EFFECT OF STORAGE AND PROCESSING ON PHYTIC ACID LEVELS IN LEGUMES AND ITS INTERFERENCE WITH THE UTILISATION OF PROTEIN AND IRON

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Ms. **UMA CHITRA,** has satisfactorily prosecuted the course of research and that the thesis entitled, "**EFFECT OF STORAGE AND PROCESSING ON PHYTIC ACID LEVELS IN LEGUMES AND ITS INTERFERENCE WITH THE UTILISATION OF PROTEIN AND IRON**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

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CERTIFICATE

This is to certify that the thesis entitled, **"Effect of storage and processing on phytic acid levels in legumes and its interference with the utilisation of protein and iron"** submitted in partial fulfilment of the requirements for the degree of **"DOCTOR OF PHILOSOPHY IN HOME SCIENCE"** of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Ms. UMA CHITRA** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I, **UMA CHITRA**, hereby declare that the thesis entitled, "**EFFECT OF STORAGE AND PROCESSING ON PHYTIC ACID LEVELS IN LEGUMES AND ITS INTERFERENCE WITH THE UTILISATION OF PROTEIN AND IRON**" submitted to Andhra Pradesh Agricultural University for the degree of Doctor of Philosophy in Home Science is the result of original research work done by me. I also declare that the material contained in the thesis has not been published earlier.

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ABSTRACT

Grain legumes are important sources of proteins, minerals and vitamins for millions of people in the world, particularly in the developing countries. Low digestibility of legume protein is one of the main drawbacks limiting the nutritional quality of food legumes. Even after cooking, the digestibility of legume seed protein is quite low. Among various antinutritional factors, phytic acid may account partly for the low digestibility of proteins. The present study was undertaken to determine the variability in phytic acid content of legumes, to investigate the effect of processing on phytic acid, *in vitro* protein digestibility,

and iron availability (*in vitro*) of legumes and to study the effect of storage on phytic acid, protein digestibility and cooking quality.

Phytic acid contents and *in vitro* protein digestibility (IVPD) values differed significantly among and within the legume species. Phytic acid content (mg/g) was the highest in soybean (36.4) followed by black gram (13.7), pigeonpea (12.7), green gram (12.0) and chickpea (9.5). There was a significant negative correlation between phytic acid and IVPD of these genotypes implying that phytic acid would adversely influence the protein quality of legumes. Phytic acid was negatively and significantly correlated with the percent ionisable iron. The present findings strongly suggest that phytic acid is a major factor inhibiting iron absorption in legumes.

Germination reduced phytic acid in chickpea and pigeonpea by over 60%,and in green gram, black gram, and soybean by about 40%. Fermentation reduced phytic acid contents by 26-40% in these legumes except pigeonpea and chickpea where it was more than 60%. Autoclaving and roasting were more effective in reducing phytic acid in chickpea and pigeonpea as compared to black gram, green gram and soybean. The processing treatments also increased the *in vitro* protein digestibility (IVPD), but the effect was more pronounced in case of germination and fermentation which also remarkably decreased the total dietary fiber (TDF) content in all the legumes. Processing treatments showed little effects on calcium, magnesium and iron contents. Germination and fermentation appeared to be most beneficial in lowering phytic acid content and increasing IVPD of these legumes.

During prolonged storage of the legumes studied, the most notable change observed was the loss of phytic acid. Chickpea, green gram, and soybean stored at 25 and 37°C showed marked decreases in protein digestibility (*in vitro*). The legumes stored at 25 and 37°C required prolonged cooking times; however, legumes stored at 5°C showed only a slight increase in cooking time. PCMP number relating the contents of pectin, calcium, magnesium and phytin increased during storage. When phytic acid disappears during prolonged storage, chelation diminishes and Ca and Mg are freed as cations. Probably free Ca and Mg associated with pectic substances causing the hard-to-cook phenomena. However, under storage conditions of low temperature (5°C), these changes were minimised. The phytic acid level can thus be indicative of the cookability of legumes.

Results indicate that the genotypes of pulses with low phytic acid content could be identified and used in breeding programs to improve their nutritive value and utilisation.

SYMBOLS AND ABBREVIATIONS

C	Centigrade
mg	milligram
mg/g	milligram per gram
g	gram
g/g	gram per gram
g/kg	gram per kilogram
µg	microgram
psi	pounds per square inch
meq	milli equivalents
nm	nanometer
ml	milliliter
w/v	weight by volume
v/v	volume by volume
min	minute
hrs	hours
%	percent
rpm	revolutions per minute
RH	Relative Humidity
M	Molar
N	Normal
N	Nitrogen
TCA	Trichloroacetic acid
IVPD	*In Vitro* Protein Digestibility
TAA	Technicon Auto Analyser
TADD	Tangential Abrasive Dehulling Device
NSI	Nitrogen Solubility Index
TDF	Total Dietary Fiber
Temp	Temperature
SE	Standard Error

INTRODUCTION

CHAPTER I

INTRODUCTION

India is the major legume producing country in the world and legume crops continue to occupy an important place from nutrition point of view in daily diets of the people in the country. It is a matter of great concern that the supply of proteins in the daily diets is the lowest in India and no significant improvement has taken place during the last three decades. Consumption of legumes in India is restricted due to the scarcity caused by their present low yields and consequent higher cost, and due to certain drawbacks in their nutritional and food use qualities.

Legume proteins do not fulfil their potential even after cooking because the digestibility of protein and availability of amino acids particularly sulphur containing amino acids are quite low (Singh, 1985). The nutrient bioavailability from legumes depends on nutrient content and factors such as post-harvest handling, processing methods and conditions, presence or absence of antinutritional factors and possible interaction of nutrients with other food components (Salunkhe, 1982). Legumes contain a variety of antinutritional factors which directly or indirectly interfere with their nutritional quality. Phytic acid is one of the widespread occurrences in legumes, accounting for about 80% of the total phosphorus (Lolas and Markakis, 1975). Complexing between phytate and proteins has been reported for several proteins of cereals and legumes and this might affect the protein digestibility and bioavailability (Reddy *et al.*, 1982). Such

complex formation is believed to obstruct or inhibit the enzymatic degradation of the protein. Phytic acid has been linked to the inhibition of digestive enzymes such as protease (O'Dell and de Boland, 1976), pepsin (Knuckles *et al.*, 1985) and trypsin (Singh and Krikorian, 1982). The interaction between phytate and proteins leads to decreased solubility of proteins (Sathe and Salunkhe, 1984). The reduced solubility of proteins as a result of protein-phytate complex can adversely affect certain functional properties of proteins which are dependent on their hydration and solubility, such as hydrodynamic properties (viscosity, gelation etc), emulsifying capacity, foaming and foam performance, and dispersibility of aqueous media (Reddy *et al.*, 1989).

Phytate has a strong binding capacity to form complexes with divalent minerals. Most of the phytate-mineral complexes are insoluble at physiological pH and make the minerals like calcium, zinc, magnesium and iron biologically unavailable. There exists a general consensus that phytic acid will decrease zinc uptake in animals and humans (Davies and Olpin, 1979; Turnlund *et al.*, 1984). There is strong support for the prevailing opinion that phytates inhibit iron absorption in man (Hallberg *et al.*, 1989; Sandberg and Svanberg, 1991).

Several suitable processing practices such as soaking, cooking, germination, fermentation and autoclaving are employed to eliminate or reduce the levels of various antinutritional factors in grain legumes. An interaction between storage and processing also affects the nutritional quality of food legumes. The storage-induced hard-to-cook defect is a major constraint associated with consumption of legumes. It is established that prolonged cooking results in a decrease in protein

digestibility. Hence, storage of legumes under optimum conditions is important. Improved storage and processing practices will play an important role in enhancing the availability and nutritive value of legumes in the daily diets of the people.

The nutritional quality with reference to chemical composition including minerals and amino acids, antinutritional factors and protein digestibility of grain legumes particularly of beans and field peas have been the subject of numerous studies in the past. The extensive reviews on phytate indicate the need for a better understanding of the interactions of phytate with protein and minerals. The objectives of this study were:

1) to examine the variability in the phytic acid content of the commonly grown and consumed pulses in India mainly pigeonpea, chickpea, green gram and black gram genotypes

2) to study the association between phytic acid and protein digestibility (*in vitro*) and to determine the influence of phytic acid on cooking time and utilisation of protein

3) to determine the correlation between phytic acid and iron availability using *in vitro* method in these legume species

4) to study the effect of processing methods on *in vitro* protein digestibility and ionisable iron of legume genotypes varying in phytic acid content

5) to compare the results of these above mentioned studies with that of soybean processed and analyzed in similar ways to know how much of these changes in pulses differ from soybean and to

6) study the effect of storage on phytic acid, *in vitro* protein digestibility and cooking quality of these legumes.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The Indian subcontinent seems to be the area of greatest dependence on legumes (Deshpande and Deshpande, 1991). The important legumes grown extensively in India include pigeonpea, chickpea, green gram, black gram and lentils. Chickpea, commonly called bengal gram, is India's most important pulse crop. Pigeonpea is the second most important crop in India (Singh, 1991). Green gram and black gram also contribute considerably to the total production of pulses in India. Notwithstanding the nutritional benefits that pulses confer on an otherwise predominantly cereal-based diet, and also the agronomic advantages that legume crops lend to the production system, neither their output nor consumption levels have improved (Rao and Sastry, 1991). Even though increasing attention has been paid to grain legume crops in India, there has been little impact on improving production and productivity (FAO, 1992). In recent years developing countries have recognized the potential of soybean as a source of proteins supplementing the traditional cereal staples with much needed protein and calories (Wijeratne and Nelson, 1991). As a result of this, considerable increase in soybean production has been recorded in developing countries, particularly in India (Prasad, 1994).

The increasing population growth has resulted in a sharp decline in the per capita availability of pulses in India (Table 1). The daily per capita availability of pulses was 42.2 g in 1980-81 and it decreased to 38.0 g in 1987-88 (Table 1). At

present, per capita availability is nearly 33 g/day against the FAO/WHO (1991) recommended dose of 80 g/day. The daily intake of pulses in the different regions of India ranges from 14 to 140 g/day/person (Salunkhe, 1982). The per capita availability of pulses is quite variable and the trends for different pulses have changed over the years (Table 1). The per capita availability of chickpea is the highest, followed by pigeonpea, black gram, and green gram (Table 1).

Table 1. Availability of pulses (g per caput per day) in India

Year	Chickpea	Peas and beans	Pigeonpea	Green gram	Black gram	Total Pulses
1980-81	17.2	1.2	7.8	3.9	3.8	42.2
1981-82	18.0	1.2	8.7	4.1	3.9	44.7
1982-83	20.1	1.3	7.6	4.4	3.8	45.1
1983-84	17.7	1.4	9.6	5.1	4.4	48.0
1984-85	16.6	1.2	9.4	3.8	4.2	43.6
1985-86	20.7	1.5	8.7	7.2	4.4	47.8
1986-87	15.9	1.4	8.0	3.8	4.4	41.0
1987-88	12.5	1.4	7.7	4.4	4.5	38.0

Source : Agricultural Statistics Compendium 1990, Techno Economic Research Institute, New Delhi.

Grain legume proteins are rich sources of lysine, but are usually deficient in sulphur containing amino acids, methionine and cystine. Cereal grain proteins are low in lysine, but have adequate amounts of sulphur amino acids. Therefore, the supplementation of cereals with legumes has been advocated as a way of combatting protein-calorie malnutrition problems in developing countries (Eggum and Beames, 1983). Several traditional novel food products are prepared from food legumes in India. Legumes are consumed in the form of a variety of food

products which are prepared by using them as whole seed, cotyledons or *dhal*, flours, and protein-rich products such as protein concentrates and isolates (Kadam *et al.*, 1989).

To ensure appropriate attention to grain quality improvement in pulses, two approaches need to be emphasized: 1) development of nutritionally superior genotypes by plant breeding, and 2) use of economical and improved processing methods to enhance nutritive value of the product. Appropriate processing is probably more important for legumes than for any other food crops, primarily due to the presence of antinutrients and lower digestibility of raw legumes. Dry whole-seeds of pulses possess a fibrous seed coat (also called husk). Most of the pulses are consumed after dehusking. Pulses are processed by two traditional processing methods: 1) primary processing which is also called dehulling that converts whole seed into *dhal*, i.e., decorticated dry split cotyledons, and 2) secondary processing which is generally referred to cooking which often includes such treatments as soaking, boiling, frying, roasting, puffing, fermentation, germination, etc., depending on the type of food and the region of consumption (Singh and Singh, 1992).

In India, mature dry whole seeds of pigeonpea are mostly consumed after dehulling largely in the form of *dhal*. Chickpea is popular with all sections of the Indian population because of its taste and flavour. Chickpeas are consumed as whole dehulled grain, sprouted grain, immature pods, mature green seed, or as *dhal* and flour. Both the grain and the flour are further processed into several types of products. Traditionally, green gram is cooked either as whole seed or sprouted as a vegetable dish. Black gram is consumed in split, boiled or roasted

forms. The green pods are eaten as a vegetable. Soybeans are used in a variety of forms as human food. Soybean curd or *tofu* is commonly consumed in China. Fermented soybean foods, such as *miso, tempeh,* and *natto* are essential dietary items in the Orient. Several meat analogs are prepared from soybean by the method of continuous extrusion under heat. Several traditional Indian products can be prepared by using soybeans as one of the ingredients. In recent years attempts have been made to popularise soy-based products in India.

One of the main drawbacks limiting the nutritional quality of legumes is the presence of antinutritional factors. Of the various antinutritional factors, phytic acid is one of the widespread occurrences in legumes accounting for about 80% of the total phosphorus in most legumes (Lolas and Markakis, 1975). Because of its complex nature and its interaction with proteins and minerals, it is becoming increasingly important from nutrition point of view.

The variability in phytic acid content of legumes, its effect on protein digestibility, iron bioavailability and cooking quality and changes in these parameters during storage and processing are reviewed under the following headings.

2.1 Variability in phytic acid content of legumes

2.2 Nutritional implications of phytate

2.3 Effect of processing on phytic acid and protein digestibility

2.4 Effect of storage on phytic acid and cooking quality

2.1 VARIABILITY IN PHYTIC ACID CONTENT OF LEGUMES

Phytic acid, myo-inositol-1, 2, 3, 4, 5, 6,-hexakis (dihydrogen-phosphate) is one of the widespread occurrences in legumes. It is the chief storage form of phosphorus in cereals, legumes and other crops. Phytic acid contains six strong acid groups which are completely dissociated in solution, three weak acid protons and three very weak acid protons (Fig. 1). At the pH values encountered in foods and in the gastrointestinal tract, phytic acid will be strongly negatively charged and have immense potential for binding positively charged species, such as cations or proteins (Cheryan, 1980).

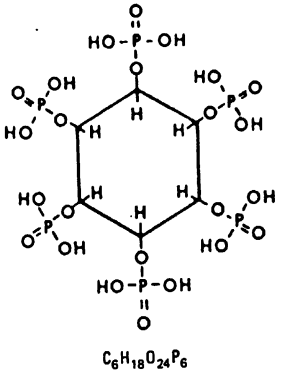

Fig. 1. Structure of phytic acid.

Source : Reddy *et al.* (1989).

The phytate content of legumes varies from 0.40 to 2.0% depending upon the species and the variety and most of it is present in the outer aleurone layers of the cotyledons or the endosperm (Deshpande *et al.*, 1982). Phytate rapidly accumulates in seeds during the ripening period, accompanied by other storage substances such as starch and lipids (Reddy *et al.*, 1989). The phytic acid content is not considered to be absolute and may vary, depending on the variety or cultivar, climatic conditions, location, irrigation conditions, type of soil, and year during which they are grown (Bassiri and Nahapetian, 1977).

Phytic acid content of eighteen varieties of mature dry limabeans (*Phaseolus lunatus*) ranged between 0.76-1.14% showing considerable variation among the varieties (Ologhobo and Fetuga, 1982). This study further indicated that phytic acid phosphorus represented 31.3-59.4% of total phosphorus with an average of 47.2%. These results are partly consistent with a view that phytic acid is the principal form of phosphorus in many seeds and that about 40-80% of the total phosphorus contents of dry legume seeds are in the form of phytic acid phosphorus (Lolas and Markakis, 1975).

Ologhobo and Fetuga (1984) observed significant differences in phytate anion, total phosphorus and phytate phosphorus in several Nigerian varieties of cowpeas, lima beans and soybeans. Their results indicated that the soybean dry seeds were the richest source of phytate (1.47% dry weight basis) followed in descending order by cowpeas (1.37%) and lima beans (0.88%). The ratio of phytate phosphorus as percentage of total phosphorus was highest in soybeans and lowest in lima beans.

Raboy *et al.* (1984) reported a mean phytic acid concentration of 17.6 g/kg for 38 soybean lines [*Glycine max* (L.)], with lines ranging from 13.9-23.0 g/kg. Among the soybean lines studied, phytic acid and seed total phosphorus were highly and positively correlated (r = 0.94), as was total protein with phosphorus (r = 0.74) and phytic acid (r = 0.74).

Khokhar and Chauhan (1986a) observed significant varietal differences in the phytic acid content of four varieties of moth bean (*Vigna aconitifolia*). Phytic acid among moth bean cultivars ranged from 0.85 to 0.90%. The level of phytic acid in moth bean cultivars seemed to be lower than that reported for black gram (Reddy *et al.*, 1978), kidney beans (Lolas and Markakis, 1975), and soybeans (de Boland *et al.*, 1975) suggesting that nutritive value of moth bean seeds would be impaired to a comparatively lesser extent.

Ologhobo (1989) analysed six varieties of soybean [*Glycine max* (L.)], for phytic acid and total phosphorus content. There did not appear to be much variation between the soybean varieties in phytic acid and phytic-phosphorus which ranged between 0.32-0.44% and 0.09-0.12% respectively. Phytic acid and phytic-phosphorus represented 63.2-83.9% and 17.9-23.5% of total phosphorus respectively.

Duhan *et al.* (1989) reported significant varietal differences in the phytic acid content of chickpea and black gram grains. These workers found that phytic acid content varied significantly from 7.58 to 8.10 g/kg in chickpea varieties, and from 6.47-6.68 g/kg in black gram varieties.

Hybridization has been known to be the most potent tool for increasing genotype variability among food crops through new recombinations. When the

variability within species is exhausted or some characters sought are not present, interspecific hybrids are attempted with the view of producing new species through amphidiploidy. The amphidiploids, in addition to showing various characters of economic importance, also exhibit a wide range of variability and desirable genotypes could be selected by using suitable breeding techniques. A large variability in phytic acid content of different varieties of amphidiploids (black gram x green gram) was observed by Kataria *et al* (1989). Phytic acid content in grains of various varieties of black gram (*Vigna mungo*) and green gram (*Vigna radiata* L.) amphidiploids ranged from 697 to 750 mg/100 g.

Farinu and Ingrao (1991) reported that phytic acid content of cowpea showed large varietal differences. Phytic acid contents of thirteen cowpea cultivars varied from 5.1 g/kg to 10.27 g/kg.

Singh *et al.* (1991) analyzed several varieties of groundnuts for protein, phytic acid, total phosphorus, nitrogen solubility and *in vitro* protein digestibility (IVPD). Phytic acid content ranged from 2.89 to 3.96 mg/g. Phytic acid constituted from 61.2 to 76.0 per cent of the total phosphorus. Nitrogen solubility of these varieties ranged from 49.7 to 60.5 per cent and *in vitro* protein digestibility between 66.8 and 77.5 percent. The highest protein digestibility was observed in 'TP 178-3' which contained the lowest amount of phytic acid.

2.2 NUTRITIONAL IMPLICATIONS OF PHYTATE

Phytic acid is normally found in the form of complexes with essential minerals or/and proteins (Erdman, 1979). *In vitro* studies have shown that phytate-protein complexes are formed by electrostatic interactions involving the

terminal beta-amino groups, the epsilon-amino group of lysine, the imidazole group of histidine, and the guanidyl groups of arginine. Many of these complexes are insoluble and are not biologically available for humans under normal physiological conditions (Cheryan, 1980). In addition, these proteins are less subject to attack by proteolytic enzymes than the free proteins.

As the pH increases and under certain phytate concentrations, phytic acid can interact with minerals and/or minerals and proteins. Since phytate cannot be absorbed and humans have a limited capacity to hydrolyze the phytate molecule, a negative effect of phytic acid on mineral bioavailability can be expected. In addition, phytate phosphorus may not be nutritionally available (Torre *et al.*, 1991).

2.2.1 *Protein-Phytate Interactions*

The ability of phytic acid to complex with proteins has been a subject of investigation for several reasons predominantly from chemical and nutritional viewpoints.

O'Dell and de Boland (1976) investigated the extent of phytate-protein interaction in aqueous extracts of a high lysine and a commercial hybrid corn germ, soybean flakes and sesame meal. The authors suggested that phytate interacts with some proteins to form insoluble products or complexes, which are not easily dissociated by electrophoresis at a high pH. Several soybean albumins formed strong associations with phytate while those of corn germ did not. The amino acid composition of the crude extracts did not explain the differences in phytate binding inasmuch as there was no correlation with the concentrations of

the basic amino acids. The insoluble phytate had a composition (Na_2 Mg_5 phytate) which suggested that phytate exists in sesame seeds, and perhaps most seeds, as a magnesium phytate.

Complexing between phytate and proteins has been reported for several proteins of cereals and legumes and this might affect the protein digestibility. Complex formation between protein and phytate is believed to obstruct or inhibit the enzymatic degradation of the protein. Phytic acid has been linked to the inhibition of digestive enzymes such as protease (O'Dell and de Boland, 1976), trypsin (Singh and Krikorian, 1982) and pepsin (Knuckles *et al.*, 1985). Others who use nitrogen solubility as a criteria for assessing digestibility believe that during protein hydrolysis, phytate forms peptide-phytate complexes that are insoluble, thus reducing the production of soluble nitrogen, which in turn appears to give a lower rate of hydrolysis.

Effect of phytic acid on protein digestibility

Reddy and Salunkhe (1981) employed whey fractions containing proteins (albumins), phytate, and minerals prepared from black gram (*Phaseolus mungo* L.) cotyledons to study the interactions between protein, phytate and minerals at pH 2.80, 6.40, and 8.40. Black gram cotyledons contained 1.7% phytate, of which 88.7% existed in water-soluble form. Phytate phosphorus represented about 89% of total phosphorus in black gram cotyledons. Recovery of phytate in fraction I (pH 2.80), fraction II (pH 8.40), and fraction III (pH 6.40) was 45%, 69% and 4%, respectively, after 48 hr. dialysis. At pH 2.80, complexation occurred between phytate and proteins. Complexation between phytate and proteins at pH 8.40 was

mediated by divalent cations such as calcium, magnesium, and zinc. Fraction II had higher concentrations of divalent cations (calcium, magnesium, and zinc) than the other two fractions I and III.

The rate of *in vitro* casein digestibility with and without phytic acid at concentrations found in legumes was determined at pH 8 and 37°C using multienzyme technique (Lathia *et al.*, 1987). Addition of 5 mg Na-phytate reduced the casein digestibility up to 20% compared to the control (Table 2). However, only 25% reduction of casein digestibility was observed in the presence of 25 mg of Na- phytate. Higher concentration of Na-phytate had no significant effect on the rate of casein digestibility. Data strongly suggested the formation of protein phytate complex at alkaline pH of small intestine.

Table 2. Influence of different phytic acid concentrations on *in vitro* digestibility expressed in %

Test	Digestibility in %
Casein	100a
Casein + 5 mg phytic acid	81
Casein + 15 mg phytic acid	79.3
Casein + 25 mg phytic acid	75.9

a. Percentage of digestibility of control was taken as 100%.

Source : Lathia *et al.* (1987).

Ritter *et al.* (1987) studied the effect of removal of phytate and phenolic compounds on the *in vitro* digestibility of soy protein isolates by trypsin and pronase enzymes at pH 8.0 and by pepsin at pH 2.0. Results generally agreed with those of the previous workers who reported that endogenous phytate exhibited a small, but consistent inhibitory effect on *in vitro* protein digestibility.

Carnovale *et al.* (1988) studied several cultivars of faba bean and pea and their protein products obtained by different methods to determine the interaction of phytic acid with protein and its effect on protein availability. *In vitro* digestibility of faba bean and pea samples decreased in the presence of exogenous phytic acid; a reduction of 6.8, 5.7, and 8.7%, respectively occurred in whole flour, protein concentrate and protein isolate following the addition of 10 mg of phytic acid. A reduction in protein digestibility (*in vitro*) of five protein sources (lactalbumin, casein, serum albumin, zein, and soy protein isolate) occurred when 1, 3, 5, or 10 mg of phytic acid was added. These data support the hypothesis that phytic acid protein interaction affects the protein availability of legumes negatively and that the nature of the protein source plays a prominent role.

Singh *et al.* (1991) reported that there was a negative and significant correlation between phytic acid and *in vitro* protein digestibility (IVPD) values in groundnut. It appeared that phytic acid reduced the protein digestibility by interfering with protease enzymes. The results suggested that phytic acid possibly inhibits the enzyme activity.

Effect of phytic acid on enzyme activity

Singh and Krikorian (1982) reported that *in vitro* activity of the proteolytic enzyme trypsin using casein as the substrate was substantially inhibited by low levels of phytic acid. The inhibition of trypsin activity by phytate varied with the phytate concentration. Trypsinogen, the inactive precursor of trypsin, is known to bind calcium ions at two sites, one being located in the body of the molecule whereas trypsin has only one binding site for calcium. Calcium ion retards trypsin

autolysis and promotes activation of trypsinogen to form trypsin. Authors further suggested that the inhibitory effect of phytic acid on trypsin might be due to the binding of trypsin with calcium and that under some circumstances *in vivo* it could well affect the conversion of inactive trypsinogen into trypsin by virtue of its binding affinity for calcium. The possibility of the direct interaction of protein, either enzymatic or nonenzymatic, with phytic acid could also, to some extent, be responsible for the phytate-induced inhibition of trypsin.

Knuckles *et al.* (1985) evaluated the effects of sodium phytate and partially hydrolyzed sodium phytate on pepsin digestion of casein and bovine serum albumin by an *in vitro* procedure using dialysates of pepsin digestion over a period of 0-23 hrs. This study indicated that the inhibitory effect of phytate differed with substrate and increased with dose level. At the highest phytate level, the digestion of casein and bovine serum albumin was reduced by 14% and 7% respectively. The inhibitory effect of phytate was universally correlated with the degree of phytate hydrolysis. Hydrolysis for 16 hrs. almost eliminated the inhibitory effect of phytate.

The effect of phytate on the *in vitro* activity of digestive proteinases was studied by Vaintraub and Bulmaga (1991), who observed that phytate inhibits the action of pepsin on the proteins but does not affect the pepsin hydrolysis of a low molecular weight substrate. The inhibition was maximal at pH 2-3 and dropped to zero when the pH was increased to 4.0-4.5. Trypsin hydrolysis of both a low molecular weight substrate and proteins was insensitive to the presence of phytate. The proteins tested included RNase and lysozyme charged positively at

the pH of the tryspin hydrolysis. The authors concluded that the inhibitory action of phytate is manifested only when it is bound with the protein substrate.

Caldwell (1992) investigated the kinetics of the activation of trypsinogen to trypsin under different combinations of calcium (II) and phytic acid. The complexation of phytic acid and calcium increased the rate of formation of catalytically inactive protein. The effect of phytate on the *in vitro* activation of trypsinogen was considerable and it was proposed that the reason for such effects may be due either to the formation of ternary complexes or competitive sequestration of Ca^{2+} ions between phytate and protein.

Effect of phytic acid on nitrogen solubility

The interaction between phytate and proteins leads to decreased solubility of proteins. Protein-phytate interactions in soybeans were studied by Prattley *et al.* (1982) who showed the association of protein and phytic acid to be highly pH dependent. Their data showed that insoluble protein-phytate complexes form below the isoelectric point of protein. These are created through the direct electrostatic attraction between the phosphate groups of phytic acid and the cationic residues on the protein. Although stable to heat, these complexes are disrupted by the competitive action of calcium ions. Above the isoelectric point, association of protein with phytic acid occurs only in the presence of divalent cations. The stability of these soluble complexes increases upto pH 10.0. Above this pH, the loss of positive charges and the high ionic strength of the environment is responsible for the dissolution of the complex. Under alkaline conditions, divalent cations (e.g. calcium, magnesium and zinc) mediate an

interaction between protein and phytic acid and this protein-phytate association is strengthened with increasing pH up to pH 10.0.

In their study on nitrogen solubility profiles in winged bean flour, Kantha *et al.* (1986) reported that nitrogen solubility dropped from 32% at pH 2.0 to 12% at pH 4.0. Conversely, phytic acid was 25% soluble at pH 2.0 and 48.0% soluble at pH 4.0; at neutral pH, the solubility of nitrogen and phytic acid were 50 and 80%, respectively.

