavailability, altered protein solubility	Cheryan, 1980; Jaffe, 1981
Lathyrogens	Lathyrism (Nervous paralysis of lower limbs), death	Liener, 1979
Flatulence factors (Oligosaccharides)	Flatulence production (hydrogen, carbon dioxide, and methane)	Murphy, 1973; Olson *et al.*, 1975, Eskin *et al.*, 1980, Reddy *et al.*, 1980
Trypsin inhibitor	Trypsin inhibition, pancreatic hypertrophy, dietary loss of cysteine	Liener, 1975, 1977
Chymotrypsin inhibitor	Chymotripsin inhibition	Liener, 1979
α-Amylase inhibitor	α-Amylase inhibition, may hinder carbohydrate utilization	Power and Whitaker, 1977 Jaffe *et al.*, 1973; Marshall, 1975
Off-flavours	Damage to amino acids, unacceptable odours	Gardner, 1979; Rackis *et al.*, 1979
Antivitamins	Liver necrosis, oxidation of vitamin E, muscular dystrophy, increased B_{12} requirement	Hogue *et al.*, 1962; Desai, 1966

2.4.1 Dehulling

Since tannins are mainly concentrated in the testa or seed coats of peas, the physical removal of seed coat by either dehulling or milling and separating hulls may reduce the tannin content in pea meals and improve their nutritional quality (Salunkhe *et al.*, 1982b). Tannins have been partially removed from pea seeds by dehulling and improved *in-vitro* protein digestibility and ionizable iron absorption (Deshpande *et al.*, 1982). Removal of the seed coat decreases the cooking time and facilitates improved palatability.

2.4.2 Extraction with chemicals

Extraction of one or more antinutritional components from seeds may be achived by employing a single or multiple solvent(s) and extraction with chemicals. Treatment of peas, beans and cereals with chemicals (dilute alkali, ammonia, hydrogen peroxide, formaldehyde, ferrous sulphate or ferric chloride) apparently reduces assayable tannins (Wah *et al.*, 1977; Price *et al.*, 1978b). De Lumen and Salamat (1980) have used 1N NaOH, 0.01N $NaHCO_3$, 4% acetic acid and 1% $CaCO_3$ and 10% ash as soaking media with the resultant removal of 65, 49, 52, 49 and 63% of tannins from winged beans. Alkanol-ammonia-water extractions (Shahidi *et al.*, 1988; Shahidi and Gabon, 1989; Wanasundara and Shahidi, 1994b) have been used to remove antinutritional/toxic compounds from canola/rapeseed, flaxseed, and mustard. Azeotropic mixtures of hexane-methanol, hexane-ethanol, and hexane-isopropanol or ethanol have been suggested to remove lipid-derived off-flavour compounds (Rackis *et al.*, 1979). Deshpande and Campbell (1992a) used NaOH (0.02N) or salts (NaCl, Na_2SO_4, NaOAc,

Na_2CO_3, KCl or K_2SO_4) at 2% (w/v) for the extraction of protein isolates from grass pea. A two-phase solvent extraction system consisting of alcohol with or without 10% (w/w) ammonia, possibly containing 5% water (v/v) and hexane was effective in the removal of flatulence-causing sugars from both oilseeds and legume seeds (Shahidi *et al.*, 1990; Naczk *et al.*, 1992a). The extraction of soybean, cottonseed and legume seeds with methanol-ammonia/hexane solvent system removed 5 to 69% of raffinose, 6 to 55% of stachyose and up to 8% of verbascose originally present in the seeds (Naczk *et al.*, 1992a). Price *et al.* (1978b) reported that when seeds/meals were treated with chemicals, tannins may become altered in some manner to become nutritionally unreactive , perhaps by forming phlobaphenes. Under alkaline conditions, the hydrolyzed tannins may become permanently bound to some compounds, especially proteins in the seeds, and render them insoluble and nutritionally inert.

2.4.3 Alkanol-ammonia-water extraction process

Removal of some undesirable plant constituents by ammonia treatment is well documented. Ammoniation has been found to inactivate aflatoxin contaminants in cottonseed and peanut meals (Gardner *et al.*, 1971; Mann *et al.*, 1971) as well as shelled corn (Brekke *et al.*, 1978). The tannin content of high-tannin sorghum grains was considerally reduced after treatment with ammonia and the treated products supported growth of chicks similar to that shown by low-tannin sorghum (Price *et al.*, 1979).

Use of different alcohols, water contents in alcohols for a two-phase solvent extraction (alkanol-ammonia-water/hexane) of rapeseed and mustard was described by Rubin *et al.* (1986), Shahidi *et al.* (1988) and Wanasundara *et al.* (1993). The effectiveness of removal of antinutritional factors (tannins, phytic acid and glucosinolates) by alkanol and alkanol-ammonia solutions was in the order of methanol >> ethanol > isopropanol > t-butanol. These authors reported that isopropanol and t-butanol without water dissolved small amounts of ammonia; however, when more than 5% (v/v) water was added in the alkanol phase, a sticky, dark-coloured meal was produced. Only ethanol and methanol gave two separate phases in the extraction system, thus allowing simultaneous extraction of oil and polar materials from oilseeds (canola) while no phase separation was noted when t-butanol was used (Shahidi *et al.*, 1988).

The effectiveness of glucosinolate removal from different canola cultivars depends 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.*, 1986a; Shahidi *et al.*, 1988; Wanasundara *et al.*, 1993). The two-phase solvent extraction system was also effective in partially removing phenolic compounds (phenolic acids and condensed tannins) of canola and rapeseed (Shahidi and Naczk, 1989; Naczk and Shahidi, 1989; Wanasundara and Shahidi, 1994a).

2.5 Laboratory preparation of pea seed meal

There has been considerable interest in detoxifying and upgrading *Lathyrus* seed meals (Padmanaban, 1980; Deshpande and Campbell, 1992b). Preparation of detoxified

Lathyrus seed meals by soaking, steeping, and boiling of the seeds in water followed by draining (Tekle-Haimanot *et al.*, 1993; Urga *et al.*, 1994; Padmajaprasad *et al.*, 1997) and also preparation of a protein isolates has been reported (Deshpande and Campbell, 1992b). The resultant protein isolates showed an increase in the trypsin inhibitor activity, but β-N-oxalylamino-L-alanine (BOAA) levels decreased by about 50-85% depending on the extractant used. The amino acid profile of the extracted product was not very different from that of the original meal; however, there was a reduction in the content of available lysine and tryptophan. Most of the works reported on *Lathyrus* seeds have not referred to the removal of lathyrogenic compounds even though they are the main limiting antinutritional factors.

Investigations by different research groups have so far shown that *Lathyrus* seeds may serve as a potential ingredient for feed or food product formulation. However, effective methods of detoxification and meal or protein preparation and their industrial applications have not been thoroughly studied. *Lathyrus* seeds need to be upgraded in order to be used as an animal feed or human food ingredient.

2.6 Chemical composition of pea seeds

A wide variation in the chemical composition of different pea varieties has been reported (Salunkhe *et al.*, 1985). Peas are characterized by a relatively large content of carbohydrates, ranging from 24 to 68% (Salunkhe and Kadam, 1989) which include water-soluble components such as sugars and certain pectins and insoluble fractions such

as starch and cellulose. The starch content of pea seeds range from 24 to 49% (Reddy *et al.*, 1984) and their crude protein content vary from 19 to 35% (Pant and Kapur, 1963). The proteins are located in the cotyledons and the embryonic axis of peas and beans with only a small amount being present in the seed coat (Singh *et al.*,1968).

Pea seeds generally have a higher concentration of lipids than cereals. Oleic and linoleic acids are the main unsaturated fatty acids present in most pea legumes (Exler *et al.*, 1977; Table 2.4). Oils from legumes in the temperate zone tend to have more unsaturated components than those of the tropics; pea legumes also contain a considerable proportion of linolenic acid (Doughty and Walker, 1982). The crude fibre content of peas range between 0.9 and 4.9% and it concentrated in the seed coat. Pea hulls constitute approximately 8.2% of pea seeds and contain approximately 55.2% cellulose and 23.1% hemicellulose by weight (Vose *et al.*, 1976).

Pea seeds are a good source of vitamins like thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, and folic acid (Ogunmodede and Oyenuga, 1970). *Lathyrus* seeds contain 0.39, 0.17, and 2.9 mg/100g thiamine, riboflavin, and niacin, respectively, and 120 μg/100g β-carotene (Gopalan *et al.*, 1982; Deosthale, 1984). Meanwhile, the mineral content (ash) of pea legumes ranges from 2.5 to 4.2% (Bressani and Elias, 1974). Apata and Ologhobo (1994) reported that potassium was the most abundant mineral followed by phosphorus in selected Nigerian legume seeds. Singh *et al.* (1968) showed that more calcium and less phosphorus were present in the seed coats than in the cotyledons. Most important thing is that the chemical composition of pea seeds is

Table 2.4 Fatty acid (%) composition of some common pea seeds[1]

Fatty acid	Grass pea	Green pea	Chickpea	Cowpea
16:0	25.00	12.92	9.22	23.50
18:0	2.00	2.08	1.20	5.60
20:0	NR	NR	NR	0.60
22:0	NR	NR	NR	2.20
18:1	1.00	15.42	21.84	8.40
18:2	67.00	36.25	43.29	34.00
18:3	3.00	6.67	2.00	25.70
Lipid (%)	1.00	2.41	4.99	2.05

[1]Adapted from Salunkhe *et al.* (1982a); NR, Not reported.

governed by cultivar, geographic location, and growth conditions (Krober, 1968).

2.6.1 Proteins

Legume seeds show very large variation in protein content ranging from 14.9 to 45% (Salunkhe *et al.*, 1985) and are important sources of protein from a nutritional and economical point of view (Anon, 1975; Orr, 1978). The protein content of *Lathyrus* seeds ranges from 21 to 33% (Gupta, 1982). The crude protein content based on nitrogen determination of legumes includes a mixture of different nitrogenous compounds, besides proteins, such as free amino acids, amines, complex lipids, purine and pyrimidine bases, nucleic acids, and alkaloids. The proportion of NPN to the total seed nitrogen is practically in the range of 10 to 20% (Earle and Jones, 1982). A higher seed protein content may be obtained by increasing the application of nitrogen fertilizers. Nitrogen deficiency lowers the content of total and NPN compounds in pea seeds. The sulphur-containing amino acids also increase by application of nitrogen-containing fertilizers.

Plant proteins are primarily of two types, namely storage proteins of the seed and metabolic proteins of the vegetative part of the plant. The storage proteins occur in distinct organelles of the seed, known as protein bodies/vacuole/granule or aleurone grains. It has also been reported that besides storage proteins, protein bodies contain other proteins such as enzymes, lectins, etc. (Prakash and Narasinga Rao, 1986). Albumins, globulins, glutelins and prolamines are four classes of proteins of the seed storage proteins. Albumins are water soluble, globulins are soluble in dilute salt solutions, but are relatively insoluble in water, glutelins are dilute acid- or alkali-soluble

and prolamines are soluble in aqueous ethanol. Globulins and prolamines function primarily as carbon and nitrogen sources for germinating seeds. Albumins are generally considered as metabolic proteins (Dieckert and Dieckert, 1985). Albumin fraction of pea protein contains two major polypeptides with molecular weights of 8,000 and 22,000 Da. Schroeder (1984) reported that these two polypeptides constitute 34% of the albumin protein fraction and are rich in sulphur-containing amino acids. Bhatty (1982) resolved the albumin proteins into 20 to 25 bands on SDS-Polyacrylamide gel electrophoresis. These albumins consist of many subunits ranging from 18,000 to 90,000 Da (Grant *et al.*, 1976). The major globulins of peas are referred to as legumin and vicilin. Millerd *et al.* (1978) showed that legumin fraction varies from 25 to 80% of the total globulins of peas. According to Boutler and Derbyshire (1978) globulins make up 65 to 80% of the extractable proteins of pea cotyledons.

2.6.2 Physico-chemical properties of pea seed proteins

Solubility characteristics and recovery of legume proteins depend on several factors, including meal to solvent ratio, particle size of the flour, temperature and length of extraction time, pH, ionic strength, type and concentration of the extractant as well as the hydration properties of various proteins (Sathe and Salunkhe, 1981a). The broad pattern of nitrogen extractability of pea seeds in different solvents and at varying pH and ionic strength is comparable with other legume seed meals. The minimum extractability of nitrogen from pea seed meals lies between pH 4.0 and 4.5 (Thompson, 1977; Sumner *et*

al., 1981; Deshpande and Campbell, 1992b). Approximately 20 to 25% of total nitrogen of pea seed meal is soluble at the minimum solubility pH due to the presence of high levels of non-protein nitrogen compounds (Singh and Jambunathan, 1982; Deshpande and Campbell, 1992b). According to the solubility of pea seed proteins in different solvent systems, approximately 12 to 15% were water-soluble, 55 to 65% salt-soluble, 2.5 to 4% soluble in ethanol and 18 to 20% soluble in 0.2% (w/v) dilute alkali (Singh and Jambunathan, 1981, 1982; Singh *et al.*, 1981).

As in other legume seed, albumins and globulins are the major groups of proteins of pea and *Lathyrus* species. Gwiazda *et al.* (1980) separated pea globulins and found sedimentation coefficients of 11.9S and 6.4S for the two fractions. They reported that legumin and vicilin were the two major groups of globulins in pea. Legumin has a molecular weight of 330,000 Da while vicilin has a molecular weight of 180,000 Da. Casey (1979) purified legumin by immunoaffinity chromatography and electrofocusing (Gatehouse *et al.*, 1980). Legumin has a glycinin-like quaternary structure involving oligomerization of several different acidic and basic subunits. Croy *et al.* (1980a, b) reported that the disulphide bond subunits in legumin (40,000 and 20,000 Da) are synthesized as a unique 60,000 Da polypeptide chain. Sumner *et al.* (1981) showed that the isolated protein was 100% soluble at pH 10.0. Deshpande and Campbell (1992b) reported that grass pea has three types of storage protein fractions generally associated with food legumes; the 11S legumin-type and two 7S, vicilin (subunit molecular weight 43-47 kDa) and convicilin (subunit molecular weight 64-66 kDa) types.

2.6.3 Amino acid composition

Nutritive values of proteins are mainly dependent upon their amino acid profile because their utilization depends on the content of limiting amino acids. The amino acid composition of different peas and *Lathyrus* seed proteins has been studied extensively (Salunkhe and Kadam, 1989). The sulphur-containing amino acids are limiting in different pea seeds (Table 2.5). Different protein fractions of various peas differ in their amino acid composition. Murray (1979), Schroeder (1984) and Singh and Jambunathan (1982) reported that the albumin fraction is relatively rich in sulphur-containing amino acids. However, Holt and Sosulski (1979) showed that the albumin proteins of pea contain more tryptophan, methionine, lysine and threonine, but less arginine, leucine, and phenylalanine than the globulin fraction. Glutelin contained a considerably higher concentration of sulphur-containing amino acids than the globulin fraction of chickpea and pigeonpea (Singh and Jambunathan, 1982). Gwiazda *et al.* (1980) observed that globulins isolated from pea flours had a higher amount of glutamic acid, aspartic acid and arginine than albumin and glutelin.

2.6.4 Nutritive value

Nutritive value of proteins depends mostly upon their digestibility and the availability of essential amino acids. Kumar *et al.* (1991) reported that the biological value of six promising varieties of pigeonpea ranged from 57.28 to 59.38%. Patwardhan (1962) showed that peas have a biological value of 48 to 49% and a protein efficiency ratio of

Table 2.5 Amino acid composition of some pea seed and FAO/WHO reference values (g/16 g N)

Amino acid	Grass pea[1]	Green pea[2]	FAO/WHO Refernce[3]
Isoleucine	6.7	7.4	4.0
Leucine	6.6	9.5	7.0
Lysine	7.4	8.9	5.5
Methionine + Cysteine	1.8	1.3	3.5
Phenylalanine + Tyrosine	4.2	4.6	6.0
Threonine	2.3	4.2	4.0
Tryptophan	0.4	0.7	1.0
Valine	4.7	6.5	5.0
Alanine	NR	NR	NR
Arginine	7.8	13.4	2.0
Aspartic acid	NR	NR	NR
Glycine	NR	NR	NR
Gtutamic acid	NR	NR	NR
Histidine	2.5	2.7	2.4
Proline	NR	NR	NR
Serine	NR	NR	NR

Adapted from [1]Bressani and Elias (1974). [2]Gopalan *et al.* (1982). [3]FAO/WHO (1973); NR. Not reported.

0.6 to 1.2. The Tetrahymena relative nutritive value (T-RNV) for peas was 44 to 45% (Davis, 1981). Payne (1978) reported that pea seeds have a higher protein utilization value than soybean. The protein fractions of peas differ in their amino acid profile and nutritional quality. Chen and Thacker (1978) noted that the albumin fraction of pea cotyledons was nutritionally superior to the globulin fraction. Similar observations were made by Singh and Jambunathan (1982) for chickpea and pigeonpea.

2.7 Utilization of pea seed proteins

The potential applications of legume flours, protein concentrates, and isolates have been summarized by Gwiazda *et al.* (1979). Mizrah *et al.* (1977) reported that the isolated proteins often improve the appearance and taste of foods and therefore can be better utilized as nutritional and functional ingredients in certain products. Sumner *et al.* (1981) reported that protein isolates can be prepared from different peas by sodium hydroxide extraction and subsequent isoelectric precipitation. Deshpande and Campbell (1992b) used different solvents for extraction of proteins from grass pea and found that alkali extraction was the best method for efficient recovery and enhanced protein content of the product.

Vose *et al.* (1976) fractionated pea flour by air classification into two protein-rich (P_I and P_{II}) and two starch-rich (S_I and S_{II}) fractions. The yields of protein- and starch-rich fractions were about 35 and 65%, respectively. Protein-rich fractions (P_I and P_{II}) contained more than 50% protein and very little starch, while the starch-rich fractions (S_I

and S_{II}) contained more than 70% starch and very low protein (6 to 12%). The protein-rich fractions also had a relatively high level of minerals, fat and fibre.

Protein rich peas are generally blended with cereals; the blends generally have a higher nutritive value than either the cereals or legumes alone (Phansalkar *et al.*, 1957). D' Appolonia (1977) showed that 5 to 20% pea flour can be blended with wheat flour for bread making without affecting the loaf volume, crumb grain ratings and palatability; the nutritive value of the product was also improved. Protein concentrates and isolates have potential uses in a variety of food formulations (Anon., 1974). Pasricha and Rebella (1982) showed the utilization of peas, pea protein concentrates and isolates in different food products such as vegetable biryani, khichri, savian uppama, pea-suji, bhat and samosa. The cereal products, particularly breads and biscuits, appear to be a choice vehicle for legume seed protein prorogation due to their universal acceptability.

2.8 Legume seed protein products

Legume seeds serve as a good source of protein, carbohydrates and certain minerals. Among the seed parts, cotyledons contribute to 96% of total proteins, 90% of lipids, 77% of carbohydrates, and 89% of minerals of the seeds. The meal and protein isolates may be used as protein supplements in animal rations and human food, respectively. However, there is a continuous desire to use more of the proteins for human foods in order to address religious concerns about composition of certain animal sources of protein.

Plant proteins from soybean, peanut, cottonseed, legume seeds, and sunflower meals have been used in a variety of applications. However, the success of including these plant proteins in different traditional foods depends on their sensory quality, nutritive value, and functional properties. Pea seed proteins may be used in several meat products such as beef patties, poultry rolls, luncheon loaves, dairy products such as whipped toppings, frozen deserts, cheeses, coffee whiteners, beverages such as fruit flavoured products, bakery products like bread, layer type cakes, pancakes, cookies, pies, doughnuts, biscuits, and some other specialized medical as well as nutritional products (D' Appolonia, 1977; McWatters, 1977; Jeffers *et al.*, 1978; Gwiazda *et al.*, 1979). Current research interests about legume seed proteins have been targeted for their application in human foods in order to increase the protein level of vegetarian diets as well as application in different industrial products.

2.9 Preparation of legume seed protein products

The production of legume seed protein products may be categorized into physical separation of the protein-rich fraction(s) (Figure 3.2) or solubilization of proteins using appropriate solvents followed by precipitation and/or lyophilization (Figure 3.4). Pretreatments are generally given to the legume seeds prior to their conversion into protein products. These pretreatments include cleaning, washing, soaking, cracking, dehulling, removal of oil, and other components, depending upon the type of legume used for processing. The moisture content of pea grains or dehulled grains is adjusted to 8 to

10% prior to milling. After milling, the grains in a pin mill are classified in a spiral air stream with a cut off point of 80 mesh between the fine and coarse particles. The remilling and air classification of the coarse fraction yields an additional 10% protein concentrate and starch fraction. Third time milling of the coarse fraction and subsequent air classification yields a high starch fraction. Sosulski and Youngs (1979) have reported that protein recoveries vary between 43.3 and 66.6% in several legumes upon air classification. Wright *et al.* (1984) found that air classification may lead to the production of pea protein concentrates with 60 to 70% protein.

Protein isolates may also be prepared by solubilizing them in alkali solutions and eliminating the insoluble constituents by centrifugation. Proteins are recovered by isoelectric precipitation and dehydration after washing; the pH of the acid precipitate may then be adjusted to neutrality.

Several types of processing options are available which use industrial membranes. An ultrafiltration system of 20 kDa molecular weight cut off keeps proteins in the retentate, while water-soluble components pass through as permeate. The permeate can be processed by reverse osmosis to obtain pure water and the concentrated soluble compounds. The ultrafiltration system can achieve fractionation while the reverse osmosis system can only concentrate the proteins. Lawhon *et al.* (1978) reported that protein concentrates produced by ultrafiltration and reverse osmosis process yield a protein product of 90% nitrogen solubility. Sumner *et al.* (1981) reported that the yield of protein isolates from field pea was 59 to 65% and the protein content of the isolates ranged from 91 to 98%. On a seed protein basis, over 70% protein was recovered under

alkaline conditions, 61% using isoelectric protein precipitation and about 50% in sulphate salts of sodium and potassium from grass pea (Deshpande and Campbell, 1992b). Various combinations of an aqueous extraction process and industrial membrane processing techniques have been studied in order to prepare vegetable protein concentrates and isolates (Lawhon *et al.*, 1981; Lusas and Rhee, 1986).

2.10 Functional properties of food proteins

Functional properties denote the physico-chemical properties of proteins that determine their behaviour in food during processing, preparation, and storage. From a food application viewpoint, colour, flavour, taste, hydration, emulsion, gelation, viscosity, water absorption, solubility, dispersibility, adhesion, flow behaviour, buffering capacity and film-forming properties, together with nutritional quality, are important. These physico-chemical properties and the manner in which proteins interact with other food components affect processing applications, quality and ultimately acceptance of food, both directly and indirectly. The type of functional properties required for a protein or a protein mixture varies according to the particular food system in which it is present (Cherry and McWaters, 1981). Tables 2.6 and 2.7 list typical functional properties of seed proteins and their importance in food applications along with examples. It is also important to note that there are no standardized tests or guidelines to evaluate each functional property of food proteins.

The functional properties which are important for food proteins are fundamentally

Table 2.6 Functional properties of food proteins and their functional criteria in food applications[1]

Functional property	Functional criteria
Organoleptic	Colour, texture, flavour, smoothness, grittiness, mouthfeel
Hydration	Water absorption, wettability, swelling, thickening, gelling, solubility, syneresis
Surface	Foaming (aeration-whipping), emulsification, protein-lipid film formation, flavour binding, lipid binding
Structural/Rheological	Elasticity, viscosity, chewiness, cohesiveness, grittiness, gelation, aggregation, adhesion, stickiness, dough formation, network cross-binding, extrudability, fibre formation, texturizability
Others	Compatibility with other food additives, antioxidant activities, enzymatic activity, compatibility with other food components

[1]Adapted from Kinsella (1979, 1982).

Table 2.7 Functional properties of food proteins in actual food system[1]

Functional property	Mode of action	Food system
Colour control	Bleaching by lipoxygenase	Breads
Water absorption and binding	Hydrogen-bonding of water, entrapment of water without dripping	Sausages, meats, cakes, breads
Foaming	Forms stable films to entrap gas	Whipped toppings, chiffon desserts, gel cakes
Fat adsorption	Binding of free fat	Sausages, meats, donuts
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soup, cakes
Flavour-binding	Adsorption, entrapment, release	Simulated meats, bakery products
Viscosity	Thickening, water binding	Soups, gravies
Cohesion-adhesion	Protein acts as adhesive material	Meats, sausages, baked goods, pasta products
Gelation	Protein matrix formation and setting	Cheese, curds, meats
Elasticity	Disulphide links in gels, hydrophobic bonding in gluten	Meats, bakery goods
Solubility	Protein solvation	Beverages

[1]Adapted from Kinsella (1979).

related to their physico-chemical, structural and conformational characteristics. These characteristics include, size, shape, amino acid composition and sequence, charge and charge distribution, hydrophilicity/hydrophobicity ratio, secondary structures and their distribution (e.g. α-helix, β-sheet and a periodic structure), tertiary and quaternary structures of polypeptide segments, inter- and intra- subunit cross links (e.g. Disulphide bonds) and the rigidity/flexibility of the protein in response to external conditions (Kinsella, 1979, 1982). Functional properties of food proteins may be regarded as manifestations of their hydrodynamic and surface-related molecular properties (Damodaran, 1989). Both gelation and viscosity are primarily dependant on the hydration and solubility of proteins. These properties also depend on the shape and size of the macromolecule and are independent of the composition and distribution of amino acids and the presence or absence of non-protein components. Foaming and emulsifying properties, fat- and flavour-binding and solubility are surface-related properties that are largely affected by the amino acid composition/distribution and molecular flexibility rather than size and shape of the macromolecule. Factors such as processing conditions, the method of isolation, environmental factors such as temperature, humidity, pH and ionic strength as well as interactions with other food components (e.g. carbohydrates, lipids, proteins, ions, flavours, phenolics, and water, etc.) may alter the functional properties of a protein (Kinsella, 1979).

The structure-function relationships of some food proteins are well known and has been exploited in industry; e.g. hydrolyzed plant proteins in household products. There has been a continuous interest among food scientists and researchers to investigate the

molecular basis for the expression of functional properties of food proteins which helps to increase utilization of novel preparations in conventional foods. Therefore, it is necessary to develop better processing techniques to retain or enhance protein functionality and also to develop genetic engineering strategies by altering the conformational characteristics of underutilized food proteins in order to improve their functionality.

2.10.1 Water binding ability

The ability of water to bind and immobilize is by itself one of the most important functional properties in many food systems. Water absorption, water holding and water binding are terms that are used interchangeably in the literature. It is known that water binding by proteins is a function of several parameters including size, shape, conformational characteristics, steric factors, hydrophilic-hydrophobic balance of amino acids in the protein molecule, lipids and carbohydrates associated with the proteins, thermodynamic properties of the system, physicochemical environment, solubility of the protein molecules and others (Chou and Morr, 1979). However, polar amino groups of protein molecules are the primary sites of protein-water interactions. Cationic, anionic, and non-ionic sites bind different amounts of water (Kuntz, 1971). Water absorption provides critical information about the functional properties of proteins such as those in meat processing which change the colour, taste, cooking loss, juiciness, tenderness and drip on frying and thawing. Binding of water to protein molecules increases the hydrodynamic volume of the protein molecule, leading to decreased density of hydrated

proteins compared to those in the dehydrated stages. Conformational changes in the protein molecules can affect the nature and availability of hydration sites. Transition from globular to random coil conformation may expose previously burried amino acid side chains, thereby making them available to interact with the aqueous medium; the unfolded proteins bind more water than their globular counterparts (Hutton and Campbell, 1977, 1981). Water holding or water binding usually refers to the water that is retained by the sample after centrifugation, gravitational filtration, or filter paper press (Nakai and Li-Chan, 1988). Kinsella (1976) defined water absorption as water taken up spontaneously by a dry powder after equilibration with water vapours of a known relative humidity. Functional properties, which depend on protein-water interactions, include wettability, dispersibility, swelling, solubility, viscosity, thickening, gelation, coagulation, and the sorption behaviour.

2.10.2 Fat binding ability

Fat binding ability of proteins is considered as an important functional property since it enhances flavour retention and improves mouthfeel. The mechanism of fat absorption by different proteins is not fully understood, but it appears to be affected by lipid-protein complexes and protein content (Kinsella, 1979). Fat binding ability of proteins depends primarialy on the hydrophilic and hydrophobic groups on the surface of protein molecules (Sosulski *et al.*, 1976; Naczk *et al.*, 1985). Ryan (1977) reported that hydrophobic interactions are most important in stabilizing interactions of both polar and non-polar lipids with proteins. Voutsinas and Nakai (1983) showed a strong interaction between

surface hydrophobicity of food proteins and their fat binding capacity. The surface-related properties of protein molecules also affect their fat binding capacity (Damodaran, 1989). Lin *et al.* (1974) reported that the availability of lipophilic groups may have an important role in contributing to higher absorption of fat. Protein-protein interaction, protein conformation, lipid-lipid interaction due to the spatial arrangement of the lipid phase are responsible for affecting protein-lipid interactions (Hutton and Campbell, 1981). Hydrophobic, electrostatic and hydrogen bonding are non-covalent interactions that also take part in protein-lipid interactions. Generally, hydrogen bonding is of secondary importance in protein-lipid mixtures though it is indirectly involved in hydrophobic bonding (Karel, 1973) as water-water interactions by hydrogen bonding in aqueous media are much stronger than the interaction between water and non-polar compounds. Nakai and Li-chan (1988) have shown that electrostatic attraction can occur between negatively charged phosphate groups of phospholipids and positively charged protein groups (lysyl and guanidyl) or between positively charged groups in the phospholipid (e.g. choline) and negatively charged amino acid side chains (glutamyl, aspartyl). Kinsella (1979) reported that fat binding capacity is mostly affected by lipid-protein complexes and protein content of the sample (protein flour, protein isolates, protein concentrates).

2.10.3 Foam and film forming properties

Foaming is usually considered separately from the other functional properties of food macromolecules because it relies on the behaviour of the biopolymers at interfaces rather than in bulk solution. Food foams are usually dispersions of gas bubbles in a continuous liquid or semi-solid phase that contains a soluble surfactant. Protein foams are important in preparation of several food products such as meringue, chiffon desserts, fudges, confectionery products, whipped toppings, whipped creams, ice creams, beer froth, bakery products, soufflés and mousses. In foaming, the gas is air (occasionally carbon dioxide) and the continuous phase is an aqueous solution or suspension containing proteins. In food foams the kinetic barrier to bubble coalescence and rupture is typically provided by a protein film surrounding the bubble. During foam formation, soluble proteins are subjected to an interfacial exposure that alters their structure and allows their subsequent association with other proteins in the interface (German *et al.*, 1985).

Foam stability is closely related to protein solubility; therefore, a good foam stability is observed at higher nitrogen solubility. Non-protein nitrogen compounds, carbohydrates and minerals also affect foam stability (Cherry and McWaters, 1981).

Foaming and film formation depend on several parameters such as concentration, solubility, type of protein, pH, ionic strength, pretreatment and different types of forces. It has been reported that extensively heat-denatured proteins show poor foam stability (Bickermann, 1953; Narayanan and Narasingha Rao, 1982; Tasneem *et al.*, 1982).

Data on foaming properties of proteins from many legumes are lacking. Susheelamma and Rao (1978a, 1978b) showed that globulins of black gram have

excellent surface activity and serve important foaming properties in the preparation of idli, with the help of arabinogalactan polysaccharides.

Several methods may be used for measuring foaming properties of proteins. Generally, shaking, sparing (gas bubbles are forced through a rather dilute protein solution and a column of foam is allowed to form above the solution) or whipping to incorporate air into the protein solution, and injection methods are employed. One widely used method is to determine the foam volume that can be achived with a given amount of protein as a function of time of aeration (Ross and Miles, 1953). The expansion usually goes through a maximum, reflecting the dynamic balance between bubble formation and destruction by severe whipping. However, whipping of a highly concentrated protein solution in a standard mixture requires a large amount of protein. Therefore, this method is not suitable for purified or concentrated proteins. When the concentrated protein solution is completely incorporated into the foam, the volume of the overrun (ratio of volume of foam to the initial liquid volume as a percentage; Halling, 1981) is measured and the maximum overrun developed is used as a measure of foaming ability. For small sample size sparing or bubbling method is often used. This method has been improved by Waniska and Kinsella (1979) using water jacketed columns and a lesser volume of protein solution. Halling (1981) reported that foam formation by shaking tends to be slower than bubbling or whipping under similar conditions.

There are two obvious characteristics of foam stability, first is the leakage of liquid (syneresis) and second is the collapse of air bubbles. These visible processes do not

correspond directly to lamellae drainage and rupture and measurement of foam volume is difficult. Halling (1981) and German *et al.* (1985) reported that foaming by bubbling or whipping and then measuring the stability of the foams by drainage/leakage or syneresis rate is very common. In another method, specific surface area from photographs as a function of time was used for determining foam stability. In this method the side of a foam should properly represent the whole foam, and that the bubbles should be spheres, and not miscellaneous polyhedrous. Some researchers have determined the short lifetime of single bubbles against a planar interface for foam stability (Mita *et al.*, 1978; Stainsby, 1986).

2.10.4 Emulsifying properties

The formation of an emulsion by a protein is mainly due to solubilized protein molecules and is enhanced by the surface activity and the ability of the protein to stabilize oil-water emulsions (Sosulski and Fleming, 1977). The emulsifying capacity of soluble proteins is based on the hydrophilic-lipophilic balance in the molecules which determines their affinity for oil and water. Sosulski (1979b) reported that the amino acid composition, protein conformation in the solution, pH and the ionic strength of the aqueous phase influence the emulsifying properties of proteins. Emulsions of fats and water are thermodynamically unstable due to a positive free energy caused by interfacial tension. Formation of a charged layer around the fat globules causes mutual repulsion, and/or possible formation of a membrane film around the droplets by solutes (such as by proteins) which may lead to a decrease in interfacial energy and prevention of

coalescence, thereby stabilizing the emulsion. Therefore, solubility of proteins is important for emulsion properties. In foods, a number of substances that are partly soluble in both the oil and water can stabilize the emulsion. Proteins are capable of unfolding at the interface and may also function as emulsifiers and emulsion stabilizers. For stable emulsion, protein molecules must be able to unfold enough to expose hydrophobic groups to act as stable emulsifiers. Protein molecules that contain cross links, such as disulphide bonds, are more rigid and remain folded, therefore these types of protein molecules are less effective in emulsion formation (Haque and Kinsella, 1988; Mangino, 1989).

The formation and stability of an emulsified oil droplet depend on the formation of a charged layer around the droplet causing droplet repulsion and the formation of a film around the droplet by solutes such as proteins (Kinsella, 1976). Hydrophobic regions of protein molecules associate at the lipid interface while polar and ionic regions interact with the aqueous phase (Johnson and Brekke, 1983). McWatters and Cherry (1977) showed that total protein composition (soluble plus insoluble) as well as components other than proteins (possibly carbohydrates) in various test material may contribute substantially to emulsification properties of protein-containing products.

The legume proteins have good potential as food emulsifiers (Sathe and Salunkhe, 1981a; Satterlee *et al.*, 1975). Sathe and Salunkhe (1981a) reported that albumins (the water-soluble proteins) appear to be more promising than globulins as emulsifying agents.

Three main tests have been devised to assess the effectiveness of proteins to serve as emulsifiers in food systems; a number of which measure emulsifying capacity (Swift *et al.*, 1961; Carpenter and Saffle, 1964; Inklaar and Fortuin, 1969). These methods generally involve adding lipids to an aqueous solution of the protein to be tested until phase inversion occurs. Thus, the test measures emulsifying capacity of the protein expressed as millilitres of oil emulsified per gram of protein.

Another means of estimating emulsion stability is to form an emulsion under conditions that resemble those in the actual food product. The emulsion is then allowed to separate either under the influence of gravity or after exposure to a centrifugal force. Within a centrifugal force the fat globules are compacted into a cream layer and an aqueous layer devoid of fat is formed. The ratio of either the cream layer or the aqueous layer formed to the volume of the initial emulsion is often utilized as an indicator of emulsion stability. The change in lipid distribution throughout the sample with time can be measured and the phase separation with time is taken into account as emulsion stability (Inklaar and Fortuin, 1969). The size distribution of the particles in an emulsion can also be used as an indicator of the effectiveness of the emulsifier but determination of the size distribution is very tedious. This method requires the use of electron microscopy (Mangino, 1989). Pearce and Kinsella (1978) described a method based on light scattering which is easy and requires a very small amount of sample. Nakai and Li-Chan (1988) demonstrated that the emulsifying activity index and emulsion stability index per min, measured according to this procedure, correlate well with surface hydrophobicity of food proteins.

2.10.5 Gelation and Viscosity

Gelation is defined as the formation of an infinite network by aggregation of trifunctional and bifunctional units or denatured protein molecules. Proteins form gels by polymerizing into a three-dimensional matrix which converts a viscous liquid into a viscoelastic solid. Gelation has been classified as a hydration, structural, textural and rheological property of proteins (Kinsella, 1976). Gelation is a very important functional property and plays a major role in preparation of various coagulated egg white, gelatin gels, soybean protein gels, dairy products, vegetable proteins texturized by extrusion or spinning, bread dough and heated comminuted fish and meat products. Protein gels are also utilized for the formation of solid viscoelastic gels as well as for improving water absorption, particle binding, emulsion and foam stabilizing and thickening (Cheftel *et al.*, 1985).

Protein gels are characterized by a relatively high viscosity, plasticity, and elasticity. The ability of proteins to form gels and to provide a structural matrix for holding water, flavours, sugars and other food ingredients is useful in food applications, and in new product development which adds a new dimension to protein functionality. Several researcher have shown that protein denaturation and unfolding prior to the step of ordered protein-protein interaction and aggregation is important in gelation of denatured proteins which involves a two step process of activation and association. The activation is caused when heat produces a change in protein structure so that interactions can occur intermolecularly. The three-dimensional matrices or networks of intertwined, partially

associated polypeptides, results from the protein-protein and protein-solvent (generally water) interactions as well as attractive and repulsive forces of adjacent polypeptide chains in the system. The attractive forces are hydrophobic and electrostatic interactions, disulphide cross linkings and hydrogen bonding.

The most important food processing techniques relative to protein gelation involve divalent cations (such as calcium) and/or heat treatment. Heat treatment is effective for establishment of covalent disulphide cross links which leads to the formation of heat irreversible gels. For the formation of a highly ordered gel matrix, it is imperative that the aggregation step after heating proceeds at a slower rate than the unfolding step (Hermansson, 1979). Different types of proteins can form gels when heated together or through interaction with gelling agent polysaccharides. Many gels have shown highly expanded and hydrated structures with water and other food constituents entrapped in the protein network. Circle *et al.* (1964) established the basic factors affecting gelation properties of soybean protein. Thus, the method of protein preparation its concentration, rate, temperature and duration of heating, cooling conditions, the presence of salts, thiols, sulphites and lipids all influence the properties of gel formation. Gelation and viscosity also depend on the presence/absence of non-protein components. Sathe and Salunkhe (1981a) indicated that smallest gelation concentrations for the Great Northern bean flour, albumins, protein concentrates, and isolates and noted that globulins did not form a firm gel up to a concentration of 20% (w/v). They also noted that the viscosity was concentration-dependent. Albumins (the water-soluble proteins) are more viscous due to water solubility than globulins which are more compact in structure and need the

presence of denaturing/dissociating agents to increase their solubility.

Gelation properties of proteins are complex and very difficult to interpret due to the extremely specific conditions required for gel formation. Heat-induced gel formation generally involves heating of protein dispersions in gel tubes, or in sausage casings. After gel formation, gel strength or hardness, adhesiveness, cohesiveness and elasticity may be measured. Dynamic rheology is currently being extensively used for the study of flow behaviour and gelation. Kinsella (1976) reported that microscopic analyses may prove extremely useful in evaluating protein gels.

2.10.6 Solubility

Solubility of protein is essential for determining their usefulness in various types of food formulations. Solubility and viscosity are two experimentally measurable properties that may afford information about the functional behaviour as well as physicochemical nature of proteins. The degree of insolubility is probably the most practical measure of protein denaturation and aggregation, because proteins that initially exist in a denatured and partially aggregated state, often exhibit impaired ability to participate effectively in gelation, emulsification and foaming (Kinsella, 1976). Solubility of proteins at neutral or isoelectric pH is the primary functional property measured at each stage of preparation and processing of a protein product. Most users rely on nitrogen solubility index (NSI) or protein dispersibility index (PDI) as a quick test of the functional properties of food proteins (Johnson, 1970) which are a function of pH, ionic strength and temperature

(Kinsella, 1979; Cheftel *et al.*, 1985) as well as presence or absence of other components which are capable to bind with proteins as well as solvent used for protein extraction and food product preparation. Dilute alkali provides a desirable solvent for effectively solubilizing seed proteins (Sathe *et al.*, 1984). A protein with a high initial solubility enhances rapid and extensive dispersion of its molecules, thus leading to a finely dispersed colloidal system. Cheftel *et al.* (1985) reported that initial solubility enhances protein diffusion to air/water and oil/water interfaces, thus improving their surface activity. Shen (1981) showed that proteins interact with the solvent by dipole-dipole and hydrogen or ionic bonding in order to become more soluble.

The solubility of a protein under a given set of conditions may be expressed as the manifestation of equilibrium between the protein-solvent [hydrophobic and the protein-protein (hydrophilic)] interactions. Many of the molecular and functional properties of food proteins are related to the proportion of hydrophobic and hydrophilic amino acids and their distribution in their primary structure. The average hydrophobicity and charge distribution are the most important molecular features that influence physical properties, such as solubility of proteins (Bigelow, 1967; Nakai and Li-Chan, 1988). The extent of exposure of hydrophobic surfaces at the exterior of protein molecules determines the solubility as well as other solution properties related to physico-chemical characteristics of the protein.

Proteins at pH values, higher or lower than their isoelectric point, carry a net negative or positive charge, respectively. Water molecules interact with these charges and contribute to the solubilization of proteins and dissociation of aggregates or unfolding of

their native structures. The solubility of a given protein as a function of pH is U- or V-shaped reaching a minimum in the vicinity of the isoelectric pH (pI). At this point, protein molecules show minimum interactions with water and their net charges are sufficiently minimal to prevent aggregate formation and protein precipitation (Shen, 1981; Cheftel *et al.*, 1985).

The ions of neutral salts at 0.5 to 1.0 M may increase the solubility of proteins due to a salting-in effect. The ions interact with the charges of proteins and decrease the electrostatic attraction between opposite charges of the neighbouring molecules. Moreover, the solvation connected with these ions serves to increase protein solubility. At concentrations greater than 1 M, neutral salts reduce protein solubility and may cause their precipitation due to a salting-out effect. At high salt concentrations, water molecules are occupied by solvating ions not enough water molecules are available for protein solvation. Thus, protein-protein interactions become stronger than protein-water interactions which may lead to the aggregation followed by precipitation of protein molecules.

2.10.7 Other functional properties

Other functional properties of proteins include their microscopic properties, adhesiveness, cohesion, flow properties as well as bulk and packing densities. Chang and Satterlee (1981) studied the major proteins from Great Northern beans and reported that 50% of the native proteins have α-helix character. Sathe (1981) employed scanning

electron microscopy to study the surface topography of Great Northern bean albumins and globulins and reported that albumins had a rod-like character while globulins had irregular shapes. Padhye (1979) studied the UV spectra of black gram proteins and found that albumins, globumins, prolamines, and glutelins had similar UV spectral profiles. However, information available is insufficient on these aspects of legume seed proteins. Therefore, more intensive efforts are needed to appreciate and understand these functional properties of legume seed proteins.

2.11 *In-vitro* protein digestibility

In-vitro protein digestibility (IVPD) of pea proteins depends mostly upon the size and nature of proteins, their amino acid composition and processing conditions (Hsu *et al.*, 1977). Ortega-Nieblas *et al.* (1996) have shown that IVPD of wild legume seeds from the Sonora desert (*Acacia farnesiana, Cercidium microphyllium, Cercidium sonorae, Mimosa grahamii, Olneya tesota, Parkinsonia aculeata* and *Prosopis juliflora*) ranges between 67 and 84%. These values are higher than those reported for conventional legumes such as common beans (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), green pea (*Pisum sativum*), and soybean (*Glycin max*) proteins (64 to 67%) (Elias and Bressani, 1976). Vijayakumari *et al.* (1997) reported *in-vitro* protein digestibility of 59.5% for *Bauhinia purpurea* legume seeds. Ortega-Nieblas *et al.* (1996) noticed that the *in-vitro* digestibility of legume seeds of Sonora desert increased by 5 to 10% upon heat treatment at 75 °C for 5 min. The improvement of legume IVPD due to autoclaving or heat treatment may be attributed to the denaturation of native proteins

including enzyme inhibitors.

Johnson and Brekke (1983) reported that protein isolates from yellow peas had *in-vitro* protein digestibility of up to 84%. They also reported that when protein isolates were acetylated at 1 mM and 3 mM anhydride/g protein significant improvement were brought about for *in-vitro* protein digestibility (from 84 to 90%). Wanasundara and Shahidi (1997) reported that flaxseed protein isolates had 90% *in-vitro* digestibility with pepsin-trypsin and pepsin-pancreatin. They also reported that acetylation and succinylation at higher concentrations reduced protein digestibility.

2.12 Starch

The major reserve carbohydrate of most legume plants is starch, representing up to 45% of the total seed weight. Starch is the only universally produced polysaccharide in small individual packets called granules.

Structural and functional properties of the main starches such as those of wheat, potato, rice and corn have been extensively studied because these starches are mostly utilized in food applications. However, legume starches have not been subjected to intensive research and neither have they been used widely in the food industry. Nonetheless pea starch has been used as a model to understand the relationship between starch biosynthesis, structure and functional properties (Borgracheva *et al.*, 1998). Jenkins *et al.* (1980) reported that legume starches are of great interest to nutritionists since they exhibit a lower glycemic index than cereals, thereby helping in the dietary

control of diabetes as well as arterial diseases. Most of the studies have so far been on tuber and cereal starches, hence only limited information is available on legume starches.

2.12.1 Starch isolation and purification

Starch isolation from legume seeds is difficult due to the presence of insoluble flocculent protein, fine fibre, and phenolic compounds, which decrease sedimentation and cosettle with the starch to give a brownish deposit (Schoch and Maywald, 1968; Hoover and Manuel, 1996). Generally, most of the legume starches are isolated using pin milling as well as air classification and aqueous extraction techniques (Wright *et al.*, 1984). Vose *et al.* (1976) have shown that air classified starch-rich fractions contain more than 70% starch and very low amounts of protein. Reichert and Youngs (1978) have reported that remilling and reclassification of air-classified pea starch granules, followed by water washing, removes most of the residual protein attached to starch granules. Hoover and Sosulski (1985a) also pointed out that repeated filtration through polypropylene screens (202 and 70 μm) combined with alkali treatment brings about a substantial reduction in the protein content of wet-process extracted legume starches belonging to the species *Phaseolus vulgaris*. Broad bean and smooth pea starches could be extracted in high yields of 93.8 - 96.7% from their respective flours after protein extraction at pH 9.0 using different sieving (200-60 μm) and washing conditions.

2.12.2 Granule size

Most of the legume starch granules are oval, although some are also spherical, round,

elliptical, and irregular in shape. The granule size is variable and ranges from 4 to 85 μm depending on the starch source. The size and shape of starch granules may vary from plant to plant, each different and recognizable as to the plant source. The surfaces of all granules are mostly smooth with no evidence of any fissures when observed under scanning electron microscope (SEM). Some legume starches show the presence of grooves (Pigeonpea starch; Hoover *et al.*, 1993). Several types of legume starches are simple granules, the exception being wrinkled pea starch and green arrow pea starch which appear to be a mixture of simple and compound granules, the latter being composed of 3-10 individual subunits joined together (Colonna *et al.*, 1982; Hoover and Manuel, 1996).

2.12.3 Chemical composition

Protein and ash contents in the isolated legume starches are 0.10 - 1.12% and 0.03 - 0.81%, respectively. Meanwhile, the lipid content in different legume starches range from 0.01 to 0.9%, when extracted by acid hydrolysis as well as hot n-propanol-water (3:1, v/v) (Hoover *et al.*, 1988a). Most legume starches are characterized by a high amylose content (24 - 65%). The amylose content of legume starches varies and this may be due to the different methods used for their determination, varietal differences (Shahen *et al.*, 1978), physiological state of the seed (Banks *et al.*, 1974), or the amount of amylose in lipid complexed during estimation (Morrison and Laignelet, 1983).

2.12.4 Molecular structure of starch

Starch is generally composed of two polysaccharides, namely amylose and amylopectin. Amylose, the less prevelant component, consists of much longer linear chains of α-D-glucose residues linked by (1→ 4) bonds. However, it is now accepted that amylose is not completely linear (Hizukuri *et al.*, 1981) although its solution properties are typical of linear polymers. Amylopectin is the major component of starch and contains α-(1→ 6)-D-linkages and linear chains of α-(1 → 4)-D-glucose residues. In addition to the fine structure, amylose and amylopectin differ in many respects as summarized in Table 2.8. Differences in solution behaviour and susceptibility to various degrading enzymes are mainly a consequence of structural features and molecular size of these polymers. Starch owes much of its functionality (such as thickening agent, flavour carrier, and binder in food related applications) to its (amylose and amylopectin) components as well as to the physical organization of these macromolecules in the granular structure. Therefore, structure of starch needs to be considered at two distinct levels: (i) at a molecular level that refers to the amount, fine structure, size, and shape of the component molecules and (ii) the supermolecular structure of the granules. Much information concerning the fine structure of amylose and amylopectin has been accumulated from studies on cereal and tuber starches, but little information is available on legume starches.

Physico-chemical characteristics of some legume starches have been described by Hoover and Sosulski (1991). The iodine binding capacity (IBC), limiting viscosity number (n), degree of polymerization (DP) and β-amyolysis limit for amylose from

Table 2.8 Physico-chemical properties of starch polymers[1]

Property	Amylose	Amylopectin
Molecular structure	Essentially linear, α-(1 → 4)	Branched, α-(1 → 4) and α-(1 → 6)
Degree of polymerization	~10^3	~10^4 - 10^5
Molecular weight	1.5 x 10^5 - 10^6	(50 - 500) x 10^6
Average chain length	~10^3	~20 - 25
Iodine complex, λ_{max} (colour)	640 - 600, nm, (blue)	530 - 550, nm, (purple)
Digestibility (%)		
β-Amylase	~70	~55
β-Amylase and debranching enzyme	~100	~100
Stability of dilute aqueous solution	Unstable (retrogrades)	Stable
Gel texture	Stiff, irreversible (T_m > 100 °C)	Soft (thermally reversible at < 100 °C)
Film properties	Strong, coherent	Brittle

[1]Adapted from Biliaderis (1991).

legume starches are in the range of 16-22, 136-280, 1000-1900, and 79-86%, respectively. The molecular weights of only selected legume amyloses have been determined and these range from 165,000 to 312,000 Da. The viscosity of legume amyloses is lower than those reported (Banks and Greenwood, 1967) for cereals (330-435 BU) and potato (410BU). Legume amyloses treated with β-amylase and pullulanase undergo incomplete conversion to maltose, thus providing evidence of the existence of α-(1 → 6) linkages in amylose and that these linkages constitute the only barrier to the action of β-amylase on amylose (Biliaderis *et al.*, 1981).

Biliaderis *et al.* (1981) and Colonna and Mercier (1984), using gel chromatography and light scattering techniques, reported that amylopectins from legume starches have average molecular weights greater than 1.8×10^7 Da. Iodine affinities, average chain lengths, and chain molar ratios have been shown to range from 1.0 to 5.3, 20 to 26 and 4.2 to 9.6, respectively, for amylopectins from legume starches (Biliaderis *et al.*, 1981). Colonna *et al.* (1981) have shown that the fine structure of amylose and amylopectin of broad bean and smooth pea starches resemble those of normal cereal starches. Colonna and Mercier (1984) reported the presence of a branched intermediate fraction of low molecular weight in wrinkled pea and smooth pea.

2.12.5 Solubility and swelling power

Starches are insoluble in water below their gelatinization temperature. However, they may swell sometime in cold water up to 10-20%, owing to diffusion and absorption

of water into the amorphous regions; this type of swelling is reversible upon drying. When granules are heated to progressively higher temperatures in water, a point is reached where granule swelling becomes irreversible and structural order disappears. As the granules continue to expand, amylose leaches out into the aqueous intergranular phase. These processes result in a substantial increase in viscosity.

Legume starches have been shown to exhibit a single stage restricted swelling and low leaching patterns (Wankhede and Ramteke, 1982; Hoover and Manuel, 1996). Cereal starches such as maize starch show multiple temperature relaxation, but legume starches show only one relaxation temperature due to strong bonding forces between their components (Schoch and Maywald, 1968). The strong intermolecular attraction between starch components may reflect an orderly arrangement of polymer chains within the starch granule, permitting close parallel alignment, thus favouring maximum interaction via hydrogen bonding. Legume starches show a wide variation in their swelling power and also solubility, mostly in the range of 60 to 90 °C (Hoover *et al.*, 1993; Hoover and Manuel, 1996). This characteristic of legume starches may be due to the melting of crystallites which involves a solvation-assisted helix coil transition of their chains (Biliaderis *et al.*, 1980). Helix coil trasition change may be responsible for increase in entropy that would offset the hydrogen bonding occuring in the crystalline regions leading to increased swelling and solubility. Deshpande *et al.* (1982) and Hoover and Sosulski (1985b) have reported that physically- and chemically-modified legume starches show a higher solubility and a lower swelling power than unmodified starches, perhaps due to the

loss of granular structure and extensive amylose leaching.

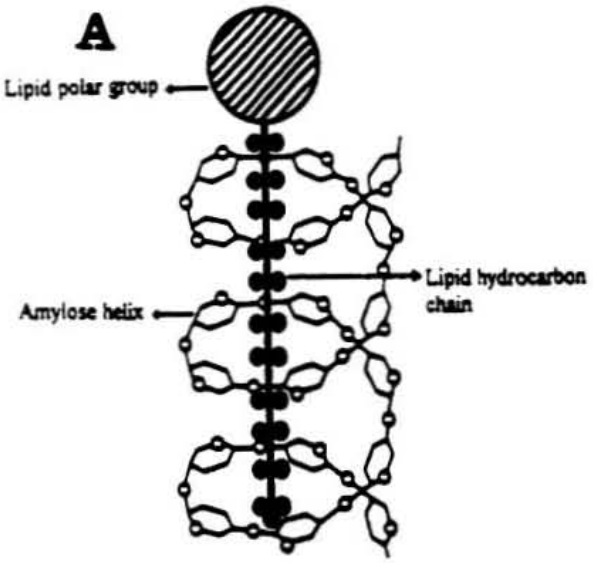
2.12.6 Starch-lipid interactions

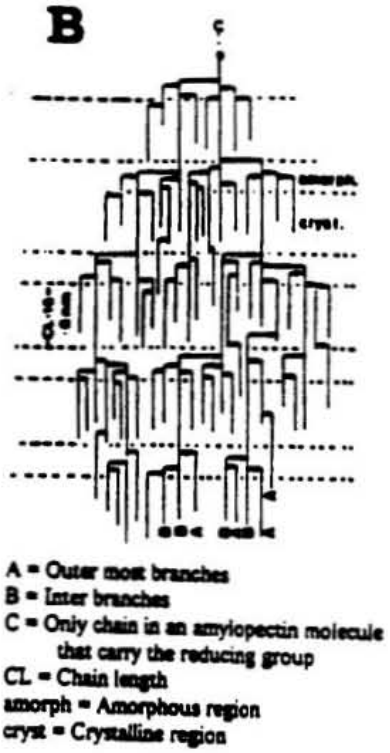
Lipids as well as surfactants are well known to act as texture modifiers in many starch food products. Lipids mostly retard firming and retrogradation properties of starch. Anti-staling properties of lipids is mostly attributed to the formation of helical inclusion complexes between starch molecules (mainly amylose) and the relevant emulsifiers (Figure 2.12A). Lipids also play an important role in the gelatinization and rheological properties of starch, which depend mostly on the type of starch and lipid as well as the time, temperature-shear history during gelation and storage (Takahashi and Seib, 1988). Hoover and Hadziyev (1982) reported that lipids present on the surface of the starch granules may also affect the water diffusion into the granules. Lipids coating the surface of granules have been shown to retard the distribution of starch granules during gelatinization (Eliasson *et al.*, 1981).

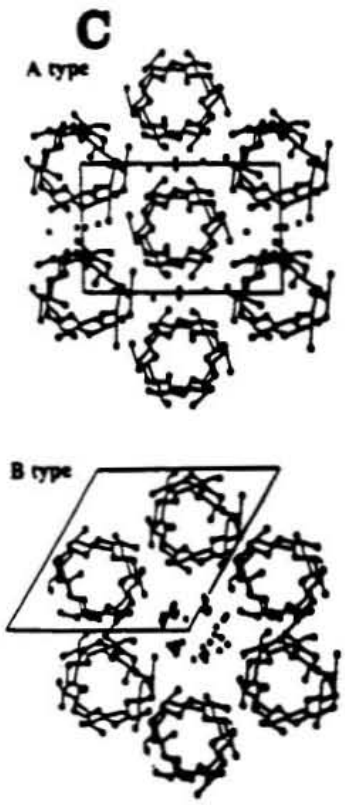
2.12.7 Crystallinity of starches

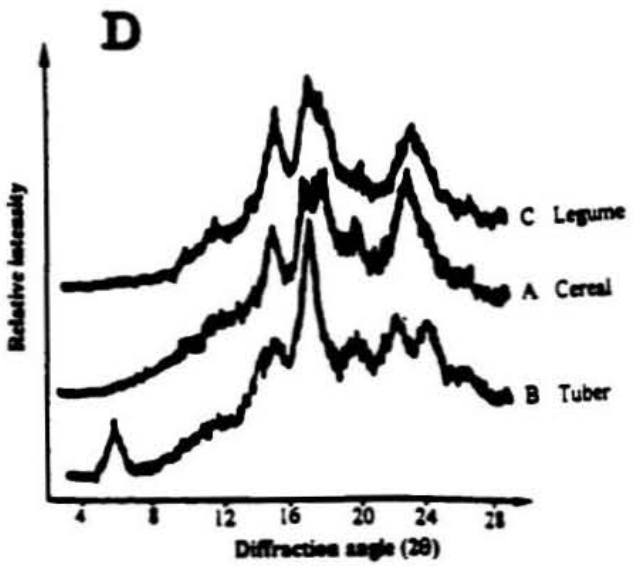
X-ray diffractometry has been used to reveal the presence and characteristics of crystalline structures of starch granules (Sarko and Wu, 1978). Katz and Van Itallie (1930) have distinguished three types of crystalline structures in intact starch granules using X-ray diffraction which gave patterns designated as A-, B-, and C- type (Figure 2.12D). The structural type depends on the botanical source of the starch: the A-type is observed for most starches of cereal origin (wheat, rice and corn); the B-type is prevalent

Figure 2.12 Characteristics of starches: (A) lipid-amylose complex; (B) cluster model of amylopectin; (C) double helices structure of amylose; (D) X-ray diffraction patterns for starches.
(adapted from Senanayake, 1995).


A
Lipid polar group
Lipid hydrocarbon chain
Amylose helix


B
C
A = Outer most branches
B = Inter branches
C = Only chain in an amylopectin molecule that carry the reducing group
CL = Chain length
amorph = Amorphous region
cryst = Crystalline region


C
A type
B type


D
Relative intensity
C Legume
A Cereal
B Tuber
4
8
12
16
20
24
28
Diffraction angle (2θ)

in tuber, fruit, and high amylose corn (>40%) starches as well as in retrograded starch; and C-type which is intermediate between A- and B-polymorphs, is observed for legume seed starches (peas and beans). Slight differences in the chain length and profile of amylopectin molecules may be responsible for differences in the X-ray patterns (Hizukuri, 1985). Starch crystallites are due to sequential packing of double helices (Wu and Sarko, 1978a, b) that are formed between the flexible "A" chains of amylopectin (French, 1972). A-chains are unbranched and linked to B-chains through α-(1 $\rightarrow$ 6) bond at its reducing end; B-chains can be either linked to another B-chain or to a C-chain in the same manner; C-chains function like B-chains and carry one functional reducing group in the molecule (Robin, 1974, Figure 2.12B). Most legume starches exhibit a "C" type X-ray diffraction pattern (Figure 2.12D), (Kawamura, 1969; Sarko and Wu, 1978; Colonna *et al.*, 1981). Hoover and Sosulski (1985a) have shown that most legume starches are characterized by two very strong intensity lines centred at 17.2 and 18.1° 2θ angle, which correspond, respectively, to the interplaner spacings of 5.15 and 4.98 Å (1 Å = 0.1 nm). In contrast, Colonna *et al.* (1982) reported that wrinkled pea starch exhibits a "B" type X-ray pattern with peaks that are both broad and weak, with the two main reflections centred at 5.5 and 17 Å 2θ angles. Gernat *et al.* (1990) have shown that the legume starch "C" crystalline polymorph is a mixture of "A" and "B" unit cells, and that these starches contain pure 'A' and 'B' polymorphs in varying proportions. For example, pea starch is composed of 61.4% type 'A' and 38.6% type 'B'unit cells, whereas broad bean starch is composed of 83.0% type 'A' and 17.0% type 'B'unit cells. This indicates that the unit cell ratio (A/B) may differ widely among legume starches.

The arrangements of duplexes into the crystal lattice is nearly the same in type 'A' and type 'B' structures, except that the unit cell of 'B' type amylose contains 36 water molecules in a channel formed by the hexagonally packed double helices; in type 'A' amylose this channel is formed by another double helix and the water molecules (eight/unit cell) are distributed in interstitial spaces between strands (Figure 2.12C). Levels of crystallinity, determined by integration of the areas under the crystalline diffraction peaks, range between 15 and 45%, depending on the source of starch (Zobel *et al.*, 1988).

2.12.8 Gelatinization

Starch, in the presence of excess water and heat, undergoes disruption of its granular structure, swelling, hydration, solubilization and an order-disorder phase transition called gelatinization (Donovan, 1979; Biliaderis *et al.*, 1980). Gelatinization temperature range is a characteristic of the source of starch and is associated with the diffusion of water into the starch granule, its hydration and swelling, uptake of heat, loss of crystallinity, decreased relaxation time of water molecules, and amylose leaching (Stevens and Elton, 1981; Lelievre and Mitchell, 1975). Gelatinization is of great importance to many food processing operations; several analytical techniques have been used to probe this phenomenon and quantitatively determine the amount of gelatinized starch in processed foods.

Differential scanning calorimetry (DSC) has been widely used in the study of starch gelatinization. Of the various methods presently available for determination of starch gelatinization, namely Kofler hot stage microscope (Watson, 1964), X-ray diffraction (Zobel *et al.*, 1988), DSC (Donavan, 1979), pulsed nuclear magnetic resonance (Lelievre and Mitchell, 1975), enzymatic digestibility (Shiotsuba, 1983), viscoamylography (Sathe and Salunkhe, 1981b), and small-angle light scattering photometer (Marchant and Blanshard, 1980), only the Kofler hot stage microscope and DSC have been widely used in studying the gelatinization temperatures of legume starches. The gelatinization temperatures, onset (T_o), midpoint (T_p) and conclusion (T_c), as determined by Kofler hot stage and DSC, are not in agreement in many cases. This discrepancy may be due to varietal differences or to differences in water content of the starch-water slurries used in the experiments. Decreases in gelatinization temperatures and a widening of the gelatinization temperature range were found to occur when legume starches were subjected to modification by acetylation (Comer and Fry, 1978), hydroxypropylation (Hoover *et al.*, 1988b), and cross-linking (Deshpande *et al.*, 1982).

Using a small angle light-scattering technique, Blanshard and co-workers (Marchant and Blanshard, 1978; Bhuiyan and Blanshard, 1980;) have suggested that rearrangements of the polymer chains in the amorphous regions and the remaining crystallites might occur in partially gelatinized granules. They have also examined the dynamics of the gelatinization in terms of the three constituent processes; (a) diffusion of water into the granule; (b) hydration-facilitated melting of crystallites; and (c) swelling owing to further hydration of the disordered polymer chains.

The gelatinization of starch is also influenced by the presence of small molecular weight solutes (non-ionic and electrolytes) as well as by hydrophilic hydrocolloids. Understanding these effects is important for better process control and for improving the texture and other quality attributes of starch-based foods.

2.12.9 Retrogradation

Starch granules, when heated in excess water above their gelatinization temperature, undergo irreversible swelling, resulting in amylose leaching into the solution. In the presence of a high starch concentration, this suspension will form an elastic gel on cooling. The molecular interactions that occur after cooling are known as retrogradation. The extent of swelling and granule disintegration as well as exudation of amylose depend on the type of starch, starch concentration, temperature, presence of other solutes, and the shear or agitation applied during heating. Miles *et al.* (1985a) reported that amylose gelation occurs as a result of a phase separation, which produces regions that are rich and deficient in polymer and that, if the amylose concentration is sufficiently high, the region rich in polymer forms a three dimensional network. Amylose crystallization was found to be a secondary process, occuring in the region rich in polymer. Rates of retrogradation can also be determined using a light-scattering equipment (Paschall and Foster, 1952; Foster and Sterman, 1956) or a spectrophotometer (reduction in transmitted light) (Craig *et al.*, 1989). Effects of storage on the microstructure of potato starch pastes (Svegmark and Hermansson, 1993) and oat and barley starch pastes (Autio *et al.*, 1992; Virtanen *et*

al., 1993) have been evaluated using light microscopy of iodine-stained pastes. The size of swollen starch granules (determined microscopically and by swelling power) has been correlated with the melting enthalpy of crystalline amylopectin (Ellis *et al.*, 1988 Jacobson *et al.*, 1997). Heterogeneous acid hydrolysis of waxy maize amylopectin gels followed by gel permeation chromatographic studies of the residue, has shown that amylopectin crystallization occurs by association of amylopectin molecules with a degree of polymarization of 15 (Ring *et al.*, 1987). The crystallization of amylopectin was shown to be reversible at temperatures below 100 °C, whereas the initial gelation and crystallization of amylose was irreversible even at 100 °C (Ring *et al.*, 1987). This shows a greater degree of molecular interaction in the latter process which was found to be more important at high starch concentrations.

The molecular structures and transformations that occur during gelation and retrogradation of starch and its components have been reported by several investigators (Clark *et al.*, 1989). Miles *et al.* (1985a, b) reported that retrogradation consists of two separate processes: (a) exuding of amylose molecules from the granules during gelation and (b) recrystallization of amylopectin. The initial development in firmness is attributed to a rapid establishment of a cross-linked network of amylose chains at concentrations above the coil overlap concentration. Subsequent increase in rigidity of starch gels are linked to recrystallization of amylopectin by short degree of polymerization chain clusters (Ring *et al.*, 1987).

2.12.10 Digestibility

Legume starches in foods have been found to be more digestible than those from tubers, but less than those of cereals (Socorro *et al.*, 1989). Ring *et al.* (1988) have shown that during a 24 h digestion with porcine pancreatic α-amylase, the percentage hydrolysis of native starches from wheat, maize, smooth pea, and potato were 100, 95, 67 and 15%, respectively. A similar observation was made by Hoover and Sosulski (1985a) during a 6 h digestion of corn starch hydrolyzed to 75% by porcine pancreatic α-amylase, whereas the corresponding values for legume starches of the *Phaseolus vulgaris* species ranged from 26 to 35%. These differences in the *in-vitro* digestibility of starches among and within species have been attributed to the interplay of many factors such as starch source (Ring *et al.*, 1988), granule size (Snow and O'Dea, 1981), starch-protein interaction (Würsch *et al.*, 1986), amylose/amylopectin ratio (Dreher *et al.*, 1984), percentage of retrograded starch (Ring *et al.*, 1988), extent of molecular association between starch components (Holm and Björck, 1988), physical distribution of starch in relation to dietary fibre components (Rao, 1969), antinutrients (Thompson and Gabon, 1987), α-amylase inhibitors (Puls and Keup, 1973), degree of crystallinity (Ring *et al.*, 1988), amylose chain length (Jood *et al.*, 1988), amylose lipid-complexes (Holm *et al.*, 1983), and the influence of drying methods and storage conditions (Kayisu and Hood, 1979). Differences in the observed digestibility of starch samples could also be attributed to differences in the α-amylase activity of enzyme preparations and their source (Rosenthal and Nakamura, 1972).

Starch granules from legumes generally exhibit only roughened surfaces indicating surface erosion (Ramadas Bhat *et al.*, 1983). El Faki *et al.* (1983) reported that salivary α-amylase attacks cowpea and horse gram starch causing pitting as well as surface erosion resulting in onion-type layering of degraded granules.