Grynspan and Cheryan (1989) observed that calcium ions interact with protein and phytate to further decrease the solubility of proteins. The interaction of phytic acid with proteins is closely dependent on pH. At a pH of 7 or more, phytate and most proteins do not interact. In the presence of multivalent cations such as calcium, insoluble protein/cation/phytate complexes occur.

2.2.2 *Effect of Phytate on Iron Bioavailability*

The iron bioavailability is the result of a complex mosaic of factors which are dependent upon the type of iron (heme or non heme), solubility, charge density, environment, reactivity, enhancers, inhibitors, and a host of physiological variables. According to Baynes and Bothwell (1990), the actual amount of iron absorbed from the gastro intestinal tract is determined by the iron content of the meal, the chemical form of the iron, the iron status of the individual, and the composition of the ingested food. About 40% of the iron in meat, poultry, and fish is heme iron, whereas the rest is nonheme iron. The iron in dairy products, eggs, and all plant foods is nonheme iron. Because heme iron is generally much better absorbed (15%-35%) than is nonheme iron (2-20%), it has been suggested that

vegetarians may be at a greater risk of iron deficiency (Monsen, 1988). Nonheme-iron absorption is strongly influenced by many inhibitory and enhancing factors in the diet whereas heme-iron absorption is very little affected by other dietary components. The efficient absorption of heme iron is because of specific heme-binding sites in the intestinal tract (Craig, 1994).

The solubility of non-heme iron in the small intestine is a major factor in determining its absorption. Polyphenolics and phytates in the plant foods are known to bind with nonheme iron and thus inhibit its absorption. Phytates in whole-grain cereals, legumes, nuts, and seeds can bind nonheme iron and greatly reduce its absorption (Hallberg, 1981). Phytic acid forms complexes with numerous divalent and trivalent cations. The insolubility of the mineral-phytates is generally considered to be a major reason for phytic acid leading to reduced mineral bioavailability. The solubilities of the mineral-phytate complexes depend on the following: 1) pH value, 2) the specific mineral, 3) mineral and phytate concentrations, 4) phytate : mineral molar ratio, and 5) the presence of other minerals (Champagne, 1988).

Prabhavathi and Narasinga Rao (1979) observed a two-fold increase in ionisable iron when legumes (bengal gram and green gram) were germinated for different periods. Germination beyond 48 hrs. was accompanied by a reduction in phytic phosphorus and an increase in ascorbic acid content of the seeds. It was indicated that a decrease in phytin phosphorus content may be partly responsible for an increase in ionisable iron observed on germination.

Subba Rao and Narasinga Rao (1983) reported that the presence of Ca^{++} or Mg^{++} ions was found to decrease the iron solubilising ability of phytate (Table 3).

Ca^{++} and Mg^{++} presumably bind to soluble iron-phytate complex, forming insoluble complexes. It was suggested that phytate *per se* may not be the only factor which limits iron availability in foods, but it is the relative concentration of phytate binding minerals like Ca, Mg and Zn and their interaction with iron phytate complex.

Table 3. Simulation of mineral and phytate contents as existing in foods

	Percent iron solubility at pH 7.5	
	Bengal gram	Red gram
Iron + phytate	92	98
Iron + phytate + Ca^{++}	11	16
Iron + phytate + Mg^{++}	0	0
Iron + phytate + Ca^{++} + Mg^{++}	0	0

Source : Subba Rao and Narasinga Rao (1983).

Lynch *et al.* (1984) observed that absorption of iron from a variety of commonly eaten legumes (lentils, split peas, green grams, black beans, and soybeans) prepared as soups was observed to be only 0.84 to 1.91%. It was concluded that legumes, which are the important dietary components in many developing countries, are all poor sources of bio-available iron.

Hazell and Johnson (1987) observed that the iron diffusibility from a group of eighteen cereals, legumes and nuts was very low (0.4-5.8%). Phytate phosphorus was negatively correlated with diffusible iron in the cereals, legumes, and nuts. When sodium phytate was added to selected foods in amounts corresponding to the endogenous levels, there was a marked inhibition of iron

diffusibility. It was concluded that phytate is a major inhibitor of iron diffusibility in cereals, legumes and nuts.

Hazell (1988) related iron availability estimated *in vitro* using a simulated digestion system to the concentration of protein, sugar, fiber, phytate, ascorbate and citrate in 33 different plant foods. Protein, sugar and fiber showed no significant correlation with iron availability. Phytate was significantly and negatively correlated with diffusible iron in cereals, legumes and nuts. These results suggested that phytate was responsible on a quantitative basis for the depression of iron availability in foods such as cereals and legumes.

Macfarlane *et al.* (1988) measured the effects on iron absorption of nuts, an important source of dietary protein in many developing countries in 137 Indian women. When the absorption from bread and nut meals i.e. (walnuts, almonds, peanuts and hazelnuts) and coconut was compared with that from bread meals, the overall geometric mean absorption from the nut meals (1.8%) was significantly less than that from the bread meals alone (6.6%). In contrast, coconut did not reduce absorption significantly. All the nuts tested contained significant amounts of two known inhibitors of iron absorption (phytates and polyphenols) but the amounts in coconut were significantly less than in the other nuts. A small reduction in the phytate content of peanuts as a result of germination resulted in a modest improvement in the iron absorption.

Brune *et al.* (1989) examined the possibility that a high bran and phytate intake over a long period would induce changes in the intestines or its microflora leading to a reduction of the inhibitory effect of dietary phytates on iron absorption. The inhibitory effect of bran on iron absorption was compared

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between a group of strict vegetarians with a regular high phytate intake and a control group by use of wheat rolls with and without bran labelled with ^{55}Fe and ^{59}Fe. The average individual decrease of the iron absorption from adding the bran was 92 and 93% in the two groups, respectively. No intestinal adaptation to a high phytate intake could be observed. It was suggested that this finding has wide nutritional implications. It is well known that the absorption of iron from the diet is a net effect of the balance between the factors enhancing iron absorption (eg, ascorbic acid, meat, and fish) and those inhibiting (eg. phytates and polyphenols). The intake of phytates is thus a main determinant of the iron nutrition especially in groups with a regular high intake. These results imply that a high phytate content in the diet must always be considered to impair iron absorption even if intake has been high for several years. It was concluded that the iron-balance situation in subjects with a high phytate consumption can only be satisfactory if the diet also contains sufficient amounts of foods counteracting the inhibition of phytates, such as those with a high content of ascorbic acid.

Latunde-Dada (1991) investigated ten Nigerian soybean varieties for iron levels and dialyzable iron and noticed significant decreases in the diffusibility of iron. Germination and fermentation enhanced the availability of iron. Although the percentage of iron absorbed from soy may be reduced, the total amount of iron absorbed is modest because soy beans naturally contain relatively large amounts of iron.

Hurrel *et al.* (1992) studying iron absorption from liquid-formula meals that contained a number of soy-protein isolates with different phytate contents observed a four-to five fold increase in iron absorption when phytic acid was

essentially removed from the soy-protein isolate. They observed that the phytic acid content of the soy-protein isolates should be reduced to lower amounts (< 10 mg phytic acid/meal) to ensure a meaningful and substantial increase in iron absorption.

2.3 EFFECT OF PROCESSING ON PHYTIC ACID AND PROTEIN DIGESTIBILITY

Grain legumes in India are processed and consumed in a variety of forms. The most common method of preparation is usually to soak them overnight and then to cook until they are soft. Other traditional methods include pressure cooking, sprouting, cooking of sprouted seeds, parching and fermentation. Processes such as germination, soaking, cooking, fermentation and other autolytic treatments are known to reduce and/or eliminate the phytate in legume seeds. Although not common in many parts of the world, dehulling of pulses seems to be the choice method of processing in the Indian subcontinent. Not only does dehulling improve palatability and digestibility of legume seeds, it also remarkably reduces their cooking time. In India, dehulling of dry seeds of pigeonpea, chickpea, black gram and green gram is very important because the major portion of these legumes is consumed in the form of *dhal*.

2.3.1 Dehulling

Reddy and Salunkhe (1980a) reported increased phytic acid content of black gram cotyledons (1.70%) compared to whole black gram seeds (1.46%). Desphande *et al.* (1982) investigated the effects of dehulling on phytic acid content

of ten cultivars of dry beans (*Phaseolus vulgaris* L.). They observed that phytic acid content of whole beans ranged from 1.16-2.93%, and dehulling significantly increased the phytic acid content of beans (range 1.63-3.67%). The increase in relative content of phytic acid after dehulling of all ten cultivars was attributed to two factors. Firstly, phytic acid may be characteristically present in the cotyledon fractions of the beans and secondly, the seed coat contributes a substantial portion of the whole seed weight. Removing the seed coat may lead to an increase in the concentration of phytic acid on a unit weight basis in the cotyledons.

2.3.2 *Soaking and Cooking*

Soaking of food legumes usually forms an integral part of bean processing methods such as cooking, germination, fermentation and toasting. Certain legumes such as green gram and black gram absorb water at a faster rate when soaked in water, whereas soybeans exhibit very slow water uptake. Soaking of beans facilitates quicker cooking. The soaking water may or may not be discarded prior to cooking, depending on the regional consumers' preferences. Such practices might influence the nutritional quality of beans. Certain water soluble and nutritionally important minerals and vitamins may also be lost to the soaking water, if discarded, along with undesirable components such as flatulence-causing oligosaccharides, phytate and tannins.

According to Chang *et al* (1977), steeping or incubation of California small white beans in water followed by cooking in boiling water increased inorganic phosphorus concentration with 50% hydrolysis of the bean phytate. This was

mainly attributed to the phytase activity during the steeping of seeds or incubation and the effect of heat treatment.

The cooking processes decrease both water- and acid-extractable phytate phosphorus in legumes. Poor extractability of phytate phosphorus with water and HCl in cooked legumes was noticed by Kumar *et al.* (1978) and it was attributed to the formation of insoluble complexes between phytate phosphorus and other components in legumes during cooking, which subsequently could not be extracted with water or HCl.

Reddy *et al.* (1978) did not notice any breakdown of phytate phosphorus during cooking of black gram seeds and cotyledons. They observed some losses in total phosphorus and phytate phosphorus during short time cooking, due to leaching of these components into the cooking water.

Iyer *et al.* (1980) also investigated the effects of soaking in mixed salt solution and distilled water on phytic acid of the Great Northern, pinto and kidney beans. They reported a reduction of 8.7% to 69.6% in phytate content of the above three beans.

Desphande and Cheryan (1983) investigated the changes in phytic acid of four dry bean (*Phaseolus vulgaris* L.) cultivars on soaking in distilled water, sodium bicarbonate, and a mixed salt solution. Quick-cooking bean processes usually involve soaking of beans in mixed salt solutions as a preliminary step. A greater reduction in phytic acid content of beans was observed on soaking in sodium bicarbonate or mixed salt solutions than on soaking in distilled water.

Ologhobo and Fetuga (1984) observed that cooking and autoclaving were only slightly effective in decreasing the phytic acid content of ten cowpea varieties.

Khokhar and Chauhan (1986a) observed that soaking of seeds in plain water or mixed salt solution was the most effective method of lowering phytic acid of moth bean grains. They further found that the loss of phytic acid was the highest during cooking of sprouted seeds and pressure cooking of seeds presoaked in mixed salt solution. The obvious decrease in phytate of moth bean during soaking was attributed to leaching of phytate ions into soaking water under the influence of concentration gradient. Such losses may be taken as a function of change permeability of seed coat. Absorption of water in seeds may also activate phytase resulting in hydrolysis and hence loss of phytic acid.

Since protein digestibility of legume grain is affected by the contents of antinutritional factors, the various processing and cooking methods affecting the levels of the antinutritional factors will influence the protein digestibility of the legume grains Khokhar and Chauhan (1986b) observed that soaking of seeds for 12 hrs. in plain water improved to a reasonable extent the *in vitro* digestibility of moth bean proteins. The higher digestibility of soaked seeds was attributed to leaching of certain antinutritional factors like phytate, trypsin inhibitor, tannins etc. from the soaked seeds. Soaking for a relatively longer time, may also initiate activation of certain enzymes, eventually leading to improvement of protein digestibility.

Jood *et al.* (1989) reported that the *in vitro* protein digestibility of grains of chickpea (*Cicer arietinum*) and black gram (*Vigna mungo*) cultivars varied from 48-

53% and 52-58% respectively. Soaking significantly improved protein digestibility of both chickpea and black gram cultivars. Improved digestibility of the soaked legumes was attributed to leaching out of antinutrients such as protease inhibitors, polyphenols, phytic acid etc. during soaking. Ordinary cooking of soaked as well as unsoaked seeds and autoclaving of soaked seeds of both the legumes significantly increased their protein digestibility. Heat processing increases protein digestibility of legume grains most likely by destroying heat labile protease inhibitors and also by denaturing globulins, highly resistant to proteases in the native state.

Kataria *et al.* (1992) observed an increase of 22 to 25% in protein digestibility when the seeds of amphidiploids (black gram x green gram) were soaked for 18 hrs. They further noticed that the cooking of both unsoaked and soaked seeds significantly improved the protein digestibility of all the varieties.

2.3.3 *Germination*

The phytate is utilized as a source of inorganic phosphate during seed germination and the inorganic form becomes available for purposes of plant growth and development. The liberation of phosphate from phytate occurs by enzyme hydrolysis. Phytase is the currently accepted enzyme, which is responsible for the complete hydrolysis of phytate (inositol hexaphosphate) into inositol and phosphate.

Reddy *et al* (1978) observed that phytate phosphorus decreased gradually during initial stages of germination of black gram, with simultaneous liberation of inorganic phosphorus as one of the final products.

Tabekhia and Luh (1980) reported a slight decrease in phytic acid during the first 48 hrs. of germination, but a much faster decrease during the 72-120 hrs. period. The first two days represented a latent period during which the phytase activity was not prevalent. After 72 hrs. of germination, the decrease in phytic acid was more rapid, and much faster still after 96 and 120 hrs.

Borade *et al.* (1984) reported that the phytate phosphorus in horse gram and moth bean seeds accounted for 57% and 55%, of the total phosphorus respectively. They further reported that the phytate phosphorus significantly decreased with germination and it accounted for only 20% in horse gram and 26% in moth bean, of the total phosphorus of the 48 hour germinated seeds.

Ologhobo and Fetuga (1984) determined phytate anion, total phosphorus (P), phytate phosphorus, inorganic and residual phosphorus in different varieties of cowpeas, lima beans and soybeans. They subjected the dry beans to different processing methods which included cooking, autoclaving, soaking and germination. Germination and soaking were most effective in decreasing phytate contents. The obvious decrease in phytate and phytate-P contents of the germinated legumes was attributed mainly to phytase activity during germination of seeds.

The hydrolysis of protein during germination is one of the factors accounting for the better digestibility of proteins. Khokhar and Chauhan (1986b) reported that the protein digestibility of moth bean improved significantly when the seeds were sprouted. Germination for 60 hrs. increased protein digestibility by 23-28% as compared to the control. Jood *et al.* (1989) observed that the protein digestibility (*in vitro*) of chickpea and black gram varieties increased significantly

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when the seeds were germinated to a desirable size of sprouts. A marginal increment in the protein digestibility occurred when the sprouts of both the legumes were cooked. Increase in protein digestibility was attributed to metabolism of seed proteins and catabolism of antimetabolites including protease inhibitors, phytate and polyphenols.

2.3.4 *Fermentation*

The legume-based fermented foods have become more popular because of their desirable changes in texture, organoleptic characteristics such as flavor, aroma, appearance or consistency, keeping quality and partial and/or complete elimination of antinutritional factors and toxic factors. Fermentation of legumes appreciably reduces the phytate content due to endogenous phytase of legumes and that of added yeast and other useful microorganisms. The fermentative microorganisms contain enzymes phytase and phosphatase which hydrolyze phytate into inositol and orthophosphate (Reinhold, 1975), and it has been suggested that the loss of phytate during fermentation might be due to the activity of the enzyme phytase naturally present in cereals and legumes (Faridi *et al.*, 1983).

Sudarmadji and Markakis (1977) studied the changes in phytic acid during *tempeh* preparation by fermenting boiled soybeans with *Rhizopus oligosporus*. Boiling of soybeans resulted in a reduction (14.0%) of phytic acid (Table 4). About one third of the phytic acid was reduced in soybeans as a result of fermentation with mold (*R. oligosporus*). The decrease of phytic acid was accompanied by an increase in inorganic phosphorus. They concluded that the reduction in phytic

acid obtained was due to the action of the enzyme, phytase, which was produced by mold during fermentation.

Table 4. Phytic acid content of soybeans and *tempeh*[a]

Sample	Phytic acid (%)	Phytic acid hydrolyzed (%)
Soybeans, raw	1.41	0.00
Soybeans, soaked	1.43	0.00
Soybeans, boiled	1.23	13.99
Tempeh	0.96	32.88

[a]Data expressed on a dry weight basis.

Source : Sudarmadji and Markakis (1977).

The effect of natural fermentation on phytic acid in black gram, rice, and a black gram and rice blend was investigated by Reddy and Salunkhe (1980a). At 45-hrs. fermentation about 13.3% of phytic acid was hydrolyzed in black gram.

Akpapunam and Achinewhu (1985) studied the effects of fermentation on the chemical composition of Nigerian cowpea (*Vigna unguiculata*). Fermentation caused significant decreases in both phytic acid and phytate phosphorus. 34%, 61% and 69% reduction in phytic acid was observed after fermentation for 24, 48 and 72 hrs. respectively. There was no significant change in total phosphorus for beans fermented for 24 hours; after fermentation for 48 hours and 72 hours, significant increases in total phosphorus were observed. The decrease in phytic acid content was attributed to microbial degradation of phytic acid.

Marfo *et al.* (1990) reported substantial reduction in phytate levels of soybean and cowpea after seventy-two hours of fermentation. Lowering of

phytate levels was most rapid within the first 48 hrs. of fermentation. The loss of phytate during fermentation was attributed to the activity of the enzyme phytase naturally present in the legumes. The drop in pH from 6.21 to 3.10, which was observed in the doughs during fermentation, probably contributed to the slow breakdown of phytate after 48 hrs. of fermentation.

Generally, legume fermentations involve the action of proteolytic and lipolytic enzymes with the result that the final products are more digestible (Hesseltine, 1983). Locust beans fermented for 3 days were more digestible than raw locust beans (Umoh and Oke, 1974). The protein digestibility, protein efficiency ratio (PER), net protein utilization (NPU), and biological value (BV) of fermented locust beans were also higher than that of raw seeds (Fetuga *et al.*, 1973).

According to Boralkar and Reddy (1985), a progressive improvement in the protein digestibility with increasing periods of fermentation of soybean batter was observed as indicated in Table 5.

Table 5. Effect of fermentation on the *in vitro* protein digestibility of soybean

Fermentation time	*In vitro* protein digestibility[a]
Raw	58
8 hours fermenting	80
12 hours fermenting	84
16 hours fermenting	86
LSD 0.05/0.01	0.98/0.99

[a]Non-protein nitrogen expressed as per cent of total protein nitrogen.

Source : Boralkar and Reddy (1985).

2.4 EFFECT OF STORAGE ON PHYTIC ACID AND COOKING QUALITY

Legumes are generally stored for domestic consumption. Several changes occur during storage which influence the cooking behaviour of legumes. Long storage periods under tropical conditions result in hard-to-cook defect. The loss of cookability during storage of legumes has been attributed to the decrease in hydration rate, changes in chemical composition, changes in microstructure of the seeds and chemical and/or enzymatic changes that occur in the seed coat and the cotyledon during storage (Moscoso *et al.*, 1984).

2.4.1 *Physical and Structural Changes During Storage*

Of the various physical characters, water absorption, solid dispersibility during cooking, and texture (hardness) seem to be reliable indicators of the cooking quality of legumes. Moisture content, temperature, and storage time are the three important variables which influence the cooking quality of beans. The hard-to-cook defect results from deterioration during storage and reduced water absorption and cookability of cotyledons accompanied by deleterious changes in texture and flavour. The use of a low storage temperature (4°C) or the practice of storing beans with a low moisture content (around 8-10%) in a relatively low humidity environment has been shown to minimize the development of the hard-shell condition in legume seeds, including black beans (Burr *et al.*, 1968).

The levels of initial moisture content, seed coat thickness, texture and permeability, and storage temperature have been shown to affect water uptake in cowpea (Sefa-Dedeh *et al.*, 1979). Cowpeas stored at 0°C, 80% RH; 21°C, 35% RH and 29°C, 85% RH for up to 12 months indicated that the rate of cooking of

the beans decreased with increasing storage temperature and that the storage at 29°C introduced the formation of the 'hard-to-cook' defect. The microstructure of the defective beans showed an incomplete breakdown of the middle lamella which may partially explain this defect.

Varriano-Marston and Jackson (1981) reported that black beans stored for a short time period at high temperature and humidity exhibited a disintegration of cytoplasmic organelles and loosening of the attachment between cell wall and plasma lemma. The cytoplasmic changes during storage affect bean cookabilities. The middle lamella consists of insoluble salts of pectates and pectinates as well as protein components, with aging the middle lamella often becomes lignified. An early stage of lignification may be due to the cross-linking of hydroxyproline-rich proteins of the middle lamella in a reaction catalyzed by cell wall bound peroxidase. During the autolysis of cytoplasmic organelles lignin monomers formed from tyrosine and phenylalanine may be secreted in the middle lamella where lignification can proceed under the influence of H_2O and peroxidases. Hence, lignification of the middle lamella may be one of the explanations for decreased cookability.

Jones and Boulter (1983a) investigated the interrelationships between reduced cell separation rate, reduced imbibition value and reduced pectin solubility with reference to reduced cooking rate in *Phaseolus vulgaris*. It was found that the reduced imbibition value and reduced pectin solubility can both cause a reduction in the rate of cell separation during cooking of beans and hence an increase in their cooking time and that these two factors act synergistically. Accompanying symptoms were solute leakage during soaking due to membrane

breakdown, phytin catabolism and pectin demethylation, all of which are key factors in the development of hardbean.

Liu *et al.* (1993) studied the mechanism of pectin changes during soaking and heating as related to hard-to-cook defect in cowpeas which were aged at 30°C and 64% relative humidity for 6, 12, and 18 months. Their results suggested that the lack of cell separation resulted from resistance of pectin to beta-eliminative degradation in addition to solubilization and that the hard-to-cook defect was caused partly by reduced beta-degradation during cooking, which apparently results from decreased tissue pH during aging.

2.4.2 *Chemical Constituents and Their Influence on Cookability of Pulses*

Certain storage conditions can result in an increase in the cooking time required to properly soften legumes. Storing legumes at high temperatures and relative humidities, conditions normally encountered in the humid tropics, accelerate the problem. This suggests that chemical or biochemical factors are responsible. Moscoso *et al.* (1984) indicated that the loss of cookability in mature bean seeds after storage results from a decrease in phytic acid content.

Among chemical factors, calcium, magnesium, phytin, and pectin seem to be the important ones for cooking quality of some pulses. The composition of the cell wall relating the contents of phytin, Ca^{2+}, Mg^{2+}, and free pectin (PCMP number) as described below affects the cooking quality of legumes (Muller, 1967).

$$\text{PCMP number} = \frac{\text{Free pectin} \times (Ca^{2+} + 1/2\ Mg^{2+})}{\text{phytin}}$$

In several species of legumes, a good correlation between phytic acid content and cookability have been observed (Kon, 1968; Kumar *et al.*, 1978). Narasimha and Desikachar (1978) reported a high correlation between cooking time and the contents of calcium, magnesium, pectin, phytin and the PCMP number. Generally there was trend of an increase in the cooking time of the pulse with an increase in calcium, magnesium and pectin and a decrease in the total and phytin phosphorus. The mechanism proposed is that phytic acid chelates calcium, reduces the formation of calcium - pectic complexes responsible for hard texture, and exhibits a texture-softening effect. When the phytic acid content is low, the pectin in the middle lamella forms insoluble calcium and magnesium pectates which contribute to the poor cooking quality.

Kon and Sanshuck (1981) reported that the storage of dry beans under conditions of relatively high moisture and temperature increased about 5-fold the cooking time of beans (*Phaseolus vulgaris*). Among the changes that occurred in beans stored in this way, the reduction (about 65%) in phytic acid content was the best indicator of the increased cooking time. Jones and Boulter (1983b) suggested that the increase in moisture content due to high relative humidity during storage was one of the key factors in initiation of the hardening phenomenon in legumes. It permits restricted metabolism which leads to membrane breakdown which in turn causes reduced leakage and imbibition values also allowing access to bivalent cations from hydrolyzed phytin to the pectin.

Moscoso *et al.* (1984) investigated the effect of storage at high-temperature, and high-humidity on cooking quality and physicochemical properties of dry, mature red kidney beans stored for 9 months. The apparent softening rate

decreased with increasing storage time. Beans stored at 2°C showed no significant change in phytic acid phosphorus content during 9 months of storage. The beans stored at 32°C showed a consistent decrease in the phytic acid phosphorus content during storage with higher moisture samples showing the greatest decrease. They suggested that the loss of cookability of dry bean seeds in storage is associated with a decrease in phytic acid phosphorus content and an alteration in the ratio of monovalent to divalent cations in soaked beans. The seeds are left with less phytic acid and less monovalent cations which can solubilize the pectic substances through chelation and ion exchange during the cooking process.

Hernandez-Unzon and Ortega-Delgado (1989) studied the phytic acid in stored common bean seeds (*Phaseolus vulgaris* L.). Four varieties of common bean seeds stored at 4°C, 80% relative humidity, for one to eight years showed no differences in proximal chemical composition. Seeds 5-6 years old absorbed more water than 1 to 4 year-old seeds. Water absorption in the older beans was faster than in the younger seeds. Freshly harvested seeds were more resistant to water absorption because their membranes were still intact. The cooking time required for five year old seeds was 6 hours, while the fresh seeds needed 3/4-to-one hour cooking time. The most remarkable difference was in phytic acid content, which decreased 94% to 98% during long storage.

Hentges *et al.* (1991) monitored the effects of storage temperature and humidity on several physical and chemical components of cowpeas and beans. Seeds stored at 29°C, 65% RH (relative humidity) required prolonged cooking times; however, seeds stored in other conditions (5°C, 30% RH; 29°C 30% RH; and 5°C, 65% RH) maintained short, stable cooking times throughout the storage

period. In general, legume seeds stored at 29°C, 65% RH had higher water absorption values than seeds stored under other conditions for the first 9-12 months of storage. As cooking time increased, phytate, amylase solubility, high methoxyl pectin and protein solubility decreased. Solids leached during storage and low methoxyl pectin increased as cooking time increased. It was suggested that membrane degradation would allow released divalent cations from phytate hydrolysis to be redistributed to the middle lamella where an insoluble complex could form with pectin and contribute to the increased cooking time. These results were consistent with the proposed theory that the hard-to-cook defect involves interactions between phytate, minerals, and pectin.

2.4.3 *Effect of Storage on Protein Digestibility of Legumes*

The hard-to-cook characteristic as a result of storage under unfavourable conditions may affect the nutritional quality of legume seeds. Molina *et al.* (1975) found a negative relationship between storage and the protein digestibility, protein efficiency ratio, and protein solubility of black bean stored at 25°C for three and six months. Although the storage increased methionine and available lysine contents of the raw and processed beans, it had, in general, a detrimental effect on protein quality. Storage had an opposite effect on the protein digestibility, and on the nitrogen solubility, in 1N NaCl, 0.05N NaOH and H_2O, of the processed beans. Preliminary results indicated that the Nitrogen fraction soluble in 1N NaCl was capable of lowering bean protein digestibility *in vitro* when using pepsin as the digestive enzyme.

Antunes and Sgarbieri (1979) reported that storage of dry beans at high temperature (37°C) and high relative humidity (76%) not only had an adverse effect on the rate of hardening but also lowered the protein quality and the availability of essential amino acids.

An interaction between storage and processing also affects the nutritional quality of food legumes. Bressani (1983) reported that after a 6-month storage at 5°C, beans soaked in water suffered a 6% loss of protein digestibility. At 30 and 40°C storage, there was a 15% loss. Even after 240 min. of cooking time, digestibility of hard-to-cook beans was significantly lower than recently harvested beans.

Sievwright and Shipe (1986) demonstrated a marked increase in seed hardness and a decrease in protein digestibility (*in vitro*), accompanied by changes in tannins and phytates, in black beans stored at 30 or 40°C and 80% relative humidity. According to these workers, protein digestibility was reduced by interactions between protein and tannins, especially high molecular weight tannins. Firmness increased and protein digestibility decreased as the phytic acid content decreased.

Tuan and Phillips (1991) studied the effect of the hard-to-cook defect on protein digestibility of cowpeas. Storage of cowpeas under conditions that produced the hard-to-cook defect reduced protein digestibility as determined by both an *in vitro* technique and analysis of rat ileal contents. The observed exacerbation of the negative effect of hard-to-cook development on protein digestibility was attributed to interaction between proteins and phenolic acids.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

Grain samples of 3-16 genotypes of 5 legumes (pigeonpea, chickpea, green gram, black gram and soybean) were selected based on the availability of the samples during that season. The study was designed to cover the following aspects.