Attempts have been made to improve the *in-vitro* digestibility of legume starches by cooking (Rao, 1969), soaking (Jood *et al.*, 1988), and germination (Kataria and Chauhan, 1988). Kataria and Chauhan (1988) have shown that soaking of mung beans in water for 18 h increased starch digestibility by 40%. However, cooking of soaked and unsoaked seeds increased starch digestibility by 623 and 555%, respectively. The corresponding values were 207 and 145% for black gram and 163 and 100% for chickpeas (Jood *et al.*, 1988), respectively. Heat treatments substantially enhance legume starch digestibility (Socorro *et al.*, 1989). *In-vitro* digestibility of baked products is improved with increasing degree of gelatinization (Wootton and Chaudhry, 1979). Amylose content and yield of resistant starch increased by wet autoclaving-cooling of starches and these positively correlated with *in-vitro* digestibility (Sievert and Pomeranz, 1989). Commercial production of resistant starch from corn, potato, and leguminous purees, intended for infant food, includes appreciable amounts of legume starches (Siljestrom, 1989), perhaps due to the higher amylose content of legume starches as compared to cereals.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Samples

The fresh green (premature), immature and mature pods of beach pea (*Lathyrus maritimus* L.) were collected from Bellevue Beach, Salmon Cove and Sandy Cove in September and October of 1995 and 1996, in the province of Newfoundland and Labrador. The grains and pod shells were manually separated and the mature and immature seeds were further segregated according to their size, shape and colour. The fully developed seeds were large and dark green and are referred to as mature seeds, the immature seeds, on the other hand, were small, light and green. The fresh green or premature pods were collected from the above locations and their seeds and pod shells separated manually. Leaves and branches plus stems were also collected from these locations. The total fresh weight and recovery of components were recorded immediately after harvesting and before samples were freeze-dried (Free zone 6 litres, Freeze dry system models 7730, 1994, Labconco Corporation 79510 ECO 7526 Kansas, MI), ground (Moulinex coffee grinder), sieved using 60 mesh sieve (250 μm), and stored in air-tight containers for the analyses of their chemical composition. Seeds of green pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L., variety code X 850002) were obtained from Crop Science and Plant Ecology Department, University of Saskatchewan (Saskatoon, SK) and Agriculture and Agri-Food Canada (Morden, Manitoba), respectively. A sample of grass pea was also procured from a local market in Calcutta, India.

3.1.2 Chemicals

All chemicals used in the present studies were of the American Chemical Society (ACS) grade or better. Electrophoretic, spectroscopic and high performance liquid chromatographic (HPLC) grade chemicals were used for analyses and preparation of reagents as required. Glass-distilled water was used for preparation of reagents. Demineralized and Ultrapure water was used for elemental and HPLC analyses. Water was demineralized and its organic matter removed using the Ultrapure Bärnstead Reverse Osmosis system (Bärnstead, Boston, MA) coupled with organic removal, demineralization and submicron filtration connected to the Nanopure II system. Weight of samples and chemicals was measured using a Mettler AE100 or AE200 (Mettler Instrument AG. Greifensee, Switzerland) balance, while all volumes were measured using appropriate measuring devices.

3.2 Methods

3.2.1 Physico-chemical properties

Fully mature beach pea seeds were analyzed for their physico-chemical properties and results were compared with those of grass pea and green pea. The colour of seeds was determined subjectively (using six panel member). The weight of one hundred seeds was measured and their density determined using a cylinder containing 100 mL of deionized water. The seeds were kept in water in order to sink and the volume of displaced water was recorded. The mass and volumes were used to calculate the density. The hydration

capacity of seeds was determined in the following manner. Seeds (100 g) were transferred to a 250 mL measuring cylinder along with 100 mL of water. The cylinder was covered with aluminium foil and left overnight at room temperature. Seeds swollen at this stage were separated and weighed. Hydration capacity per seed was calculated from the ratio of the weight difference data between the soaked and unsoaked seeds and the number of seeds. Hydration index was subsequently calculated as the ratio of the hydration capacity per seed and the weight of one seed. For determination of swelling capacity, the volume of seeds (100 g) were measured in a graduated cylinder before and after soaking overnight. The volume of the soaked seeds was noted in a graduated cylinder. Swelling capacity per seed was calculated from the volume difference data of soaked and unsoaked seeds and the number of seeds used. Swelling index was then calculated as the ratio of the swelling capacity per seed to the volume of one seed (Akinyele *et al.*, 1986; Bishnoi and Khetarpaul, 1993).

3.2.2 Chemical analyses

3.2.2.1 Moisture

Moisture content was determined by weighing exactly 2 to 3 g ground seeds into a pre-weighed aluminium moisture determination pan (57 mm, Fisher Scientific Unionville, ON). The sample was then dried in a forced-air convection oven (Fisher Isotemp Oven 300 Series, Model 338F, Fair Lawn, NJ) at 105 °C overnight or until a constant weight was obtained (AOAC, 1990). The moisture content was calculated from the weight

difference data.

3.2.2.2 Crude protein

The crude protein content of each sample was determined by the Kjeldahl method (AOAC, 1990). Approximately 0.2 to 0.3 g of dried material, or 5 to 10 mL of liquid sample, were digested in 20 mL concentrated H_2SO_4 (Fisher Scientific Co., Fair Lawn, NJ) in the presence of two catalyst pellets (Kjeltabs Profamo Analytical Service Inc., Dorval, PQ) containing 5 g of K_2SO_4 and 0.25 g of HgO in a digester (Büchi 430, Büchi Laboratoriums-Technik AG, Schweiz, Switzerland) until a clear solution was obtained (380 °C 1h) on digestion. During digestion, the nitrogen was converted to ammonia in the form of ammonium sulphate. To the cooled digested samples, 50 mL of distilled water and 150 mL of 25% (w/v) NaOH were added in a distillation tube (Büchi 321, distillation unit, Büchi Laboratoriums-Technik AG, Schweiz, Switzerland). Samples were then steam-distilled and the distillate was collected into 50 mL of a 4% (w/v) boric acid solution (Fisher Scientific Co., Fair Lawn, NJ) containing 1 mL of indicator (N point indicator, EMSCT, Gibbstown, NJ). One hundred and fifty millilitres of distillate were collected and subsequently titrated with a standard 0.1 N H_2SO_4 solution to reach the end point. A sample blank (distillate collected with distilled water and NaOH only) determination was also carried out. Nitrogen contents of samples were calculated and reported as crude protein content using the following formula (AOAC, 1990).

Dried samples

$$\% \text{ Nitrogen} = \frac{\text{Volume of } H_2SO_4 \text{ (sample-blank), mL x Normality of } H_2SO_4 \text{ X } 14.007}{\text{Sample weight, mg}} \times 100$$

Liquid samples

$$\% \text{ Nitrogen} = \frac{\text{Volume of } H_2SO_4 \text{ (sample-blank), mL x Normality of } H_2SO_4 \text{ x } 14.007 \text{ x Total volume, mL}}{\text{Sample weight, mg x Volume taken for digestion, mL}} \times 100$$

Percent crude protein was calculated as % Nitrogen x 6.25. However, it should be noted that proper nitrogen conversion factor for field pea is 5.2 (Moossé, 1990; Sosulski and Imafidon, 1990).

3.2.2.3 Lipid

The lipid content of beach pea, green pea and grass pea was determined according to the standard method of AOAC (1990). Ten grams of finely ground sample were weighed into a thimble which was then covered with a piece of cotton. The thimble was then placed in the Soxhlet extraction apparatus (Fisher Scientific Co., Fair Lawn, NJ) along with 250 mL of hexane. The extraction flask was pre weighed with several glass beads. The extraction was continued for 8 h. The hexane was removed using a rotary evaporator at 40 °C and then placed in a vacuum drier at 40 °C for 2 h to ensure complete removal of any residual moisture and then weighed. The oil content of the sample was calculated as percentage weight of the extracted oil to that of the original sample.

3.2.2.4 Fatty acid composition

Fatty acid composition of extracted lipids from beach pea, green pea and grass pea lipids was determined using gas chromatography (GC) (Hewlett-Packard Series II, Type 5890, Hewlett-Packard Mississuaga, ON) as described by Wanasundara and Shahidi (1994a). Ten to sixty milligrams of lipid were placed into a transmethylation vial and transmethylated overnight in an oven (Thelco, Model 2, Precision Scientific Co., Chicago, IL) at 61.8 °C in 6% (v/v) H_2SO_4 in 99.9 mol % methanol (HPLC grade) along with 15 mg of BHA (butylated hydroxyanisole) as an antioxidant. After incubation, 1.0 mL of distilled water was added and the solution then extracted three times with 1.5 mL of hexane. During the first extraction, a few more crystals of BHA were added. The hexane layer was removed into a clean tube and then washed twice with 1.5 mL of H_2O by vortexing. On the first wash, H_2O layer was discarded. On the second wash, the hexane layer was transferred into a clean tube. The hexane was then evaporated under N_2 in a fumehood. The dried matter was dissolved in 1 mL of CS_2 prior to GC analysis. Fatty acid methyl esters (FAMEs) were separated using a gas chromatograph equipped with a fused silica capillary column (Supelcowax 10, 0.25 mm x 60 m, 0.25 μm film thickness; Supelco, Oakville, ON), a flame ionization detector and a split/splitless injector. The chromatographic parameters were: detector and injector temperatures, 250 °C; oven temperature was initially 220 °C for 10.25 min and then ramped to 240 °C at 30 °C/min followed by a hold period of 9 min. Total run time was 19.92 min and helium was used as a carrier gas. The fatty acid methyl esters were identified by comparison of their

relative retention times with those of reference fatty acid methyl esters (Supelco, Oakville, ON). The content of each identified fatty acid in the sample was calculated from the integration data of the chromatographed fatty acids.

3.2.2.5 Ash

Ash content was determined by charring exactly weighed 2 to 5 g of sample into a pre-weighed crucible over a Bünsen burner flame and then heating in a muffle furnace (Blue M Electric Co., Blue Island, IL) at 550 °C overnight or until the ash had a white appearance (AOAC, 1990). The ash content was calculated from difference in weight.

3.2.2.6 Carbohydrate

The carbohydrate content, as percentage, was determined as the difference of the weight percent of moisture, crude protein, lipid and ash content from 100 (AOAC, 1990).

3.2.2.7 Crude fibre

Exactly 2-3 g of finely ground seed or plant material were weighed and transferred to a 500 mL beaker, avoiding fibre contamination from paper or brush. Then 200 mL of a 1.25% (v/v) solution of H_2SO_4 was added into the beaker and heated on a hot plate to boiling for exactly 30 min, while rotating the beaker periodically to keep solids from adhering to the sides of the beaker. After 30 min, the content was filtered through 8 layers of cheese cloth (Muslin cloth) and the residue washed with 50-75 mL hot water;

water from the residue was completely drained. The residue on the cheese cloth was returned to the 500 mL beaker with the help of a small spoon in order to avoid any loss and subsequently 200 mL of a solution of 1.25% (w/v) NaOH were added to it and boiled on a hot plate for exactly 30 min. The content was filtered through the same cheese cloth, the residue was washed with 50 mL of hot 1.25% (v/v) H_2SO_4 and then with hot water to remove any residual sulphuric acid. The water was drained from the residue which was subsequently transferred to a pre-weighed aluminium foil which was then kept in an oven at 105 °C until a constant weight was obtained. The content of crude fibre in the sample was calculated as the weight percentage of the residue to that of the original sample.

3.2.2.8 Determination of soluble sugars and starch from different peas

3.2.2.8.1 Extraction of soluble sugars and starch

3.2.2.8.1.1 Soluble sugars

Dried peas were ground in a coffee grinder (Moulinex coffee grinder) and passed through a 60 mesh screen. Flour (~1 g) was weighed into a 50 mL centrifuge tube and few drops of 80% (v/v) ethanol were added to prevent clumping and stirred thoroughly. Twenty five millilitres of 80% (v/v) ethanol were then added and heated in a water bath at 100 °C for 20 min. After cooling to room temperature and centrifuging at 10,000 x g for 10 min, the supernatant was collected in a 150 mL beaker. Again 25 mL of 80% (v/v) ethanol were added to the centrifuge tubes, stirred well and heated in a boiling water

bath for 20 min, cooled and centrifuged as above, and supernatants collected in the same beaker. This washing treatment was repeated two more times for a total of four washings or until it gave a negative test with anthrone reagent. The combined supernatants were used for the determination of total soluble, reducing and non-reducing sugars (Nelson, 1944) and also for soluble proteins.

3.2.2.8.1.2 Starch

To the residue left after sugar extraction, 5 mL of water were added, and while stirring, 6.5 mL of diluted (52%, v/v) perchloric acid were introduced. The mixture was stirred for about 5 min with a glass rod and occasionally thereafter for 15 min. After that, 20 mL of water were added and the content centrifuged at 10,000 x g for 10 min. The aqueous starch solution was transferred into a 100 mL volumetric flask. This procedure was repeated two more times and supernatants were collected in the same flask. The combined extract was diluted to a 100 mL, filtered and used for determination of starch (McCready *et al.*, 1950).

3.2.2.8.2 Determination of the content of soluble sugars

3.2.2.8.2.1 Reducing sugars

The above sugar solution (Section 3.2.2.8.1.1), extracted with 80% (v/v) ethanol and collected in a 150 mL beaker, was evaporated to 8 - 10 mL on a hot plate and then diluted with distilled water and depigmented using activated charcoal, filtered and 2 mL

of saturated $Pb(OAC)_2$ solution were added to it and filtered again followed by the addition of 2 mL of potassium oxalate to precipitate any excess lead acetate used to clarify the solution. The final volume was made to 100 mL with distilled water. The clear solution was then used for the estimation of sugars. One millilitre of the filtrate was pipetted into a narrow test tube of 20 to 25 mL capacity. One millilitre of alkaline reagent [25 parts of Reagent A + 1 part of Reagent B, Reagent A: 25 g of Na_2CO_3 (anhydrous), 25 g of Rochelle salt, 20 g of $NaHCO_3$, and 200 g of Na_2SO_4 (anhydrous) were dissolved in about 800 mL of water and diluted to 1 litre. Reagent B: 15 per cent $CuSO_4.5H_2O$ containing one or two drops of concentrated sulphuric acid per 100 mL] was then added to it. One millilitre portions of appropriate standards and 1 mL of distilled water were used, as blanks. The solutions were mixed and heated for 20 min in a boiling water bath. At the end of 20 min, the tubes were cooled and 1 mL of arsenomolybdate reagent was added to each tube (For preparation of arsenomolybdate solution 25 g of ammonium molybdate were dissolved in 450 mL of distilled water, to which 21 mL of concentrated H_2SO_4 were added, mixed and 3 g of $Na_2HAsO_4.7H_2O$ dissolved in 25 mL of water, mixed well and placed in an incubator at 37 °C for 24 to 48 h). The mixture was then diluted to 10 mL (adding 7 mL distilled water), mixed well and the colour intensity was read at 520 nm using a diode array spectrophotometer (Hewlett Packard 8452A Diode Array Spectrophotometer Montereal, PQ). Glucose was used as a standard in these determinations (Figure A1).

3.2.2.8.2.2 Total soluble sugars

A twenty five millilitre aliquot from clarified and deleaded solution, prepared according to section 3.2.2.8.2.1, was transferred into a 100 mL beaker, to which 5 mL of HCl:water (1:1, v/v) were added and allowed to stand at room temperature for 24 h for inversion. The sample was neutralized with a 5 N NaOH solution and diluted to 100 mL. An aliquot was taken for determination of total soluble sugars, as given above (Section 3.2.2.8.2.1), and non-reducing sugars were determined by subtracting the content of reducing sugars from the total amount of soluble sugars.

3.2.2.8.2.3 Determination of starch content

Five to ten millilitres of the filtered starch solution (Section 3.2.2.8.1.2) were diluted to 100 to 500 mL. The diluted solution (0.1 - 1.0 mL) was pipetted into a 25 x 250 mm borosilicate glass tube, cooled in a water bath, and 10 mL of fresh anthrone reagent (0.2%, w/v in conc. H_2SO_4) were added to it. After the anthrone solution was added to all sample tubes followed by cooling in a water bath, content in each tube was mixed thoroughly and heated for 7.5 min at 100 °C. The tubes were then rapidly cooled to 25 °C in a water bath and the colour intensity of each solution was read at 620 nm.

A standard curve (Figure A2), using 0 - 100 µg of glucose containing the same amount of perchloric acid as those in the starch aliquots, was constructed each day in order to obtain the yield of glucose from starch (Figure A2). The colour intensities from the anthrone-sugar reaction, using 0 to 100 µg or more of glucose, were linearly

dependent on concentration. To obtain the content of starch, the content of glucose was multiplied by a factor of 0.90 (McCready *et al.*, 1950).

3.2.2.9 Soluble proteins

The soluble proteins were estimated by Folin-Ciocalteau method (1951). The solution of sugars extracted in 80% (v/v) ethanol, as explained in Section 3.2.2.8.1 was used for determination of soluble proteins.

Five millilitres of the alkaline solution [reagent (3)] were added [Reagents were: (1) Alkaline sodium carbonate solution (2% Na_2CO_3 in 0.1 N NaOH); (2) Copper sulphate, sodium potassium tartrate solution, (0.5% $CuSO_4$ in 1% Na, K tartrate). Solutions were prepared fresh by mixing stock solutions; and (3) Alkaline solution: Prepared on the day of use by mixing 50 mL of reagent (1) and 1 mL of reagent (2), respectively] to 1 mL of the test solution followed by thorough mixing. The mixture was then allowed to stand at room temperature for 10 min or longer. Diluted Folin-Ciocalteau reagent (0.5 mL) was rapidly added to the test solution with immediate mixing (the commercial Folin-Ciocalteau reagent diluted with an equal volume of water on the day of use. This was a solution of sodium tungstate and sodium molybdate in phosphoric and hydrochloric acids). After 30 min, the colour intensity of the solution was read against the appropriate blank at 750 nm. A standard curve of bovine serum albumin (BSA, 0.2 mg/mL) was used (Figure A3) and the results expressed as mg soluble proteins per 100 g sample.

3.2.2.10 Total amino acid composition

Total amino acids were determined as described by Shahidi *et al.* (1992). Samples were freezed-dried (Free zone 6 litres, Freeze dry system models 7730, 1994, Labconco Corporation 79510 ECO 7526 Kansas, MI) and then hydrolysed for 24 h in 1 mL of 6 N HCl containing 0.05% phenol at 110 °C using Corning 16 x 100 mm culture tubes with teflon lined screw caps (Blackburn, 1978). The tubes were purged for 5 min with nitrogen before capping. The HCl was removed under vacuum, and the dried samples were reconstituted using a lithium citrate buffer at pH 2.2 for analysis.

Tryptophan was determined separately by hydrolysis of the sample with 1 mL of 3 M mercaptoethanesulphonic acid for 22 h at 110 °C in nitrogen purged Corning culture tubes with teflon lined caps as described by Penke *et al.* (1974) and then neutralized with LiOH and adjusted to pH 2.2. Cysteine and methionine were determined after performic acid oxidation prior to hydrolysis in 6 N HCl solution. Cysteine and methionine were measured as cysteic acid and methionine sulphone, respectively (Blackburn, 1978).

The individual amino acids were separated, identified and quantified using a Beckman 121 MB amino acid analyzer (Beckman Instruments, Inc., Palo Alto, CA) equipped with a cation exchanger resin column (Benson D - x 8.25 bed size 200 x 2.8 mm) at the Amino Acid Facility, Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF.

3.2.2.11 Evaluation of nutritional parameters of peas

The amino acid composition of samples, determined according to the procedure described in Section 3.2.2.10, was used for calculation of the nutritional value of pea proteins as summarized below.

(a) The proportion of essential amino acids (E) to the total amino acids (T) of the protein:

$$E/T\ \% = \frac{\text{Ile + Leu + Lys + Met +Cys + Phe + Tyr + Thr + Trp + Val + His}}{\text{Ala + Asp + Arg + Gly + Glu + His + Ile + Leu + Lys + Met + Cys + Phe + Tyr + Pro + Ser + Thr + Trp + Val}} \times 100$$

$$\text{(b) Amino acid score} = \frac{\text{mg of amino acid per g test protein}}{\text{mg of amino acid per g of FAO/WHO standard pattern}} \times 100$$

Limiting essential amino acids were determined based on their amino acid scores. The first limiting amino acid is the one with the least amino acid score. Essential amino acid (g amino acid/16 g N) pattern of the FAO/WHO standard protein is Ile=4.00, Leu=7.04, Lys=5.44, Met+Cyst=3.52, Phe+Tyr=6.08, Thr=4.00, Trp=0.96 and Val=4.96.

(c) Predicted biological value (BV)

The following regression equation (Mørup and Olesen, 1976) was used for prediction of BV.

$$BV = 10^{2.15} \times q^{0.41}_{Lys} \times q^{0.60}_{Phe+Tyr} \times q^{0.77}_{Met+Cys} \times q^{2.4}_{Thr} \times q^{0.21}_{Trp}$$

Where,

$$q = \frac{a_i \text{ sample}}{a_i \text{ reference}} \qquad \text{for } a_i \text{ sample} \leq a_i \text{ reference}$$

$$q = \frac{a_i \text{ reference}}{a_i \text{ sample}} \qquad \text{for } a_i \text{ sample} \geq a_i \text{ reference}$$

a_i = mg of the amino acid per g of total essential amino acids

(d) The predicted protein efficiency ratio (PER) value

The predicted PER values of different peas and plant parts as well as treated samples of peas were calculated from their amino acid composition based on three equations developed by Alsmeyer *et al.* (1974), as given below.

(1) PER = -0.684 + 0.456 (LEU) - 0.047 (PRO)

(2) PER = -0.468 + 0.454 (LEU) - 0.105 (TYR)

(3) PER = -1.816 + 0.435 (MET) + 0.780 (LEU) + 0.211 (HIS) - 0.944 (TYR)

3.2.2.12 Free amino acids

For determination of free amino acids, 1 to 2 g of samples were homogenized using a Polytron PT 3000 (Brinkman Instruments, Rexdale, ON) homogenizer in a 50 mL centrifuge tube with 20 mL of ice-cold 6 % (v/v) perchloric acid for 2 min (10,000 rpm) in an ice bath. The homogenized samples were then incubated in ice for 30 min before centrifugation (IEC Centra MP4 centrifuge, International Equipment Co., Needham Heights, MA) at 4000 x g for 20 min. The residue was then re-extracted with 20 mL 6%

(v/v) perchloric acid and centrifuged, as described above. The supernatants from the first and second extraction were combined and filtered through a Whatman No. 4 filter paper. The pH of the filtrate was adjusted (Accumet pH meter, Model 810, Fisher Scientific Co., Fair Lawn, NJ) to 7.0 using a 33% (w/v) KOH solution and then centrifuged at 4000 x g for 20 min to remove precipitates of potassium perchlorate. The extract was filtered through a 0.45 μm nylon filter to eliminate any turbidity. The supernatant was then acidified to pH 2.2 using a 10 N HCl solution, and diluted to 50 mL with distilled water. Two millilitres of the extract were taken into a clean tube and 1.0 mL of lithium citrate buffer (pH 2.2; Beckman Instruments, Inc., Palo Alto, CA) was added to it. Samples were then analyzed on a Beckman 121 MB amino acid analyzer using Benson D - X 8.25 cation exchange resin and a single column employing three-buffer lithium method as per Beckman 121 MB-TB-017 application notes. Results were calculated and reported as mg of amino acid/100 g of sample.

3.2.2.13 Determination of mineral constituents

3.2.2.13.1 Preparation of samples for mineral analysis

Dried and ground samples (1 to 2 g) were subjected to dry ashing in well cleaned porcelain crucibles at 550 °C in a muffle furnace (Blue M Electric Co., Blue Island, IL). The resultant ash was dissolved in 5 mL of $HNO_3/HCl/H_2O$ (1:2:3:, v/v/v) and warmed on a hot plate until brown fumes disappeared. To the remaining content in each crucible 5 mL of deionized water were added and heated until a colourless solution was obtained.

The mineral solution in each crucible was transferred to a 100 mL volumetric flask by filtering through a Whatman No. 42 filter paper and further diluted to 100 mL with deionized water.

3.2.2.13.2 Elemental analysis by Atomic Absorption spectrophotometry

The concentration of elements (Al, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Si, and Zn) in each solution, prepared as described in section 3.2.2.13.1, was determined using a Perkin Elmer 8650 atomic absorption spectrophotometer (Perkin Elmer Co., Montreal, PQ). The parameters used for the analysis of each element are given in Table 3.1. Calibration curves of absorbance values versus concentration for each element at appropriate concentrations (to obey Beer's-Lambert Law) were constructed using their respective standards of 0 - 1000 µg/L (Fisher Scientific Co., Unionville, ON). A cell length of 10 cm was used and concentration of each element in samples was calculated as mg per 100 g of dry matter.

3.2.2.14 Phosphorus

Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri (1975). To 1 mL of the diluted digest (Section 3.2.2.13.1), 4 mL of demineralized water, 3 mL of 1.5 N H_2SO_4, 0.4 mL of 10% (w/v) $(NH_4)_6Mo_7O_{24}.4H_2O$ and 0.4 mL of 2% (w/v) ascorbic acid were added and mixed well. The solution was allowed to stand for 20 min and absorbance was recorded at 660

Table 3.1 Analytical parameters used in atomic absorption spectrophotometry

Element	Wavelength (nm)	Slit width (nm)	Oxidant-fuel of premix
Al	309.3	0.2	N_2O-acetylene
Ca	422.7	0.7	Air-acetylene
Cu	324.8	0.7	Air-acetylene
Fe	248.3	0.2	Air-acetylene
K	766.5	0.7	Air-acetylene
Li	670.8	0.7	Air-acetylene
Mg	285.2	0.7	Air-acetylene
Mn	279.5	0.2	Air-acetylene
Na	589.0	0.2	Air-acetylene
Si	251.6	0.2	N_2O-acetylene
Zn	213.9	0.2	Air-acetylene

nm. The content of phosphorus in the extracts was determined using a standard curve obtained for KH_2PO_4 (Figure A4) and expressed as mg phosphorus per 100 g of sample.

3.2.2.15 Vitamins

Ascorbic acid, β-carotene, folic acid, riboflavin, thiamin, and niacin of beach pea seeds were determined according to the standard procedures of AOAC (1990). These experiments were carried out by Labstat Incorporated, Kitchener, ON.

3.2.2.16 Total phenolics

The total phenolics in 1 g sample of beach pea, green pea, and grass pea were extracted three times with 10 mL of 70% (v/v) aqueous acetone containing 1% concentrated HCl at room temperature using a Polytron PT 3000 (Brinkman Instruments, Rexdale, ON) homogenizer for 1 min at 10,000 rpm. The slurry was centrifuged at 5000 x g for 10 min, the supernatants were collected, combined and evaporated to dryness at 30 °C under vacuum. The extracted total phenolics were then dissolved in 25 mL of methanol, centrifuged again and the clear solution was used for determination of total phenolics.

The total content of phenolics in methanol was determined colorimetrically according to the method of Swain and Hillis (1959). To 0.5 mL of methanol solution of phenolics, 0.5 mL Folin-Denis reagent, 1 mL saturated solution of sodium carbonate and 8 mL water were added and mixed well. Absorbance was read at 725 nm after 30 min standing at

room temperature and centrifugation; trans-sinapic acid (Sigma Chemicals Co., St. Louis, MO) was used as a standard in these experiments (Figure A5). The content of phenolics was expressed as mg trans-sinapic acid equivalents per 100 g of dry meal.

3.2.2.17 Phenolic acids

The free, esterified and insoluble-bound phenolic acids of pea meals were isolated using the procedure of Krygier *et al.* (1982). Meals (2 g) were extracted six times with 40 mL of water/methanol/acetone (6:7:7, v/v/v) at room temperature using a Polytron PT 3000 (Brinkman Instruments, Rexdale, ON) homogenizer for 1 min at 10,000 rpm. After each extraction, samples were centrifuged for 15 min at 5000 x g and supernatants were collected (residue kept for further analysis). Combined supernatants were evaporated at 30 °C under vacuum to 40 mL and the pH of the mixture was adjusted to 2 using 6 N HCl. The samples were centrifuged at 5,000 x g 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 residue after centrifugation were mixed together and then treated with 30 mL 4 N NaOH under nitrogen for 4 h at room temperature to release esterified phenolic acids. The resultant hydrolysate was acidified to pH 2 using 6 N HCl and extracted into diethyl ether/ethyl acetate 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 4 N NaOH under nitrogen at room temperature, then acidified with 6 N HCl to pH 2 and centrifuged at 5000 x g 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 mg trans-sinapic acid equivalents per 100 g sample using a standard curve (Figure A5). The total phenolic acid content was calculated as the sum of free, esterified and insoluble-bound fractions.

3.2.2.17.1 UV spectra

UV spectra of phenolic acids in methanol were recorded using a Hewlett Packard 8452A Diode Array spectrophotometer.

3.2.2.17.2 Thin layer chromatography

Thin layer chromatograms of different phenolic fractions (1, Insoluble; 2, Free and

3, Esterified); separated from beach pea and grass peas, were developed using (A) acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v); (B) water-acetic acid-n-butanol (1:1:3, v/v/v) and sprayed with a solution of potassium ferricyanide-ferric chloride, followed by hydrochloric acid (Egon, 1969).

3.2.2.18 Condensed tannins

3.2.2.18.1 Extraction of tannins

Pea flours (1 - 2 g) together with 40 mL water or acidified water (1%, v/v, HCl in water) were heated in a boiling water bath for 30 min, centrifuged (4000 x g) and the supernatant collected in a clean beaker. This procedure was repeated two more times; the combined extracts were freeze dried and, after solubilization in absolute methanol, centrifuged (4000 x g) and the volume made to 100 mL prior to performing the vanillin-HCl assay.

Pea flours (1 - 2 g) were extracted three times with 10 - 20 mL absolute methanol, absolute acetone, 90%, 80% and 70% methanol and also acetone. In another experiment, 100%, 90%, 80%, and 70% methanol as well as acetone acidified by adding 1% concentrated HCl, respectively were used as extraction solvents. Samples were homogenized using a PT 3000 Polytron homogenizer for 1 min at 10,000 rpm and subsequently centrifuged (4000 x g) and supernatants collected in a clean flask. This procedure was repeated two more times and combined extracts were evaporated using a rotary evaporator at 40 °C to dryness and the dry residue was then dissolved in 25 mL

absolute methanol prior to performing the vanillin-HCl assay.

3.2.2.18.2 Determination of condensed tannins

The condensed tannins were assayed colorimetrically by the method of Price *et al.* (1978a), as follows. To 0.2 to 1 mL of methanolic solutions of condensed tannins, 5 mL of 0.5% vanillin reagent were added; a 5 mL volume of 4% concentrated HCl in methanol was used as a blank. The absorbances of sample and blank were read at 500 nm after standing for 20 min at room temperature. Catechin (+) (3.5 moles of water per mole of catechin, Sigma Chemical Co., St. Louis, MO) was used as a standard in these experiments (Figure A6). The content of condensed tannins in the meal was expressed as g catechin equivalents per 100 g sample.

3.2.2.19 Phytic acid

Phytic acid from the prepared meals was extracted according to the method of Tangkongchitr *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 5000 x g. Five millilitres of the supernatant were mixed with 5 mL of distilled water and 6 mL of 0.4% $FeCl_3.6H_2O$ in a 0.07 N 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 5000 x g for 15 min and the supernatant discarded. The precipitate

was mixed thoroughly with 5 mL of 4% Na_2SO_4 in 0.07 N HCl and the mixture was centrifuged (5000 x g) again. The recovered ferric phytate was digested using 6 mL of a 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. Ten millilitres of distilled water were added to the warm digest and the solution was then heated in a boiling water bath for 30 min to destroy pyrophosphate; the mixture was subsequently diluted to 50 mL with distilled water. The phytate phosphorus was determined according to the method described by Nahapetian and Bassiri (1975). To 1 mL of diluted digest, 4 mL distilled water, 3 mL 1.5 N H_2SO_4, 0.4 mL 10% $(NH_4)_6$ $Mo_7O_{24}.4H_2O$ and 0.4 mL 2% ascorbic acid were added and mixed. The solution was allowed to stand for 20 min and the absorbance read at 660 nm. The content of phosphorus in the mixture was calculated from a standard curve using KH_2PO_4 as the standard (Figure A4). The phytic acid content was calculated by multiplying the phytate phosphorus content of the meal by a factor of 3.55 which is derived from the empirical formula $C_6P_6H_{18}O_{24}$.

3.2.2.20 Different forms of Phosphorus

The mass-balance determination of phosphorus is outlined in Figure 3.1. All phosphorus determinations were done according to the method of Tangkongchitr *et al.* (1981) in triplicate.

The inorganic phosphorus (Pi) in the sample (3.0 g) was extracted with 50 mL of a 12.3% aqueous trichloroacetic acid solution using a mechanical shaker for 12 h at room

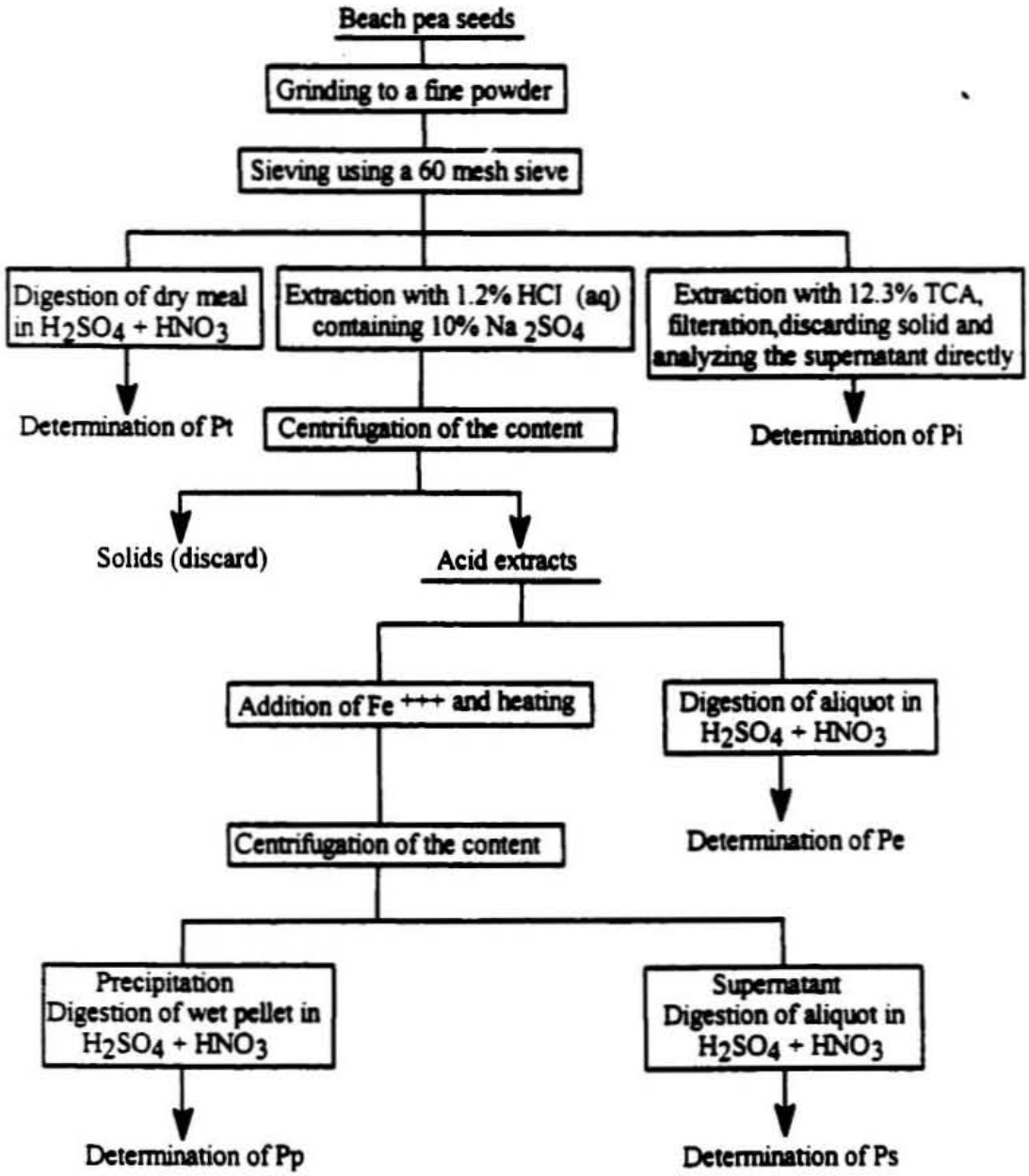


Figure 3.1 Flow chart for determination of different types of phosphorus (Pt, Total phosphorus; Pi, Inorganic phosphorus; Pe, Phosphorus extractable in 1.2% aqueous HCl + 10% Na_2SO_4; Pp, Fraction of Pe precipitated by ferric ion; Ps, Fraction of Pe not precipitated by ferric ion).

temperature (Pons *et al.*, 1946). After centrifugation at 2000 x g for 20 min, an aliquot was diluted to 100 mL volume with distilled water. The solution was then analyzed directly for its content of inorganic phosphorus.

Total phosphorus (Pt) in the original dry sample was determined as described in Section 3.2.2.14.

For determination of Pe (Pe, phosphorus extractable in 1.2% aqueous HCl + 10% Na_2SO_4); Ps (Ps, fraction of Pe not precipitated by ferric ion); and Pp (Pp, fraction of Pe precipitated by ferric ion); a sample of dried pea flour (2.0 g) was extracted with 40 mL of a 1.2% (w/v) aqueous HCl solution containing 10% Na_2SO_4 (Earley 1944; Nahapetian and Bassiri, 1975) as modified by Naczk *et al.* (1986a). The mixture was shaken at room temperature for 2 h, centrifuged at 2000 x g for 40 min and the supernatant collected for further analyses. An aliquot (5.0 mL) of the acid extract was used to determine Pe. The phytic acid in 5 mL of the acid extract was precipitated by adding 5 mL of distilled water and 6 mL of 0.4% $FeCl_3.6H_2O$ in a 0.07 N HCl solution. The mixture was heated in a boiling water bath for 45 min and then cooled to room temperature. The ferric phytate precipitate was collected by centrifugation at 5000 x g for 20 min and the supernatant was collected in a separate beaker. The precipitate was mixed thoroughly with 5 mL of 4% Na_2SO_4 in 0.07 N HCl and the mixture was centrifuged (5000 x g) again and the supernatant transferred to the above beaker. Five millilitres of the combined supernatant were used for determination of Ps (Ps, fraction of Pe not precipitated by ferric ion). The recovered ferric phytate was digested using 6 mL of a 1:1 (v/v) mixture of concentrated

H_2SO_4 and HNO_3 in a micro-Kjeldahl flask. 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 any pyrophosphate; the mixture was then diluted with distilled water to 50 mL. After digestion, Pe, the Ps, and Pp (Pp, fraction of Pe precipitated by ferric ion) (quantitatively 50 mL) were determined colorimetrically as orthophosphate by employing the method of Lindberg and Ernster (1956) as modified by Nahapetian and Bassiri (1975), as described above for phytic acid (3.2.2.19).

3.2.2.21 Non-protein nitrogen (NPN)

The content of NPN 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 a 10% trichloroacetic acid (TCA) solution at 20 °C for one hour using a wrist-action shaker (Burrel, Pittsburgh, PA). The insoluble residue was removed by centrifugation at 5000 x g for 10 min and the residue was treated three times with 15 mL of a 10% (w/v) TCA solution. The supernatant was collected as before and brought to 100 mL with distilled water; an aliquot was taken for determination of soluble nitrogen using the Kjeldahl procedure (AOAC, 1990).

3.2.2.22 β-N-Oxalylamino-L-Alanine (BOAA)

For determination of BOAA, 2 g of sample were homogenized using a Polytron PT

3000 (Brinkman Instruments, Rexdale, ON) homogenizer in a 50 mL centrifuge tube, with 20 mL of ice-cold 6 % perchloric acid (v/v) for 2 min (at 10,000 rpm) in an ice bath. The homogenized samples were then incubated in ice for 30 min before centrifugation (IEC Centra MP4 centrifuge, International Equipment Co., Needham Heights, MA) at 4000 x g for 20 min. The residue was then re-extracted with 20 mL ice-cold 6% perchloric acid (v/v) and centrifuged, as described above. The supernatants from the first and second extraction were combined and filtered through a Whatman No. 4 filter paper. The pH of the filtrate was adjusted (Accumet pH meter, Model 810, Fisher Scientific Co., Fair Lawn, NJ) to 7.0 using a 33% (w/v) KOH solution and then centrifuged at 2000 x g for 10 min to remove precipitates of potassium perchlorate. The supernatant was then acidified to pH 2.2 using a 10 N HCl solution, and diluted to 50 mL with distilled water. Two millilitres of the extract were taken into a clean tube and 1.0 mL of lithium citrate buffer (pH 2.2; Beckman Instruments, Inc., Palo Alto, CA) was added to it. Samples were then analyzed on a Beckman 121 MB amino acid analyzer using Beckman W-2 cation exchange resin 80 x 2.8 mm. Elution buffers were 0.01 M trifluoroacetic acid (TFA) for 30 min (Capony and Demaille, 1983) and 0.2 M sodium citrate pH 3.25, for 20 min at a flow rate of 10 mL/h. The post column reactant was ninhydrin at a flow of 5 mL/h. The peak of BOAA was eluted at 46.5 min (Figure A7). Quantitation was achieved using a Hewlett Packard computing integrator Model 3395 A. The final results were calculated and reported as mg BOAA per 100 g dry meal.

3.2.2.23 Extraction, isolation and detection of saponins from beach pea seeds

Extraction, isolation and detection of saponins were carried out according to the procedure of Shiraiwa *et al.* (1991) with the following modification. Twenty five grams of ground pea meal were introduced into a 1000 mL dark glass bottle and suspended in 200 mL of ethanol-water (80:20, v/v). The tightly capped bottle was placed in a water bath at 80 °C. After 15 min, during which the content was twice shaken, the extract was cooled and filtered under partial vacuum. The material left on the filter paper was transferred back to dark glass bottle for further extraction with 200 mL of the same extraction solution. This procedure was repeated three more times over 15, 30 and 30 min of extraction, respectively. Supernatants were combined and evaporated using a rotary vacuum evaporator to remove any remaining solvent; the water was then removed by lyophilization.

One gram of the extract was dispersed in n-butanol-water (1:1, v/v). After standing overnight, the butanol layer was separated and evaporated to dryness under reduced pressure. Isolated saponins were dissolved in small quantities of methanol and injected onto the HPLC column (Ultrasphere-ODS Altex, 7 µm, 4.6 x 150 mm) for analytical detection. The mobile phase consisted of acetic acid-n-propanol-water-methanol (0.1:6:23.9:70, v/v/v/v). The HPLC system used for analysis consisted of a Waters 600E pump, a Waters 715 ULTRA WISP autosampler set at an injection volume of 10 µL, and a Waters 996 photodiode array detector acquiring spectra in the wavelength range of 200-400 nm with a resolution of 1.2 nm and a data acquisition rate of 1 spectra/4 s. Peak

heterogeneity was assessed by the photodiode array software option of Millennium chromatography Manager V 2.10 software. Saponins were detected by UV-monitoring at 205 nm, and the flow rate was 0.5 mL/min. The reference sample of soybean saponin "group B" was obtained according to Shiraiwa *et al.* (1991).

3.2.2.24 Chlorophyll

Five grams of ground sample of beach pea, green pea or grass pea seeds were weighed in a centrifuge tube; 25 mL 85% (v/v) acetone were added and the mixture was shaken using a mechanical shaker for 20 min. The mixture was then centrifuged at 10.000 x g for 10 min; supernatants were collected in a clean volumetric flask. The procedure was repeated three more times. The combined extracts were made to 50 mL. A known quantity of sample solution (1 mL) was taken into another 60 mL reagent bottle to which a teaspoonful (5 g) of anhydrous Na_2SO_4 was added and filled with diethyl ether. After obtaining an optically clear solution, the absorbance was read at 660 and 642.5 nm; diethyl ether was used as a blank. The aliquot was adjusted in such a way that 0.6 absorbance unit could be obtained at 660 nm. The total contents of chlorophyll and its "**a**" and" **b**" components were calculated using the following formula; results were expressed as mg chlorophyll per g dry meal.

Total chlorophyll = $7.12\ A_{660.0} + 16.8\ A_{642.5}$

Chlorophyll "**a**" = $9.93\ A_{660.0} - 0.777\ A_{642.5}$

Chlorophyll "**b**" = $17.6\ A_{642.5} - 2.81\ A_{660.0}$

3.2.3 Air classification

Mature dry beach pea seeds were dehulled using a Seedburo hand grinder (Seedburo Equipment Company, Chicago, IL). Ground seed fines were first separated using a mesh 30 sieve (600 μm) on a Seedburo portable sieve shaker; the hulls were separated on a 757 South Dakota Seed Blower (Seedburo Equipment Company, Chicago, IL) equipped with a large (4") tube set. Pending on the set up of air pressure, clean hulls and cotyledons were obtained (Figure 3.2). The pressure of air in the tube was controlled by the size of opening at the top. The separated fractions were ground into a fine powder (60 mesh) using a coffee grinder and stored in "Nasco" whirl pack plastic bags or air tight glass bottles and kept at room temperature for further chemical analyses.

3.2.3.1 Chemical analyses

Chemical analyses of separated cotyledons and hulls (Section 3.2.3) were carried out essentially in a similar manner to that described in Section 3.2.2.

3.2.3.1.2 Proanthocyanidin

The condensed tannins were assayed by the proanthocyanidin method, as described by Mole and Waterman (1987). One millilitre of methanolic solution of condensed tannins was added to 10 mL of the n-butanol-HCl reagent (The n-butanol-HCl reagent was prepared by dissolving 0.7 g of ferrous sulphate heptahydrate in 25 mL of concentrated HCl containing a small volume of n-butanol. This solution was then made

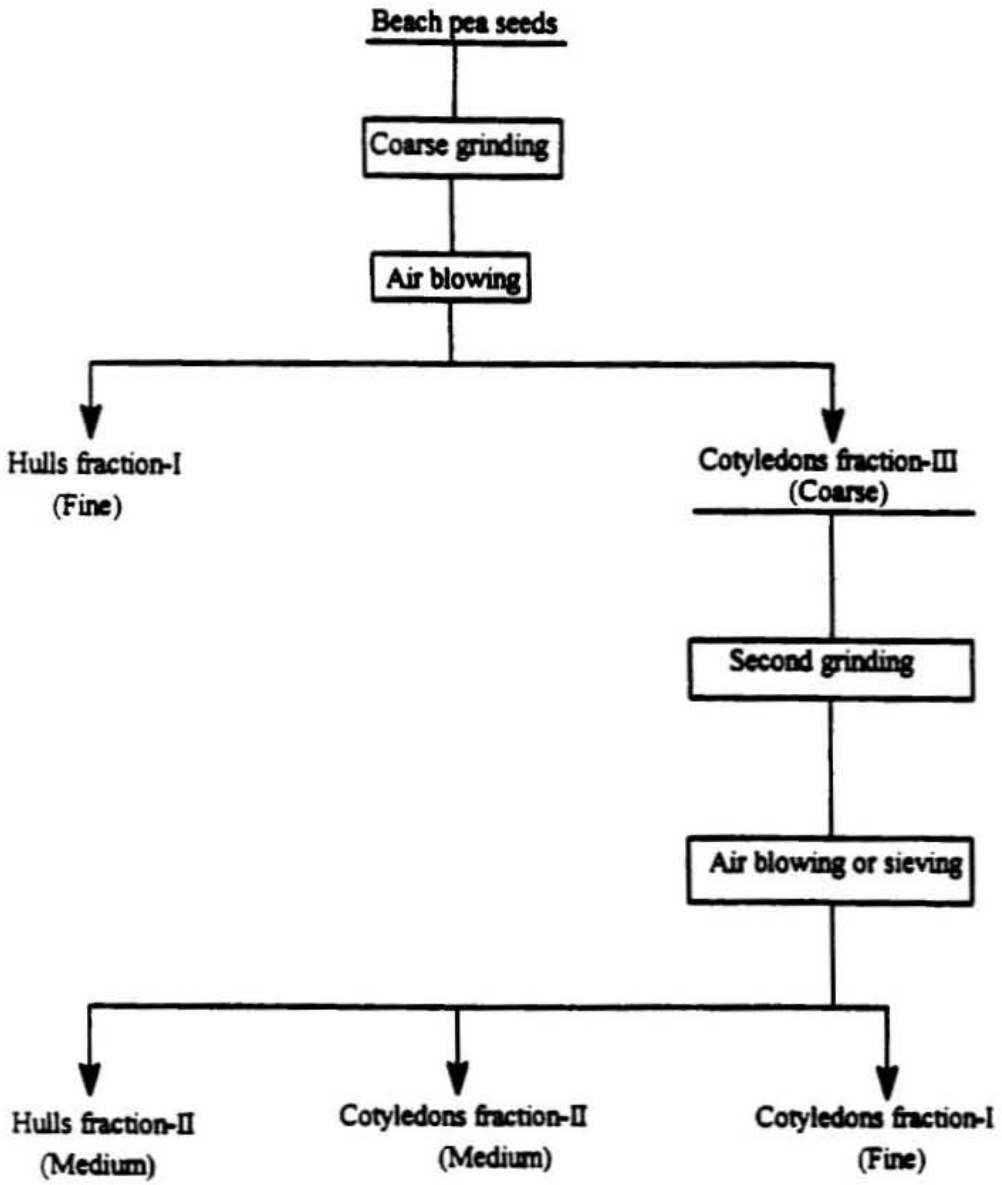


Figure 3.2 Flow chart for air classification of cotyledons and hulls of beach pea.

to 1 litre with n-butanol). This mixture was heated in a sealed ampule for 2 h in a boiling water bath and then allowed to cool. The absorbance of the solution was read at 550 nm against a reagent blank. For A > 0.75, the reaction mixture was diluted with n-butanol. The content of tannins was expressed as ΔA_{550} per gram of hulls.

3.2.3.1.3 Protein precipitation assay

The protein precipitating capacity of condensed tannins of beach pea hulls was assayed as described by Hagerman and Butler (1978) with the following modification. To 1 mL of crude tannin extract in methanol, 2 mL of a standard BSA (Sigma, fraction V, initial fractionation by cold alcohol precipitation) solution was added (1 mg of protein/mL in 0.2 M acetate buffer, pH 4.0 and containing 0.17 M sodium chloride) and mixed well. After 15 min standing at room temperature, the solution was centrifuged at 10,000 x g for 15 min. The supernatant was discarded and the surface of the pellet and the tube walls were carefully washed with acetate buffer (pH 4.0) without disturbing the pellet. The pellet was then dissolved in 4 mL of sodium dodecyl sulphate (SDS)-triethanolamine solution [1% SDS and 5% (v/v) triethanolamine in distilled water] and 1 mL of ferric chloride reagent (0.01 M ferric chloride in 0.01 M HCl) was added to it mixed and the absorbance was measured at 510 nm against a reagent blank (4 mL of SDS solution + 1 mL of ferric chloride reagent) after 15 min of equilibration. The protein precipitating capacity of tannins was expressed as A_{510}/g hulls.

3.2.3.1.4 Biological activity of condensed tannins

The biological activity of condensed tannins of beach pea hulls was assessed by the dye-labled protein assay of Asquith and Butler (1985). One millilitre of methanolic solution of crude tannin extract was added to 4 mL of blue BSA solution containing 2 mg of protein/mL in 0.2 M phosphate buffer, pH 3.5, as modified by Naczk et al. (1994). The mixture was vigorously mixed at 1000 rpm for 5 min at room temperature. The protein-tannin complex was then separated by centrifugation at 4000 x g for 20 min. The supernatant was carefully discarded and the pellet dissolved in 3.5 mL of a 1% (w/v) solution of sodium dodecyl sulphate containing 5% (v/v) triethanolamine and 20% (v/v) 2-propanol. The absorbance was read at 590 nm against an appropriate blank. The biological activity of tannins was expressed as milligrams of BSA precipitated per gram of hulls.

3.2.3.1.5 Scanning electron microscopy (SEM)

Structural morphology of beach pea hulls were studied by scanning electron microscopy. Hull samples were mounted on circular aluminum stubs with double sticky tape, and then coated with 20 nm of gold using Edwards S150A sputter coater, examined and photographed in a Hitachi (S-570) Scanning Electron Microscope (Hitachi S-570 Scanning Electron Microscope Hitachi, Ltd. Tokyo, Japan) at an accelerating potential of 20 kV.

3.2.3.2 Phenolics and natural antioxidants from beach pea hulls

3.2.3.2.1 Extraction

Fine powders (60 mesh) of beach pea hulls were extracted with 70% (v/v) acetone containing 1% concentrated HCl (meal to solvent ratio was 1:10) at room temperature using a Polytron homogenizer (Brinkman PT 3000) for 1 min at 10,000 rpm. The slurry was centrifuged at 4000 x g for 10 min, the supernatant filtered through a Whatman No. 41 filter paper and the residue was extracted two more times using the same procedure. Supernatants were combined and evaporated under vacuum using a rotary evaporator to remove acetone and the water was then removed by lyophilization.

3.2.3.2.2 Phenolic compounds

A known quantity of lyophilized sample was dissolved in absolute methanol and used for the determination of total phenolic compounds and condensed tannins as described by Naczk *et al.* (1992b; see Section 3.2.2.16 and 3.2.2.18).

3.2.3.2.3 Column chromatography

A 1.5 g portion of the acetone extract was dissolved in 5 mL methanol and applied to a chromatographic column (3.4 x 50 cm) packed with Sephadex LH-20 and eluted with absolute ethanol. Fractions (8 mL) were collected using a LKB Bromma 2112 Redirac fraction collector (Pharmacia, Uppsala, Sweden) and their absorbance at 280 nm, in ethanol was read; the absorbance was lowest and constant in tube numbers 60 - 66. Then

the elution solvent was changed to acetone:water (50:50, v/v) to remove the tannin fraction, the absorbance of which was read at 280 nm. Absorbance value of all fractions at 500 nm was measured after colour development with 0.5% vanillin reagent for condensed tannins (Price *et al.*, 1978a). Eluates were then pooled into three major fractions based on their absorbance at 280 nm and vanillin positive test. Pooled eluates were lyophilized and weighed. The contents of total phenolic compounds and condensed tannins in each major fraction were then estimated (Naczk *et al.*, 1992b; see Section 3.2.2.16 and 3.2.2.18). Trans-sinapic acid and catechin were used as standards.

3.2.3.2.4 UV spectra

The UV spectrum of each separated fraction from 220 to 400 nm was recorded using a Hewlett Packard 8452A Diode Array Spectrophotometer (Hewlett Packard Canada, Ltd., Montreal, PQ).

3.2.3.2.5 Thin layer chromatography

The separated fractions and crude extracts were examined on silica gel TLC plates (Silica gel, 60 Å mean pore diameter, 2-25 μm mean particle size, 250 μm thickness, Sigma Chemical Co., St. Louis, MO). Plates were developed in a glass chamber 22 x 22 x 10 cm (Fisher Scientific Co., Toronto, ON) using acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v) and water-acetic acid-n-butanol (1:1:3, v/v/v) mixtures as developing systems (Amarowicz *et al.*, 1995). To visualize phenolic compounds, each

plate was sprayed with a solution of ferric chloride (Reio, 1958). Compounds with antioxidant activity were visualised after spraying of each plate with a solution of β-carotene and linoleic acid (Philip, 1974).

3.2.3.2.6 Antioxidant activity

The antioxidant activity of isolated fractions and the crude extract was evaluated using a β-carotene-linoleate model system as described by Miller (1971) with the following modifications. A solution of β-carotene (Sigma Chem. Co., St. Louis, MO) was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. One millilitre of this solution was then pipetted into a round bottom flask. After removing the chloroform under vacuum, using a rotary evaporator at 40 °C, 20 mg of purified linoleic acid, 200 mg of Tween 40 emulsifier (Aldrich Chem. Co., Milwaukee, WI) and 50 mL of aerated distilled water were added to the flask with vigorous manual shaking. Aliquots (5 mL) of this prepared emulsion were transferred into a series of tubes containing 2 mg of each fraction (Fraction I-III), the crude extract, or 2 mg of BHA which was used for comparative purposes. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470 nm. Subsequent absorbance readings were recorded at 15 min intervals by keeping the samples in a water bath at 50 °C until the colour of β-carotene in the control sample devoid of any extract or synthetic antioxidant had disappeared (approximately 120 min).

3.2.3.2.7 HPLC analyses

The vanillin positive fraction (III) was used for purity testing by HPLC using a standard sample of catechin (Sigma Chemicals Co., St. Louis, MO). Individual catechins from fraction number III were separated from the Sephadex-isolated fractions by analytical HPLC. A Shimadzu (Japan) chromatographic system was used; it consisted of a LC-6A pump, SPD-6AV UV-VIS spectrophotometric detector, SCL-6B system controller, CR 501 chromatopac and a CSL-Spherisorb-ODS-2 analytical column (4.5 mm x 250 mm) (Chromatographic Specialities, Inc., Brockville, ON). The mobile phase was acetic acid-methanol-dimethylformamide-water (1:3:40:157, v/v/v/v) (Höefler and Coggon, 1976) and the flow rate was 1.5 mL/min with an injection volume of 20 µL. For analytical methods, the detector wavelength was set at 280 nm. The standard catechin and epicatechin were run on the same semi-preparative HPLC column under the same conditions compared to the unknowns from beach pea hulls.

3.2.4 Determination of concentration of phenolic compounds and sugars in beach pea by different solvent extraction methods

3.2.4.1 Extraction

Extraction of phenolic compounds and sugars was carried out as described previously for saponins (3.2.2.23).

3.2.4.2 Sugars

Following evaporation of the organic solvent in a rotary vacuum evaporator at 40 °C, the remaining water was removed by lyophilization followed by colorimetric determination of sugars by the method of Dubois *et al.* (1956). Lyophilized extract (0.5 g) was dissolved in distilled water (100 mL), 2 mL of the sugar solution were pipetted into a test tube and 0.05 mL of an 80% (w/v) phenol were added to the mixture. Subsequently 5 mL of concentrated sulphuric acid were added rapidly, the stream of acid being directed against the liquid surface rather than against the side of the test tube in order to obtain good mixing. The tubes were allowed to stand for 10 min, shaken and allowed to stand for 20 min at room temperature (25-30 °C). The absorbance of the characteristic yellow-orange colour was read at 490 nm for hexoses. Blanks were prepared by substituting the sugar solution by distilled water. The amount of sugar present in the sample was determined by constructing a standard curve using glucose (Figure A8).

3.2.4.3 Phenolic compounds

A known quantity of lyophilized sample was dissolved in absolute methanol and used for the determination of total phenolics and condensed tannins as described by Naczk *et al.* (1992b). The results were expressed as mg trans-sinapic acid equivalents per 100 g sample for phenolics and g catechin equivalents per 100 g dry meal for condensed tannins. The same colorimetric methods were used to analyze the degree of extraction

of sugar and phenolic compounds after extractions.

3.2.4.4 UV spectra

Extraction of sugars and phenolic compounds by different solvent systems was monitored by means of UV absorption at 280 nm. UV spectra of the extracts in methanol were also measured.

3.2.4.5 Thin layer chromatography

The extracts were also characterized by means of thin layer chromatography on silica gel plates (Merck) using the following developing systems; A: acetic acid-water-n-butanol (10:10:30, v/v/v) (Zadernowski, 1987); B: acetic acid-petroleum ether-diethyl ether, 1:20:80, v/v/v); and C: water-methanol-chloroform (10:35:65, v/v/v) (Amarowicz *et al.*, 1992b). Following developing of chromatograms, plates were sprayed with, A and B, an aqueous solution of ferric chloride to visualized phenolic compounds (Barton *et al.*, 1952). Sugars, glucosides and some other organic compounds were visualized on plate "C" by spraying with an aqueous solution of H_2SO_4 (10 g/100 mL) and heating at 120 °C for 10 min (Amarowicz *et al.*, 1992b).

3.2.4.6 Column chromatography

A 1.0 g portion of acetone extract was dissolved in 5 mL of methanol and applied to a chromatographic column (3.4 x 50 cm) packed with Sephadex LH-20 and eluted with

methanol. Fractions (6 mL) were collected using a fraction collector and their absorbance in methanol was read at 280 nm.

3.2.4.7 UV absorption and thin layer chromatography

Absorbance value at 500 nm was read after colour development for condensed tannins (Price *et al.*, 1978a). The eluted fractions were also characterized by means of thin layer chromatography on silica gel plates (Merck) using water-methanol-chloroform (10:35:65, v/v/v) as the developing system followed by spraying with 0.5% vanillin solution in methanol containing 4% HCl. Based on the above information, and absorbance at 280 nm, eluates were then pooled into two major fractions; the purity of each was then tested using catechin as a standard (Sigma Chemical Co., St. Louis, MO).

3.2.4.8 HPLC analyses

Individual catechins from tube numbers 23 to 30 (Figure 4.15) were separated from the Sephadex-isolated fractions by semi-preparative HPLC, as described in Section 3.2.3.2.7.

3.2.5 Methanol-ammonia-water extraction

Beach pea and grass pea seeds were first ground using a Moulinex coffee grinder before solvent extraction. Ground seeds (60 mesh, 75 g) were blended with 500 mL of absolute or 95% (v/v) methanol, (R=6.7; volume of solvent in mL/weight of seed in g)

with or without ammonia (10%, w/w) for 2 min at low speed (approximately 2000 rpm) in a 4 L commercial Waring blender, Model 33BL34 (Waring Commercial Blender, Dynamics Corporation of America, New Hartford, CT). Ammonia was bubbled into a 95% (v/v) methanol at 0 °C. The final concentration of ammonia in the solution was adjusted by mixing of the resultant solution with enough methanol 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 were added and the slurry was blended again for 2 min. The meal was separated by vacuum filtration using a Whatman No. 41 filter paper, rinsed 3 times with 125 mL of methanol and dried at 40 °C under vacuum. The meal was extracted two more times with 95% (v/v) methanol containing 10% (w/w) ammonia (Figure 3.3). The two liquid phases from the first extraction were separated and the hexane layer was evaporated to recover the oil. The methanol phase was re-extracted three times with hexane at a methanol to hexane ratio of 2:1 (v/v) to recover additional oil. The combined methanol extracts were evaporated using a rotary vacuum evaporator to recover the dissolved solids. The recovery of meal and mass balance of the materials due to the process were evaluated. The recovered meals were stored in "Nasco" whirl pack plastic bags (Polycello, Amherst, NS) or air tight glass bottles and kept at room temperature until used for analyses.

3.2.5.1 Chemical analyses

Chemical analyses of methanol-ammonia-water/hexane treated meals (Section

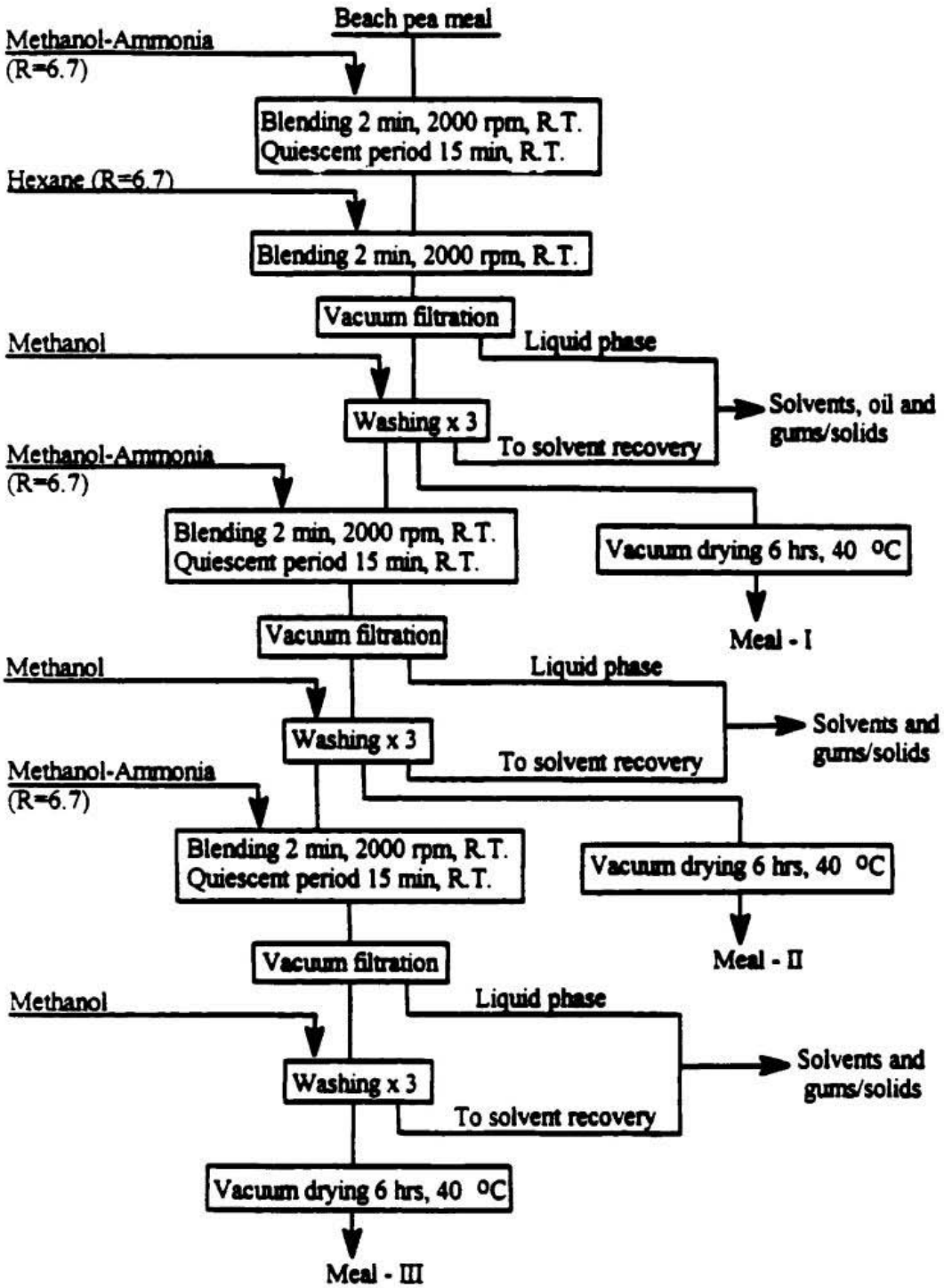


Figure 3.3 Flow chart for methanol-ammonia-water/hexane solvent extraction system.

3.2.5) were carried out in a similar manner to that described in Section 3.2.2.

3.2.5.2 Oligosaccharides

The content of oligosaccharides was determined according to the method of Southgate (1991). Approximately 3 - 5 g beach pea, grass pea and methanol-ammonia-water treated samples were weighed into 100 mL beakers, to which 25 mL of 85% (v/v) methanol were added. Beakers containing the sample and solvent were placed on an electric hot-plate and the mixture brought to boil while stirring with a glass rod. This procedure was carried out in a fumehood. The mixture, while hot, was filtered through a Whatman No. 41 filter paper into a 100 mL volumetric flask. This process was repeated three more times with aqueous methanol, allowing the filtrate to drain between successive extractions. In the second and subsequent extractions, it was essential to stir the mixture continuously while heating in order to avoid losses through bumping. The final volume of the combined filtrates was made to 100 mL with 85% (v/v) methanol.

Oligosaccharides in the extracts were separated on a LiChroCART NH_2 analytical column (250 x 4 mm, particle size 7 µm; Merck, Darmstadt, Germany). The mobile phase consisted of a mixture of acetonitrile-water (65:35, v/v). The system used for the analysis was a Shimadzu HPLC system, consisting of an LC-10 AD pump, RID-6A column oven, SCL-6B system controller and a computer system C-R4A Chromatopac. An injection volume of 20 µL sample and a flow rate of 1 mL/min were used for the analysis. Oligosaccharides in the extract were detected using a refractive index detector

and tentatively identified by comparing their retention times with those of known standards. Standards used were: sucrose, raffinose and stachyose (Sigma Chem. Co., St. Louis, MO). Verbascose was obtained from Faba bean according to Amarowicz *et al.* (1992a).

3.2.6 Separation of protein classes based on solubility characteristics

Protein classes of beach pea, green pea and grass pea seeds were separated according to their solubility using a modified Osborne classification procedure as described by Lund and Sandstrom (1943). Defatted and dried pea samples (approximately 2 - 3 g) were dispersed and extracted with 25 mL of distilled water over a 15 min period at room temperature (25±1 °C) using a Gyratory shaking water bath (Gyratory water bath shaker Model G76, New Brunswick Scientific Co., Inc. New Brunswick, NJ). The suspension was then centrifuged at 4000 x g for 10 min and the supernatant was recovered and saved. The residues were re-extracted two more times with the same solvent under similar conditions and recovered supernatants were combined and regarded as the water-soluble fraction. The residue was then extracted successively with 5% (w/v) NaCl, 70% (v/v) ethanol at 65 °C in a shaking water bath and 0.2% (w/v) NaOH in a similar manner as for the water-soluble fraction; respective soluble fractions were collected separately. The total nitrogen contents of the supernatants collected and the residue left after sequential extractions were determined using the Kjeldahl method as described in Section 3.2.2.2. The content of each protein fraction was calculated as a

percentage of the total nitrogen content (as sum of nitrogen content of all fractions including residue) of the meal.

3.2.7 Determination of protein and soluble nitrogen

Approximately 0.5 g of different pea meals were dispersed in distilled water (1:100, w/v) and pH of the dispersion was adjusted between 2.0 and 12.0 using 1 N solutions of HCl and NaOH. The dispersions were shaken at 200 rpm in an orbital shaker for 30 min and pH values were recorded after mixing. Samples were centrifuged at 3500 x g for 20 min and nitrogen content of the supernatant was determined by Kjeldahl analysis (AOAC, 1990; see Section 3.2.2.2). The content of soluble nitrogen was expressed as the percent ratio of nitrogen in the supernatant to that in the meal.

The pH of the remaining supernatant was adjusted to 4.5±0.1. It was then centrifuged at 4000 x g for 20 min and nitrogen content of an aliquot of it was determined by Kjeldahl nitrogen analysis (Section 3.2.2.2). The difference in the soluble nitrogen content of supernatants before and after pH adjustment to 4.5±0.1 was considered as protein nitrogen content and expressed as percentage of protein nitrogen recovered from total nitrogen of the meal. Volume of aliquots recovered for nitrogen determination was considered in the calculations. Percentages of soluble nitrogen and protein nitrogen were calculated using the formula given below.

$$\text{Soluble nitrogen \%} = \frac{\text{mg of nitrogen}_{\text{extract}}}{\text{mg of nitrogen}_{\text{sample}}} \times 100$$

$$\text{Protein nitrogen \%} = \frac{\text{mg of nitrogen}_{\text{extract}} - \text{mg of nitrogen}_{\text{supernatant}}}{\text{mg of nitrogen}_{\text{sample}}} \times 100$$

3.2.8 Separation of different protein fractions and Polyacrylamide gel electrophoresis

3.2.8.1 Separation of protein fractions

Defatted and dried pea samples (1 g) were dispersed and extracted into 25 mL of distilled water over a 30 min period at room temperature using a magnetic stirrer. The suspension was then centrifuged at 4000 x g for 20 min and the resultant supernatant recovered and saved. The residues were re-extracted three more times with the same solvent under similar conditions and recovered supernatants were combined and regarded as the water-soluble fraction. The residue was then extracted successively with a 0.5 M solution of sodium chloride in a 0.01 M phosphate buffer (pH 7.0), 70% ethanol at 65 °C in a shaking water bath and 0.1 N sodium hydroxide to separate the total seed proteins into albumin, globulin, prolamine and glutelin fractions, respectively. Proteins from respective soluble fractions were precipitated by isoelectric precipitation at pH 4.5, using 1 N HCl or NaOH and separated by centrifugation at 12,000 x g for 20 min (Sorvall Superspeed RC2-B, Automatic Refrigerated Centrifuge Newtown, CT). The precipitate was washed with distilled water at pH 4.5, redispersed in distilled water, neutralized at pH 7.0, and then freeze dried. These freeze dried fractions were used for determination of total crude protein (see Section 3.2.2.2) and amino acid composition (see Section 3.2.2.10).

3.2.8.2 Scanning electron microscopy (SEM)

Structural morphology of protein fractions were studied by scanning electron microscopy as described in Section 3.2.3.1.5.

3.2.8.3 Polyacrylamide gel electrophoresis

Protein fractions, separated as described in above section (3.2.8.1), and protein isolates (Section 3.2.9.1) were used for gel electrophoresis studies.

For comparison, non-denatured proteins present in protein isolates of beach pea, green pea and Canadian grass pea were prepared by extracting the meal with distilled water (pH adjusted at 9.0 with 1N sodium hydroxide) and sodium hexametaphosphate (SHMP; 2.8%, w/v) at a meal to solvent ratio of 1:5 (w/v) at pH 9.0. The extracted protein precipitated at pH 4.5 was then redissolved in distilled water, adjusted to pH 7.0 and extensively dialyzed against ten changes of distilled water at 4 °C for 72 h. The dialyzed extract was centrifuged at 12,000 x g for 20 min and then freeze dried prior to use for gel-electrophoresis studies.

Non-denaturing polyacrylamide gel electrophoresis (NPAGE) was performed on 12% (w/v) vertical polyacrylamide Bio-Rad gels (13.5 cm, length and 2 mm thick) at pH 6.8 using a 20 mM Tris-glycine buffer containing 1% bromophenol blue and 0.01% sodium azide. Protein samples 100 µg, were loaded onto each well and electrophoresed (Electrophoresis Apparatus Bio-Rad Protean™) at a constant current at 80 volts for stacking gels and 180 volts for resolving gels (for 240 min) supplied by Pharmacia

electrophoresis constant power supply unit (ECPS 2000/300, Pharmacia Fine Chemicals, Uppsala, Sweden).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on Bio-Rad gels composed of stacking gel (4%, w/v, 1.5 cm) and resolving gel (12%, w/v, 12 cm) as given above. Protein samples were dissolved in 65 mM Tris-HCl (pH 6.8) containing 10% (w/v) SDS and 1% bromophenol blue. Reduction of disulphide bridges was performed by the addition of a small quantity of dithiothreitol (0.8M) (DTT) at 100 °C for 3 min. Protein samples (100 μg) were loaded onto each well and electrophoresis was conducted at a constant current of 80 volts for stacking gels and 180 volts for resolving gels until the dye travelled the same distance for all wells to the end of the gel (for 240 min). The molecular weight markers used were myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45, kDa), carbonic anhydrase (29 kDa), and α-lactalbumin (14.2 kDa). The relationship between the molecular weight of the protein standards and their mobility on the gel is provided in Figure A9.

Following electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 in acetic acid-water-methanol (1:4:5, v/v/v) and destained in a mixture of methanol-acetic acid-water (1:1.5:17.5, v/v/v) until a desired background colour was obtained. The gels were fixed and stored in a 7% (v/v) acetic acid solution.

3.2.9 Preparation of beach pea, green pea, and grass pea protein isolates

3.2.9.1 Extraction of proteins and preparation of isolates

Beach pea, green pea and grass pea meals (25 - 50 g) were added to distilled water or 2.8% (w/v) sodium hexametaphosphate (Albright and Wilson Americas Toronto, ON]; a meal-to-solvent ratio of R=1:5 was used. The mixture was stirred with a magnetic stirrer for 10 min, then pH was adjusted to 9.0 using 1 N HCl or NaOH and continued stirring for another 30 min at room temperature. Each extract was separated by centrifugation at 4000 x g for 20 min. The residues were re-extracted two more times with the same solvent under similar conditions. The extracts were combined and the protein precipitated by adjusting the pH to 4.5 with 1 N HCl and subsequent separation by centrifugation at 4000 x g for 20 min. The precipitate was redispersed in 100 mL distilled water at pH 9.0 and reprecipitated at pH 4.5. After separation of the protein by centrifugation, the precipitate was washed two times with distilled water (R=1:2). The precipitated protein was re-suspended in distilled water and the pH was adjusted to 7.0 with 1 N NaOH prior to freeze drying. The freeze-dried protein isolates were stored in air-tight glass bottles at room temperature for further analyses. All extractions were carried out in triplicate. Sufficient quantities of beach pea, green pea, and grass pea protein isolates were prepared as outlined in Figure 3.4.

3.2.9.2 Chemical analyses

Chemical analyses of protein isolates were carried out essentially in a similar

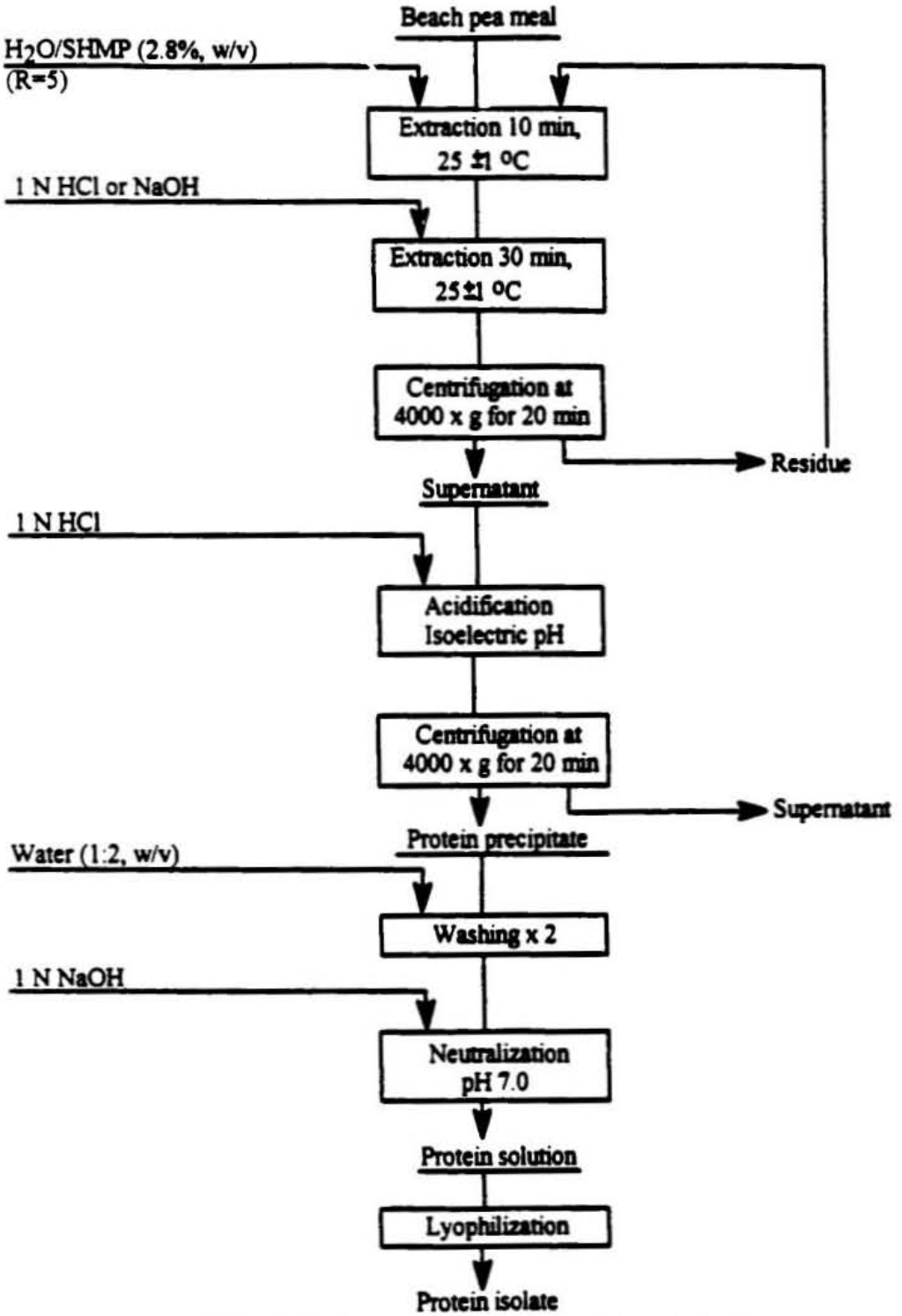


Figure 3.4 Flow chart for preparation of protein isolates.

manner to those described in Section 3.2.2.

3.2.10. Evaluation of functional properties of protein isolates

3.2.10.1 Water absorption capacity

Water absorption capacity was determined by a combination of the AACC (1995) method and those of Sosulski (1962) and Rutkowski and Kozlowska (1981). A 2 g sample was dispersed in 20 mL of distilled water. The contents were mixed for 30 s every 10 min using a glass rod and after mixing five times, centrifuged at 4000 x g for 20 min. The supernatant was carefully decanted, then the tube was drained at a 45 degree angle for 10 min and weighed. The water absorption capacity of the protein isolate was expressed as the percentage increase of the sample weight.

3.2.10.2 Whippability and foam stability

One hundred millilitres of a dispersion of protein isolates (1%, w/v) in distilled water were homogenized for 60 s using a Polytron homogenizer at 10,000 rpm. The mixture was then immediately transferred into a 250 mL measuring cylinder and the foam volume was recorded. The percentage ratio of the volume increase to that of the original volume of protein solution was calculated and expressed as foam capacity or whippability (Naczk *et al.*, 1985). Foam stability was expressed (on the basis of 100 mL of a 1%, w/v dispersion) as the volume of the foam remaining after 0, 15, 30, and 60 min of quiescent period.

3.2.10.3 Fat binding capacity

Fat binding capacity of the protein isolates was determined by a turbidimetric method as described by Voutsinas and Nakai (1983). Lyophilized sample (40 mg) in a centrifuge tube (1.5 mL) pure corn oil was added to and the mixture was homogenized for 1 min at 8,000 rpm using a Polytron homogenizer. The protein dispersion was centrifuged at 3020 x g for 20 min after holding for 30 min at room temperature. The free oil separated after centrifugation was pipetted off and 2 mL of distilled water were added to the contents of the tube. Oil adhered to the sides of the tube was then removed with the help of a glass rod. Any oil trapped below the protein precipitate was removed by forcing it to the surface of the water. To the content of the tube was added 1 mL of 0.1 M metaphosphoric acid [$(HPO_3)_n$, 35% HPO_3, pH 2.1) followed by centrifugation at 4.200 x g for 15 min. The supernatant was pipetted off and the precipitate was washed with distilled water (3 to 4 mL) without dispersing it. Finally, the tube walls were cleaned with a cotton swab to remove any excess oil deposits. The protein precipitate was mixed well with 0.3 mL of distilled water and then 20 mL of the digestion medium (7 M urea in 50% H_2SO_4) were added to the mixture in 2 mL portions. The mixture was homogenized using a Polytron homogenizer for 30 s at 4000 rpm. The homogenate was held at room temperature for 30 min and the absorbance was then read at 600 nm using the digestion mixture as blank. The aqueous supernatants, removed in the previous steps, were used for determination of lost protein during handling of the precipitate as given in Section 3.2.2.9 (Lowry *et al.*, 1951).

The standard curve given in Figure A10 for determination of bound oil content was prepared as follows. In a series of proteins (40 mg) and 0 to 100 mg of pure corn oil (specific gravity = 0.89) were added followed by subsequent mixing with a glass rod. While the mixture was being mixed, 0.3 mL of distilled water and then 20 mL of digestion mixture were added to the tube. The mixture was homogenized at 4000 rpm for 30 s and held at room temperature for 30 min before reading the absorbance at 600 nm.

3.2.10.4 Emulsifying activity

Emulsifying activity of protein isolates was determined according to the modified method of Pearce and Kinsella (1978), as described by Wanasundara and Shahidi (1997) for succinylated and acetylated flaxseed protein isolates. Protein dispersions (0.5%, w/v, 4 mL) in a Britton-Robinson Universal buffer (Britton, 1956) in the pH range of 2.0 to 12.0 and NaCl concentration of 0, 0.35 and 0.70 M, 4 mL of pure corn oil were added. The mixture was then homogenized for 1 min at 2000 rpm using a Polytron homogenizer. A 50 μL volume of the emulsion formed was immediately taken from the bottom of the container and diluted in 10 mL of the same buffer containing 0.10% (w/v) SDS. Absorbances of the diluted samples were read at 500 nm using a diode array UV/VIS spectrophotometer and recorded as emulsifying activity values.

3.2.10.5 Emulsion stability

Samples for determination of emulsion stability were prepared in a similar manner to those for determination of emulsifying activity. The absorbance at 500 nm was read as soon as the emulsion was formed, i.e. zero time. Subsequently, aliquots were removed at appropriate time intervals and absorbance values read at 500 nm. Emulsion stability was determined as the time, in min, required for absorbance at 500 nm to reach one half of that for the emulsion at zero time (i.e. half-life; Paulson and Tung, 1988).

3.2.10.6 Solubility

To study the effect of pH and salt concentration on solubility, 1% (w/v) protein dispersions were prepared by mixing 0.25 g of the isolate with 0, 2.0 or 4.0 mL of a 25.6% (w/v) NaCl solution in order to make a final NaCl concentration of 0, 0.35, or 0.70 M. The pH was then adjusted with 2 N NaOH or 2 N HCl followed by addition of distilled water to reach a 25 mL volume. The protein dispersion was centrifuged at 4000 x g for 15 min and protein content of the supernatant after centrifugation was determined using the Kjeldahl method (AOAC, 1990) as described in Section 3.2.2.2. The solubility was expressed as percent ratio of protein content of the supernatant to that of the suspension.

3.2.11 *In-vitro* digestibility

In-vitro digestibility of protein isolates was determined using trypsin-pepsin and

pepsin-pancreatin enzyme system according to the method of Saunders *et al.* (1973) with minor modifications. In a centrifuge tube, 1 g of protein material was suspended in 20 mL of 0.10 N HCl and mixed with 50 mg pepsin (from porcine stomach mucosa, 570 AU/mg solid) in 1 mL of 0.01 N HCl. The mixture was gently shaken at 37 °C for 48 h and then centrifuged (4000 x g for 10 min). After removing the supernatant, solids were suspended in a solution made of 10 mL of water and 10 mL of a 0.10 M phosphate buffer (pH 8.0) containing 5 mg trypsin (from porcine pancreas, 1870 BAEE units/mg solid). The mixture was gently shaken for 16 h at 23 °C in a water bath shaker. The digested mixture was then centrifuged and trichloroacetic acid (TCA) was added to the supernatant to reach a concentration of 8 M in the solution. The supernatant previously obtained from pepsin digestion was also treated in a similar manner. Precipitated proteins were removed by centrifugation at 10,000 x g for 25 min. The TCA-soluble nitrogen content of the supernatant was determined by Kjeldahl nitrogen analysis.

For pepsin-pancreatin digestion, 250 mg of the sample were suspended in 15 mL of 0.1 N HCl containing 1.5 mg of pepsin followed by gentle shaking for 15 min at 37 °C. The resultant solution was then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin (from porcine pancreas, activity equivalent to 4 x U.S.Pharmacopoeia) in 7.5 mL of phosphate buffer (0.1 M, pH 8.0). The mixture was shaken for 24 h at 37 °C in a water bath shaker and the undigested solids were separated by centrifugation, as given above. The supernatant was treated in a similar manner as described earlier for trypsin-pepsin digestion; nitrogen content was then determined by Kjeldahl analysis. *In-vitro*

digestibility was expressed as percentage enzymatic digestion as given below.

$$\text{Enzymatic digestion, \%} = \frac{\text{Nitrogen (non-protein nitrogen) released by enzyme}}{\text{Total nitrogen content of undigested sample}} \times 100$$

3.2.12 Starch isolation and characterization

3.2.12.1 Starch isolation and purification

Beach pea, green pea and Canadian grass pea seeds were divided into two lots representing the whole samples. Each lot was further subdivided into two parts and starch was extracted from each. Beach pea seeds were placed into 1000 mL beakers containing distilled water (seeds to water ratio was 1:3, w/v) and kept in a water bath at 50 °C for 48 h. One part steeped grains or seeds and three parts distilled water were blended for 3 min in a Waring blender (Waring Products Division Dynamics Corporation of America) at low speed followed by a further 3 min at the medium speed. The resultant slurry was passed through a double layer of cheese cloth and then centrifuged at 5000 x g for 20 min. The supernatant was discarded and the sediment re-suspended in excess distilled water containing 0.04% NaOH to remove any residual proteins and phenolic compounds (supernatant becomes dark green or red). After standing for 4 h, the supernatant was discarded. This procedure was repeated six to eight times (or until the supernatant became colourless). The final sediment was suspended in distilled water, and then subjected to filtration through a 70 μ polypropylene screen, neutralized to pH 7.0, filtered on a Büchner funnel and thoroughly washed with distilled water. The filter cake was

dried overnight at room temperature (25 °C).

Starch isolation and purification from green pea and grass pea was carried out according to the procedure of Hoover and Manuel (1996). Seeds were steeped overnight in water (seed to water ratio was 1:3, w/v) at room temperature. Sodium hydroxide (0.02%) was used to remove the residual proteins. Other procedures used were the same as those employed for beach pea starch isolation and purification. Dried starch was stored in air-tight glass bottles for further analyses.

3.2.12.2 Moisture

Moisture content in starch was determined as described in Section 3.2.2.1.

3.2.12.3 Ash

Ash content of starch was determined essentially in a similar manner to that described in Section 3.2.2.5.

3.2.12.4 Lipids

3.2.12.4.1 Surface lipids

Starch lipids were analyzed by first extracting 5 g (db) of the starch with 100 mL of chloroform-methanol (2:1, v/v) under vigorous agitation in a wrist action shaker for 1 h. The content was then filtered through a thimble into a preweighed round bottom flask and the residue was rinsed with a small quantity of chloroform-methanol. The

solvent was then removed at 30 °C using a rotary vacuum evaporator.

3.2.12.4.2 Bound lipids

The residues after chloroform-methanol extraction were extracted with 100 mL of n-propanol-water (3:1, v/v) at 90-100 °C for 7 h in a Soxhlet extraction apparatus. Extracted lipids were isolated from n-propanol-water mixture after solvent removal.

3.2.12.4.3 Total lipids

Two gram of starch sample were placed in a screw capped tube which was wetted with about 2 mL of 95% (v/v) ethanol. Subsequently, 25 mL 24% (w/v) HCl was added to the tube which was then placed in a water bath at 70-80 °C for 30 min, with periodic shaking (till a clear suspension obtained). The hydrolysate was then extracted four times with hexane in a round bottom flask; the solvent was then removed using a rotary vacuum evaporator.

3.2.12.4.4 Purification of lipids

Surface lipids, bound lipids, and total lipids were purified according to Bligh and Dyer (1959). The volumes of chloroform, methanol, and water before and after dilution were kept in the proportions of 1:2:0.8 and 2:2:1.8, (v/v/v), respectively, at room temperature. The chloroform layer was then diluted with benzene and brought to dryness using a rotary evaporator. The flask was then kept in a desiccator for 2 h and weighed.

The percent lipid in the sample was calculated.

3.2.12.5 Nitrogen

Nitrogen content of starch was determined essentially in a similar manner to that described in Section 3.2.2.2.

3.2.12.6 Estimation of starch damage

The extent of starch damage was determined enzymatically (AACC, 1995). The method determines the percentage of starch granules which are susceptible to hydrolysis by α-amylase. The enzyme used was a fungal α-amylase from *Aspergillus oryzae*, 44 units per milligram solid (Sigma Chemicals Co., St. Louis, MO). One unit of enzyme will liberate one milligram of maltose from starch in 3 min at pH 6.9 at 20 °C, respectively. The starch (1.0 g, db) was digested with α-amylase (12,500 Sigma units) in a 30 °C water bath for exactly 15 min, followed by addition of 3.68 N H_2SO_4 (3.0 mL) and 12% (w/v) sodium tungstate dihydrate ($Na_2WO_4 . 2H_2O$; 2.0 mL). The mixture was allowed to stand for 2 min, and then filtered. Sample aliquots (1 mL) of the carbohydrate solution were mixed with 2.0 mL of chilled 3,5-dinitrosalicylic acid (2% in 1N NaOH) and then diluted to 4.0 mL with distilled water. The tubes were subsequently heated in a boiling water bath for 5 min and the reaction mixture was diluted with 8 mL of distilled water. The absorbance was then read at 540 and 590 nm (for absorbance above 1.5) against a blank in which no enzyme was present (Bruner, 1964). A calibration curve was

established (at 540 and 590 nm) with maltose (0.2-2.0 mg in 2 mL H_2O). The percentage of damaged starch was calculated as: % damaged starch = (M x 1.64)/(W x 1.05) x 100 Where M = mg maltose equivalents in the digests; W = mg starch (db). 1.05 = molecular weight conversion of starch to maltose and 1.64 = the reciprocal of the mean percentage maltose yield from gelatinized starches. The latter is an empirical factor which assumes that under the conditions of the experiment, the maximum hydrolysis is 61%.

3.2.12.7 Amylose content

The apparent amylose content of native starches was determined by the method of Chrastil (1987) after complete dispersion of samples in a 0.5 N KOH solution followed by neutralization with 0.1 N HCl at room temperature. The total amylose content of the starches was determined by the same procedure.

3.2.12.7.1 Preparation of starch dispersions

Starch samples (20 mg, db) were dispersed in 10 mL of 0.5 N KOH in 20 mL screw capped glass tubes. The contents were transferred into volumetric flasks and diluted to 100 mL. Aliquots (10 mL) were then neutralized with 5 mL of 0.1 N HCl prior to dilution to 50 mL.

3.2.12.7.2 Chrastil's method of amylose determination

Sample aliquots (0.1 mL) of the neutralized solution were mixed with 5.0 mL of

0.5% trichloroacetic acid (TCA) and 0.05 mL of 0.01 M I_2-KI solution (1.27 g of I_2 per litre = 3 g of KI per litre). The absorbance of the blue colour was read at 620 nm (after 30 min at 25 °C) using a diode array spectrophotometer. The absorbance of the reaction blanks with water was zero, and with pure amylopectin (corn amylopectin from Sigma Chem. Co., St. Louis, MO purified by precipitation with acetone) was less than 0.03. The amylose content was calculated using the standard curve for amylose (Figure A11).

3.2.12.8 Swelling factor

The swelling factor (SF) of starches when heated at 50-95 °C in excess water was measured in triplicate according to the method of Tester and Morrison (1990). Starch samples (50-200 mg on a dry weight basis, depending on the anticipated SF) were weighed exactly to 50 mg into 10 mL screw capped tubes; 5.0 mL of water were added, and the sealed tubes were incubated with constant shaking in a water bath at the required temperature for 30 min. The tubes were then cooled rapidly to 20 °C, 0.5 mL of blue dextran (0.5%, w/v) (Pharmacia, M_r $2x10^6$, 5 mg/mL) was added and the contents were mixed gently by inverting the closed tubes several times. After centrifugation at 1,500 x g for 5 min the absorbance of the supernatant (A_s) was read at 620 nm. The absorbance of the reference (A_r) tube devoid of any starch was also recorded. Calculation of SF was based on starch weight, corrected to 10% moisture content, and assuming a density of 1.4 mg/mL.

Free or interstitial plus supernatant water (FW) is given by $FW = 5.5 (A_r/A_s) - 0.5$.

A_r and A_s are absorbances of the reference and the sample, respectively. The initial volume of the starch (V_o) of weight W (in milligrams) is $V_o = W/1,400$ and the volume of the absorbed intragranular water (V_i) is thus $V_i = 5.0 - FW$; hence, the volume of the swollen starch granules (V_z) is $V_z = V_o + V_i$ and $SF = V_z/V_o$. This can also be expressed by a single equation as given below.

$SF = 1 + \{(7700/W) \times ([A_s - A_r]/A_s)\}$

3.2.12.9 Extent of amylose leaching

Starch samples (20-25 mg) in distilled water (10 mL) were heated (50-95 °C) in sealed tubes for 30 min. The tubes were then cooled to ambient temperatures and centrifuged at 4000 x g for 20 min. An aliquot (0.1 mL) of the supernatant was assayed for solubilized amylose, using the method of Chrastil (1987) (Section 3.2.12.7.2). Percentage amylose leaching was calculated and presented as mg of amylose leached per 100 mg of dry starch.

3.2.14.10 X-ray diffraction

X-ray diffractions were recorded using a Rigaku RU 200 R X-ray Diffractometer (Rijaku-Denki Co., Japan) connected to a data acquisition and processing station. The starch powder (≈10% moisture, wet basis) was scanned through the 2θ range of 3-35°. Traces were obtained using Cu-Kα radiation detector with a nickel filter and a scintillation counter operating under the following conditions: 40 kV, 50 mA, 1°/1°

divergence slit/scattering slit, 0.3 mm receiving slit, 1 s time constant and scanning rate of 3°/min.

3.2.12.11 Differential scanning calorimetry (DSC)

Gelatinization temperature of starches was measured and recorded on a Perkin-Elmer Differential Scanning Calorimeter-2 (DSC-2) (Perkin-Elmer Corporation Norwalk, CT) equipped with a thermal analysis data acquisition and processing station. Water (9.0 µL) was added with a microsyring to starch (3.0 mg) in DSC pans which were then sealed, reweighed and allowed to stand overnight at room temperature. The scanning temperature range and the heating rate were 30 - 100 °C and 10 °C per min, respectively. The thermogram was recorded using water as a reference. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c) temperatures of the gelatinization endotherm. Indium was used for calibration. The enthalpy (ΔH) was estimated by integrating the area under the peak (Figure A12) and expressed as calories per unit weight of dry starch. All DSC experiments were replicated at least three times.

3.2.12.12 Brabender viscoamylography

Pasting characteristics of starch slurries at a concentration of 7% (w/v) and pH 5.5 were determined using the Brabender viscoamylograph, Model VA-V (C. W. Brabender instruments, Inc., South Hackensack, NJ), equipped with a 700-cm. g sensitivity cartridge, operating at a bowl speed of 75 rpm. The starch slurry was heated from 30 to 96 °C at

the rate of 1.5 °C/min, maintained at 96 °C for 30 min, and then cooled to 50 °C at the same rate. The viscosity was measured in Brabender units (BU).

3.2.12.13 Scanning electron microscopy (SEM)

Granule morphology of starches were studied by scanning electron microscopy as described in Section 3.2.8.2.

3.2.12.14 Enzymatic hydrolysis

Enzymatic digestion studies on starches were carried out using crystalline porcine pancreatic α-amylase (Sigma Chemical Co., St. Louis, MO) in 2.9 M NaCl containing 3 mM $CaCl_2$, in which the concentration of α-amylase was 30.0 mg ml^{-1}, and the specific activity was 790 units per milligram of protein. One unit activity was defined as the amount of α-amylase which liberated 1 mg maltose in 3 min at 20 °C and pH 6.9.

The procedure used for enzyme hydrolysis was essentially that of Knutson *et al.* (1982). However, a higher concentration of enzyme was used in this study. Starch granules (100 mg) were suspended in distilled water (25 mL) and 5 mL aliquots were placed in a constant temperature water bath at 37 °C. Then 4.0 mL of 0.1 M phosphate buffer (pH 6.9) containing 0.006 M NaCl were added to the slurry. The mixture was gently stirred before adding 5 μL α-amylase suspension. The reaction mixtures were shaken manually on an hourly basis to resuspend the deposited granules. Then 1.0 mL aliquots were removed at specified time intervals, pipetted into 0.2 mL of 95% (v/v)

ethanol, and centrifuged. Aliquots of the supernatant were analyzed for soluble carbohydrates (Bruner, 1964). Percentage hydrolysis was calculated as the amount (mg) of maltose released per 100 mg of dry starch using a standard curve for maltose (Figure A13). Controls without enzyme, but subjected to the above experimental conditions, were run concurrently.

3.2.12.15 Acid hydrolysis

The starches were hydrolysed with 2.2 N HCl at 35 °C (1.0 g starch/40 mL acid) for 20 days. The starch slurries were shaken manually on a daily basis to resuspend the deposited granules. At specified time intervals, aliquots (1.0 mL) of the reaction mixtures were neutralized and centrifuged at 4000 x g for 10 min; the supernatant was then assayed for its total carbohydrate content (Bruner, 1964). Controls without acid, but subjected to the above experimental conditions, were run concurrently. The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial dry starch.

3.2.13 Statistical analyses

All experiments were replicated three to six times. Mean values with standard deviations (SD) were reported. Analysis of variance (ANOVA) was performed and differences in mean values were determined using Tukey's studentized test at $p<0.05$ and employing ANOVA and TUKEY's Procedures of Statistical Analytical System (SAS,

1990), respectively. Linear regression analyses were also performed using the same software. Experimental designs used in the study are described in appropriate sections.

For testing significance of data when expressed as percentage and their range is below 30 or above 70 then the experimental error variance will not be constant over all observations. Such a situation will affect both the significance levels and the sensitivity of the t- and F-tests. In such cases, the appropriate error variance for comparing one pair of treatments might be four times as large as that for another pair and the use of the same estimated variance for both comparisons would lead to completely erroneous t- and F-tests. To avoid such types of errors a transformation can be performed that will place the data on a scale on which the error variance is nearly constant. Fortunately, it often happens that such transformations also bring the distribution of errors closer to normality. Therefore, prior to statistical analysis, data that was reported as percentages were transformed as given below (Snedecor and Cochram, 1980).

$$\text{transformed } x = \arcsin\sqrt{x/100}$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physico-chemical properties of beach pea, green pea and grass pea seeds

Physico-chemical properties of beach pea seeds, namely grain weight, density, hydration capacity, hydration index, swelling capacity, swelling index and colour are presented in Table 4.1. These results are also compared with those of green pea and Canadian grass pea. The grain weight of beach pea was 3.01 g/100 seeds while green pea had the highest seed weight (25.27 g/100 seeds). Meanwhile, the seed density for beach pea was less than half of those of green pea and Canadian grass pea.

Hydration capacity and indix of immature beach pea was one order of magnitude lower than those of mature seeds. Hydration capacity and hydration index of beach pea were significantly lower than those of green pea and Canadian grass pea. Among pea samples examined, beach pea had the lowest hydration capacity (0.005 g/seed) and hydration index (0.16), while green pea had the highest hydration capacity (0.24 g/seed) and hydration index (0.95).

Swelling capacity and swelling index of beach pea was lower than green and Canadian grass pea samples. Since beach pea had the lowest density, hydration capacity, hydration index, swelling capacity, and swelling index, it may require more time in order to germinate or cook. Physico-chemical properties of beach pea and other peas in the present study were similar to that observed for other leguminous seeds such as cowpea (Ojomo, and Chheda, 1972), faba bean (Ahmed and Shehata, 1982), vicia faba (Sharma, 1989), soybean (Latunda Dada, 1991) and vegetable pea (Bonneville and Arke) and field

Table 4.1 Physico-chemical properties of beach pea, green pea and grass pea[1]

Parameter	Beach pea, Immature	Beach pea, Mature	Green pea	Grass pea[2]
Seed colour	Green	Black	Green	Light brown
Grain weight (g/100 seeds)	3.09 ± 0.06^{cd}	3.01 ± 0.07^{d}	25.27 ± 0.05^{a}	16.33 ± 0.47^{b}
Density (g/mL)	0.59 ± 0.07^{bc}	0.56 ± 0.05^{c}	1.27 ± 0.00^{a}	1.21 ± 0.02^{a}
Hydration capacity (g/seed)	0.0005 ± 0.0001^{d}	0.005 ± 0.001^{c}	0.24 ± 0.00^{a}	0.15 ± 0.002^{b}
Hydration Index	0.016 ± 0.003^{d}	0.16 ± 0.03^{c}	0.95 ± 0.00^{a}	0.89 ± 0.007^{b}
Swelling capacity (mL/seed)	0.003 ± 0.0003^{d}	0.008 ± 0.003^{cd}	0.04 ± 0.00^{a}	0.015 ± 0.005^{bc}
Swelling Index	0.07 ± 0.006^{b}	0.16 ± 0.07^{ab}	0.20 ± 0.00^{a}	0.11 ± 0.03^{ab}

[1]Results are mean values of four determinations, ± standard deviation. Means in the same row with different superscripts are significantly ($p<0.05$) different.

[2]Canadian grass pea.

pea (HFP4 and Rachna) (Bishnoi and Khetarpaul, 1993). Akinyele *et al.* (1986) and Bishnoi and Khetarpaul (1993) noted that the above parameters were important for judging the cooking time for seeds and preference by consumers and processors.

4.2 Chemical composition of seeds of beach pea, green pea and grass pea

4.2.1 Chemical composition of pea seeds

The proximate compositions of beach pea, green pea and grass pea are presented in Table 4.2. Moisture content of beach pea seeds was 9.69% which within the range of 8.20 to 10.41% observed for other pea cultivars examined. Meanwhile, beach pea had the highest crude protein content (29.16%) as compared to green pea (23.51%), Canadian grass pea (23.64%) and Indian grass pea (21.33%). Beach pea had a significantly higher amount of crude fibre almost two fold (12.00%), reducing sugar (171.82 mg/100g) and total phenolics (1.19%) over 4 fold than green and grass pea samples. The differences in total crude fibre content among beach pea, grass pea and green pea may perhaps originate from the existing differences in testa structure and thick walled, leathery skins of seeds used. Ene-Obong and Carnovale (1992) obtained similar results for African yam bean, pigeonpea and cowpea legumes. Beach pea contained 3.34% soluble sugars while green pea contained a higher amount of soluble sugars (5.68%) followed by Canadian grass pea (3.79%), and Indian grass pea (2.22%). Beach pea contained the lowest amount of starch and lipid when compared with other peas. The ash and soluble protein contents

Table 4.2 Chemical composition of beach pea, green pea and grass pea[1]

Constituent, %	Beach pea[2]	Green pea	Grass pea[3]	Grass pea[4]
Moisture	9.69±0.29^{b}	8.20±0.23^{d}	8.60±0.05^{cd}	10.41±0.05^{a}
Protein (% N x 6.25)	29.16±0.15^{a}	23.51±0.39^{c}	23.64±0.07^{bc}	21.33±1.21^{d}
Soluble proteins (mg/100g)	305.72±2.72^{c}	456.19±6.90^{a}	344.19±6.09^{b}	218.88±4.90^{d}
Lipid	1.11±0.14^{b}	1.48±0.09^{a}	1.34±0.15^{a}	1.20±0.02^{a}
Ash	3.04±0.03^{a}	2.61±0.01^{d}	2.89±0.01^{b}	2.73±0.01^{c}
Crude fibre	12.00±0.24^{a}	5.53±0.31^{cd}	5.00±0.53^{d}	6.43±0.39^{b}
Carbohydrates[5]	57.00±0.39^{b}	64.20±0.28^{a}	63.53±0.72^{a}	64.33±0.32^{a}
Soluble sugars	3.34±0.04^{c}	5.68±0.13^{a}	3.79±0.04^{b}	2.22±0.05^{d}
Reducing sugars (mg/100g)	171.82±3.19^{a}	122.44±3.01^{b}	105.15±1.02^{cd}	103.41±5.92^{d}
Non-reducing sugars	3.17±0.01^{c}	5.55±0.13^{a}	3.69±0.04^{b}	2.12±0.05^{d}
Starch	24.70±0.46^{d}	34.12±0.06^{b}	39.01±0.46^{a}	29.00±0.16^{c}
Phenolics	1.19±0.001^{a}	0.26±0.003^{bc}	0.25±0.001^{c}	0.20±0.002^{d}

[1]Results are mean values of triplicate determinations, ± standard deviation. Results other than moisture content are on a dry weight basis. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.

[2]Values are for composite seed samples as harvested, containing both mature and immature seeds.

[3]Canadian grass pea.

[4]Indian grass pea.

[5]By difference from 100-(moisture + crude protein + lipid + ash).

of beach pea were at par with the Canadian and Indian varieties of grass pea but green pea contained a slightly higher amount of these constituents than those present in beach pea and grass pea. Beach pea possessed 57.00% total carbohydrates as compared to 64.33% for Indian grass pea, 64.20% for green pea and 63.53% for Canadian grass pea. These values are within the range reported in the literature for different peas such as field pea (Moran *et al.*, 1968), green pea (Gueguen and Barbot, 1988; Savage and Deo, 1989), pigeon pea (Kumar *et al.*, 1991), cowpea (Giami, 1993), chickpea and green bean (Barrado *et al.*, 1994), bambara groundnut and pigeon pea (Igbedioh *et al.*, 1994).

4.2.2 Total and free amino acid composition of beach pea, green pea and grass peas

Beach pea as well as grass pea and green pea were somewhat deficient in sulphur-containing (cysteine and methionine) amino acids (Table 4.3). The content of total sulphur-containing amino acids in beach pea (2.71 g/16g N) was higher than that of other pea seeds. The relatively low content of methionine and cysteine in legumes has been reported by many investigators for several pulses and beans (Patwardhan, 1962), Ghanian legumes (Owusu-Domfeh *et al.*, 1970), yam bean, bambara groundnut, kidney bean, lima bean, pigeonpea and jackbean (Apata and Ologhobo, 1990, 1994). Meanwhile, in beach pea, valine was lower (4.75 g/16g N) and tryptophan (0.25 g/16g N) higher than other legume seeds examined. Beach pea contained 17.41 g/16g N glutamic acid as compared to 18.38 g/16g N for Indian grass pea, 17.32 g/16g N for Canadian grass pea and 16.91 g/16g N for green pea. Arginine content was lower in beach pea (7.93 g/16g N) than

Table 4.3 Total amino acid composition of beach pea, green pea and grass pea (g/16g N)[1]

Amino acid	Beach pea[2]	Green pea	Grass pea[3]	Grass pea[4]
Isoleucine	4.11±0.06^{d}	4.32±0.06^{c}	4.75±0.08^{b}	5.13±0.09^{a}
Leucine	7.67±0.14^{cd}	7.62±0.12^{d}	7.78±0.56^{bcd}	8.60±0.10^{a}
Lysine	7.67±0.13^{a}	7.56±0.10^{a}	7.56±0.50^{a}	7.85±0.18^{a}
Cysteine[5]	1.63±0.03^{a}	1.41±0.06^{b}	0.66±0.10^{cd}	0.54±0.01^{d}
Methionine[5]	1.08±0.02^{a}	0.98±0.01^{b}	0.40±0.01^{c}	0.37±0.01^{d}
Total sulphur amino acids	2.71	2.39	1.06	0.91
Tyrosine	3.29±0.05^{a}	3.49±0.50^{a}	3.68±0.12^{a}	3.69±0.06^{a}
Phenylalanine	4.73±0.10^{a}	4.92±0.11^{a}	4.95±0.14^{a}	5.20±0.60^{a}
Total aromatic amino acids	8.02	8.41	8.63	8.89
Threonine	4.29±0.08^{a}	3.84±0.07^{b}	4.24±0.11^{a}	4.16±0.12^{a}
Tryptophan[5]	0.25±0.01^{a}	0.21±0.01^{b}	0.06±0.01^{d}	0.07±0.01^{cd}
Valine	4.75±0.07^{b}	4.92±0.40^{a}	5.30±0.16^{a}	5.47±0.09^{a}
Histidine	2.63±0.03^{ab}	2.43±0.04^{b}	2.84±0.09^{a}	2.74±0.30^{ab}
Total essential amino acids	42.10	41.70	42.22	43.82
Arginine	7.93±0.19^{b}	9.34±0.90^{a}	9.00±0.22^{ab}	9.78±0.21^{a}
Aspartic acid + Asparagine	13.12±0.69^{a}	12.34±1.06^{a}	13.22±0.60^{a}	13.47±0.40^{a}
Glutamic acid + Glutamine	17.41±0.26^{a}	16.91±1.10^{a}	17.32±1.06^{a}	18.38±0.64^{a}
Serine	5.04±0.09^{bc}	4.92±0.13^{c}	5.33±0.41^{abc}	5.78±0.21^{a}
Proline	4.20±0.12^{bc}	4.07±0.12^{c}	4.47±0.06^{a}	1.68±0.01^{d}
Glycine	4.24±0.10^{a}	4.39±0.11^{a}	4.55±0.56^{a}	4.48±0.35^{a}
Alanine	4.34±0.11^{a}	4.45±0.09^{a}	1.90±0.06^{c}	2.08±0.04^{bc}
Total non-essential amino acids	56.28	56.42	55.79	55.65
E/T, %	42.79	42.50	43.08	44.05
Amino acid score	109.64	109.08	109.39	114.11
BV	61.18	40.09	21.24	15.56

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.

[2]Composite flour of beach pea seeds, [3]Canadian grass pea, [4]Indian grass pea, [5]Limiting amino acid.

other peas. The present results of amino acids are similar to those reported by earlier investigators for other legumes (Moran *et al.*, 1968; Hsu *et al.*, 1980; Abdus Sattar *et al.*, 1989; Singh *et al.*, 1990; Kumar *et al.*, 1991). However, legumes in this study contained higher amounts of lysine than those reported previously (Evans and Bendemer, 1967; Meredith and Thomas, 1982). Methionine, methionine/glycine ratio, and lysine/arginine ratio in beach pea were higher than those of green pea and grass pea and can therefore be considered to have higher hypocholesterolemic effect compared to other peas (Chau *et al.*, 1998).

Percentage of essential amino acids to total amino acids of beach pea (42.79%) was similar to that of green pea (42.50%) and Canadian grass pea (43.08%), but slightly less than Indian grass pea (44.05%). A similar trend was observed for amino acid scores of all peas studied (Table 4.3). Predicted biological value of beach pea meal was 61.18 as compared to 40.09, 21.24, and 15.56 for green pea, Canadian grass pea, and Indian grass pea, respectively. These results indicate that beach pea is superior in its nutritional value as compared to those of other peas examined. These differences in the biological values are due to the existing differences in the amount of specific amino acids present in pea proteins. The biological value of chickpea cultivars ranged from 52.0 to 85.0 (Khan *et al.*, 1979). Similar results were reported by Kumar *et al.* (1991) for pigeonpea varieties (57.09 - 59.38) and Boulter *et al.* (1972) for cowpeas (45 - 72). The biological value of *Lathyrus sativus* protein ranged from 42 to 53 (Eggum and Beames, 1983). The protein efficiency ratio (PER) of peas was in the range of 1.55 to 3.16. Beach pea had

a PER between those of grass pea and green pea (Table 4.4). The present PER values for beach pea, green pea and grass pea are higher than those for chickpea (1.2 - 2.64) and cowpea (0.5 - 1.4) (Khan *et al.*, 1979).

The content of free amino acids in beach pea as compared to green pea and grass pea is shown in Table 4.5. Beach pea possessed 124.42 mg/100g asparagine, 115.24 mg/100g glutamic acid and 91.93 mg/100g arginine as compared to 64.23, 147.72, and 220.54 mg/100g for green pea, respectively. Corresponding values for Canadian grass pea and Indian grass pea were 53.66, 52.82, and 27.75 mg/100g and 45.22, 38.02 and 98.37 mg/100g, respectively. The content of total free amino acids was highest in beach pea (571.40 mg/100g) followed by green pea (546.68 mg/100g), Indian grass pea (297.73 mg/100g) and Canadian grass pea (235.88 mg/100g).

4.2.3 Lipid

The lipid fatty acids of beach pea, green pea and Canadian grass pea are presented in Table 4.6. Beach pea consisted of 14.83% saturated and 76.24% polyunsaturated fatty acids. Linoleic (69.12%), palmitic (12.54%), oleic (7.89%) and linolenic (5.18%) acids constituted the major fatty acids present in beach pea seed lipids. Oleic (26.54%) and linolenic (11.07%) acids were present in higher amounts in green pea than in beach pea and Canadian grass pea. Thus, the content of unsaturated fatty acids in beach pea (85.15%), Canadian grass pea (84.94%) and green pea (83.48%) were similar. The content of unsaturated fatty acids in lipids of cowpea (68.10%) and chickpea (67.13%)

Table 4.4 Predicted PER values of different pea meals

Pea	Predicted PER values using equation[1]		
	1	2	3
Beach pea (Composite flour)	2.62	2.67	2.09
Green pea	2.60	2.62	1.77
Grass pea[2]	2.65	2.68	1.55
Grass pea[3]	3.16	3.00	2.15

[1]Alsmeyer *et al.* (1974).

[2]Canadian grass pea.

[3]Indian grass pea.

Table 4.5 Free amino acid composition of beach pea, green pea and grass pea (mg/100g)[1]

Free amino acid	Beach pea[2]	Green pea	Grass pea[3]	Grass pea[4]
Alanine	20.26±0.32^{a}	12.88±0.60^{b}	7.14±0.33^{d}	8.66±0.18^{c}
Arginine	91.93±1.24^{c}	220.54±2.23^{a}	27.75±1.23^{d}	98.37±2.10^{b}
Asparagine	124.42±3.27^{a}	64.23±0.98^{b}	53.66±0.83^{c}	45.22±1.20^{d}
Aspartic acid	19.78±0.33^{d}	20.92±1.03^{cd}	29.72±1.15^{b}	38.37±2.10^{a}
Cysteine	15.53±0.95^{bc}	12.16±0.56^{d}	25.39±0.83^{a}	13.60±0.60^{cd}
Glutamic acid	115.24±2.32^{b}	147.72±2.60^{a}	52.82±1.73^{c}	38.02±1.13^{d}
Glutamine	2.07±0.73^{cd}	1.83±0.12^{d}	2.52±0.31^{bcd}	5.68±0.18^{a}
Glycine	20.88±0.41^{a}	7.18±0.43^{bc}	4.49±0.12^{d}	6.89±0.20^{c}
Histidine	11.56±1.03^{a}	2.69±0.52^{cd}	3.36±0.18^{bcd}	2.56±0.06^{d}
Hydroxyproline	1.71±0.30^{a}	0.94±0.10^{c}	1.20±0.03^{bc}	1.30±0.08^{abc}
Isoleucine	5.11±0.26^{a}	1.20±0.14^{b}	0.88±0.06^{d}	0.90±0.02^{cd}
Leucine	8.91±0.18^{a}	2.04±0.52^{d}	2.12±0.04^{cd}	8.03±0.23^{b}
Lysine	12.70±0.59^{a}	8.20±0.18^{c}	5.76±0.18^{d}	9.26±0.36^{b}
Methionine	30.46±0.16^{a}	3.10±0.06^{c}	1.47±0.10^{d}	4.13±0.22^{b}
Phenylalanine	4.17±0.07^{a}	4.38±0.26^{a}	3.49±0.23^{b}	2.26±0.09^{c}
Proline	44.36±1.32^{a}	11.34±1.20^{b}	1.46±0.08^{cd}	1.12±0.03^{d}
Serine	20.73±0.12^{a}	7.93±0.24^{b}	2.03±0.03^{d}	2.87±0.08^{c}
Tyrosine	2.96±0.52^{b}	4.22±0.08^{a}	1.91±0.08^{cd}	1.89±0.05^{d}
Threonine	6.10±0.10^{a}	5.40±0.04^{b}	2.05±0.38^{d}	2.40±0.08^{cd}
Tryptophan	6.41±0.42^{a}	3.29±0.03^{cd}	3.16±0.42^{d}	3.63±0.18^{bcd}
Valine	6.11±0.13^{a}	4.49±0.10^{b}	3.50±0.23^{c}	2.57±0.30^{d}
Total	571.40	546.68	235.88	297.73

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.

[2]Composite flour of mature and immature seeds of beach pea.

[3]Canadian grass pea.

[4]Indian grass pea.

Table 4.6 Fatty acid composition of beach pea, green pea and grass pea (% area)[1]

Fatty acid	Beach pea	Green pea	Grass pea[2]
C8:0	0.12±0.09	ND	ND
C10:0	0.11±0.08	ND	ND
C12:0	0.06±0.01	ND	ND
C14:0	0.35±0.01^{c}	0.49±0.00^{b}	0.54±0.01^{a}
C15:0	0.42±0.02^{a}	0.16±0.00^{c}	0.29±0.01^{b}
C16:0	12.54±0.42^{a}	11.23±0.01^{b}	8.43±0.03^{c}
C17:0	0.14±0.02^{c}	0.18±0.01^{b}	0.23±0.00^{a}
C18:0	0.88±0.03^{c}	3.72±0.01^{b}	4.21±0.01^{a}
C20:0	ND	0.54±0.00^{b}	0.96±0.01^{a}
C22:0	0.21±0.03^{bc}	0.20±0.00^{c}	0.39±0.01^{a}
C14:1	ND	ND	0.09±0.00
C16:1	0.17±0.01^{c}	0.19±0.00^{b}	0.26±0.01^{a}
C17:1	ND	ND	0.15±0.00
C18:1	7.89±0.22^{c}	26.54±0.07^{a}	16.66±0.02^{b}
C18:2	69.12±1.88^{a}	45.06±0.07^{c}	55.99±0.03^{b}
C18:3	5.18±0.11^{c}	11.07±0.00^{a}	10.56±0.06^{b}
C20:1	0.82±0.12^{a}	0.45±0.01^{c}	0.52±0.00^{bc}
C20:2	0.24±0.02^{a}	ND	0.15±0.00^{b}
C20:3	1.70±0.01	ND	ND
C22:1	0.03±0.01^{c}	0.17±0.00^{b}	0.56±0.02^{a}
Total saturated fatty acids	14.83	16.52	15.05
Total monounsaturated fatty acids	8.91	27.35	18.24
Total polyunsaturated fatty acids	76.24	56.13	66.70

[1]Results are means of triplicate determinations, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]Canadian grass pea.

was also lower than that of beach pea (Salunkhe *et al.*, 1982a) and this corresponds with the results obtained for some of the other legumes (Salunkhe and Kadam, 1989).

4.2.4 Minerals

The mineral composition of different peas are shown in Table 4.7. Potassium was the most abundant mineral, ranging from 475.83 mg/100g in beach pea to 1098.08 mg/100g in the Canadian grass pea. The phosphorus (413.16 mg/100g) and sodium (84.14 mg/100g) contents of beach pea seeds were higher than green pea; sodium was the least abundant macroelement present in pea samples examined. The calcium content of beach pea was lower (144.18 mg/100g) than the Indian grass pea (187.40 mg/100g) and the Canadian grass pea (155.56 mg/100g), but higher than green pea (128.74 mg/100g). Thus, all pea samples contained higher amounts of phosphorus and calcium than other macroelements. Furthermore, the content of magnesium was 179.73 mg/100g for beach pea, 181.40 mg/100g for green pea, 178.11 mg/100g for the Indian grass pea and 149.98 mg/100g for the Canadian grass pea. Beach pea seeds contained the lowest amount of total microelements than other pea seeds examined. Silicon was absent in beach pea seeds but was found in highest amounts in grass pea (Indian grass pea; 22.72 mg/100 g) and was present in green pea seeds at 6.44 mg/100g. The contents of minerals in seeds examined were similar to those of other legumes such as the African locust bean, groundnut (Oyenuga, 1968), *Lathyrus* and medicago (Varnaite, 1984), field pea (Acikgoz *et al.*, 1985), cowpea (Jagadi *et al.*, 1987), and bambara groundnut, kidney bean, lima

Table 4.7 Mineral composition of beach pea, green pea and grass pea (mg/100g)[1]

Mineral	Beach pea[2]	Green pea	Grass pea[3]	Grass pea[4]
Macroelement				
Calcium	144.18±0.61^{c}	128.74±0.21^{d}	155.56±0.41^{b}	187.40±0.68^{a}
Magnesium	179.73±1.28^{ab}	181.40±0.98^{a}	149.98±1.37^{c}	178.11±1.26^{b}
Phosphorus	413.16±1.22^{b}	400.69±1.34^{c}	482.32±0.96^{a}	384.22±0.27^{d}
Potassium	475.83±1.00^{d}	1045.33±2.33^{b}	1098.08±2.15^{a}	987.59±2.17^{c}
Sodium	84.14±0.43^{b}	73.47±0.30^{c}	60.53±0.13^{d}	93.78±1.13^{a}
Microelement				
Aluminum	4.49±0.29^{d}	5.09±0.31^{cd}	6.71±0.10^{b}	20.53±0.80^{a}
Copper	0.85±0.16^{b}	2.41±0.30^{a}	2.39±0.18^{a}	2.16±0.16^{a}
Iron	9.37±0.21^{ab}	7.48±0.85^{c}	9.71±0.40^{a}	8.16±0.41^{bc}
Lithium	0.90±0.12^{d}	4.15±0.55^{bc}	3.06±0.65^{c}	5.93±0.35^{a}
Manganese	3.50±0.58^{b}	1.23±0.15^{d}	1.46±0.16^{cd}	8.68±0.39^{a}
Silicon	ND	6.44±0.13^{c}	15.92±0.25^{b}	22.72±0.13^{a}
Zinc	2.97±0.08^{d}	5.10±0.25^{c}	6.72±0.09^{a}	5.41±0.10^{bc}

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]Composite flour of beach pea seeds.

[3]Canadian grass pea, [4]Indian grass pea.

bean and pigeon pea (Apata and Ologhobo, 1994). However, compared to the Mexican and North American beans (Meiners *et al.*, 1976; D' mello *et al.*, 1985; Apata and Ologhobo, 1989; Zacharie and Ronald, 1993; Barrado *et al.*, 1994), all minerals were present in slightly higher amounts in beach pea, grass pea (both Canadian and Indian varieties) and green pea seeds examined in this study. Such variations in the content of minerals for peas might be due to their genetic and geographical origin as well as soil condition.

Aluminum content in beach pea (4.5 mg/100g) was significantly lower than those of grass peas. High level of aluminum can cause toxicity to the biological system. Aluminum (5.6 mg/kg) fed to rats resulted in kidney function damage and high levels of aluminum storage in the brain. Aluminum may decrease the absorption of other elements, calcium, phosphorus, iron and possibly cholesterol by forming an aluminum-pectin complex that binds fats to nondigestive vagetable fibres, in the gastroestinal tract (Liu *et al.*, 1996). Aluminum also has cholesterol lowering effect in chickens (Shoremi *et al.*, 1996).

4.2.5 Vitamins

The contents of vitamins/provitamins of beach pea, green pea and the Indian grass pea are shown in Table 4.8. A close scrutiny of the results indicate that beach pea seeds contain higher amounts of β-carotene (0.17 mg/100g), folic acid (0.08 mg/100g), and thiamine (0.59 mg/100g) than green pea (0.04, 0.008, and 0.47 mg/100g, respectively) and

Table 4.8 Vitamin content of beach pea, green pea and the Indian grass pea seeds (mg/100g)[1]

Vitamin	Beach pea	Green pea[2]	Grass pea[2]
Ascorbic acid	1.60	6.50[3]	ND
β-Carotene	0.17	0.04	0.12
Folic acid	0.08	0.008	ND
Riboflavin (B_2)	0.06	0.19	0.17
Thiamine (B_1)	0.59	0.47	0.39
Niacin	3.44	3.40	2.90

[1]Results are means of duplicate determinations, on a dry weight basis. ND, Not detected.

[2]Gopalan *et al.* (1982).

[3]Fordham *et al.* (1975).

grass pea (0.12, not detected, 0.39 mg/100g, respectively). Ascorbic acid and riboflavin contents of beach pea (1.60 and 0.06 mg/100g, respectively) were lower than those of green pea (6.50, and 0.19 mg/100g); no ascorbic acid was detected in the Indian grass pea. These results indicate that beach pea seeds serve as a good source of vitamins/provitamins for human and animal nutrition. These vitamins/provitamins function *in-vivo* in several ways in different biological system such as co-enzymes or their precursors, components of the antioxidative defense system, factor involved in genetic regulation and specialized function such as vision. Uzogara *et al.* (1991) showed that cowpea contains 0.77 mg/100g thiamin, 0.25 mg/100g riboflavin and 3.48 mg/100g niacin. However, the present results for niacin (beach pea, green pea and the Indian grass pea, 3.44, 3.40, and 2.90 mg/100g, respectively) were higher than those reported for beans (1.19 mg/100g), lentils (1.23 mg/100g), chickpea (1.33 mg/100g) but lower than green beans (4.53 mg/100g; Vidal-Valverde and Reche, 1991). It is important to note that genetic, environmental factors as well as processing and storage conditions might result in considerable variations in the vitamin content of pea samples (Lynch *et al.*, 1959; Uzogara *et al.*, 1991).

4.2.6 Non-protein nitrogen

Non-protein nitrogen (NPN) content of seeds and plant parts of beach pea as well as seeds of green pea and grass peas is presented in Table 4.9. The NPN content was highest in beach pea (23.29%) and lowest in green pea (10.92%). Branches plus stems

Table 4.9 Non-protein nitrogen content in different plant parts of beach pea and green pea and grass pea seeds[1]

Type of pea	Non- Protein Nitrogen (% Total nitrogen basis)
Beach pea (composite seed sample)	23.29±0.55^{ab}
Mature seeds	21.76±0.68^{ab}
Immature seeds	23.80±1.13^{ab}
Fresh green seeds	24.99±1.95^{a}
Mature pod shells	7.67±0.13^{g}
Fresh green pod shells	9.23±1.33^{f}
Leaves	16.25±0.18^{cd}
Branches plus stems	20.90±0.23^{b}
Green pea seeds	10.92±1.27^{ef}
Grass pea seeds[2]	14.99±0.59^{d}
Grass pea seeds[3]	23.14±0.37^{ab}

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts are significantly ($p<0.05$) different from one another.

[2]Canadian grass pea.

[3]Indian grass pea.

of beach pea contained a higher amount of non-protein nitrogen (20.90%) than other plant parts (leaves 16.25%, fresh green pod shells 9.23%, mature pod shells 7.67%). Pod shells and leaves were the primary source of non-protein nitrogen, possibly for easy transport of these material to seeds where they will be used for protein synthesis. Alternatively, the metabolic rate of non-protein nitrogen synthesis may be different in each part of the plant. The content of NPN in beach pea seeds decreased as the maturity progressed (from 23.80 to 21.76%), perhaps due to their conversion to protein nitrogen. At maturity the rate of protein nitrogen synthesis may decrease, thus leading to accumulation of non-protein nitrogen. This might be the reason that mature seeds had more non-protein nitrogen than other plant parts. Singh and Jambunathan (1982) reported that chickpea and pigeonpea contain 11.20 and 12.80% NPN, respectively. Bhatty et al. (1973) reported 10.2 - 13.2% non-protein nitrogen for 3 pea varieties using NaOH-TCA as extractant. The values were 12.5 to 19.5% when the extractant was TCA only.

4.2.7 Phenolic acids

The content of total phenolic acids of peas, obtained as the sum of free, esterified and insoluble-bound fractions, is presented in Table 4.10. Beach pea meal contained 1012.25 mg/100g of total phenolic acids, on a dry weight basis, followed by green pea (254.57 mg/100g), Canadian grass pea (231.74 mg/100g) and the Indian grass pea (198.54 mg/100g). Sosulski and Dabrowski (1984) reported that the total free phenolic acids in legume flours (mung beans, field peas, lentils, faba beans, pigeonpeas, nava beans, lupins,

Table 4.10 Phenolic acids content in different types of peas (mg/100g)[1]

Type of pea	Free phenolic acids	Esterified phenolic acids	Insoluble phenolic acids	Total phenolic acids
Beach pea	179.74±2.22[a]	751.23±4.31[a]	81.28±0.57[a]	1012.25±7.10[a]
Green pea	18.32±1.22[c]	226.56±0.53[b]	9.69±0.60[c]	254.57±2.35[b]
Grass pea[2]	11.38±0.81[d]	215.55±0.94[c]	4.81±0.36[d]	231.74±2.11[c]
Grass pea[3]	32.68±1.19[b]	130.06±1.57[d]	35.80±0.37[b]	198.54±1.31[d]

[1]Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another.

[2]Canadian grass pea.

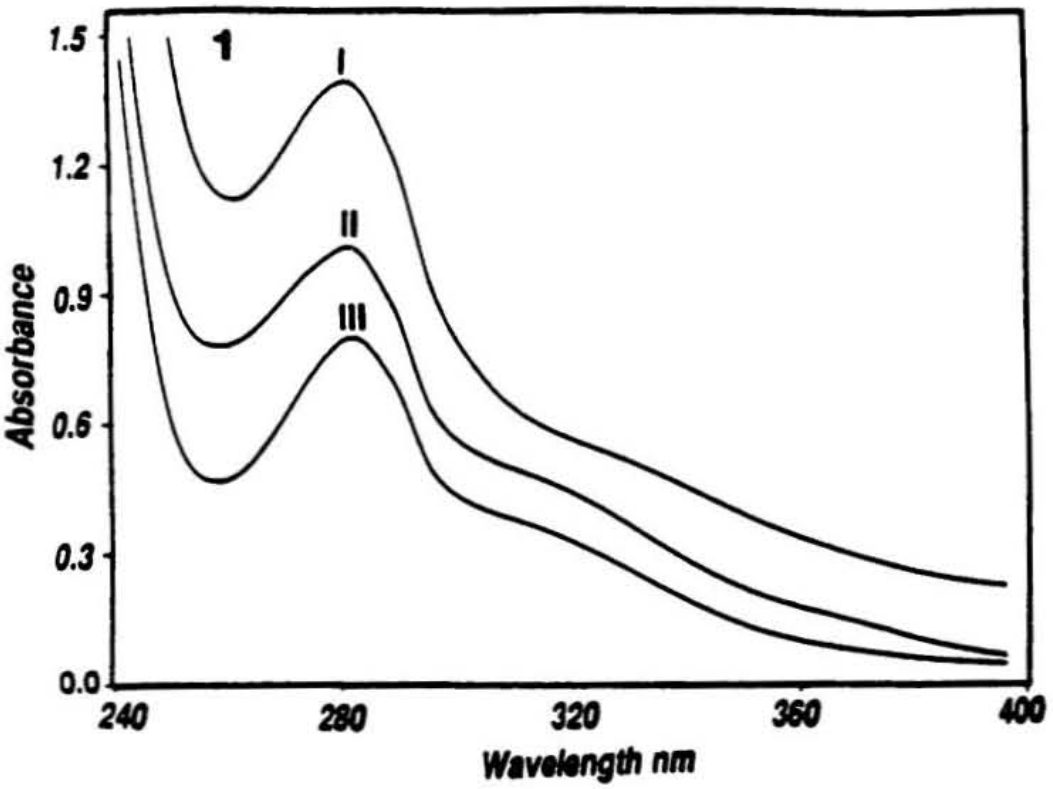
[3]Indian grass pea.

lima beans, chickpeas, and cowpeas) ranged from 1.80 to 16.30 mg/100g. In the present study esterified phenolic acids were higher than the free phenolic acids and insoluble-bound phenolic acids. In fact beach pea has one order of magnitude higher levels of free phenolic acids, 3 times as much esterified, and at least 2 to 8 fold of insoluble phenolic acids. It was also demonstrated that total phenolic acids of beach pea were higher than those of other legumes (dehulled samples), possibly due to the presence of hulls, and/or cultivar difference and the method employed for their determination.

Separation of phenolic acids from beach pea, and both grass pea meals was achieved by different solvents and the UV spectra of the extracts were recorded using a spectrophotometer (Figures 4.1 and 4.2). UV spectra for beach pea phenolics showed a maximum at 280 nm for all extracts, but in case of grass pea only esterified phenolic acids showed a maximum at 280 nm while insoluble-bound phenolics absorbed at 270 nm and free phenolics at 265 nm. Separated phenolic acid fractions were used for further TLC studies. Chromatograms were developed using (A) acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v); (B) acetic acid-water-n-butanol (1:1:3, v/v/v) and sprayed with a solution of potassium ferricyanide-ferric chloride, followed by hydrochloric acid [Figure 4.1, 2A and 2B, (beach pea) and 4.2, 2A and 2B (grass pea)]. Beach pea showed 8 spots and grass pea showed 7 spots in the insoluble fractions. In the second developing system (2B), beach pea showed three spots and grass pea showed only two spots for free phenolic fractions and same developing system showed three spots for esterified phenolic acids for beach pea and grass pea. These results indicate that each phenolic acid fraction

Figure 4.1 1-UV spectra of individual (I) insoluble, (II) free, and (III) esterified phenolic acids from beach pea meal.

2-TLC chromatograms of different phenolic fractions (1-Insoluble, 2-Free and 3-Esterified) separated from beach pea meal; chromatograms were developed using (A) acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v); (B) acetic acid-water-n-butanol (1:1:3, v/v/v) and sprayed with a solution of potassium ferricyanide-ferric chloride, followed by hydrochloric acid.


1
I
II
III
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1.2
0.9
0.6
0.3
0.0
240
280
320
360
400
Wavelength nm

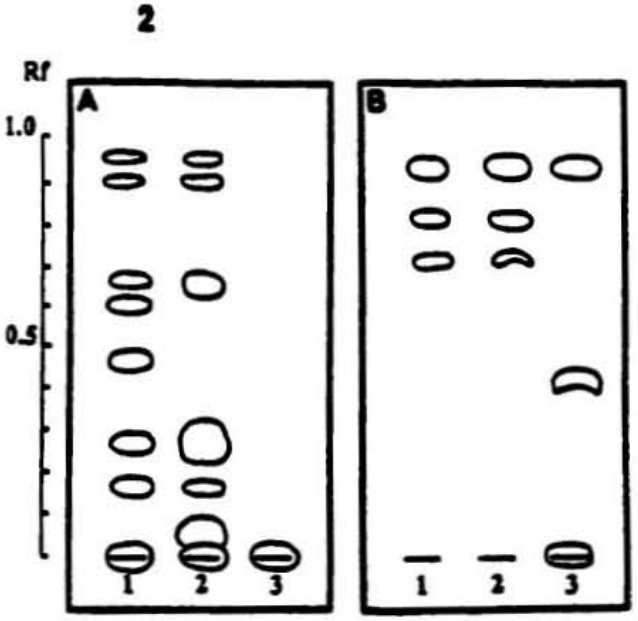
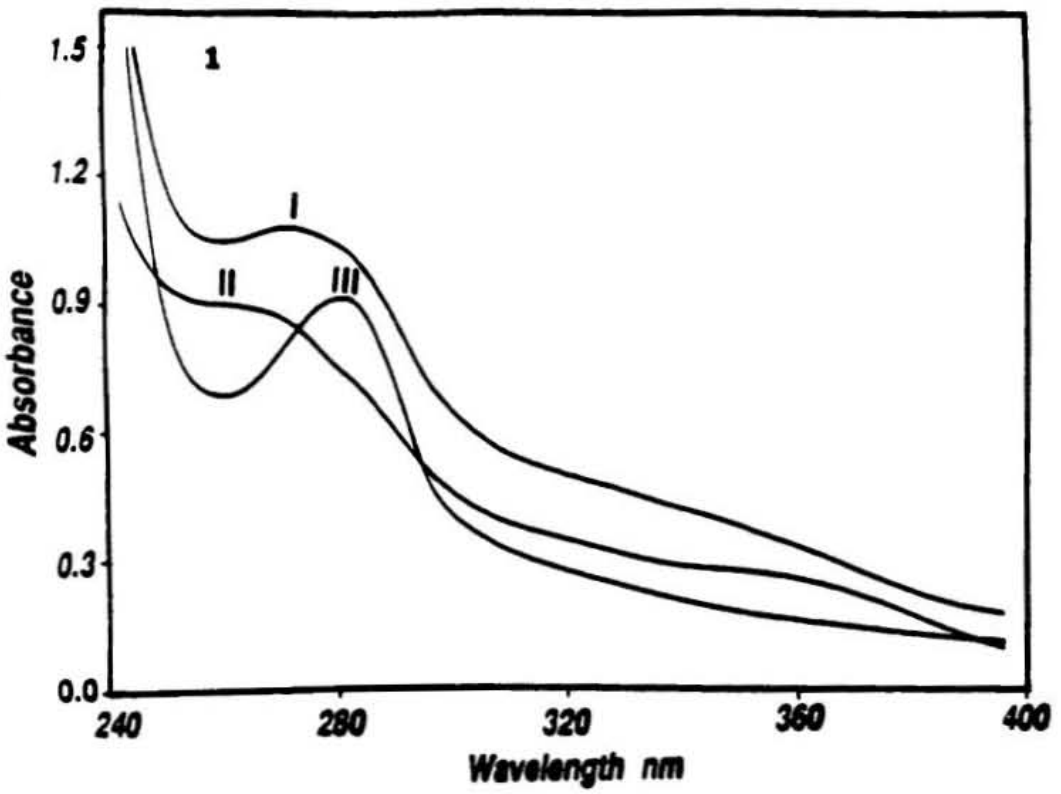
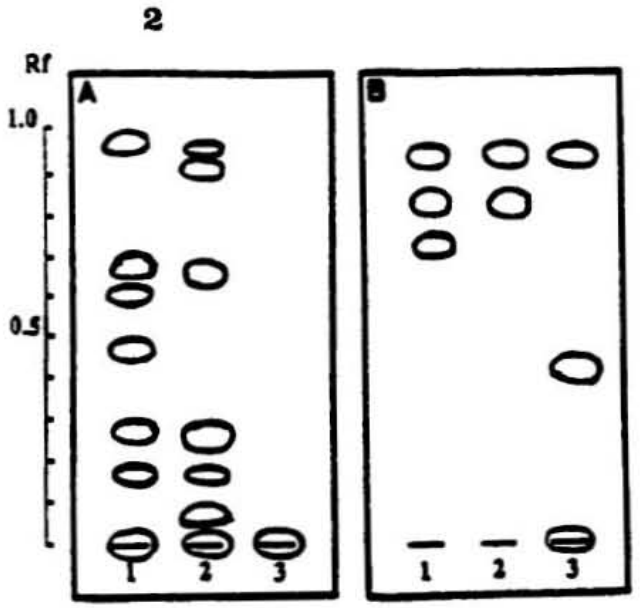

2
Rf
1.0
0.5
A
B
1
2
3
1
2
3

Figure 4.2 1-UV spectra of individual (I) insoluble, (II) free, and (III) esterified phenolic acids from grass pea meals.