1. Screening the phytic acid level and determining the range in each legume.
2. Testing the effect of processing methods namely germination, fermentation, autoclaving and roasting on *in vitro* protein digestibility, phytic acid, total dietary fiber content, and ionisable iron.
3. Testing the effect of storage on phytic acid, *in vitro* protein digestibility and cooking quality.

3.1 MATERIALS

The legumes selected for the present study consisted of pigeonpea, chickpea (sixteen genotypes each), green gram (3 genotypes), black gram (four genotypes) and soybean (six genotypes). Grain samples of pigeonpea genotypes were supplied by the Pigeonpea breeding unit of Legumes Program at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Patancheru,

Andhra Pradesh, India. Grain samples of chickpea genotypes were provided by the Chickpea breeding unit of Legumes Program at ICRISAT. Samples of green gram and black gram were obtained from the Andhra Pradesh State Seeds Development Corporation, Hyderabad, India. Soybean samples were procured from the University of Agricultural Sciences, Dharwad, Karnataka, India. The genotypes selected for the study are given in Table 6.

The grain samples were cleaned and stored in a cold room at 5°C until processed for physical and chemical analysis.

Table 6. Genotypes selected for the study

Pigeonpea (*Cajanus cajan*)	Chickpea (*Cicer arietinum*)	Green gram (*Vigna radiata*)	Black gram (*Vigna mungo*)	Soybean (*Glycine max*)
ICPL 87051	Desi	PS 16	T 9	MONETTA
ICPL 87119	ICCV 89211	ML 267	LBG 611	MACS 58
ICP 8094	ICCV 89214	LGG 407	LBG 22	MACS 124
ICP 8863	ICCV 89217		LBG 17	JS 335
ICPL 88046	ICCV 89405			PK 472
ICPL 85012	ICCV 88202			KhSB2
UPAS 120	ICCC 37			
ICPL 85010	ICCV 10			
ICPL 4	ICCV 89303			
ICPL 366	ICCV 89304			
BDN 1	ICCV 89302			
ICPL 87	ICCV 89424			
ICPL 151	ICCV 88108			
ICPL 84031	ICCV 89230			
ICPL 84052	Kabuli			
C 11	ICCV 6			
	ICCV 3			
	ICCV 2			

3.2 PROCESSING

3.2.1 *Primary Processing*

Pre-treatment of whole grain for dehulling

For dehulling the seed samples were soaked in distilled water for 4 hrs. at room temperature (25°±1°C) and this was followed by drying in the oven as the pre-treatment employed for dehulling pigeonpea. In case of green gram and black gram, the samples were moistened with distilled water and kept aside as a heap for 4 hrs. at room temperature (25±1°C). The treated samples of pigeonpea, green gram and black gram were dried in an oven at 55°C for 16 hrs. and used for dehulling.

Dry method of dehulling was used for chickpea and soybean genotypes which were dried in an oven at 55°C for 4 hrs. without any pretreatment and used for dehulling. Whole seed and *dhal* samples of the five legumes studied (genotypes selected for secondary processing studies) are given in plates 1, 2, 3, 4 and 5).

Dehulling

For the preparation of *dhal* samples (decorticated split cotyledons), a tangential abrasive dehulling device (TADD) was used. After standardising the TADD for dehulling of the different legumes, a 100-g grain sample was dehulled for the required time (1 min for chickpea and pigeonpea, 1 1/2 min. for green gram and black gram and 30 seconds for soybean) by putting an approximately equal mass of grain material in 12 cups/holes of the TADD plate. After dehulling,

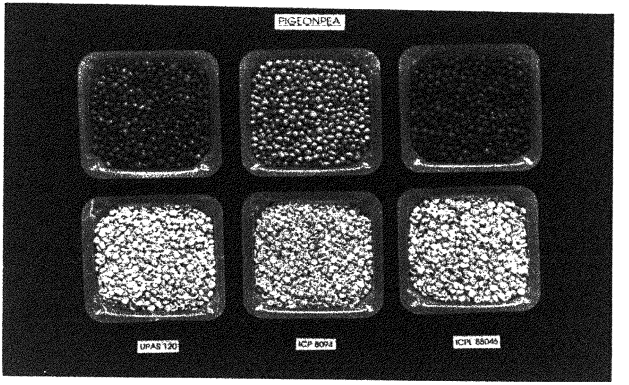

Plate 1. Whole seed and dhal of pigeonpea genotype UPAS 120, ICP 8094 and ICPL 88046.

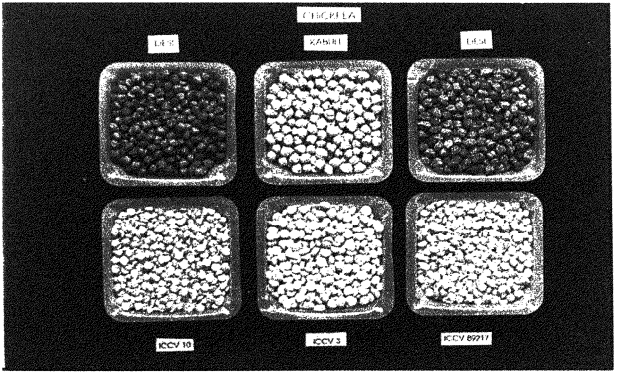

Plate 2. Whole seed and dhal of chickpea genotypes - ICCV 10, ICCV 3 and ICCV 89217.

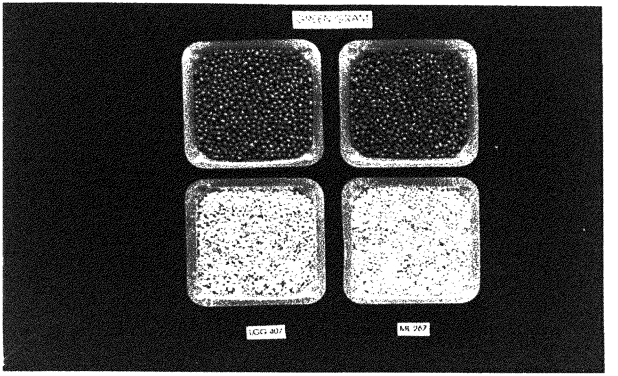

Plate 3. Whole seed and dhal of green gram genotypes - LGG 407 and ML 267.

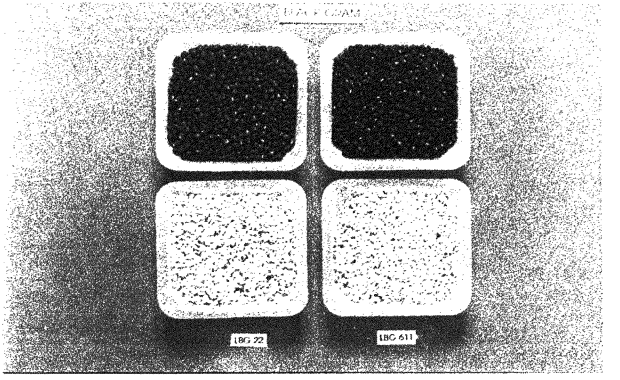

Plate 4. Whole seed and <u>dhal</u> of black gram genotypes - LBG 22 and LBG 611.

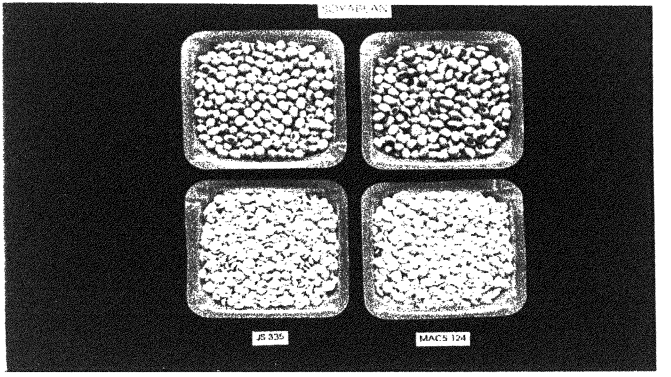

Plate 5. Whole seed and dhal of soybean genotypes - JS 335 and MACS 124

the processed material was manually separated into *dhal*, brokens, powder, and husk fractions. Both unsplit and split decorticated cotyledons were included as *dhal*. Powder fraction in the present study is defined as the fine flour obtained as a result of successive removal of the outer layers of cotyledons during the dehulling operation in TADD. The brokens, powder and husk fractions of dehulling were discarded and *dhal* fractions were used for further study. Dehulling fractions recovery (i.e. % undehulled seed, % *dhal*, % brokens, % powder and % husk) was calculated.

For chemical analyses, the *dhal* (decorticated cotyledons) samples thus obtained were ground in a Udy cyclone mill using a 0.4 mm screen and defatted using n-hexane. All chemical analyses were made in duplicate. All chemicals used in the study were of Analar grade and obtained locally.

3.2.2 *Secondary Processing*

For secondary processing studies, three genotypes each of pigeonpea (ICPL 88046, ICP 8094, and UPAS 120) and chickpea (ICCV 89217, ICCV 10 and ICCV 3) having high, moderate and low phytic acid levels and two genotypes each of green gram (ML 267 and LGG 407), black gram (LBG 611 and LBG 22) and soybean (JS 335 and MACS 124), with high and low phytic acid content were selected. The processing methods employed were germination, fermentation, autoclaving (wet-heating) and roasting (dry-heating).

Germination

Seeds were soaked in distilled water for 12 hrs. at room temperature (25°C). The soaked seeds were germinated in sterile petri dishes lined with wet filter paper. To obtain a sprout measuring 1.5 cm, the seeds of pigeonpea and soybean were germinated for 72 hrs. (Plates 6 and 10) and those of chickpea, green gram, and black gram were germinated for 48 hrs (Plates 7, 8 and 9). Seed coat was removed manually from the sprouted samples, which were then freeze-dried and ground to a fine powder in a Waring blendor.

Fermentation

Dhal samples of pigeonpea, chickpea, and soybean were soaked in distilled water for 16 hrs. at room temperature (25°C±1°C) and those of green gram and black gram were soaked in distilled water for 2 hrs. at room temperature. A seed-to-water ratio of 1:2 (w/v) was used for soaking the samples. The soaked *dhal* samples were ground to a batter in a Waring blendor. The batter was thoroughly mixed with 1.5% inoculum (w/v) and allowed to ferment for 24 hrs. in an incubator at 30°C. The natural curd sample containing lactic acid bacteria was used as an inoculum. The fermented batter was freeze-dried and ground to a fine powder in a Waring blendor.

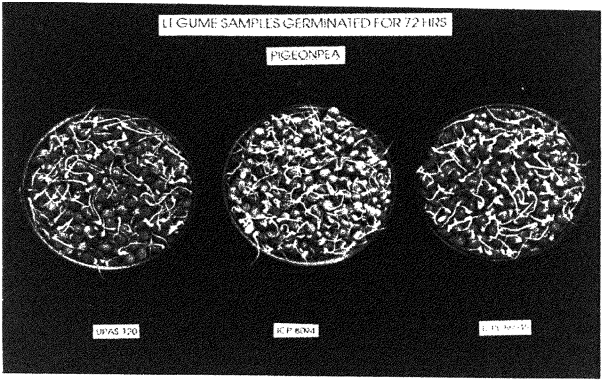

Plate 6. Germinated samples of pigeonpea genotypes - UPAS 120, ICP 8094 and ICPL 88046.

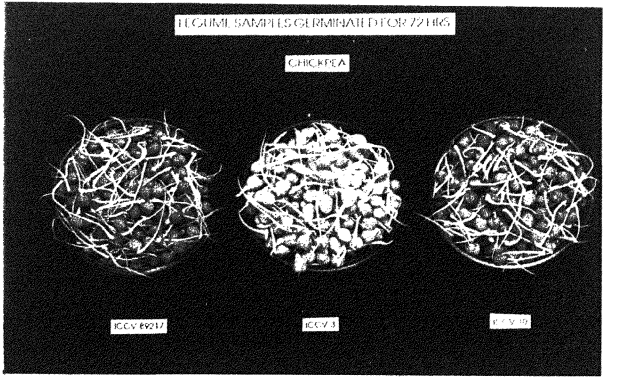

Plate 7. Germinated samples of chickpea genotypes - ICCV 89217, ICCV 3 and ICCV 10.

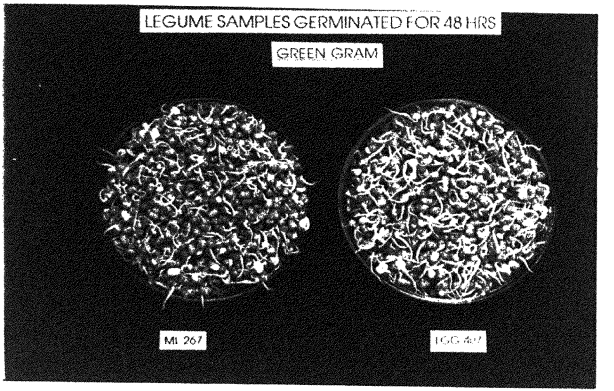

Plate 8. Germinated samples of green gram genotypes - MI 267 and LGG 407.

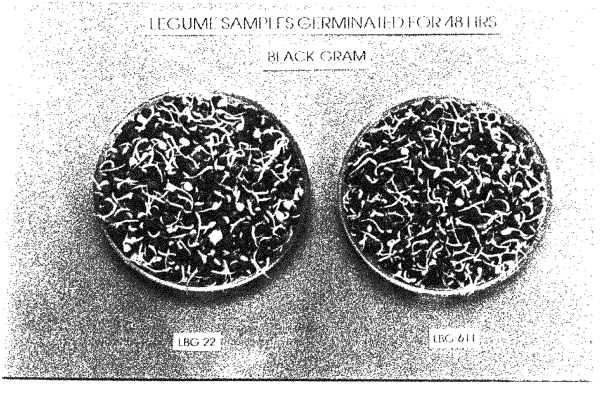

Plate 9. Germinated samples of black gram genotypes - LBG 22 and LBG 611.

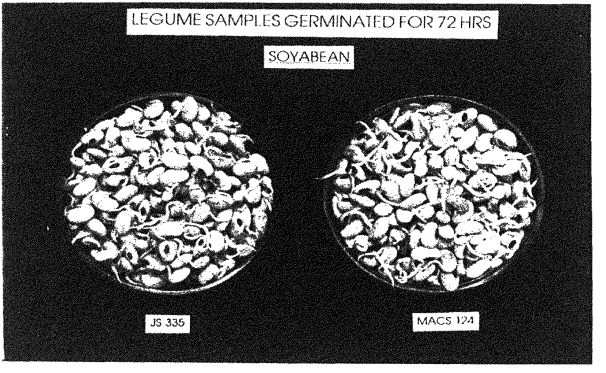

Plate 10. Germinated samples of soybean genotypes – JS 335 and MACS 124.

Autoclaving (wet-heating)

Dhal samples were autoclaved in an autoclave at 15 psi pressure for 15 min. for pigeonpea, chickpea and soybean and 10 min. for green gram and black gram, as these being their normal cooking times. *Dhal*-to-water ratio of 1:2 (w/v) was used for autoclaving. The whole cooked broth was freeze-dried and ground to a fine powder in a Waring blendor.

Roasting (dry-heating)

Whole-seed samples were roasted in a sand bath at 200°C for 2 min. The roasted material was separated from the sand by sieving, dehulled by TADD and ground to a fine powder in a Waring blendor.

3.3 STORAGE

Dhal sample of one genotype each of pigeonpea (ICPL 87119), chickpea (ICCC 37), green gram (PS 16), black gram (T 9), and soybean (MONETTA) was stored for a period of 12 months at 5°C, 25°C, and 37°C to study the effect of storage on phytic acid, *in vitro* protein digestibility and cooking quality. Samples were drawn periodically (every 3 months) for chemical analyses and evaluation of cooking quality.

3.4 PHYSICAL ANALYSES

3.4.1 100-Seed Mass

For determination of seed mass 100-seeds were randomly selected, counted manually and average weight of five replicates of each genotype recorded.

3.4.2 Seed Coat Content

Seed coat content was determined by manually separating the seed coat from the cotyledons. Seed samples were soaked in excess distilled water at room temperature (25°C ± 1°C) for 16 hrs. After this treatment, excess water was discarded and seed coat was removed manually using forceps. Seed coat fractions were dried in the oven at 55°C for 16 hrs. and weighed to calculate seed coat percentage.

3.5 CHEMICAL ANALYSES

3.5.1 Moisture

Moisture content was determined by drying the samples overnight (16 hrs.) in a hot air oven at 110°C (AOAC, 1984).

3.5.2 Ash

For estimation of ash, previously weighed samples were incinerated in a muffle furnace at 600°C for 2 hrs. Ash (%) was obtained by difference in weight (AOAC, 1984).

3.5.3 *Fat*

Ground samples (2 g) were packed in a Whatman No. 2 filter paper and the oil was extracted in a Soxhlet apparatus with n-hexane for 16 hrs. The extracted oil was transferred to a previously weighed beaker (250 ml) containing 3-4 boiling chips and the solvent was evaporated on a sand bath. The beaker was reweighed. From the difference in weight, fat percent of the sample was calculated (AOCS, 1981).

3.5.4 *Protein*

For the determination of protein, a suitable amount of defatted sample (70 mg) was placed in a digestion tube. One Kjel-tab (Thompson and Capper Ltd. Runcorn, England) (each tablet containing 1.5 g potassium sulphate and 7.5 mg selenium) and 3 ml of sulphuric acid-phosphoric acid mixture [95 parts conc. sulphuric acid, 5 parts of 85% phosphoric acid (v/v)] were added to the digestion tube and the sample was digested at 370°C for 1 hour. After cooling, distilled water was added to bring the volume to 75 ml. A suitable aliquot was used for nitrogen estimation in Technicon Auto Analyser (TAA) using ammonium sulphate as a standard (Singh and Jambunathan, 1981a). Nitrogen values were converted into protein by multiplying by a factor of 6.25.

3.5.5 *In Vitro Protein Digestibility Assay*

The *in vitro* protein digestibility (IVPD) was determined by employing the pepsin and pancreatin enzymes (Singh and Jambunathan, 1981b). An amount of

defatted sample containing 6.75 ± 0.1 mg N was mixed with pepsin solution (pH 2.0) (Sigma Chemical Co.) and incubated at 37°C for 16 hrs. in a water bath shaker. After incubation, pancreatin solution (porcine pancreas, Sigma Chemical Co.) in 0.1 M borate buffer (pH 6.8) was added and the contents were further incubated for 24 hrs. The reaction was stopped by adding 10% trichloroacetic acid. The contents were centrifuged and the supernatants pooled for determination of nitrogen content. 5 ml of the aliquot was dried in a digestion tube and nitrogen content determined by Technicon auto analyser as described above.

Protein digestibility (*in vitro*) was calculated by using the following formula:

$$\text{Protein digestibility (\%)} = \frac{\text{Digested protein}}{\text{Total protein}} \times 100$$

3.5.6 Nitrogen Solubility Index

The nitrogen solubility was determined by employing AACC method (AACC, 1982) with minor modifications as follows. Suitable amounts (500 mg) of defatted sample was weighed in 50 ml centrifuge tubes. Distilled water (20 ml) was measured, a small portion was used to disperse the sample using a vortex mixer and then the remainder of the water was added. The contents were shaken on a mechanical shaker for 1 hour at room temperature and centrifuged at 7500 rpm for 15 min. Supernatant was collected and the residue was suspended and centrifuged again with 20 ml of water. The supernatants were pooled and the

final volume made to 50 ml. Suitable aliquots (5 ml) were analyzed for nitrogen content by using Technicon Auto Analyser as described above.

Nitrogen solubility index was calculated by using the following formula:

$$\% \text{ Nitrogen Solubility Index (NSI)} = \frac{\% \text{ water-soluble N}}{\% \text{ total N}} \times 100$$

3.5.7 *Total Dietary Fiber*

Total dietary fiber (TDF) as an index of unavailable carbohydrates was determined according to the method described by Southgate (1978).

Principle

This method is based on the enzymatic removal of protein and starch to give an 'indigestible residue' and the procedure is based on extraction with neutral detergent solutions.

Reagents

Standard reagents used for protein determination

Ethyl alcohol

2 M Sodium acetate buffer, pH 4.8

Ethanol (90% v/v)

5% Phenol

96% Sulphuric acid

Procedure

1 g of sample was packed in a Whatman No. 2 filter paper and inserted into a thimble. The thimble was kept in a Soxhlet extractor and the sample was extracted with 90% ethanol overnight (16 hrs.). The residue insoluble in the ethanol was dried, weighed and ground into a fine powder and analysed for protein and starch. Protein was determined by the Technicon Auto Analyser procedure as described above. The starch content in the residue was determined by enzymatic hydrolysis (Singh *et al.*, 1980). Dried residue (50 mg) was placed in a conical flask and a few drops of ethyl alcohol and 10 ml of water were added. Contents were dispersed and then autoclaved for 90 min. at 19 psi pressure. The suspension was cooled to room temperature and 1 ml of 2 M sodium acetate buffer (pH 4.8) was added and the final volume was made to 25 ml with distilled water. This suspension was incubated with 25 mg of amyloglucosidase (Sigma Chemical Co.) for 2 hrs. at 55°C. The glucose thus released was estimated, using the phenol-sulphuric acid method. The percentage of starch present was obtained by multiplying the glucose concentration with 0.9.

Calculations

Total dietary fiber (TDF) was calculated by using the following formula:

$$\% \text{ TDF} = \frac{\text{Residue insoluble in alcohol-starch + protein}}{\text{Weight of the sample taken}} \times 100$$

3.5.8 Phytic Acid

Phytic acid was assayed as ferric-phytate by the extraction method of Wheeler and Ferrel (1971).

Principle

The phytic acid and phytic acid chelates react with ferric chloride and form ferric phytate. The available ferric ion after reaction is determined by developing blood-red color with potassium thiocyanate.

Reagents

Trichloroacetic acid (TCA), 5% : 5 g TCA was dissolved in glass distilled water and volume made upto 100 ml.

Potassium thiocyanate, 29% : 29 g potassium thiocyanate was dissolved in glass distilled water and volume made upto 100 ml.

Ferric chloride, 0.25% : 25 mg ferric chloride was dissolved in 100 ml 5% TCA.

Phytic acid : 50 mg sodium salt of phytic acid was dissolved in 100 ml 5% TCA.

Procedure

200 mg defatted sample was weighed into a centrifuge tube and extracted with 5% TCA at 60°C and centrifuged at 5000 rpm for 15 min. The precipitate was washed twice with 5% TCA and centrifuged. The supernatants were pooled and volume made up to 50 ml. An aliquot (20 ml) was pipetted into a digestion tube and 5 ml of 0.25% $Fecl_3$ $6H_2O$ (Ferric chloride) was added. The tube was heated

in a block digestor at 95°C for 45 min. The contents were cooled and volume made up to 75 ml. The available ferric ion after reaction was determined by reaction with potassium thiocyanate which developed a blood-red colour compound. The absorbance was read at 485 nm against a reagent blank. To determine the volume of ferric chloride solution which will represent 1 mg phytic acid, a standard curve of absorbance against standard sodium salt of phytic acid (Sigma Chemical Co.) was plotted and used for calculation. Phytate content was calculated from the iron concentration by assuming a constant Fe : P molecular ratio of 4:6 in the precipitate.

3.5.9 Calcium, Magnesium and Iron

Samples were digested using a tri-acid mixture which contained nitric acid, perchloric acid and sulphuric acid in the ratio of 20:4:1. Defatted samples (0.5 g) were weighed and transferred to glass tubes and digested in a block digestor. After adding 10 ml of tri-acid mixture, the mixture was digested first at 70°C for 30 min, then at 180°C for 30 min and finally at 220°C for 30 min. After digestion, the mixture was cooled, dissolved in glass distilled water and the volume made to 50 ml. Suitable aliquots were analysed for determination of calcium, magnesium, and iron in an atomic absorption spectrophotometer (Varian Tectron Model - 1200) (Piper, 1966).

3.5.10 Total Phosphorus

The determination of total phosphorus was based on the reaction between phosphorus and molybdovanadate to form a phosphomolybdovanadate complex. To 80 mg of defatted sample 4 ml conc. sulphuric acid containing 1.5% Se was added and the sample was digested on a block digestor at 360°C for 75 min. The volume was made up to 75 ml after cooling. Suitable aliquots were used for the determination of Total phosphorus using Technicon Auto Analyser (Technicon Industrial Systems, 1972).

3.5.11 Ionisable Iron

Availability of iron was estimated in terms of ionisable iron using a simulated *in vitro* gastrointestinal digestion procedure. The method adopted was a combination of two methods. The extraction of ionisable iron was performed according to the method of Narasinga Rao and Prabhavati (1978); ionisable iron in the filtrate was measured by the method of Miller *et al.* (1981).

Principle

The iron present in the food is released by pepsin. Protein precipitant precipitates protein and reduces Fe^{3+} to Fe^{2+}, Fe^{2+} reacts with bathophenanthroline to give pink color which is read in a UV-VIS spectrophotometer.

Reagents

0.1 N Hydrochloric acid solution

0.5% Pepsin hydrochloric acid solution : 0.5 g pepsin was dissolved in 0.1 N HCl and volume made upto 100 ml with the same.

Protein precipitant solution: 10 g trichloroacetic acid, 10 g hydroxylamine hydrochloride (Sigma Chemical Co.) and 10 ml concentrated HCl was dissolved in water and made to a volume of 100 ml.

Chromagen solution: 50 mg bathophenanthroline sulfonate was dissolved in 2 M sodium acetate and the volume was made upto 100 ml with the same.

Iron standard solution

Ferrous ammonium sulphate [$FeSO_4$ $(NH_4)_2SO_4$, $6H_2O$] was used for the preparation of stock iron standard solution (1000 µg/ml). An amount of salt weighing 0.7021 g was dissolved in a minimum volume of glass distilled water, 2 to 3 drops of concentrated hydrochloric acid was added and the volume was made upto 100 ml with glass distilled water. The second stock solution (10 µg/ml) was prepared from the original stock solution (1000 µg/ml) by suitably diluting with glass distilled water. The working standard solution (1 µg/ml) was prepared from the second stock solution (10 µg/ml) again by diluting with glass distilled water.

A series of standards were prepared from the working standard solution (1 µg/ml). To 0.1 ml to 0.6 ml of working standard solution in a series of tubes,

0.9 ml to 0.4 ml of glass distilled water was added to make up the volume to 1 ml. To each tube, 1 ml of protein precipitant solution was added, mixed thoroughly, kept for 5 min. at room temperature and then centrifuged at 3000 rpm for 20 min. From each tube, 0.5 ml of supernatant was transferred to another series of tubes, 0.5 ml of chromagen solution was added, mixed and allowed to stand for 20 min. at room temperature.

A blank solution was prepared along with the standard, with 1 ml of glass distilled water and 1 ml protein precipitant and processed in the same way as described for standards.

Simulated gastro intestinal digestion of samples

Weighed quantity (2 g) of powdered sample was first incubated with 25 ml pepsin-HCl solution. The pH of the mixture was adjusted to 1.35 and incubated in a 100 ml conical flask at 37°C in a shaker water bath for 90 min. At the end of this incubation, pH was adjusted to 7.5 using 10 to 20% NaOH and incubated at 37°C for 90 min. in a shaker water bath. At the end of this incubation period the contents of the flask were centrifuged at 3000 rpm for 45 min. The supernatant was filtered through Whatman No. 44 filter paper. An extract blank was run simultaneously.

Estimation of ionisable iron

Bathophenanthroline reactive iron was measured in the filtrate immediately after the incubation. The protein precipitant solution was added to the filtrate at

a ratio of 1:1 and thoroughly mixed in a vortex mixer. The mixture was centrifuged at 5000 rpm for 10 min. An aliquot of the clear supernatant was transferred to a clean test tube and the chromagen solution was added with thorough mixing at a ratio of 1:1. After 10 min. the absorbance was read at 533 nm in a Shimadzu spectrophotometer (UV 160). The ionisable iron present in the extract was calculated from a standard curve of absorbance against the standard iron concentration. The ionisable iron content in foods was expressed in :

1. mg/100 g (absolute amount) and
2. Percentage (relative amount)

3.5.12 Pectic Substances

The method described by Dekker and Richards (1972) was used to determine pectic substances.

Principle

Pectic substances are solubilized in a buffer followed by reaction with polygalacturonase to break into individual units of galacturonic acid. The galacturonic acid thus obtained is colorimetrically estimated using carbazole reaction.

Reagents

Sulphuric acid : water (6:1) v/v : 6 volumes of sulphuric acid was diluted in 1 volume of water.

Carbazole 0.1% in ethanol (w/v) : 100 mg of carbazole was dissolved in ethyl alcohol and volume made up to 100 ml.

Ammonium oxalate and oxalic acid (oxalate buffer), 2.5% (w/v) : 2.5 g each of ammonium oxalate and oxalic acid was dissolved in 80 ml distilled water, pH adjusted to 4.0 with dilute sodium hydroxide solution and volume made upto 100 ml.