2-TLC chromatograms of different phenolic fractions (1-Insoluble, 2-Free and 3-Esterified) separated from grass pea meals; chromatograms were developed using (A) acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v); (B) acetic acid-water-n-butanol (1:1:3, v/v/v) and sprayed with a solution of potassium ferricyanide-ferric chloride, followed by hydrochloric acid.


1
1.5
1.2
0.9
0.6
0.3
0.0
Absorbance
I
II
III
240
280
320
360
400
Wavelength nm


2
Rf
1.0
0.5
A
B
1
2
3
1
2
3

had several components. Beach pea as well as grass pea showed higher numbers of free and insoluble-bound phenolic fractions than the esterified phenolic fraction.

4.2.8 Tannins

The content of condensed tannins of beach pea seeds ranged from 7.19% in fresh green seeds to 11.70% in fully mature dark green seeds (Table 4.11). The contents in green pea, Canadian grass pea and Indian grass pea were 0.07, 0.11, and 1.54%, respectively. The content of condensed tannins in beach pea was nearly 100 times more than that in green pea and Canadian grass pea and 7.5 times that of Indian grass pea. This might be due to the very thick coat of beach pea seeds. The cotyledons to seed coat ratio of beach pea was also higher than the other peas. The content of condensed tannins in different plant parts of beach pea was significantly different ($p<0.05$); the highest amount was present in dark green seeds (11.70%) followed by leaves 2.68%, mature pod shells 2.05% and branches plus stems (0.95%). The synthesis of tannins in different plant parts may depend on the metabolic rate of tannin synthesis in a particular site. Another reason may be higher polymerization of existing polyphenolic compounds in the seed coat to high molecular weight compounds during maturation. The proportion of condensed tannins in beach pea seeds increased from 7.19% (fresh green seeds) to 11.70% (mature seeds), but in case of pod shells the reverse was observed; condensed tannins decreased from 9.13% (fresh green pod shells) to 2.05% (mature pod shells). These results indicate that as the maturity progressed the concentration of condensed tannins increased in seeds,

Table 4.11 Condensed tannins content of different plant parts of beach pea and seeds of green pea and grass pea[1]

Name of sample	Condensed tannins (%)
Beach pea (mature seeds)	11.70±0.39^{a}
Beach pea (immature seeds)	9.29±0.48^{bc}
Beach pea (fresh green seeds)	7.19±0.26^{d}
Beach pea (composite seed sample)	11.58±0.20^{a}
Leaves	2.68±0.20^{e}
Branches plus stems	0.95±0.07^{h}
Mature pod shells	2.05±0.08^{fg}
Premature pod shells	9.13±0.25^{c}
Green pea seeds	0.07±0.001^{j}
Grass pea seeds[2]	0.11±0.009^{ij}
Grass pea seeds[3]	1.54±0.15^{g}

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts are significantly ($p<0.05$) different from one another.

[2]Canadian grass pea.

[3]Indian grass pea.

with a concurrent decreased in pod shells, possibly due to the delocalization of condensed tannins from pod shells to seeds during maturation and polymerization into high molecular weight compounds. Price *et al.* (1980) analyzed ten varieties of each of cowpea, chickpea, pigeonpeas, and mung beans for their tannin content by vanillin assay and reported values ranging from 0.0 to 0.7%. Several factors such as plant type, cultivar, age of the plant or plant parts, stage of development, and environmental conditions govern the tannin content in plants. The changes observed during development or maturation were mostly due to metabolism of polyphenolic compounds or polymerization of existing phenolic compounds.

4.2.9 β-N-Oxalylamino-L-alanine

β-N-Oxalylamino-L-alanine (BOAA) content in mature beach pea seeds was more (4.02 mg/100g) than that in fresh green seeds (2.90 mg/100g) on a dry weight basis. Branches plus stems also contained BOAA (0.65 mg/100g), but other plant parts (leaves and pod shells) were devoid of it (Table 4.12). The BOAA content of six strains of *Lathyrus* grown at six different locations varied from 245 to 551 mg/100g (Anon, 1980). Radha Ayyagari *et al.* (1989) reported that *Lathyrus* seeds contain up to 11.0 g BOAA per kg of seed. Somayajulu *et al.* (1975) reported that location, environmental conditions (drought) and cultivars mostly affect the content of BOAA in *Lathyrus* seeds.

Table 4.12 β-N-Oxalylamino-L-alanine (BOAA) content in different plant parts of beach pea[1]

Plant part	BOAA (mg/100g)
Beach pea (composite seed sample)	4.02
Fresh green seeds	2.90
Leaves	ND
Branches plus stems	0.65
Mature pod shells	ND
Fresh green pod shells	ND

[1]Results are means of duplicate determinations, on a dry weight basis. ND, Not detected.

4.2.10 Presence of saponins in beach pea seeds

The 80% aqueous ethanol separated saponins of beach pea were subjected to a semi-preparative HPLC; soybean saponins were used as references. The separated saponins from beach pea gave two peaks in their HPLC chromatogram which were similar to those of soybean saponins with identical retention times (Figure 4.3). The UV spectra of these two separated compounds were further recorded. Beach pea saponins showed similar spectra (Figure 4.4 A and B) to those of soybean saponins (Figure 4.4 C and D). Based on these results and comparison with the literature data, it is concluded that beach pea has two types of saponins belonging to the group "B" saponins. These results are also similar to soybean saponin group "B" as shown by Shiraiwa *et al.* (1991) and faba bean saponins reported by Amarowicz *et al.* (1997).

4.2.11 Different forms of phosphorus and phytic acid

The content of different forms of phosphorus in beach pea, green pea and grass peas is presented in Table 4.13. The inorganic phosphorus content of beach pea was 34.88 mg/100g followed by 31.24 mg/100g in green pea, 23.39 mg/100g in the Canadian grass pea and 35.80 mg/100g in the Indian grass pea. Beach pea contained 189.14 mg/100g phosphorus which was extractable in 1.2% HCl containing 10% Na_2SO_4, significantly higher than that in green pea (184.73 mg/100g) and the Indian grass pea (174.21 mg/100g), but lower than that in the Canadian grass pea (229.46 mg/100g). Phosphorus extractable in 1.2% HCl containing 10% Na_2SO_4 and precipitated by ferric

Figure 4.3 Chromatograms of the analytically separated patterns of group "B" saponins from soybean (A) and beach pea (B) by semi-preparative HPLC.

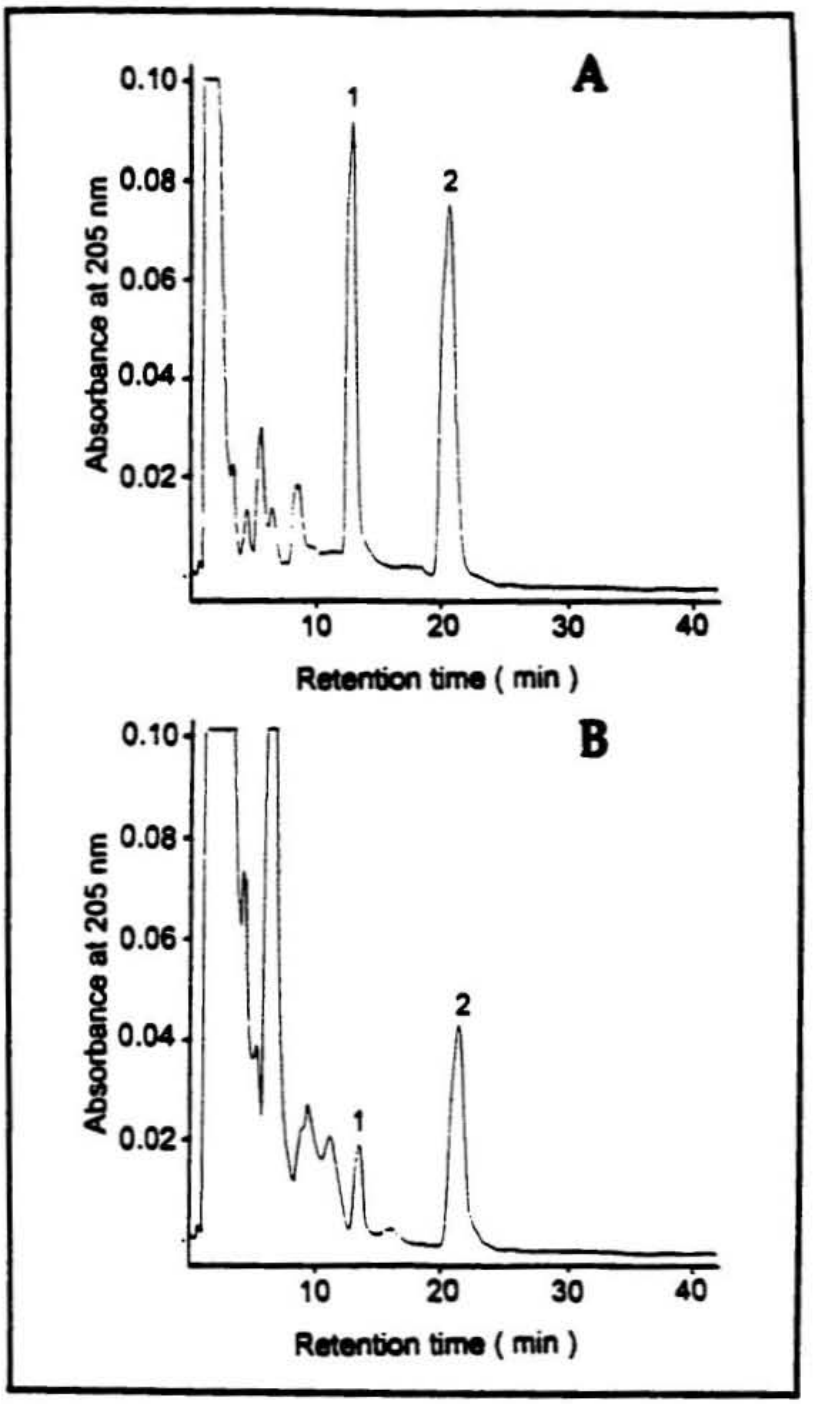
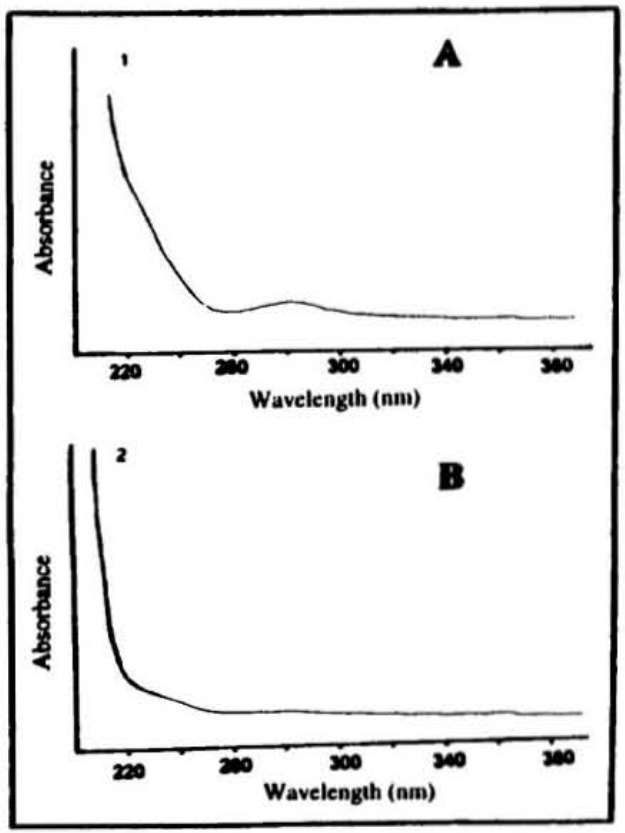

A
0.10
0.08
0.06
0.04
0.02
Absorbance at 205 nm
1
2
10
20
30
40
Retention time (min)
B
0.10
0.08
0.06
0.04
0.02
Absorbance at 205 nm
1
2
10
20
30
40
Retention time (min)

Figure 4.4 UV spectra of compound one (A) and compound two (B) of beach pea and compound one (C) and compound two (D) of soybean saponins separated by analytical HPLC.


A
1
Absorbance
220
260
300
340
380
Wavelength (nm)
B
2
Absorbance
220
260
300
340
380
Wavelength (nm)

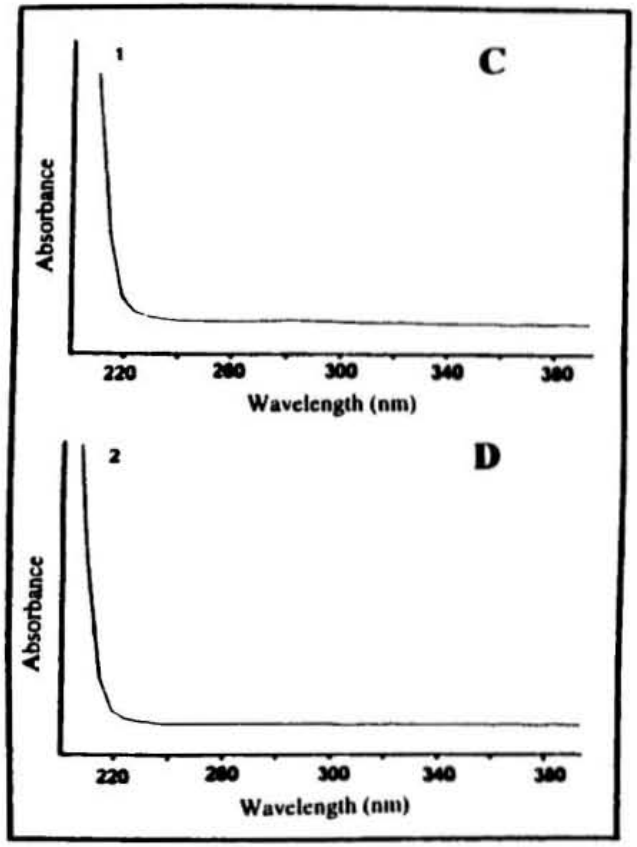

C
1
Absorbance
220
260
300
340
380
Wavelength (nm)
D
2
Absorbance
220
260
300
340
380
Wavelength (nm)

Table 4.13 Content of different types of phosphorus in peas (mg/100g)[1]

Type of Phosphorus	Type of pea			
	Beach pea	Green pea	Grass pea[2]	Grass pea[3]
Total Phosphorus (Pt)	413.16±1.22[b]	400.69±1.34[c]	482.32±0.96[a]	384.22±0.27[d]
Inorganic Phosphorus (Pi)	34.88±0.43[b]	31.24±0.35[c]	23.39±0.53[d]	35.80±0.20[a]
Phosphorus extractable in 1.2% HCl and 10% Na_2SO_4 (Pe)	189.14±1.18[b]	184.73±1.23[c]	229.46±1.99[a]	174.21±1.71[d]
Fraction of Pe not precipitated by ferric ion (Ps)	87.99±1.95[b]	69.40±1.14[d]	79.56±1.27[c]	97.13±1.35[a]
Fraction of Pe precipitated by ferric ion (Pp)	101.15±1.80[c]	115.32±1.19[b]	149.91±1.98[a]	77.08±1.04[d]
Phytic acid (Pa)	359.09±2.76[c]	409.39±2.20[b]	532.18±2.83[a]	273.63±1.07[d]

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. Phytic acid (Pa) = Fraction of Pe precipitated by ferric ion X 3.55, see Section 3.2.2.19.

[2]Canadian grass pea.

[3]Indian grass pea.

ion was 101.15, 115.32, 149.91 and 77.08 mg/100g in beach pea, green pea, Canadian grass pea and Indian grass pea, respectively.

Phytic acid content in beach pea was 359.09 mg/100g followed by that in the Indian grass pea (273.63 mg/100g). Beach pea and Indian grass pea had lower amounts of phytic acid than those present in green pea and Canadian grass pea (409.39 and 532.18 mg/100g, respectively) (Table 4.14). Reddy *et al.* (1982) reported that total phosphorus content of chickpeas, black gram, black-eyed beans and red kidney beans was 3.51, 5.20, 5.40 and 5.45 mg/g, respectively. However, these authors also noted that the phytic acid content in these legumes was 4.40, 14.56, 11.48 and 11.70 mg/g, respectively. The phytic acid content in seeds as well as plant parts increased as the maturity of seeds and plant parts progressed. Igbedioh *et al.* (1994) reported that pigeonpea contains 220 mg/100g phytic acid, while Carnovale *et al.* (1988) reported phytic acid contents of 0.85, 0.94, and 0.75% in pea cultivars Imposant, Finale and Rondo. Phytic acid content in pea seeds in the present study was lower than those reported in the literature for other peas, perhaps due to genetic variation in cultivars as well as climatic and soil conditions.

4.2.12 Chlorophyll

The chlorophyll content of beach pea, green pea and grass pea seeds is presented in Table 4.15. The content of chlorophylls A and B and their total amount in beach pea was 1.16, 0.62, and 1.78 mg/g, respectively. These values were higher than those for green pea (0.73, 0.32, and 1.05 mg/g, respectively). Chlorophyll was not detected in both

Table 4.14 Phytic acid content in different plant parts of beach pea, and seeds of green pea and grass pea[1]

Type of pea	Phytic acid (mg/100g)
Beach pea (composite seed sample)	359.09±2.76^{d}
Mature seeds	369.87±6.20^{c}
Immature seeds	324.74±4.07^{e}
Fresh green seeds	276.68±1.35^{fg}
Mature pod shells	97.96±2.05^{j}
Fresh green pod shells	41.70±1.68^{k}
Leaves	170.68±1.75^{h}
Branches plus stems	149.06±1.37^{i}
Green pea seeds	409.39±2.20^{b}
Grass pea seeds[2]	532.18±2.83^{a}
Grass pea seeds[3]	273.63±1.03^{g}

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts are significantly ($p<0.05$) different from one another. Phytic acid (Pa) = Fraction of Pe precipitated by ferric ion X 3.55, see Section 3.2.2.20.

[2]Canadian grass pea.

[3]Indian grass pea.

Table 4.15 Chlorophyll content of beach pea, green pea and grass pea seeds (mg/g)[1]

Component	Beach pea	Green pea	Grass pea[2]	Grass pea[3]
Chlorophyll				
A	1.16±0.01[a]	0.73±0.01[b]	ND	ND
B	0.62±0.01[a]	0.32±0.005[b]	ND	ND
Total	1.78±0.02[a]	1.05±0.01[b]	ND	ND

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]Canadian grass pea.

[3]Indian grass pea.

grass pea cultivars examined. Due to the presence of higher amounts of chlorophyll, beach pea seeds were dark green as compared to the light colour of green peas (pale pink). This difference in chlorophyll content may be due to wide variation in genetic regulations (synthesis of chlorophyll) and difference in species.

4.3 Chemical composition of beach pea plant parts

4.3.1 Chemical composition

Chemical compositions of mature seeds, leaves, branches plus stems and mature pod shells are shown in Table 4.16. Seed constituents were dominated by crude protein (28.03%) and starch (26.50%) while generally lower levels were present in the leaves (23.48% and 0.78%, respectively), branches plus stems (14.59% and 1.37%, respectively) and mature pod shells (10.69% and 2.20%, respectively). Singh and Jambunathan (1980) and Earle and Jones (1982) reported a wide variation in seed protein content of all cultivated species of legumes and their protein content ranging from 14.9 to 45.0%. The mature pod shells contained higher amounts of carbohydrate (81.49%) and crude fibre (35.54%) than other plant parts. The total content of phenolics was 2.97% in leaves, 1.35% in seeds, 0.61% in pod shells and 0.52% in branches plus stems. In addition, green plant parts served as a good source of minerals and soluble sugars (Table 4.16). The ash content of seeds (3.07%) was similar to those reported for other legumes (Platt, 1980; Apata and Ologhobo, 1994).

Consideration of the overall chemical composition of leaves, branches plus stems

Table 4.16 Chemical composition of different plant parts of beach pea[1]

Constituent, %	Seeds[2]	Leaves	Branches & Stems	Mature pod shells
Moisture (wet weight basis)	11.83±0.27^{c}	77.09±1.74^{a}	76.55±1.56^{a}	21.95±0.84^{b}
Protein	28.03±1.37^{a}	23.48±0.07^{b}	14.59±0.15^{c}	10.69±0.18^{d}
Soluble proteins (mg/100g)	289.58±3.30^{c}	372.53±1.58^{b}	708.99±3.02^{a}	190.22±1.59^{d}
Lipid (hexane extractable)	1.27±0.14^{d}	5.98±0.01^{a}	2.33±0.21^{b}	1.41±0.03^{cd}
Ash	3.07±0.03^{c}	6.82±0.06^{a}	5.05±0.10^{b}	2.19±0.09^{d}
Crude fibre	10.68±0.16^{c}	27.27±0.67^{b}	34.48±0.15^{a}	35.54±0.48^{a}
Carbohydrates[3]	55.80±0.78^{d}	57.27±0.11^{c}	70.15±0.18^{b}	81.49±0.17^{a}
Soluble sugars	2.77±0.04^{c}	4.02±0.49^{b}	12.23±0.14^{a}	0.07±0.01^{d}
Reducing sugars (mg/100g)	165.40±0.02^{c}	1071.00±1.12^{b}	5471.30±2.80^{a}	39.14±0.01^{d}
Non-reducing sugars	2.60±0.04^{c}	2.95±0.49^{bc}	6.76±0.35^{a}	0.03±0.01^{d}
Starch	26.50±0.46^{a}	0.78±0.07^{d}	1.37±0.04^{cd}	2.20±0.06^{b}
Phenolics	1.35±0.01^{b}	2.97±0.02^{a}	0.52±0.07^{d}	0.61±0.01^{cd}

[1]Results are mean values of triplicate determinations, ± standard deviation and are expressed on a dry weight basis, unless otherwise specified. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.
[2]Values are for mature seeds. [3]By difference.

and mature pod shells reveals that beach pea is a good source of nutrients for animal feed, as a fodder, or ensiled forage. These results are similar to those for green pea plant parts (Trevino *et al.*, 1987). The protein, ash, crude fibre and phenolics contents of beach pea were slightly higher than those reported for other legumes (Moran *et al.*, 1968; Wills *et al.*, 1984, 1987; Kumar *et al.*, 1991).

4.3.2 Total and free amino acid composition of different plant parts of beach pea

The amino acid composition of seeds, leaves, branches plus stems and pod shells of beach pea are given in Table 4.17. The content of lysine (7.43 g/16g N), arginine (7.97 g/16g N) and glutamic acid (16.56 g/16g N) in dried beach pea seeds was higher than other parts of the plant. The content of these amino acids is fairly similar to those of faba bean (Hsu *et al.*, 1980; Khalil and Mansour, 1995), field pea (Acikgoz *et al.*, 1985), mung bean (Abdus Sattar *et al.*, 1989), pigeonpea (Singh *et al.*, 1990) and Nigerian legume seeds namely bambara groundnut, kidney bean, lima bean, pigeonpea and jack bean (Apata and Ologhobo, 1994). The amounts of methionine and cysteine in seeds, branches plus stems, leaves and pod shells were lower than reference values given by FAO/WHO (1985). Tryptophan content was higher in leaves (1.35 g/16g N) than the FAO/WHO (1985) reference value, but lower in all other parts of the plant. Total essential amino acids were higher in branches plus stems (45.81 g/16g N) and lowest in mature pod shells (39.79 g/16g N). The non-essential amino acids were abundant in seeds (54.89 g/16g N), but less prevelat in mature pod shells (43.61 g/16g N). The high content of leucine,

Table 4.17 Total amino acid composition of different plant parts of beach pea (g/16g N)[1]

Amino acid	Seeds[2]	Leaves	Branches & Stems	Mature Pod Shells
Isoleucine	3.97±0.01^{bc}	4.17±0.14^{ab}	4.39±0.26^{a}	3.66±0.02^{c}
Leucine	7.53±0.16^{a}	7.59±0.16^{a}	6.57±0.30^{bc}	6.15±0.01^{c}
Lysine	7.43±0.15^{a}	6.38±0.10^{b}	7.03±0.33^{a}	8.21±1.72^{a}
Cysteine	1.70±0.04^{b}	1.47±0.02^{d}	1.49±0.06^{cd}	1.80±0.02^{a}
Methionine	1.13±0.03^{cd}	1.59±0.03^{a}	1.34±0.08^{b}	1.08±0.02^{d}
Total sulphur amino acids	2.83	3.06	2.83	2.88
Tyrosine	3.25±0.05^{bc}	3.88±0.35^{a}	4.43±0.30^{a}	3.19±0.05^{c}
Phenylalanine	4.60±0.10^{bc}	5.32±0.13^{a}	4.58±0.29^{c}	3.69±0.01^{d}
Total aromatic amino acids	7.85	9.20	9.01	6.88
Threonine	4.17±0.10^{d}	4.89±0.06^{b}	5.69±0.08^{a}	4.20±0.02^{cd}
Tryptophan	0.33±0.02^{d}	1.35±0.02^{a}	0.56±0.02^{b}	0.43±0.01^{c}
Valine	4.65±0.01^{d}	5.46±0.21^{b}	6.17±0.38^{a}	4.86±0.01^{cd}
Histidine	2.59±0.01^{bcd}	2.44±0.02^{d}	3.56±0.12^{a}	2.52±0.01^{cd}
Total essential amino acids	41.35	44.54	45.81	39.79
Arginine	7.97±0.19^{a}	4.69±0.18^{cd}	5.05±0.22^{bc}	4.52±0.10^{d}
Aspartic acid + Asparagine	13.04±0.13^{b}	15.13±0.74^{a}	3.80±0.16^{d}	10.33±0.21^{c}
Glutamic acid + Glutamine	16.56±0.31^{a}	9.70±0.17^{bc}	9.49±0.38^{cd}	8.82±0.09^{d}
Serine	4.98±0.14^{d}	5.43±0.06^{c}	8.63±0.31^{a}	5.77±0.02^{bc}
Proline	3.95±0.14^{d}	6.94±0.15^{b}	8.50±0.09^{a}	5.01±0.20^{c}
Glycine	4.12±0.12^{b}	4.69±0.09^{a}	4.95±0.18^{a}	4.75±0.03^{a}
Alanine	4.27±0.13^{c}	5.47±0.09^{a}	5.51±0.20^{a}	4.41±0.05^{bc}
Total non-essential amino acids	54.89	52.05	45.93	43.61
E/T, %	42.97	46.11	49.93	47.71
Amino acid score	107.67	116.94	117.36	96.56
BV	67.24	82.86	46.55	65.35

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.

[2]Values are for mature seeds.

lysine, arginine and glutamic acid in beach pea seeds is similar to previous reports for other legume seeds (Evans and Bendemer, 1967; Meredith and Thomas, 1982; Khalil and Mansour, 1995; Mohan and Janardhanan, 1995).

The percent ratio of essential to total amino acids of beach pea branches plus stems was higher (49.93) than that for mature pod shells (47.71), leaves (46.11), and mature seeds (42.97). Calculated biological value of beach pea plant parts was in the order of leaves (82.86), mature seeds (67.24), mature pod shells (65.35) and branches plus stems (46.55). These results demonstrate good nutritional value of beach pea plant parts for use as animal feed ingredients. The protein efficiency ratio of mature seeds of beach pea was in the range of 2.03 to 2.61. The protein efficiency ratio of leaves (2.45, 2.57, and 1.65) was higher than branches plus stems (1.91, 2.05, 2.28) and mature pod shells (1.88, 1.99, 0.97) (Table 4.18).

The content of free amino acids in different parts of beach pea plant are presented in Table 4.19. Seeds contained mainly arginine (128.64 mg/100g), but the amount was less in branches plus stems (76.01 mg/100g), leaves (23.88 mg/100g) and mature pod shells (12.35 mg/100g). Leaves and branches plus stems had higher amounts of asparagine, proline, valine, serine, alanine, glutamine and histidine than seeds and mature pod shells. Total free amino acid content was highest in branches plus stems (3147.88 mg/100g), followed by leaves (280.28 mg/100g), mature seeds (503.92 mg/100g) and mature pod shells (150.73 mg/100g). Anabolism of proteins is directly related to the availability of their precursors such as free amino acids. These results indicate that the

Table 4.18 Predicted PER values of different plant parts of beach pea

Plant part	Predicted PER values using equation[1]		
	1	2	3
Beach pea (mature seeds)	2.56	2.61	2.03
Leaves	2.45	2.57	1.65
Branches & Stems	1.91	2.05	2.28
Mature pod shells	1.88	1.99	0.97

[1]Alsmeyer *et al.* (1974).

Table 4.19 Free amino acid composition of different plant parts of beach pea (mg/100g)[1]

Free amino acid	Seeds[2]	Leaves	Branches & Stems	Mature Pod Shells
Alanine	20.68±0.54^{c}	110.25±1.20^{a}	91.87±2.95^{b}	4.72±0.12^{d}
Arginine	128.64±1.24^{a}	23.88±0.15^{c}	76.01±1.63^{b}	12.35±0.28^{d}
Asparagine	109.29±7.23^{c}	991.92±2.37^{b}	1764.94±7.70^{a}	24.64±0.33^{d}
Aspartic acid	32.67±2.20^{bc}	32.31±2.22^{c}	43.08±1.75^{a}	4.20±0.17^{d}
Cysteine	16.45±1.95^{b}	31.41±1.41^{a}	33.58±1.82^{a}	2.93±0.57^{c}
Glutamic acid	11.81±4.67^{d}	45.21±1.24^{a}	28.62±2.00^{c}	31.90±0.32^{bc}
Glutamine	1.84±0.76^{d}	97.97±2.98^{a}	67.03±0.90^{b}	21.64±0.23^{c}
Glycine	12.89±0.59^{a}	7.60±0.04^{b}	6.01±0.53^{c}	1.53±0.23^{d}
Histidine	10.64±0.75^{c}	88.43±2.90^{a}	64.35±2.15^{b}	2.77±0.03^{d}
Hydroxyproline	1.80±0.34^{a}	ND	0.73±0.05^{bc}	0.71±0.05^{c}
Isoleucine	7.50±0.41^{b}	44.31±0.51^{a}	45.32±1.01^{a}	0.89±0.09^{c}
Leucine	10.23±1.05^{c}	45.31±0.29^{a}	32.31±0.33^{b}	1.11±0.16^{d}
Lysine	20.20±0.27^{b}	26.89±0.42^{a}	18.41±0.07^{c}	4.27±0.16^{d}
Methionine	27.92±1.82^{a}	12.38±0.23^{b}	8.46±0.19^{c}	2.77±0.51^{d}
Phenylalanine	4.13±0.21^{c}	139.10±1.76^{a}	47.43±1.09^{b}	0.60±0.00^{d}
Proline	42.15±1.33^{c}	682.65±1.86^{a}	344.20±1.53^{b}	22.95±0.27^{d}
Serine	16.02±0.10^{c}	86.48±2.82^{b}	279.21±7.91^{a}	4.53±0.25^{d}
Tyrosine	5.13±0.10^{c}	41.59±0.22^{a}	11.09±0.10^{b}	0.36±0.03^{d}
Threonine	10.48±0.55^{c}	111.10±2.45^{a}	101.08±4.34^{b}	3.13±0.04^{d}
Tryptophan	7.07±0.41^{b}	69.86±1.36^{a}	3.93±0.03^{c}	0.70±0.48^{d}
Valine	6.38±0.04^{c}	120.63±1.19^{a}	80.22±1.24^{b}	2.03±0.53^{d}
Total	503.92	2809.28	3147.88	150.73

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]Values are for mature seeds.

concentration of free amino acids depends mostly on their metabolism in specific parts of beach pea plant and their mobilization in different plant organs according to their specific requirement.

4.3.3 Minerals

The mineral contents of dry seeds and different plant parts of beach pea are shown in Table 4.20. Potassium was the most abundant macroelement present, ranging from 626.50 mg/100g in mature pod shells to 450.82 mg/100g in seeds, followed by calcium which was present at 1630.44 mg/100g in leaves and 138.69 mg/100g in seeds. The content of phosphorus in seeds (434.04 mg/100g) and magnesium in leaves (393.36 mg/100g) was highest as compared to other plant parts. Sodium content was highest in branches plus stems (354.68 mg/100g) and lowest (112.71 mg/100g) in seeds. Khalil and Mansour (1995) reported that faba beans contain 297 mg/100g sodium. However, sodium content in beach pea seeds and other parts of plant was higher than that of other legumes (from 11.5 to 40.1 mg/100g), as reported by Salunkhe *et al.* (1985). Among microelements, manganese was present at 1.69 - 5.72 and iron at 8.80 - 34.23 mg/100g in mature pod shells, branches plus stems and seeds. The highest level of aluminum was present in branches plus stems (25.99 mg/100g) and lowest in seeds (3.09 mg/100g). Silicon was absent in seeds but was present at highest amount in branches plus stems (75.62 mg/100g). Seeds contained 3.06 and 0.90 mg/100g zinc and copper, respectively. Thus, all parts of beach pea plant may serve as a valuable source of essential minerals for

Table 4.20 Mineral composition of beach pea plant parts (mg/100g)[1]

Mineral	Seeds[2]	Leaves	Branches & Stems	Mature Pod Shells
Macroelement				
Calcium	138.69±0.31^{d}	1630.44±2.94^{a}	386.98±1.34^{b}	262.82±1.06^{c}
Magnesium	182.86±1.42^{c}	393.36±0.61^{a}	200.08±1.47^{b}	156.81±1.45^{d}
Phosphorus	434.04±0.82^{a}	230.65±1.28^{b}	184.02±1.52^{c}	50.85±0.52^{d}
Potassium	450.82±2.02^{d}	503.96±1.91^{c}	553.79±1.85^{b}	626.50±1.10^{a}
Sodium	112.71±2.79^{c}	350.90±0.93^{a}	354.68±1.84^{a}	264.39±1.07^{b}
Microelement				
Aluminum	3.09±0.36^{d}	17.33±0.46^{b}	25.99±0.19^{a}	11.26±0.60^{c}
Copper	0.90±0.19^{a}	0.90±0.19^{a}	0.70±0.11^{a}	0.70±0.11^{a}
Iron	8.80±0.37^{d}	25.93±0.66^{b}	12.80±0.73^{c}	34.23±0.41^{a}
Lithium	1.05±0.07^{bc}	2.51±0.54^{a}	3.14±0.59^{a}	0.78±0.50^{c}
Manganese	3.77±0.51^{bc}	3.36±0.60^{c}	1.69±0.18^{d}	5.72±0.95^{a}
Silicon	ND	41.09±0.22^{b}	75.62±0.28^{a}	23.01±0.86^{c}
Zinc	3.06±0.04^{a}	2.06±0.12^{c}	2.48±0.17^{b}	0.62±0.07^{d}

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]Values are for mature seeds.

human and animal nutrition. Results for the contents of minerals in beach pea are comparable with those of tropical legumes such as African locust bean, groundnut (Oyenuga, 1968), *Lathyrus* and medicago (Varnaite, 1984), field pea (Acikgoz *et al.*, 1985), cowpea (Jagadi *et al.*, 1987), chickpea, green pea, pigeonpea, cowpea and *Lathyrus* beans (Salunkhe and Kadam, 1989), bambara groundnut, kidney bean, lima bean, jack bean and pigeon pea (Apata and Ologhobo, 1994), as well as other Mexican and North American beans (Meiners *et al.*, 1976; Barrado *et al.*, 1994). However, the lower sodium content of beach pea as compared to other leguminous seeds might be an added advantage due to the direct relationship of sodium intake with hypertension in humans (Dahl, 1972).

4.4 Chemical composition of beach pea seeds and pod shells as affected by maturity stage

4.4.1 Chemical composition

Chemical composition of beach pea seeds and pod shells, as affected by the stage of maturity, is shown in Table 4.21. The fresh weight of seeds increased consistently over the whole period of plant growth. Seeds and pod shells were classified into fresh green (premature), immature and mature during their developmental stage as previously described by Le Deunff and Rachidian (1988) and Rochat and Boutin (1989) for green pea. The maturation stage was characterized by percent seed recovery and rapid senescence of the pods. This order of development of seeds and pod shells has been described previously for green pea, field pea, and pigeonpea (McKee *et al.*, 1955a; Rochat

Table 4.21 Chemical composition of beach pea seeds and pod shells at different maturity stages[1]

Constituent, %	Fresh green seeds	Immature seeds	Mature seeds	Fresh green pod shells	Mature pod shells
Recovery[2]	49.15±0.98^{a}	46.48±1.12^{b}	15.60±0.29^{d}	50.85±0.98^{a}	37.92±0.29^{c}
Moisture in fresh sample	66.22±0.71^{b}	8.98±0.31^{e}	11.83±0.27^{d}	76.83±0.93^{a}	21.95±0.84^{c}
Protein	30.50±0.22^{a}	29.60±0.26^{ab}	28.03±1.37^{b}	11.74±0.19^{cd}	10.69±0.18^{d}
Soluble proteins (mg/100g)	415.18±1.02^{a}	311.14±4.56^{b}	289.58±3.30^{d}	296.40±2.88^{cd}	190.22±1.59^{e}
Lipid	1.76±0.07^{a}	1.06±0.02^{d}	1.27±0.14^{c}	1.75±0.05^{a}	1.41±0.03^{bc}
Ash	3.46±0.01^{b}	3.03±0.03^{d}	3.07±0.03^{cd}	6.56±0.16^{a}	2.19±0.09^{e}
Crude fibre	10.51±0.49^{e}	12.45±0.32^{c}	10.68±0.16^{de}	27.61±0.13^{b}	35.54±0.48^{a}
Carbohydrates[3]	59.28±0.18^{c}	57.33±0.33^{d}	55.80±0.78^{e}	77.06±0.13^{b}	81.49±0.17^{a}
Soluble sugars	4.76±0.06^{b}	3.56±0.06^{c}	2.77±0.04^{d}	8.13±0.16^{a}	0.07±0.01^{e}
Reducing sugars (mg/100g)	700.00±11.00^{b}	173.98±0.07^{cd}	165.40±0.02^{d}	6210.01±60.0^{a}	39.14±0.87^{e}
Non-reducing sugars	4.06±0.07^{a}	3.39±0.05^{b}	2.60±0.04^{c}	1.92±0.17^{d}	0.03±0.01^{e}
Starch	17.74±0.24^{c}	24.09±0.74^{b}	26.50±0.46^{a}	3.32±0.04^{d}	2.20±0.06^{e}
Phenolics	1.45±0.02^{bc}	1.13±0.05^{d}	1.35±0.01^{c}	2.44±0.09^{a}	0.61±0.01^{e}

[1]Results are mean values of three determinations, ± standard deviation and are expressed on a dry weight basis, unless otherwise specified. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.

[2]Relative content in percentage (immature seeds, mature seeds and mature pod shells; fresh green seeds and fresh green pod shells).

[3]By difference.

and Boutin, 1989).

Protein content in fresh green beach pea seeds on a dry weight basis was 30.50% and decreased to 28.03% in mature seeds. Fresh green pod shells contained 11.74% crude protein as compared to 10.69% in mature pod shells. Crude and soluble protein contents of seeds and pod shells decreased with maturity. Thus, synthesis and turnover of proteins into other components occurred simultaneously, thereby confirming previous observations of decrease in protein content as maturity stages proceeded in pigeon pea and peas (Rao and Rao, 1974; Khatra *et al.*, 1986; Daveby *et al.*, 1993). In the present study, the content of crude and soluble protein decreased from 30.50 to 28.03% and 415.18 to 289.58 mg/100g of seeds, respectively, thus confirming that protein is laid down during the early stages in seed development for increasing the size (Holl and Vose, 1980). During the period of rapid starch synthesis, protein concentration is diluted as shown by the lower protein content at the time of harvest. Similar results were reported by Rochat and Boutin (1989) for green pea. The lipid content in seeds decreased during growth while the amount of ash remained unchanged, but decreased in pod shells from 6.56 to 2.19%. The change in crude fibre content in the seeds was insignificant ($p>0.05$), but was significant ($p<0.05$) for pod shells which changed from 27.61 to 35.54%.

Total carbohydrate in fresh green (premature) seeds was 59.28% and this decreased to 55.80% in mature seeds; the corresponding values for pod shells were 77.06 and 81.49%, respectively. The total soluble sugars decreased in both seeds and pod shells during maturity from 4.76 to 2.77 and 8.13 to 0.07%, respectively. As maturation

progressed, the content of starch in seeds increased from 17.74 to 26.50%. It has been reported that synthesis of both amylose and amylopectin, the two major components of starch, increases rapidly at the maturity stage of marrowfat and fodder peas (Haeder, 1989) as well as Swedish peas (Daveby *et al.*, 1993). Soluble carbohydrates accumulated before the rapid increase in starch, suggesting that soluble sugars reaching the seeds are transformed to starch, as shown by Bisson and Jones (1932). Turner (1953) has demonstrated that extracts from pea seeds can synthesize sucrose from a mixture of glucose-1-phosphate and fructose. In the developing pea seeds, sucrose is probably being broken down into glucose and fructose. If the synthetic mechanism was working in reverse, each sucrose molecule could thus form one molecule of glucose-1-phoshate, capable of direct condensation to starch and one molecule of fructose, whose transformation to starch or use in the respiration would require preliminary phosphorylation. A corresponding increase in ester phosphate was followed by a decrease during the early stages of active starch synthesis. The content of phenolic compounds ranged from 1.45 to 1.35% in the seeds and 2.44 to 0.61% in the pod shells of beach pea during their maturity stages. With advancing maturity, the seeds remobilize protein, soluble sugars and phenolic compounds and gain starch and crude fibre. Mature pod shells also remobilized ash, crude protein, sugars, starch and phenolic compounds and gained carbohydrates and crude fibre (Table 4.21). Results obtained in this study are in accord with those in the literature for other pea seeds and pod shells (Mckee *et al.*, 1955b; Turner and Turner, 1957; Trevino *et al.*, 1987; Daveby *et al.*, 1993).

4.4.2 Total and free amino acid composition at different maturity stages of beach pea

The amino acid profile during development of the seeds and pod shells of beach pea are presented in Table 4.22. Levels of arginine, serine and alanine were highest in fresh green seeds and later generally stabilised to a lower level. The content of sulphur-containing amino acids as well as tryptophan increased in beach pea seeds during development. However, levels of tyrosine, threonine, valine and histidine did not change significantly at different maturity stages. This may be due to the synthesis and their utilization in different plant parts was similar. The proportion of essential amino acids was higher (39.79 g/16g N) in mature pod shells as compared to fresh green ones (37.21 g/16g N). Daveby *et al.* (1993) have shown an increase in the proportion of essential amino acids of proteins as the maturity stage progressed in Timo and Vreta pea cultivars.

Immature seeds of beach pea showed slightly higher percentage ratio of essential to total amino acids and also amino acid score (Table 4.22), but their biological value was lower. This may be due to physiological immaturity of the seeds. Physiologically mature seeds have higher biological value (67.24) than the fresh green (58.40) and immature (54.83) seeds. In case of mature and fresh green pod shells the situation was reversed as the biological value was lower in mature (65.35) than fresh green pod shells (78.02). This situation might be due to the migration of amino acids from pod shells to seeds during development. Protein efficiency ratio generally marginally decreased in both seeds and pod shells as the maturity progressed (Table 4.23).

The content of each free amino acid was higher at fresh green (premature) stage

Table 4.22 Total amino acid composition of fresh green, immature, mature seeds, fresh green pod and mature pod shells of beach pea (g/16 g N)[1]

Amino acid	Fresh green seeds	Immature seeds	Mature seeds	Fresh green pod shells	Mature pod shells
Isoleucine	4.15±0.18^{a}	4.19±0.11^{a}	3.97±0.01^{a}	3.62±0.03^{c}	3.66±0.02^{bc}
Leucine	7.59±0.40^{a}	7.78±0.13^{a}	7.53±0.16^{a}	6.31±0.03^{bc}	6.15±0.01^{c}
Lysine	7.44±0.49^{ab}	7.82±0.12^{a}	7.43±0.15^{ab}	5.64±0.05^{b}	8.21±1.72^{a}
Cysteine[2]	1.47±0.05^{e}	1.61±0.02^{d}	1.70±0.04^{c}	1.71±0.02^{bc}	1.80±0.02^{a}
Methionine[2]	0.99±0.04^{b}	1.07±0.02^{a}	1.13±0.03^{a}	1.09±0.02^{a}	1.08±0.02^{a}
Tyrosine	3.01±0.13^{a}	3.33±0.05^{a}	3.25±0.05^{a}	3.17±0.28^{a}	3.19±0.05^{a}
Phenylalanine	4.55±0.21^{a}	4.83±0.10^{a}	4.60±0.10^{a}	3.87±0.00^{bc}	3.69±0.01^{c}
Threonine	4.40±0.43^{a}	4.28±0.06^{a}	4.17±0.10^{a}	4.33±0.03^{a}	4.20±0.02^{a}
Tryptophan[2]	0.23±0.01^{de}	0.21±0.01^{e}	0.33±0.02^{c}	0.35±0.00^{bc}	0.43±0.01^{a}
Valine	4.86±0.19^{a}	4.84±0.13^{a}	4.65±0.01^{a}	4.67±0.18^{a}	4.86±0.01^{a}
Histidine	2.58±0.22^{a}	2.66±0.06^{a}	2.59±0.01^{a}	2.45±0.01^{a}	2.52±0.01^{a}
Arginine	8.15±0.56^{a}	7.99±0.20^{a}	7.97±0.19^{a}	4.62±0.05^{bc}	4.52±0.10^{c}
Aspartic acid + Asparagine	13.72±0.67^{a}	13.15±1.26^{a}	13.04±0.13^{a}	10.69±0.15^{bc}	10.33±0.21^{c}
Glutamic acid + Glutamine	15.82±1.03^{b}	17.15±0.22^{a}	16.56±0.31^{ab}	10.53±0.12^{c}	8.82±0.09^{d}
Serine	6.35±0.01^{a}	5.11±0.05^{de}	4.98±0.14^{c}	5.46±0.10^{c}	5.77±0.02^{b}
Proline	4.24±0.22^{cd}	4.34±0.11^{bcd}	3.95±0.14^{d}	4.64±0.15^{abc}	5.01±0.20^{a}
Glycine	4.24±0.23^{cd}	4.32±0.09^{bcd}	4.12±0.12^{d}	4.86±0.06^{a}	4.75±0.03^{a}
Alanine	5.54±0.18^{a}	4.43±0.07^{bcde}	4.27±0.13^{c}	4.32±0.03^{de}	4.41±0.05^{cde}
E/T, %	41.55	43.00	42.97	45.20	47.71
Amino acid score	107.47	111.00	107.67	103.53	96.56
BV	58.40	54.83	67.24	78.02	65.35

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. [2]Limiting amino acid.

Table 4.23 Predicted PER values of different maturity stages of beach pea seeds and pod shells

Maturity stage	Predicted PER values using equation[1]		
	1	2	3
Fresh green seeds	2.58	2.66	2.24
Immature seeds	2.66	2.71	2.14
Mature seeds	2.56	2.61	2.03
Fresh green pod shells	1.98	2.06	1.10
Mature pod shells	1.88	1.99	0.97

[1]Alsmeyer *et al.* (1974).

of seeds and pod shells. As the maturity stage was reached, the content of free amino acids in seeds and pod shells decreased (Table 4.24). Earlier studies on the free amino acids in developing peas have shown an abundance of alanine, glutamine, threonine and homoserine during the early stages of seed development (Macnicol, 1977; Rochat and Boutin, 1991). At later stages, the amino acids are mainly incorporated into storage proteins (Pate and Flinn, 1977; Murray, 1983; Rochat and Boutin, 1989). The storage proteins, which comprise up to 80% of the seed nitrogen, have different amino acid patterns and are synthesized at different rates (Pernollet, 1985). Free amino acids occurring in the seed during filling appear to play various roles according their nature and stage of seed development. They represent the first temporary reserve material in the embryo sac fluid and are then translocated in the apoplast of the seed coat and the cotyledon where they are used for synthesis of storage proteins. Subsequently, as seed development reaches completion free amino acids, such as asparagine and arginine, represent nitrogen reserve material. The results obtained in the present study are in accord with literature values for other pea seeds (McKee *et al.*, 1955a; Macnicol, 1983; Murray, 1992; Khatra *et al.*, 1986).

4.4.3 Minerals

The effect of maturity stage on the mineral content of seeds and pod shells is presented in Table 4.25. Fresh green (premature) seeds and pod shells had higher amounts of all minerals as compared to mature seeds and pod shells, especially for

Table 4.24 Free amino acid composition of fresh green (premature) seeds, immature (green seeds), mature (dark seeds), fresh green pod shells and mature pod shells of beach pea (mg/100g)[1]

Free amino acid	Fresh green seeds	Immature seeds	Mature seeds	Fresh green pod shells	Mature pod shells
Alanine	235.02±5.83^{a}	14.77±0.11^{d}	20.68±0.54^{c}	28.03±0.36^{b}	4.72±0.12^{e}
Arginine	230.56±0.67^{a}	79.60±1.48^{d}	128.64±1.24^{b}	86.18±2.58^{c}	12.35±0.28^{e}
Asparagine	293.83±0.77^{a}	129.50±5.08^{b}	109.29±7.23^{c}	53.18±0.96^{d}	24.64±0.33^{e}
Aspartic acid	31.81±1.66^{a}	15.45±0.61^{b}	32.67±2.20^{a}	10.19±1.12^{c}	4.20±0.17^{d}
Cysteine	29.47±0.21^{a}	31.31±0.46^{a}	27.92±1.82^{b}	19.99±0.61^{c}	2.93±0.57^{d}
Glutamic acid	279.29±1.21^{a}	149.95±2.66^{b}	11.81±4.67^{e}	34.12±0.16^{cd}	31.90±0.32^{d}
Glutamine	25.69±0.69^{b}	2.15±1.44^{de}	1.84±0.76^{e}	80.76±0.98^{a}	21.64±0.23^{c}
Glycine	47.10±0.19^{a}	7.53±0.16^{c}	12.89±0.59^{b}	2.09±0.07^{de}	1.53±0.23^{e}
Histidine	30.20±0.39^{a}	11.87±4.81^{cd}	10.64±0.75^{d}	14.42±0.47^{bcd}	2.77±0.03^{e}
Hydroxyproline	1.04±0.33^{bcd}	1.68±0.32^{ab}	1.80±0.34^{a}	0.74±0.05^{cd}	0.71±0.05^{d}
Isoleucine	26.58±0.19^{a}	4.32±0.22^{d}	7.50±0.41^{c}	11.22±0.02^{b}	0.89±0.09^{e}
Leucine	35.30±0.07^{a}	8.47±0.18^{d}	10.23±1.05^{c}	12.48±0.14^{b}	1.11±0.16^{e}
Lysine	53.04±0.74^{a}	10.18±0.13^{c}	20.20±0.27^{b}	7.69±0.24^{d}	4.27±0.16^{e}
Methionine	20.88±1.03^{a}	15.22±0.95^{d}	16.45±1.95^{cd}	17.21±0.05^{bcd}	2.77±0.51^{e}
Phenylalanine	21.91±0.21^{a}	4.18±0.07^{cd}	4.13±0.21^{d}	6.93±0.00^{b}	0.60±0.00^{e}
Proline	146.26±0.50^{a}	45.11±1.73^{cd}	42.15±1.33^{d}	74.53±2.07^{b}	22.95±0.27^{e}
Serine	325.92±4.02^{a}	22.30±0.11^{c}	16.02±0.10^{d}	55.33±0.60^{b}	4.53±0.25^{e}
Tyrosine	17.01±0.07^{a}	2.23±0.54^{d}	5.13±0.10^{c}	6.07±0.30^{b}	0.36±0.03^{e}
Threonine	57.17±1.38^{a}	4.64±0.52^{de}	10.48±0.55^{c}	24.23±0.40^{b}	3.13±0.04^{e}
Tryptophan	12.51±0.07^{a}	6.19±0.54^{c}	7.07±0.41^{bc}	12.70±0.30^{a}	0.70±0.48^{d}
Valine	35.37±0.13	6.02±0.11^{d}	6.38±0.04^{cd}	20.67±0.39^{b}	2.03±0.53^{e}
Total	1955.96	572.67	503.92	578.76	150.73

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.

Table 4.25 Mineral composition of fresh green, immature, mature seeds, fresh green pod shells and mature pod shells of beach pea (mg/100g)[1]

Mineral	Fresh green seeds	Immature seeds	Mature seeds	Fresh green pod shells	Mature pod shells
Macroelement					
Calcium	147.87±1.17^{cd}	146.03±1.13^{d}	138.69±0.31^{e}	365.86±2.02^{a}	262.82±2.06^{b}
Magnesium	206.40±1.30^{a}	178.68±1.14^{c}	182.86±1.42^{b}	157.99±1.15^{de}	156.81±1.45^{c}
Phosphorus	438.00±1.77^{a}	405.93±1.62^{c}	434.04±0.82^{b}	118.02±1.85^{d}	50.85±0.52^{e}
Potassium	1329.76±2.95^{b}	484.22±1.89^{d}	450.82±2.02^{e}	2385.17±3.50^{a}	626.50±2.10^{c}
Sodium	126.58±1.37^{c}	74.56±0.95^{e}	112.71±2.79^{d}	705.05±2.02^{a}	264.39±1.07^{b}
Microelement					
Aluminum	3.45±0.32^{de}	4.96±0.23^{c}	3.09±0.36^{e}	57.10±0.32^{a}	11.26±0.60^{b}
Copper	2.26±0.12^{a}	0.83±0.05^{cd}	0.90±0.19^{bcd}	2.39±0.15^{a}	0.70±0.11^{d}
Iron	9.98±0.25^{cd}	9.56±0.35^{de}	8.80±0.37^{e}	25.26±0.30^{b}	34.23±0.41^{a}
Lithium	1.26±0.17^{a}	0.85±0.26^{a}	1.05±0.07^{a}	1.83±0.75^{a}	0.78±0.50^{a}
Manganese	4.58±0.30^{bcd}	3.41±0.65^{d}	3.77±0.51^{cd}	6.98±1.13^{a}	5.72±0.95^{abc}
Silicon	3.28±0.23 ^{c}	ND	ND	91.90±0.53^{a}	23.01±0.86^{b}
Zinc	3.77±0.09^{b}	2.94±0.11^{d}	3.06±0.04^{cd}	19.26±0.09^{a}	0.62±0.07^{e}

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

potassium, but with the exception of iron in pod shells. Results in the present study show that beach pea would constitute a valuable source of essential minerals. similar to other legumes, for human nutrition (Mieners *et al.*, 1976; Jagadi *et al.*, 1987; Apata and Ologhobo, 1994; Barrado *et al.*, 1994). Calcium content was higher than the phosphorus in pod shells during all stages of maturation and thus provides an ideal balance for adequate growth and bone formation in animals (Apata and Ologhobo, 1989).

4.5 Effect of different solvents on the recovery of condensed tannins from beach pea, green pea and grass peas

Effect of organic solvents, addition of water, as well as concentrated HCl to the extraction solvent on the efficiency of removal of pea seed condensed tannins is shown in Table 4.26. Results indicate that pure solvents were poor extraction media for the removal/recovery of tannins. Pure water or water plus 1% concentrated HCl was also a very poor solvent for the extraction of tannins. Addition of water, up to 30% (v/v), and 1% concentrated HCl in acetone, improved the effectiveness of tannin recovery from beach pea (11.58%), Indian grass pea (1.54%), Canadian grass pea (0.11%) and green pea (0.07%). Acetone containing 30% water and 1% concentrated HCl was more efficient for the recovery of tannins as compared to 70% (v/v) acetone, 70% (v/v) methanol and 70% (v/v) methanol containing 1% concentrated HCl. Results indicate that the presence of HCl in the extraction medium increased the recovery of condensed tannins from beach pea seed meals from 0.20% (in water) to 11.58% (in 70% acidified acetone). Absolute

Table 4.26 Effect of different solvents on extraction of condensed tannins from different peas[1]

Solvent	Beach pea (g/100g meal)	Grass pea[2] (g/100g meal)	Green pea (mg/100g meal)	Grass pea[3] (mg/100g meal)
Water	0.20±0.01	0.04±0.02	15.15±0.82	19.14±0.51
Water + HCl	0.46±0.08	0.12±0.03	39.98±0.10	52.99±0.49
Methanol				
100%	2.20±0.21	0.17±0.12	83.54±0.54	70.96±0.48
90%	2.20±0.12	0.12±0.04	34.03±0.03	66.89±0.47
80%	1.58±0.12	0.11±0.02	25.74±0.34	56.22±0.72
70%	0.92±0.07	0.21±0.05	52.72±0.96	50.79±0.25
Acidified Methanol				
100%	2.46±0.07	0.23±0.12	58.49±0.16	49.25±0.50
90%	2.85±0.13	0.43±0.18	65.95±0.27	90.52±0.21
80%	4.40±0.13	0.57±0.03	69.02±0.19	77.93±0.61
70%	4.54±0.67	0.65±0.04	69.76±0.24	75.06±0.13
Acetone				
100%	0.23±0.11	0.14±0.05	33.37±0.88	34.78±0.89
90%	4.12±0.22	0.75±0.20	32.45±0.61	59.06±0.54
80%	8.47±0.20	0.99±0.50	42.96±0.80	57.17±0.91
70%	10.15±0.88	1.04±0.30	58.82±0.59	45.14±0.37
Acidified Acetone				
100%	0.35±0.14	0.39±0.07	57.39±0.76	43.42±0.37
90%	6.59±0.43	0.93±0.53	65.76±0.59	64.88±0.17
80%	10.66±0.58	1.43±0.24	69.74±0.74	66.95±0.47
70%	11.58±0.19	1.54±0.15	71.98±0.12	109.11±0.73

[1]Results are means of six determinations, on a dry weight basis, ± standard deviation.
[2]Indian grass pea. [3]Canadian grass pea.

methanol, methanol containing 1% concentrated HCl and 70% acetone are common solvent systems used for the recovery of plant tannins. Maxson and Rooney (1972) as well as Price *et al.* (1978b) demonstrated that sorghum tannins were effectively extracted with methanol containing 1% concentrated HCl. Leung *et al.* (1979) used acetone containing 30% water for the extraction of tannins from rapeseed hulls. Gupta and Haslam (1980) used different solvent systems such as water, methanol, ethanol, propanol, acetone and dimethylformamide for the extraction of polyphenolics from sorghum grain. These authors have also suggested that methanol was the most effective solvent for the recovery of polyphenolics. These results are similar to those reported by Price *et al.* (1978b) who found that the addition of concentrated HCl to methanol improved the extraction of sorghum tannins. Tenfold less tannin was removed from green pea and grass pea. This is because of grass pea and green pea have different levels of tannin content than those of beach pea. The present study suggests that the nature of tannins as well as their solubility in different solvent systems differs from sample to sample, depending on their origin.

This variation in the condensed tannins content may be due to genetic variations in different peas. Beach pea and grass peas are from same genus, but their content of condensed tannins was significantly different due to different seed characteristics.

4.6 Nutrient distribution in air classified fractions of cotyledons and hulls of beach pea

4.6.1 Chemical composition

Chemical composition of mechanically separated cotyledons and hulls fractions of beach pea are presented in Table 4.27. The crude protein content ranged from 32.82 to 35.28% in cotyledons and from 14.73 to 16.81% in hulls. Similarly, soluble proteins, soluble sugars and starch were present in higher amounts in cotyledons. Ash content of cotyledons decreased from fraction I to fraction III, but in case of hulls it increased, indicating a higher ash content in cotyledons compared to hulls. Similar results for ash content in cotyledons and hulls were reported for cowpea, green pea, and pigeonpea (Singh *et al.*, 1968). Crude fibre content was higher in hull fraction I (37.13%) and fraction II (36.85%) than those of cotyledons (Fraction I, II, and III, 2.83%, 2.99%, and 3.08%, respectively). The levels of carbohydrates, total phenolics and condensed tannins was higher in hulls compared to cotyledons. The results indicate that those components are mostly concentrated in the seed coats and might be easily removed by dehulling. Total phenolics and condensed tannins in beach pea were high than those reported in the literature for other seeds, perhaps due to the presence of some hulls in the cotyledons fraction. Condensed tannins of cotyledons and hulls ranged from 5.76 to 6.90% and 52.49 to 57.24%, respectively, expressed as catechin equivalents. Reddy *et al.* (1985) reported that the tannin content in cotyledons of cowpeas was 28 mg/100g, peas 460 - 560 mg/100g, pigeonpea 22 - 43 mg/100g and chickpea 16 -38 mg/100g, as catechin

Table 4.27 Chemical composition of air classified fractions of cotyledons and hulls of beach pea[1]

Constituent, %	Cotyledons			Hulls	
	Fraction-I	Fraction-II	Fraction-III	Fraction-I	Fraction-II
Moisture	4.57±0.08^{ab}	4.73±0.13^{a}	4.64±0.06^{ab}	4.22±0.09^{c}	4.49±0.05^{b}
Ash	3.92±0.05^{a}	3.77±0.11^{ab}	3.53±0.07^{bc}	2.99±0.13^{d}	3.29±0.20^{cd}
Lipid	1.08±0.04^{a}	0.98±0.02^{b}	0.92±0.01^{c}	0.45±0.02^{ed}	0.48±0.01^{d}
Crude fibre	2.83±0.05^{d}	2.99±0.08^{cd}	3.08±0.10^{bcd}	37.13±0.60^{a}	36.85±0.92^{a}
Protein	35.28±0.98^{a}	34.49±0.53^{a}	32.82±1.02^{a}	14.73±0.92^{c}	16.81±1.56^{bc}
Soluble proteins (mg/100g)	318.72±2.92^{a}	306.13±3.06^{bc}	302.37±4.31^{c}	105.49±0.62^{e}	134.85±1.77^{d}
Carbohydrates[2]	55.15±1.43^{d}	56.03±1.82^{cd}	58.09±1.60^{bcd}	77.61±1.47^{a}	74.93±1.74^{a}
Soluble sugars	2.97±0.12^{a}	2.86±0.07^{ab}	2.73±0.10^{b}	0.08±0.00^{d}	0.17±0.01^{cd}
Reducing sugars (mg/100g)	302.13±2.27^{a}	287.25±4.91^{b}	267.61±5.34^{c}	66.77±1.74^{e}	136.88±1.28^{d}
Non-reducing sugars	2.67±0.11^{a}	2.57±0.37^{a}	2.46±0.52^{a}	0.01±0.00^{c}	0.03±0.01^{bc}
Starch	37.12±1.23^{a}	34.18±2.03^{a}	34.57±1.43^{a}	3.56±0.22^{c}	7.15±0.12^{b}
Phenolics	0.93±0.005^{d}	1.05±0.006^{cd}	1.12±0.005^{bcd}	15.80±1.20^{a}	14.92±1.08^{a}
Condensed tannins	5.76±0.11^{e}	6.46±0.16^{de}	6.90±0.13^{cde}	57.24±1.67^{a}	52.49±0.83^{b}

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed different superscripts in each row are significantly ($p<0.05$) different from one another.

[2]By difference.

equivalents. The present results indicate that beach pea hulls contain high amounts of total phenolics and condensed tannins. This might be due to the genetic characteristics of beach pea and the fact that tannins provide a barrier for seeds against harsh and humid environmental conditions in the shorelines.

There are several chemicals such as Tween-80, polyethylene glycol, caffeine and iron that may complex with tannins (Armstrong *et al.*, 1973). The complexation of these components with tannins may be enhanced as the number of hydroxyl groups of tannins increases. When these agents are used as supplements in the diet, they may form tannin-protein complex or may release protein from such complexes. Marquardt *et al.* (1977) and Ford and Hewitt (1979) found that addition of Tween-80, polyethylene glycol, caffeine and iron in the high tannin field bean diets eliminates the growth depressing effect of tannins and increases protein digestibility in chicks. Price and Butler (1980) reported that methionine and choline play an important role in detoxification of tannins in experimental animals. Additional methionine and choline in the diet react with tannins by the formation of monomethyl ethers and avoid depletion of the methyl donors, methionine and choline, in the body. Several studies have shown that addition of methionine and choline to the tannin-rich diets can counteract the antinutritional effects of condensed as well as hydrolyzable tannins. Addition of these two amino acids to legume-based diets with high tannins can detoxify tannins and improve their nutritional quality. Bressani *et al.* (1983) reported that methionine plays an important role in metabolic detoxification of bean tannins in animals.

4.6.2 Minerals

Minerals in air classified fractions of beach pea cotyledons and hulls are shown in Table 4.28. Minerals in beach pea cotyledons were dominated by phosphorus, potassium, and zinc, while hulls were rich in calcium, sodium, aluminum, iron and manganese. Silicon was not detected in cotyledons as well as in hulls of beach pea. Calcium content in hulls was lower than the reported values for peas, cowpeas, and pigeonpeas (900, 853, and 917 mg/100g, respectively; Singh *et al.*, 1968); other elements in cotyledons and hulls were present in similar amounts.

4.6.3 Non-protein nitrogen (NPN) and phytic acid content

The content of NPN and phytic acid in different fractions of beach pea cotyledons and hulls is presented in Table 4.29. The ratio of NPN to total nitrogen was significantly higher in hulls (Fraction I, 23.79% and Fraction II, 22.73%) than in cotyledons (from 17.27 to 20.13%). Most of the protein nitrogen stored in the cotyledons were synthesized from non-protein nitrogen mobilized from hulls. Ultimately the content of non-protein nitrogen was lower in cotyledons. Singh and Jambunathan (1982) also observed that the proportion of NPN was very high in seed coat than cotyledons of chickpea and pigeonpea (21.3, 10.7, 27.4, and 9.5% to total nitrogen, respectively).

Phytic acid was present significantly ($p<0.05$) higher amounts in cotyledons than in hulls, 439.46 mg/100g (Fraction III) to 483.92 mg/100g (Fraction I) of cotyledons, compared to 68.69 and 67.44 mg/100g in fractions I and II of hulls, respectively (Table

Table 4.28 Mineral content of air classified fractions of cotyledons and hulls of beach pea (mg/100g)[1]

Mineral	Cotyledons			Hulls	
	Fraction-I	Fraction-II	Fraction-III	Fraction-I	Fraction-II
Macroelement					
Calcium	111.34±1.07^{e}	121.89±1.32^{d}	124.83±1.24^{cd}	270.85±1.62^{a}	237.32±2.06^{b}
Magnesium	220.42±1.11^{a}	216.42±1.21^{bc}	215.98±1.05^{c}	195.84±1.20^{e}	202.18±1.08^{d}
Phosphorus	645.23±3.52^{a}	590.12±2.76^{b}	523.17±2.31^{c}	86.95±0.38^{e}	93.67±0.73^{d}
Potassium	1401.22±8.07^{a}	1255.99±9.66^{b}	1177.16±9.06^{c}	1016.25±10.36^{e}	1100.09±10.12^{d}
Sodium	178.86±0.68^{e}	188.27±0.29^{d}	195.40±0.30^{b}	191.96±0.73^{c}	203.37±0.92^{a}
Microelement					
Aluminum	3.07±0.28^{d}	3.29±0.36^{cd}	5.47±0.12^{ab}	6.41±0.78^{a}	4.69±0.91^{bc}
Copper	1.84±0.16^{ab}	1.72±0.20^{b}	1.89±0.10^{ab}	1.79±0.21^{ab}	2.25±0.18^{a}
Iron	9.79±0.76^{a}	10.02±1.00^{a}	10.19±0.93^{a}	11.23±1.03^{a}	9.63±0.65^{a}
Lithium	1.48±0.35^{a}	1.22±0.30^{a}	1.22±0.63^{a}	1.01±0.11^{a}	1.04±0.06^{a}
Manganese	3.07±0.24^{cd}	2.97±0.06^{d}	2.97±0.08^{d}	5.02±0.20^{a}	4.56±0.07^{b}
Silicon	ND	ND	ND	ND	ND
Zinc	5.26±0.70^{a}	4.52±0.20^{ab}	4.13±0.13^{bcd}	3.42±0.23^{cd}	3.41±0.11^{d}

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

Table 4.29 Non-protein nitrogen and phytic acid content in air classified fractions of cotyledons and hulls of beach pea[1]

Component	% NPN to total nitrogen	Phytic acid (mg/100g)
Cotyledons		
Fraction-I	17.27±1.96^{c}	483.92±3.76^{a}
Fraction-II	18.38±1.07^{bc}	478.00±2.99^{a}
Fraction-III	20.13±0.92^{abc}	439.46±2.78^{b}
Hulls		
Fraction-I	23.79±1.74^{a}	68.69±1.08^{e}
Fraction-II	22.73±1.83^{a}	67.44±1.66^{cd}

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another.

4.29). Phytic acid accumulates with protein bodies in the cotyledons and cotyledons contain higher amounts of protein bodies than hulls, thus leading to higher contents of phytic acid in cotyledons. These results are in agreement with similar observations in black gram (Duhan *et al.*, 1989) and cowpea (Uzogara *et al.*, 1990).

4.6.4 Tannins and their properties

The content and properties of beach pea hull tannins from air classified fractions I, II and manually separated hulls are presented in Tables 4.30. Condensed tannins, expressed as catechin equivalents, were present at very high levels in fraction I (572.4 mg/g) as compared to fraction II (524.9 mg/g) and 12 h soaked seed hulls (396.8 mg/g). The content of proanthocyanidin of mechanically separated hulls was also very high for fraction I and fraction II as compared to soaked and manually separated hulls from beach pea. Protein precipitation capacity of hull fraction I (91.3) was higher while that from fraction II was lower (57.8) compared to the 12 h soaked sample (64.3). The biological and ecological role of tannins is attributed to their abilities to bind or precipitate proteins (Bate-Smith, 1973; Hagerman and Butler, 1978; Salunkhe *et al.*, 1990). Bovine serum albumin (BSA) assay allows for direct measurement of protein precipitation by tannins used in the present study. Condensed tannins extracted from beach pea hulls fractions precipitated 793 to 1131 mg of BSA/g hulls. Fraction II gave lower values for all assays than those for fraction I, possibly due the presence of some cotyledons in the hulls fractions. The lower values for the content of tannins of soaked hulls might be due to

Table 4.30 Content and properties of tannin from beach pea hull fractions[1]

Assay	Fraction I		Fraction II		Soaked	
	Absorbance/g	Content mg/g	Absorbance/g	Content mg/g	Absorbance/g	Content mg/g
Vanillin	340±9.0	572.4 (catechin equiv.)	311±5.0	524.9 (catechin equiv.)	500±3.0	396.8 (catechin equiv.)
Proanthocyanidin	822±17.0		614±23.0		212±15.5	
Protein precipitation	91.3±7.5		57.8±1.5		64.3±1.3	
Dye-labeled BSA	232±20.0	1131 mg BSA/g	175±26.0	851.8 mg BSA/g	163±6.3	793.4 mg BSA/g
Total phenol (Folin-Denis)	444±15.0		284±5.0		28.5±2.0	
No-tannin fraction (Folin-Denis)	35±9.0		29±8.0		7±3.0	
Tannin fraction (Folin-Denis)	322±17.0		228±30.0		46±3.0	

[1]Results are means of three determinations, on a dry weight basis, ± standard deviation.

their leaching out during soaking. Beach pea hulls contained very high amounts of condensed tannins than those reported earlier for other legumes such as pigeonpea (11.41 mg/g), chickpea (1.65 mg/g) (Udayasekhara Rao and Deosthale, 1982), cowpea (2.62 mg/g) (Chang *et al.*, 1994), green pea (5-10 mg/g) (Reddy *et al.*, 1985).

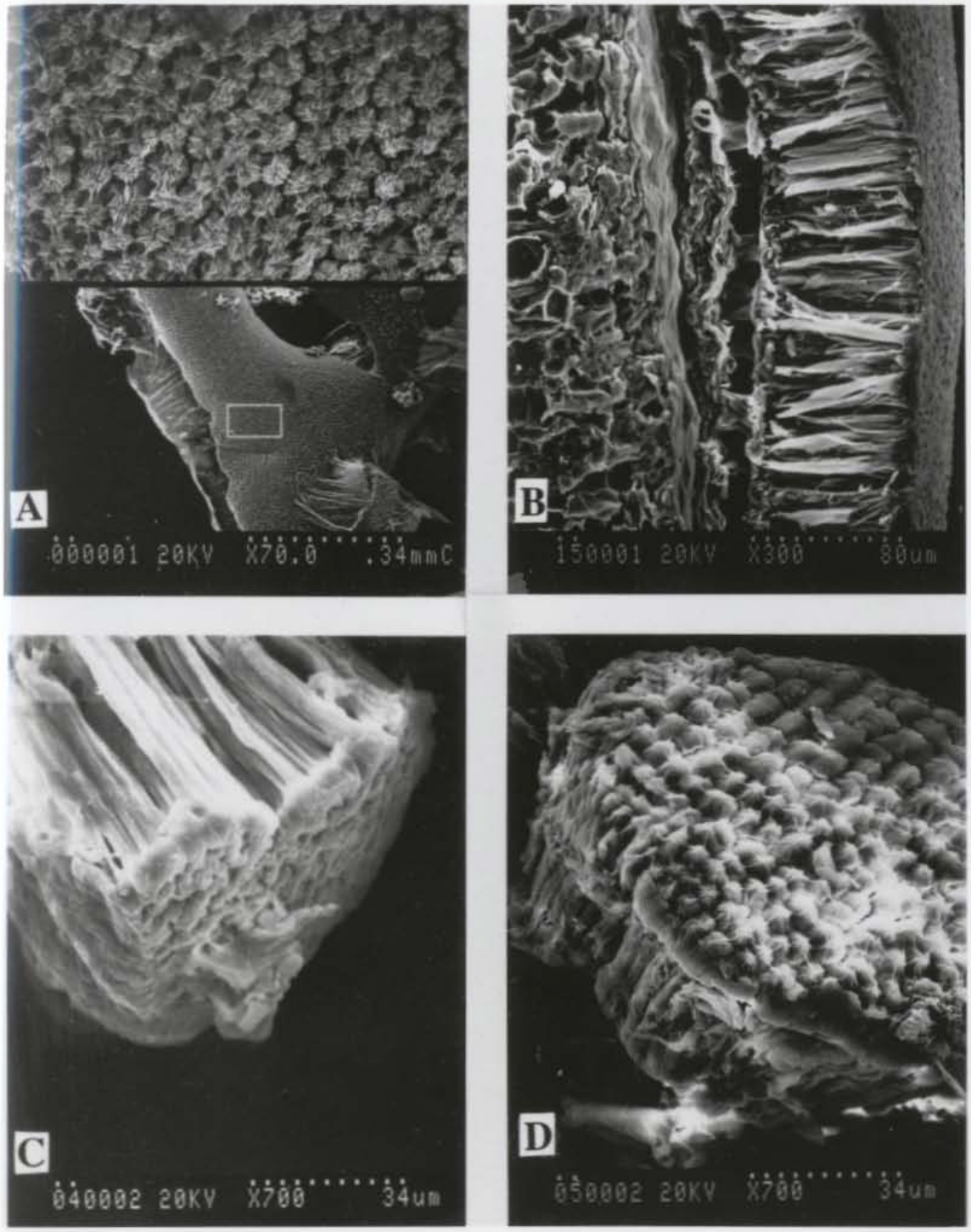
4.6.5 Seed coat structure of beach pea

Beach pea seed coat structure studied using electron microscope (SEM) is shown in Figure 4.5A; the cross section of the seed coats, shown in Figure 4.5B, indicate a highly compact and a very hard structure. Preliminary studies showed that beach pea seeds are very hard to imbibe (Table 4.1). Effect of soaking in concentrated H_2SO_4 for 30 min and then in distilled water for 12 h at room temperature showed complete destruction of the compact structure of beach pea seed coat (Figure 4.5C). Beach pea seeds that were heat processed for 30 min in boiling water also were swollen and showed loosened seed coat structure (Figure 4.5D). These two treatments improve beach pea seed coat permeability to water and help imbibition as well as lowering of the antinutritional factors by diffusion and were found to be useful for separation of hulls from cotyledons.

4.7 Antioxidant activity of phenolic fractions of beach pea hull extract

4.7.1 Separation of phenolic fractions from beach pea hull extract

Three phenolic fractions (Fraction I-iii) were separated from acetone extract (70%, v/v, containing 1% concentrated HCl) of beach pea hulls via Sephadex LH-20 column


A
000001 20KV X70.0 .34mmC
B
150001 20KV X300 80um
C
040002 20KV X700 34um
D
050002 20KV X700 34um

chromatography and each fraction was tested for the presence of condensed tannins (Figure 4.6). One major peak (III) and two minor peaks (I and II) were observed when absorbances were measured at 280 nm (Figure 4.6). For beach pea hulls, the highest content of phenolic compounds and condensed tannins was observed in fraction III. Furthermore, the crude extract possessed considerably more phenolic compounds than fractions I and II, while condensed tannins were not detected in fractions I and II (Table 4.31). Phenolic compounds from Polish white bean, pea, everlasting pea, lentil, broad bean and faba bean seed hulls showed higher amount of crude extract compared with the whole seeds but less than beach pea hulls (Amarowicz *et al.*, 1996b). They also had high antioxidative properties, compared with those of seeds from mustard, canola, rape, and flax (Amarowicz *et al.*, 1996a). These authors have also reported that the extract obtained from seed coats contained 2.5 - 13 times higher amounts of total phenolic than those from whole seeds.

The absorption maxima of separated fractions (Figure 4.7) occurred mainly in the range of 280 to 290 nm. Fractions I, II and III had only one maximum at 284, 284 and 282 nm, respectively. This suggest that flavonoids are potentially the main phenolics present in beach pea hulls extracts. Mabry *et al.* (1970) reported that flavones and flavonols produced two major absorption peaks in the 240 to 400 nm range in methanol. Amarowicz and Raab (1997) separated five phenolic fractions from the acetone extract of everlasting pea, faba bean and broad bean using Sephadex LH-20 column chromatography. They also reported that UV spectra of separated fractions from these

Figure 4.6 Separation of phenolic fractions of beach pea hulls extracts by Sephadex LH-20 column chromatography: UV absorbance of phenolics (280 nm) and condensed tannins (500 nm) following colour development (Fraction I and II ethanol mobile phase and III fraction with acetone-water, 50:50 (v/v) mobile phase).

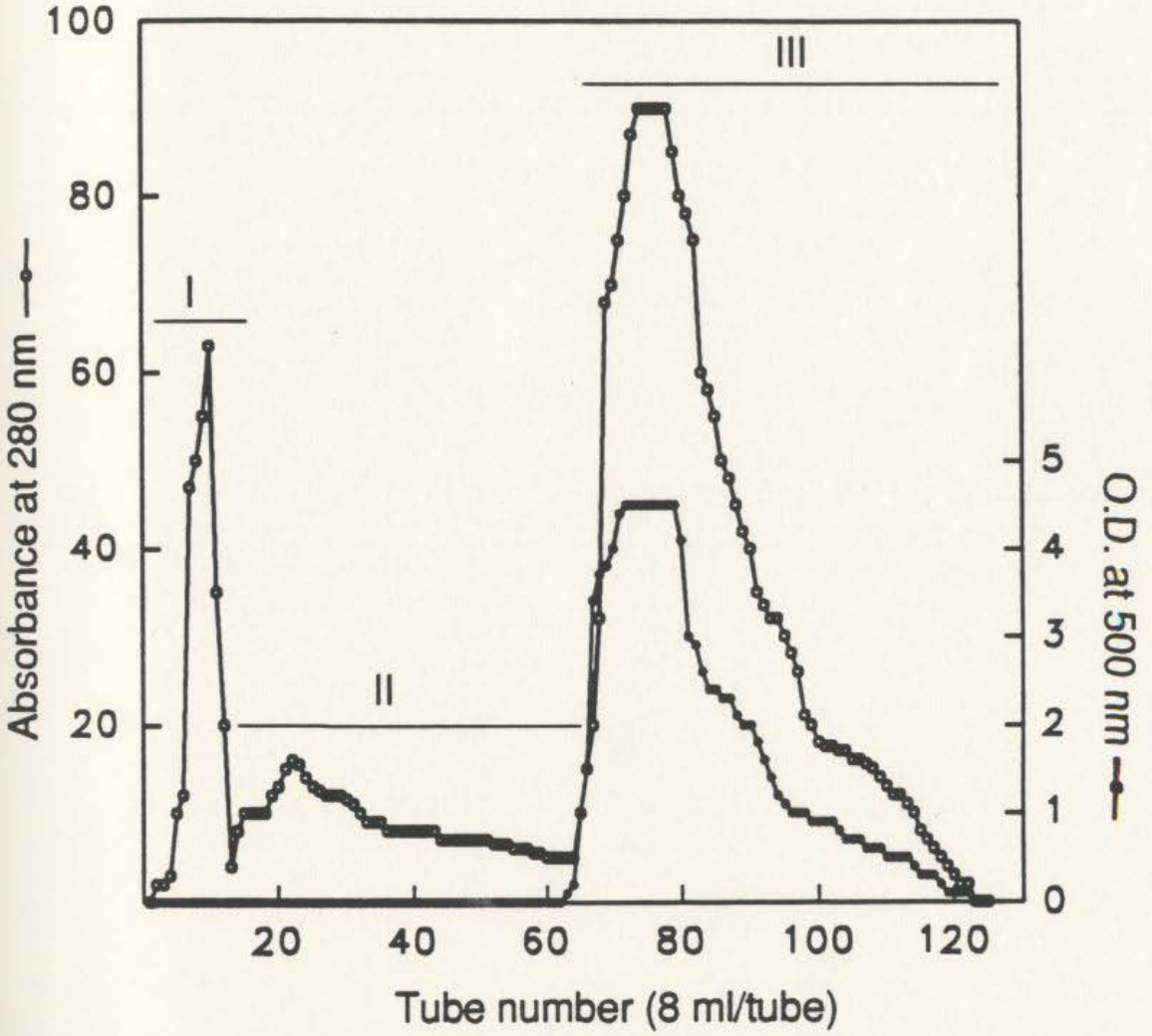

100
80
60
40
20
Absorbance at 280 nm
I
II
III
5
4
3
2
1
0
O.D. at 500 nm
20
40
60
80
100
120
Tube number (8 ml/tube)

Table 4.31 Percentage recovery of beach pea hulls extract and their total phenolic and condensed tannin contents[1]

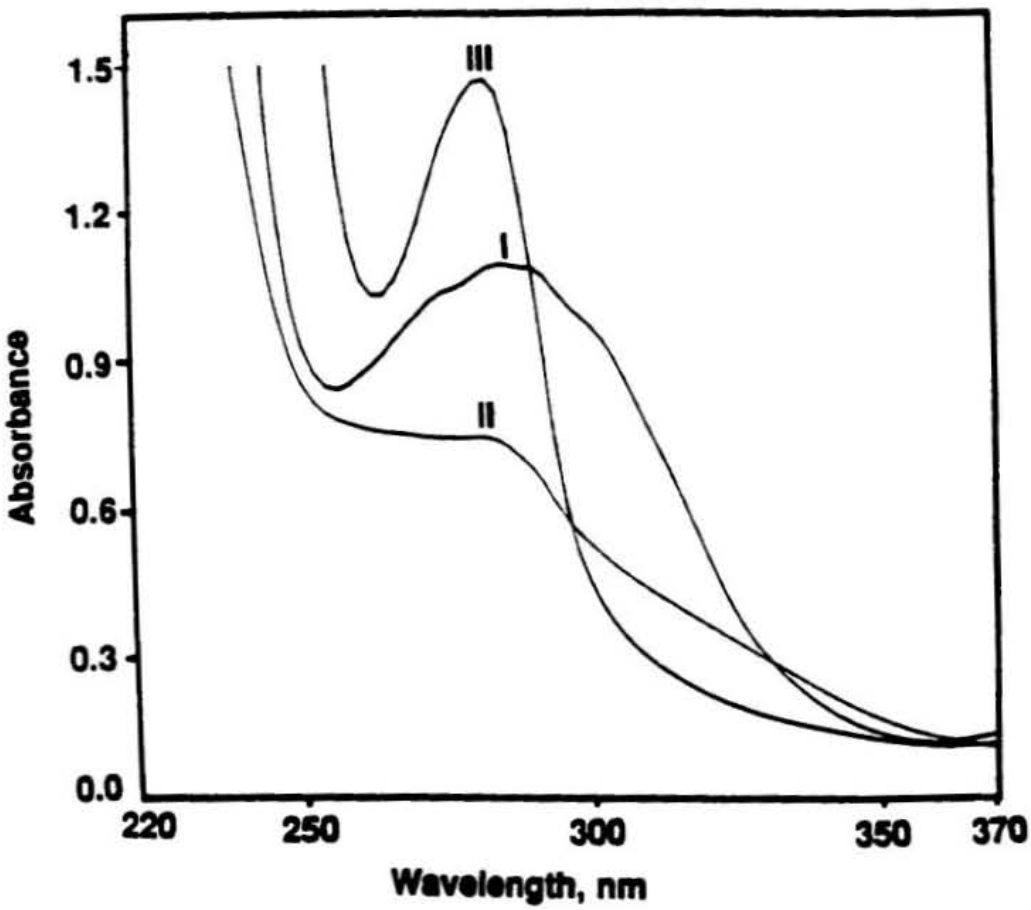
Fraction	Relative content (% of total)	Total phenolic (% of extract)[2]	Condensed tannins (% of extract)[3]
Crude extract	21.53 ± 1.23[c]	54.15 ± 1.26[b]	156.73 ± 1.53[b]
Fraction-I	23.87 ± 0.87[b]	3.11 ± 0.41[d]	ND
Fraction-II	19.22 ± 0.42[d]	19.21 ± 0.23[c]	ND
Fraction-III	56.89 ± 1.03[a]	79.06 ± 1.06[a]	220.19 ± 3.83[a]

[1]All fractions separated on Sephadex LH-20 column. Results are means of three determinations, ± standard deviation. Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]As sinapic acid equivalents.

[3]As catechin equivalents.

Figure 4.7 UV spectra of individual fractions of beach pea hulls extracts separated on a Sephadex LH-20 column (I and II fraction from ethanol as mobile phase and fraction III from acetone-water, 50:50 (v/v) as mobile phase).


1.5
1.2
0.9
0.6
0.3
0.0
Absorbance
III
I
II
220
250
300
350
370
Wavelength, nm

legumes had most of their absorption bands in the 270 to 280 nm range.

4.7.2 TLC separation of phenolic fractions of beach pea hull extract

The TLC plates after development of the three isolated fractions and crude extract from beach pea hulls following spraying with different reagents are shown in Figure 4.8. Thin layer chromatography indicated that the separated fractions contained several phenolic compounds. Compounds from fraction III of beach pea hulls extract, close to the solvent front in both developing systems, possessed maximum antioxidant activity. Several phenolic compounds were visualized on the TLC plate. Therefore, fraction III with the highest antioxidant activity may contain several antioxidative phenolics. The total number of hydroxyl groups present in compounds of fraction III may be higher than those of compounds in other fractions or different additional compound(s) may be present: this may partially be responsible for better antioxidative properties of this fraction as shown in the inhibition of the bleaching of β-carotene.

4.7.3 Antioxidant activity of phenolic fractions of beach pea hull extract

The antioxidant activity of each fraction and crude extract, as compared with BHA is presented in Figure 4.9. Fraction III exhibited the highest antioxidative activity against bleaching of β-carotene. The crude extract had a better antioxidative effect than fractions I and II. The activity of fraction III was higher than that of fractions I, II and the crude extract. Fraction III which exhibited the best antioxidative activity, contained 79.1%

Figure 4.8 TLC of phenolic fractions separated from beach pea hulls extracts: chromatograms were developed using (1) acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v) and (2) acetic acid-water-n-butanol (10:10:30, v/v/v); plates were sprayed with a solution of ferric chloride (A) to give spots of phenolic compounds and (B) β-Carotene-linoleate in order to evaluate antioxidant activity of crude extract (C) and fractions (I-III).

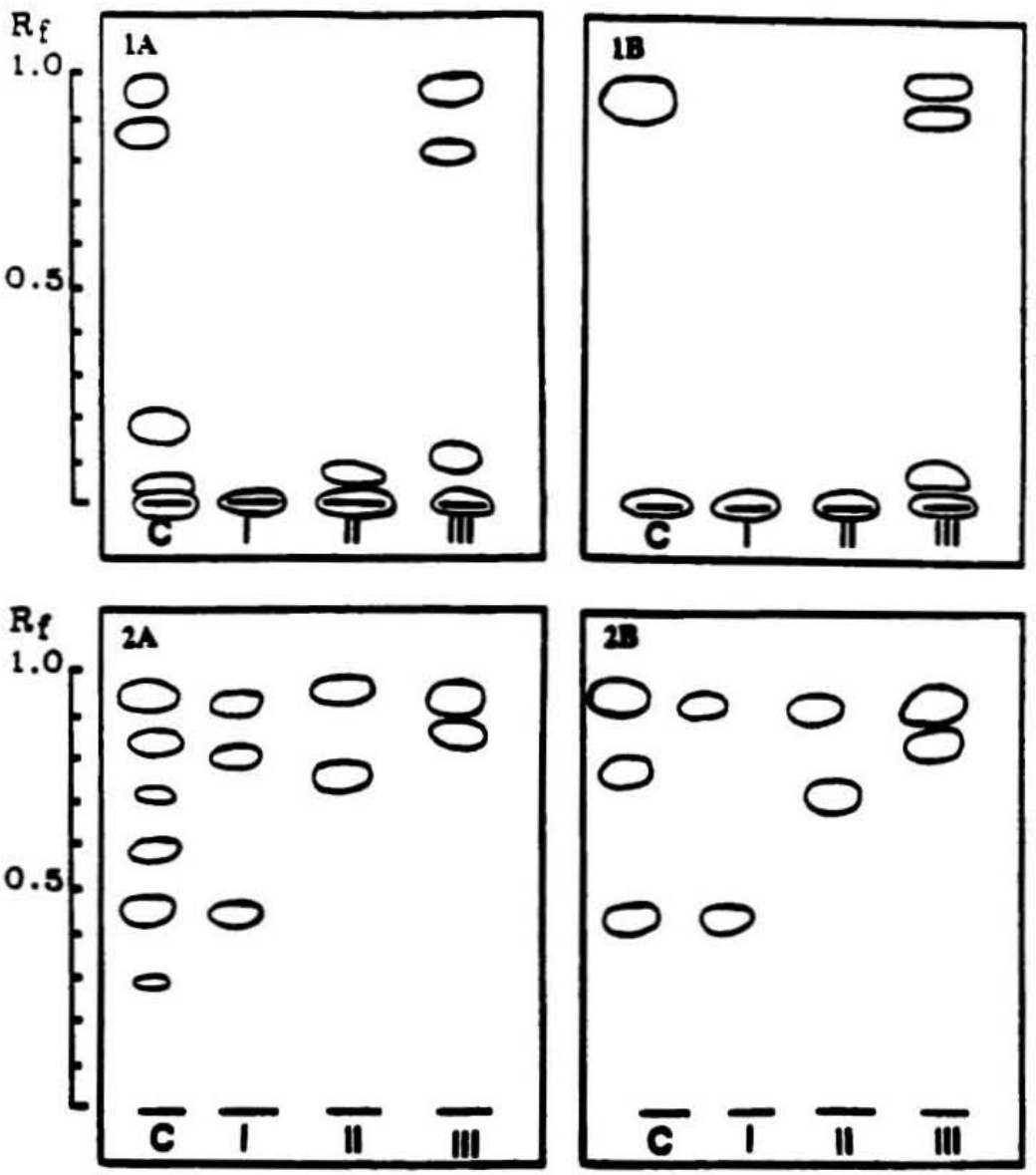
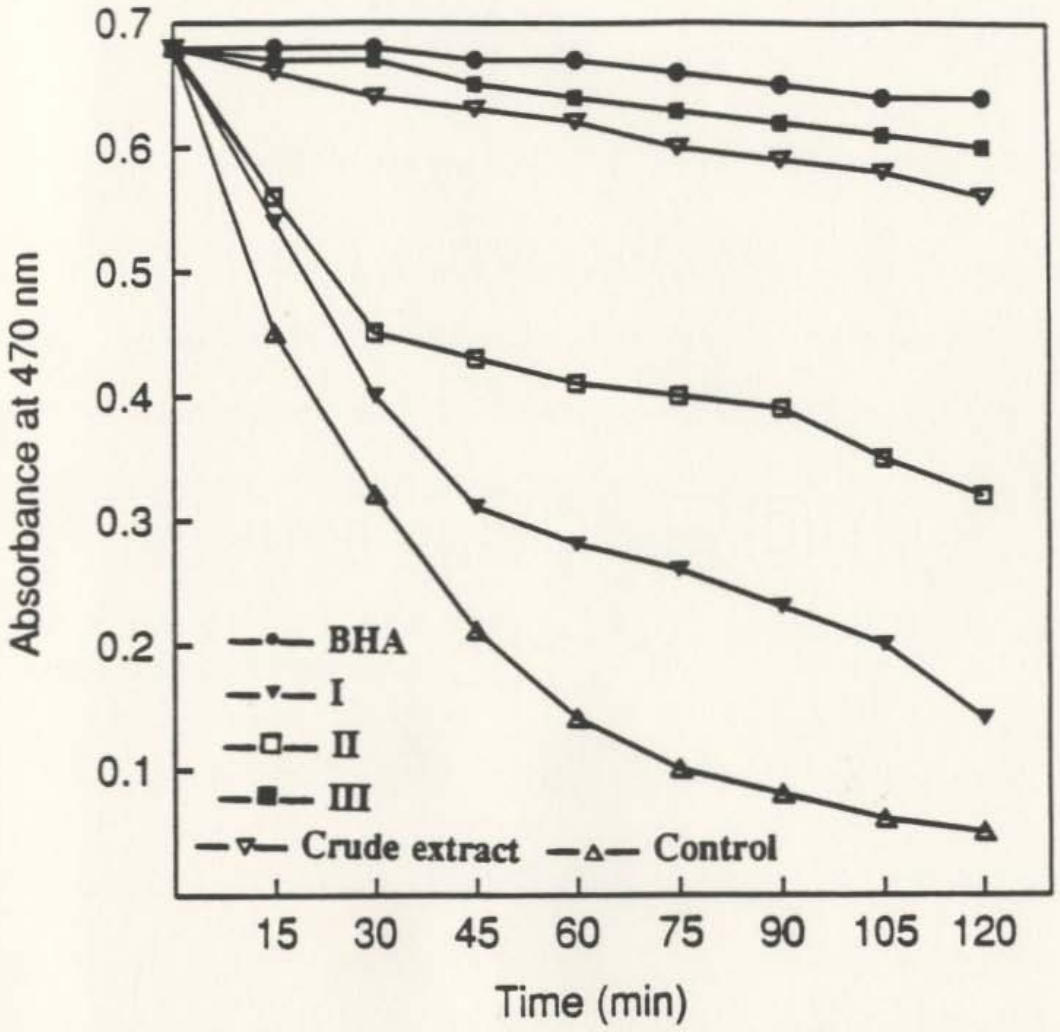

Rf
1.0
0.5
1A
C
1B
C
Rf
1.0
0.5
2A
C
I
II
III
2B
C
I
II
III

Figure 4.9 Inhibition of bleaching of β-carotene in a model β-carotene-linoleate system containing the crude extract or individual fractions of beach pea hulls extracts separated on a Sephadex LH-20 column.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbance at 470 nm
BHA
I
II
III
Crude extract
Control
15
30
45
60
75
90
105
120
Time (min)

phenolics, as sinapic acid equivalents, and 220.2 % condensed tannins, as catechin equivalents (Table 4.31). This indicates that the amount of phenolic compounds and their molecular structures play an important role in their antioxidative activity (Wanasundara *et al.*, 1996).

4.7.4 Detection of phenolic/tannin compound present in fractions of beach pea hull extract

Fraction III of the beach pea hull extract which contained the highest amount of phenolics, condensed tannins and possessed strong antioxidant activity, was further separated on a semi-preparative HPLC. Presence of (+) catechin and (-) epicatechin as main phenolic compounds in this fraction was detected (Figure 4.10).

4.8 Effect of different solvent extractions on phenolic compounds, tannins and sugars of beach pea seeds

4.8.1 Effect of different solvents on extraction capability of phenolics, tannins and sugars

The extraction capability of different solvents of phenolic compounds, tannins and sugars with time of extraction is presented in Figure 4.11 and Table 4.32, respectively. UV data after consecutive stages of extraction at 280 nm show that acetone-water (80:20, v/v) is most effective in extracting a maximum amount of phenolic compounds and tannins from beach pea. Acetone-water mixture extracted almost 1.5 times more phenolics and condensed tannins from beach pea seeds than methanol-water or ethanol-

Figure 4.10 Chromatograms of HPLC separated (+) catechin and (-) epicatechin from Sephadex LH-20 fractions (tube numbers 66-120, Figure 4.6).

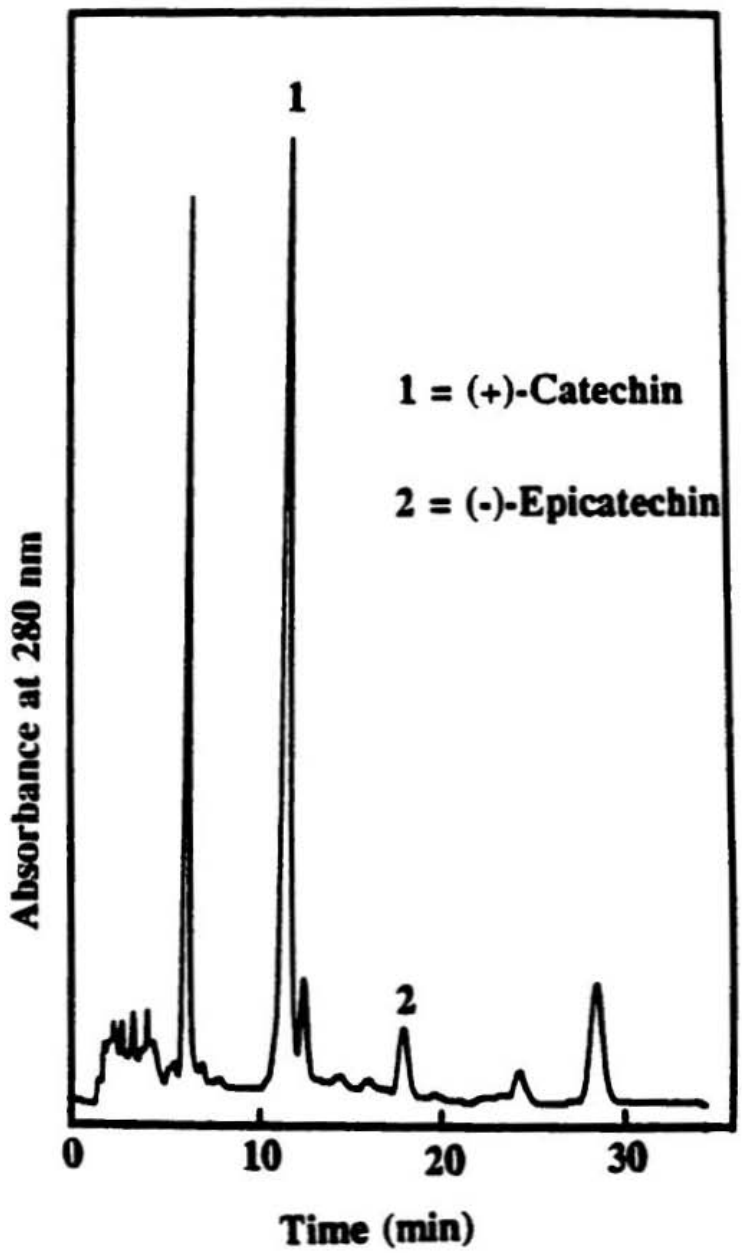

1
1 = (+)-Catechin
2 = (-)-Epicatechin
2
Absorbance at 280 nm
0
10
20
30
Time (min)

Figure 4.11 Comparison of extracting capability of the solvents used: extraction of compounds with UV absorbance at 280 nm; —△— methanol-water; —○— ethanol-water; —□— acetone-water extracts; all solvents at 80:20, (v/v) ratio.

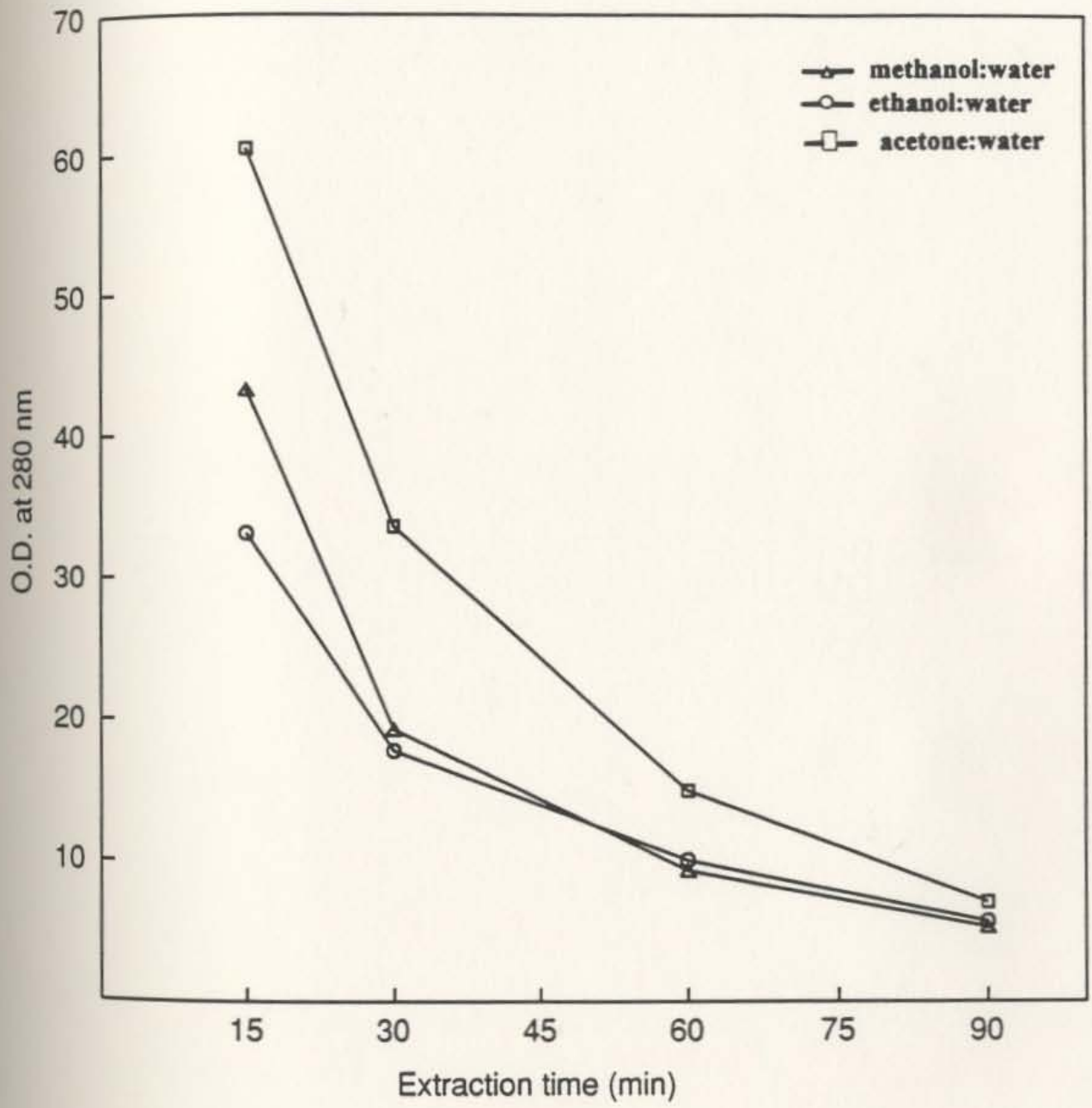

methanol:water
ethanol:water
acetone:water
O.D. at 280 nm
70
60
50
40
30
20
10
15
30
45
60
75
90
Extraction time (min)

Table 4.32 Comparison of extraction efficiency of methanol, ethanol and acetone on beach pea as percentage of total amounts of sugars, phenolics and tannins[1]

Extraction, solvent and time (min)	Soluble sugars	Phenolic compounds[2]	Condensed tannins[3]
Methanol			
15	57.65	32.00	31.98
30	23.86	26.90	27.08
60	10.54	23.32	24.43
90	7.95	17.78	16.51
Ethanol			
15	42.03	32.96	30.15
30	30.63	27.35	27.85
60	19.24	22.78	23.55
90	8.10	16.91	18.45
Acetone			
15	38.19	49.34	52.51
30	31.21	30.32	21.35
60	19.92	14.39	17.55
90	10.68	5.95	8.59

[1]Results are means of two determinations.

[2]As sinapic acid equivalents.

[3]As catechin equivalents.

water mixtures. However, methanol-water was most effective in extracting a maximum amount of sugars from beach pea seeds only at 15 min extraction. This might be due to simple sugars and oligosaccharides which dissolve more easily in methanol-water than in ethanol-water or acetone-water. Price and Spiro (1985) and Price and Spitzer (1993) reported that the highest extraction of phenolic compounds from plant material with methanol-water (80:20, v/v) was achieved during the first stage of extraction. Acetone-water (80:20, v/v) was most effective in extracting the phenolic compounds from lentil seeds (Amarowicz *et al.*, 1995), but this solvent system was less effective in the removal of sugars.

4.8.2 UV spectra of beach pea seed extracts

The beach pea seeds were extracted 4 times (I-IV) with methanol:water (A) ethanol:water (B) and acetone:water (C); their UV spectra are shown in Figure 4.12. Four extractions (I-IV) of each solvent combined with their UV spectra recorded between 240 and 400 nm are shown in Figure 4.13 (ethanol:water, 1; methanol:water, 2; acetone:water, 3). The UV spectra of ethanol-water extract showed a maximum at 292 nm. The first methanol-water extraction showed a UV absorption maximum at 284 while the next three extractions and combined extracts (I-IV) exhibited a maximum at 292 nm. When acetone-water was used for the extraction, a maximum was observed at 284 nm. These UV spectral data indicate that methanol-water and ethanol-water solvent systems extract the same types of compound, but in case of acetone-water different compounds

Figure 4.12 UV spectra of each extraction (I-IV); A, methanol-water (80:20, v/v); B, ethanol-water (80:20, v/v); C, acetone-water (80:20, v/v) from beach pea seed extracts.

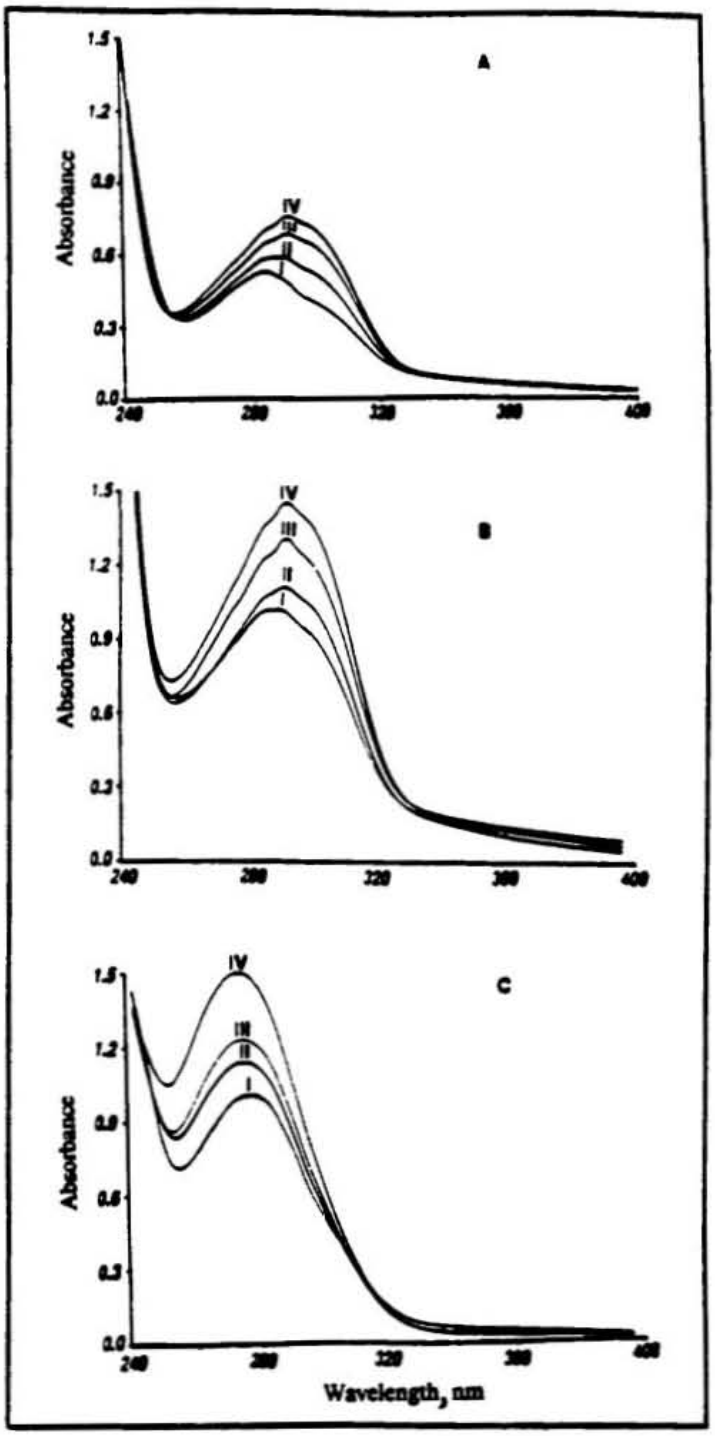
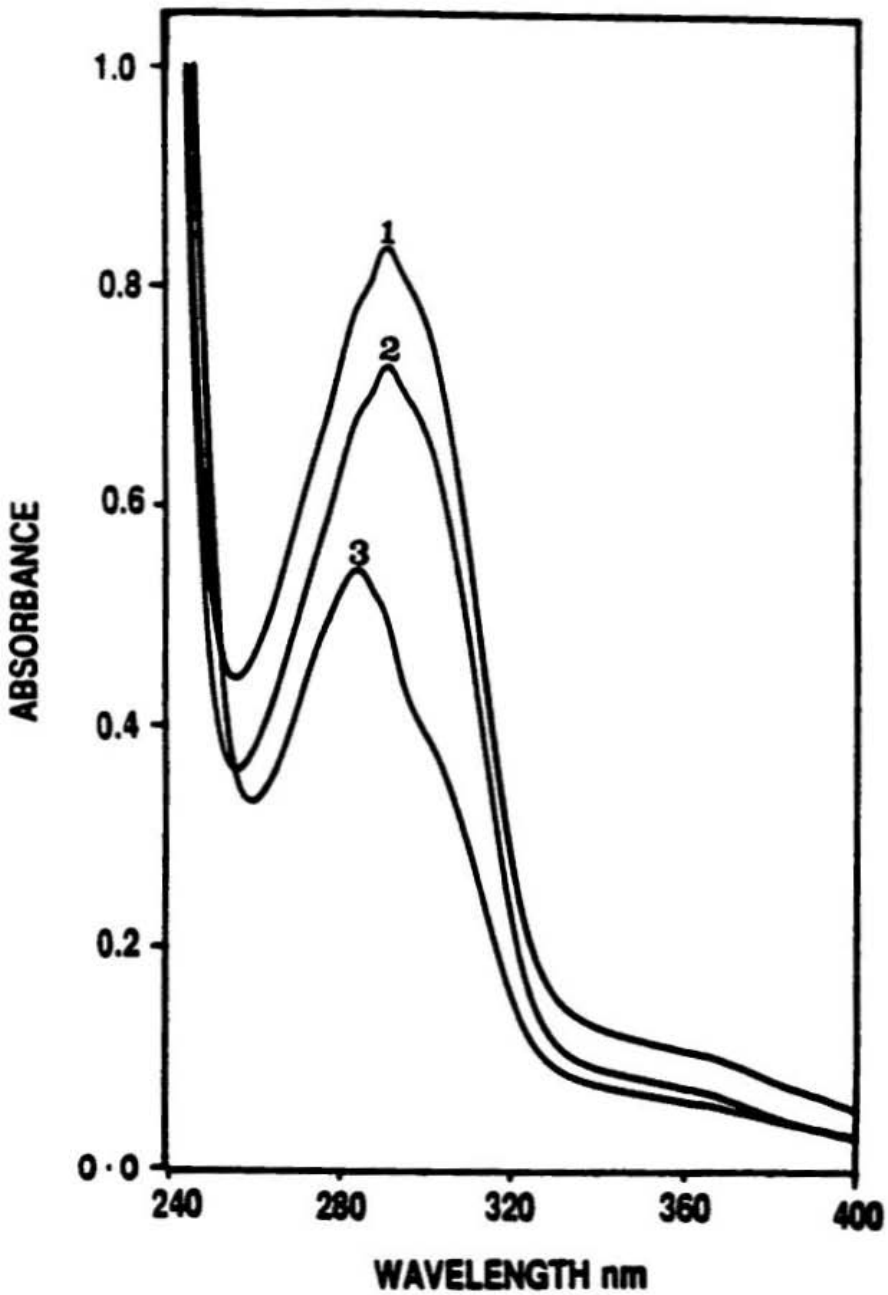

A
B
C
Absorbance
Wavelength, nm
I
II
III
IV

Figure 4.13 UV spectra of combined extracts (Extractions I-IV) of beach pea seed obtained using (1) ethanol-water (80:20, v/v); (2) methanol-water (80:20, v/v); and (3) acetone-water (80:20, v/v) solvents.


1.0
0.8
0.6
0.4
0.2
0·0
ABSORBANCE
1
2
3
240
280
320
360
400
WAVELENGTH nm

may be extracted from the seeds. Frejnagelt *et al.* (1994) reported that the absorption maxima of phenolic compounds obtained from the faba bean seed coats ranged from 264 to 280 nm. Amarowicz *et al.* (1995) used similar conditions for extraction of phenolic compounds and sugars from lentil and reported that UV spectrum of the acetone extract had a maximum at 274 nm and compounds in methanol and ethanol extracts had a maximum at 272 nm. Acetone-water (8:2, v/v), used for the extraction of phenolic compounds from everlasting pea, faba bean, and broad bean afforded absorption maximum in the range of 260 to 282 nm (Amarowicz *et al.*, 1996b).

4.8.3 Total extracts and the content of phenolics, condensed tannins and sugars extracted by different solvent systems

The amounts of extract recovered with methanol (80:20, v/v), ethanol (80:20, v/v) and acetone (80:20, v/v) are shown in Table 4.33. The amount of extract recovered by acetone as a solvent was significantly ($p<0.05$) higher than when methanol and ethanol solvent systems were used. Extraction of sugars was highest when methanol was employed and the content of phenolic compounds extracted from beach pea seeds by acetone was twice that recovered with methanol or ethanol. Extraction of condensed tannins with acetone-water was also ten time more effective than methanol or ethanol. However, the sugar content in methanol extract was higher than that in ethanol and acetone extract. These results indicate the importance of choice of solvent in quantification of different components of the extracts.

Table 4.33 The content of extract (%), sugars (%), phenolic compounds (mg/100g) and condensed tannins (mg/100g) in beach pea seeds using different solvent systems[1]

Extraction solvent	Extract	Sugars	Phenolic compounds[2]	Condensed tannins[3]
Methanol (80:20, v/v)	16.62±0.60^{ab}	5.03±0.35^{a}	619.64±0.31^{b}	748.93±2.29^{b}
Ethanol (80:20, v/v)	15.01±0.65^{b}	4.87±0.29^{a}	421.06±0.54^{c}	381.06±1.70^{c}
Acetone (80:20, v/v)	19.32±0.90^{a}	2.95±0.15^{b}	1283.87±3.90^{a}	7485.74±3.01^{a}
Difference significance	A=M, A>E, M=E	M=E>A	A>M>E	A>M>E

[1]Results are means of triplicate determinations, ± standard deviation. Means followed by different supercripts in each column are significantly ($p<0.05$) different from one another. M, Methanol; E, Ethanol; and A, Acetone.

[2]As sinapic acid equivalents.

[3]As catechin equivalents.

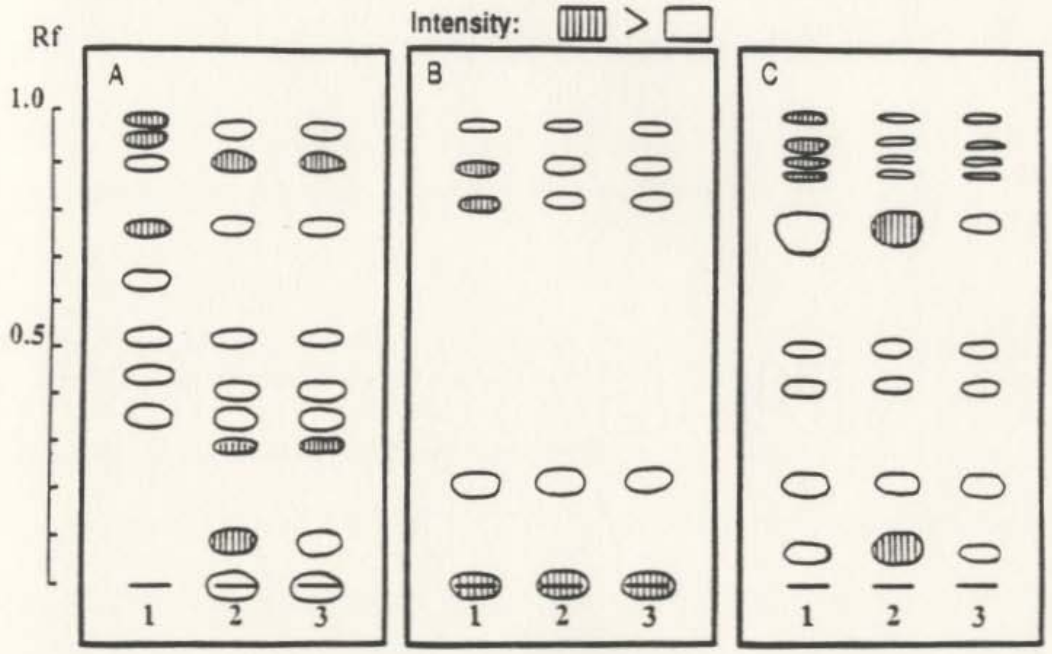
4.8.4 TLC separation of beach pea seed extracts

TLC plates, with beach pea seed extracts developed using different solvent systems and sprayed with different reagents are presented in Figure 4.14. Chromatograms of extracts developed with acetic acid-water-n-butanol (10:10:30, v/v/v), a high polar solvent system, showed three intense spots from ethanol extract close to the solvent front. Methanol extract also showed three intense spots, one very close to the origin and the other two at R_f values of 0.3 and 0.9. Acetone extract also showed two intense spots at R_f values of 0.3 and 0.9 (Figure 4.14A). Chromatograms developed with acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v), a non-polar solvent (Figure 4.14B), and sprayed with a ferric chloride solution showed only two intense spots for ethanol extract at R_f values of 0.8 and 0.9. The same extracts used for chromatogram development in water-methanol-chloroform (10:35:65, v/v/v), a polar solvent, (Figure 4.14C), and sprayed with 10% sulphuric acid and heated at 120 °C showed four very intense spots close to the solvent front for ethanol extract, two spots for the methanol extract at R_f values of 0.1 and 0.8, respectively, for the presence of sugars and no intense spots were observed for acetone extract. Ferric chloride reacts with phenolic compounds and gives blue or greenish coloured spots while sulphuric acid char the sugars at higher temperatures to yield black spots.

4.8.5 Separation of phenolic/tannin fraction of beach pea extract

The phenolics of beach pea extracted by acetone-water were further separated

Figure 4.14 TLC of beach pea seed extracts; developed using A: acetic acid-water-n-butanol (10:10:30, v/v/v); B: acetic acid-petroleum ether-diethyl ether (1:20:80, v/v/v); C: water-methanol-chloroform (10:35:65, v/v/v). Plates were sprayed with, A and B-ferric chloride solution and C-10% sulphuric acid and heated at 120 °C.
(1, ethanol-water; 2, methanol-water; 3, acetone-water extracts).


Intensity: >
Rf
1.0
0.5
A
B
C
1
2
3
1
2
3
1
2
3

using Sephadex LH-20 column chromatography. UV absorbances of extracted phenolics at 280 nm and condensed tannins at 500 nm, following colour development, are presented in Figure 4.15. Major phenolics were found in tube numbers 10-23 and condensed tannins in tube numbers 23-30. The condensed tannins fraction, separated on Sephadex LH-20, was further fractionated by semi-preparative HPLC. The major fraction (tube numbers 23-30) of condensed tannins of beach pea seed extract contained (+) catechin and (-) epicatechin as its major compounds (Figure 4.16). Similar separation of (+) catechin and (-) epicatechin were reported for pea and bean extract (Tsuda *et al.*, 1993).

4.9 Effects of methanol-ammonia-water/hexane extraction on the nutrients and antinutrients of beach pea and grass pea

4.9.1 Effect on β-N-oxalylamino-L-alanine (BOAA)

In preliminary experiments methanol, methanol/hexane, methanol-water/hexane, methanol-ammonia/hexane and methanol-ammonia-water/hexane solvents were used for extraction of beach pea and grass pea seed meals. The solvent extraction system consisting of methanol-ammonia-water/hexane was superior to other solvent systems for reducing the content of β-N-oxalylamino-L-alanine, phenolics as well as condensed tannins and non-protein nitrogen from beach pea and grass peas. This solvent system also performed very well in enrichment of protein in processed meals of beach pea and grass peas. Therefore, methanol-ammonia-water/hexane solvent system was used in subsequent solvent extraction of beach pea and grass peas.

Figure 4.15 Separation of phenolic fractions of beach pea seed extracts by Sephadex LH-20 column chromatography: UV absorbance of phenolics (280 nm) and condensed tannins (500 nm) following colour development.

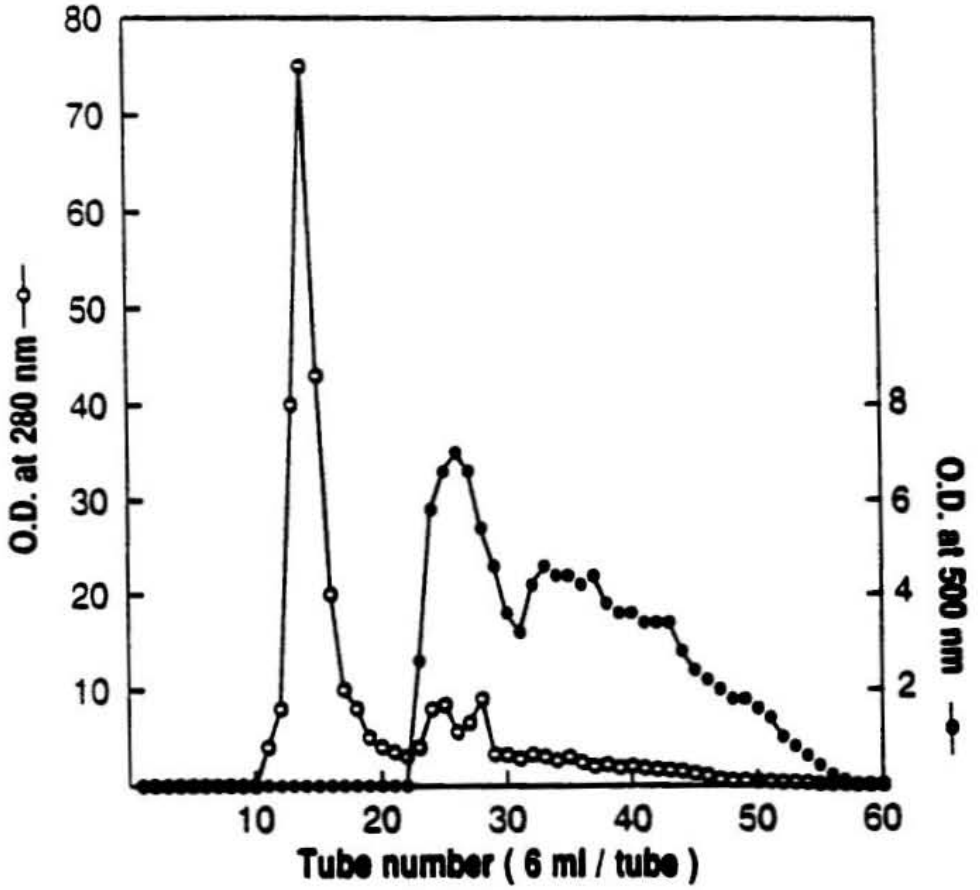
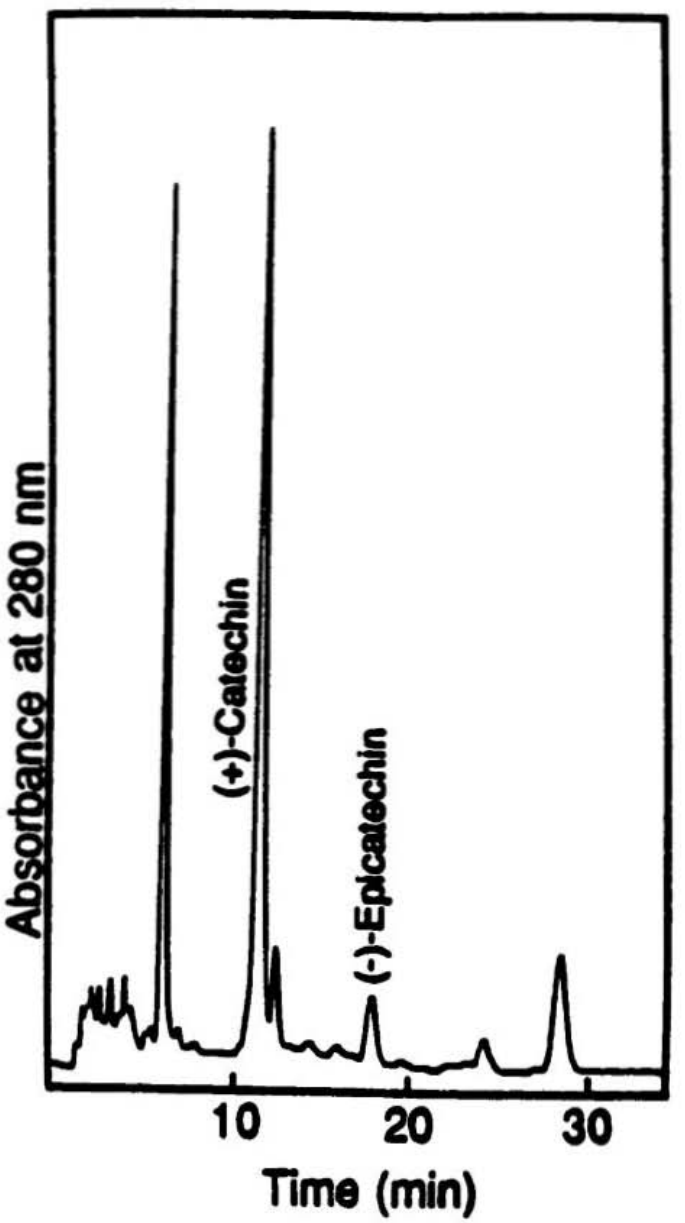

80
70
60
50
40
30
20
10
O.D. at 280 nm
8
6
4
2
O.D. at 505 nm
10
20
30
40
50
60
Tube number (6 ml / tube)

Figure 4.16 Chromatograms of the analytically separated pure (+) catechin and (-) epicatechin obtained from Sephadex LH-20 fractions (tube numbers 23-30, Figure 4.15) followed by semi-preparative HPLC separation.


Absorbance at 280 nm
(+)-Catechin
(-)-Epicatechin
10
20
30
Time (min)

The mass balance and BOAA in laboratory prepared beach pea and grass pea meals after I, II, and III extractions with methanol-ammonia-water are summarized in Tables 4.34 and 4.35, respectively. The amount of meal recovered after three extractions varied from 91.0 to 94.3% in beach pea , 91.1 to 94.4% in the Canadian grass pea and 91.9 to 94.2% in the Indian grass pea, on a dry weight basis. The recovery of meal decreased with increase in the number of extractions for all peas examined. Losses during extraction varied from 1.07 to 2.98% in beach pea, 2.48 to 3.23% in the Canadian grass pea and 1.95 to 2.65% in the Indian grass pea. These losses may be considered reasonable because it is difficult to transfer the slurries or solvent mixtures from one unit operation to another in sequential extractions in the laboratory scale process. The recovery of solids (gums) was significantly ($p<0.05$) higher as a result of first extraction. As the number of extractions increased the recovery of solids in polar phase decreased. Shahidi *et al.* (1988) reported that solids (gums) recovered in the polar phase may include phenolic compounds, soluble sugars, phospholipids as well as some breakdown products of polar compounds present. Beach pea contained 4.02 mg/100g BOAA, while the Canadian grass pea and the Indian grass pea had 86.20, 434.65 mg BOAA/100g sample, respectively. Extraction of beach pea meal with methanol-ammonia-water/hexane (first extraction) showed complete removal of BOAA. Methanol containing 5% (v/v) water and 10% (w/w) ammonia was very effective in reducing the content of BOAA from the Canadian (79%) and the Indian grass pea, (74%) following three extractions. Diosady *et al.* (1985) reported that methanol-ammonia containing 15% water removed 86% of

Table 4.34 Effect of methanol-ammonia-water extraction on the mass balance and BOAA content of beach pea[1]

Treatment/ Constituent, %	Recovery	Loss	Lipids	Gums/Solids	BOAA (mg/100g)
Control	100[a]	NP	1.11±0.14[a]	NP	4.02±0.01
I	91.04±1.02[d]	1.07±0.10[b]	1.14±0.18[a]	6.95±0.80[a]	ND
II	93.67±0.95[c]	2.35±0.13[a]	NA	3.98±0.31[b]	ND
III	94.25±0.83[bc]	2.98±0.65[a]	NA	2.77±0.12[c]	ND

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another. NA, Not analyzed; ND, Not detected; NP, Not applicable.

I, II, and III these are 1st, 2nd and 3rd methanol-ammonia-water extractions.

Table 4.35 Effect of methanol-ammonia-water extraction on the mass balance and BOAA content of grass pea[1]

Treatment/Constituent, %	Recovery	Loss	Lipid	Gums/Solids	BOAA (mg/100g)
Grass pea[2]					
Control	100[a]	NP	1.34±0.15[a]	NP	86.20±0.23[a]
I	91.12±2.10[d]	2.48±0.65[a]	1.12±0.13[a]	5.28±0.40[a]	32.43±0.12[b]
II	94.37±1.21[bcd]	2.53±0.32[a]	NA	3.10±0.15[bc]	23.12±0.09[c]
III	94.35±1.30[cd]	3.23±0.41[a]	NA	2.42±0.21[c]	17.69±0.05[d]
Grass pea[3]					
Control	100[a]	NP	1.20±.02[a]	NP	434.65±0.68[a]
I	91.94±1.62[d]	1.95±0.28[c]	0.98±0.05[b]	5.13±0.36[a]	318.03±0.49[b]
II	94.74±1.15[bcd]	2.00±0.13[b]	NA	3.26±0.25[bc]	162.69±0.32[c]
III	94.18±1.36[cd]	2.65±0.15[a]	NA	3.17±0.81[c]	112.01±0.12[d]

[1]Results are means of triplicate determinations, ± Standard deviation. Means followed by different superscripts in each column for each variety are significantly ($p<0.05$) different from one another. NA, Not analyzed; NP, Not applicable. I, II, and III these are 1st, 2nd and 3rd methanol-ammonia-water extractions.

[2]Canadian grass pea.

[3]Indian grass pea.

glucosinolates originally present in rapeseed meal. A two-stage process, using methanol-ammonia-water, removed 98% of glucosinolates originally present in rapeseed meal (Shahidi *et al.*, 1988). Wanasundara *et al.* (1993) treated flaxseed meal with methanol-ammonia-water /hexane and found that this solvent system removed 90% of its cyanogenic glucosides. Deshpande and Campbell (1992a) used sodium chloride, sodium acetate, sodium carbonate, potassium chloride, potassium sulphate and sodium hydroxide as a solvent for preparation of grass pea protein isolates and reported that the level of BOAA decreased by approximately 50 - 85% in protein isolates, depending on the solvent extraction system used.

4.9.2 Effect on oligosaccharides of beach pea and grass pea

Results on the removal of oligosaccharides from beach pea, Canadian and Indian grass pea meals are presented in Table 4.36. Beach pea, in general, contained more sucrose and its α-galactosides (sucrose, 0.67%; stachyose, 1.37%; and verbascose, 0.73%) than those of Indian grass pea (0.30, 0.78 and 0.52%, respectively) and more galactosides than Canadian grass pea (0.67, 0.86 and 0.48%, respectively). Retention times of beach pea sucrose, raffinose, stachyose and verbascose were 3.8, 4.5, 5.3 and 6.3 min, respectively (Figure 4.17). Naczk *et al.* (1992a) reported that field pea contained 0.80% raffinose, 1.28% stachyose and 2.35% verbascose, while Abdel-Gawad (1993) showed that cowpea had 1.51% sucrose, 0.77% raffinose, 3.00% stachyose and 0.30% verbascose. The contents of oligosaccharides in samples examined in this study are within the range

Table 4.36 Effect of methanol-ammonia-water extraction on oligosaccharide content (%) of different peas[1]

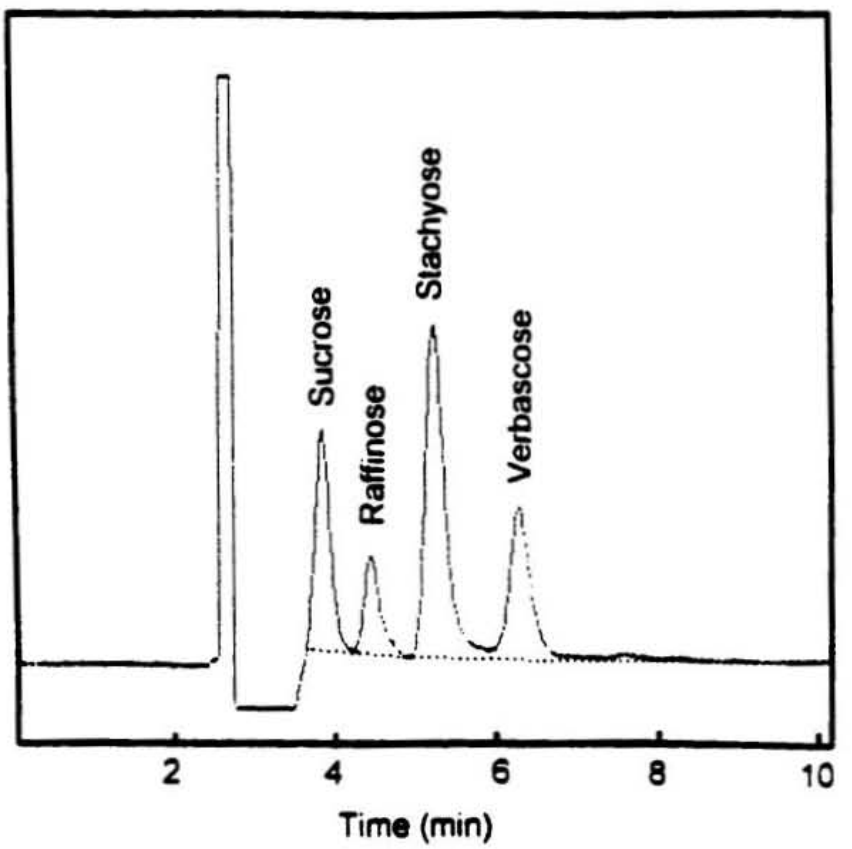
Pea	Sucrose	Raffinose	Stachyose	Verbascose
Beach pea				
Control	0.67	0.33	1.37	0.73
M-A-W/H Treated	0.25	0.10	0.48	0.69
Grass pea[2]				
Control	0.67	0.57	0.86	0.48
M-A-W/H Treated	0.41	0.53	0.81	0.42
Grass pea[3]				
Control	0.30	0.58	0.78	0.52
M-A-W/H Treated	0.11	0.36	0.58	0.46

[1]Results are means of duplicate determinations, on a dry weight basis. M-A-W/H, Methanol-ammonia-water/hexane extraction.

[2]Canadian grass pea.

[3]Indian grass pea.

Figure 4.17 Chromatogram of the analytically separated sucrose, raffinose, stacyose and verbascose from beach pea meal by semi-preparative HPLC.


Sucrose
Raffinose
Stachyose
Verbascose
2
4
6
8
10
Time (min)

of reported values for other legumes (Phillips and Abbey, 1989; Revilleza *et al.*, 1990; Su and Chang, 1995a). Several investigators have demonstrated that raffinose, stachyose and verbascose are principle causes of flatulence (production of carbon dioxide, hydrogen, and methane) in human and animals (Calloway *et al.*, 1971; Fleming, 1981; Jood *et al.*, 1985; Olson *et al.*, 1994). Therefore, it would be desirable to reduce the content of these sugars in legumes to improve their acceptability as an inexpensive protein source. Different processing methods have been evaluated to reduce the content of these undesirable carbohydrates for improving the quality of many legumes (Jood *et al.*, 1986). In the present study, the level of sucrose and its oligosaccharides was reduced 51% for beach pea, 16% for Canadian grass pea and 31% for Indian grass pea by by methanol-ammonia-water treatment. Reduction in stachyose was very high in beach pea compared to the other two pea cultivars. Upadhyay and Garcia (1988) reported that soaking and cooking of cowpea reduced oligosaccharide levels from 47 - 77%. Cowpea seeds soaked in water for 12 h showed a 27.5% reduction in oligosaccharides (Abdel-Gawad, 1993). The methanol-ammonia-water/hexane extraction system removed 20% of total flatulence causing sugars from field pea and 27% from mung bean (Naczk *et al.*, 1992a). Comparison of these results with those reported for oilseeds indicate that methanol-ammonia-water/hexane treatment was more effective in the removal of flatulence causing sugars from peas than from oilseeds (Shahidi *et al.*, 1990). The structural organization and intra-molecular interaction of the seed components as well as polarity of solvent extraction systems are important factors affecting the removal of oligosaccharides from

legume seeds.