Galacturonic acid (standard) : 7.5 mg galacturonic acid was dissolved in water and volume made upto 100 ml.

Polygalacturonase 0.5 mg/ml solution : 50 mg polygalacturonase was dissolved in 100 ml oxalate buffer.

Procedure

For determination of pectic substances, 500 mg of defatted sample was weighed into a 250 ml flat bottom flask. The sample was refluxed for 1 hr. with oxalate buffer, pH 4.0 [Ammonium oxalate and oxalic acid, 2.5% (w/v)]. The contents were cooled, volume made up to 100 ml and a portion of it centrifuged at 5000 rpm for 10 min. 20 ml of supernatant was pipetted into a 10 ml erlenmeyer flask, 5 ml polygalacturonase solution (0.5 mg/ml, Sigma Chemical Co.) was added and the flask was incubated at 30°C for 20 hrs. At the end of the incubation period the contents of the flask were boiled for 2 min. to inactivate the enzyme (An enzyme blank was also processed simultaneously). The galacturonic acid thus obtained was colorimetrically determined with carbazole reagent at 520 nm. Pectic substances in terms of galacturonic acid was estimated by comparison

with a standard curve made by plotting absorbance against standard D-galacturonic acid monohydrate (Sigma Chemical Co.).

3.6 DETERMINATION OF COOKING QUALITY PARAMETERS

Cooking time

Cooking time was determined by boiling the *dhal* in distilled water in a BD-20 heating block digestor (Tecator, Sweden). The *dhal* sample (10.0 ± 0.5 g) was boiled in 50 ml distilled water. During boiling, samples were removed at 1 min. intervals and examined for their softness by pressing them between the forefinger and the thumb to determine the cooking time.

Water absorption

For water absorption, *dhal* samples (5.0 ± 0.5 g) were boiled for the average cooking time (of that particular pulse) in excess distilled water (35 ml) in the BD-20 block digestor. The excess water after boiling was decanted and the *dhal* weighed. The amount of water taken up by the *dhal* was calculated and the results were expressed as an increase in *dhal* weight per gram of sample.

Solid dispersion

The percentage of solids dispersed into the cooking water was determined by boiling the *dhal* sample (5.0 ± 0.5 g) for the average cooking time. The boiled material was passed through a 20 (850 μm) mesh sieve and the residue thoroughly washed with distilled water. After washing, the residue was dried at

110°C for 3 hrs. The loss in weight of *dhal* after boiling was calculated and expressed as percentage of solids dispersed into the cooking water.

PCMP Number

PCMP number was calculated according to the formula of Muller (1967). 'PCMP' number is an index of cooking quality relating the contents of phytin, calcium, magnesium and pectin. The contents of these components were expressed as meq (milli equivalents) per 100 g of sample for calculation of PCMP No.

'PCMP' number was calculated by using the following formula:

$$\text{PCMP No.} = \frac{\text{Pectin} \times (\text{Ca} + 1/2\text{Mg})}{\text{Phytin}}$$

3.7 STATISTICAL ANALYSES

For all chemical analyses, two replicates were used for the determination of each constituent. Standard errors (SE) were determined by one way analysis of variance (Snedecor and Cochran, 1967). Nutritional and cooking quality parameters were statistically analysed for correlation coefficients. The statistical analyses were carried out using the GENSTAT program. Graphics were prepared using Freelance package.

RESULTS

CHAPTER IV

RESULTS

Grain legumes are rich and less expensive sources of dietary proteins and contribute substantially to the protein content of the diets of a large part of the Indian population. Presence of antinutritional factors is one of the main drawbacks limiting the nutritional and food qualities of legumes. Phytic acid, widely distributed in legumes inhibits several proteolytic enzymes and lowers the bioavailability of minerals. Genetic manipulation implied in evolving new high yielding varieties may produce wide variation in the phytic acid content.

Grain legumes in India are processed and consumed in a variety of forms. Traditional methods of processing include germination, fermentation, pressure cooking and roasting. It is important to know the extent to which phytic acid survives these domestic processing treatments and finally remains in the food form in which it is eaten.

Certain storage conditions can result in an increase in the cooking time required to properly soften legumes. Storing legumes at high temperatures and relative humidities, conditions normally encountered in the humid tropics, accelerate the problem. Phytic acid is one of the factors that has been implicated in the loss of cookability. The results of the present study on the variability in phytic acid and protein digestibility of legumes, the effects of processing practices on phytic acid, protein digestibility, dietary fiber, and mineral contents of pulse

crops and the effects of storage on phytic acid, cooking quality and *in vitro* protein digestibility are presented as follows.

4.1 DEHULLING QUALITY OF DIFFERENT LEGUMES

In India, dehulling of legumes is a primary process that converts whole seed into *dhal* (decorticated, dry, split cotyledons); various procedures, ranging from commercially operated *dhal* mills in cities to manually operated stone *chakkis* in the villages, are employed for this purpose. Dehulling was carried out using a Tangential Abrasive Dehulling Device (TADD). Although, it is difficult to compare the TADD with a commercially operated *dhal* mill employed for dehulling legumes, the abrasive action involved in this TADD dehulling equipment appears to be comparable with that of the commercial *dhal* mill.

Thirty three varieties of different legumes studied (i.e. pigeonpea, chickpea, green gram, black gram and soybean) were dehulled as above and results are given in Tables 7 and 8. *Dhal* yield differed significantly among and within the legume species studied. *Dhal* yield was highest in soybean (85.2%) and lowest in green gram (67.2%). *Dhal* yield of pigeonpea genotypes ranged from 68.0 to 85.9% with mean being 78.9%, showing a large variation among the genotypes (Table 7). Among chickpea genotypes, *dhal* yield was the highest (89.5%) for ICCV 3 and the lowest (77.1%) for ICCC 37. This indicated significant ($P < 0.01$) differences in dehulling quality of chickpea genotypes. Among chickpea genotypes, *dhal* recovery was higher in kabuli than in desi genotypes (Table 7).

Table 7. **Percentage *dhal* yield, brokens, powder and husk fractions of pigeonpea and chickpea[a]**

Genotype	*Dhal* yield (%)	Brokens (%)	Powder (%)	Husk (%)
Pigeonpea				
ICPL 87051	77.5	1.8	4.4	9.7
ICPL 87119	80.7	2.3	6.8	9.0
ICP 8094	75.1	2.8	6.0	10.4
ICP 8863	78.8	2.1	7.0	8.1
ICPL 88046	84.5	1.2	4.0	9.7
ICPL 85012	80.8	1.4	5.5	9.3
UPAS 120	76.4	1.7	5.1	9.5
ICPL 85010	81.6	1.4	4.9	9.5
ICPL 4	68.0	4.7	10.5	10.6
ICPL 366	85.9	1.9	4.4	8.8
Mean	78.9	2.1	5.9	9.5
SE	±0.79	±0.11	±0.12	±0.11
Chickpea				
Desi				
ICCV 89211	86.9	0.27	3.6	9.1
ICCV 89214	79.0	0.65	5.7	10.8
ICCV 89217	79.7	0.56	6.4	9.5
ICCV 89405	77.8	0.57	6.7	10.7
ICCV 88202	78.4	0.75	6.9	11.7
ICCC 37	77.1	1.18	6.7	10.1
ICCV 10	81.8	0.55	6.5	9.5
Kabuli				
ICCV 6	89.4	0.02	8.7	2.2
ICCV 3	89.5	0.42	9.5	0.6
ICCV 2	89.1	0.41	6.8	3.3
Mean	82.9	0.54	6.7	7.7
SE	±0.85	±0.071	±0.52	±0.36

[a]Values are mean of two independent determinations.

In green gram, dehulling quality was generally poor as compared to other legumes as the dehulling losses in terms of powder fraction were the highest (19.9%) in green gram followed by black gram (17.5%). Recovery of *dhal* was the highest (mean 85.2%) in soybean. No significant difference in *dhal* yield of soybean genotypes was observed (Table 8).

4.1.2 *Relationships Between the Dhal Yield and the Grain Physical Characteristics*

The results of 100-seed mass and seed coat content of different legumes are summarised in Tables 9 and 10. The 100-seed mass of pigeonpea genotypes ranged between 5.1 and 12.3 g with mean being 9.0 g (Table 9). The seed size of chickpea genotypes differed significantly ($P < 0.01$). The mean 100-seed mass of green gram and black gram was 3.3 and 4.9 g, respectively (Table 10). The 100-seed mass of soybean genotypes showed significant differences ($P < 0.01$). The seed coat content of pigeonpea and chickpea genotypes showed a large variation (Table 9). *Dhal* yield was positively correlated ($r = 0.72$) with 100 seed mass and negatively correlated ($r = 0.45$) with seed coat content.

4.2 CHEMICAL COMPOSITION OF LEGUMES

As expected, the protein content was the highest (mean 54.6%) for soybean. Pigeonpea had the lowest protein content. *Dhal* protein content in pigeonpea genotypes ranged from 19.6 to 27.5% indicating a large variation among genotypes (Table 11). Among chickpea genotypes protein content was the highest (28.1%) for ICCV 6 and the lowest (18.7%) for ICCV 3 indicating a large

Table 8. **Percentage *dhal* yield, brokens, powder and husk fractions of green gram, black gram, and soybean[a]**

Genotype	*Dhal* yield (%)	Brokens (%)	Powder (%)	Husk (%)
Green gram				
PS 16	71.4	3.4	15.5	7.5
ML 267	67.5	4.9	24.3	1.8
LGG 407	62.5	2.6	19.8	3.9
Mean	67.2	3.7	19.9	4.4
SE	±0.44	±0.89	±0.64	±0.44
Black gram				
T 9	72.6	3.7	16.0	4.8
LBG 611	66.3	3.3	20.5	6.8
LBG 22	77.9	1.3	14.7	6.2
LBG 17	69.3	2.3	18.8	4.2
Mean	71.5	2.7	17.5	5.5
SE	±1.00	±0.52	±0.76	±0.28
Soybean				
MONETTA	85.2	1.9	7.1	5.0
MACS 58	86.6	2.5	4.3	6.2
MACS 124	84.6	2.4	6.0	6.3
JS 335	84.6	1.9	7.4	4.9
PK 472	86.1	2.3	4.5	6.2
KhSB 2	84.2	2.3	6.8	5.9
Mean	85.2	2.2	6.0	5.8
SE	±0.84	±0.26	±0.59	±0.09

[a]Values are means of two independent determinations.

Table 9. Physical characteristics of pigeonpea and chickpea[a]

Pigeonpea			Chickpea		
Genotype	100-seed mass (g)	Seed coat %	Genotype	100-seed mass (g)	Seed coat %
ICPL 87051	12.3	10.95	Desi		
ICPL 87119	10.6	11.45	ICCV 89211	22.4	10.93
ICP 8094	6.4	11.36	ICCV 89214	20.8	13.38
ICP 8863	9.0	12.92	ICCV 89217	18.7	13.31
ICPL 88046	9.0	12.93	ICCV 89405	17.5	14.77
ICPL 85012	9.8	11.32	ICCV 88202	20.5	15.42
UPAS 120	8.4	14.55	ICCC 37	17.9	12.10
ICPL 85010	8.7	11.58	ICCC 10	17.0	12.43
ICPL 366	5.1	11.47	Kabuli		
ICPL 4	10.3	16.45	ICCV 6	19.7	5.50
			ICCV 3	22.9	5.31
			ICCV 2	26.4	5.78
Mean	9.0	12.50		20.4	10.89
SE±	±0.18	±0.561		±0.46	±1.237

[a]Results are mean of two independent determinations.

Table 10. Physical characteristics of green gram, black gram and soybean[a]

Green gram			Black gram			Soybean		
Genotype	100-seed mass (g)	Seed coat %	Genotype	100-seed mass (g)	Seed coat %	Genotype	100-seed mass (g)	Seed coat %
PS 16	3.3	10.11	T 9	4.7	10.71	MONETTA	13.3	6.46
ML 267	3.1	10.02	LBG 611	4.5	10.34	MACS 58	12.7	7.55
LGG 407	3.4	10.39	LBG 22	4.9	10.2	MACS 124	14.4	7.04
			LBG 17	5.3	10.39	JS 335	14.6	6.51
						PK 472	15.3	7.48
						KhSB 2	12.5	8.43
Mean	3.3	10.17		4.9	10.41		13.8	7.25
SE	±0.09	0.152		±0.16	±0.108		±0.48	±0.74

[a]Results are mean of two independent determinations.

variation among genotypes. Protein content of green gram and black gram did not show significant differences among the genotypes (Table 12).

Among the legumes studied soybean had the highest fat content with mean being 25.1% and green gram had the lowest fat content with mean being 1.5%. Fat content of pigeonpea genotypes ranged between 1.3 and 2.9% with mean being 2.0%. Among the chickpea genotypes fat content ranged between 5.1 and 7.6% indicating a large variation among genotypes (Table 11). The mean fat content of black gram genotypes was 1.6%. Among the legumes studied calcium content was the highest for soybean (mean 210.1 mg/100 g) and the lowest for green gram (mean 40.2 mg/100 g). Calcium content was the highest (68.0 mg/100 g) for ICP 8094 and the lowest (29.5 mg/100 g) for ICPL 4 indicating a large variation among pigeonpea genotypes. Similar variations among chickpea genotypes were noticed for calcium content (Table 11). Among the chickpea genotypes kabuli varieties had a higher calcium content than the desi varieties. Mean calcium content of black gram genotypes was 56.3 mg/100 g (Table 12). The calcium content of soybean genotypes ranged between 163.1 and 292.2 mg/100 g indicating a large variation.

Magnesium and iron content of soybean were also higher compared to the other legumes and this was supported by the higher values for ash content (Table 12). The iron content was the highest for soybean (6.4 mg/100 g) and the lowest for pigeonpea (3.8 mg/100 g).

Significant differences ($P < 0.01$) were observed for iron content among all the legume genotypes. Mean iron content of chickpea (6.1 mg/100 g) and

Table 11. Chemical composition of pigeonpea and chickpea[a]

Genotype	Protein	Fat	Ash	Total dietary fiber	Calcium	Magnesium	Iron
	(%)				mg/100 g		
Pigeonpea							
ICPL 87051	20.3	1.9	3.7	17.7	49.6	127.4	4.0
ICPL 87119	19.6	2.9	3.8	18.7	36.2	99.6	2.9
ICP 8094	27.5	1.6	3.9	18.6	68.0	120.7	4.5
ICP 8863	23.4	1.6	4.4	19.7	35.8	124.1	4.3
ICPL 88046	23.5	1.3	4.4	19.0	48.4	122.8	3.8
ICPL 85012	20.5	2.0	4.3	18.2	39.6	97.0	4.1
UPAS 120	24.3	1.7	5.4	18.3	51.4	129.9	3.8
ICPL 85010	25.1	2.6	5.8	18.7	40.2	90.4	3.1
ICPL 4	23.7	1.9	4.9	17.9	29.5	107.7	4.7
ICPL 366	25.7	2.3	5.5	18.9	37.1	132.2	3.0
Mean	23.4	2.0	4.6	18.6	43.6	115.2	3.8
SE	±0.28	±0.08	±0.09	±0.32	±0.76	±3.62	±0.11

Table 11 (Continued)

Chickpea							
Desi							
ICCV 89211	24.3	7.0	2.5	21.3	33.8	102.0	6.4
ICCV 89214	26.1	6.9	2.7	19.9	33.1	109.1	6.1
ICCV 89217	25.9	6.0	2.8	17.7	44.3	120.2	6.7
ICCV 89405	27.1	5.9	3.0	18.0	34.6	128.3	6.2
ICCV 88202	27.0	6.2	3.6	17.2	28.7	103.2	7.1
ICCC 37	24.0	7.4	3.2	17.7	42.6	96.7	6.5
ICCV 10	20.4	7.6	3.6	16.1	47.4	123.1	5.6
Kabuli							
ICCV 6	28.1	5.1	3.0	17.5	68.8	135.6	5.6
ICCV 3	18.7	7.6	3.4	14.4	72.5	122.6	6.3
ICCV 2	19.0	7.2	3.4	18.0	60.6	118.0	5.1
Mean	24.1	6.7	3.1	17.8	46.6	115.9	6.1
SE	±0.58	±0.07	±0.03	±0.42	±1.29	±2.63	±0.32

[a]Based on two independent determinations for each constituent and results expressed on dry weight basis.

Table 12. Chemical composition of green gram, black gram and soybean[a]

Genotype	Protein	Fat	Ash	Total dietary fiber	Calcium	Magnesium	Iron
	(%)				mg/100 g		
Green gram							
PS 16	26.4	1.5	4.2	16.6	37.0	155.8	4.5
ML 267	26.0	1.6	4.6	15.7	48.6	169.4	4.7
LGG 407	24.4	1.5	3.7	18.5	35.1	151.6	3.7
Mean	25.6	1.5	4.1	16.9	40.2	158.9	4.3
SE	±0.31	±0.10	±0.04	±0.27	±0.44	±0.89	±0.09
Black gram							
T 9	28.4	1.4	4.2	21.0	60.5	197.0	4.1
LBG 611	27.6	1.7	3.8	22.4	67.8	212.7	4.2
LBG 22	27.8	1.6	3.4	23.5	55.4	210.0	4.0
LBG 17	28.2	1.6	3.7	21.8	41.7	179.4	5.5
Mean	28.0	1.6	3.8	22.2	56.3	199.8	4.4
SE	±0.10	±0.03	±0.06	±0.41	±1.13	±0.80	±0.14

Table 12 (Continued)

Soybean							
MONETTA	52.0	22.3	7.4	20.4	181.2	425.6	6.6
MACS 58	53.2	27.5	8.2	22.8	239.1	421.1	7.7
MACS 124	55.7	24.1	8.0	20.2	195.1	432.7	6.2
JS 335	56.1	24.8	7.4	20.0	190.1	477.5	5.6
PK 472	53.8	25.3	7.5	19.3	163.1	391.9	6.3
KhSB 2	57.1	26.6	7.7	19.3	292.2	389.1	6.5
Mean	54.6	25.1	7.7	20.3	210.1	423.0	6.4
SE	±0.85	±0.14	±0.09	±0.31	±2.28	±5.00	±0.13

[a]Based on two independent determinations for each constituent and results expressed on dry weight basis.

soybean (6.4 mg/100 g) was higher than that of the other legumes. Mean iron content for green gram was 4.3 mg/100 g and for black gram 4.4 mg/100 g.

Total dietary fiber (TDF) content was the highest in black gram (22.2%) and the lowest in green gram (16.9%). Mean TDF (Total dietary fiber) content of pigeonpea genotypes was 18.6%. TDF content of chickpea genotypes ranged from 14.4% to 21.3% indicating a significant variation (Table 11). TDF content of soybean genotypes ranged between 19.3 and 22.8% (Table 12).

4.3 VARIABILITY IN PHYTIC ACID AND PROTEIN DIGESTIBILITY

Among the present legumes, mean phytic acid was the highest in soybean (36.4 mg/g) and the lowest in chickpea (9.5 mg/g). Phytic acid content of pigeonpea genotypes ranged between 6.8 mg/g for ICPL 88046 and 17.5 mg/g for UPAS 120 (Table 13). This indicated a large variation and significant differences in phytic acid content of pigeonpea genotypes. Phytic acid constituted 63.3 to 85.2% of the total phosphorus in pigeonpea. IVPD values of these varieties ranged between 60.4 and 74.4% with mean being 65.5%. The highest IVPD was observed in 'ICPL 88046' which contained the lowest amount of phytic acid. Similarly, phytic acid content of chickpea genotypes differed significantly ($P < 0.01$). As shown in Table 14, nearly two fold differences in phytic acid content of chickpea genotypes was observed. Two of the three kabuli genotypes of chickpea included in the study had lower phytic acid contents than did the desi genotypes. When calculated, phytic acid as percent of total phosphorus, results indicated that

Table 13. **Protein, IVPD, phytic acid and phosphorus contents of *dhal* of pigeonpea[a]**

Genotype	Seed size/ 100-seed mass (g)	Protein (%)	IVPD (%)	Phytic acid (mg/g)	Phosphorus		
					Total (mg/100 g)	Phytic acid (mg/100 g)	Phytic acid (as % of Total P)
Pigeonpea							
ICPL 87051	12.3	20.4	68.8	10.8	363.1	294.9	81.3
ICPL 87119	10.6	19.7	64.6	9.7	317.5	264.8	83.5
ICP 8094	6.4	27.5	69.1	11.7	402.2	318.1	79.1
ICP 8863	9.0	23.5	64.9	13.9	529.2	379.5	71.9
ICPL 88046	9.0	23.6	74.4	6.8	291.9	184.3	63.3
ICPL 85012	9.8	20.6	67.3	12.3	405.8	337.2	83.1
UPAS 120	8.4	24.3	63.7	17.5	559.9	476.4	85.1
ICPL 85010	8.7	25.1	63.5	15.6	528.0	427.3	80.9
ICPL 4	5.1	23.8	60.4	17.2	549.3	468.2	85.2
ICPL 366	10.3	25.7	64.1	14.4	469.6	393.1	83.7
BDN 1	9.6	28.2	63.7	14.9	488.3	406.8	83.4
ICPL 87	8.9	21.4	65.1	11.1	446.8	303.1	67.7
ICPL 151	7.5	23.0	62.6	13.1	520.2	357.6	68.8
ICPL 84031	9.2	23.0	68.6	10.4	377.0	282.5	75.0
ICPL 84052	6.8	23.8	61.6	14.1	452.3	385.2	85.1
C 11	10.7	24.9	66.0	10.4	369.5	282.6	76.5
Mean	8.9	23.6	65.5	12.7	441.9	347.6	78.3
SE	±0.20	±0.029	±0.43	±0.57	±6.50	±15.46	±3.83

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 14. Protein, IVPD, phytic acid and phosphorus contents of dhal of chickpea[a]

Genotype	Seed size/ 100-seed mass (g)	Protein (%)	IVPD (%)	Phytic acid (mg/g)	Phosphorus		
					Total (mg/100 g)	Phytic acid (mg/100 g)	Phytic acid (as % of Total P)
Chickpea							
Desi							
ICCV 89211	22.4	24.3	73.5	8.4	311.3	229.4	73.7
ICCV 89214	20.8	26.2	68.8	10.1	338.0	275.8	81.5
ICCV 89217	18.7	25.9	65.6	12.3	391.1	334.5	88.6
ICCV 89405	17.5	27.2	69.1	12.2	418.6	333.1	79.6
ICCV 88202	20.5	27.0	65.3	11.3	391.0	308.5	79.0
ICCC 37	17.9	24.0	73.7	7.8	284.6	211.6	74.3
ICCV 10	17.0	20.4	75.4	9.2	354.6	249.8	70.4
ICCV 89303	15.4	23.7	69.0	10.9	354.3	296.2	83.7
ICCV 89304	15.5	25.3	70.5	11.0	356.1	300.4	84.3
ICCV 89302	27.5	19.8	70.8	9.9	324.0	269.0	83.1
ICCV 89424	16.4	19.0	68.2	11.7	383.2	319.4	83.5
ICCV 88108	28.2	23.3	72.8	7.8	345.7	211.6	61.3
ICCV 89230	27.9	20.1	70.5	7.7	318.5	210.3	66.2
Kabuli							
ICCV 6	19.7	28.1	76.1	7.0	311.7	189.8	60.9
ICCV 3	22.9	18.7	79.4	5.4	210.9	147.4	70.0
ICCV 2	26.4	19.0	72.5	11.4	375.3	311.2	83.0
Mean	20.4	23.2	71.3	9.5	341.8	262.3	76.4
SE	±0.48	±0.57	±0.46	±0.59	±5.54	±15.95	±5.03

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

phytic acid represents 60.9 to 88.6% of the total phosphorus in chickpea. The mean values for protein digestibility of *dhal* of desi and kabuli chickpea were 70.2 and 76%, respectively.

Table 15 summarises the *dhal* phytic acid contents and IVPD values of green gram, black gram and soybean genotypes. The phytic acid content of green gram genotypes ranged between 10.2 and 14.8 mg/g. Among the three green gram genotypes studied, phytic acid phosphorus constituted 61.9 to 79.6% of the total phosphorus. The mean values for protein digestibility of green gram and black gram *dhal* were 70.1 and 59.9% respectively. Among the black gram genotypes, 'T 9' had the highest phytic acid content (15.4 mg/g) and exhibited the lowest protein digestibility. Phytic acid constituted 77.2 to 84.0% of the total phosphorus in black gram genotypes.

Soybean had the highest level of phytic acid (Table 15). *In vitro* protein digestibility of soybean genotypes ranged between 62.7 and 71.6% with mean being 65.6%. Phytic acid content in soybean genotypes differed significantly ($P < 0.01$) as it ranged between 32.4 and 41.3 mg/g. The results in Table 15 also showed that all the soybean genotypes contained high amounts of phosphorus with most of it present in the form of phytic acid. Phytic acid phosphorus ranged from 84.1 to 86.4% of the total phosphorus.

There was a significant positive correlation ($r = 0.99$) between phytic acid and total phosphorus content in all the legumes (Table 16). Protein content was also positively and significantly correlated with phytic acid ($r = 0.94$). The magnitude of correlation between phytic acid and protein was low, although it

Table 15. **Protein, IVPD, phytic acid and phosphorus contents of *dhal* of green gram, black gram, and soybean[a]**

Genotype	Seed size/ 100-seed mass (g)	Protein (%)	IVPD (%)	Phytic acid (mg/g)	Phosphorus		
					Total (mg/100 g)	Phytic acid (mg/100 g)	Phytic acid (as % of Total P)
Green gram							
PS 16	3.3	26.4	67.2	10.2	447.8	277.1	61.9
ML 267	3.1	26.0	70.8	14.8	507.7	404.1	79.6
LGG 407	3.4	24.4	72.2	10.9	403.9	298.9	74.0
Mean	3.3	25.6	70.1	12.0	453.1	326.7	71.8
SE	±0.09	±0.31	±0.48	±0.25	±7.26	±6.80	±0.91
Black gram							
T 9	4.7	28.4	55.7	15.4	499.1	419.1	84.0
LBG 611	4.5	27.6	60.4	13.8	486.5	375.4	77.2
LBG 22	4.9	27.8	60.4	12.9	456.1	350.8	77.0
LBG 17	5.3	28.2	63.3	13.0	433.9	353.6	81.5
Mean	4.9	28.0	59.9	13.7	468.9	374.7	79.9
SE	±0.16	±0.10	±0.34	±0.18	±3.31	±4.92	±1.27

Table 15 (Continued)

Soybean							
MONETTA	13.3	52.0	71.6	32.4	1036.1	884.5	85.4
MACS 58	12.7	53.2	62.7	41.3	1305.4	1127.5	86.4
MACS 124	14.4	55.7	63.3	39.9	1292.1	1089.3	84.3
JS 335	14.6	56.1	65.5	33.1	1072.4	903.7	84.3
PK 472	15.3	53.8	66.2	33.5	1087.8	914.5	84.1
KhSB 2	12.5	57.1	64.6	38.2	1228.6	1042.8	84.9
Mean	13.8	54.6	65.6	36.4	1170.4	993.7	84.9
SE	±0.48	±0.85	±0.39	±0.70	±6.24	±19.04	±1.65

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

was positive in pigeonpea and chickpea (Tables 17 and 18). There was a negative correlation between the seed size and phytic acid content in both pigeonpea and chickpea genotypes which was not significant. There was a significant negative correlation (r = 0.39**) between phytic acid and IVPD of all the legumes (Table 16). A highly significant and negative correlation was observed between the *in vitro* protein digestibility and the concentration of phytic acid in both pigeonpea (r = 0.80) and chickpea (r = 0.83) (Fig. 2 and Fig. 3).

4.4 PHYTIC ACID, NITROGEN SOLUBILITY AND IRON AVAILABILITY OF DIFFERENT LEGUMES

The nitrogen solubility index was the highest in soybean (78.4%) and the lowest in black gram (32%). The nitrogen solubility index, phytic acid, total and ionisable iron of pigeonpea and chickpea are given in Table 19. Nitrogen solubility of pigeonpea genotypes ranged between 68.3 and 78.2%, with mean being 73.2%. Nitrogen solubility of chickpea cultivars ranged between 60.1 and 75.5% indicating a large variation between the genotypes. Percent mean value for nitrogen solubility was higher for pigeonpea than chickpea. Mean nitrogen solubility in green gram genotypes was 65.7%. The nitrogen solubility of soybean genotypes ranged from 72.8 to 79.2% (Table 20).

Mean per cent ionisable iron was the highest in chickpea (25.7%) and the lowest in black gram (16.8%). Percent ionisable iron of pigeonpea genotypes varied from 19.1 to 27.6% with mean being 22.7% and chickpea genotypes from

Table 16. Correlation coefficients between seed size, protein, phytic acid, phosphorus content, and IVPD of different legumes

	All Legumes (n=45)			
Constituent	Seed size	Protein	Phytic acid	Phosphorus
Seed size	-			
Protein	-0.07			
Phytic acid	-0.14	0.94**		
Phosphorus	-0.15	0.95**	0.99**	
IVPD	0.57	-0.24	-0.39**	-0.37*

* Significant at 5% level.
** Significant at 1% level. n = number of genotypes.

Table 17. Correlation coefficients between seed size, protein, phytic acid, phosphorus content, and IVPD of pigeonpea

	Pigeonpea (n=16)			
Constituent	Seed size	Protein	Phytic acid	Phosphorus
Seed size	-			
Protein	-0.35	-		
Phytic acid	-0.45	0.38	-	
Phosphorus	-0.49*	0.36	0.92**	-
IVPD	0.37	-0.12	-0.80**	-0.76**

* Significant at 5% level.
** Significant at 1% level. n = number of genotypes.

Table 18. **Correlation coefficients between seed size, protein, phytic acid, phosphorus content, and IVPD of chickpea**

	Chickpea (n=16)			
Constituent	Seed size	Protein	Phytic acid	Phosphorus
Seed size	-			
Protein	-0.40			
Phytic acid	-0.39	0.22	-	
Phosphorus	-0.28	0.32	0.90	
IVPD	0.21	-0.35	-0.83	-0.77**

* Significant at 5% level.
** Significant at 1% level. n = number of genotypes.

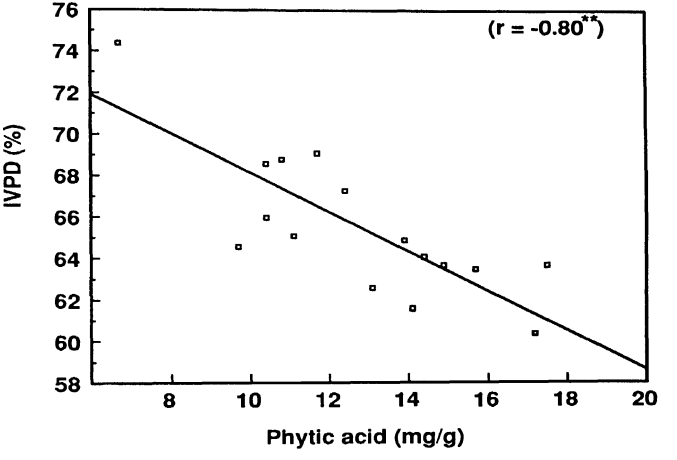

Fig. 2. Relationship between phytic acid and *in vitro* protein digestibility in pigeonpea.

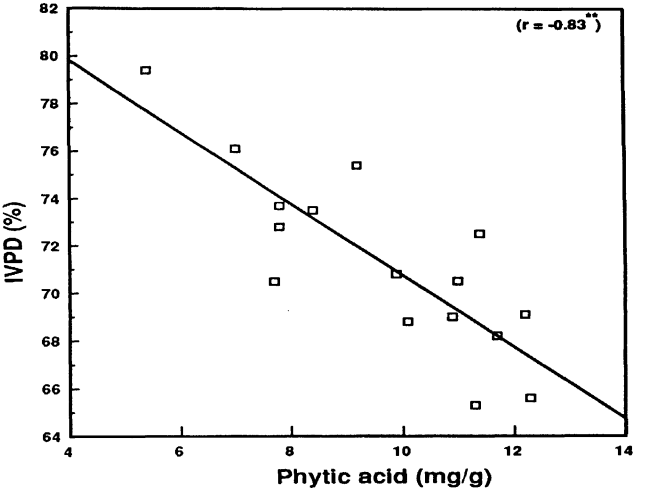

Fig. 3. Relationship between phytic acid and *in vitro* protein digestibility in chickpea.

22.5 to 31.7% with mean being 25.7% (Table 19). This indicated that ionisable iron of chickpea is higher than that of pigeonpea.

Mean per cent ionisable iron in green gram genotypes was 21.9% (Table 20). Per cent ionisable iron in soybean genotypes ranged from 16.9 to 22.7% (Table 20) showing a large variation.

4.4.1 Effect of pH on Nitrogen Solubility

One genotype of each legume was selected to study the effect of pH on nitrogen solubility. The nitrogen solubility index (NSI) value for different legume genotypes differed at different pH levels of the extracting solvent. Nitrogen solubility index (NSI) of different legumes in solvent over a range of pH 1.0-8.0 showed that the solubility was higher at alkaline than at the acidic pH. The lowest NSI values for all the legume species was observed at pH 3.0 (Fig. 4). Highest solubility was observed at pH 7.0. In general, increasing pH from 4.0 to 7.0 increased solubility considerably. At pH 7.0 the lowest nitrogen solubility was observed for black gram and the highest for pigeonpea (Table 21).

4.5 RELATIONSHIPS BETWEEN PHYTIC ACID, PROTEIN, IVPD, NITROGEN SOLUBILITY, TOTAL DIETARY FIBER (TDF) AND IONISABLE IRON OF DIFFERENT LEGUMES

There was no significant correlation between phytic acid and nitrogen solubility in all the legumes (Table 22). However, phytic acid was negatively and significantly ($P < 0.01$) correlated with the percent ionisable iron. Total dietary

Table 19. Nitrogen solubility index, phytic acid, total and ionisable iron in pigeonpea and chickpea[a]

Genotype	Nitrogen solubility index (%)	Phytic acid mg/g	Iron		
			Total mg/100 g	Ionisable mg/100 g	Ionisable (as % of Total)
Pigeonpea					
ICPL 87051	73.1	10.8	4.0	0.9	22.5
ICPL 87119	70.1	9.7	2.9	0.8	27.6
ICP 8094	77.4	11.7	4.5	1.0	22.2
ICP 8863	75.9	13.9	4.3	1.0	23.3
ICPL 88046	78.2	6.8	3.8	1.0	26.3
ICPL 85012	68.7	12.3	4.1	0.9	22.0
UPAS 120	68.3	17.5	3.8	0.8	21.1
ICPL 85010	76.0	15.6	3.1	0.6	19.4
ICPL 4	70.3	17.2	4.7	0.9	19.1
ICPL 366	74.3	14.4	3.0	0.7	23.3
Mean	73.2	13.0	3.8	0.8	22.7
SE	±0.90	±0.48	±0.11	±0.06	±0.96
Chickpea					
Desi					
ICCV 89211	71.1	8.4	6.4	1.6	25.0
ICCV 89214	61.6	10.1	6.1	1.5	24.6
ICCV 89217	75.5	12.3	6.7	1.6	23.9
ICCV 89405	66.6	12.2	6.2	1.5	24.2
ICCV 88202	65.4	11.3	7.1	1.6	22.5
ICCC 37	62.5	7.8	6.5	1.7	26.2
ICCV 10	73.1	9.2	5.6	1.4	25.0
Kabuli					
ICCV 6	60.1	6.9	5.6	1.5	26.8
ICCV 3	68.5	5.4	6.3	2.0	31.7
ICCV 2	70.7	11.4	5.1	1.4	27.5
Mean	67.5	9.5	6.1	1.6	25.7
SE	±0.82	±0.50	±0.32	±0.09	±0.58

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 20. **Nitrogen solubility index, phytic acid, total and ionisable iron in green gram, black gram, and soybean[a]**

Genotype	Nitrogen solubility index (%)	Phytic acid mg/g	Iron		
			Total mg/100 g	Ionisable mg/100 g	Ionisable (as % of Total)
Green gram					
PS 16	69.0	10.2	4.5	1.0	22.2
ML 267	79.7	14.8	4.7	0.9	19.1
LGG 407	48.3	10.9	3.7	0.9	24.3
Mean	65.7	12.0	4.3	0.9	21.9
SE	±0.82	±0.25	±0.09	±0.03	±0.51
Black gram					
T 9	26.1	15.4	4.1	0.5	12.2
LBG 611	39.9	13.8	4.2	0.7	16.7
LBG 22	32.8	12.9	4.0	0.8	20.0
LBG 17	29.2	13.0	5.5	1.0	18.2
Mean	32.0	13.7	4.4	0.8	16.8
SE	±0.92	±0.18	±0.14	±0.07	±1.20
Soybean					
MONETTA	72.8	32.4	6.6	1.5	22.7
MACS 58	73.2	41.3	7.7	1.3	16.9
MACS 124	74.4	39.9	6.2	1.1	17.7
JS 335	73.7	33.1	5.6	1.2	21.4
PK 472	79.2	33.5	6.3	1.4	22.2
KhSB 2	76.8	38.2	6.5	1.2	18.5
Mean	78.4	36.4	6.4	1.3	19.9
SE	±0.77	±0.70	±0.13	±0.08	±1.45

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 21. Effect of pH on nitrogen solubility index (NSI) of *dhal* of different legumes[a]

Genotype	Nitrogen solubility index (%)							
	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8
Pigeonpea								
ICPL 88046	72.7	63.7	32.1	59.3	65.3	69.4	78.2	63.9
Chickpea								
ICCV 3	66.6	56.9	36.7	50.1	59.9	64.4	68.5	64.1
Green gram								
LGG 407	47.1	44.1	37.6	41.8	43.8	45.6	48.3	43.1
Black gram								
LBG 22	33.0	31.2	28.0	30.2	31.7	32.4	32.9	30.1
Soybean								
JS 335	69.5	51.0	45.0	55.1	60.8	64.2	73.7	66.3
SE	±0.76	±0.55	±0.91	±0.54	±1.07	±0.53	±0.97	±0.55

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

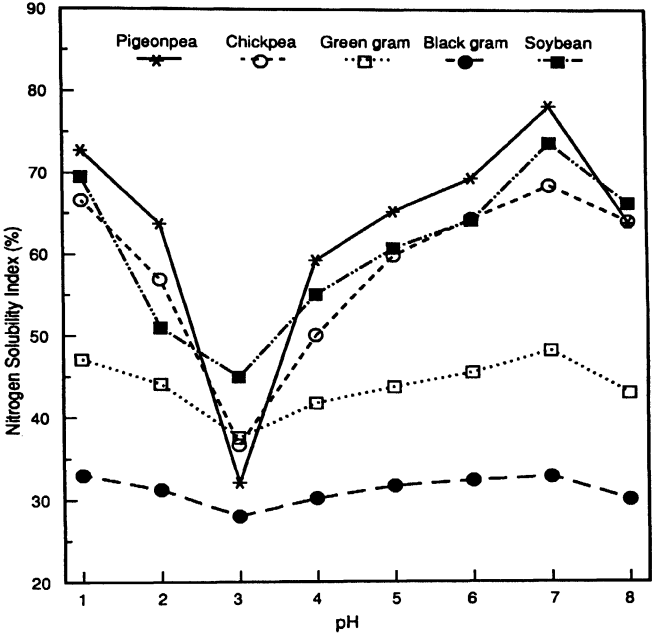

Fig.4. Effect of pH on Nitrogen Solubility Index (NSI) of dhal of different legumes.

fiber was negatively correlated with IVPD, nitrogen solubility and ionisable iron (Table 22).

There was a significant negative correlation between phytic acid and ionisable iron in both pigeonpea (r = 0.81) and chickpea (r = 0.73) genotypes tested separately (Tables 23 and 24). There was no correlation between TDF and IVPD, between TDF and nitrogen solubility and between TDF and ionisable iron in pigeonpea (Table 23). There was a negative correlation between TDF and nitrogen solubility and between TDF and percent ionisable iron in chickpea genotypes, although the magnitudes of correlations were low and non significant (Table 24).

4.6 COOKING QUALITY

Soybean required the longest cooking time (mean being 15 min.). The phytic acid, protein content, and cooking quality parameters of pigeonpea and chickpea are reported in Table 25. Cooking time of pigeonpea genotypes (*dhal*) varied from 18 to 31 min. Similar variations were observed for solid dispersion of these genotypes. The water absorbing capacity of pigeonpea genotypes did not show large differences. Cooking time of *dhal* sample of chickpea genotypes ranged between 27 and 45 min (Table 25). These differences in cooking time were supported by the differences in the amounts of solids dispersed during cooking of these genotypes. There were no large differences in water absorbing capacity of chickpea genotypes. Cooking time of green gram genotypes ranged between 17 and 21 min. (Table 26).

Table 22. Correlation coefficients between phytic acid, protein, IVPD, nitrogen solubility, total dietary fiber (TDF) and ionisable iron of different legumes[a] (n=33)

Constituent	Phytic acid	Protein	IVPD	Nitrogen solubility	TDF
Phytic acid	-				
Protein	0.94**	-			
IVPD	-0.39*	-0.26	-		
Nitrogen solubility	0.24	0.19	0.37*	-	
TDF	0.40*	0.41*	-0.58**	-0.48**	-
Ionisable iron	-0.51**	-0.42*	0.81*	0.37*	-0.55**

[a]Based on analysis of *dhal* samples.
n = number of genotypes.
* Significant at 5% level.
** Significant at 1% level.

Table 23. Correlation coefficients between phytic acid, protein, IVPD, nitrogen solubility, total dietary fiber (TDF) and ionisable iron of pigeonpea[a] (n=10)

Constituent	Phytic acid	Protein	IVPD	Nitrogen solubility	TDF
Phytic acid					
Protein	0.36				
IVPD	-0.85**	-0.07			
Nitrogen solubility	-0.41	0.53	0.53		
TDF	-0.18	0.30	0.14	0.56	
Ionisable iron	-0.81**	-0.40	0.53	0.17	0.42

[a]Based on analysis of *dhal* samples.
n = number of genotypes.
* Significant at 5% level.
** Significant at 1% level.

Table 24. Correlation coefficients between phytic acid, protein, IVPD, nitroger solubility, total dietary fiber (TDF) and ionisable iron of chickpea[a] (n=10)

Constituent	Phytic acid	Protein	IVPD	Nitrogen solubility	TDF
Phytic acid	-				
Protein	0.31	-			
IVPD	-0.87**	-0.58	-		
Nitrogen solubility	0.34	-0.48	-0.10	-	
TDF	-0.31	0.44	0.38	-0.10	-
Ionisable iron	-0.73*	-0.67	0.83**	-0.04	-0.52

[a]Based on analysis of *dhal* samples.
n = number of genotypes.
* Significant at 5% level.
** Significant at 1% level.

Table 25. Phytic acid, protein content, and cooking quality parameters of pigeonpea and chickpea[a]

Genotype	Phytic acid (mg/g)	Protein (%)	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)
Pigeonpea					
ICPL 87051	10.8	20.3	27	1.3	34.7
ICPL 87119	9.7	19.6	29	1.2	39.1
ICP 8094	11.7	27.5	25	1.3	42.5
ICP 8863	13.9	23.4	18	1.4	68.3
ICPL 88046	6.8	23.5	22	1.3	61.7
ICPL 85012	12.3	20.5	31	1.2	36.7
UPAS 120	17.5	24.3	22	1.2	46.9
ICPL 85010	15.6	25.1	24	1.3	40.5
ICPL 4	17.2	23.7	22	1.3	50.0
ICPL 366	14.4	25.7	26	1.2	40.8
Mean	13.0	23.4	24.6	1.3	46.1
SE	±0.48	±0.028	±0.47	±0.03	±0.98
Chickpea					
Desi					
ICCV 89211	8.4	24.3	30	0.99	23.6
ICCV 89214	10.1	26.1	29	0.99	26.0
ICCV 89217	12.3	25.9	28	1.04	25.9
ICCV 89405	12.2	27.1	29	1.03	30.1
ICCV 88202	11.3	27.0	28	1.0	24.7
ICCC 37	7.8	24.0	32	1.0	25.5
ICCV 10	9.2	20.4	27	1.0	27.4

Table 25 (Continued)

Kabuli					
ICCV 6	6.9	28.1	36	0.92	24.9
ICCV 3	5.4	18.7	40	0.92	24.1
ICCV 2	11.4	19.0	45	0.98	25.4
Mean	9.5	24.1	32.4	0.99	25.7
SE	±0.50	±0.58	±0.63	±0.03	±0.42

[a]Based on two independent determinations for each constituent.

Table 26. Phytic acid, protein content, and cooking quality of green gram, black gram, and soybean[a]

Genotype	Phytic acid mg/g	Protein (%)	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)
Green gram					
PS 16	10.2	26.4	23	1.2	29.1
ML 267	14.8	26.0	11	1.4	40.5
LGG 407	10.9	24.4	11	1.5	36.2
Mean	12.0	25.6	15	1.4	35.2
SE	±0.25	±0.31	±0.70	±0.04	±1.2
Black gram					
T 9	15.4	28.4	17	1.6	25.9
LBG 611	13.8	27.6	17	1.7	28.8
LBG 22	12.9	27.8	19	1.8	27.9
LBG 17	13.0	28.2	21	1.6	27.0
Mean	13.7	28.0	19	1.7	27.4
SE	±0.18	±0.10	±0.50	±0.02	±0.66

Table 26 (Continued)

Soybean					
MONETTA	32.4	52.0	88	0.96	30.7
MACS 58	41.3	53.2	77	0.93	29.2
MACS 124	39.9	55.7	75	0.97	28.6
JS 335	33.1	56.1	88	1.01	29.2
PK 472	33.5	53.8	84	1.01	30.4
KhSB 2	38.2	57.1	94	1.00	27.8
Mean	36.4	54.6	84.3	0.95	29.3
SE	±0.70	±0.85	±0.68	±0.017	±0.15

[a]Based on two independent determinations for each constituent.

4.6.1 *'PCMP Number' as an Index of Cooking Quality of Legumes*

Since any of the parameters, viz., phytates, Ca^{++}, Mg^{++} and pectin content cannot individually account for the cooking pattern in legumes, Muller (1967) has suggested the cumulative effect of these as PCMP number in the following mathematical formula:

PCMP number = Free pectin + (Ca^{++} + 1/2 Mg^{++}) - Phytin

The PCMP number of the different legumes used in this study is given in Tables 27 and 28. Green gram which was easily cooked had the lowest PCMP number (mean 2.7) while soybean with prolonged cooking had the highest PCMP value (mean 9.4). PCMP number of pigeonpea genotypes ranged from 2.3 to 7.8 with mean being 4.0. The kabuli type of chickpea which required longer cooking time had higher PCMP number (Table 27). PCMP number of black gram genotypes varied between 3.1 and 4.3 (Table 28). A significant positive correlation ($P < 0.01$) was observed between cooking time and PCMP No. in pigeonpea, chickpea, green gram, and black gram (Table 29).

4.6.2 *Relationships Between Cooking Time and Various Physicochemical Characteristics*

The cooking time was positively and significantly ($P < 0.01$) correlated with the phytic acid content in pigeonpea, chickpea, green gram and black gram (Table 29). Water absorption and solids dispersion were negatively and significantly correlated with the cooking time in these legumes. The cooking time was negatively and significantly ($P < 0.01$) correlated with the protein content.

Table 27. **'PCMP number' as an index of cooking quality of *dhal* of pigeonpea and chickpea[a]**

Genotype	Calcium	Magnesium	Phytic acid	Pectin	PCMP Number
	..... (meq/100 g)				
Pigeonpea					
ICPL 87051	2.5	10.5	9.5	7.1	5.7
ICPL 87119	1.9	8.2	8.6	5.9	4.1
ICP 8094	3.4	9.9	10.3	5.5	4.5
ICP 8863	1.8	10.2	12.2	6.6	3.7
ICPL 88046	2.4	10.1	6.0	6.2	7.8
ICPL 85012	2.0	8.0	10.9	5.2	2.8
UPAS 120	2.6	10.7	15.4	6.0	3.1
ICPL 85010	2.1	7.4	13.8	6.3	2.7
ICPL 4	1.5	8.9	15.1	5.8	2.3
ICPL 366	1.9	10.8	12.7	6.6	3.8
Mean	2.2	9.4	11.4	6.1	4.0
SE	±0.04	±0.31	±0.43	±0.21	±0.30
Chickpea					
Desi					
ICCV 89211	1.7	8.4	7.4	8.4	6.7
ICCV 89214	1.7	9.0	8.9	9.0	6.3
ICCV 89217	2.2	9.9	10.8	9.1	6.0
ICCV 89405	1.7	10.6	10.8	9.7	6.3
ICCV 88202	1.5	8.5	10.0	9.4	5.4
ICCC 37	2.1	8.0	6.8	7.4	6.6
ICCV 10	2.4	10.1	8.1	8.2	7.5
Kabuli					
ICCV 6	3.5	11.1	6.2	7.7	11.3
ICCV 3	3.6	10.1	4.8	6.5	12.0
ICCV 2	3.0	9.7	10.1	7.9	6.2
Mean	2.3	9.5	8.4	8.3	7.4
SE	±0.07	±0.21	±0.45	±0.15	±0.52

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 28. 'PCMP number' as an index of cooking quality of *dhal* of green gram, black gram and soybean[a]

Genotype	Calcium	Magnesium	Phytic acid	Pectin	PCMP Number
	..... (meq/100 g)				
Green gram					
PS 16	1.9	12.8	8.9	3.2	3.0
ML 267	2.4	13.9	13.1	3.0	2.1
LGG 407	1.8	12.4	9.7	3.5	2.9
Mean	2.0	13.0	10.5	3.2	2.7
SE	±0.04	±0.09	±0.21	±0.07	±0.06
Black gram					
T 9	3.0	16.2	13.5	4.0	3.3
LBG 611	3.4	17.4	12.2	4.3	4.3
LBG 22	2.8	17.2	11.3	4.3	4.3
LBG 17	2.1	14.7	11.4	3.8	3.1
Mean	2.8	16.4	12.1	4.1	3.7
SE	±0.04	±0.06	±0.14	±0.24	±0.19
Soybean					
MONETTA	9.1	41.1	28.5	10.2	10.6
MACS 58	12.0	34.6	36.4	11.6	9.3
MACS 124	9.8	35.5	35.2	12.0	9.4
JS 335	9.5	39.1	29.2	9.6	9.6
PK 472	8.2	32.2	29.5	9.6	7.9
KhSB 2	14.6	31.9	33.7	10.8	9.9
Mean	10.5	35.7	32.1	10.6	9.4
SE	±0.13	±1.01	±0.62	±0.34	±0.46

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 29. Correlation coefficients between physicochemical characteristics and cooking quality of different legumes[a] (n=27)

Constituent	Phytic acid	Protein	Cooking time	Water absorption	Solid dispersion	Calcium	Magnesium	Iron
Phytic acid	-							
Protein	0.31	-						
Cooking time	-0.53**	-0.48**	-					
Water absorption	0.51**	0.38**	-0.78**	-				
Solid dispersion	0.33	-0.14	-0.41*	0.27	-			
Calcium	-0.20	-0.01	0.20	0.12	-0.21	-		
Magnesium	0.26	0.52**	-0.56**	0.75**	-0.17	0.42*	-	
Iron	-0.42*	0.11	0.44*	-0.59**	-0.56**	-0.05	-0.21	-
PCMP No.	-0.81**	-0.24	0.67**	-0.62**	-0.35	0.44	-0.22	0.54**

[a]Based on analysis of *dhal* samples.
n = number of genotypes.
* Significant at 5% level.
** Significant at 1% level.

There was a negative correlation ($P < 0.01$) between cooking time and magnesium content (Table 29).

4.7 EFFECT OF PROCESSING ON PHYTIC ACID, PROTEIN DIGESTIBILITY, NITROGEN SOLUBILITY, DIETARY FIBER AND MINERALS OF LEGUMES

For processing studies three genotypes each of pigeonpea (UPAS 120, ICP 8094, and ICPL 88046) and chickpea (ICCV 89217, ICCV 10 and ICCV 3) having high, moderate and low phytic acid levels and two genotypes each of green gram (ML 267 and LGG 407), black gram (LBG 611 and LBG 22) and soybean (MACS 124 and JS 335) with high and low phytic acid content were selected.

4.7.1 Phytic Acid

Table 30 shows the effect of germination, fermentation, autoclaving and roasting on the total phosphorus and phytate contents of pigeonpea. Germination effected the most pronounced loss in ICP 8094 and UPAS 120 genotypes followed by fermentation while autoclaving and roasting only slightly decreased phytate contents. In ICPL 88046 germination and fermentation resulted in similar reduction of phytic acid (Fig. 5).

The effect of processing on phytic acid, total phosphorus and phytic acid phosphorus of chickpea genotypes is given in Table 31. Phytic acid contents of chickpea genotypes were greatly reduced by germination (64-87%). Fermentation

Table 30. Effect of processing on phytic acid, total phosphorus and phytic acid phosphorus of pigeonpea genotypes[a]

Treatment	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid as % of Total P
ICPL 88046				
Control	6.8	291.9	184.3	63.3
Germination	2.1 (69.1)	286.5	55.9	19.6
Fermentation	2.1 (69.1)	289.7	57.3	19.8
Autoclaving	4.9 (27.9)	289.5	133.8	46.3
Roasting	5.0 (26.5)	288.4	135.2	46.9
SE	±0.17	±4.18	±4.69	±2.13
ICP 8094				
Control	11.7	402.2	318.1	79.1
Germination	4.0 (65.8)	398.4	109.2	27.5
Fermentation	5.4 (53.8)	394.9	147.4	37.4
Autoclaving	7.2 (38.5)	400.7	195.2	48.7
Roasting	7.8 (33.3)	379.6	212.9	56.1
SE	±0.26	±4.07	±6.98	±1.85

Table 30 (Continued)

UPAS 120				
Control	17.5	559.9	476.4	85.1
Germination	9.7 (44.6)	548.7	264.8	48.3
Fermentation	12.0 (31.4)	524.7	326.2	62.2
Autoclaving	12.7 (27.4)	486.1	345.4	71.1
Roasting	13.5 (22.9)	486.2	368.5	75.8
SE	±0.17	±4.54	±4.70	±1.23

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.
Figures in parenthesis indicate percentage decrease in phytic acid content.

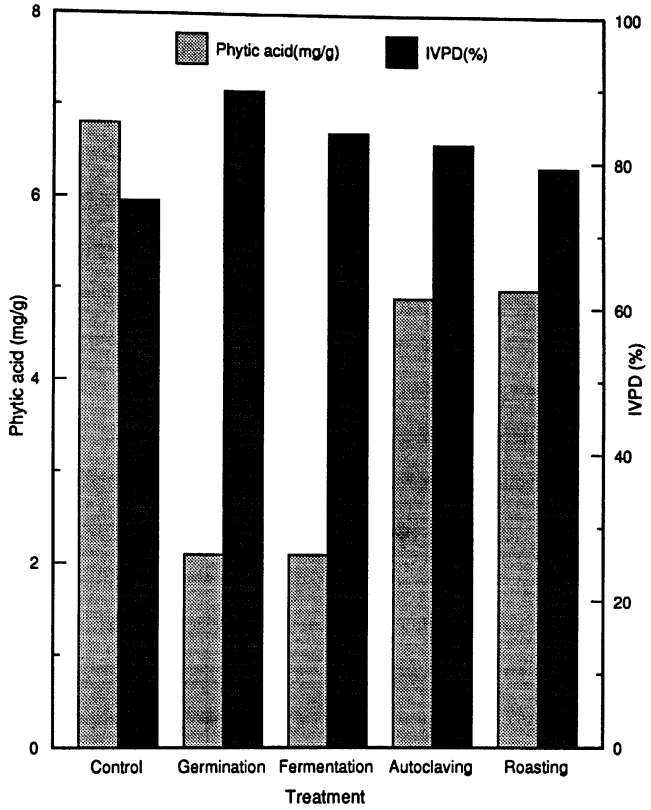

Fig.5. Effect of processing on Phytic acid and IVPD of Pigeonpea (ICPL 88046).

also reduced considerable amounts (39-66%) of phytic acid in chickpea. Autoclaving of chickpea genotypes reduced phytic acid levels more effectively (35-51% reduction) than roasting (20-32% reduction) (Fig. 6). The processing treatments also effectively decreased total phosphorus and phytate phosphorus contents in all the genotypes.

In terms of absolute losses of phytic acid during different processing methods (Table 32), it was observed that germination and fermentation were the most effective methods of lowering phytic acid of green gram genotypes (Fig. 7). Autoclaving and roasting of green gram genotypes decreased phytate by 17-40% and 15-21%, respectively.

Germination reduced phytic acid content of black gram genotypes to a greater extent than the other treatments in the present study (Table 33). Fermentation also resulted in a considerable reduction of phytic acid in black gram (30-31%). In LBG 22 autoclaving reduced phytic acid content more effectively than when seeds were roasted (Fig. 8). In LBG 611 autoclaving reduced phytic acid content by 21% while roasting decreased it by 30%.

The results presented in Table 34 clearly show that germination was very effective in lowering phytic acid and phytate phosphorus contents of the soybean genotypes. The decrease in phytic acid amounted to 46% and 38% in the germinated samples of JS 335 and MACS 124, respectively. Fermentation and autoclaving also effectively decreased (32-39% and 30% respectively), phytic acid contents of the soybean genotypes. Roasting was not as effective as the other processing methods but the losses in phytic acid were significant (Fig. 9).