4.9.3 Effect on crude protein, non-protein nitrogen (NPN), phenolics and condensed tannins of beach pea and grass pea

The content of crude protein, NPN, phenolics and condensed tannins in the extracted meals of beach pea, Canadian and Indian grass peas is presented in Tables 4.37 and 4.38. The crude protein content of the meals increased from 29.16 to 37.13% in beach pea, from 23.64 to 31.37% in the Canadian grass pea and from 21.33 to 32.52% in the Indian grass pea after three extractions with methanol containing 5% water (v/v) and 10% (w/w) ammonia. The process reduced non-protein nitrogen from 23.29 to 12.10% in beach pea, 14.99 - 9.06% in the Canadian grass pea and 23.14 - 12.67% in the Indian grass pea. The extraction system performed well in reducing phenolic compounds and condensed tannins by 50 and 68% in beach pea, respectively. The corresponding reduction in Canadian grass pea were 93 and 100% while 45 and 94% phenolics and condensed tannins were removed from the Indian grass pea. Naczk *et al.* (1985) and Shahidi *et al.* (1988) reported that canola meal extracted with methanol-ammonia-water/hexane contained 25% more crude protein than its untreated counterpart. These authors also reported that the non-protein nitrogen content of extracted meal was reduced by about 50%. The decrease in the non-protein nitrogen content was due to their partial dissolution in the extraction medium. Wanasundara and Shahidi (1994a) reported that crude protein content of flaxseed increased from 5.3 to 13%, depending on the solvent

Table 4.37 Effect of methanol-ammonia-water extraction on protein, non-protein nitrogen, phenolics and tannin contents (%) of beach pea[1]

Treatment	Protein	NPN (as a % total N)	Phenolics	Condensed tannins
Control	29.16±0.15^{d}	23.29±0.55^{a}	1.19±0.01^{a}	11.58±0.19^{a}
I	34.37±0.30^{c}	15.51±1.34^{bc}	0.90±0.01^{b}	5.49±0.29^{b}
II	35.52±0.63^{b}	13.67±0.33^{cd}	0.76±0.03^{c}	4.52±0.20^{c}
III	37.13±0.51^{a}	12.10±0.21^{d}	0.59±0.02^{d}	3.65±0.12^{d}

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another.

I, II, and III these are 1st, 2nd and 3rd methanol-ammonia-water extractions.

Table 4.38 Effect of methanol-ammonia-water extraction on protein, non-protein nitrogen, phenolics and tannin contents of grass peas[1]

Treatment	Grass pea[2]				Grass pea[3]			
	Protein (%)	NPN (as a % total N)	Phenolics mg/100g	Condensed tannins mg/100g	Protein (%)	NPN (as a % total N)	Phenolics mg/100g	Condensed tannins mg/100g
Control	23.64±0.07^{d}	14.99±0.59^{a}	248.95±1.22^{a}	109.11±0.91	21.33±1.21^{c}	23.14±0.37^{a}	200.94±1.45^{a}	1541.1±15.0^{a}
I	27.23±0.13^{c}	12.18±1.15^{bc}	44.39±0.14^{b}	ND	28.68±0.62^{b}	16.74±1.07^{bc}	166.52±2.30^{b}	211.83±1.32^{b}
II	29.38±0.29^{b}	10.67±0.42^{cd}	22.13±0.35^{c}	ND	31.29±0.53^{a}	14.97±0.90^{c}	146.32±0.90^{c}	151.17±0.83^{c}
III	31.37±0.28^{a}	9.06±0.23^{d}	16.67±0.26^{d}	ND	32.52±0.36^{a}	12.67±0.55^{d}	110.13±1.78^{d}	93.67±0.35^{d}

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each column for each variety are significantly ($p<0.05$) different from one another. ND, Not detected. I, II, and III these are 1st, 2nd and 3rd methanol-ammonia-water extractions.

[2]Canadian grass pea.

[3]Indian grass pea.

employed. These authors found that methanol-ammonia-water was most effective in reducing the content of phenolic compounds, condensed tannins and soluble sugars of flaxseed meal. Shahidi *et al.* (1988) extracted canola meal with methanol-ammonia-water and found that quantity of ammonia remaining in the meal was very low (1.5 mg/g of meal) and had little effect on the nitrogen content of the preparation. The increase of crude protein content was mainly due to the dissolution of polysaccharides, phospholipids and other non-protein nitrogen compounds in the methanol-ammonia-water phase. Wanasundara and Shahidi (1994a) showed that 48% of total phenolic acids may be removed from flaxseed by employing methanol in combination of ammonia and water as a solvent system. Methanol-ammonia-water/hexane system also reduced the condensed tannin content of flaxseed meals by 74% (Wanasundara and Shahidi, 1994a). Shahidi and Naczk (1989) reported a reduction of condensed tannins in canola meals from 67 to 96% upon methanol-ammonia-water/hexane extraction.

An effective chemical treatment employing ammonia was considered important for the removal of tannins from sorghum seeds (Price *et al.*, 1979), sal seed meal (Gandhi *et al.*, 1975) and cassava leaves (Padamaja, 1989). Ammoniation mostly extracts the polar compounds (phenolic compounds and condensed tannins) into a polar solvent. Phenolic compounds in solution may form phenolic anions which are more soluble in the extraction medium. Sosulski (1979a) reported that the removal of phenolic compounds and condensed tannins from legumes and oilseeds is important because they may prevent the formation of dark colour, off-flavour and binding of minerals; thus allowing better

utilization of legume and oilseed meals in different food formulations as a cheap source of protein.

4.9.4 Effect on the total and free amino acids content of beach pea and grass pea

The amino acid composition of beach pea, Canadian and Indian grass pea meals, before and after extraction with methanol-ammonia-water three times are presented in Tables 4.39 and 4.40. The content of amino acids of the meals decreased slightly as the number of extractions increased. The reduction in amino acid content was lower in beach pea than the Canadian and the Indian grass pea after three extractions. This lowering of the amino acid values might be due to their alteration during processing. Nearly 50% of tryptophan was lost after three extractions of beach pea while it was not detected after the second extraction in both grass pea types. Amino acid score as well as biological value of methanol-ammonia-water extracted meals of beach pea and grass peas decreased as the number of extractions increased over that of the control. The predicted protein efficiency ratios (PER) were also reduced due to extraction of pea samples (Table 4.41). Shahidi *et al.* (1992) have shown that when canola and rapeseed meals were treated with methanol-ammonia-water/hexane their amino acid composition was slightly altered compared to that of the untreated meals; a similar result was obtained by Wanasundara and Shahidi (1994a) for flaxseed.

The effect of methanol-ammonia-water/hexane extraction on free amino acid content of beach pea, Canadian and Indian grass peas is presented in Tables 4.42 and

Table 4.39 Effect of methanol-ammonia-water extraction on total amino acid composition of beach pea meal (g/16g N)[1]

Amino acid	Treatment			
	Control	I	II	III
Isoleucine	4.11±0.06^{a}	4.03±0.03^{a}	3.85±0.02^{bc}	3.76±0.02^{c}
Leucine	7.67±0.14^{a}	7.51±0.15^{a}	7.37±0.08^{ab}	7.31±0.11^{b}
Lysine	7.67±0.13^{a}	7.48±0.12^{ab}	7.43±0.11^{ab}	7.30±0.13^{b}
Cysteine	1.63±0.03^{a}	1.50±0.02^{b}	1.44±0.03^{cd}	1.39±0.01^{d}
Methionine	1.08±0.02^{a}	1.06±0.01^{a}	1.01±0.01^{b}	0.83±0.01^{c}
Total sulphur amino acids	2.71	2.56	2.45	2.22
Tyrosine	3.29±0.05^{a}	3.20±0.03^{bc}	3.13±0.01^{c}	3.05±0.01^{d}
Phenylalanine	4.73±0.10^{a}	4.60±0.06^{ab}	4.48±0.05^{bc}	4.41±0.03^{c}
Total aromatic amino acids	8.02	7.80	7.61	7.46
Threonine	4.29±0.08^{a}	4.25±0.10^{a}	4.16±0.12^{a}	4.11±0.05^{a}
Tryptophan	0.25±0.01^{a}	0.20±0.01^{b}	0.16±0.01^{c}	0.10±0.00^{d}
Valine	4.75±0.07^{a}	4.63±0.03^{b}	4.52±0.02^{cd}	4.46±0.03^{d}
Histidine	2.63±0.03^{bcd}	2.87±0.07^{a}	2.60±0.05^{cd}	2.56±0.04^{d}
Total essential amino acids	42.10	41.33	40.15	39.28
Arginine	7.93±0.19^{a}	7.24±0.13^{bcd}	7.17±0.10^{cd}	7.14±0.11^{d}
Aspartic acid + Asparagine	13.12±0.69^{a}	12.39±0.12^{a}	12.35±0.14^{a}	12.31±0.10^{a}
Glutamic acid + Glutamine	17.41±0.26^{a}	16.37±0.15^{bcd}	16.31±0.11^{cd}	16.28±0.12^{d}
Serine	5.04±0.09^{a}	4.90±0.10^{abc}	4.82±0.05^{bc}	4.78±0.03^{c}
Proline	4.20±0.12^{a}	4.13±0.11^{a}	4.11±0.03^{a}	4.08±0.02^{a}
Glycine	4.24±0.10^{a}	4.19±0.03^{a}	4.17±0.01^{a}	4.13±0.01^{a}
Alanine	4.34±0.11^{a}	4.21±0.12^{a}	4.16±0.11^{a}	4.14±0.03^{a}
Total non-essential amino acids	56.28	53.43	53.09	52.86
E/T, %	42.79	43.62	43.06	42.63
Amino acid score	109.64	106.83	104.31	102.00
BV	61.18	58.26	55.54	46.91

[1]Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another.

I, II, and III these are 1st, 2nd and 3rd methanol-ammonia-water extractions.

Table 4.40 Effect of methanol-ammonia-water extraction on total amino acid composition of grass peas (g/16g N)[1]

Amino acid/ Treatment	Grass pea[2]				Grass pea[3]			
	Control	I	II	III	Control	I	II	III
Isoleucine	4.75±0.08^{a}	4.52±0.10^{bcd}	4.41±0.06^{cd}	4.35±0.04^{d}	5.13±0.09^{a}	4.78±0.05^{b}	4.52±0.03^{cd}	4.39±0.02^{d}
Leucine	7.78±0.56^{a}	7.45±0.31^{a}	7.26±0.15^{a}	7.14±0.12^{a}	8.60±0.10^{a}	8.02±0.08^{bcd}	7.88±0.10^{cd}	7.82±0.11^{d}
Lysine	7.56±0.50^{a}	7.18±0.23^{a}	6.98±0.20^{a}	6.83±0.15^{a}	7.85±0.18^{a}	7.32±0.20^{bcd}	7.26±0.21^{cd}	7.11±0.15^{d}
Cysteine	0.66±0.10^{a}	0.65±0.05^{a}	0.61±0.02^{a}	0.58±0.01^{a}	0.54±0.01^{a}	0.51±0.02^{b}	0.46±0.01^{cd}	0.44±0.01^{d}
Methionine	0.40±0.01^{a}	0.38±0.02^{a}	0.35±0.01^{b}	0.31±0.01^{c}	0.37±0.01^{a}	0.36±0.01^{a}	0.33±0.01^{b}	0.29±0.01^{c}
Total sulphur amino acids	1.06	1.03	0.96	0.89	0.91	0.87	0.79	0.73
Tyrosine	3.68±0.12^{a}	3.34±0.03^{bc}	3.28±0.02^{cd}	3.16±0.03^{d}	3.69±0.06^{a}	3.10±0.03^{bc}	3.02±0.02^{cd}	2.94±0.02^{d}
Phenylalanine	4.95±0.14^{a}	4.73±0.10^{a}	4.59±0.11^{a}	4.48±0.04^{a}	5.20±0.60^{a}	4.84±0.10^{a}	4.73±0.04^{a}	4.67±0.03^{a}
Total aromatic amino acids	8.63	8.07	7.87	7.64	8.89	7.94	7.75	7.61
Threonine	4.24±0.11^{a}	4.04±0.02^{bc}	3.88±0.03^{a}	3.77±0.10^{d}	4.16±0.12^{a}	3.79±0.11^{bcd}	3.72±0.12^{d}	3.73±0.03^{cd}
Tryptophan	0.06±0.01^{a}	0.02±0.00^{a}	ND	ND	0.07±0.01^{a}	0.02±0.01^{b}	ND	ND
Valine	5.30±0.16^{a}	5.14±0.12^{a}	5.06±0.13^{a}	5.02±0.10^{a}	5.47±0.09^{a}	5.16±0.12^{bcd}	5.01±0.10^{cd}	4.98±0.12^{d}
Histidine	2.84±0.09^{a}	2.74±0.10^{ab}	2.68±0.06^{ab}	2.64±0.05^{b}	2.74±0.30^{a}	2.57±0.02^{a}	2.56±0.04^{a}	2.53±0.02^{a}
Total essential amino acids	42.22	40.19	39.10	38.28	43.82	40.47	39.49	38.90
Arginine	9.00±0.22^{a}	8.19±0.09^{bcd}	8.14±0.11^{cd}	8.12±0.08^{d}	9.78±0.21^{a}	8.45±0.13^{bcd}	8.38±0.15^{cd}	8.35±0.05^{d}
Aspartic acid + Asparagine	13.22±0.60^{a}	13.16±0.20^{a}	13.14±0.13^{a}	13.12±0.38^{a}	13.47±0.40^{a}	13.33±0.21^{a}	13.29±0.18^{a}	13.21±0.08^{a}
Glutamic acid + Glutamine	17.32±1.06^{a}	17.11±0.38^{a}	17.06±0.23^{a}	17.01±0.15^{a}	18.38±0.64^{a}	17.94±0.03^{a}	17.87±0.10^{a}	17.82±0.04^{a}
Serine	5.33±0.41^{a}	5.06±0.13^{a}	5.00±0.11^{a}	4.94±0.12^{a}	5.78±0.21^{a}	5.32±0.06^{bcd}	5.29±0.11^{cd}	5.23±0.13^{d}
Proline	4.47±0.06^{a}	4.26±0.02^{b}	4.08±0.04^{cd}	4.02±0.02^{d}	1.68±0.01^{a}	1.61±0.03^{abc}	1.57±0.05^{bc}	1.56±0.02^{c}
Glycine	4.55±0.56^{a}	4.39±0.10^{a}	4.32±0.10^{a}	4.26±0.08^{a}	4.48±0.35^{a}	4.20±0.13^{a}	4.15±0.30^{a}	4.11±0.04^{a}
Alanine	1.90±0.06^{a}	1.92±0.12^{a}	1.88±0.03^{a}	1.85±0.05^{a}	2.08±0.04^{a}	2.03±0.04^{abc}	2.00±0.01^{bc}	1.98±0.01^{c}
Total non-essential amino acids	55.79	54.09	53.62	53.32	55.65	52.88	52.55	52.26
E/T, %	43.08	42.65	42.17	41.79	44.05	43.35	42.91	42.67
Amino acid score	109.39	104.11	101.17	99.00	114.11	105.28	102.58	101.03
BV	21.24	17.64	NC	NC	15.56	12.29	NC	NC

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row for each variety are significantly ($p<0.05$) different from one another. [2]Canadian grass pea, [3]Indian grass pea. ND, Not detected; NC, Not calculated.

Table 4.41 The predicted PER values of methanol-ammonia-water treated beach pea and grass pea meals

Treatment	Predicted PER values using equation[1]		
	1	2	3
Beach pea			
Control	2.62	2.67	2.09
I	2.55	2.61	2.09
II	2.48	2.55	1.97
III	2.46	2.53	1.91
Grass pea[2]			
Control	2.65	2.68	1.55
I	2.51	2.56	1.57
II	2.43	2.48	1.47
III	2.39	2.44	1.46
Grass pea[3]			
Control	3.16	3.00	2.15
I	2.90	2.85	2.21
II	2.84	2.79	2.16
III	2.81	2.77	2.17

[1]Alsmeyer *et al.* (1974). I, II and III these are 1st, 2nd, and 3rd methanol-ammonia-water extractions.

[2]Canadian grass pea.

[3]Indian grass pea.

Table 4.42 Effect of methanol-ammonia-water extraction on free amino acid composition of beach pea meal (mg/100g)[1]

Free amino acid	Treatment			
	Control	I	II	III
Alanine	20.26±0.32^{a}	10.76±0.13^{b}	6.35±0.08^{c}	3.08±0.11^{d}
Arginine	91.93±1.24^{a}	51.63±1.08^{b}	30.19±1.20^{c}	10.36±0.20^{d}
Asparagine	124.42±3.27^{a}	82.33±1.30^{b}	60.59±0.38^{c}	32.78±0.34^{d}
Aspartic acid	19.78±0.33^{a}	10.66±1.11^{b}	7.30±0.04^{cd}	6.00±0.05^{d}
Cysteine	15.53±0.95^{a}	9.47±0.14^{b}	5.93±0.20^{c}	3.12±0.06^{d}
Glutamic acid	115.24±2.32^{a}	96.04±1.91^{b}	72.04±0.53^{c}	56.82±1.04^{d}
Glutamine	2.07±0.73^{a}	1.73±0.08^{a}	1.34±0.11^{ab}	0.65±0.13^{b}
Glycine	20.88±0.41^{a}	11.28±0.22^{b}	7.63±0.13^{c}	2.48±0.16^{d}
Histidine	11.56±1.03^{a}	6.25±0.08^{b}	3.41±0.04^{cd}	2.29±0.03^{d}
Hydroxyproline	1.71±0.30^{a}	0.65±0.01^{b}	ND	ND
Isoleucine	5.11±0.26^{a}	3.21±0.10^{b}	2.63±0.14^{c}	1.23±0.10^{d}
Leucine	8.91±0.18^{a}	4.02±1.13^{b}	1.28±0.03^{c}	ND
Lysine	12.70±0.59^{a}	3.95±0.09^{b}	1.93±0.12^{cd}	1.20±0.06^{d}
Methionine	30.46±0.16^{a}	14.76±1.05^{b}	10.22±0.73^{c}	5.68±0.12^{d}
Phenylalanine	4.17±0.07^{a}	2.10±0.08^{b}	1.09±0.02^{c}	ND
Proline	44.36±1.32^{a}	25.66±1.20^{b}	14.18±0.33^{c}	9.38±0.41^{d}
Serine	20.73±0.12^{a}	9.40±0.10^{b}	4.32±0.09^{c}	2.42±0.11^{d}
Tyrosine	2.96±0.52^{a}	1.05±0.02^{bc}	0.33±0.01^{c}	ND
Threonine	6.10±0.10^{a}	3.56±0.23^{b}	2.11±0.04^{c}	0.98±0.03^{d}
Tryptophan	6.41±0.42^{a}	4.43±0.14^{b}	2.19±0.16^{cd}	1.72±0.02^{d}
Valine	6.11±0.13^{a}	3.63±0.04^{b}	1.92±0.09^{c}	ND
Total	571.40	356.57	236.98	140.19

[1]Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

I, II, and III these are 1st, 2nd and 3rd methanol-ammonia-water extractions.

4.43. There was a sigificant reduction in the content of nearly all individual amino acids present and some amino acids such as hydroxiproline, leucine, phenylalanine, typrosine, valine were also totally eliminated. The removal of free amino acids affects the formation of Maillard reaction products during heat processing, thus improving the nutritional and sensory quality of the resultant meals.

4.9.5 Effect on mineral components of beach pea and grass pea

Mineral composition of methanol-ammonia-water extracted meals of beach pea and grass peas are presented in Tables 4.44 and 4.45. The content of macro- and micro-elements decreased slightly as the number of extractions increased for all pea seed meals. The highest reduction was observed for sodium in all three peas tested. The percent loss of minerals in beach pea and both grass peas was similar after three extractions. The decrease in minerals might be due to their dissolution in the polar solvents used during processing.

4.10 Fractionation, isolation and characterization of beach pea, green pea and grass pea proteins

4.10.1 Nitrogen solubility of beach pea, green pea and grass pea

Protein fractionation of beach pea, green pea and grass peas, separated according to their solubility in different solvent systems are presented in Table 4.46. The content of water-soluble fraction was not similar ($p>0.05$) for all pea types. The amount of salt-

Table 4.43 Effect of methanol-ammonia-water extraction on free amino acid composition of grass peas (mg/100g)[1]

Free amino acid /Treatment	Grass pea[2]				Grass pea[3]			
	Control	I	II	III	Control	I	II	III
Alanine	7.14±0.33^{a}	5.93±0.21^{b}	3.11±0.12^{c}	1.20±0.13^{d}	8.66±0.18^{a}	5.93±0.20^{b}	3.12±0.13^{c}	2.35±0.08^{d}
Arginine	27.75±1.23^{a}	18.62±0.33^{b}	7.09±0.41^{c}	4.32±0.11^{d}	98.37±2.10^{a}	53.44±0.86^{b}	39.67±0.23^{c}	19.85±0.15^{d}
Asparagine	53.66±0.83^{a}	41.35±0.43^{b}	30.25±0.12^{c}	21.36±0.32^{d}	45.22±1.20^{a}	33.48±0.33^{b}	18.52±0.18^{c}	13.38±0.19^{d}
Aspartic acid	29.72±1.15^{a}	15.13±0.30^{b}	11.43±0.21^{c}	8.53±0.13^{d}	38.37±2.10^{a}	26.08±0.85^{b}	20.88±0.65^{c}	13.54±0.50^{d}
Cysteine	25.39±0.83^{a}	2.54±0.35^{bc}	1.32±0.30^{cd}	0.89±0.21^{d}	13.60±0.60^{a}	8.32±0.22^{b}	2.03±0.18^{cd}	1.85±0.04^{d}
Glutamic acid	52.82±1.73^{a}	35.87±0.65^{b}	23.15±0.41^{c}	11.23±0.10^{d}	38.02±1.13^{a}	22.68±0.25^{b}	18.18±0.32^{c}	12.68±0.18^{d}
Glutamine	2.52±0.31^{a}	0.10±0.03^{b}	ND	ND	5.68±0.18^{a}	3.13±0.20^{bc}	2.78±0.16^{cd}	2.63±0.10^{d}
Glycine	4.49±0.12^{a}	2.92±0.10^{b}	1.36±0.02^{c}	0.92±0.01^{d}	6.89±0.20^{a}	3.02±0.23^{b}	2.10±0.15^{c}	1.02±0.16^{d}
Histidine	3.36±0.18^{a}	1.65±0.11^{b}	ND	ND	2.56±0.06^{a}	1.07±0.05^{b}	ND	ND
Hydroxyproline	1.20±0.03	ND	ND	ND	1.30±0.08^{a}	0.98±0.03^{b}	ND	ND
Isoleucine	0.88±0.06	ND	ND	ND	0.90±0.02^{a}	0.18±0.02^{b}	ND	ND
Leucine	2.12±0.04^{a}	0.78±0.01^{b}	ND	ND	8.03±0.23^{a}	4.36±0.13^{b}	2.12±0.10^{cd}	1.93±0.20^{d}
Lysine	5.76±0.10^{a}	2.50±0.02^{b}	1.68±0.03^{c}	0.85±0.01^{d}	9.26±0.36^{a}	4.13±0.21^{b}	3.30±0.08^{cd}	2.87±0.05^{d}
Methionine	1.47±0.10^{a}	0.81±0.01^{b}	0.24±0.01^{c}	ND	4.13±0.22^{a}	1.20±0.03^{b}	0.65±0.01^{c}	0.21±0.02^{d}
Phenylalanine	3.49±0.23^{a}	2.13±0.11^{b}	1.38±0.10^{c}	0.72±0.03^{d}	2.26±0.09^{a}	0.96±0.01^{b}	0.73±0.02^{c}	0.45±0.01^{d}
Proline	1.46±0.08^{a}	0.99±0.04^{b}	0.81±0.02^{c}	0.39±0.01^{d}	1.12±0.03^{a}	0.82±0.04^{b}	ND	ND
Serine	2.03±0.03^{a}	1.43±0.01^{b}	1.10±0.01^{c}	0.38±0.01^{d}	2.87±0.08^{a}	2.35±0.05^{b}	2.00±0.01^{c}	1.36±0.01^{d}
Tyrosine	1.91±0.08^{a}	1.30±0.04^{b}	0.59±0.05^{cd}	0.56±0.02^{d}	1.89±0.05^{a}	0.73±0.01^{b}	0.59±0.01^{c}	0.50±0.02^{d}
Threonine	2.05±0.38^{a}	1.73±0.10^{ab}	1.39±0.01^{b}	0.86±0.02^{d}	2.40±0.08^{a}	2.00±0.10^{b}	1.38±0.11^{c}	1.00±0.03^{d}
Tryptophan	3.16±0.42^{a}	2.39±0.11^{b}	1.82±0.03^{c}	0.90±0.01^{d}	3.63±0.18^{a}	2.25±0.02^{b}	ND	ND
Valine	3.50±0.23^{a}	2.85±0.08^{b}	1.42±0.01^{c}	0.85±0.20^{d}	2.57±0.30^{a}	2.11±0.05^{bc}	1.85±0.10^{c}	1.37±0.03^{d}
Total	235.88	141.02	88.14	53.96	297.73	179.22	119.90	76.99

[1]Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row for each variety are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]Canadian grass pea. [3]Indian grass pea.

Table 4.44 Effect of methanol-ammonia-water extractaion on mineral content of beach pea (mg/100g)[1]

Mineral	Treatment			
	Control	I	II	III
Macroelement				
Calcium	144.18±0.61^{a}	141.86±1.83^{a}	132.78±2.01^{b}	126.95±1.79^{c}
Magnesium	179.73±1.28^{a}	172.97±2.33^{b}	166.97±2.13^{c}	161.10±1.08^{d}
Phosphorus	413.16±1.22^{a}	387.06±3.02^{bc}	381.07±3.18^{c}	370.65±3.42^{d}
Potassium	475.83±1.00^{a}	392.69±2.30^{b}	380.85±1.68^{cd}	378.15±2.86^{d}
Sodium	84.14±0.43^{a}	53.09±0.12^{b}	50.96±0.09^{c}	47.68±0.72^{d}
Microelement				
Aluminum	4.49±0.29^{a}	3.93±0.19^{b}	3.76±0.34^{c}	3.43±0.15^{d}
Copper	0.85±0.16^{a}	0.77±0.08^{ab}	0.60±0.03^{bc}	0.52±0.06^{c}
Iron	9.37±0.21^{a}	8.98±0.39^{b}	8.67±0.70^{c}	8.36±0.67^{d}
Lithium	0.90±0.12^{a}	0.72±0.25^{a}	0.66±0.03^{a}	0.54±0.13^{a}
Manganese	3.50±0.58^{a}	3.37±0.90^{a}	3.15±0.23^{a}	3.02±0.28^{a}
Silicon	ND	ND	ND	ND
Zinc	2.97±0.08^{a}	2.43±0.23^{bcd}	2.13±0.15^{cd}	1.92±0.29^{d}

[1]Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

I, II, and III these are 1st, 2nd and 3rd methanol-ammonia-water extractions.

Table 4.45 Effect of methanol-ammonia-water extraction on mineral content of grass peas (mg/100g)[1]

Mineral/ Treatment	Grass pea[2]				Grass pea[3]			
	Control	I	II	III	Control	I	II	III
Macroelement								
Calcium	155.56±0.41^{a}	140.22±1.61^{b}	131.09±1.30^{c}	124.86±1.08^{d}	187.40±0.68^{a}	169.79±1.21^{b}	157.12±1.42^{c}	151.22±1.67^{d}
Magnesium	149.98±1.37^{a}	140.67±2.01^{b}	130.89±2.11^{c}	118.26±2.43^{d}	178.11±1.26^{a}	162.13±2.01^{b}	156.48±1.08^{c}	141.95±1.78^{d}
Phosphorus	482.32±0.96^{a}	467.13±3.10^{b}	459.02±2.92^{cd}	452.85±3.82^{d}	384.22±0.27^{a}	372.23±2.08^{b}	363.94±3.60^{cd}	358.91±3.21^{d}
Potassium	1098.08±2.15^{a}	1039.99±9.72^{b}	966.34±7.80^{c}	927.05±8.95^{d}	987.59±2.17^{a}	886.03±5.12^{b}	824.13±4.19^{c}	808.22±8.11^{d}
Sodium	60.53±0.13^{a}	58.67±0.76^{b}	51.03±0.65^{c}	46.13±0.93^{d}	93.78±1.13^{a}	90.99±0.92^{b}	82.25±0.53^{c}	73.93±0.38^{d}
Microelement								
Aluminum	6.71±0.10^{a}	6.03±0.31^{b}	5.86±0.13^{c}	5.61±0.16^{d}	20.53±0.80^{a}	18.35±0.96^{b}	16.93±0.75^{c}	15.35±0.53^{d}
Copper	2.39±0.18^{a}	1.89±0.20^{a}	1.67±0.33^{ab}	1.03±0.42^{b}	2.16±0.16^{a}	1.05±0.20^{bcd}	0.91±0.08^{cd}	0.88±0.04^{d}
Iron	9.71±0.40^{a}	9.07±1.03^{b}	8.26±0.67^{c}	7.30±0.25^{d}	8.16±0.41^{a}	7.74±0.32^{b}	7.23±0.22^{c}	5.96±0.30^{d}
Litium	3.06±0.65^{a}	1.32±0.04^{bcd}	1.06±0.03^{cd}	0.96±0.05^{d}	5.93±0.35^{a}	3.93±0.61^{b}	2.70±0.36^{cd}	2.13±0.20^{d}
Manganese	1.46±0.16^{a}	1.00±0.05^{b}	0.73±0.10^{c}	0.42±0.09^{d}	8.68±0.39^{a}	7.97±0.78^{b}	6.29±0.81^{c}	5.14±0.42^{d}
Silicon	15.92±0.25^{a}	13.32±1.00^{b}	12.61±0.22^{c}	10.90±0.48^{d}	22.72±0.13^{a}	20.49±0.85^{b}	18.65±0.82^{c}	17.95±0.65^{d}
Zinc	6.72±0.09^{a}	2.65±0.20^{b}	2.13±0.23^{cd}	1.83±0.08^{d}	5.41±0.10^{a}	3.59±0.60^{bcd}	3.23±0.45^{cd}	2.91±0.28^{d}

[1]Results are means of triplicate determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row for each variety are significantly ($p<0.05$) different from one another.

[2]Canadian grass pea.

[3]Indian grass pea.

Table 4.46 Solubility fractionation of beach pea, green pea and grass pea proteins[1]

Pea	Percent of total protein nitrogen				
	Water-soluble	Salt-soluble	Alcohol-soluble	Alkali-soluble	Residue
Beach pea	43.04±1.23[a]	41.04±1.03[a]	4.50±0.08[ab]	6.35±0.10[c]	5.07±0.32[a]
Green pea	44.01±1.67[a]	38.89±1.13[ab]	4.79±0.13[a]	8.51±0.11[a]	3.80±0.08[c]
Grass pea[2]	44.81±1.33[a]	38.25±1.23[b]	4.23±0.20[b]	8.47±0.08[a]	4.23±0.04[bc]
Grass pea[3]	43.75±1.25[a]	39.67±1.63[ab]	4.85±0.26[a]	6.89±0.32[b]	4.84±0.28[a]

[1]Results are means of four determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another.

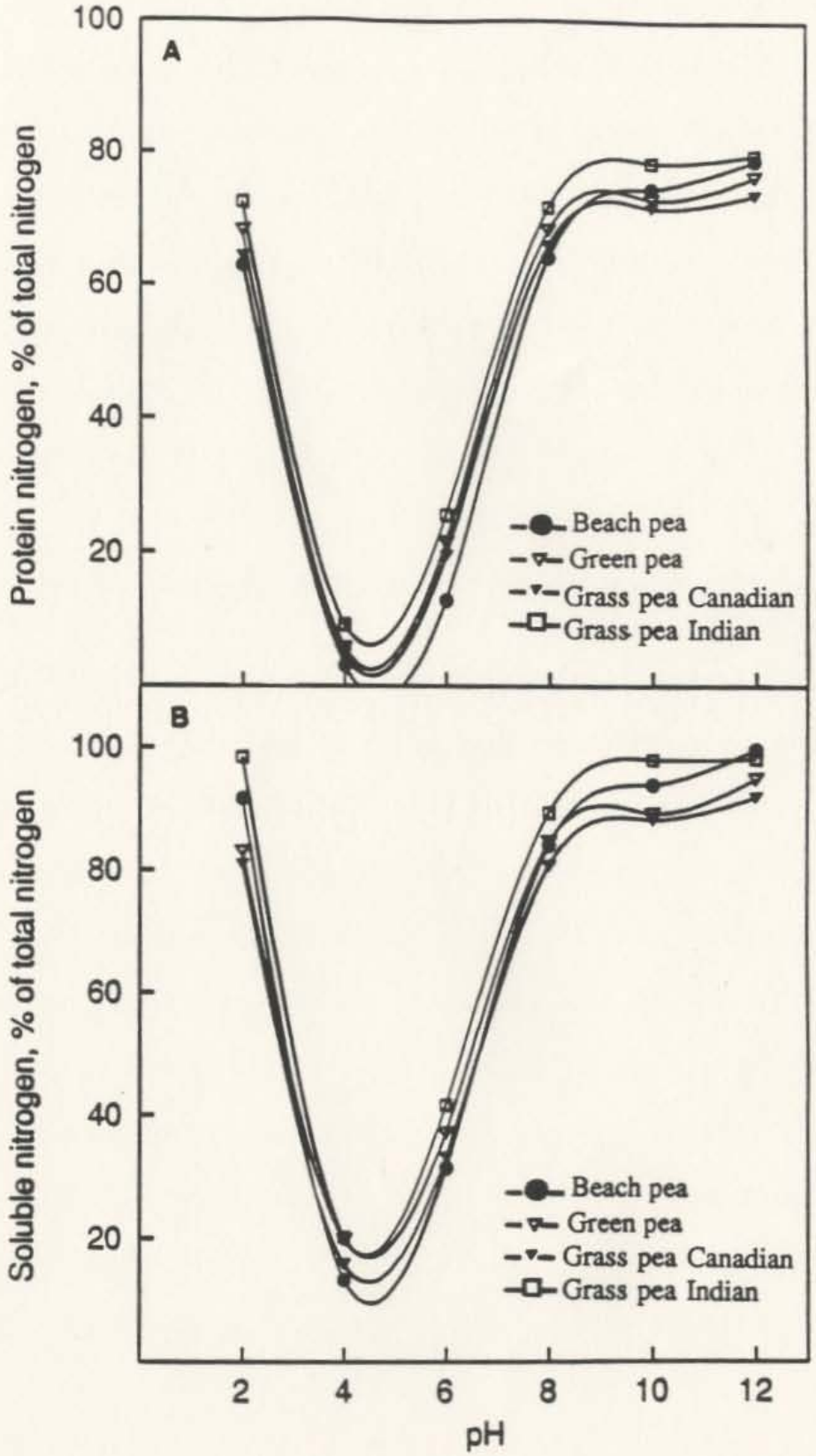
[2]Canadian grass pea.

[3]Indian grass pea.

soluble fraction was higher in beach pea than other peas tested. Alkali-soluble fraction of total nitrogen was significantly ($p<0.05$) lower in beach pea than green pea, Canadian and Indian grass peas. Alcohol-soluble fraction (prolamine) was lowest contributor to total proteins of peas studied. Residual nitrogen fraction was significantly ($p<0.05$) higher in beach pea and Indian grass pea than green pea and Canadian grass pea. The results indicate that water and salt soluble (albumin and globulin) are the major soluble proteins of peas. These results indicate that no major differences existed among beach pea, green pea and grass peas as far as the distribution of seed protein nitrogen fractions is concerned (Singh *et al.*, 1981; Singh and Jambunathan, 1982). The nitrogen content in the residue may be due to the presence of other proteins which might be complexed with phenolic compounds, and tannins and could remain in the residue.

The percentage of protein nitrogen and soluble nitrogen of beach pea, green pea, and two grass pea cultivars at different pH conditions is presented in Figures 4.18A and 4.18B, respectively. Approximately 50% of the total nitrogen content of all pea seeds examined was soluble at pH 6 - 7. The nitrogen solubility of pea seeds was lowest at pH 4.5 and increased below and above this pH; the maximum solubility was observed at pH 10 and above. These results are similar to those of green gram, yellow pea and moth bean as reported by Krishnamurthy and Rama Rao (1976), Hsu *et al.* (1982) and Borhade *et al.* (1984), respectively. Prinyawiwatkul *et al.* (1997) reported that cowpea proteins solubility was minimum at pH 4.0, but increased in both the acidic and alkaline regions. Most of the legumes have a protein solubility of about 10% or less at their isoelectric pH

Figure 4.18 Perceentage of protein nitrogen (A) and soluble nitrogen (B) of beach pea, green pea, Canadian grass pea and Indian grass pea meals as affected by pH of the extraction medium.


A
B
Protein nitrogen, % of total nitrogen
Soluble nitrogen, % of total nitrogen
100
80
60
40
20
2
4
6
8
10
12
pH
Beach pea
Green pea
Grass pea Canadian
Grass pea Indian

(Sefa-Dedeh and Stanley, 1979). Padmashree *et al.* (1987) and Sosulski *et al.* (1987) have shown that solubility of cowpea protein at pH 4.0 ranges from 17 to 40%. Similar results for nitrogen solubility of *Phaseolus angularis*, *Phaseolus calcaratus*, and *Phaseolus lablab* legume seeds were shown by Chau *et al.* (1997). Taha (1987) reported that pigeonpea proteins exhibit a maximum precipitation (92%) at pH 4.4 when extracted with a 0.05N NaOH solution. The solubility of proteins or their extraction at the isoelectric point is generally at its lowest due to the overall neutral charge of protein molecules.

4.10.2 Protein distribution in anatomical parts of beach pea, green pea and grass pea

The separation of different protein fractions from beach pea, green pea and Canadian grass pea was carried out using different solvents, namely water, salt, alcohol and alkali and their distribution in seeds as well as cotyledons, hulls and residues is presented in Table 4.47. Globulin was the major fraction of cotyledons and whole seeds of peas. The content of albumin and globulin in beach pea seeds, cotyledons and hulls was lower than those of other seeds. However, glutelin contents in beach pea seed, its cotyledons, and hulls were higher than those of green pea and Canadian grass pea. Hulls were the major storage sites of glutelin fraction and non-protein nitrogen and prolamine as compared to other components, but contained a much smaller proportion of albumin and globulin. Similar results are reported in the literature for chickpea, pigeonpea, kidney bean, Great Northern bean and black gram (Singh *et al.*, 1981; Singh and Jambunathan,

Table 4.47 Distribution of protein fractions in different anatomical parts of beach pea, green pea and grass pea seeds[1]

Pea/Component	Protein fractions (%)				
	Albumin	Globulin	Prolamine	Glutelin	Residue
Beach pea					
Whole seed	13.79±1.12^{e}	57.17±2.14^{c}	3.08±0.26^{bcde}	19.14±1.07^{bcdefg}	6.82±0.36^{de}
Cotyledons	14.79±1.45^{bcde}	61.89±2.82^{abc}	2.87±1.20^{cde}	18.85±2.11^{cdefg}	1.60±0.98^{ghi}
Hulls	3.26±0.88^{h}	23.73±1.94^{f}	3.48±0.78^{abcde}	35.87±2.52^{a}	33.66±1.63^{a}
Green pea					
Whole seed	18.73±2.05^{a}	58.56±1.89^{bc}	2.58±0.38^{de}	16.23±1.52^{efg}	3.90±1.02^{fghi}
Cotyledons	16.96±1.78^{abcde}	62.23±2.65^{abc}	2.48±1.40^{e}	16.82±1.23^{defg}	1.51±0.33^{hi}
Hulls	3.82±0.56^{fgh}	28.50±0.75^{ef}	3.76±0.23^{abcde}	34.22±1.67^{a}	29.70±0.97^{bc}
Grass pea[2]					
Whole seed	13.89±1.73^{de}	59.49±2.11^{abc}	5.63±1.08^{a}	15.73±1.67^{fg}	5.26±1.22^{ef}
Cotyledons	14.76±1.16^{cde}	64.85±2.19^{a}	3.64±1.06^{abcde}	15.57±1.33^{g}	1.18±0.76^{i}
Hulls	3.57±0.83^{gh}	29.89±1.25^{de}	5.77±0.36^{a}	33.79±0.72^{a}	26.98±0.77^{c}

[1]Results are means of three determinations, on a dry weight basis, and are expressed as percentage of total protein (%N x 6.25). Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another.

[2]Canadian grass pea.

1982; Sathe *et al.*, 1984).

4.10.3 Amino acid composition of protein fractions of beach pea, green pea and grass pea

The various protein fractions of beach pea, green pea and Canadian grass pea seeds were analyzed for their amino acid composition and results are shown in Tables 4.48, 4.49, and 4.50, respectively. Albumin fraction contained the highest amount of sulphur-containing amino acids followed by glutelin, globulin, and prolamine in all samples studied and the amount of these amino acids was higher in beach pea as compared to green pea and Canadian grass pea. Leucine, lysine, aspartic acid, glutamic acid and alanine contents were higher in albumin than in other fraction of beach pea, green pea and Canadian grass pea seed proteins. Globulin was the major protein fraction with a lower proportion of sulphur-containing amino acids than those of albumin and glutelin. The total essential amino acids, the ratio of essential to total amino acids, amino acid score and biological value (BV) of the albumin fraction of beach pea were higher than those of Canadian grass pea. Predicted biological value of albumin and glutelin fractions of beach pea was higher than green pea and Canadian grass pea protein fractions, while BV of globulin and prolamine protein fractions of beach pea was lower than Canadian grass pea but higher than green pea protein fractions. These results are in agreement with the literature values for chickpea, pigeonpea, red bean, mung bean and broad bean (Singh and Jambunathan, 1982; Liang *et al.*, 1988). It may also be concluded that the selection of cultivars in which the albumin and glutelin fractions are higher would result in improved

Table 4.48 Total amino acid composition of seed protein fractions of beach pea (g/16 g N)[1]

Amino acid	Albumin	Globulin	Prolamine	Glutelin
Isoleucine	4.64±0.11^{a}	4.09±0.13^{bc}	4.09±0.12^{bc}	4.06±0.13^{c}
Leucine	8.48±0.12^{a}	7.73±0.12^{bc}	7.40±0.11^{d}	7.57±0.15^{cd}
Lysine	9.16±0.10^{a}	7.06±0.13^{b}	6.52±0.12^{cd}	6.20±0.18^{d}
Cysteine[2]	1.32±0.08^{a}	0.93±0.06^{bc}	0.77±0.10^{c}	1.20±0.11^{a}
Methionine[2]	1.20±0.04^{a}	0.70±0.03^{c}	0.43±0.08^{d}	0.76±0.06^{bc}
Total sulphur amino acids	2.52	1.63	1.20	1.96
Tyrosine	3.69±0.11^{a}	3.30±0.10^{c}	3.79±0.12^{a}	3.33±0.12^{bc}
Phenylalanine	5.41±0.09^{a}	4.73±0.21^{cd}	4.30±0.21^{d}	4.76±0.15^{bc}
Total aromatic amino acids	9.10	8.03	8.09	8.09
Threonine	4.41±0.13^{b}	3.52±0.09^{d}	5.15±0.14^{a}	3.88±0.12^{c}
Tryptophan[2]	0.80±0.05^{b}	0.94±0.06^{a}	0.65±0.05^{c}	0.40±0.03^{d}
Valine	5.53±0.16^{a}	4.88±0.11^{bcd}	4.67±0.13^{d}	4.87±0.16^{cd}
Histidine	2.59±0.03^{c}	2.97±0.08^{a}	2.62±0.10^{bc}	3.08±0.10^{a}
Total essential amino acids	47.23	40.85	40.39	40.11
Arginine	8.42±0.17^{b}	8.73±0.21^{ab}	7.51±0.15^{c}	8.97±0.23^{a}
Aspartic acid + Asparagine	12.07±0.18^{a}	10.47±0.15^{c}	11.59±0.24^{ab}	11.21±0.28^{b}
Glutamic acid + Glutamine	17.15±0.24^{a}	16.43±0.23^{b}	14.34±0.26^{c}	17.14±0.30^{a}
Serine	5.33±0.12^{a}	4.78±0.13^{b}	5.30±0.13^{a}	5.13±0.11^{a}
Proline	4.28±0.15^{a}	4.05±0.10^{ab}	3.70±0.18^{b}	4.26±0.10^{a}
Glycine	4.39±0.10^{c}	4.00±0.11^{d}	4.87±0.11^{a}	4.46±0.13^{bc}
Alanine	5.11±0.13^{a}	4.15±0.12^{bc}	3.61±0.14^{d}	4.00±0.20^{c}
Total non-essential amino acids	56.75	52.61	50.92	55.17
E/T, %	45.42	43.71	44.23	42.10
Amino acid score	124.08	105.22	104.92	102.86
BV	51.01	38.95	26.77	52.21

[1]Results are mean values of triplicate determinations, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. [2]Limiting amino acid.

Table 4.49 Total amino acid composition of seed protein fractions of green pea (g/16 g N)[1]

Amino acid	Albumin	Globulin	Prolamine	Glutelin
Isoleucine	5.00±0.13^{a}	4.32±0.11^{bc}	4.15±0.21^{c}	4.93±0.29^{a}
Leucine	8.97±0.15^{a}	8.08±0.13^{c}	8.15±0.16^{bc}	8.99±0.25^{a}
Lysine	8.98±0.20^{a}	6.71±0.12^{c}	6.08±0.12^{d}	8.07±0.33^{b}
Cysteine[2]	1.21±0.08^{a}	0.96±0.05^{b}	0.71±0.10^{c}	0.71±0.05^{c}
Methionine[2]	1.16±0.10^{a}	0.57±0.03^{cd}	0.41±0.07^{d}	0.90±0.05^{b}
Total sulphur amino acids	2.37	1.53	1.12	1.61
Tyrosine	4.26±0.10^{a}	3.50±0.13^{cd}	3.20±0.13^{d}	3.82±0.23^{bc}
Phenylalanine	5.79±0.11^{a}	5.08±0.14^{c}	5.16±0.18^{bc}	5.83±0.26^{a}
Total aromatic amino acids	10.05	8.58	8.36	9.65
Threonine	4.40±0.18^{a}	3.14±0.18^{cd}	3.12±0.19^{d}	3.80±0.10^{b}
Tryptophan[2]	1.03±0.08^{a}	0.79±0.04^{b}	0.60±0.03^{c}	0.97±0.04^{a}
Valine	5.65±0.20^{a}	4.66±0.10^{bc}	4.38±0.22^{c}	5.25±0.25^{a}
Histidine	2.49±0.11^{a}	2.23±0.08^{ab}	2.05±0.10^{b}	2.38±0.20^{ab}
Total essential amino acids	48.94	40.04	38.00	45.65
Arginine	9.07±0.24^{bc}	9.33±0.20^{abc}	9.80±0.21^{a}	9.03±0.40^{c}
Aspartic acid + Asparagine	12.10±0.30^{ab}	11.76±0.25^{ab}	12.50±0.18^{a}	11.70±0.38^{b}
Glutamic acid + Glutamine	17.58±0.22^{a}	16.51±0.13^{c}	16.88±0.25^{bc}	17.59±0.28^{a}
Serine	5.61±0.15^{a}	4.69±0.10^{c}	4.75±0.14^{bc}	5.35±0.15^{a}
Proline	4.78±0.12^{a}	4.20±0.12^{cd}	4.16±0.20^{d}	4.38±0.12^{bcd}
Glycine	4.70±0.10^{a}	3.82±0.18^{cd}	3.79±0.11^{d}	4.24±0.11^{b}
Alanine	5.04±0.21^{a}	4.04±0.14^{b}	3.33±0.12^{c}	4.63±0.31^{a}
Total non-essential amino acids	58.88	54.35	55.21	56.92
E/T, %	45.39	42.42	40.77	44.51
Amino acid score	129.03	105.03	99.86	120.19
BV	42.82	26.17	23.41	28.41

[1]Results are mean values of triplicate determinations, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. [2]Limiting amino acid.

Table 4.50 Total amino acid composition of seed protein fractions of Canadian grass pea (g/16 g N)[1]

Amino acid	Albumin	Globulin	Prolamine	Glutelin
Isoleucine	4.74±0.11^{ab}	4.65±0.12^{ab}	4.33±0.35^{b}	5.08±0.21^{a}
Leucine	8.10±0.28^{ab}	8.48±0.11^{a}	7.62±0.19^{b}	8.17±0.30^{ab}
Lysine	6.93±0.13^{a}	6.79±0.10^{a}	5.49±0.11^{b}	6.96±0.22^{a}
Cysteine[2]	1.27±0.11^{a}	0.75±0.02^{c}	0.89±0.09^{bc}	0.89±0.02^{bc}
Methionine[2]	0.90±0.03^{a}	0.70±0.05^{c}	0.49±0.02^{d}	0.80±0.03^{b}
Total sulphur amino acids	2.17	1.45	1.38	1.69
Tyrosine	3.86±0.16^{a}	4.10±0.08^{a}	3.29±0.07^{b}	4.08±0.12^{a}
Phenylalanine	5.04±0.10^{a}	5.02±0.31^{a}	4.87±0.13^{b}	5.44±0.13^{a}
Total aromatic amino acids	8.90	9.12	8.16	9.52
Threonine	3.72±0.18^{bc}	4.28±0.19^{a}	3.60±0.20^{c}	3.95±0.19^{abc}
Tryptophan[2]	0.96±0.10^{ab}	1.27±0.20^{a}	0.81±0.10^{b}	1.09±0.03^{ab}
Valine	5.14±0.21^{ab}	5.18±0.16^{ab}	4.71±0.11^{b}	5.36±0.31^{a}
Histidine	2.60±0.05^{a}	2.53±0.09^{a}	2.25±0.04^{b}	2.49±0.10^{a}
Total essential amino acids	43.26	43.75	38.35	44.31
Arginine	9.96±0.40^{a}	8.04±0.12^{c}	9.47±0.18^{a}	8.62±0.33^{bc}
Aspartic acid + Asparagine	12.22±0.36^{a}	10.57±0.41^{b}	11.20±0.40^{ab}	11.67±0.52^{ab}
Glutamic acid + Glutamine	17.66±0.26^{a}	14.07±0.36^{d}	16.44±0.62^{bc}	15.80±0.38^{c}
Serine	5.46±0.17^{a}	5.17±0.12^{ab}	5.00±0.08^{b}	5.32±0.20^{ab}
Proline	4.53±0.08^{a}	4.14±0.26^{a}	4.08±0.12^{a}	4.49±0.18^{a}
Glycine	4.02±0.12^{a}	4.29±0.16^{a}	3.89±0.22^{a}	4.24±0.21^{a}
Alanine	4.32±0.06^{b}	5.18±0.14^{a}	3.78±0.19^{c}	4.76±0.23^{ab}
Total non-essential amino acids	58.17	51.46	53.86	54.90
E/T, %	42.65	45.95	41.59	44.66
Amino acid score	122.94	114.50	100.28	116.17
BV	43.66	43.65	42.65	38.27

[1]Results are mean values of triplicate determinations, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. [2]Limiting amino acid.

methionine and cysteine content in the seeds of pea legumes. Predicted PER values for albumin, globulin, prolamine and glutelin fractions were found to be at par in beach pea and Canadian grass pea, but were lower than those for green pea protein fractions (Table 4.51).

4.10.4 UV spectra of different protein fractions

UV spectra of individual protein fractions from seeds of beach pea, green pea, and Canadian grass pea are shown in Figure 4.19. The absorption maxima of separated fractions occurred mostly in 260 - 282 nm range. Beach pea seed protein fractions showed typically different spectra than those of green pea and Canadian grass pea (Figure 4.19 and Table 4.52). This difference in spectral data shows that beach pea protein fractions might contain different proportions of amino acids or may contain extracted phenolics and condensed tannins which could potentially change their UV spectra. Green pea and Canadian grass pea showed similar UV spectra for all protein fractions. Padhye (1979) studied the UV spectra of black gram proteins and found that albumins, globulins, prolamines and glutelins had similar UV spectral features.

4.10.5 Surface topography of protein isolates and protein fractions

The scanning electron microscopic (SEM) structures of pea flours, protein isolates, albumins, globulins, prolamines and glutelins of beach pea, green pea and Canadian grass pea proteins are shown in Figures 4.20, 4.21, and 4.22, respectively. The SEM results

Table 4.51 The predicted PER values of protein fractions of beach pea, green pea, and grass pea

Pea/Protein fraction	Predicted PER values using equation[1]		
	1	2	3
Beach pea			
Albumin	2.98	2.99	2.38
Globulin	2.65	2.69	2.03
Prolamine	2.52	2.49	1.12
Glutelin	2.57	2.62	1.93
Green pea			
Albumin	3.18	3.16	2.19
Globulin	2.80	2.83	1.90
Prolamine	2.84	2.90	2.13
Glutelin	3.21	3.21	2.48
Grass pea[2]			
Albumin	2.80	2.80	1.80
Globulin	2.99	2.95	1.77
Prolamine	2.60	2.65	1.71
Glutelin	2.83	2.81	1.58

[1]Alsmeyer *et al.* (1974).

[2]Canadian grass pea.

Figure 4.19 UV spectra of individual seed protein fractions: beach pea, green pea and Canadian grass pea (A, Albumin; B, Globulin; C, Prolamine; and D, Glutelin).

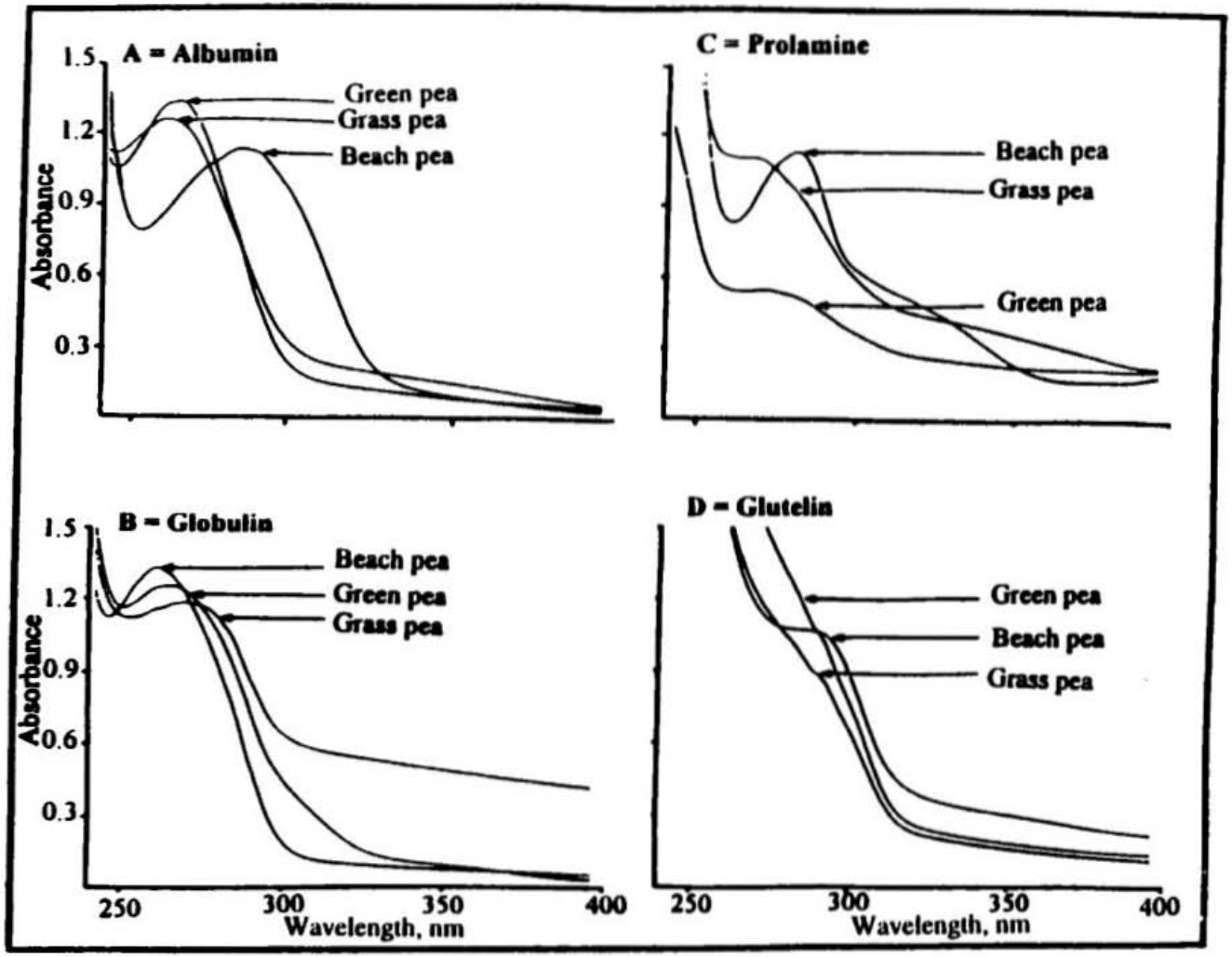

A = Albumin
Green pea
Grass pea
Beach pea
C = Prolamine
Beach pea
Grass pea
Green pea
B = Globulin
Beach pea
Green pea
Grass pea
D = Glutelin
Green pea
Beach pea
Grass pea
Absorbance
1.5
1.2
0.9
0.6
0.3
250
300
350
400
Wavelength, nm

Table 4.52 UV spectral data of different seed protein fractions of beach pea, green pea and grass pea[1]

Protein fraction	Beach pea (λ_{max}, nm)	Green pea (λ_{max}, nm)	Grass pea[2] (λ_{max}, nm)
Albumin	282	264	260
Globulin	260	266	270
Prolamine	280	270	266
Glutelin	282	-	-

[1]Protein fractions extracted in different solvent system. -, No peak.

[2]Canadian grass pea.

Figure 4.20 Scanning electron micrographs of pea flours and protein isolates (NaOH-extracted): A, beach pea flour; B, green pea flour; C, Canadian grass pea flour; D, beach pea protein isolate; E, green pea protein isolate; and F, Canadian grass pea protein isolate.

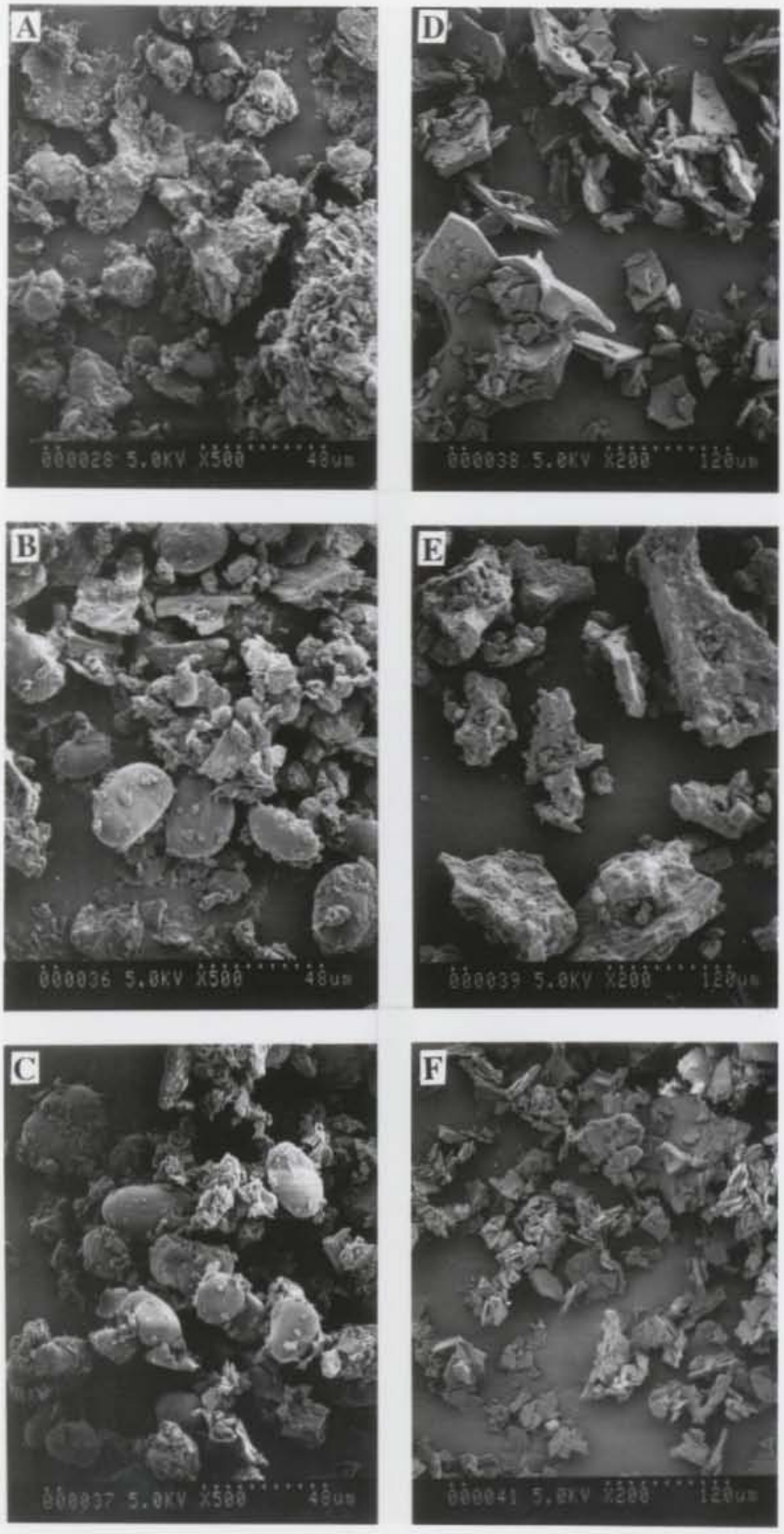
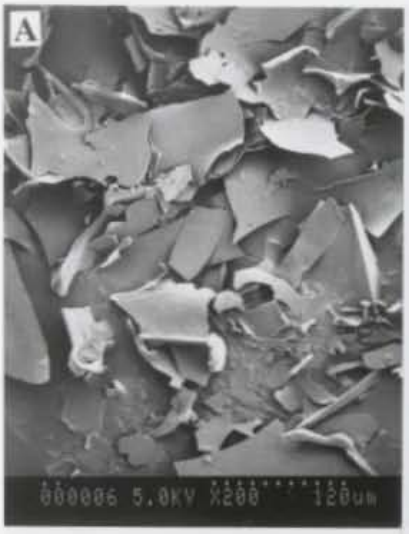
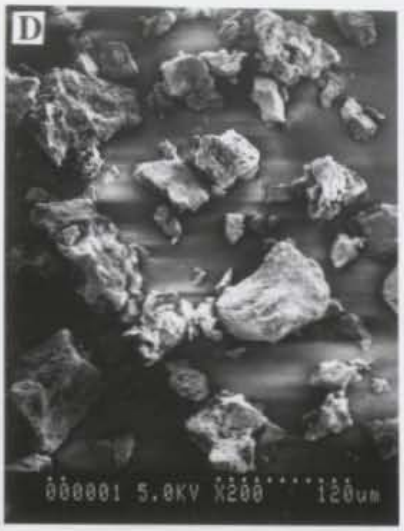
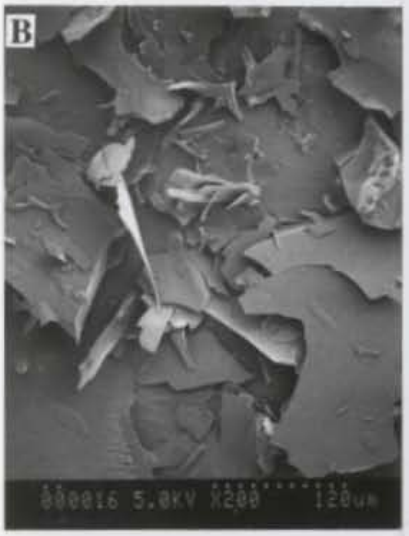
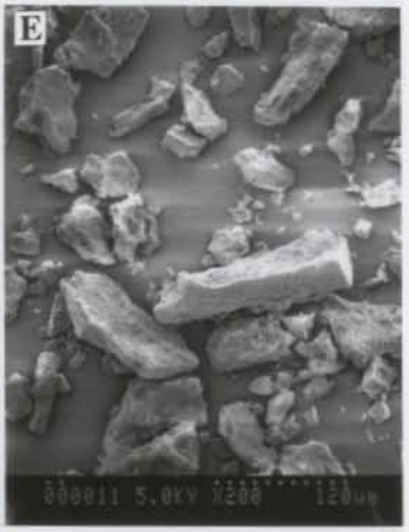

A
D
B
E
C
F

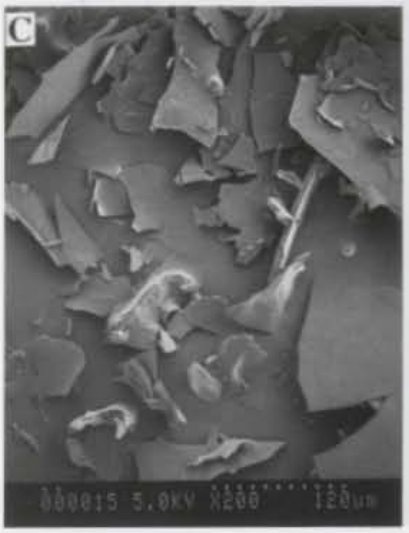
Figure 4.21 Scanning electron micrographs of pea protein fractions (A, beach pea albumin; B, green pea albumin; C, Canadian grass pea albumin; D, beach pea globulin; E, green pea globulin; and F, Canadian grass pea globulin).


A
000006 5.0KV X200 120um


D
000001 5.0KV X200 120um


B
000016 5.0KV X200 120um


E
000011 5.0KV X200 120um


C
000015 5.0KV X200 120um

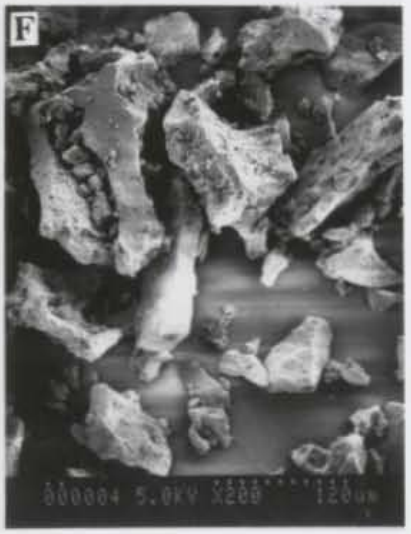
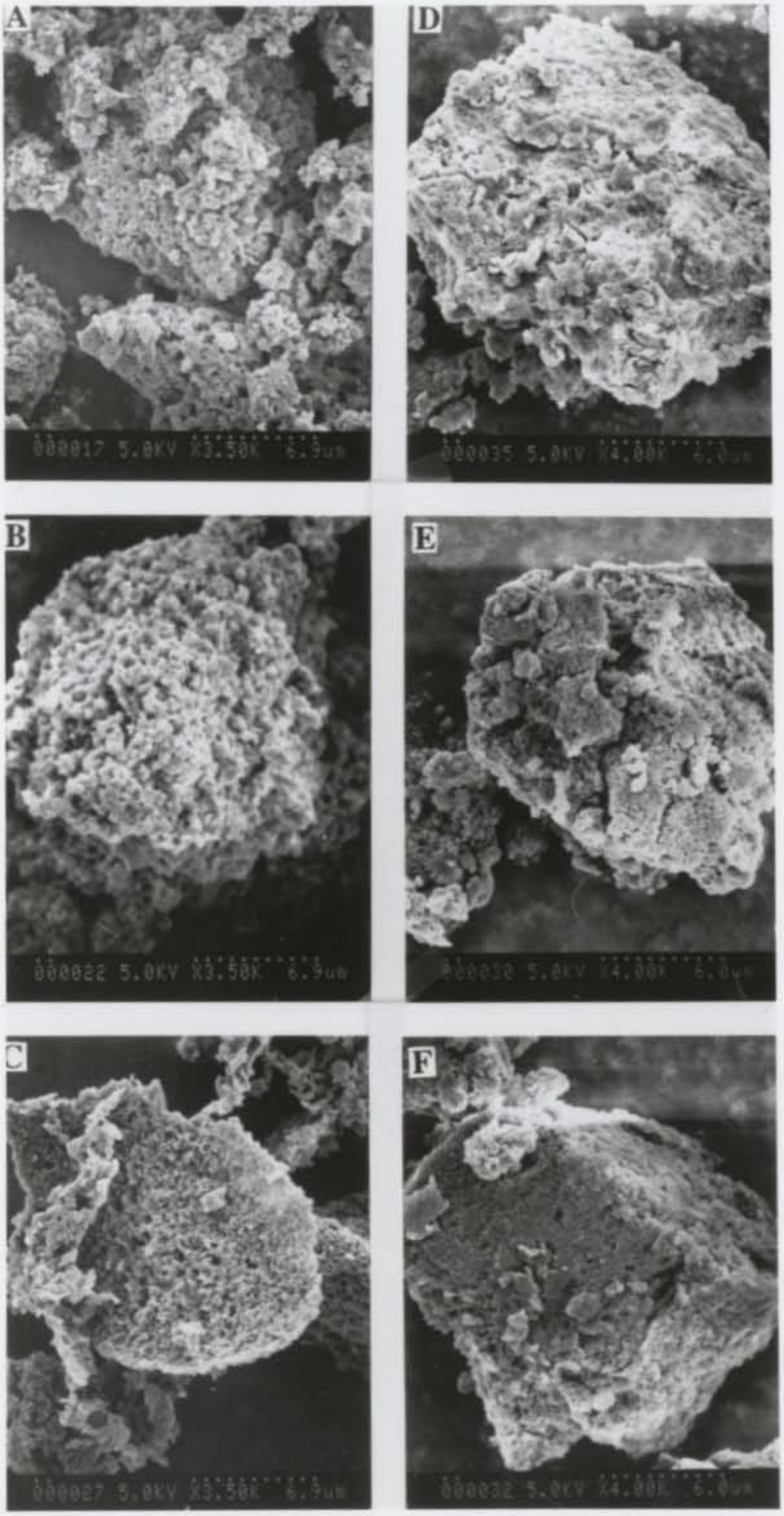

F
000004 5.0KV X200 120um

Figure 4.22 Scanning electron micrographs of pea protein fractions (A, beach pea prolamine; B, green pea prolamine; C, Canadian grass pea prolamine; D, beach pea glutelin; E, green pea glutelin; and F, Canadian grass pea glutelin).