The decrease in phytic acid as a result of germination was the highest in chickpea (87%), followed by pigeonpea (69%), black gram (48%), green gram and soybean (46%). Fermentation also resulted in the reduction of phytic acid of

Table 31. Effect of processing on phytic acid, total phosphorus and phytic acid phosphorus of chickpea genotypes[a]

Treatment	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid as % of Total P
ICCV 3				
Control	5.4	210.9	147.4	70.0
Germination	0.7 (87.0)	207.0	19.1	9.2
Fermentation	1.8 (66.7)	208.8	47.8	22.9
Autoclaving	2.6 (51.9)	205.0	69.6	34.0
Roasting	4.3 (20.4)	209.4	116.0	55.4
SE	±0.29	±2.85	±7.90	±3.74
ICCV 10				
Control	9.2	354.6	249.8	70.4
Germination	3.3 (64.1)	349.8	90.1	25.8
Fermentation	5.6 (39.1)	351.3	152.9	43.6
Autoclaving	5.9 (35.9)	347.6	161.1	46.4
Roasting	7.0 (23.9)	348.2	191.1	54.9
SE	±0.28	±6.24	±7.56	±1.88

Table 31 (Continued)

ICCV 89217				
Control	12.3	391.1	334.5	85.6
Germination	3.8 (69.1)	368.6	102.3	27.8
Fermentation	5.7 (53.7)	380.6	155.6	40.9
Autoclaving	6.6 (46.3)	335.7	180.2	53.8
Roasting	8.3 (32.5)	373.1	225.3	60.4
SE	±0.21	±4.68	±5.69	±1.67

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.
Figures in parenthesis indicate percentage decrease in phytic acid content.

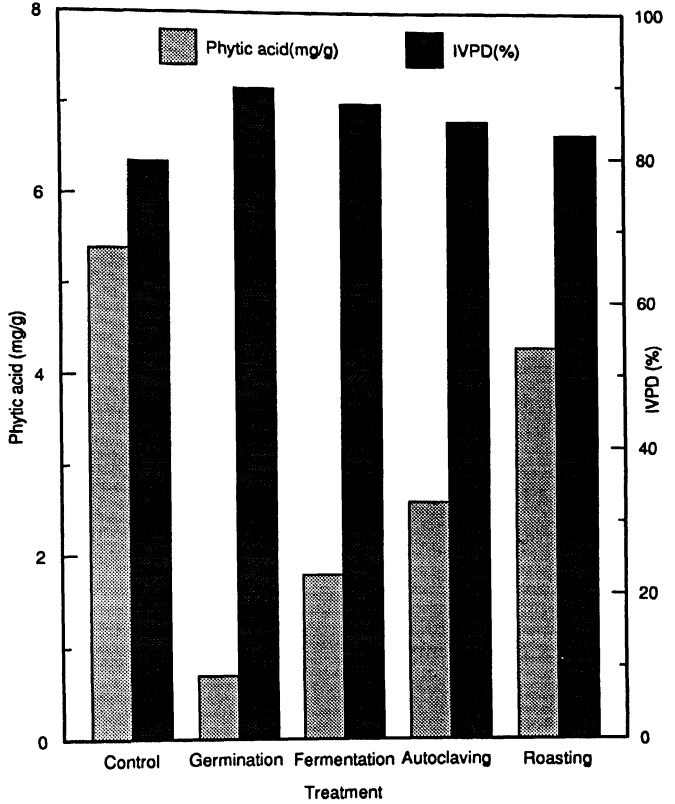

Fig.6. Effect of processing on Phytic acid and IVPD of Chickpea (ICCV 3).

Table 32. Effect of processing on phytic acid, total phosphorus and phytic acid phosphorus of green gram genotypes[a]

Treatment	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid as % of Total P
LGG 407				
Control	10.9	403.9	298.9	74.0
Germination	5.9 (46.4)	389.6	161.1	41.4
Fermentation	6.4 (41.8)	386.6	173.4	44.9
Autoclaving	6.6 (40.0)	393.8	180.2	45.8
Roasting	9.3 (15.5)	400.1	252.6	63.2
SE	±0.18	±5.09	±4.85	±1.13
ML 267				
Control	14.8	507.7	404.1	79.6
Germination	9.3 (37.2)	491.0	253.9	51.7
Fermentation	10.9 (26.4)	497.4	297.6	59.9
Autoclaving	12.2 (17.6)	505.8	331.7	65.6
Roasting	11.6 (21.6)	501.0	315.4	63.0
SE	±0.22	±4.36	±6.06	±1.17

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.
Figures in parenthesis indicate percentage decrease in phytic acid content.

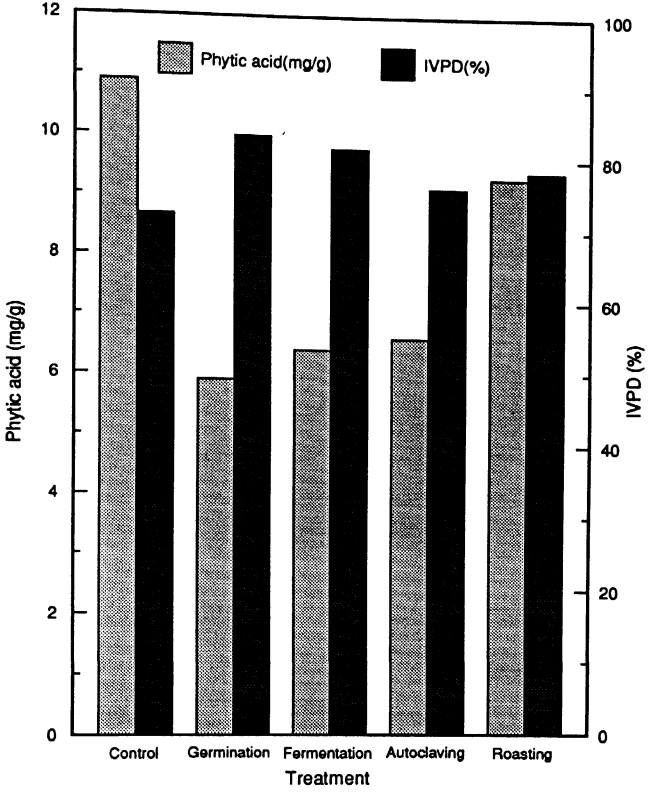

Fig.7. Effect of processing on Phytic acid and IVPD of Green gram (LGG 407).

Table 33. Effect of processing on phytic acid, total phosphorus and phytic acid phosphorus of black gram genotypes[a]

Treatment	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid as % of Total P
LBG 22				
Control	12.9	456.1	350.8	77.0
Germination	6.7 (48.1)	441.4	182.9	41.5
Fermentation	8.8 (31.8)	448.3	238.8	53.4
Autoclaving	8.6 (33.3)	439.6	233.4	53.2
Roasting	9.3 (27.9)	447.8	253.9	56.7
SE	±0.45	±7.72	±12.20	±3.11
LBG 611				
Control	13.8	486.5	375.4	77.2
Germination	8.2 (40.6)	473.5	223.9	47.3
Fermentation	9.6 (30.4)	480.6	260.7	54.3
Autoclaving	10.8 (21.7)	482.3	293.5	60.9
Roasting	9.6 (30.4)	481.9	260.8	54.1
SE	±0.13	±4.97	±3.65	±0.90

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.
Figures in parenthesis indicate percentage decrease in phytic acid content.

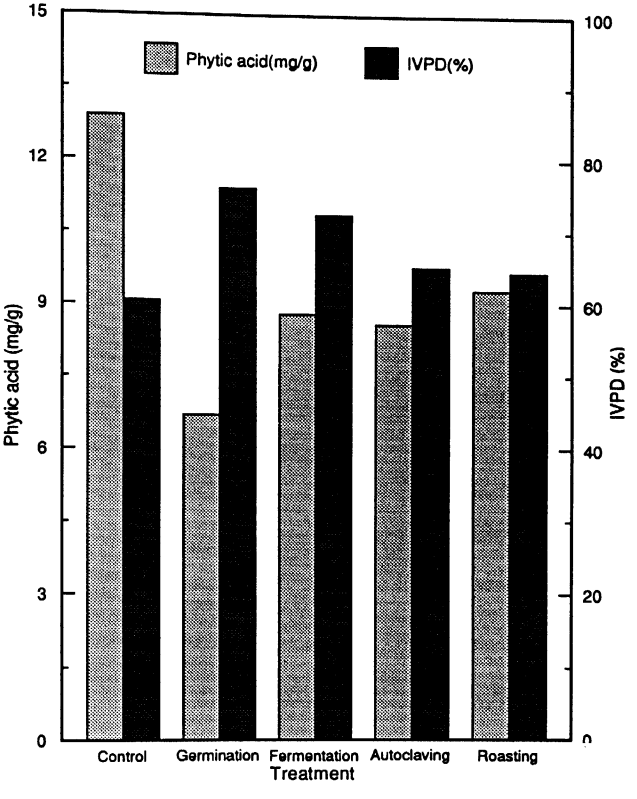

Fig.8. Effect of processing on Phytic acid and IVPD of Black gram (LBG 22).

Table 34. Effect of processing on phytic acid, total phosphorus and phytic acid phosphorus of soybean genotypes[a]

Treatment	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid as % of Total P
JS 335				
Control	33.1	1072.4	903.7	84.3
Germination	17.7 (46.5)	936.3	483.3	51.6
Fermentation	20.1 (39.3)	915.7	548.8	60.0
Autoclaving	22.9 (30.8)	914.8	625.2	68.4
Roasting	25.2 (23.9)	873.5	688.0	78.8
SE	±0.39	±9.49	±10.69	±1.54
MACS 124				
Control	39.9	1292.1	1089.3	84.3
Germination	24.4 (38.8)	1153.1	666.1	57.8
Fermentation	27.0 (32.3)	1172.5	737.1	62.9
Autoclaving	27.9 (30.1)	1107.9	760.3	68.6
Roasting	33.1 (17.0)	1100.5	901.8	82.1
SE	±0.62	±12.42	±16.90	±1.67

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.
Figures in parenthesis indicate percentage decrease in phytic acid content.

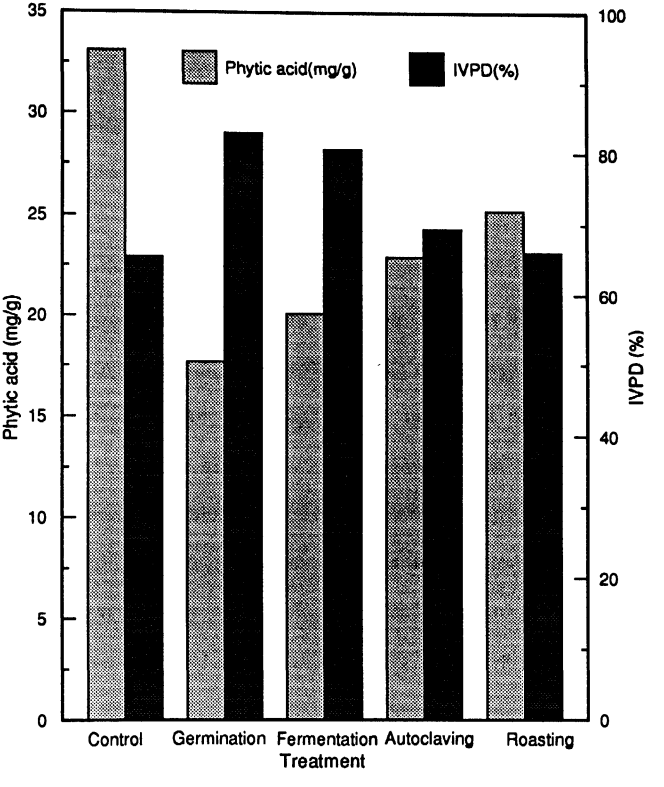

Fig.9. Effect of processing on Phytic acid and IVPD of Soybean (JS 335).

these legumes, even though it was less effective as compared to germination. Both, wet-heating and dry-heating also reduced the phytic acid levels in these legumes ranging from 15-51%. However, no striking differences were observed between the wet-heating and dry-heating for these legumes.

4.7.2 *Protein Digestibility*

The *in vitro* protein digestibility (IVPD) values were greatly influenced by these processing practices. Both germination and fermentation significantly ($P < 0.01$) increased the IVPD in all legume species (Tables 35-39). Effects were more pronounced in pigeonpea as compared to other legumes (Table 35). Both germination and fermentation appeared to be equally effective in increasing IVPD of these legumes. Germination also significantly increased protein values in pigeonpea (Table 35), green gram, black gram, and soybean (Tables 37-39) whereas fermentation was more effective in increasing protein content of pigeonpea (Table 35) and soybean (Table 39). Roasting and autoclaving did not noticeably change the levels of protein in these legumes, but resulted in considerable increases in IVPD values in pigeonpea, chickpea, green gram and black gram (Tables 35-38).

4.7.3 *Nitrogen Solubility*

Germination significantly increased nitrogen solubility index of all the legume species (Tables 35-39). In pigeonpea, fermentation did not noticeably change the nitrogen solubility (Table 35). Fermentation resulted in significant

Table 35. Effect of processing on phytic acid, protein, IVPD, nitrogen solubility and total dietary fiber (TDF) of pigeonpea genotypes[a]

Treatment	Phytic acid mg/g	Protein (%)	IVPD (%)	Nitrogen solubility (%)	TDF (%)
ICPL 88046					
Control	6.8	23.6	74.4	78.2	19.0
Germination	2.1	25.8	89.5	82.6	7.8
Fermentation	2.1	24.6	83.8	79.3	11.0
Autoclaving	4.9	23.1	82.4	71.3	20.9
Roasting	5.0	23.4	79.3	72.7	20.9
SE	±0.17	±0.23	±0.38	±0.83	±0.83
ICP 8094					
Control	11.7	27.5	69.1	77.4	18.7
Germination	4.0	30.2	85.1	79.5	7.8
Fermentation	5.4	29.1	83.9	76.2	8.6
Autoclaving	7.2	27.7	73.0	73.5	20.4
Roasting	7.8	26.9	74.9	72.7	21.0
SE	±0.26	±0.18	±0.39	±1.07	±0.74
UPAS 120					
Control	17.5	24.3	63.7	68.3	18.3
Germination	9.7	27.7	78.0	73.1	8.1
Fermentation	12.0	25.4	72.4	70.3	7.5
Autoclaving	12.7	24.7	67.3	63.2	23.4
Roasting	13.5	24.5	68.0	62.3	23.1
SE	±0.17	±0.15	±0.80	±1.29	±0.20

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 36. Effect of processing on phytic acid, protein, IVPD, nitrogen solubility and total dietary fiber (TDF) of chickpea genotypes[a]

Treatment	Phytic acid mg/g	Protein (%)	IVPD (%)	Nitrogen solubility (%)	TDF (%)
ICCV 3					
Control	5.4	18.7	79.4	68.5	14.4
Germination	0.7	19.0	89.6	76.1	9.1
Fermentation	1.8	18.6	87.5	72.8	8.9
Autoclaving	2.6	17.4	85.1	66.9	24.9
Roasting	4.3	18.2	83.3	64.0	20.1
SE	±0.29	±0.31	±0.64	±0.83	±0.61
ICCV 10					
Control	9.2	20.4	75.4	73.1	16.1
Germination	3.3	21.0	86.5	79.0	6.8
Fermentation	5.6	21.0	84.6	77.6	8.2
Autoclaving	5.9	19.6	80.5	68.5	20.8
Roasting	7.0	20.4	78.5	62.7	20.1
SE	±0.28	±0.19	±0.55	±1.19	±0.55
ICCV 89217					
Control	12.3	25.9	65.6	75.5	17.7
Germination	3.8	26.6	79.6	83.8	8.8
Fermentation	5.7	26.9	76.8	78.3	4.1
Autoclaving	6.6	25.6	67.2	68.8	21.8
Roasting	8.3	26.1	70.9	71.8	19.9
SE	±0.21	±0.19	±0.85	±1.07	±0.43

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 37. Effect of processing on phytic acid, protein, IVPD, nitrogen solubility and total dietary fiber (TDF) of green gram genotypes[a]

Treatment	Phytic acid mg/g	Protein (%)	IVPD (%)	Nitrogen solubility (%)	TDF (%)
LGG 407					
Control	10.9	24.4	72.2	48.3	18.5
Germination	5.9	28.1	83.2	59.2	7.8
Fermentation	6.4	24.8	81.5	57.7	6.9
Autoclaving	6.6	24.1	76.0	37.3	21.1
Roasting	9.3	24.3	78.4	36.4	18.4
SE	±0.18	±0.19	±0.89	±1.10	±0.56
ML 267					
Control	14.8	26.1	70.9	79.7	15.7
Germination	9.3	30.3	82.7	81.8	6.4
Fermentation	10.9	26.8	78.9	81.4	5.9
Autoclaving	12.2	25.9	73.8	74.7	21.8
Roasting	11.6	25.7	73.1	70.2	19.5
SE	±0.22	±0.22	±0.43	±0.86	±0.53

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 38. Effect of processing on phytic acid, protein, IVPD, nitrogen solubility and total dietary fiber (TDF) of black gram genotypes[a]

Treatment	Phytic acid mg/g	Protein (%)	IVPD (%)	Nitrogen solubility (%)	TDF (%)
LBG 22					
Control	12.9	27.8	60.5	32.8	23.5
Germination	6.7	30.4	76.0	41.5	7.4
Fermentation	8.8	28.6	72.4	41.0	6.2
Autoclaving	8.6	27.6	65.2	27.5	19.0
Roasting	9.3	27.2	64.5	28.6	19.8
SE	±0.45	±0.10	±0.73	±1.17	±0.41
LBG 611					
Control	13.8	27.6	60.4	39.9	22.4
Germination	8.2	30.1	74.2	47.6	7.8
Fermentation	9.6	27.9	71.3	43.6	6.3
Autoclaving	10.8	27.0	64.7	28.1	22.7
Roasting	9.6	27.0	65.9	26.0	17.5
SE	±0.13	±0.17	±0.36	±0.64	±0.45

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 39. Effect of processing on phytic acid, protein, IVPD, nitrogen solubility and total dietary fiber (TDF) of soybean genotypes[a]

Treatment	Phytic acid mg/g	Protein (%)	IVPD (%)	Nitrogen solubility (%)	TDF (%)
JS 335					
Control	33.1	56.1	65.5	73.7	20.0
Germination	17.7	58.2	82.9	77.2	9.5
Fermentation	20.1	59.2	80.6	75.5	7.6
Autoclaving	22.9	55.2	69.4	68.5	19.6
Roasting	25.2	56.6	66.1	66.9	17.3
SE	±0.39	±0.74	±0.68	±0.89	±0.32
MACS 124					
Control	39.9	55.7	63.3	74.4	20.2
Germination	24.4	63.1	73.6	80.0	9.0
Fermentation	27.0	62.1	71.7	78.0	8.0
Autoclaving	27.9	58.6	66.8	66.1	17.9
Roasting	33.1	59.9	65.3	65.1	17.5
SE	±0.62	±0.43	±0.60	±0.94	±0.39

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

increases in the nitrogen solubility profiles of chickpea, green gram, black gram and soybean (Tables 36-39). The nitrogen solubility index of all the legume species decreased after heat processing (Tables 35-39).

4.7.4 *Total Dietary Fiber*

Germination and fermentation significantly reduced the total dietary fiber (TDF) contents of all the legumes (Tables 35-39). In pigeonpea, autoclaving and roasting resulted in slight increases in TDF values of ICPL 88046 and ICP 8094, and significant increases in TDF values of UPAS 120 (Table 35). TDF values increased significantly ($P < 0.01$) as a result of autoclaving and roasting in chickpea (Table 36). In green gram autoclaving resulted in a significant increase in TDF content (Table 37). Roasting did not change the TDF content of LGG 407, but resulted in a significant increase in the TDF content of ML 267. In black gram autoclaving and roasting resulted in a slight decrease in the TDF content of LBG 22. Autoclaving did not change the TDF content of LBG 611 but roasting resulted in a significant decrease in the TDF content (Table 38). Autoclaving and roasting resulted in slight decreases in the TDF content of soybean (Table 39).

4.7.5 *Minerals*

Germination and fermentation did not bring about any apparent changes in the calcium, magnesium, and iron contents of pigeonpea, chickpea, green gram and black gram (Tables 40-44). Calcium and iron content slightly decreased as a

Table 40. Effect of processing on phytic acid, mineral content and ionisable iron of pigeonpea genotypes[a]

Treatment	Phytic acid mg/g	Ash (%)	Calcium mg/100 g	Magnesium mg/100 g	Iron		
					Total mg/100 g	Ionisable mg/100 g	Ionisable as % of Total
ICPL 88046							
Control	6.8	4.4	48.4	122.8	3.8	1.0	26.3
Germination	2.1	4.0	46.9	120.0	3.6	1.5	41.7
Fermentation	2.1	4.2	47.4	122.3	3.2	1.1	34.4
Autoclaving	4.9	4.2	47.8	120.4	3.7	1.2	32.4
Roasting	5.0	4.3	48.1	121.3	3.8	1.2	31.6
SE	±0.17	±0.08	±1.66	±1.35	±0.17	±0.06	±0.54
ICP 8094							
Control	11.7	4.0	68.0	120.7	4.5	1.0	22.2
Germination	4.0	3.9	65.6	120.8	4.2	1.3	31.0
Fermentation	5.4	3.8	67.5	121.7	4.0	1.1	27.5
Autoclaving	7.2	3.9	69.1	122.5	3.7	1.0	27.0
Roasting	7.8	3.8	66.8	119.3	3.9	0.9	23.1
SE	±0.26	±0.06	±2.17	±2.31	±0.14	±0.08	±1.13

Table 40 (Continued)

UPAS 120							
Control	17.5	5.4	51.4	129.9	3.8	0.8	21.1
Germination	9.7	4.9	48.0	128.0	3.6	1.0	27.8
Fermentation	12.0	5.1	50.2	128.3	3.4	0.8	23.5
Autoclaving	12.7	5.3	49.4	128.5	3.4	0.8	23.5
Roasting	13.5	5.2	50.0	127.4	3.7	0.8	21.6
SE	±0.17	±0.08	±1.52	±1.95	±0.08	±0.03	±0.60

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

result of germination in all the legume species. No noticeable reduction in these mineral nutrients was observed due to roasting and autoclaving processes.

Effect of Processing on Availability of Iron from Legumes

The total iron and ionisable iron in the control and processed samples of pigeonpea are given in Table 40. Germination resulted in a significant increase ($P < 0.01$) in the ionisable iron in pigeonpea. Both fermentation and autoclaving resulted in an increase in the ionisable iron of pigeonpea. Roasting resulted in an increase in ionisable iron content only in the case of ICPL 88046.

Both germination and fermentation resulted in significant increases ($P < 0.01$) in ionisable iron of chickpea (Table 41). No striking differences between the wet-heating and dry-heating processes were observed with respect to improvement in ionisable iron of ICCV 3 and ICCV 10.

The impact of germination on availability of iron was found to be more pronounced than the other processing methods in green gram (Table 42). Fermentation also improved the *in vitro* availability of iron significantly in green gram. Roasting was more effective than autoclaving in increasing ionisable iron content of green gram.

Germination and fermentation influenced the *in vitro* availability of iron positively, increasing its levels in both the black gram genotypes tested (Table 43). Both autoclaving and roasting also increased the ionisable iron content in black gram, however no significant differences between autoclaving and roasting were observed.

Table 41. Effect of processing on phytic acid, mineral content and ionisable iron of chickpea genotypes[a]

Treatment	Phytic acid mg/g	Ash (%)	Calcium mg/100 g	Magnesium mg/100 g	Iron		
					Total mg/100 g	Ionisable mg/100 g	Ionisable as % of Total
ICCV 3							
Control	5.4	3.4	72.5	122.6	6.3	2.0	31.7
Germination	0.7	2.8	69.1	119.1	5.9	2.4	40.7
Fermentation	1.8	2.6	71.3	122.2	6.0	2.3	38.3
Autoclaving	2.6	2.6	71.9	121.9	6.2	2.2	35.5
Roasting	4.3	3.3	70.7	121.5	6.1	2.2	36.1
SE	±0.29	±0.20	±1.25	±1.66	±0.16	±0.06	±0.47
ICCV 10							
Control	9.2	3.6	47.4	123.1	5.6	1.4	25.0
Germination	3.3	3.1	44.8	120.6	4.8	1.7	35.4
Fermentation	5.6	3.2	48.7	121.5	5.3	1.6	30.2
Autoclaving	5.9	3.4	47.0	121.9	4.8	1.4	29.2
Roasting	7.0	3.5	47.2	123.3	4.2	1.3	31.0
SE	±0.28	±0.06	±1.83	±1.47	±0.19	±0.05	±0.47

Table 41 (Continued)

ICCV 89217							
Control	12.3	2.8	44.3	120.2	6.7	1.6	23.9
Germination	3.8	2.6	41.3	117.8	5.9	1.8	30.5
Fermentation	5.7	2.6	43.2	119.7	6.5	1.8	27.7
Autoclaving	6.6	2.8	42.2	119.8	6.6	1.7	25.8
Roasting	8.3	2.6	43.5	120.1	6.4	1.9	29.7
SE	±0.21	±0.09	±2.14	±2.55	±0.22	±0.05	±0.22

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 42. Effect of processing on phytic acid, mineral content and ionisable iron of green gram genotypes[a]

Treatment	Phytic acid mg/g	Ash (%)	Calcium mg/100 g	Magnesium mg/100 g	Iron		
					Total mg/100 g	Ionisable mg/100 g	Ionisable as % of Total
LGG 407							
Control	10.9	3.7	35.1	151.6	3.7	0.9	24.3
Germination	5.9	3.2	30.9	147.1	3.4	1.1	32.4
Fermentation	6.4	3.0	32.4	149.2	3.7	1.1	29.7
Autoclaving	6.6	3.2	32.3	150.4	3.6	0.9	25.0
Roasting	9.3	3.5	33.2	150.1	3.5	1.0	28.6
SE	±0.18	±0.03	±0.84	±1.71	±0.07	±0.02	±0.56
ML 267							
Control	14.8	4.6	48.6	169.4	4.7	0.9	19.1
Germination	9.3	4.2	46.2	165.9	4.2	1.2	28.6
Fermentation	10.9	4.1	47.2	167.5	4.1	1.0	24.4
Autoclaving	12.2	3.8	50.7	167.6	4.4	1.0	22.7
Roasting	11.6	4.5	46.6	168.9	4.6	1.1	23.9
SE	±0.22	±0.06	±1.84	±2.63	±0.09	±0.02	±0.49

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 43. Effect of processing on phytic acid, mineral content and ionisable iron of black gram genotypes[a]

Treatment	Phytic acid mg/g	Ash (%)	Calcium mg/100 g	Magnesium mg/100 g	Iron		
					Total mg/100 g	Ionisable mg/100 g	Ionisable as % of Total
LBG 22							
Control	12.9	3.4	55.4	210.0	4.0	0.8	20.0
Germination	6.7	3.2	50.9	207.4	3.8	1.2	31.6
Fermentation	8.8	3.4	53.2	211.8	3.4	0.9	26.5
Autoclaving	8.6	3.1	53.3	208.6	3.8	0.9	23.7
Roasting	9.3	3.2	54.6	209.1	3.9	0.9	23.1
SE	±0.45	±0.11	±1.36	±1.68	±0.09	±0.03	±0.72
LBG 611							
Control	13.8	3.9	67.8	212.7	4.2	0.7	16.7
Germination	8.2	3.7	64.4	209.0	4.0	1.0	25.0
Fermentation	9.6	3.5	65.4	210.8	4.0	0.9	22.5
Autoclaving	10.8	3.3	66.6	209.6	3.3	0.7	21.2
Roasting	9.6	3.7	67.1	208.1	3.1	0.7	22.6
SE	±0.13	±0.06	±1.05	±2.01	±0.08	±0.05	±1.08

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 44. Effect of processing on phytic acid, mineral content and ionisable iron of soybean genotypes[a]

Treatment	Phytic acid mg/g	Ash (%)	Calcium mg/100 g	Magnesium mg/100 g	Iron		
					Total mg/100 g	Ionisable mg/100 g	Ionisable as % of Total
JS 335							
Control	33.1	7.4	190.1	477.5	5.6	1.2	21.4
Germination	17.7	7.1	186.1	474.2	5.4	1.6	29.6
Fermentation	20.1	7.2	188.5	476.3	5.5	1.6	29.1
Autoclaving	22.9	7.1	189.9	476.7	5.3	1.4	26.4
Roasting	25.2	7.3	190.3	475.8	5.4	1.4	25.9
SE	±0.39	±0.09	±1.68	±1.80	±0.07	±0.06	±0.99
MACS 124							
Control	39.9	8.0	195.1	432.7	6.2	1.1	17.7
Germination	24.4	7.7	189.8	425.1	6.0	1.6	26.7
Fermentation	27.0	7.6	190.4	429.2	5.6	1.3	23.2
Autoclaving	27.9	7.9	192.0	426.6	5.2	1.1	21.2
Roasting	33.1	7.8	194.6	425.6	5.8	1.2	20.7
SE	±0.62	±0.05	±2.34	±2.08	±0.20	±0.06	±1.07

[a]Based on two independent determinations for each constituent. All results are expressed on a dry weight basis.

Table 44 shows the effect of processing on ionisable iron content of soybean genotypes. The *in vitro* availability of iron increased in soybean after germination and fermentation. Both wet-heating and dry-heating were found to increase the *in vitro* availability of iron in soybean however no significant differences between these two treatments were observed.

4.8 EFFECT OF STORAGE ON PHYTIC ACID, PROTEIN DIGESTIBILITY, MINERALS AND COOKING QUALITY

4.8.1 *Phytic Acid in Stored Pulses*

In general phytic acid content of legumes decreased during storage (Tables 45-49). Phytic acid content of pigeonpea stored at 5°C decreased by 11.3% after 12 months of storage while phytic acid content of pigeonpea stored at 25 and 37°C decreased by 33 and 42% respectively (Table 45). The greatest decrease in phytate was exhibited by pigeonpea samples stored at 37°C (Fig. 10).

A similar trend was observed for chickpea (ICCC 37) samples stored for 12 months (Fig. 11). Among all the legumes studied, maximum reduction in phytic acid content during storage was observed for chickpea (Table 46). Phytic acid content of chickpea stored for 12 months at 25 and 37°C decreased by 66 and 56% respectively.

Phytic acid content of green gram samples stored for 12 months are given in Table 47. Green gram samples stored at higher temperatures exhibited a greater loss in phytic acid than samples stored at 5°C (Fig. 12). The greatest decrease in phytate was exhibited by green gram stored at 37°C.

Table 45. **Effect of storage on phytic acid, total phosphorus and phytic acid phosphorus of pigeonpea *dhal* (ICPL 87119)[a]**

Storage period (months)	Temp. °C	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid (as % of Total P)
0		9.7	317.5	264.8	83.5
3	5	9.0 (7.2)	307.6	244.4	79.4
	25	8.9 (8.2)	317.4	241.6	76.2
	37	7.8 (19.6)	318.8	212.9	66.8
6	5	8.9 (8.2)	302.4	243.0	80.4
	25	8.3 (14.4)	316.4	226.6	71.7
	37	8.0 (17.5)	318.9	217.1	68.1
9	5	8.2 (15.5)	314.9	223.9	71.1
	25	6.7 (30.9)	315.4	182.9	58.0
	37	5.4 (44.3)	313.5	147.4	47.0
12	5	7.9 (11.3)	316.7	215.7	68.0
	25	6.5 (33.0)	313.9	176.0	56.1
	37	5.6 (42.3)	310.3	151.5	48.9
	SE	±0.27	±2.73	±7.27	±2.28

[a]Values are mean of two independent determinations and results expressed on a dry weight basis. Figures in parenthesis indicate percentage decrease in phytic acid content.

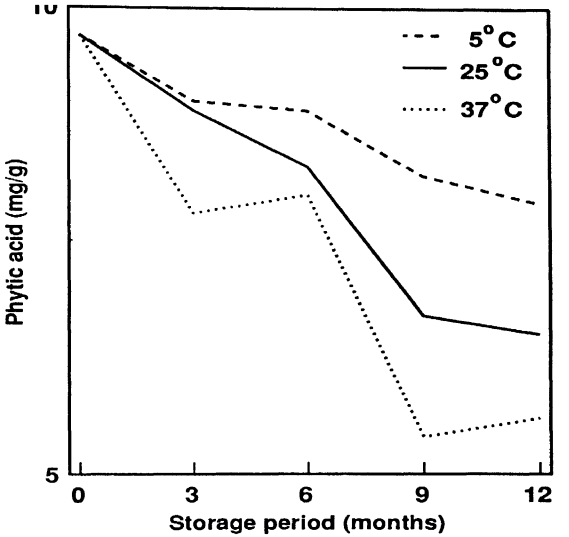

Fig. 10. Effect of storage on phytic acid content of pigeonpea dhal (ICPL 87119).

Table 46. Effect of storage on phytic acid, total phosphorus and phytic acid phosphorus of chickpea *dhal* (ICCC 37)[a]

Storage period (months)	Temp. °C	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid (as % of Total P)
0		7.8	284.6	211.6	74.3
3	5	7.6 (2.6)	283.9	207.5	73.1
	25	6.5 (16.7)	283.3	177.4	62.6
	37	7.4 (5.1)	282.5	200.6	71.1
6	5	7.3 (6.4)	276.8	198.0	71.5
	25	4.6 (41.0)	287.3	125.6	43.8
	37	5.5 (29.5)	283.2	150.1	53.0
9	5	6.5 (16.7)	283.9	176.0	62.2
	25	3.2 (59.0)	284.3	86.0	30.3
	37	3.6 (53.8)	280.1	96.9	34.6
12	5	5.9 (24.4)	283.0	161.1	56.9
	25	2.6 (66.7)	283.6	70.9	25.1
	37	3.4 (56.4)	279.2	91.4	32.8
	SE	±0.10	±2.60	±2.72	±0.93

[a]Values are mean of two independent determinations and results expressed on a dry weight basis. Figures in parenthesis indicate percentage decrease in phytic acid content.

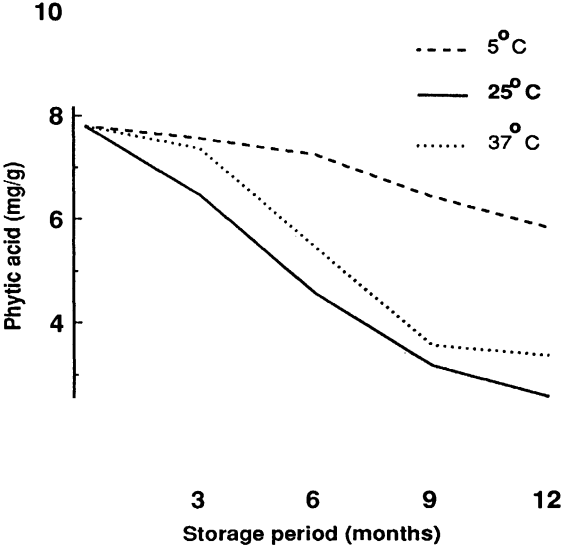

Fig. 11. Effect of storage on phytic acid content of chickpea dhal (ICCC 37).

Table 47. Effect of storage on phytic acid, total phosphorus and phytic acid phosphorus of green gram *dhal* (PS 16)[a]

Storage Period (months)	Temp. °C	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid (as % of Total P)
0		10.2	447.8	277.1	61.9
3	5	9.9 (2.9)	443.2	268.9	60.7
	25	9.7 (4.9)	441.1	263.4	59.8
	37	8.6 (15.7)	447.6	233.5	52.2
6	5	9.4 (7.8)	439.1	256.6	58.5
	25	9.3 (8.8)	445.5	252.5	56.7
	37	8.7 (14.7)	445.2	237.5	53.4
9	5	8.9 (12.7)	440.2	241.6	54.9
	25	6.8 (33.3)	444.8	185.6	41.7
	37	6.2 (39.2)	449.4	169.3	37.7
12	5	8.2 (19.6)	448.6	222.5	49.6
	25	6.9 (32.4)	443.1	187.0	42.2
	37	6.4 (37.3)	444.8	174.8	39.3
	SE	±0.15	±2.47	±3.96	±0.88

[a]Values are mean of two independent determinations and results expressed on a dry weight basis. Figures in parenthesis indicate percentage decrease in phytic acid content.

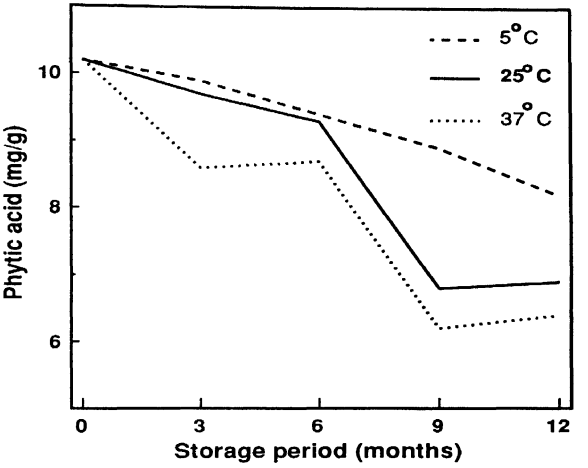

Fig. 12. Effect of storage on phytic acid content of green gram dhal (PS 16).

Table 48. **Effect of storage on phytic acid, total phosphorus and phytic acid phosphorus of black gram *dhal* (T 9)[a]**

Storage period (months)	Temp. °C	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid (as % of Total P)
0		15.4	499.1	419.1	84.0
3	5	15.0 (1.9)	491.4	408.2	83.1
	25	13.0 (15.6)	494.5	354.9	71.8
	37	13.3 (13.6)	491.8	363.1	73.9
6	5	14.7 (4.5)	480.7	400.0	80.7
	25	11.3 (26.6)	494.4	308.5	62.4
	37	12.2 (20.8)	488.5	333.1	68.2
9	5	13.4 (13.0)	496.5	364.5	73.4
	25	10.2 (33.8)	489.8	278.5	56.9
	37	9.8 (36.4)	484.0	266.2	55.0
12	5	12.9 (16.2)	494.3	352.2	71.3
	25	8.9 (42.2)	484.4	243.0	50.2
	37	9.2 (40.3)	480.1	251.2	52.3
	SE	±0.08	±3.65	±2.11	±0.56

[a]Values are mean of two independent determinations and results expressed on a dry weight basis. Figures in parenthesis indicate percentage decrease in phytic acid content.

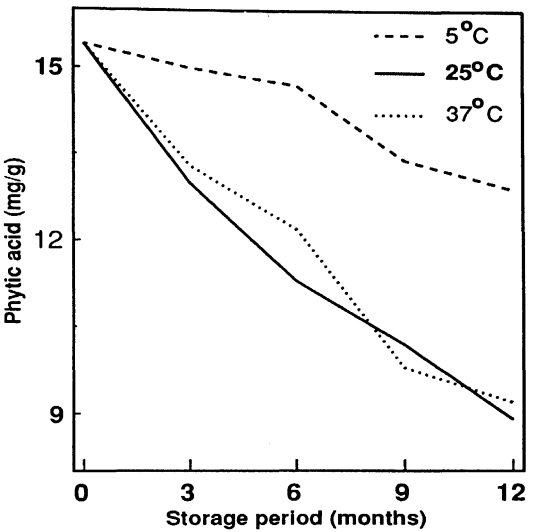

Fig. 13. Effect of storage on phytic acid content of black gram dhal (T 9).

Table 49. **Effect of storage on phytic acid, total phosphorus and phytic acid phosphorus of soybean *dhal* (MONETTA)[a]**

Storage period (months)	Temp. °C	Phytic acid mg/g	Total phosphorus mg/100 g	Phytic acid phosphorus mg/100 g	Phytic acid (as % of Total P)
0		32.4	1036.1	884.5	85.4
3	5	31.6 (2.5)	1030.3	862.7	83.8
	25	29.6 (8.6)	1031.8	808.1	78.4
	37	28.7 (11.4)	1034.2	782.2	75.7
6	5	29.8 (8.0)	1017.1	813.5	80.0
	25	28.8 (11.1)	1020.0	786.3	77.1
	37	27.9 (13.9)	1016.1	761.7	75.0
9	5	28.4 (12.3)	1019.2	775.3	76.1
	25	25.6 (21.0)	1022.8	697.6	68.2
	37	25.9 (20.1)	1017.9	705.7	69.4
12	5	27.2 (16.0)	1017.3	741.2	72.9
	25	23.0 (29.0)	1017.5	627.9	61.7
	37	23.1 (28.7)	1012.9	630.6	62.3
	SE	±0.15	±0.81	±4.14	±0.40

[a]Values are mean of two independent determinations and results expressed on a dry weight basis. Figures in parenthesis indicate percentage decrease in phytic acid content.

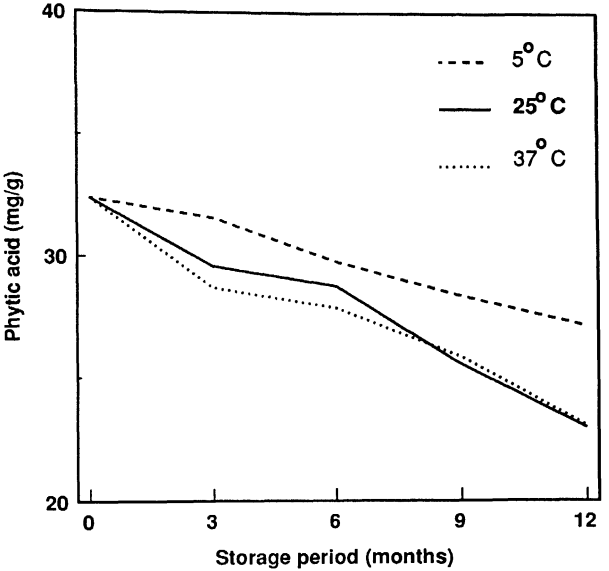

Fig. 14. Effect of storage on phytic acid content of soybean dhal (MONETTA).

Black gram samples (T 9) stored at 25 and 37°C showed greater phytic acid losses than samples stored at 5°C (Fig. 13). At the end of 12 months storage phytic acid was reduced by 40-42% in black gram stored at higher temperatures (Table 48). Conditions of storage also had a significant effect on the loss of phytic acid from stored soybeans ($P < 0.01$). Higher losses of phytic acid occurred in soybean samples stored at elevated temperatures (Fig. 14). About 29% reduction in phytic acid was observed in soybean stored at 25 and 37°C for 12 months (Table 49).

4.8.2 *Effect of Storage on Protein Digestibility and Mineral Content of Pulses*

The effect of storage on protein content and *in vitro* protein digestibility of all the legumes studied are presented in Tables 50-54. As the data show the total protein content was unaffected by storage. Chickpea, green gram, and soybean stored at 25 and 37°C showed marked decreases in digestibility of proteins (*in vitro*).

After 12 months of storage at 5°C, pigeonpea suffered a loss of 5.8% protein digestibility. At 25 and 37°C storage there was a 10% loss (Table 50). Conditions of storage had a significant effect on the loss of digestibility from stored chickpea ($P < 0.01$). *In vitro* protein digestibility of chickpea stored at 5°C decreased by 7% whereas IVPD of samples stored at 25 and 37°C decreased by 14% (Table 51). IVPD of green gram samples stored at 25 and 37°C decreased at a faster rate than samples stored at 5°C. Storage for 12 months at 5°C resulted in

Table 50. Effect of storage on nutritional quality of pigeonpea *dhal* (ICPL 87119)[a]

Storage period (months)	Temp. °C	Phytic acid mg/g	Protein (%)	IVPD (%)	Calcium	Magnesium	Iron
					..... mg/100 g		
0		9.7	19.7	64.6	36.2	99.6	2.9
3	5	9.0	19.7	63.6	38.0	102.8	2.6
	25	8.9	20.1	64.9	37.7	101.7	3.1
	37	7.8	20.4	60.8	37.4	99.0	3.1
6	5	8.9	19.8	63.0	43.3	112.8	2.4
	25	8.3	20.3	63.4	40.0	107.3	3.1
	37	8.0	20.1	60.9	41.4	110.4	2.6
9	5	8.2	19.4	59.8	48.4	108.0	3.3
	25	6.7	20.2	59.7	38.8	107.3	3.2
	37	5.4	20.4	58.5	48.9	106.3	3.4
12	5	7.9	18.9	60.8	49.3	114.6	3.7
	25	6.5	20.2	58.3	40.5	114.9	3.1
	37	5.6	20.2	58.2	50.4	112.2	3.3
	SE	±0.27	±0.09	±0.27	±0.98	±1.87	±0.13

[a]Values are mean of two independent determinations and results expressed on a dry weight basis.

Table 51. Effect of storage on nutritional quality of chickpea *dhal* (ICCC 37)[a]

Storage period (months)	Temp. °C	Phytic acid mg/g	Protein (%)	IVPD (%)	Calcium	Magnesium	Iron
					..... mg/100 g		
0		7.8	24.0	73.7	42.6	96.7	6.5
3	5	7.6	23.7	72.0	42.4	96.5	6.7
	25	6.5	23.9	72.7	44.6	97.2	7.1
	37	7.4	23.7	70.1	43.4	95.9	6.7
6	5	7.3	24.0	71.5	42.6	95.7	6.5
	25	4.6	24.1	71.4	45.6	98.0	6.8
	37	5.5	24.2	67.8	45.1	99.0	6.7
9	5	6.5	23.7	69.2	45.8	95.4	6.8
	25	3.2	24.0	65.9	50.4	98.7	6.7
	37	3.6	23.9	62.5	46.4	98.6	6.3
12	5	5.9	22.9	68.0	46.0	104.0	6.5
	25	2.6	23.9	63.7	49.5	107.8	6.1
	37	3.4	23.5	63.1	47.4	106.4	6.4
	SE	±0.10	±0.07	±0.37	±0.62	±0.69	±0.13

[a]Values are mean of two independent determinations and results expressed on a dry weight basis.

Table 52. Effect of storage on nutritional quality of green gram dhal (PS 16)[a]

Storage period (months)	Temp. °C	Phytic acid mg/g	Protein (%)	IVPD (%)	Calcium	Magnesium	Iron
					..... mg/100 g		
0		10.2	26.5	67.2	37.0	155.8	4.5
3	5	9.9	27.0	66.1	32.8	153.5	4.8
	25	9.7	27.0	65.8	31.3	151.9	4.2
	37	8.6	27.0	66.8	30.4	150.3	4.7
6	5	9.4	27.3	66.9	35.8	153.7	4.7
	25	9.3	27.1	62.5	33.4	160.4	3.9
	37	8.7	27.2	62.7	31.9	154.4	4.2
9	5	8.9	26.4	66.6	34.3	159.2	4.0
	25	6.8	26.8	58.3	34.6	166.8	3.6
	37	6.2	26.7	57.8	31.2	161.9	4.5
12	5	8.2	25.7	64.1	36.5	159.6	3.4
	25	6.9	26.1	56.3	39.4	165.1	3.5
	37	6.4	25.2	55.2	34.8	163.4	4.0
	SE	±0.15	±0.08	±0.63	±0.53	±1.23	±0.16

[a]Values are mean of two independent determinations and results expressed on a dry weight basis.

Table 53. Effect of storage on nutritional quality of black gram *dhal* (T 9)[a]

Storage period (months)	Temp. °C	Phytic acid mg/g	Protein (%)	IVPD (%)	Calcium	Magnesium	Iron
					..... mg/100 g		
0		15.4	28.4	55.5	60.5	197.0	4.1
3	5	15.0	28.2	55.1	62.3	196.5	4.5
	25	13.0	27.7	54.7	62.6	197.5	4.5
	37	13.3	28.1	55.7	64.2	199.7	4.4
6	5	14.7	29.0	54.7	62.4	197.8	4.2
	25	11.3	28.1	54.3	61.9	202.4	4.0
	37	12.2	28.1	53.5	63.6	195.5	4.6
9	5	13.4	27.8	52.4	60.3	204.7	4.1
	25	10.2	27.6	51.0	61.9	213.3	4.0
	37	9.8	28.3	51.0	61.6	220.9	4.4
12	5	12.9	26.9	51.0	61.2	221.9	4.2
	25	8.9	26.7	47.0	62.5	223.8	4.1
	37	9.2	27.0	48.0	63.3	226.9	4.4
	SE	±0.08	±0.09	±0.56	±0.40	±2.70	±0.14

[a]Values are mean of two independent determinations and results expressed on a dry weight basis.

Table 54. Effect of storage on nutritional quality of soybean *dhal* (MONETTA)[a]

Storage period (months)	Temp. °C	Phytic acid mg/g	Protein (%)	IVPD (%)	Calcium	Magnesium	Iron
					..... mg/100 g		
0		32.4	51.9	71.6	181.2	425.6	6.6
3	5	31.6	51.9	70.6	183.3	462.3	7.1
	25	29.6	51.5	70.5	181.7	461.9	6.7
	37	28.7	51.9	70.6	183.2	466.9	7.0
6	5	29.8	53.9	69.3	188.6	469.5	6.5
	25	28.8	53.5	65.3	187.6	473.6	6.5
	37	27.9	53.0	63.5	186.3	463.6	6.2
9	5	28.4	53.9	69.2	190.3	469.1	6.6
	25	25.6	52.3	61.8	189.2	469.2	6.8
	37	25.9	53.1	59.2	187.5	467.2	6.5
12	5	27.2	51.8	66.0	185.4	463.2	6.2
	25	23.0	52.8	59.4	186.4	463.9	6.6
	37	23.1	51.5	54.1	185.1	461.6	6.4
	SE	±0.15	±0.19	±0.39	±0.69	±0.93	±0.14

[a]Values are mean of two independent determinations and results expressed on a dry weight basis.

a 5% loss in protein digestibility of green gram whereas 16 and 18% loss in IVPD was observed when stored at 25 and 37°C (Table 52). Storage for 12 months resulted in decreases in IVPD of black gram and soybean. Soybean samples stored at 25 and 37°C experienced larger decreases in digestibility than samples stored at 5°C (Table 54). A similar pattern was observed for black gram (Table 53), although the extent to which IVPD decreased, as a result of storage, was lower as compared to the other legumes.

There was an increase in the calcium content of pigeonpea during storage (Table 50). The calcium content of the other legumes studied did not change noticeably during storage. The magnesium content of all the legumes studied increased significantly during storage (Tables 50-54). Iron content of the stored legumes did not differ significantly from control values.

4.8.3 Effect of Storage on Cooking Quality

The cooking time of legumes increased with an increase in storage time (Tables 55-59). The cooking rates of control and 12 months stored pigeonpea samples are given in Table 55. The samples stored at 37°C required the longest cooking time. There was only a slight increase in the cooking time of pigeonpea samples stored at 5°C. There was a slight increase in water absorption of pigeonpea during storage. There was an increase in the pectin content of pigeonpea. The rate of increase was higher for samples stored at 25 and 37°C. PCMP number of pigeonpea increased during storage.

Table 55. Effect of storage on cooking quality of pigeonpea *dhal* (ICPL 87119)[a]

Storage period (months)	Temp. °C	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)	Phytic acid (meq/100 g)	Calcium	Magnesium	Pectin	PCMP Number
						meq/100 g			
0		29	1.2	39.2	8.6	1.9	8.2	5.9	4.1
3	5	30	1.1	40.3	7.9	1.9	8.4	5.5	4.3
	25	31	1.2	41.9	7.8	1.9	8.4	5.7	4.4
	37	29	1.2	40.2	6.9	1.9	8.1	5.3	4.6
6	5	30	1.1	41.9	7.8	2.2	9.3	5.9	5.1
	25	32	1.2	44.9	7.3	2.0	8.8	6.2	5.5
	37	33	1.2	45.1	7.0	2.1	9.1	5.6	5.2
9	5	30	1.2	43.2	7.2	2.4	8.9	5.8	5.5
	25	32	1.3	47.5	5.9	2.0	8.8	6.5	7.0
	37	34	1.2	46.8	4.8	2.5	8.7	6.2	8.8
12	5	31	1.3	40.2	7.0	2.5	9.4	6.0	6.4
	25	33	1.3	41.9	5.7	2.0	9.4	6.8	8.1
	37	35	1.3	41.2	4.9	2.5	9.2	6.6	9.6
	SE	±0.35	±0.02	±0.47	±0.24	±0.05	±0.16	±0.07	±0.25

[a]Based on two independent determinations for each constituent.

Table 56. Effect of storage on cooking quality of chickpea *dhal* (ICCC 37)[a]

Storage period (months)	Temp. °C	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)	Phytic acid (meq/100 g)	Calcium	Magnesium	Pectin	PCMP Number
						meq/100 g			
0		32	0.9	25.5	6.8	2.1	8.0	7.4	6.6
3	5	33	1.0	27.7	6.7	2.1	7.9	7.5	6.8
	25	35	1.0	27.1	5.7	2.3	8.0	7.1	7.8
	37	37	1.0	29.0	6.5	2.2	7.9	7.2	6.8
6	5	36	1.0	27.1	6.4	2.2	7.9	7.3	7.0
	25	39	1.1	26.3	4.1	2.3	8.0	7.4	11.4
	37	37	1.1	27.2	4.9	2.3	8.2	7.6	9.8
9	5	36	1.1	25.7	5.7	2.3	7.8	7.6	8.4
	25	40	1.1	25.3	2.8	2.6	8.1	7.7	18.5
	37	39	1.1	26.0	3.2	2.4	8.1	7.7	15.6
12	5	38	1.1	24.7	5.2	2.3	8.5	7.8	9.8
	25	41	0.7	24.7	2.3	2.5	8.9	8.0	24.2
	37	40	1.2	23.8	3.0	2.4	8.8	8.1	18.5
	SE	±0.37	±0.09	±0.25	±0.09	±0.03	±0.06	±0.05	±0.51

[a]Based on two independent determinations for each constituent.

Table 57. Effect of storage on cooking quality of green gram *dhal* (PS 16)[a]

Storage period (months)	Temp. °C	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)	Phytic acid (meq/100 g)	Calcium	Magnesium	Pectin	PCMP Number
						..... meq/100 g			
0		23	1.2	29.1	8.9	1.9	12.8	3.2	3.0
3	5	23	1.3	34.5	8.7	1.7	12.6	3.1	2.8
	25	23	1.2	34.1	8.5	1.6	12.5	3.1	2.8
	37	26	1.3	28.5	7.6	1.6	12.4	3.2	3.3
6	5	25	1.3	37.0	8.3	1.8	12.6	3.0	2.9
	25	26	1.2	36.4	8.2	1.7	13.1	3.5	3.5
	37	28	1.4	32.9	7.7	1.6	12.7	3.5	3.6
9	5	27	1.4	37.7	7.8	1.7	13.1	3.4	3.6
	25	28	1.3	34.0	6.0	1.8	13.7	3.5	5.0
	37	29	1.4	34.1	5.5	1.6	13.3	3.6	5.4
12	5	27	1.5	33.5	7.2	1.9	13.1	3.3	3.9
	25	28	1.4	30.1	6.1	2.0	13.6	3.5	5.2
	37	30	1.5	30.4	5.6	1.8	13.4	3.9	5.9
	SE	±0.36	±0.03	±0.31	±0.13	±0.03	±0.10	±0.04	±0.12

[a]Based on two independent determinations for each constituent.

Table 58. Effect of storage on cooking quality of black gram *dhal* (T 9)[a]

Storage period (months)	Temp. °C	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)	Phytic acid (meq/100 g)	Calcium	Magnesium	Pectin	PCMP Number
						..... meq/100 g			
0		17	1.6	25.9	13.5	3.0	16.2	4.0	3.3
3	5	19	1.7	25.1	13.2	3.1	16.1	4.1	3.5
	25	20	1.7	26.2	11.5	3.2	16.2	4.1	4.0
	37	18	1.7	25.5	11.7	3.2	16.4	4.0	3.9
6	5	20	1.8	26.3	12.9	3.2	16.2	4.0	3.5
	25	21	1.8	29.1	10.0	3.1	16.6	4.5	5.1
	37	26	1.8	26.3	10.8	3.2	16.0	4.2	4.3
9	5	21	1.9	29.2	11.8	3.1	16.8	4.2	4.1
	25	21	1.8	25.9	9.0	3.1	17.5	4.8	6.3
	37	29	1.8	25.4	8.6	3.1	18.1	4.6	6.4
12	5	22	1.9	24.6	11.4	3.1	18.2	4.5	4.9
	25	25	1.8	24.8	7.9	3.2	18.3	5.0	7.8
	37	30	1.9	24.6	8.1	3.2	18.6	4.9	7.6
	SE	±0.34	±0.02	±0.27	±0.06	±0.02	±0.23	±0.11	±0.12

[a]Based on two independent determinations for each constituent.

Table 59. Effect of storage on cooking quality of soybean *dhal* (MONETTA)[a]

Storage period (months)	Temp. °C	Cooking time (min)	Water absorption (g/g)	Solid dispersion (%)	Phytic acid (meq/100 g)	Calcium	Magnesium	Pectin	PCMP Number
						..... meq/100 g			
0		88	0.9	30.7	28.5	9.1	41.1	10.2	10.6
3	5	89	1.0	30.4	27.9	9.2	37.9	10.2	10.3
	25	91	1.0	29.9	26.1	9.1	37.9	10.4	11.2
	37	93	1.0	29.9	25.3	9.2	38.3	9.8	11.0
6	5	93	1.0	28.8	26.2	9.4	38.5	9.9	10.8
	25	94	1.0	29.4	25.4	9.4	38.9	10.8	12.2
	37	102	1.0	29.6	24.6	9.3	38.0	10.1	11.6
9	5	96	1.1	28.7	25.0	9.6	38.5	10.2	11.7
	25	103	1.1	29.0	22.5	9.5	38.5	10.8	13.8
	37	103	1.1	28.6	22.8	9.4	38.3	10.7	13.5
12	5	99	1.1	28.4	23.9	9.3	38.0	10.5	12.4
	25	107	1.1	28.8	20.3	9.4	38.0	11.2	15.7
	37	112	1.1	27.5	20.4	9.3	37.9	11.5	15.9
	SE	±0.37	±0.02	±0.12	±0.14	±0.03	±0.37	±0.13	±0.21

[a]Based on two independent determinations for each constituent.