A
D
B
E
C
F

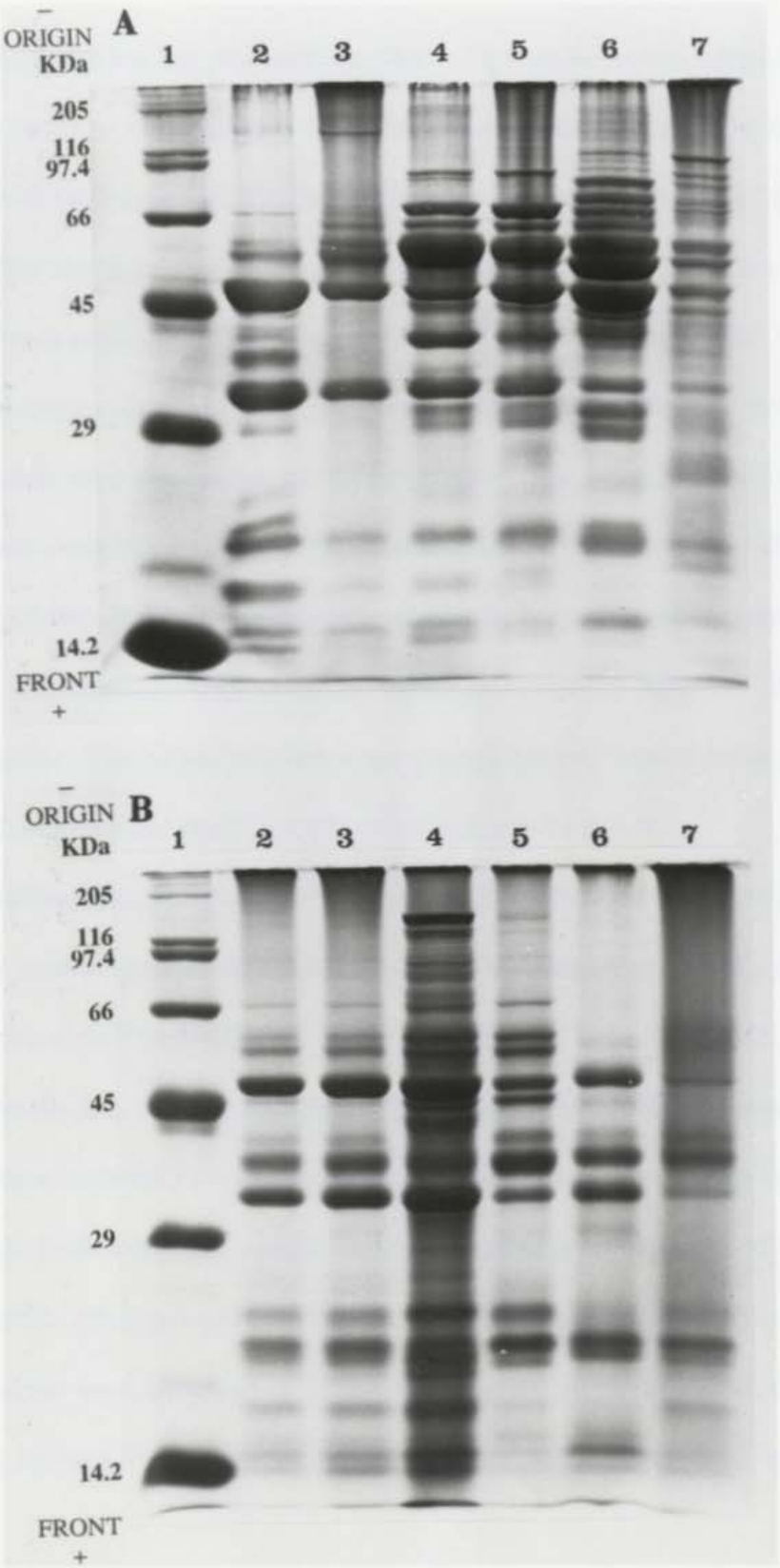
indicate that albumins of all peas had very smooth plate-like surface topography, while globulins were irregular with rougher surfaces and big size particles (Figure 4.21). Prolamines and glutelins also were irregular in shape and of large particle size. However, prolamines showed higher porosity and loose structure than glutelins even at lower magnification (Figure 4.22). Pea flours and protein isolates, as expected, consisted of all these four protein fractions. However, their morphological characteristics resembled those of the isolated fractions (Figure 4.20). The different topographical characteristics of protein fractions (albumin, globulin, prolamine and glutelin) may contribute to the overall physico-chemical and functional properties of pea seed proteins. All four protein fractions of beach pea seeds showed similar topographical characteristics as compared to those of green pea and Canadian grass pea protein fractions and protein isolates. Thus, the present results support the findings of Sathe (1981) who employed SEM to study the surface structure of albumins and globulins from Great Northern bean and reported that albumins had rod-like structures, while globulins were irregular in shape.

4.10.6 Polyacrylamide gel electrophoresis (PAGE) of protein isolates and protein fractions from beach pea, green pea and grass pea

Electrophoretic pattern of sodium hydroxide- and sodium hexametaphosphate (SHMP)-extracted and extensively dialysed protein isolates from beach pea, green pea and Canadian grass pea in a non-denatured PAGE (NPAGE) system is shown in Figure 4.23A. Hames (1981) showed that NPAGE separates proteins based on their size and negative

Figure 4.23A The NPAGE of pea proteins (1, molecular weight markers; 2, beach pea NaOH protein isolate; 3, beach pea SHMP protein isolate; 4, green pea NaOH protein isolate; 5, green pea SHMP protein isolate; 6, Canadian grass pea NaOH protein isolate; 7, Canadian grass pea SHMP protein isolate).

B The SDS-PAGE of beach pea proteins (1, molecular weight markers; 2, NaOH protein isolate; 3, SHMP protein isolate; 4, albumin; 5, globulin; 6, prolamine; 7, glutelin).

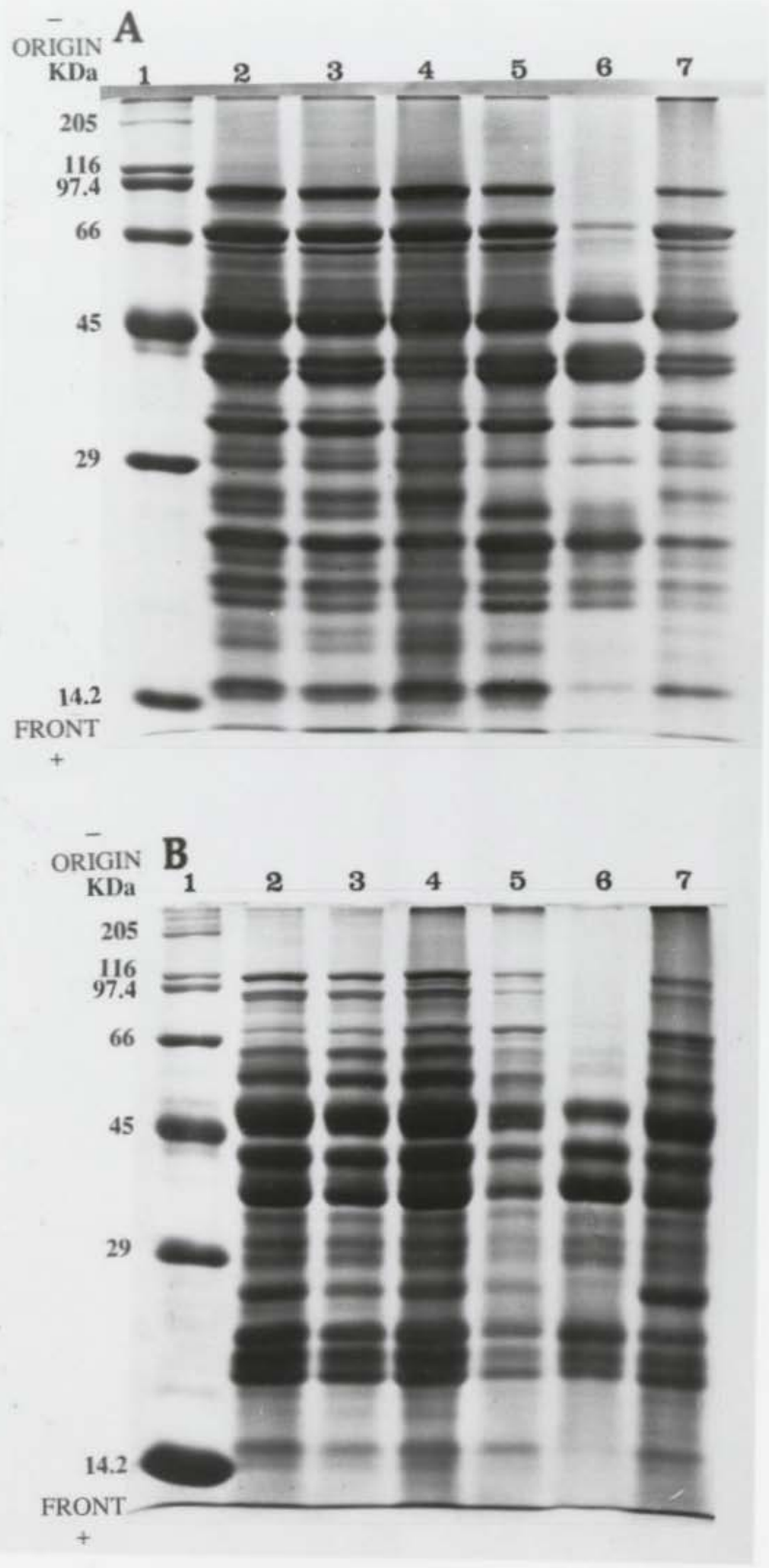

A
ORIGIN
KDa
1
2
3
4
5
6
7
205
116
97.4
66
45
29
14.2
FRONT
B
ORIGIN
KDa
1
2
3
4
5
6
7
205
116
97.4
66
45
29
14.2
FRONT

charge without having any denaturation effect. The major protein bands occur in the 30 to 45 kDa range in non-denatured proteins of beach pea. Four of the bands were not observed in SHMP-extracted protein isolates from beach pea. In case of green pea, after dialysis, Nine major bands were observed in the range of 29 to 97.4 kDa. Both NaOH- and SHMP-extracted samples of green pea showed similar protein bands. Canadian grass pea protein isolates showed 14 intense bands in the sodium hydroxide extract while low intensity bands were observed in the SHMP-extract. The major bands for Canadian grass pea were observed in the range of 29 to 30 and 40 to 95 kDa in both NaOH and SHMP extracts. Sodium hydroxide-extracted protein isolates, following extensive dialysis, invariably showed very high intensity bands as compared with the SHMP-extracted protein isolates. The NPAGE of beach pea protein isolates showed bands with much less intensity than green pea and Canadian grass pea protein isolates.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of various protein isolates and protein fractions (albumins, globulins, prolamines, and glutelins) are shown in Figures 4.23B, 4.24A, and 4.24B for beach pea, green pea and Canadian grass pea, respectively. Most polypeptides bind SDS in a constant ratio such that they have essentially the same charge densities and migrate in the polyacrylamide gel according to their molecular weight. Beach pea protein isolates showed very few bands as compared to green pea and Canadian grass pea. The major protein bands of beach pea protein isolates were observed at 35 and 47 kDa (Figure 4.23B). Albumin fraction of beach pea showed 18 polypeptide bands with two major bands at 35 and 47 kDa and

Figure 4.24A The SDS-PAGE of green pea proteins (1, molecular weight markers; 2, NaOH protein isolate; 3, SHMP protein isolate; 4, albumin; 5, globulin; 6, prolamine; 7, glutelin).

B The SDS-PAGE of the Canadian grass pea proteins (1, molecular weight markers; 2, NaOH protein isolate; 3, SHMP protein isolate; 4, albumin; 5, globulin; 6, prolamine; 7, glutelin).


–
ORIGIN
KDa
A
1
2
3
4
5
6
7
205
116
97.4
66
45
29
14.2
FRONT
+
–
ORIGIN
KDa
B
1
2
3
4
5
6
7
205
116
97.4
66
45
29
14.2
FRONT
+

minor bands in the range of 14.4 - 117 kDa. Globulin fraction showed 11 polypeptides, while prolamine and glutelin each had six polypeptide bands. All major bands were observed in the range of 35 to 47 kDa in protein isolates as well as in protein fractions for beach pea (Figure 4.23B). Green pea protein isolates, albumin, globulin and glutelin had 21 polypeptide bands with major bands at 14.2, 25, 40, 45, 66, and 95 kDa (Figure 4.24A). Polypeptide band at 66 kDa was present in green pea prolamine fraction only. Canadian grass pea protein isolates, albumin and glutelin consisted of 16 polypeptide bands on denatured polyacrylamide gel electrophoresis (Figure 4.24B). The intensive bands for protein isolates, albumin and glutelin fractions of Canadian grass pea were observed in the range of 22.5 - 27 kDa and 40 - 65 kDa. Globulin and prolamine showed major bands in the range of 22.5 - 25 kDa as well as 40 - 47 kD. No peptide band was observed in prolamine fraction between 47 and 205 kDa in beach pea and Canadian grass pea protein fractions (Figures 4.23B, 4.24A, and 4.24B). Canadian grass pea protein isolates, albumin, globulin, and glutelin showed one extra peptide band at 116 kDa which was absent in beach pea protein isolates, prolamine, glutelin as well as in green pea protein isolates and protein fractions. These results showed that beach pea proteins are structurally very simple while green pea and Canadian grass pea had complex proteins with higher number of bands and intensities. Deshpande and Campbell (1992b) reported that grass pea was characterized by the presence of all three types of storage protein fractions generally associated with food legumes, the 11S legumin type (apparent molecular weight after dissociation gives 35 - 40 and 22 - 26 kDa), and two 7S, vicilin

(subunit molecular mass 43 - 47 kDa) and convicilin (subunit molecular mass 64 - 66 kDa) types. Idouraine *et al.* (1994) showed that SDS-PAGE of tepary bean in sodium phosphate buffer and salt fractions contained 37 and 27 polypeptides, respectively, with major bands at 29, 45 and 49 kDa. Similar results were reported by Utsumi *et al.* (1980) for vicia faba, Singh *et al.* (1981) for pigeonpea species and by Sathe and Salunkhe (1981c) for Great Northern bean proteins.

4.11 Protein isolates from beach pea, green pea and grass pea and their functional properties

4.11.1 Chemical composition of protein isolates

Protein isolates of beach pea, green pea and Canadian grass pea were prepared by two different solvent extraction procedures [sodium hydroxide and sodium hexametaphosphate, (SHMP)]. Sodium hydroxide-extracted beach pea protein isolate had 86.62% protein, while SHMP-extracted contained 85.07% protein. In case of green pea and Canadian grass pea NaOH- and SHMP-extracted protein isolates had 90.57, 89.95, 90.59, and 88.26% protein content, respectively (Table 4.53). The yield of total protein extracted in various protein isolates ranged from 67.85 to 77.28% for beach pea, 62.95 to 66.96% for green pea and 59.44 to 66.38% for Canadian grass pea. These results indicate that solutions of NaOH and SHMP behave similarly for extraction of proteins from peas. Protein content of SHMP-extracted beach pea isolate was significantly ($p<0.05$) lower than those of green pea and NaOH-extracted Canadian grass pea proteins.

Table 4.53 Chemical composition of beach pea, green pea and grass pea protein isolates[1]

Constituent, %	Beach pea		Green pea		Grass pea[2]	
	NaOH	SHMP	NaOH	SHMP	NaOH	SHMP
Extract recovery	22.84±1.36^{a}	26.49±1.63^{a}	16.34±1.23^{de}	17.50±1.13^{cde}	15.51±1.03^{e}	17.78±1.83^{bcde}
Moisture	2.35±0.01^{e}	2.83±0.01^{a}	2.40±0.21^{d}	2.67±0.15^{b}	2.43±0.02^{cd}	2.92±0.01^{a}
Ash	5.99±0.08^{a}	5.85±0.02^{b}	2.96±0.23^{e}	2.58±0.16^{f}	3.43±0.03^{c}	3.94±0.01^{de}
Protein	86.62±1.34^{ab}	85.07±1.50^{b}	90.57±1.63^{a}	89.95±1.94^{a}	90.59±2.63^{a}	88.26±0.61^{ab}
Lipid	3.20±0.01^{c}	4.03±0.02^{a}	3.05±0.26^{de}	3.20±0.22^{c}	3.02±0.02^{e}	3.24±0.01^{bc}
Crude fibre	1.51±0.02^{b}	1.83±0.03^{a}	0.50±0.01^{e}	0.93±0.01^{d}	0.20±0.01^{f}	1.20±0.03^{c}
Soluble sugars	0.33±0.002^{f}	0.39±0.001^{ef}	0.52±0.06^{c}	0.67±0.05^{a}	0.33±0.002^{de}	0.44±0.002^{bc}
Phenolics (mg/100g)	76.36±0.01^{a}	50.45±0.08^{b}	32.18±0.08^{e}	20.13±0.03^{f}	40.43±0.10^{c}	34.28±0.03^{d}
Condensed tannins	ND	ND	ND	ND	ND	ND
NPN	ND	ND	ND	ND	ND	ND

[1]Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. ND, Not detected.

[2]Canadian grass pea.

During SHMP extraction coagulation of proteins at isoelectric point was observed and therefore adjustment at pH 4.5 (i.e. isoelectric point) was very difficult. This may account for the lower protein content in SHMP-extracted protein isolates compared to that of NaOH-extract. Incomplete recovery of protein may partially be due to losses during the washing process or complexation with other nutrients. The recovery of protein isolates in all preparations was higher in SHMP (17.50 to 26.49%) than NaOH extracts (15.51 to 22.84%); it was significantly higher for beach pea as compared to green pea and Canadian grass pea. The gross recovery of protein isolates ranged from 6.0% (sodium acetate solvent) to 20.6% (sodium hydroxide solvent) in grass pea. While protein content of grass pea protein isolates varied from 83.3 to 92.1% depending upon solvent used (Deshpande and Campbell, 1992b). Sumner *et al.* (1981) showed that the yield of protein in isolates ranged from 59 to 65% and the protein content of isolates ranged from 91 to 98% in different flours prepared from field pea and 8% protein remained in the residue. Ant'Anna *et al.* (1985) used different isolation conditions for preparation of protein isolate from pigeonpea to alter their functional properties and reported yields 49.7 to 63.6% of total protein in various isolates. Taha (1987) used 0.05 N NaOH for preparation of pigeonpea protein isolate and recovered 92% precipitated protein isolates at pH 4.4, with a protein content of 88%. Our results are similar to those of Sathe and Salunkhe (1981a) for protein isolate (92.43% protein) from Great Northern bean prepared by 0.5% Na_2CO_3 solvent extraction.

The ash content of beach pea protein isolate was higher than that of other peas.

All pea protein isolates contained higher amounts of ash than the whole seeds, this may be due to salt formation during protein precipitation at their isoelectric point. Lipid content in protein isolates was high as it concentrated with the protein fraction. The crude fibre content was significantly higher in protein isolates from beach pea (1.51 to 1.83%) than those from green pea (0.50 to 0.93%) and Canadian grass pea (0.20 to 1.20%). Our results are in agreement with those of mung bean, field pea, and green pea (Thompson, 1977; Sumner *et al.*, 1981; Naczk *et al.*, 1986b). Soluble sugars of protein isolates were significantly lower in all pea samples than the whole seeds. The content of phenolic compounds was also reduced in the protein isolates but these were present at higher percentage in beach pea followed by the Canadian grass pea and green pea. Condensed tannins and non-protein nitrogen were not detected in all pea protein isolates examined. These results indicate that the water-soluble constituents such as sugars and phenolic compounds were largely removed under alkaline conditions employed for preparation of protein isolates.

4.11.2 Amino acid composition of protein isolates

The profile of individual amino acids in protein isolates of beach pea, green pea, and Canadian grass pea indicated a slightly lower level of amino acids as compared to those present in the whole seeds. Sulphur-containing amino acids were found in higher quantities in SHMP extraction of beach pea and green pea isolates while sodium hydroxide extraction afforded a higher content of sulphur-containing amino acids in

Canadian grass pea isolate (Table 4.54). Tryptophan content was higher in NaOH-extracted isolates than SHMP isolates in all peas examined. The percentage ratios of essential to total amino acids (E/T, %) for NaOH and SHMP protein isolates (beach pea 43.83 and 44.42, green pea 43.00 and 44.36 and Canadian grass pea 43.10 and 43.21, respectively) were well above 36%, the value reported for an ideal protein by the FAO/WHO (1973). The present results showed that all isolates are rich in lysine, leucine, glutamic acid as well as aspartic acid but limiting in tryptophan, methionine and cysteine. Ant'Anna *et al.* (1985) have shown that the percent ratio of essential to total amino acids varies from 37.8 to 41.2 for pigeonpea protein isolates obtained under various isolation conditions. Ant'Anna *et al.* (1985) and Taha (1987) reported that tryptophan and sulphur-containing amino acids (methionine and cysteine) were limiting in all isolates. The present results show a slightly higher amount of these amino acids in beach pea protein isolates than those of mung bean protein isolates extracted by sodium hydroxide solubilization (Thompson, 1977). The results about the distribution of amino acids in beach pea indicates that it may be used to complement cereal proteins which are low in lysine.

Robaidek (1983) reported that digestibility of proteins is a major factor in protein quality assessment. Therefore, the availability of these amino acids to the body is also a determining factor in protein quality assessment. The predicted biological value of all protein isolates was lower (18 - 40) than those of the whole seed proteins (21 - 65), but the levels were higher in SHMP- than NaOH-extracted isolates. The predicted PER

Table 4.54 Total amino acid composition of beach pea, green pea and grass pea protein isolates extracted with sodium hydroxide and sodium hexametaphosphate (SHMP)[1]

Amino acid	Beach pea		Green pea		Grass pea[2]	
	NaOH	SHMP	NaOH	SHMP	NaOH	SHMP
Isoleucine	4.24±0.34[a]	4.30±0.12[a]	4.23±0.16[a]	4.30±0.29[a]	4.93±0.43[a]	4.69±0.06[a]
Leucine	7.95±0.96[a]	7.97±0.33[a]	7.58±0.33[a]	7.86±0.16[a]	7.57±0.69[a]	7.83±0.05[a]
Lysine	7.83±0.25[a]	7.03±0.10[a]	7.65±0.13[a]	7.57±0.37[a]	6.98±0.52[a]	6.33±0.35[a]
Cysteine[3]	0.87±0.05[a]	1.08±0.04[a]	0.92±0.08[a]	1.09±0.08[a]	0.27±0.10[c]	0.30±0.03[bc]
Methionine[3]	1.05±0.03[a]	0.97±0.03[ab]	0.86±0.03[ab]	0.77±0.05[b]	0.47±0.08[cd]	0.41±0.06[d]
Total sulphur amino acids	1.92	2.05	1.78	1.86	0.74	0.71
Tyrosine	3.12±0.23[a]	3.37±0.90[a]	3.75±0.25[a]	3.70±0.23[a]	3.93±0.32[a]	3.66±0.08[a]
Phenylalanine	4.97±0.13[a]	4.94±0.15[a]	5.29±0.17[a]	5.02±0.25[a]	4.81±0.37[a]	4.76±0.11[a]
Total aromatic amino acids	8.09	8.31	9.04	8.72	8.74	8.42
Threonine	3.49±0.13[a]	3.46±0.67[a]	3.48±0.21[a]	3.57±0.12[a]	3.52±0.31[a]	3.36±0.05[a]
Tryptophan[3]	1.23±0.17[a]	0.84±0.17[a]	0.98±0.05[a]	0.84±0.04[a]	1.30±0.11[a]	1.11±0.09[a]
Valine	4.87±0.29[a]	4.87±0.23[a]	4.56±0.23[a]	4.67±0.21[a]	5.20±0.31[a]	4.88±0.07[a]
Histidine	2.56±0.04[a]	2.49±0.20[a]	2.23±0.21[a]	2.28±0.13[a]	2.62±0.08[a]	2.45±0.05[a]
Total essential amino acids	42.18	41.32	41.53	41.67	41.60	40.28
Arginine	7.86±0.37[bc]	7.78±0.16[c]	8.83±0.36[abc]	8.58±0.28[abc]	9.56±0.73[a]	9.45±0.33[ab]
Aspartic acid + Asparagine	12.59±0.42[ab]	12.37±0.38[ab]	12.41±0.52[ab]	11.45±0.34[b]	13.20±0.24[a]	12.01±0.37[ab]
Glutamic acid + Glutamine	16.55±0.24[a]	15.09±0.98[a]	16.26±0.72[a]	15.65±0.42[a]	17.03±0.42[a]	16.50±0.13[a]
Serine	5.11±0.25[a]	4.82±0.29[a]	4.95±0.20[a]	4.47±0.11[a]	5.41±0.40[a]	4.79±0.17[a]
Proline	4.15±0.15[a]	3.98±0.43[a]	4.33±0.19[a]	4.06±0.16[a]	4.69±0.30[a]	4.37±0.08[a]
Glycine	3.88±0.68[a]	3.78±0.11[a]	4.03±0.11[a]	3.94±0.12[a]	3.69±0.86[a]	3.74±0.02[a]
Alanine	3.91±0.23[a]	3.88±0.12[a]	4.24±0.31[a]	4.12±0.23[a]	1.33±0.32[c]	2.07±0.49[bc]
Total non-essential amino acids	54.05	51.70	55.05	52.27	54.91	52.93
E/T, %	43.83	44.42	43.00	44.36	43.10	43.21
Amino acid score	110.06	107.86	109.17	109.42	108.28	105.08
BV	36.50	40.13	33.55	36.84	17.61	18.08

[1]Results are mean values of triplicate determinations, ± standard deviation. Means followed by different superscripts in each row are significantly ($p<0.05$) different from one another. [2]Canadian grass pea. [3]Limiting amino acid.

values were higher in beach pea protein isolates followed by those of green pea and Canadian grass pea (Table 4.55); all of which were higher than the literature values for seed proteins of cowpea (1.21), pigeonpea (1.82), and *Lathyrus sativus* (negative to 0.03) (Salunkhe and Kadam, 1989).

4.11.3 Functional properties of beach pea, green pea and grass pea protein isolates

4.11.3.1 Water binding capacity of protein isolates

The water binding properties of a protein isolate determine its degree of interaction with water, sometimes these are reported in the literature as water absorption. Beach pea protein isolates had the lowest water binding capacity (257 - 288%) among all sample studied. Canadian grass pea protein isolate had the highest water binding capacity (269 - 311%), but not significantly different from that of green pea protein isolate (263 -305%). Water binding capacity of proteins is a function of several parameters including size, shape, steric factors, conformational characteristics, hydrophilic-hydrophobic balance of amino acids in the protein molecule as well as lipids, carbohydrates and tannins associated with proteins. Thermodynamic properties of the system (interfacial tension, energy of bonding), physicochemical environment (pH, ionic strength, vapour pressure, temperature, presence or absence of surfactants); solubility of the protein molecules are most responsible factors for the water binding capacity of protein isolates (Chou and Morr, 1979). However, polar amino groups of protein molecules are the primary sites of protein-water interactions. Cationic, anionic and nonionic sites bind different amounts of

Table 4.55 The predicted PER values of beach pea, green pea and grass pea protein isolates

Protein isolate	Predicted PER values using equation[1]		
	1	2	3
Beach pea			
NaOH	2.75	2.81	2.44
SHMP	2.76	2.80	2.17
Green pea			
NaOH	2.57	2.58	1.40
SHMP	2.71	2.71	1.64
Grass pea[2]			
NaOH	2.55	2.56	1.14
SHMP	2.68	2.70	1.53

[1]Alsmeyer *et al.* (1974).

[2]Canadian grass pea.

water (Kuntz, 1971). The difference in water binding capacity of protein isolates may be due to protein concentration and possibly their conformational characteristics. Sumner *et al.* (1981) reported that freeze dried protein isolate from field pea had a water absorption capacity of 205%. Similar observations were reported for protein isolates of Great Northern bean (273%, Sathe and Salunkhe, 1981a), *Pisum sativum* (260%, Johnson and Brekke, 1983) and Woodstone pea protein preparation (278 - 293%, Naczk *et al.*, 1986b).

4.11.3.2 Foaming and foam stability of protein isolates

The foaming property and foam stability of beach pea, green pea, and Canadian grass pea, NaOH- and SHMP-extracted protein isolates are presented in Table 4.56. The values are as percentage volume increase after whipping a 1% protein isolates in 100 mL water and foam stability is the volume of foam remaining after a specified time as a percentage of initial foam volume. Beach pea protein isolates exhibited increased foam volume by 128 - 143%. This foam expansion was significantly lower than that for green pea isolates (170 - 185%) and Canadian grass pea (151 - 175%). The low foaming capacity of beach pea protein isolates could be due to inadequate electrostatic repulsions and hence excessive protein-protein interactions to form aggregates that are detrimental to foam formation. Increase in foam expansion in green pea and Canadian grass pea protein isolates might be due to increased solubility, rapid unfolding at the air-water interface, limited intermolecular cohesion and flexibility of the protein surfactant molecules (Kinsella *et al.*, 1985). Lawhon *et al.* (1972) also reported that constituents

Table 4.56 Foaming properties of beach pea, green pea and grass pea protein isolates[1]

Protein isolate	Foam expansion[2] (%)	Foam stability[3] (%)		
		15	30	60
Beach pea				
NaOH	142.82±2.30^{e}	91.55±0.85^{abc}	91.55±1.25^{a}	90.14±1.02^{a}
SHMP	128.35±3.98^{f}	90.62±1.36^{bc}	89.06±1.45^{ab}	87.50±1.83^{a}
Green pea				
NaOH	185.13±2.03^{a}	95.29±2.36^{a}	81.03±1.83^{c}	79.49±1.33^{bc}
SHMP	170.23±1.90^{c}	92.31±2.01^{abc}	76.47±1.72^{d}	64.71±1.28^{d}
Grass pea[4]				
NaOH	175.87±4.18^{bc}	90.86±1.03^{abc}	89.71±1.07^{ab}	89.14±1.67^{a}
SHMP	150.96±3.48^{de}	90.00±2.00^{c}	86.67±1.13^{b}	76.00±1.25^{c}

[1]Results are means of three determinations, ± standard deviation. Means followed by different superscripts in each column are significantly ($p<0.05$) different from one another.

[2]At pH 7.0 percentage volume increase after whipping 100 mL of 1% (w/v) protein solution.

[3]Foam left after 15, 30 and 60 min as a percentage of initial foam volume.

[4]Canadian grass pea.

other than proteins may aid in the formation of whipped foam. As seen in Table 4.58 foams from beach pea and Canadian grass pea protein isolates were more stable than those from green pea protein isolates after standing for 30 and 60 min. Foam volume decreased with increasing rest time. A similar trend was observed in cowpea protein isolate (Aluko and Yada, 1997), as well as those of soybean and sunflower (Lin *et al.*, 1974). Kinsella *et al.* (1985) and Myers (1988) have suggested that in foams, the ability to hold water in the protein film surrounding the air particle and presence of electrostatic repulsions are important for their stability.

4.11.3.3 Fat absorption capacity of protein isolates

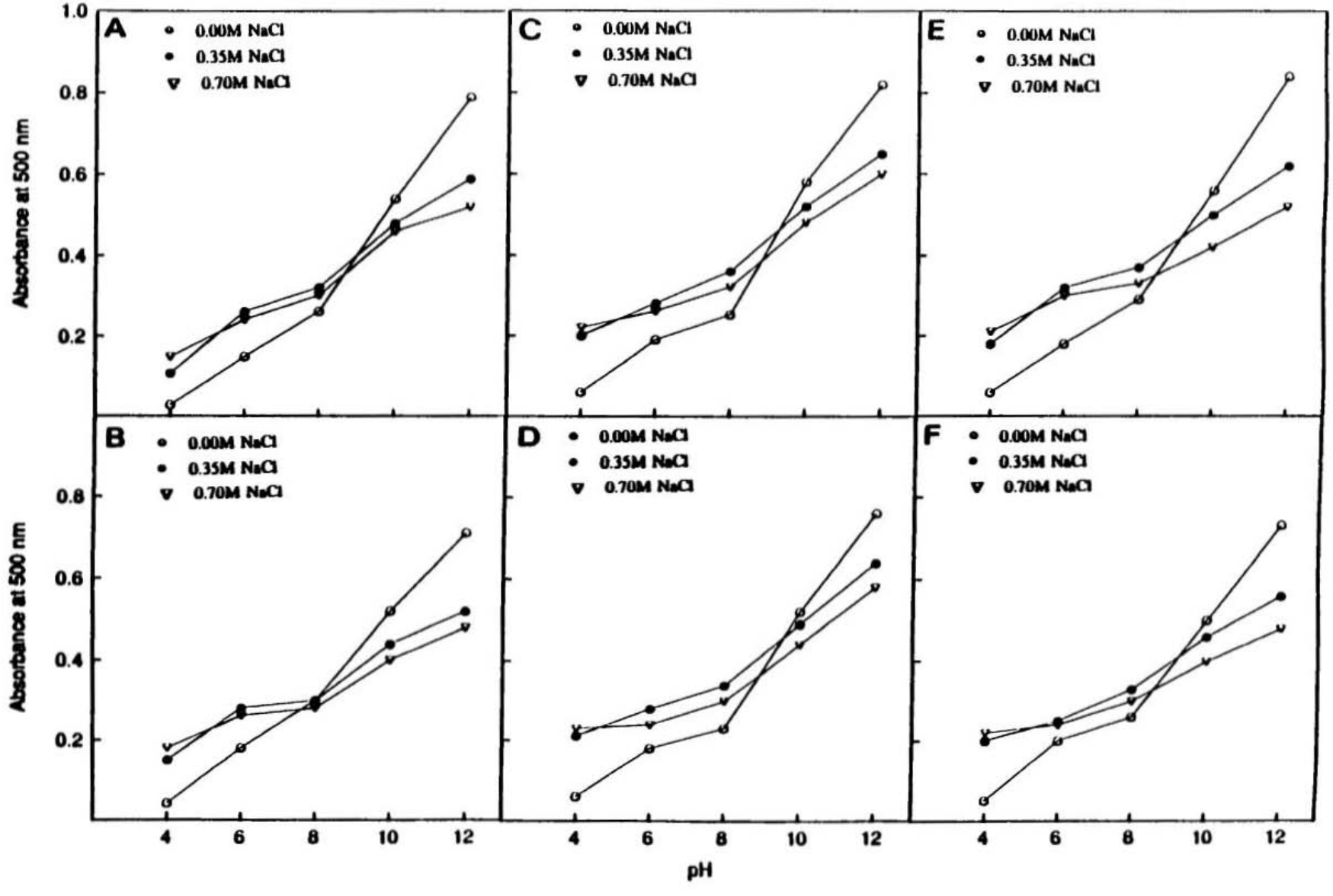
Data on fat absorption clearly showed that beach pea protein isolates had oil absorption values ranging from 64 to 82 mL/100g, while those of green pea and Canadian grass pea protein isolates varied from 86 to 94 and 62 to 85 mL/100g, respectively. Low fat binding capacity of beach pea as well as Canadian grass pea isolates suggests the presence of a large proportion of hydrophilic as compared to hydrophobic groups on the surface of the protein molecules of the isolates. In the present study green pea protein isolate could be more lipophilic than those of beach pea and Canadian grass pea. These results are comparable to those for woodstone pea-protein preparations (fat absorption was 90.1 to 94.5%; Naczk *et al.*, 1986b) and field pea protein isolates (fat absorption was 90 to 127%; Sumner *et al.*, 1981). The mechanism of fat binding by proteins is not fully understood, but it appears to be affected by lipid-protein complexes and protein content

(Kinsella, 1979). Lin *et al.* (1974) showed that the availability of lipophilic groups may also have an important role in contributing to higher binding of fat to proteins. However, low fat absorption may be desirable in some applications, such as Seviya and Chakali prepared by deep fat frying of legume-based products.

4.11.3.4 Emulsifying activity and stability of protein isolates

Emulsifying activity (EA) of beach pea, green pea and Canadian grass pea protein isolates (NaOH- and SHMP-extracted), measured as a function of pH and NaCl concentration, are shown in Figures 4.25, A and B (beach pea), C and D (green pea), and E and F (Canadian grass pea). Emulsion activity of all protein isolates increased as the with pH. NaCl at 0.35 and 0.70M increased EA compared to the samples without salt at initial stage (pH 4 - 9), but after that decreased as pH increased. At higher pH and salt concentration the EA of all protein isolates decreased. In all cases beach pea protein isolates had slightly lower emulsifying activity than Canadian grass pea but similar to green pea protein isolates. There was insignificant ($p>0.05$) difference in emulsifying activity of NaOH- and SHMP-extracted protein isolates of all three peas examined (Figure 4.25). The effect of NaCl on EA may be due to its effect on protein adsorption at the oil-water (O/W) interface. Results of the present study, where EA first increases and then decreases with addition of NaCl, are in accord with those of Waniska *et al.* (1981) and Paulson and Tung (1988). In the present study EA increased with pH, which suggests that droplet size might have decreased as the pH was increased beyond the isoelectric

Figure 4.25 Emulsifying activity of protein isolates (measured as absorbance at 500 nm) as a function of pH and NaCl concentration: beach pea (A) NaOH-extracted, (B) SHMP-extracted; green pea (C) NaOH-extracted, (D) SHMP-extracted; Canadian grass pea (E) NaOH-extracted, and (F) SHMP-extracted.


A
B
C
D
E
F
0.00M NaCl
0.35M NaCl
0.70M NaCl
Absorbance at 500 nm
pH
1.0
0.8
0.6
0.4
0.2
4
6
8
10
12

region.

The effects of pH and salt concentration on emulsion stability (ES) of beach pea, green pea and Canadian grass pea are presented in Figures 4.26. Emulsion stability of beach pea protein isolates was higher than those of green pea and Canadian grass pea. Increased with increasing pH but decreased with increase in NaCl concentration. Differences in emulsion stability of NaOH- and SHMP-extracted beach pea, green pea and Canadian grass pea protein isolates were not significanmt. The low ES at low pH and salt concentration may be attributed to increased interaction between the emulsified droplets since net charge on the proteins is decreased by the presence of chloride ions. As the pH and ionic strength increased, the columbic repulsion increased between neighbouring droplets coupled with increased hydration of the charged protein molecules which may account for the higher ES obtained. These results are in agreement with literature values for cowpea (Aluko and Yada, 1997), canola (Paulson and Tung, 1988), and green pea protein isolates (Johnson and Brekke, 1983).

4.11.3.5 Solubility of protein isolates

Solubility of protein isolates from extracted by NaOH and SHMP beach pea, green pea and Canadian grass pea, as a function of pH and NaCl concentration is presented in Figures 4.27, A and B (beach pea), C and D (green pea) and E and F (Canadian grass pea). In the absence of NaCl the protein isolates exhibited a gradual increase in solubility above and below their isoelectric points. Protein isolates from beach pea, green pea and

Figure 4.26 Emulsion stability (time required to reduce the absorbance at 500 nm by 50%) of beach pea protein isolates (A) NaOH-extracted, (B) SHMP-extracted; green pea protein isolates (C) NaOH-extracted, (D) SHMP-extracted and Canadian grass pea protein isolates (E) NaOH-extracted, (F) SHMP-extracted as a function of pH and NaCl concentration.

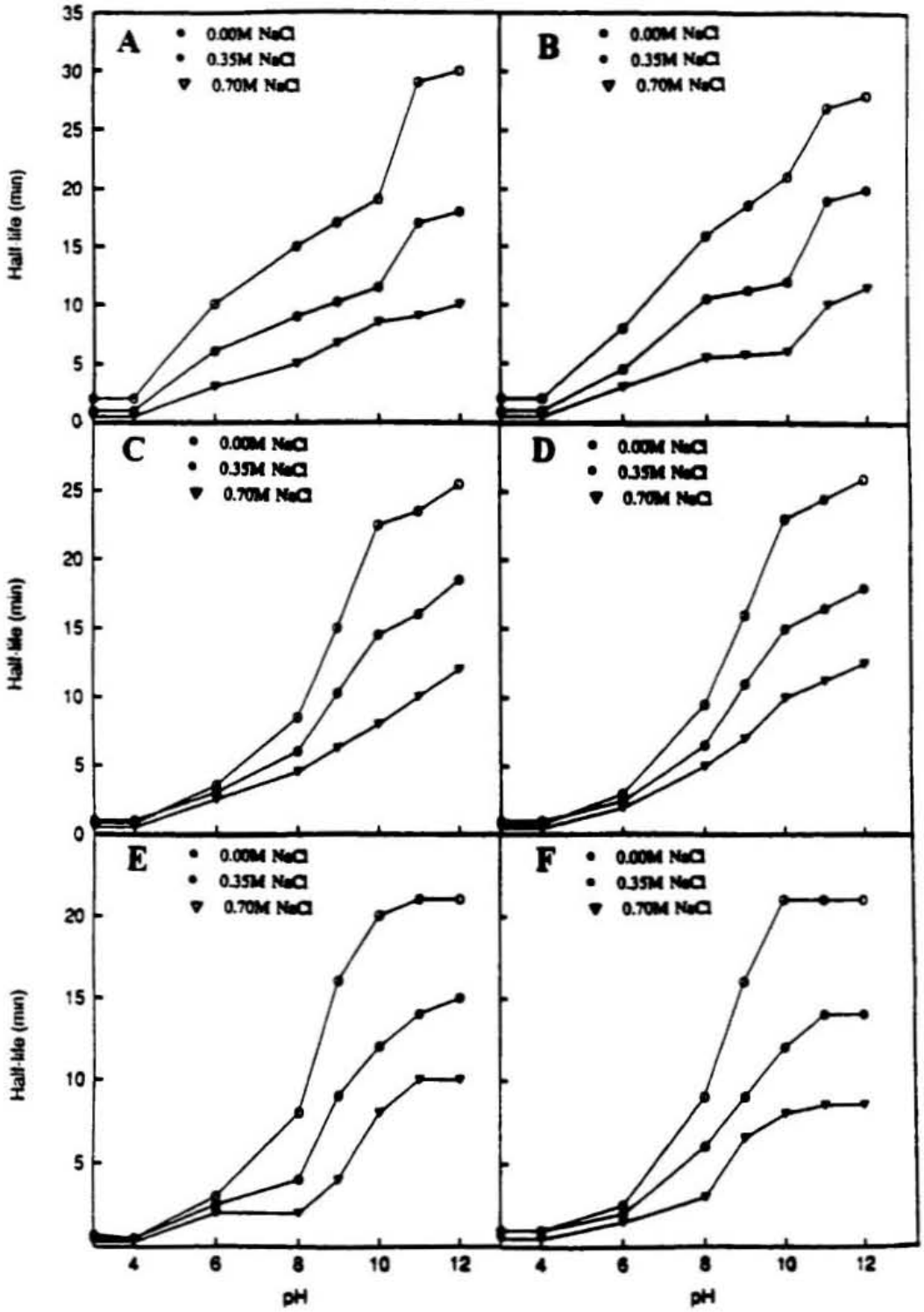
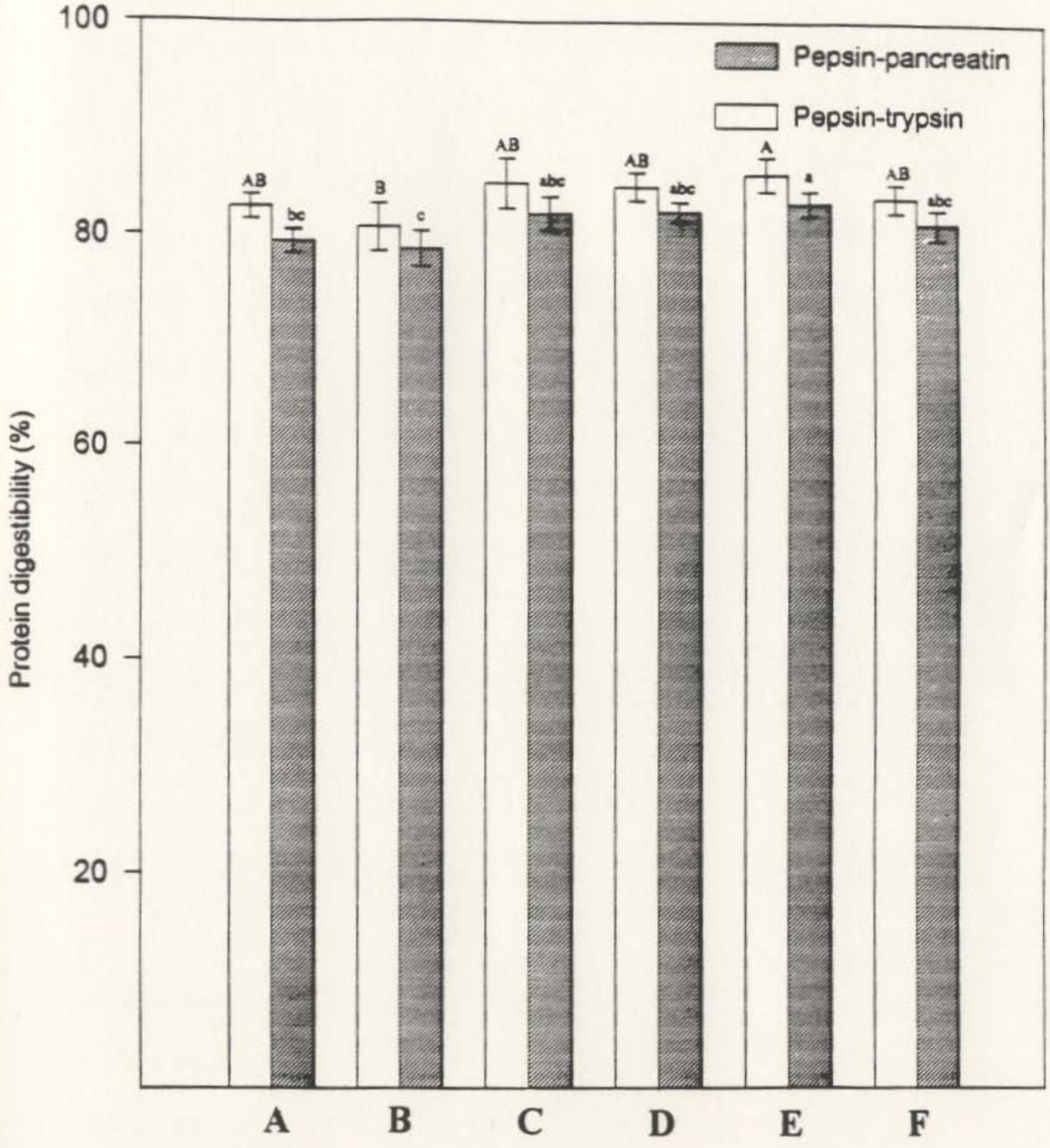

A
B
C
D
E
F
0.00M NaCl
0.35M NaCl
0.70M NaCl
Half life (min)
Half-life (min)
pH
4
6
8
10
12
0
5
10
15
20
25
30
35

Figure 4.27 Solubility of protein isolates as function of pH and NaCl concentration: beach pea (A) NaOH-extracted, (B) SHMP-extracted; green pea (C) NaOH-extracted, (D) SHMP-extracted; Canadian grass pea (E) NaOH-extracted and (F) SHMP-extracted.

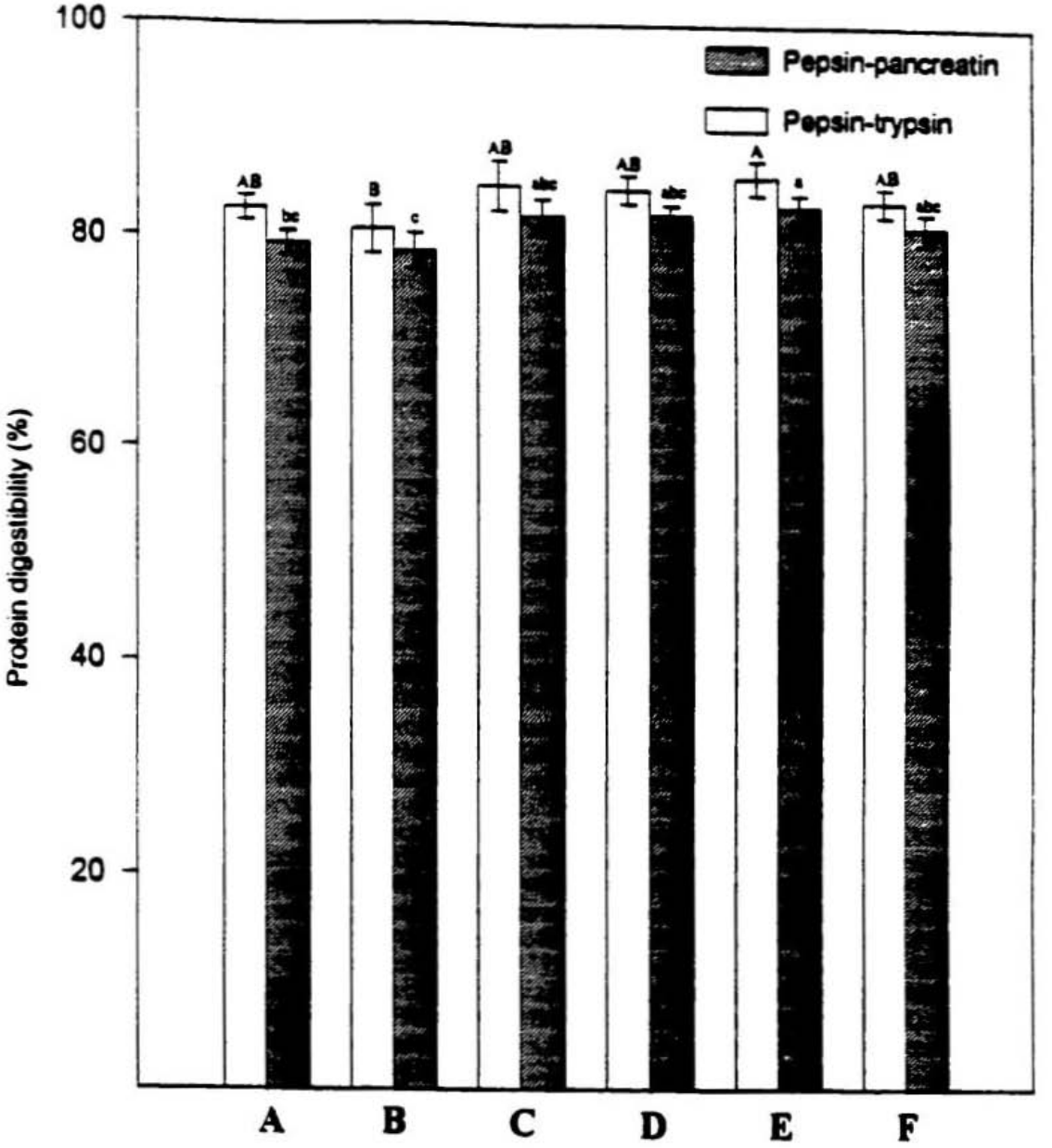

100
80
60
40
20
Protein digestibility (%)
Pepsin-pancreatin
Pepsin-trypsin
AB
bc
B
c
AB
abc
AB
abc
A
a
AB
abc
A
B
C
D
E
F

Canadian grass pea prepared with NaOH and SHMP showed a minimal solubility at pH 4.5, where they were separated using isoelectric precipitation (Figure 4.27). Beach pea protein isolates had higher solubility at their isoelectric point than those of green pea and Canadian grass pea isolates. This might be due to the presence of other compounds in the beach pea protein isolates. Åman and Gillberg (1977) reported that in addition to protein, isolates also may contain RNA, acidic polysaccharides, phytic acid and acidic polyphenols extracted from the meal. The effect of NaCl concentration on the protein isolates was to increase protein solubility at isoelectric region and decrease solubility in the higher acidic and alkaline regions. The reduction in solubility of protein isolates in beach pea was higher when pH and salt concentration increased as compared to those of green pea and Canadian grass pea. The solubility of green pea and Canadian grass pea isolates were similar, with or without salt addition. These results are similar to those obtained for green pea (Johnson and Brekke, 1983), sesame seed (Prakash, 1986), canola (Paulson and Tung, 1987), soybean flour (McWatters and Holmes, 1979), and cowpea (Aluko and Yada, 1995, 1997).

4.12 *In-vitro* digestibility of protein isolates

In-vitro digestibility of beach pea, green pea, and Canadian grass pea protein isolates with pepsin-trypsin and pepsin-pancreatin is shown in Figure 4.28. Beach pea protein isolates (NaOH- and SHMP-extracted) had lower digestibility (pepsin-trypsin 80.6 to 82.6% and pepsin-pancreatin 78.6 to 79.2%) than green pea and Canadian grass pea

Figure 4.28 *In-vitro* digestibility of protein isolates: [A, beach pea NaOH-extracted; B, beach pea SHMP-extracted; C, Canadian grass pea NaOH-extracted; D, Canadian grass pea SHMP-extracted; E, green pea NaOH-extracted; F, green pea SHMP-extracted].
Error bars having different superscripts are significantly ($p<0.05$) different from one another.


100
80
60
40
20
Protein digestibility (%)
Pepsin-pancreatin
Pepsin-trypsin
AB
B
AB
AB
A
AB
A
B
C
D
E
F

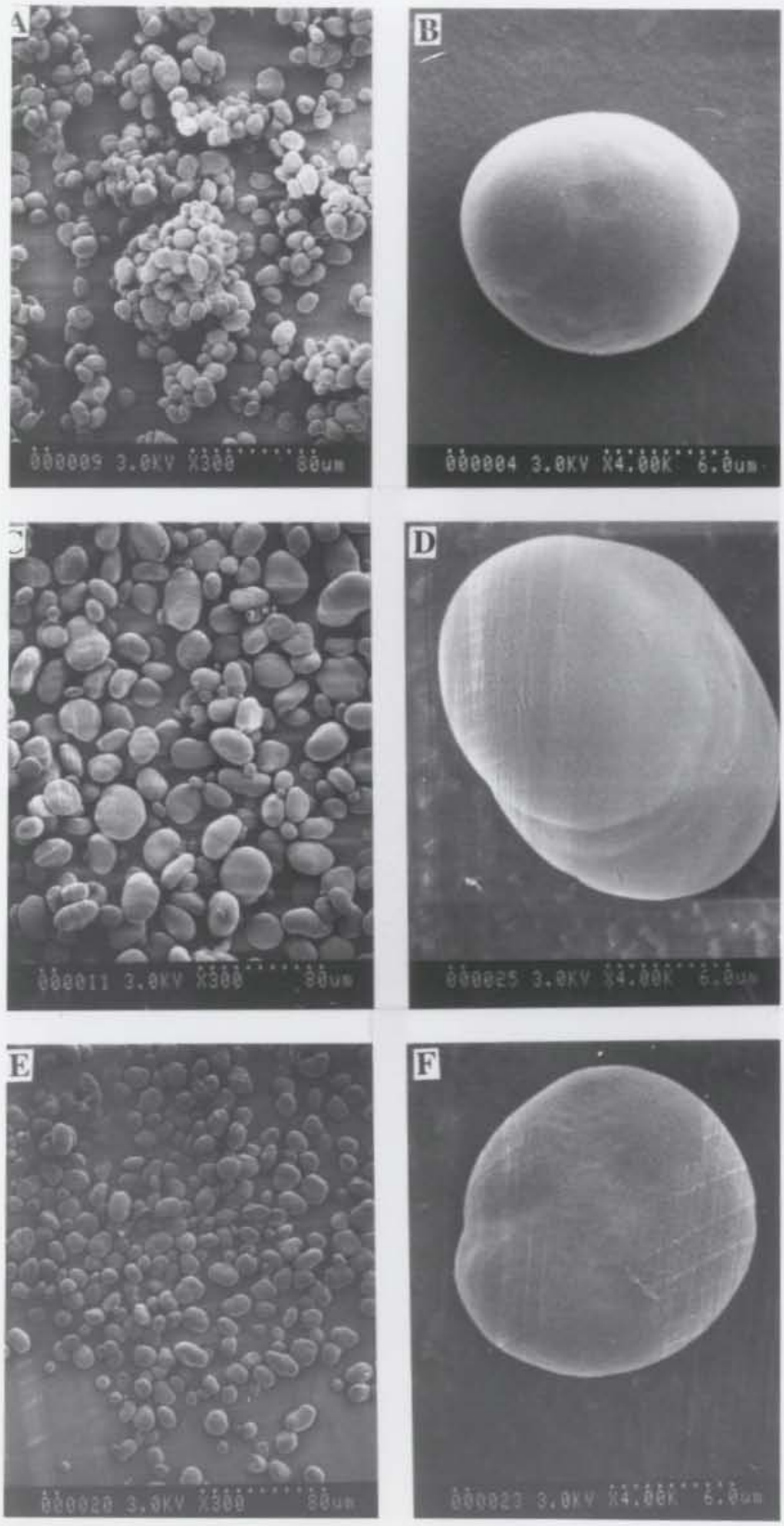
(pepsin-trypsin 83.4 to 85.7% and 84.5 to 84.8%, pepsin-pancreatin 80.9 to 82.9% and 81.9 to 82.1%, respectively) types, although the difference was not significant. SHMP-extracted protein isolates of all peas types showed similar digestibility with pepsin-trypsin as well as pepsin-pancreatin as compared to NaOH-extracted isolates. Protein digestibility of isolates was significantly ($p<0.05$) higher than the whole meal protein digestibility for cowpeas (73%) and pigeonpeas (59%) (Salunkhe and Kadam, 1989). Johnson and Brekke (1983) reported that *in-vitro* digestibility of green pea protein isolates with a multi-enzyme system was 84%. Le-Guen *et al.* (1995) studied digestibility of protein isolates in piglets from two varieties of pea (Finale and Frijaune) and reported that protein digestibility ranged from 83.7 to 85.4%. Similarly, Wanasundara and Shahidi (1997) have shown that flaxseed protein isolates had an *in-vitro* protein digestibility of 90% with pepsin-trypsin and pepsin-pancreatin enzymes.

4.13 Starch isolation and characterization from beach pea and comparison with green pea and grass pea starches

4.13.1 Morphological granular characteristics of starches

Microscopic examination showed that starch granules of beach pea, green pea and Canadian grass pea had irregular shapes which varied from oval to round (6 to 33 μm) to elliptical (shorter diameter, 11 to 22 μm; longer diameter, 17 to 35 μm) (Figure 4.29). The size of beach pea starch granules was smaller than those reported for other legume starches (Hoover and Sosulski, 1991). The surfaces of the above starches appeared to be

Figure 4.29 Scanning electron micrographs of legume starches: (A) and (B) beach pea; (C) and (D) green pea; and (E) and (F) Canadian grass pea.


A
B
C
D
E
F

smooth and showed no evidence of fissures when viewed under the scanning electron microscope (SEM) (Figure 4.29). Granule clustering was more evident in beach pea (Figure 4.29A) than in green pea (Figure 4.29C) and Canadian grass pea (Figure 4.29E) starches.

4.13.2 Chemical composition of starches

The data on composition and yield of starches from beach pea, green pea and Canadian grass pea are presented in Table 4.57. The purity of the starches was judged on the basis of composition and microscopic examination. The yield of starch from beach pea, green pea and Canadian grass pea was 12, 30 and 26%, respectively. The value for beach pea starch was much lower than the range (18 - 45%) reported (Hoover and Sosulski, 1991) for most legume starches. Isolation of starches from legumes is generally difficult due to the presence of a highly hydrated fine fibre fraction (Vose, 1977) which is derived from the cell wall enclosing the starch granules (Schoch and Maywald, 1968). The ash content (beach pea >> green pea ≈ Canadian grass pea) (Table 4.57) which reflects contamination with fine fibre, suggests that the low yield of starch from beach pea seeds is due to its higher fine fibre content. The nitrogen content was 0.08, 0.09 and 0.07% in beach pea, green pea and Canadian grass pea starches, respectively. These low values indicate the absence of non-starch lipids (lipids associated with endosperm proteins). Therefore, total lipids (obtained by acid hydrolysis) in beach pea (0.16%), green pea (0.19%) and Canadian grass pea (0.12%) starches (Table 4.57) represent the

Table 4.57 Chemical composition (%) of beach pea, green pea and grass pea starches[1]

Characteristic	Starch source		
	Beach pea	Green pea	Grass pea[2]
Yield (% initial material)	12.3±2.21^{c}	30.0±1.91^{a}	26.0±1.13^{b}
Moisture	10.57±0.07^{a}	10.60±0.42^{a}	10.87±0.03^{a}
Ash	0.22±0.03^{a}	0.07±0.01^{bc}	0.05±0.01^{c}
Nitrogen	0.08±0.01^{a}	0.09±0.02^{a}	0.07±0.01^{a}
Lipid			
Acid hydrolyzed	0.16±0.02^{ab}	0.19±0.02^{a}	0.12±0.01^{b}
Solvent extracted:			
chloroform:methanol(2:1)	0.06±0.02^{a}	0.07±0.02^{a}	0.05±0.01^{a}
n-propanol:water (3:1)	0.10±0.00^{b}	0.12±0.01^{a}	0.07±0.01^{c}
Amylose content(% of total starch)			
Apparent	27.30±0.43^{c}	32.67±0.17^{b}	34.52±0.35^{a}
Total	29.02±0.20^{b}	36.70±0.26^{a}	36.37±0.31^{a}
Amylose complexed by native lipid	5.9	11.0	5.1
Starch damage	4.9±0.11^{a}	1.9±0.11^{b}	1.7±0.12^{b}
Granule shape	Round to elliptical	Round to elliptical	Round to elliptical
Granule size (μm)			
Round	6 to 17	14 to 33	13 to 17
Elliptical			
Short diameter	11	22	13
Long diameter	17	35	21

[1]All data reported on a dry weight basis and represent the means of three determinations, ± S.D. Means in each row with different superscripts are significantly different ($p<0.05$).

[2]Canadian grass pea.

free and bound starch lipids. The total lipid content (Table 4.57) of beach pea starch was within the range reported for most legume starches (Hoover and Sosulski, 1991). The amount of bound lipids of beach pea starch (extracted with propanol-water) (Table 4.57) was higher (0.10%) than that of Canadian grass pea (0.07%) but lower than that of green pea (0.12%) starch. These values were within the range reported for other legume starches (Hoover and Manuel, 1996). A comparison of the apparent and total amylose content (Table 4.57) showed that 5.9, 11.0 and 5.1% of the total amylose was complexed with native starch lipids in beach pea, green pea and Canadian grass pea starches, respectively. The value for beach pea (5.9%) was comparable to that of CC gold lentil starch (5.6%) (Hoover and Manuel, 1995), but was lower than those reported for starches from mung bean (12.1%; Hoover *et al.*, 1997) and laird lentil (12.4%; Hoover and Manuel, 1995). The extent of starch damage during wet milling was more pronounced in beach pea (4.9%) than in Canadian grass pea (1.7%) and green pea (1.9%) (Table 4.57). This is not surprising, since the seed coat of beach pea did not soften (steeping in water at 50 °C, 48 h) to the same extent as the seed coats of the other two legume seeds.

4.13.3 X-ray diffraction

Beach pea and Canadian grass pea starches showed the characteristic 'C' pattern of legume starches (Colonna *et al.*, 1981; Hoover and Sosulski, 1985c; Gernat *et al.*, 1990; Hoover and Manuel, 1996; Hoover *et al.*, 1997). In beach pea starch the X-ray pattern was characterized by a strong intensity peak at 5.12 Å, a medium intensity peak

at 3.86 Å and a weak intensity peak at 5.80 Å (Figure 4.30, Table 4.58). In Canadian grass pea starch, the strong intensity peak occurred at 5.19 Å and the medium and weak intensity peaks occurred at 3.89 and 5.86 Å, respectively (Figure 4.30, Table 4.58). Green pea starch showed a strong intensity peak at 5.15 Å, two medium intensity peaks at 5.85 and 3.82 Å and a weak intensity peak at 15.7 Å (The peak at 15.7 Å is characteristic of tuber starches). Gernat *et al.* (1990) have shown that the legume starch 'C' crystalline polymorph is a mixture of 'A' and 'B' unit cells, and that these starches contain pure 'A' and 'B' polymorphs in varying proportions. The results suggest that beach pea and Canadian grass pea starches have a higher proportion of 'A' unit cells than green pea starch. Starch crystallites are due to sequential packing of double helices (Wu and Sarko, 1978a) that are found between the flexible 'A' chains of amylopectin (French, 1972). The difference in X-ray intensities among the starches cannot be attributed to differences in crystallite size [since all these starches exhibit sharp X-ray patterns (Figure 4.30)] or to amylopectin content [since beach pea starch with a higher amylopectin content (Table 4.57) exhibits the weakest X-ray pattern (Figure 4.30)]. Therefore, differences in X-ray intensities (Table 4.58) are probably due to the manner in which the double helices are arranged within the crystalline domains of the granule. The results indicate that the double helices of beach pea starch are less compactly packed and/or less well arranged to diffract X-rays than those of Canadian grass pea and green pea starches.

Figure 4.30 X-ray diffraction patterns of: (A) beach pea starch; (B) Canadian grass pea starch; (C) green pea starch.

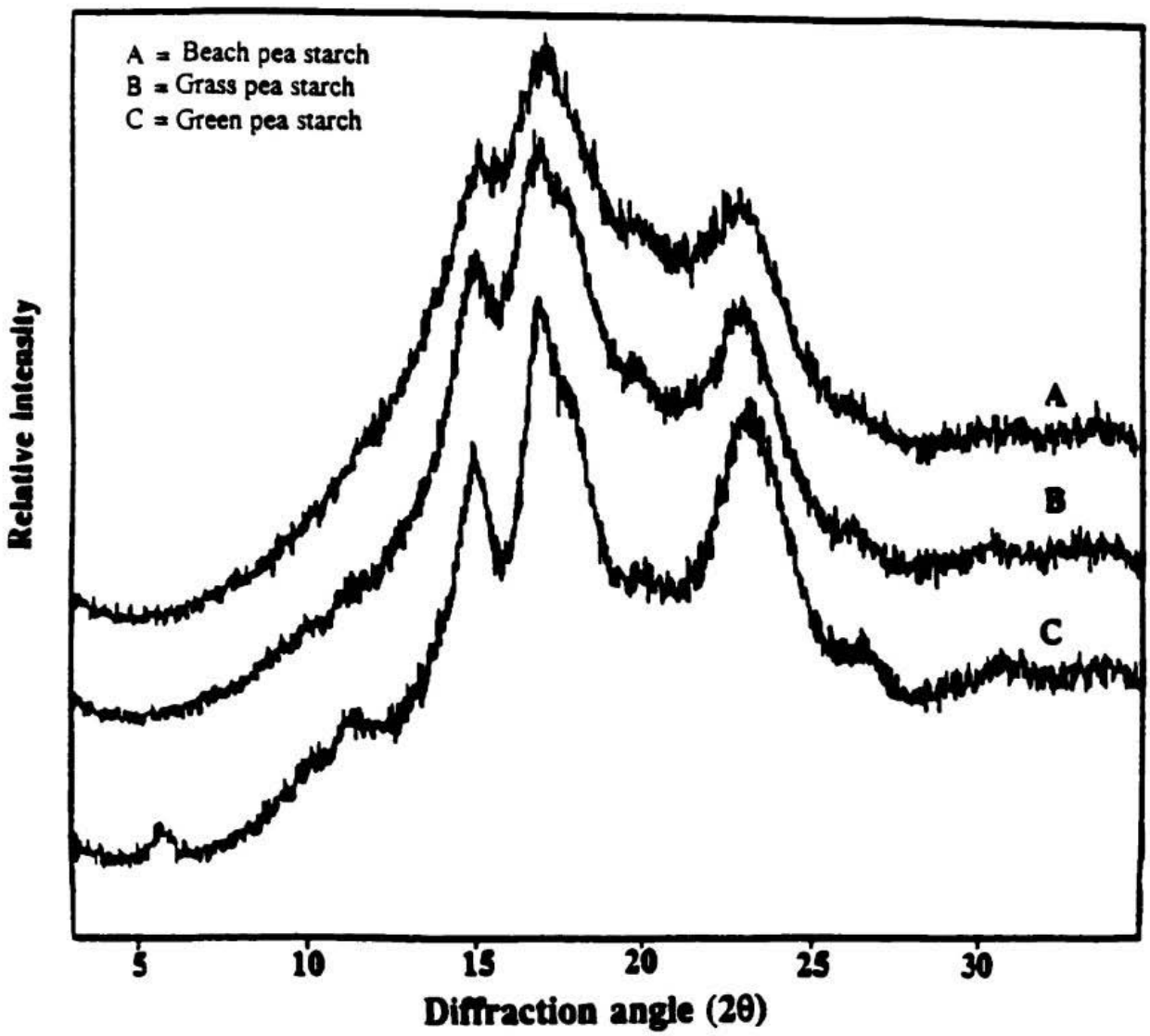

A = Beach pea starch
B = Grass pea starch
C = Green pea starch
Relative Intensity
A
B
C
5
10
15
20
25
30
Diffraction angle (2θ)

Table 4.58 X-ray diffraction intensities of the major peaks of beach pea, green pea, and grass pea starches

Starch source	Interplanar spacings (d) in Å with intensities (CPS)[1]
Beach pea[2]	5.80 (490), 5.12 (1149), 3.86 (843)
Grass pea[3]	5.86 (901), 5.19 (1478), 3.89 (1022)
Green pea[4]	15.73 (192), 5.85 (967), 5.15 (1750), 3.82 (1331)

[1]Counts per second.

[2]Moisture content 10.57%.

[3]Moisture content 10.60%, Canadian grass pea.

[4]Moisture content 10.87%.

4.13.4 Swelling factor (SF) and amylose leaching (AML)

The swelling factor (SF) and amylose leaching were investigated over the temperature range of 50 - 95 °C. The results are presented in Tables 4.59. The SF followed the order: green pea > beach pea > Canadian grass pea (Table 4.59). However, the corresponding order for AML was: Canadian grass pea ≈ green pea > beach pea (Table 4.59). The SF and AML values were within the range reported for other legume starches (Hoover and Manuel, 1996). Starch granule swelling is known to begin in the bulk of relatively mobile amorphous fraction and in the more restrained amorphous regions immediately adjacent to the crystalline region (Donovan, 1979). Furthermore, amylose-lipid complexes have been shown to inhibit granule swelling (Maningat and Juliano, 1980; Tester and Morrison, 1990; Hoover and Manuel, 1996). The observed order in SF (Table 4.59) suggests that bound lipid content (Table 4.57) is not a factor influencing granule swelling. It is likely that interactions between amylose chains within the amorphous domains of the granule (these interactions would reduce hydration of amylose chains) negate the influence of bound-lipids on granular swelling. The results indicate that the magnitude of this interaction follows the order: Canadian grass pea > beach pea > green pea. The results (Table 4.59) suggest that the extent of AML in these starches is influenced by the differences in amylose content (green pea ≈ Canadian grass pea > beach pea), bound lipid content (green pea > beach pea > Canadian grass pea) and by the magnitude of interaction between amylose chains within the native granules (Canadian grass pea > beach pea > green pea).

Table 4.59 Swelling factor and leached amylose content of beach pea, green pea and grass pea starches at different temperatures[1]

Temperature (°C)	Swelling factor			Leached amylose (% dry weight)		
	Beach pea	Green pea	Grass pea[2]	Beach pea	Green pea	Grass pea[2]
50	7.33±0.17[a]	7.52±1.02[a]	1.42±0.14[b]	———	———	———
60	8.55±0.05[a]	8.94±1.08[a]	1.56±0.20[b]	———	———	———
70	16.73±0.18[a]	17.70±1.06[a]	10.02±0.08[b]	3.43±0.03[b]	6.16±1.09[a]	6.25±0.09[a]
80	18.43±0.10[b]	21.11±1.07[a]	13.03±0.07[c]	7.54±0.08[b]	14.33±1.03[a]	15.07±0.54[a]
85	19.61±0.13[b]	22.41±1.03[a]	14.91±0.07[c]	9.84±0.29[b]	15.08±1.10[a]	15.66±0.53[a]
90	24.92±0.21[b]	28.01±0.37[a]	19.58±0.13[c]	11.55±0.52[b]	16.69±1.02[a]	17.68±0.10[a]
95	30.72±0.82[b]	34.13±0.24[a]	26.01±0.07[c]	12.94±0.18[b]	17.08±1.57[a]	19.07±0.13[a]

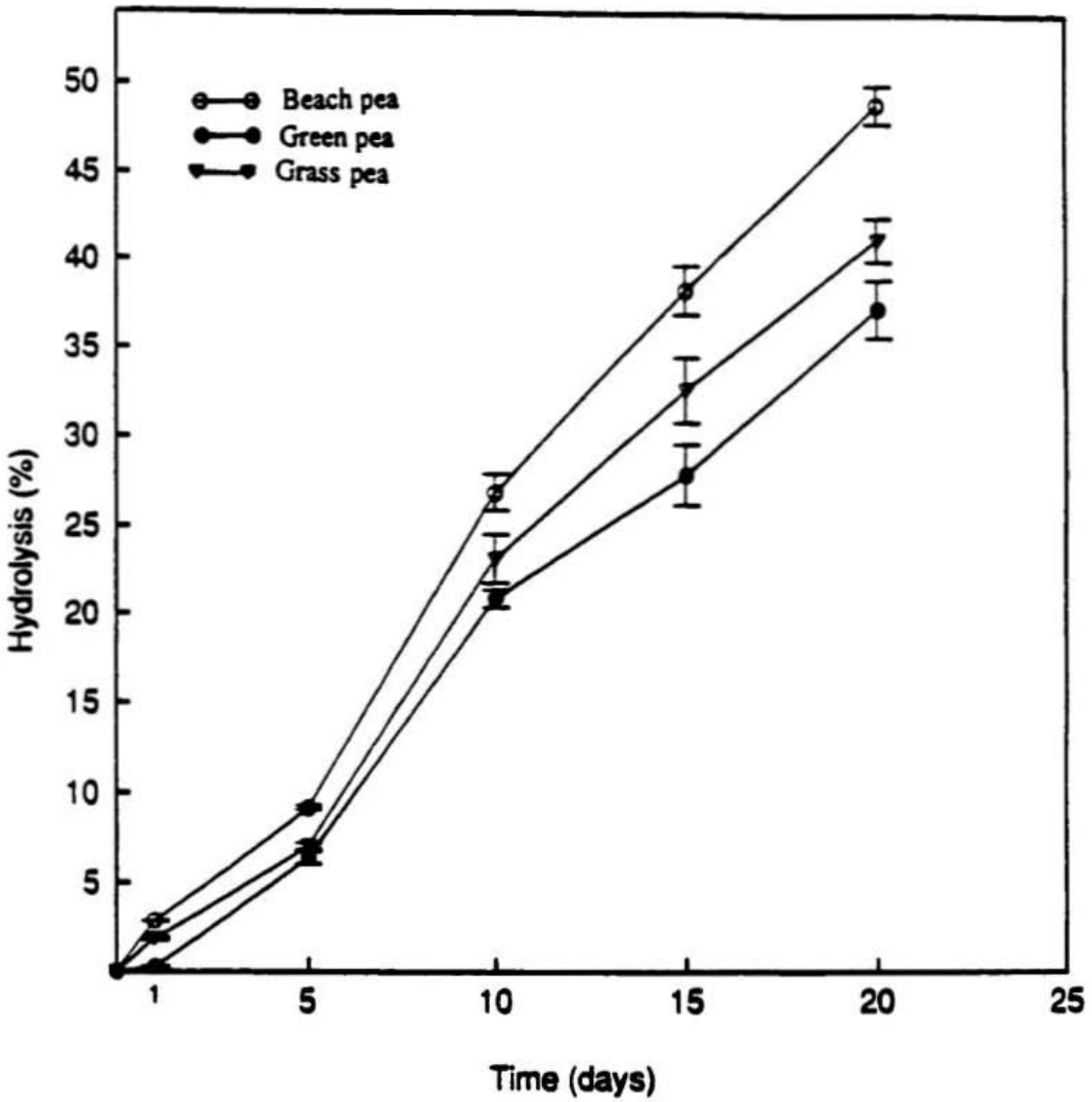
[1]The data represent the means of four determinations, ± S.D. Means in each row with different superscripts are significantly different ($p<0.05$) from one another. ——— Amylose leaching was not observed at these temperatures.

[2]Canadian grass pea.

4.13.5 Acid hydrolysis

The hydrolysis of the legume starches by 2.2 N HCl is presented in Figure 4.31. All three starches exhibited a two-stage solubilization pattern. A relatively higher rate was observed during the first 10 days, followed by a slower rate between 10 and 20 days. At the end of the 10th day of hydrolysis [corresponding to the degradation of the amorphous region of the granule (Kainuma and French, 1971; Cairns *et al.*, 1990)], beach pea, Canadian grass pea and green pea starches were hydrolyzed by 27, 23 and 21%, respectively. These values were comparable to those of other legume starches (Hoover and Manuel, 1995; Hoover *et al.*, 1993; Hoover and Manuel, 1996). The rate of increase in hydrolysis beyond day 10 [corresponding to degradation of the crystallite region (Kainuma and French, 1971; Cairns *et al.*, 1990)] followed the order: beach pea > Canadian grass pea > green pea. After 20 days, beach pea, Canadian grass pea and green pea starches were hydrolyzed by 49, 41 and 37%, respectively. Morrison *et al.* (1993) have shown, by studies on lintnerized barley starches (covering a wide range of amylose and lipid contents), that lipid complexed with amylose chains are resistant to acid hydrolysis. Furthermore, several researchers (BeMiller, 1967; Kainuma and French, 1971; Hoover and Manuel, 1996) have shown that a change in conformation of D-glucopyranose units (chair → half chair) is a pre-requisite for hydrolysis of glucosidic bonds by H_3O^+. These transformations would be more difficult in lipid complexed amylose chains (due to a decrease in chain flexibility). Thus, the higher resistance of green pea starch towards acid hydrolysis may be attributed to its higher content of amylose-lipid complexes (Table

Figure 4.31 Time course of acid hydrolysis (2.2 N HCl) of beach pea (⊶), green pea (⊷), and Canadian grass pea (▽⊷▽) starches. The data represent mean values of three determinations.


Beach pea
Green pea
Grass pea
Hydrolysis (%)
50
45
40
35
30
25
20
15
10
5
1
5
10
15
20
25
Time (days)

4.57). In this regard Canadian grass pea starch should have been hydrolyzed to a grater extent than beach pea starch due to its lower bound lipid content (Table 4.57). However, the observed extent of hydrolysis (beach pea > Canadian grass pea) suggests that this difference in hydrolysis is mainly influenced by the magnitude of interaction between amylose chains (Canadian grass pea > beach pea) within the amorphous domains of the starch granules. Strong associations between amylose chains will decrease the accessibility of the glucosidic linkages towards H_3O^+. The above results have shown that susceptibility towards acid hydrolysis during the first 10 days is influenced by the interplay of bound-lipid content and amylose chain associations within the amorphous domains of the starch granule.

The crystalline regions (consisting basically of double helices of external A and B chains of amylopectin) are generally less accessible than the amorphous regions to attack by hydrated protons (Kainuma and French, 1971; Robin *et al.*, 1974; Cairns *et al.*, 1990), due to dense packing of starch chains within the starch crystallites and to the high activation energy (Wu and Sarko, 1978a,b) required to change the conformation of the glucose units (within the starch crystallites) from a chair to a half chair form (a pre-requisite for acid hydrolysis). The increase in hydrolysis (beach pea > Canadian grass pea > green pea) beyond the 10th day (Figure 4.31) suggests that crystallites in beach pea starch are loosely packed and/or are fewer in number than in Canadian grass pea and green pea starches. This seems possible since the X-ray diffraction pattern (Figure 4.30) of beach pea starch was much weaker than those of Canadian grass pea and green pea

starches (Figure 4.30).

4.13.6 Enzyme hydrolysis

The extent of hydrolysis of starches by porcine pancreatic α-amylase is presented in Figures 4.32 and 4.33. The results indicate that beach pea is a better substrate than Canadian grass pea and green pea starches, undergoing 35% hydrolysis in 24 hours as compared to 22 and 16%, respectively. Furthermore, the rate of increase in hydrolysis during the 24 h period was more pronounced in beach pea than in the other two starches (Canadian grass pea > green pea). The mode of attack by α-amylase on native granules (after 24 h) was investigated by SEM (Figures 4.33A - F). Granules of beach pea starch were more extensively degraded (Figures 4.33A,B) than those of green pea (Figures 4.33C, D) and Canadian grass pea (Figures 4.33E, F) starches (Canadian grass pea > green pea). Attack by α-amylase on green pea starch granules manifested itself in only mild superficial surface erosion (Figures 4.33C, D). In contrast, the surface of beach pea starch granules was extensively eroded with numerous fissures on the entire granule surface (Figure 4.33B). Furthermore, many granules of beach pea starch were split open (Figure 4.33B). The surface of Canadian grass pea starch granules was also covered with fissures, but in this case the extent of erosion was less pronounced than that in beach pea starch (Figure 4.33F). Granule splitting due to α-amylase action was not evident in Canadian grass pea starch.

Thoma (1968) postulated that the enzyme-catalyzed hydrolysis of α-D-(1 → 4)

Figure 4.32 Time course of hydrolysis of beach pea (—○—), green pea (—●—), and Canadian grass pea (—▽—) starches by porcine pancreatic α-amylase. The data represent the mean of four determinations.

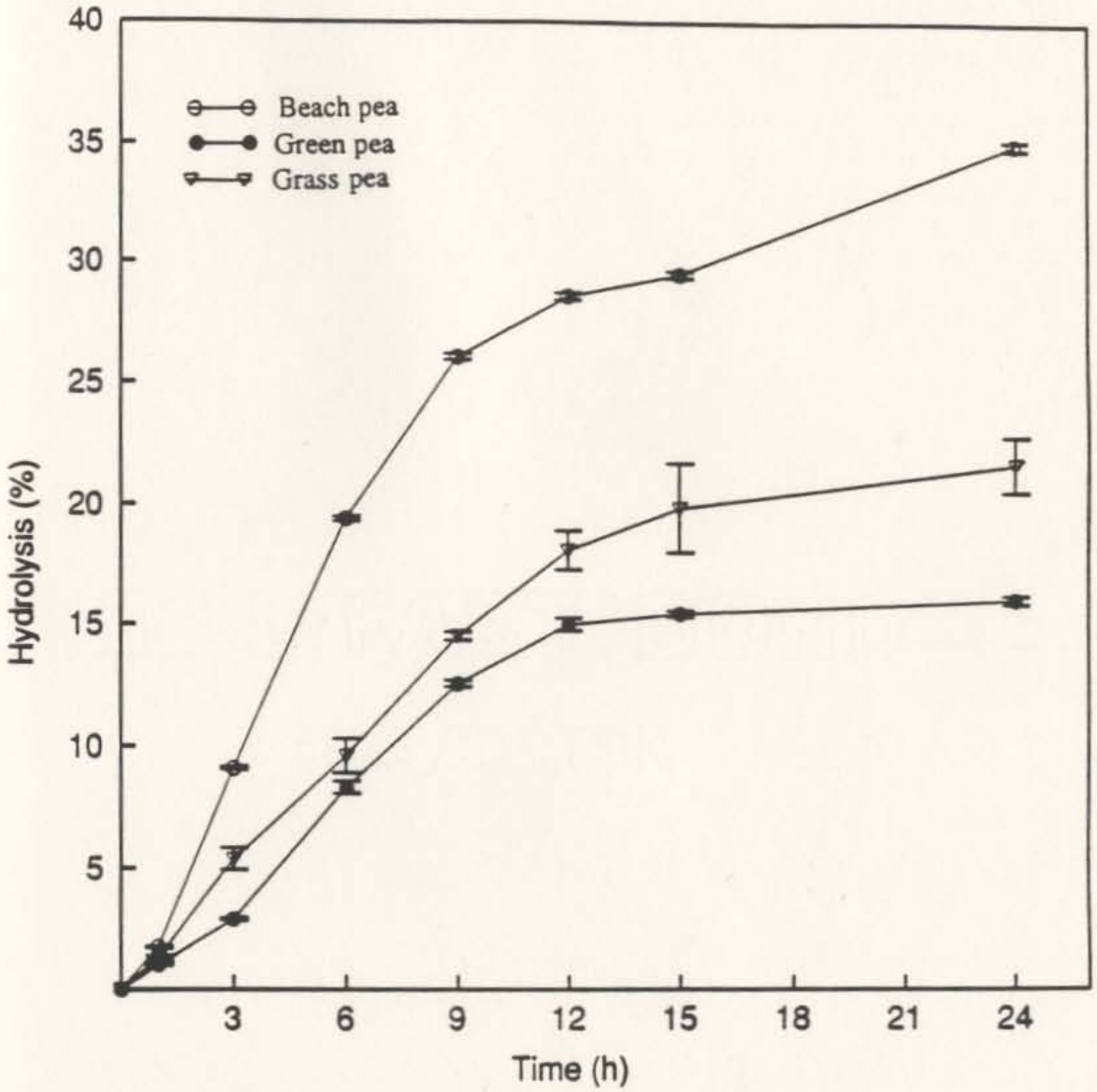
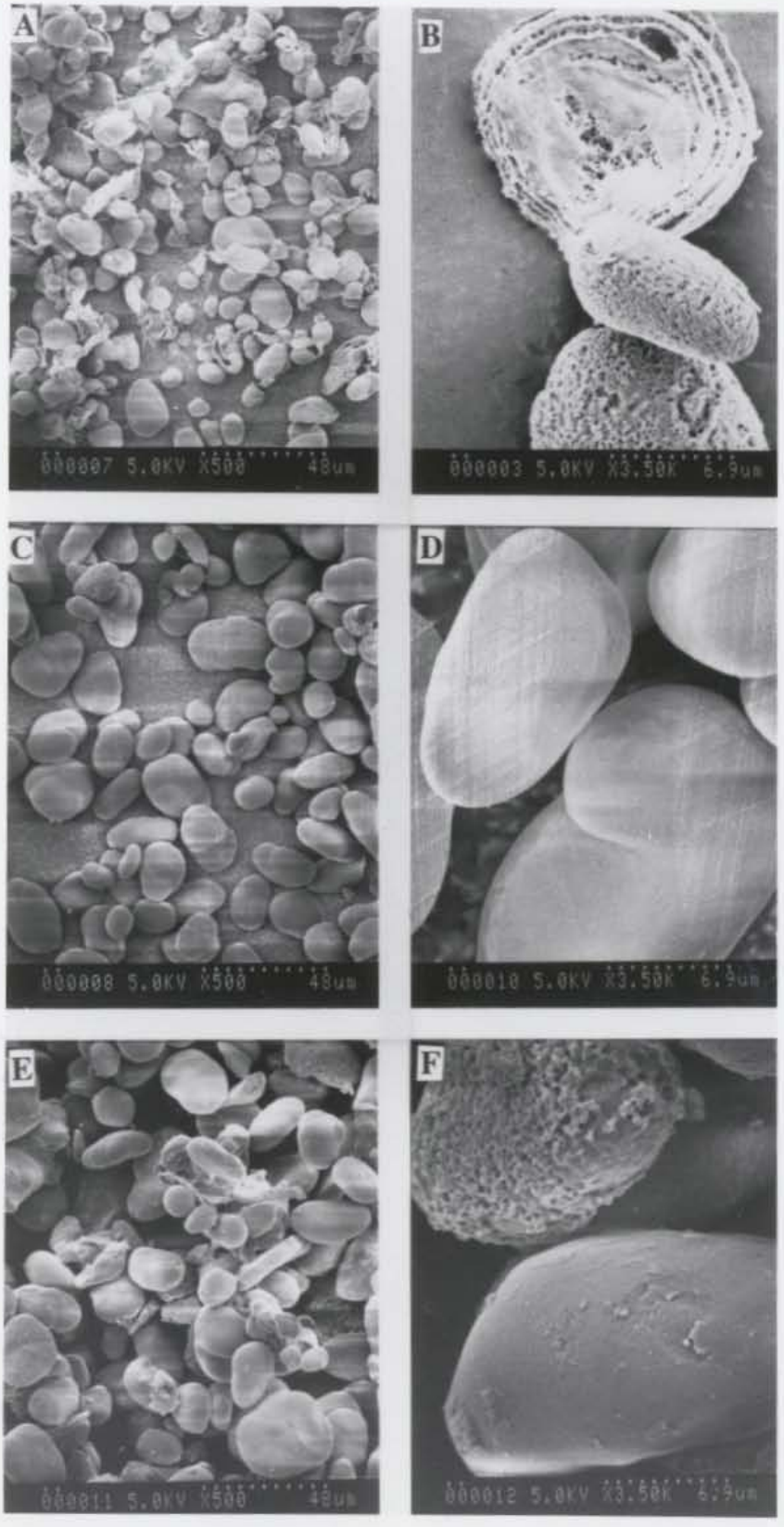

Beach pea
Green pea
Grass pea
Hydrolysis (%)
Time (h)
40
35
30
25
20
15
10
5
3
6
9
12
15
18
21
24

Figure 4.33 Scanning electron micrographs of native starches after attack (24 h) by porcine pancreatic α-amylase: (A) and (B) beach pea; (C) and (D) green pea; (E) and (F) Canadian grass pea.


A
B
C
D
E
F

glycosidic bonds of starch molecules involves enzyme-induced ring distortion of one of the D-glucosyl residues from the $^4C_1$ chair conformation to a 'half chair' conformation. This ring distortion decreases the enthalpy of activation and increases the susceptibility of the glucosyl residues to nucleophilic attack by functional groups on the enzyme and water. László *et al.* (1978) have shown that ring distortion or a 'half chair' conformation is involved in the transition state of α-amylase. It is therefore possible that conformational changes (chair → half chair) during α-amylase hydrolysis may be difficult for those amylose chains that are complexed by native lipids (due to decreased chain flexibility). This would then explain the differences in the degree of susceptibility among green pea (11.0% of amylose complexed by lipid) and the other two starches (5.1 - 5.9% amylose complexed by lipid). On this basis, Canadian grass pea starch (5.1% amylose complexed by lipid) should have been hydrolyzed to a greater extent than beach pea starch (5.9% amylose complexed by lipid). The difference in hydrolysis between beach pea and green pea starches is thus, probably due to amylose chains being more loosely organized (this increases the rate of diffusion of α-amylase into the granule interior) within the amorphous regions of beach pea starch. This seems plausible, since in spite of its low amylose content (27.3%; Table 4.57), the rate and extent of hydrolysis of beach pea starch was higher than that of Canadian grass pea starch (Figure 4.32). The results suggest that the interplay of bound lipid content and amylose chain associations within the amorphous regions influence granule susceptibility towards α-amylase hydrolysis.

4.13.7 Differential scanning calorimetry (DSC)

The gelatinization transition temperatures [at a volume fraction of water (v_1) = 0.85] and the enthalpy of gelatinization (ΔH) of beach pea, green pea and Canadian grass pea starches are presented in Table 4.60. The onset (T_0), mid-point (T_p) and conclusion (T_c) temperatures of the gelatinization endotherm of the starches followed the order: green pea > Canadian grass pea > beach pea. Whereas, ΔH followed the order: beach pea > Canadian grass pea > green pea.

Gelatinization involves the uncoiling and melting of the external chains of amylopectin that are packed together as double helices in clusters. Cooke and Gidley (1992) through studies of starches isolated at various steps of the gelatinization process have shown that the relative decrease in double helix content parallels the relative decrease in both crystallinity and residual gelatinization enthalpy, but occurs at higher temperatures than the relative decrease in granular birefringence. The above authors by studies on granular starch and model crystallites have shown that ΔH is due mainly to the disruption of the double helices rather than the long range disruption of crystallinity.

The lower T_0, T_p, T_c and the higher ΔH of beach pea starch suggests that disruption of double helices (in the amorphous and crystalline regions) during gelatinization is more pronounced in beach pea than in Canadian grass pea and green pea starches (Canadian grass pea > green pea). This indicates that the degree of association between double helical chain clusters in these starches follows the order: green pea > Canadian grass pea > beach pea. Furthermore, differences ΔT (T_c - T_o) among the

Table 4.60 Differential scanning calorimetric parameters for beach pea, green pea, and grass pea starches[1]

Starch source	Transition temperatures (°C)[2]				ΔH[4](cal/g)
	T_o[3]	T_p[3]	T_c[3]	$\Delta T(T_c - T_o)$	
Beach pea	60.0±0.6[c]	64.5±1.0[b]	74.2±1.2[a]	14.2	1.6±0.04[a]
Green pea	69.4±1.6[a]	72.0±0.8[a]	76.3±0.7[a]	6.9	1.2±0.13[c]
Grass pea[5]	65.7±1.5[b]	71.0±0.8[a]	74.2±1.0[a]	8.5	1.4±0.03[b]

[1]The data represent the means of three determinations, ± S.D. Means in each column with different superscripts are significantly different ($p<0.05$) from one another.

[2]starch:water (1:3).

[3]T_o, T_p and T_c indicate the temperature of the onset, mid-point and conclusion of gelatinization.

[4]Enthalpy of gelatinization.

[5]Canadian grass pea.

starches (beach pea >> Canadian grass pea > green pea) suggest that crystallites within the crystalline domains of beach pea starch granules have varying stability.

4.13.8 Pasting characteristics

The pasting characteristics of the beach pea starch at a concentration of 7% (w/v) and pH of 5.5 compared with green pea and Canadian grass pea were investigated using the Brabender viscoamylograph; results are presented in Table 4.61. At this concentration and pH beach pea starch exhibited a lower pasting temperature than those of green pea and Canadian grass pea starches. Beach pea starch showed a higher pasting viscosity (50 BU) at 95 °C when compared with green pea (18 BU) and Canadian grass pea (10 BU). The viscosity of beach pea starch (300 BU) increased markedly during holding period (at 95 °C) which is indicative of strong bonding forces with the granule. The viscosity of beach pea starch at 50 °C was also very high (380 BU) as compared with green pea (60 BU) and Canadian grass pea (80 BU). It has been postulated (Hoover *et al.*, 1993; Wang and White, 1994) that pasting properties are influenced by the amount of leached starch components, starch lipid content and the magnitude of bonding forces within the interior of granules. At a concentration of 6% (w/v) and pH 5.5 most legume starches exhibit pasting temperatures in the region 65 - 95 °C, their viscosities were greater than 100 BU and gradual increase in consistency (40 - 69 BU) during the holding period at 95 °C (Hoover *et al.*, 1993). The difference in viscosity in present results could be due to higher viscosity as a result of higher resistance to swelling and rupture (i.e., restricted

Table 4.61 Pasting characteristics of beach pea, green pea and grass pea starches[1]

Pea	Pasting temperature (°C)	Viscosity at 95°C (BU)[2]	Viscosity after 30 min at 95°C (BU)[2]	Viscosity at 50°C (BU)[2]
Beach pea	91.5	50.0	300.0	380.0
Green pea	93.0	18.0	30.0	60.0
Grass pea[3]	96.0	10.0	40.0	80.0

[1]Results are the means of two determinations at 7% (w/v) starch and pH 5.5.

[2]Barbender units.

[3]Canadian grass pea.

swelling). High viscosity could be an indication of relative molecular size of amylose (Naivikul and D'Appolonia, 1979).

CHAPTER 5

SUMMARY AND CONCLUSIONS

Objectives of the present study were accomplished as given below. Protein, crude fibre, reducing sugars, ash, and biological value of beach pea seeds were substantially higher than those of other peas examined. The mineral and vitamin contents were comparable or higher in beach pea seeds than those of green pea and grass pea. Thus, beach pea may potentially have a better nutritional quality than those of other peas studied (Objectives 1 and 2). Different plant parts of beach pea were examined for their nutritional quality and suitability as a feed or food item. The results indicated that beach pea seeds as well as plant parts (leaves, branches plus stem and pod shells) have very good nutritional value. Utilization of the whole beach pea plant as a green fodder, dry matter or ensiled may also be considered. The effect of maturity on nutritional quality of grains and pod shells of beach pea indicated considerable changes in the chemical composition (protein, crude fibre, starch, amino acids, and tannins) of seeds and pod shells during growth and maturation. These changes are of significance for the nutritional and technological properties of beach pea products. As the maturity of beach pea seeds progressed the content of condensed tannins increased, but with a concurrent decrease of tannins in pod shells (Objective 1).

Methanol-ammonia-water extraction of beach pea and grass peas resulted in simultaneous removal of antinutritional and/or toxic constituents and production of a protein-enriched (37%) meal. The meal of beach pea had no detectable level of β-N-oxalylamino-L-alanine (BOAA) while reduced amounts of it were detected in the

Canadian and Indian grass peas; corresponding reductions were from 86 to 18 mg/100g and 435 to 112 mg/100g sample, respectively. The content of phenolics and condensed tannins was effectively lowered/removed by methanol-ammonia-water extraction process for all pea samples examined (Objective 3).

The protein content of air classified cotyledons was enriched and the content of antinutrients such as phenolics and condensed tannins in cotyledons was significantly ($p<0.05$) reduced. Minerals in beach pea cotyledons were dominated by phosphorus, potassium, and zinc, while hulls were rich in calcium, sodium, iron and manganese (Objective 4).

The extract of beach pea hulls in acetone exhibited a strong antioxidant activity in a β-carotene-linoleate model system. The extract contained different classes of phenolic compounds with varying antioxidative strength. Among the three fractions, isolated on a Sephadex LH-20, Fraction III exhibited the highest antioxidant activity and contained several phenolic compounds when tested on a TLC plate. Further separation of Fraction III on a semi-preparative HPLC column showed both (+) catechin and (-) epicatechin as main phenolic compounds. Thus, beach pea hulls and their extracts may potentially be used as a component of functional food formulations and nutraceuticals (Additional to objective 4).

Of the different organic solvent extraction systems used 70% acetone containing 1% concentrated HCl served best in the removal of a maximum amount of condensed tannins from beach pea seeds. Methanol-water (80:20, v/v) was most effective in

removing a maximum amount of simple sugars and oligosaccharides from beach pea seeds (Objective 5).

Protein fractionation of beach pea, green pea and grass pea, separated according to their solubility in different solvents, was also achieved. Results indicated that water- and salt-soluble (albumin and globulin) protein fractions were the major contributors to the total protein content of all pea seeds examined. The solubility of protein nitrogen and total nitrogen of pea seeds was lowest at pH 4.5. The separation of different protein fractions from beach pea in comparison with green pea and Canadian grass pea, was carried out using water, salt, alcohol and alkali and their distribution in seeds, cotyledons, hulls and the final residue was determined. Globulin was the major fraction of proteins in cotyledons of pea samples examined. Albumin fraction contained the highest amount of sulphur-containing amino acids followed by glutelin, globulin, and prolamine; the proportion of sulphur-containing amino acids was higher in beach pea (Objective 6).

Protein isolates were also prepared using sodium hydroxide and sodium hexametaphosphate (2.8, w/v) at a meal to solvent ratio of 1:5. Overall, results indicated that both solvent systems employed were equally efficient in the extraction of proteins from beach pea. Functional properties and *in-vitro* digestibility of beach pea protein isolates were comparable to those from green pea and grass pea (Objective 7).

Beach pea starch was significantly different from other legume starches with respect to yield (low), amylose content (low), pasting characteristic (high), X-ray diffraction intensities (low) and the extent of interaction (weak) between starch chains

within the amorphous and crystalline domains of the native granules (Objective 8).

Future research should be focused on cultivation of beach pea on farm lands on a pre-commercial scale in order to examine the yield of products under different agronomic conditions. To develop new beach pea strains with low levels of condensed tannins (reduce the thickness of hull) by different plant breeding programmes such as germ plasm selection or genetic alteration. Application of beach pea protein isolates in different food products and examination of their functional and chemical properties is also essential. Chemical modification of beach pea protein isolates (such as succinylation or acetylation) and study of their effect on functional properties and *in-vitro* digestibility is also an important aspect. In case of beach pea starch it is necessary to study the rheological and retrogradation properties in order to assess its suitability for food and non-food related applications. It is also necessary to develop a wet or dry milling process for isolation (with increased yield and minimize starch damage) of beach pea starch to make it competitive to starch from other legume seeds. Possible derivatization of beach pea starch might also prove beneficial.

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APPENDIX

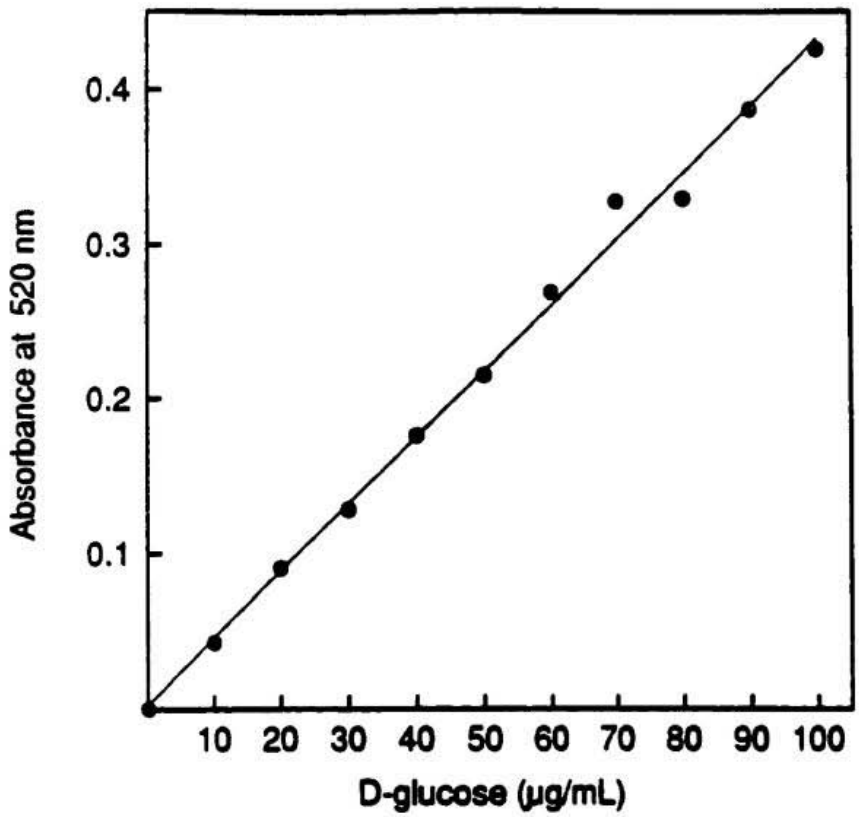


Figure A1. Concentration dependence of standard sugar solution on absorbance of D-glucose-arsenomolybdate complex

Regression coefficient (r) = 0.997
Equation of the line (Y=aX+b) where,

Y = absorbance at 520 nm (A_{520})
X = concentration of D-glucose in 1 mL solution, µg (C)
a = 0.0043
b = 0.0

A_{520} = 0.0043 x C
Therefore, C = 232.6 x A_{520}
Since extract solution has to be diluted, then
C = K(232.6 x A_{520})
Where, K = dilution factor

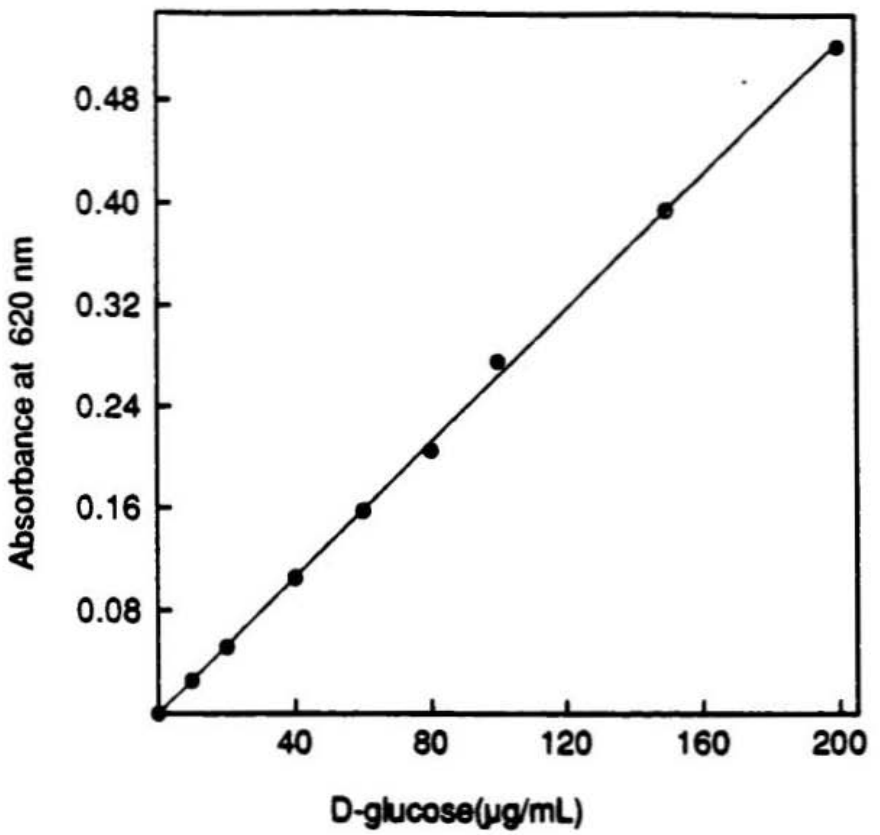


Figure A2. Concentration dependence of standard sugar solution on absorbance of D-glucose-anthrone complex

Regression coefficient (r) = 0.999
Equation of the line (Y=aX+b) where,

Y = absorbance at 620 nm (A_{620})
X = concentration of D-glucose in 1 mL solution, µg (C)
a = 0.0026
b = 0.0

A_{620} = 0.0026 x C
Therefore, C = 384.6 x A_{620}
Since extract solution has to be diluted, then
C = K(384.6 x A_{620})
Where, K = dilution factor

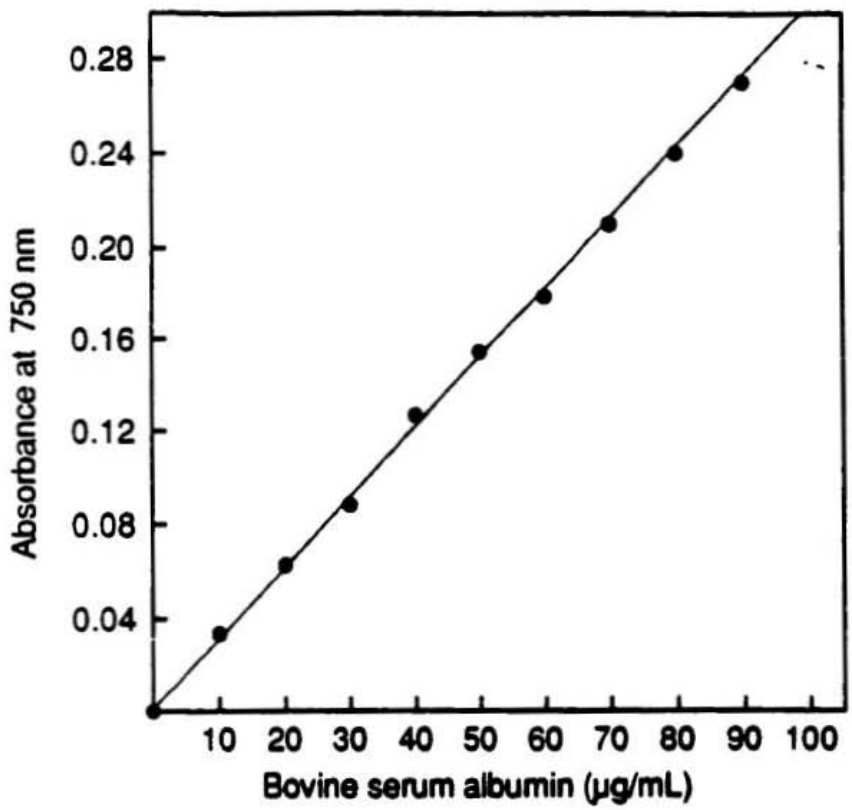


Figure A3. Concentration dependence of standard protein solution on absorbance of bovine serum albumin (BSA)-Folin Ciocalteu complex

Regression coefficient (r) = 0.997
Equation of the line (Y=aX+b) where,

Y = absorbance at 750 nm (A_{750})
X = concentration of BSA in 1 mL solution, µg (C)
a = 0.0027
b = 0.0

A_{750} = 0.0027 x C
Therefore, C = 370.4 x A_{750}
Since extract solution has to be diluted, then
C = K(370.4 x A_{750})
Where, K = dilution factor

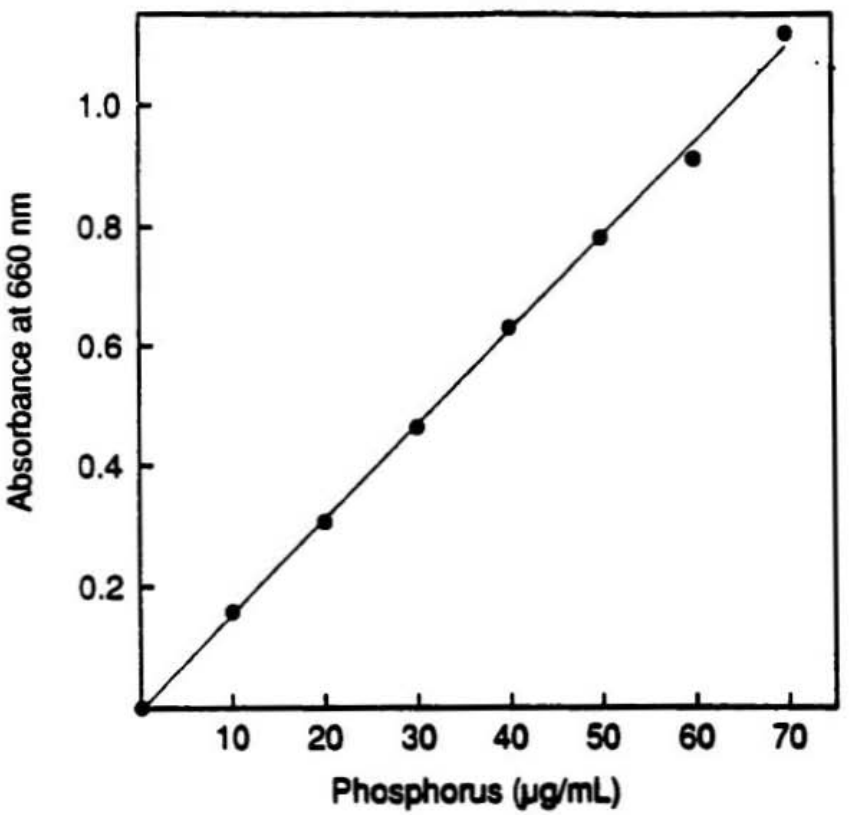


Figure A4. Concentration dependence of standard solution of phosphorus on absorbance of phosphorus-ammonium molybdate complex

Regression coefficient (r) = 0.999
Equation of the line (Y=aX+b) where,

Y = absorbance at 660 nm (A_{660})
X = concentration of phosphorus in 1 mL solution, µg (C)
a = 0.0157
b = 0.0

$A_{660} = 0.0157 \times C$
Therefore, $C = 63.7 \times A_{660}$
Since extract solution has to be diluted, then
$C = K(63.7 \times A_{660})$
Where, K = dilution factor

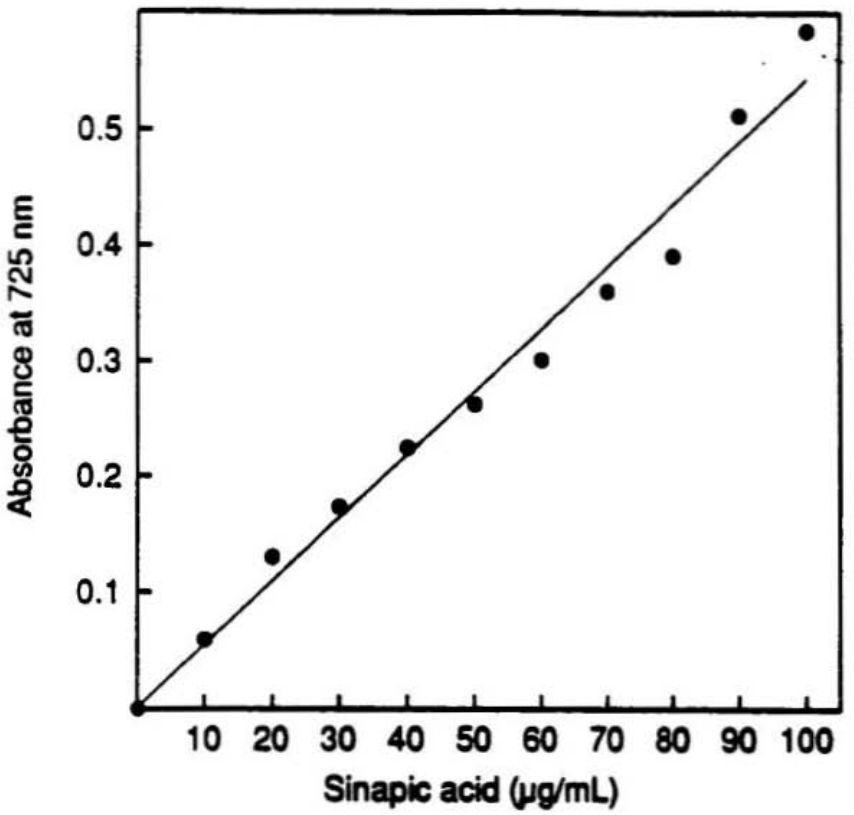


Figure A5. Concentration dependence of standard phenolic solution on absorbance of sinapic acid-Folin Denis complex

Regression coefficient (r) = 0.990
Equation of the line (Y=aX+b) where,

Y = absorbance at 725 nm (A_{725})
X = concentration of sinapic acid in 1 mL solution, µg (C)
a = 0.0054
b = 0.0

$A_{725} = 0.0054 \times C$
Therefore, $C = 185.2 \times A_{725}$
Since extract solution has to be diluted, then
$C = K(185.2 \times A_{725})$
Where, K = dilution factor

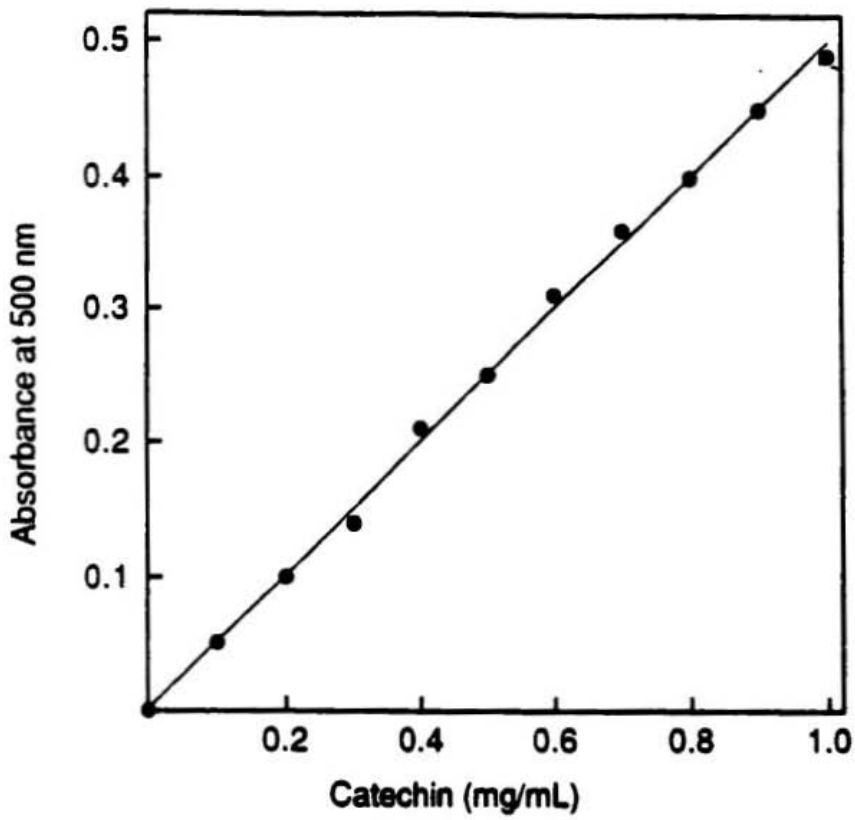


Figure A6. Concentration dependence of standard condensed tannins solution on absorbance of catechin-vanillin complex

Regression coefficient (r) = 0.998
Equation of the line (Y=aX+b) where,

Y = absorbance at 500 nm (A_{500})
X = concentration of catechin in 1 mL solution, mg (C)
a = 0.4891
b = 0.0

$A_{500} = 0.4891 \times C$
Therefore, $C = 2.0446 \times A_{500}$
Since extract solution has to be diluted, then
$C = K(2.0446 \times A_{500})$
Where, K = dilution factor

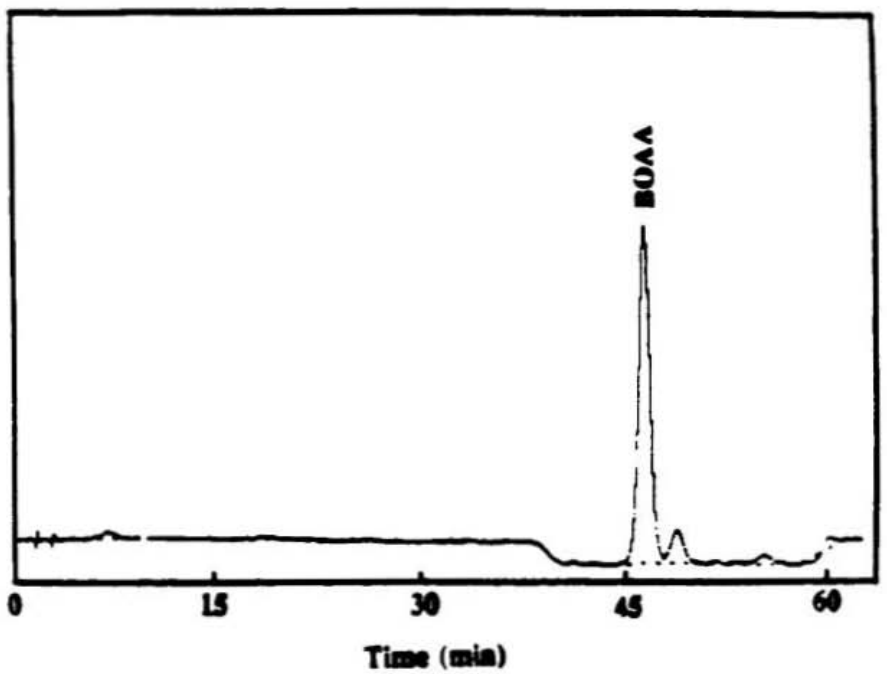


Figure A7. Chromatogram of the analytically separated BOAA from beach pea by amino acid analyzer.

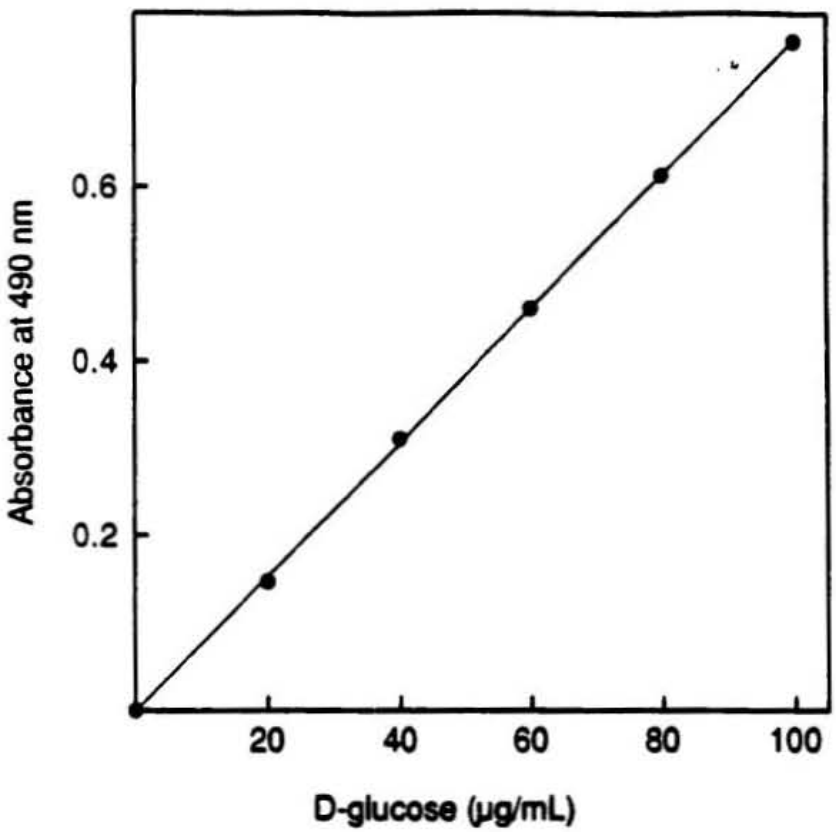


Figure A8. Concentration dependence of standard sugar solution on absorbance of D-glucose-phenol-sulphuric acid complex

Regression coefficient (r) = 0.996
Equation of the line (Y=aX+b) where,

Y = absorbance at 490 nm (A_{490})
X = concentration of D-glucose in 1 mL solution, µg (C)
a = 0.0078
b = 0.0

$A_{490} = 0.0078 \times C$
Therefore, $C = 128.2 \times A_{490}$
Since extract solution has to be diluted, then
$C = K(128.2 \times A_{490})$
Where, K = dilution factor

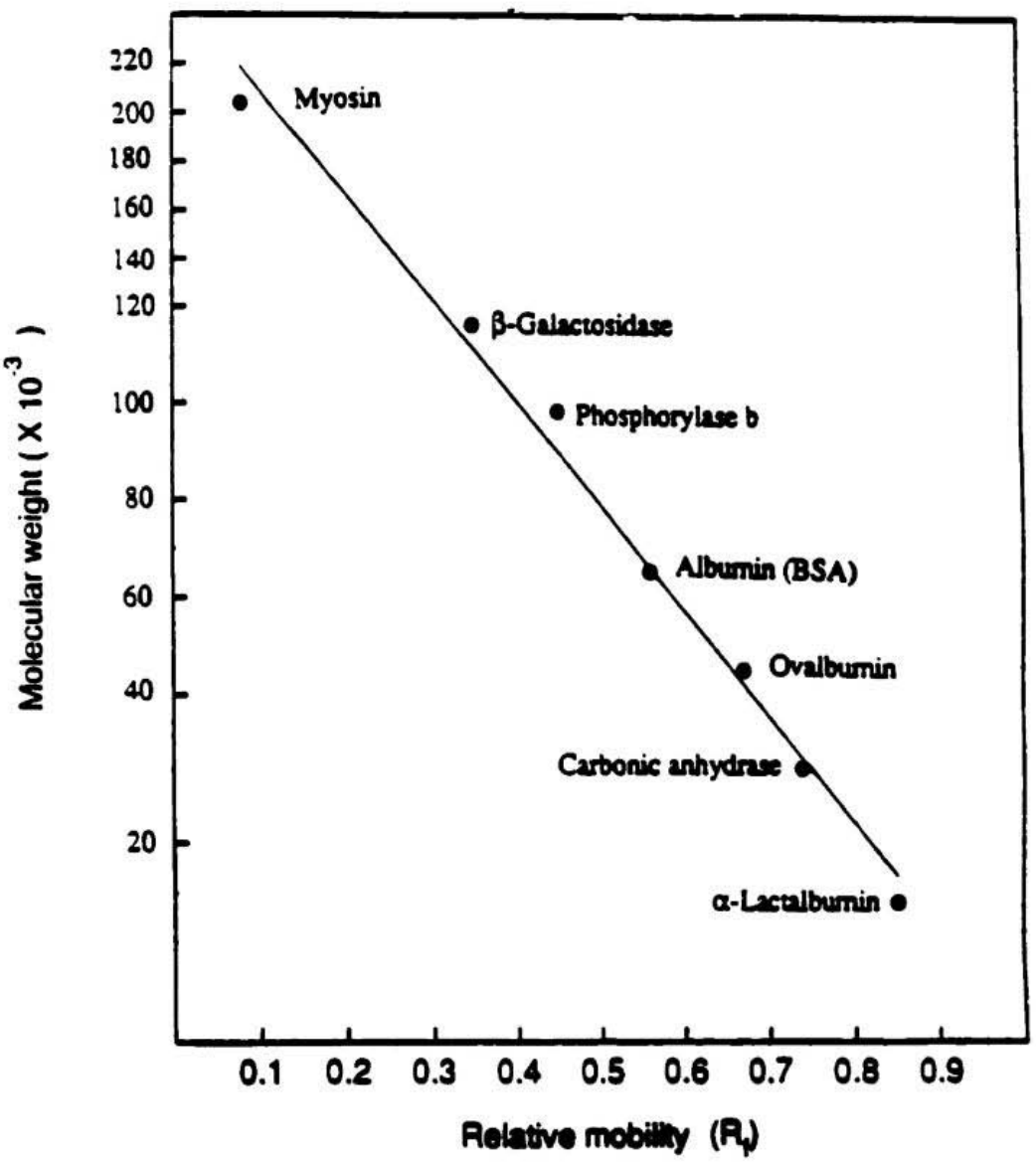


Figure A9. The relation between the molecular weight of the protein standards and relative mobility on SDS polyacrylamide gel.

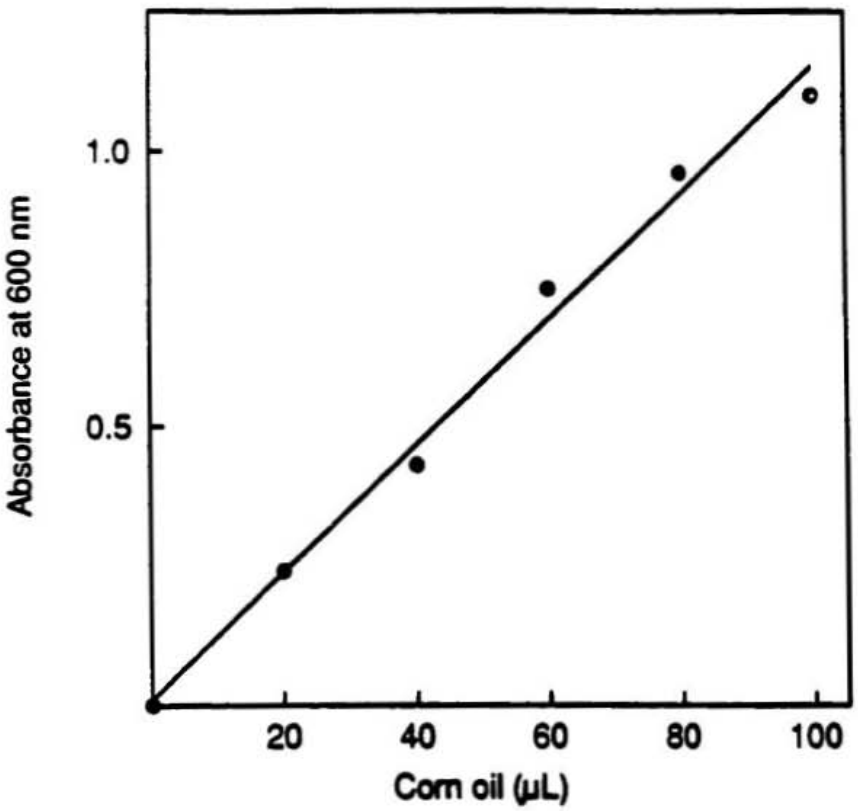


Figure A10. Standard line for determination of fat binding capacity of pea protein isolates

Regression coefficient (r) = 0.983
Equation of the line (Y=aX+b) where,

Y = absorbance at 600 nm (A_{600})
X = concentration of corn oil in 20.3 mL solution, µL (C)
a = 0.0203
b = 0.0

A_{600} = 0.0203 x C
Therefore, C = 49.26 x A_{600}
Since extract solution has to be diluted, then
C = K(49.26 x A_{600})
Where, K = dilution factor

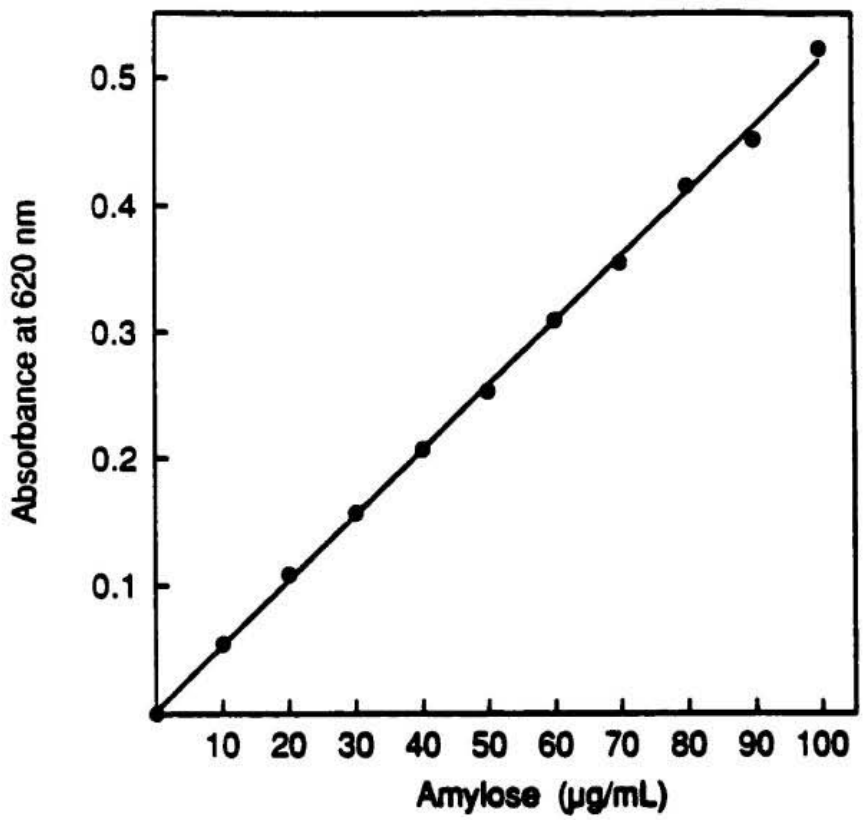
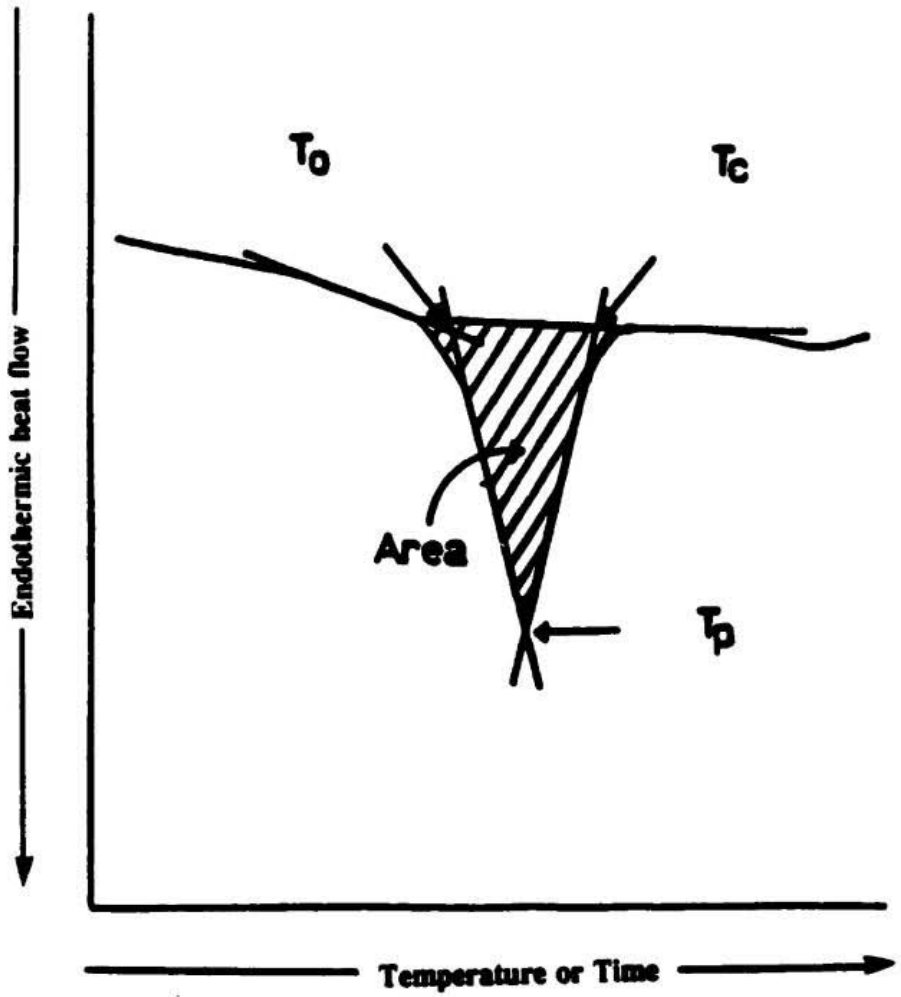


Figure A11. Concentration dependence of standard amylose solution on absorbance of amylose-potassium iodide complex

Regression coefficient (r) = 0.999
Equation of the line (Y=aX+b) where,

Y = absorbance at 620 nm (A_{620})
X = concentration of amylose in 1 mL solution, µg (C)
a = 0.0051
b = 0.0

A_{620} = 0.0051 x C
Therefore, C = 196.1 x A_{620}
Since extract solution has to be diluted, then
C = K(196.1 x A_{620})
Where, K = dilution factor



T_o = Onset temperature of gelatinization

T_p = Peak temperature of gelatinization

T_c = Conclusion temperature of gelatinization

Area: Used for enthalpy calculation

Figure A12. Thermogram for measurement of gelatinization temperatures and enthalpy by differential scanning calorimeter.

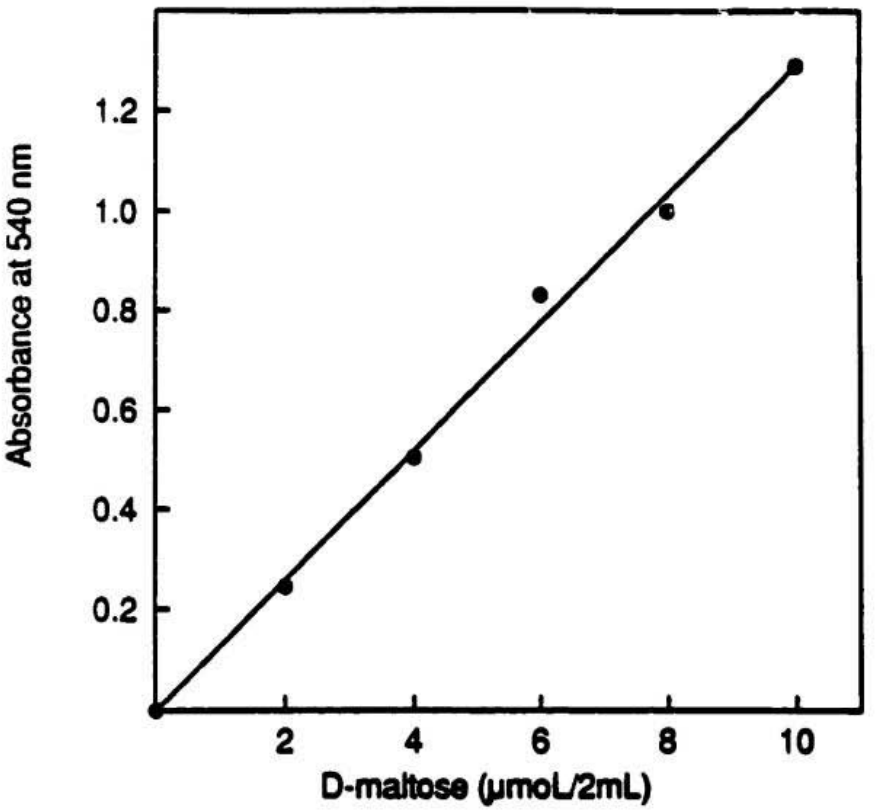


Figure A13. Concentration dependence of standard sugar solution on absorbance of sugar-3,5-dinitrosalicylic acid complex

Regression coefficient (r) = 0.998
Equation of the line (Y=aX+b) where,

Y = absorbance at 540 nm (A_{540})
X = concentration of D-maltose in 2 mL solution, µmoL (C)
a = 0.1292
b = 0.0

$A_{540} = 0.1292 \times C$
Therefore, $C = 15.48 \times A_{540}$
Since extract solution has to be diluted, then
$C = K(15.48 \times A_{540})$
Where, K = dilution factor

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1961	Born on March 25, Gursale, Malshiras, Maharashtra, India.
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1985	B.Sc. in Agriculture from Mahatma Phule Agricultural University, Rahuri, Ahmednagar, Maharashtra, India.
1987	M.Sc. in Agriculture from Mahatma Phule Agricultural University, Rahuri, Ahmednagar, Maharashtra, India.
1988-1989	Joined as a Senior Research Assistant in the Department of Biochemistry, Mahatma Phule Agricultural University, Rahuri, Ahmednagar, Maharashtra, India.
1989-1994	Senior Research Assistant in the Department of Food Science and Technology, Mahatma Phule Agricultural University, Rahuri, Ahmednagar, Maharashtra, India.
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1995-1998	Ph.D. Student at Memorial University of Newfoundland St. John's Canada.
1998	Ph.D. in Food Science from Memorial University of Newfoundland St. John's, Newfoundland Canada.