Table 56 presents the data on cooking quality parameters of stored chickpea as a function of storage time. Samples stored at 25 and 37°C required a longer cooking time. The water uptake and the dispersed solids of chickpea samples remained unaffected by the storage period. There was a slight increase in pectin content of chickpea during storage. There was a drastic increase in the PCMP number from the ninth month of storage for samples stored at 25 and 37°C.

The effect of storage on cooking quality of green gram is given in Table 57. Cooking time, water absorption and solid dispersion of green gram increased during storage. The extent of increase was greater for samples stored at 25 and 37°C. The PCMP number based on phytic acid , calcium, magnesium and pectin content increased during storage. The increase was lower for samples stored at 5°C.

Table 58 shows the effect of storage on the cooking quality of black gram. Conditions of storage had a significant effect ($P < 0.01$) on cooking time of black gram. Samples stored at 25 and 37°C registered larger increases in cooking time than sample stored at 5°C. There was a slight increase in water absorption after 12 months of storage. PCMP number increased significantly ($P < 0.01$) during storage.

A similar pattern was observed for soybean. Soybean samples stored at 25 and 37°C experienced larger increases in cooking time than samples stored at 5°C (Table 59). There was a slight decrease in solids dispersed at the end of the storage period. Generally there was a trend of an increase in the cooking time of

the pulse with an increase in calcium, magnesium and pectin content during storage and decrease in phytic acid content.

DISCUSSION

CHAPTER V

DISCUSSION

Phytic acid, a major component of all legumes, has a tremendous potential for binding positively charged molecules such as cations or proteins. The interaction between phytic acid and minerals such as iron, zinc, calcium, magnesium etc., leads to the formation of complexes that are insoluble at intestinal pH and, hence, biologically unavailable for absorption (Erdman, 1979). In addition, complex formation of phytic acid with proteins may inhibit the enzymatic digestion of the proteins (Singh and Krikorian, 1982).

Phytate levels in legumes are reduced during food-processing operations. In India, traditional methods of processing include germination, fermentation, pressure cooking and roasting. Chemical characteristics of legumes as affected by cooking have been reported to involve phytates, phosphorus, divalent cations and pectins (Moscoso *et al.*, 1984). Detailed information on the chemical characteristics affecting cooking and other processing qualities are needed to develop legumes with desirable attributes, either by genetic manipulation or by postharvest processing. The results of the present study on effect of storage and processing on phytic acid levels in legumes and its interference with the utilisation of protein and iron, are discussed in the following sections.

5.1 DEHULLING QUALITY OF DIFFERENT LEGUMES

Dry whole-seeds of pulses possess a fibrous seed coat, or testa ("husk, hull or skin"). The seed coat is often indigestible and sometimes causes a bitter taste (Singh and Singh, 1992). Therefore, pulses are mostly consumed after dehusking to improve their palatability and taste. The most beneficial effect of dehulling is the reduction of cooking time in terms of removing the impermeable nature of the seed coat of pulses which hinders water uptake during cooking. Dehulling quality describes both the rate of hull removal from the cotyledon and the yield of dehulled grain obtained. High throughput and a high yield of dehulled grain are desirable in commercial practice.

In the present study, average *dhal* yield was highest in soybean and lowest in green gram. A large variability in dehulling quality of pigeonpea genotypes was observed (Table 7). Singh *et al.* (1992) reported that *dhal* yield values primarily depend on the content of seed coat (husk) of pigeonpea genotypes. Genotypes with a higher seed coat content had significantly lower ($P < 0.01$) *dhal* yields.

In general, *dhal* yield was higher for kabuli types than desi types of chickpea and this might be due to their lower seed coat contents. However,it was observed that powder fraction was relatively higher in kabuli types indicating that kabuli genotypes might incur greater nutrient losses as a result of dehulling (Table 7).

As a result of dehulling the outer layers of the cotyledons of green gram and black gram were increasingly scarified and removed in the form of powder

fraction. The dehulling characteristics of green gram and black gram genotypes were generally poor (Table 8). The poor dehulling characteristics probably resulted from high susceptibility to seed splitting during dehulling and high seed coat adhesion. Ehiwe and Reichert (1987) reported that the dehulling quality of green gram cultivars was generally poor because of low yields and long dehulling time. The major seed factors responsible for good dehulling quality of cowpea, pigeonpea, and green gram included resistance to seed splitting during dehulling and a seed coat loosely bound to the cotyledons.

As seen in Table 8, soybean had very good dehulling qualities because of high yield, short dehulling time, and high dehulling efficiency. No large variability in *dhal* yield of soybean varieties was obtained.

5.1.1 *Relationships Between Dhal Yield and Grain Physical Characteristics*

Dhal yield showed positive association with 100-seed mass and negative association with seed coat content. It appeared that *dhal* yield in the Tangential Abrasive Dehulling Device (TADD) depended on the size of grains implying that smaller grains would reduce the *dhal* yield. Since the yield of *dhal* depends on the cotyledon content a negative association between *dhal* yield and seed coat content was observed. Ehiwe and Reichert (1987) reported that dehulling time was related to seed hardness and seed coat content. Even though the *dhal* yield primarily depends on the type of machine, abrasion techniques and other physical conditions that are employed during dehulling, other characteristics such as size,

shape and hardness of the grain seem to play an important role in determining dehulling losses (Singh *et al.*, 1992).

5.2 CHEMICAL COMPOSITION OF LEGUMES

Observed differences in chemical composition of genotypes within legume species might have been the results of environments and genetic characteristics of the crop. Protein content in pigeonpea genotypes ranged from 19.6 to 27.5% indicating a large variation among genotypes (Table 11). A similar trend was observed among chickpea genotypes (18.7 to 28.1%). Earlier studies have reported that the protein content of chickpea genotypes ranged from 20.5-29.6% (Williams and Singh, 1987). Protein content was the highest for soybean (mean 54.6%). Protein content of black gram and green gram did not show noticeable differences among the genotypes (Table 12). Expectedly, the fat content was the highest (mean 25.1%) for soybean and lowest for green gram (mean 1.5%). No significant differences were observed in fat content of black gram and green gram genotypes (Table 12). Fat content of chickpea genotypes ranged between 5.1 and 7.6%. Williams and Singh (1987) reported that the fat content of decorticated chickpeas ranged between 4.2 and 6.9%. Soybeans provide a considerable amount of fat to the human diet since the fat content in analyses ranged between 22.3 and 27.5%.

Calcium content was the highest for soybean and the lowest for green gram. Raboy *et al.* (1984) reported that calcium content of 38 soybean lines ranged between 2.2 and 3.4 g/kg. Magnesium and iron content of soybean was also higher compared to the other legumes and this was substantiated by the higher

ash content (Table 12). Among pigeonpea genotypes calcium content was the highest for ICP 8094 and the lowest for ICPL 4 indicating a large variation among genotypes (Table 11). Similar variations for calcium content were observed among chickpea genotypes. Among the chickpea genotypes, kabuli types had a higher calcium content than the desi types. Jambunathan and Singh (1981) reported that *dhal* of kabuli chickpea cultivars contained significantly more calcium than *dhal* of desi cultivars. Significant differences ($P < 0.01$) were observed among all the legume genotypes for iron content. Iron content of chickpea and soybean was higher than that of the other legumes. Jambunathan and Singh (1981) reported that iron content of chickpea *dhal* ranged between 4.9 and 6.2 mg/100 g.

Total dietary fiber (TDF) content was the lowest (16.9%) for green gram and the highest for black gram (22.2%). TDF content of chickpea genotypes differed significantly (Table 11). Singh (1984) reported that total dietary fiber content of chickpea *dhal* was significantly higher in desi than in kabuli cultivars and this was associated with a higher hemicellulose content in desi *dhal* samples. Kamath and Belavady (1980) reported that unavailable carbohydrate content of *dhal* of green gram (12.2-15.6%), black gram (10.9-17.2%), pigeonpea (12.2-14.7%) and chickpea (13.2-13.5%) showed small varietal differences.

5.3 VARIABILITY IN PHYTIC ACID AND PROTEIN DIGESTIBILITY

Phytic acid contents and *in vitro* protein digestibility (IVPD) values differed significantly among and within the legume species. Phytic acid content (mg/g) was the highest in soybean (36.4) followed by black gram (13.7), pigeonpea (12.7),

green gram (12.0), and chickpea (9.5). On an average, phytic acid constituted 78.2% of the total phosphorus content and this percentage figure was the highest in soybean and the lowest in green gram. Results have indicated a considerable genetic variability in phytic acid content of both pigeonpea and chickpea genotypes (Tables 13 and 14). Duhan *et al.* (1989) reported that phytic acid content of chickpea showed large varietal differences. These workers found that phytic acid content varied significantly from 7.58 to 8.10 g/kg in chickpea varieties. In the present study, mean phytic acid content of green gram and black gram genotypes was 10.9 and 13.0 mg/g respectively (Table 15). Duhan *et al.* (1989) reported that phytic acid content of black gram varieties ranged from 6.47-6.68 g/kg. Information on phytic acid content of pigeonpea and green gram is limited and there has been no screening of genetic stocks of these legumes for phytic acid content.

Among the present legumes, mean phytic acid was the highest in soybean (36.4 mg/g) and the lowest in chickpea (9.5 mg/g). Ologhobo and Fetuga (1984) observed soybean dry seeds to have higher phytate (1.47% dry weight basis). Raboy *et al.* (1984) reported a mean phytic acid concentration of 17.6 g/kg for 38 soybean lines [*Glycine max* (L.)], with lines ranging from 13.9 to 23.0 g/kg.

There was a significant positive correlation ($r = 0.99$) between phytic acid and total phosphorus content in all the legumes (Table 16). This observation is in agreement with the previous findings of positive and significant correlations between phytic acid and total phosphorus content (Lolas and Markakis, 1975). Protein content was also positively and significantly correlated with phytic acid

(r = 0.94). The magnitude of correlation between phytic acid and protein was low, although it was positive in chickpea and pigeonpea. Raboy *et al.* (1984) reported a positive correlation between phytic acid and protein among soybean lines. There was a negative correlation between the seed size and phytic acid content in both pigeonpea and chickpea genotypes but it was not significant.

Percent mean value for IVPD was higher for chickpea than pigeonpea indicating that protein digestibility of chickpea may be better than pigeonpea. The mean values for protein digestibility of green gram and black gram *dhal* were 70.1 and 59.9 percent respectively (Table 15). Jood *et al.* (1989) reported that protein digestibility (*in vitro*) of black gram genotypes varied from 52 to 58 percent. Among the legumes studied chickpea was the most digestible (mean 71.3%). IVPD of kabuli genotypes was more than that of desi genotypes (Table 14). Singh and Jambunathan (1981b) reported that IVPD of desi genotypes of chickpea *dhal* ranged from 63.7-76% and that of kabuli genotypes from 72.7-79.1%.

There was a significant negative correlation (r = 0.39**) between phytic acid and IVPD of all the legumes studied implying that phytic acid would adversely influence the protein quality of legumes. A highly significant negative correlation was observed between the *in vitro* protein digestibility and the concentration of phytic acid in both pigeonpea (r = 0.80) and chickpea (r = 0.83) (Figs. 2 and 3). These results are in agreement with the results of Singh *et al.* (1991) who reported a negative and significant correlation between phytic acid and IVPD values in groundnut.

Carnovale *et al.* (1988) reported that the *in vitro* protein digestibility of faba bean and pea samples decreased in the presence of exogenous phytic acid. The results of the present study, showing a negative correlation between phytic acid content and *in vitro* protein digestibility, agree with the data of other workers (Knuckles *et al.*, 1985; Serraino *et al.*, 1985).

The negative association observed between phytic acid and IVPD in the present study could be attributed to the inhibition of enzyme activity by phytate. Phytic acid probably reduced the protein digestibility by interfering with enzyme activity. *In vitro* activity of the proteolytic enzyme pepsin has been reported to be substantially inhibited by low levels of phytic acid (Vaintraub and Bulmaga, 1991). Knuckles *et al.* (1985) reported that phytate exhibited an inhibitory effect on pepsin digestion of casein.

The ability of phytic acid to complex with protein and inhibit enzyme activity has been reported by earlier workers (O'Dell and de Boland, 1976). Singh and Krikorian (1982) reported that *in vitro* activity of the proteolytic enzyme trypsin using casein as the substrate was substantially inhibited by low levels of phytic acid. The inhibition of trypsin activity by phytate varied with the phytate concentration. The present results support the hypothesis that phytic acid protein interaction affects the protein availability of legumes negatively.

5.4 PHYTIC ACID, NITROGEN SOLUBILITY AND IRON AVAILABILITY OF DIFFERENT LEGUMES

Nitrogen solubility also showed significant variation ($P < 0.01$) among and within the legume species. Among the present legumes mean nitrogen solubility index was the highest in soybean (78.4%) and the lowest in black gram (32%). Phytic acid is reported to form a complex with proteins rendering them less soluble (de Rham and Jost, 1979). The results of the present study do not appear to lend support to this observation. Soybean which had the highest phytic acid content (mean 36.4 mg/g) exhibited the highest nitrogen solubility index (Table 20). The formation of a complex with protein did not appear to be a strong factor in the present study as there was no noticeable negative correlation between nitrogen solubility (as an index of protein solubility) and phytic acid. The present results suggest that phytic acid possibly inhibits the enzyme activity.

Genotypic differences were observed in the ionisable iron content of the legumes studied. Ionisable iron expressed as percent of total iron is considered as a parameter of bioavailability of food iron (Narasinga Rao and Prabhavathi, 1978). In chickpea, nearly 26% of the total iron in the grain was ionisable (Table 19) while in black gram it was only 17% (Table 20). Among the commonly consumed pulse crops in India, chickpea contains the highest amount of total iron. However, the results of this study show that its available iron content is very low. The poor iron availability from legumes has been attributed to their high polyphenolic content (Rao and Prabhavati, 1982) and most importantly to the iron binding effects of phytate (Hazell, 1988).

Ionisable iron was significantly ($P < 0.01$) higher in kabuli than in the desi genotypes of chickpea (Table 19). The low phytate content of the kabuli genotypes may explain the high level of available iron of kabuli genotypes of chickpea. Hazell (1988) reported that phytate was significantly and negatively correlated with diffusible iron in cereals, legumes and nuts. Iron diffusibility was found to be inversely correlated with phytate levels, but it was suggested that iron availability may also be inversely related to the levels of condensed polyphenols (tannins).

Ionisable iron as percent of total iron was quite low in green gram and soybean (Table 20). The findings of Hurrel *et al.* (1992) suggest that phytic acid is a major inhibitory factor in soy protein isolates. Reduction of phytic acid to < 0.01 mg/g of isolate increased iron absorption four-to five fold whereas adding back the phytic acid reduced iron absorption to its original low value. The present results also demonstrate that phytic acid strongly inhibits iron absorption in legumes.

Ionisable iron as percent of total iron in pigeonpea ranged from 19.1 to 27.6% showing significant differences among the genotypes (Table 19). Snehalatha (1984) showed that only 20.8% of the total iron content of pigeonpea was bioavailable. Ionisable iron differed significantly among soybean genotypes. Latunde-Dada (1991) reported genotypic differences in dialyzable iron for ten Nigerian soybean varieties.

5.4.1 *Effect of pH on Nitrogen Solubility*

The pH modification markedly affected the solubility of nitrogen in the legumes studied. The lowest nitrogen solubility index (NSI) was observed at pH 3 in all the legumes (Fig. 4). Prattley *et al.* (1982) reported that the association of protein and phytic acid is highly pH dependent. Their data showed that insoluble protein-phytate complexes form below the isoelectric point of protein. The solubility curve of nitrogen in the present legumes showed a broad minimum at pH 3-4 (Table 21). Nitrogen solubility was higher at acidic and alkaline pH, but solubility decreased above pH 7. Similar nitrogen solubility patterns have been reported previously (de Rham and Jost, 1979; Kantha *et al.*, 1986). Kantha *et al.* (1986) reported that nitrogen solubility in winged bean flour dropped from 32% at pH 2.0 to 12% at pH 4.0. Conversely, phytic acid was 25% soluble at pH 2.0 and 48.0% soluble at pH 4.0; at neutral pH, the solubility of nitrogen and phytic acid were 50 and 80% respectively. Differences in solubility of nitrogen and phytic acid can be utilized to prepare protein concentrates with low phytic acid content (de Rham and Jost, 1979).

5.5 RELATIONSHIPS BETWEEN PHYTIC ACID, PROTEIN, IVPD, NITROGEN SOLUBILITY, TOTAL DIETARY FIBER AND IONISABLE IRON OF DIFFERENT LEGUMES

There was no significant correlation between phytic acid and nitrogen solubility (Table 22). The results of the present study do not appear to lend

support to the observation that phytic acid forms a complex with proteins rendering them less soluble.

Phytic acid was negatively and significantly correlated with the percent ionisable iron (Table 22). There was a significant negative correlation between phytic acid and ionisable iron in both pigeonpea ($r = 0.81$) and chickpea ($r = 0.73$) genotypes tested separately (Tables 23 and 24). These results agree with the studies of Hallberg *et al.* (1987) who showed that there was a strong relationship ($r = 0.99$) between the inhibition of iron absorption and the amount of phytate in man. The present findings strongly suggest that phytic acid is a major factor inhibiting iron absorption in legumes. The negative association of phytic acid with ionisable iron observed in the present study was probably due to the insoluble complex formation of phytic acid with iron at gastro intestinal pH.

Total dietary fiber was negatively correlated with *in vitro* protein digestibility in all the legumes studied (Table 22). This indicates that phytic acid is only one of the factors affecting *in vitro* protein digestibility of legumes. This observation is in agreement with the findings of Mongeau *et al.* (1989) who reported that the true protein digestibility in rats was negatively correlated with the total food fiber level. Their results indicated that several food fiber fractions and possibly associated substances influenced protein digestibility. Digestibility and absorption of protein occur in the upper gastrointestinal tract in the presence of mostly undegraded fiber. A decreased protein digestibility may be due to the nature of the protein, to the fiber acting as a physical barrier to enzyme diffusion

or to the presence of other substances present in the fiber source (Mongeau *et al.*, 1989).

In the present study, a negative correlation (r = 0.55) was observed between total dietary fiber and ionisable iron (Table 22). The capacity of dietary fiber to bind polyvalent mineral ions may impart a negative effect on the bioavailability of some minerals (Laszlo, 1987). Reinhold *et al.* (1986) reported that maize and wheat fibers decreased the retention of ferrous iron by binding and by promoting autoxidation and formation of poorly soluble iron polymers. Retention of ferric iron was also lowered in the presence of fiber, presumably as a result of polymerisation.

5.6 COOKING QUALITY

Cooking quality was measured as a function of cooking time, amount of water absorbed and solids dispersed. Green gram required the shortest cooking time (mean being 15 min.) and soybean the longest cooking time (mean being 84 min.) (Table 26). On the other hand, calcium content was the highest in soybean, and the lowest in green gram. Phytic acid has been implicated in influencing the cooking quality of legumes. Phytic acid chelates divalent cations (Ca, Mg) and prevents their crosslinking with pectin, thereby facilitating cell wall dissolution during the cooking process (Moscoso *et al.*, 1984). In the present study, genotypes of the different legumes having low levels of phytic acid took longer time to cook.

The amount of solids dispersed into the cooking water was highest for pigeonpea and lowest for chickpea (Table 25). Among the chickpea genotypes, the

kabuli genotypes required a longer cooking time than the desi genotypes. Williams *et al.* (1983) reported a positive and significant correlation between seed size and cooking time for chickpeas.

Water absorption is an important determinant of the rate of hydration and of cooking properties. Water absorption is to some extent determined by heredity, but it is also influenced by environmental factors, such as agronomic and storage conditions. Starch and protein are the major components involved in the hydration process, while seed anatomy and cellular structure are just as important (Gomez, 1991). In the present study mean water absorption was higher in black gram and lower in soybean (Table 26). No large differences in water absorbing capacity were observed between the genotypes of the different legumes.

5.6.1 PCMP Number as an Index of Cooking Quality of Legumes

The interaction between phytic acid, calcium, magnesium and pectin has been suggested to influence the cooking quality of legumes (Crean and Haisman, 1963). Muller (1967) has shown in peas that the hardness of the seed can be directly correlated to higher PCMP number. A similar trend was observed in the present study. Green gram which was easily cookable had the lowest PCMP number while soybean requiring prolonged cooking had higher PCMP value (Table 28). PCMP number of pigeonpea genotypes differed significantly. The kabuli genotypes of chickpea which required longer cooking time had higher PCMP number (Table 27). Generally there was a trend of an increase in the cooking time of the pulse with an increase in calcium, magnesium and pectin and

a decrease in phytin phosphorus. These findings are in agreement with the observations of Narasimha and Desikachar (1978). Their results indicated a high correlation between cooking time and the contents of calcium, magnesium, pectin and phytin and the PCMP number calculated according to the formula of Muller (1967). In the present study a good correlation between phytic acid content and cookability was observed in pigeonpea, chickpea, green gram and black gram. This suggests that high phytic acid phosphorus content in the pulses favors a rapid rate of softening and dissolution of the pectic substances making the pulses more cookable. These findings confirm the results by Singh *et al.* (1984) who found a good correlation between phytic acid content and cookability of legumes.

5.6.2 *Relationships Between Cooking Time and Various Physicochemical Characteristics*

The cooking time was positively and significantly correlated with the phytic acid content in pigeonpea, chickpea, green gram and black gram (Table 29). This observation is in agreement with the findings of Kumar *et al.* (1978) and Singh *et al.* (1984) who found a good correlation between phytic acid content and cookability. The mechanism proposed is that phytic acid chelates calcium, reduces the formation of calcium-pectic complexes responsible for hard texture, and exhibits a texture-softening effect (Moscoso *et al.*, 1984).

Water absorption and solids dispersion were negatively and significantly correlated with the cooking time (Table 29). This is similar to the findings of Narasimha and Desikachar (1978) who observed that easy cooking varieties of

pigeonpea had higher water uptake and more solids got dispersed while poor cooking varieties had lower water uptake and less solid dispersion. Based on these data the level of dispersed solids appears to be a better index of cookability than water uptake during cooking.

The cooking time was negatively and significantly correlated with the protein content in pigeonpea, chickpea, green gram and black gram. Narasimha and Desikachar (1978) reported that there was trend of an increase in the cooking time of pigeonpea with a decrease in protein content.

5.7 EFFECT OF PROCESSING ON PHYTIC ACID, PROTEIN DIGESTIBILITY, NITROGEN SOLUBILITY, DIETARY FIBER AND MINERALS OF LEGUMES

5.7.1 Phytic Acid

The decrease in phytic acid as a result of germination was higher in chickpea (87%) followed by pigeonpea (69%), black gram (48%), green gram and soybean (46%). This indicated that the treatment will be more beneficial in chickpea and pigeonpea as compared with other legumes. These figures are considerably higher than those reported for black gram (Reddy *et al.*, 1978); and beans (Tabekhia and Luh, 1980). The breakdown of phytate during germination could be attributed to an increase in the activity of the endogenous phytase as reported in case of faba bean cultivars (Eskin and Wiebe, 1983).

Fermentation also resulted in the reduction of phytic acid of these legumes, though it was less effective as compared to germination. In India black gram and

chickpea are the choice ingredients in the fermented steamed food products 'idli' and 'dhokla' respectively. As a result of fermentation, phytic acid was reduced by 30% in black gram (Table 33) and 53% in chickpea (Table 31). Seventy two hours of fermentation substantially reduced phytic acid in several foodstuffs ranging from 52% to 65% for soybean and cowpea (Marfo *et al.*, 1990). On the other hand about 10% reduction was reported due to 24 hrs. of fermentation of black gram (Reddy and Salunkhe, 1980a). The fermentative microorganisms contain enzymes phytase and phosphatase which hydrolyze phytate into inositol and orthophosphate (Reinhold, 1975), and it has been suggested that the loss of phytase during fermentation might be due to the activity of the enzyme phytase naturally present in legumes (Marfo *et al.*, 1990).

Both, wet-heating and dry-heating also reduced the phytic acid levels in these legumes. No striking differences between the wet-heating and dry-heating processes were observed for pigeonpea (Table 30). In chickpea and soybean autoclaving resulted in significantly higher reductions in phytic acid than roasting (Tables 31 and 34). The reduction figures observed in the present study are slightly higher than those reported for chickpea and black gram by Duhan *et al.* (1989). The heat-processing might have reduced the extractability of phytic acid in the present study. Kumar *et al.* (1978) observed that the cooking process decreased both water- and acid-extractability of phytate phosphorus in green gram, cowpea and chickpea. Poor extractability of phytate phosphorus with water and HCl was attributed to the formation of insoluble complexes between phytic phosphorus and other components during cooking.

5.7.2 *Protein Digestibility*

Both germination and fermentation remarkably increased the *in vitro* protein digestibility (IVPD) in all legume species. Effects were more pronounced in pigeonpea as compared to other legumes (Table 35). Both germination and fermentation appeared to be equally effective in increasing IVPD of these legumes. Germination has been reported to increase the protein digestibility of green gram (Kataria *et al.*, 1988), moth bean (Khokhar and Chauhan, 1986b), soybean (Boralkar and Reddy, 1985) and chickpea and black gram (Jood *et al.*, 1989). The hydrolysis of seed proteins, protease inhibitors, phytic acid, and polyphenols during germination may considerably account for increased IVPD in legumes (Duhan *et al.*, 1989). Boralkar and Reddy (1985) reported an improvement in IVPD of soybean with increasing fermentation period. Production of certain proteolytic enzymes by microflora during fermentation may be responsible for improved protein digestibility of legumes (Reddy *et al.*, 1982). Germination also significantly increased protein values in pigeonpea, black gram, green gram and soybean whereas fermentation was more effective in increasing protein content of pigeonpea and soybean.

Roasting and autoclaving did not noticeably change the levels of protein in these legumes, but resulted in considerable increases in IVPD values in pigeonpea, chickpea, green gram and black gram (Tables 35-38). Heat processing was reported to increase protein digestibility of grain legumes (Jood *et al.*, 1989; Khokhar and Chauhan, 1986b) most likely by destroying heat labile protease inhibitors, and also by denaturing globulin proteins which are highly resistant to

proteases in the native state (Walker and Kochar, 1982). In the present study, an increase in IVPD was associated with a decrease in phytic acid due to various processing practices indicating that phytic acid interferes with protein digestibility. *In vitro* protein digestibility (IVPD) was negatively and significantly correlated with the phytic acid content in these legumes.

5.7.3 *Nitrogen Solubility*

The nitrogen solubility index of all the legumes studied showed a decreasing trend after heat processing (Tables 35-39). This observation supported the similar finding when heating decreased nitrogen solubility in winged bean (Narayana and Narasinga Rao, 1982) and in soy and peanut (Mc Watters and Holmes, 1979). Narayana and Narasinga Rao (1982) reported that autoclaving denatured the proteins of winged bean flour and reduced their solubility. Reduction in nitrogen solubility due to heat processing observed in the present study may be attributed to denaturation of proteins.

Germination process increased the nitrogen solubility of all the legume species. The fermentation process also resulted in significant increases in the nitrogen solubility profiles of chickpea, green gram, black gram and soybean (Tables 36-39). Quinn and Beuchat (1975) reported an increased nitrogen solubility of solvent defatted peanut flour as a result of fungal fermentation. The increased nitrogen solubility was attributed to protein hydrolysis by fungal acid proteases to form peptides and free amino acids.

5.7.4 *Total Dietary Fiber*

Germination and fermentation processes significantly reduced the total dietary fiber (TDF) contents of all the legumes (Tables 35-39). Increased alpha galactosidase activity during germination and fermentation has been reported to cause a decrease in the oligosaccharide content of black gram and a black gram/rice blend leading to reduced levels of dietary fiber (Reddy and Salunkhe, 1980b).

In pigeonpea, chickpea and green gram (Tables 35, 36 and 37) autoclaving and roasting resulted in slight increases in TDF values. Valverde and Frias (1991) have reported a considerable increase in the neutral detergent fiber (NDF) content in processed chickpeas and kidney beans.

5.7.5 *Minerals*

Germination and fermentation did not bring about any apparent changes in the calcium, magnesium, and iron contents of pigeonpea, chickpea, green gram and black gram (Tables 40-43). Calcium and iron content were slightly decreased as a result of germination in all the legume species. Reddy and Salunkhe (1980a) did not find a significant decrease in calcium, magnesium, zinc and iron contents of black gram after fermentation. Generally, mineral losses have been reported to occur during the soaking of legumes when soaking water is discarded before germination or sprouting (Kumar *et al.*, 1978). The heating processes noticeably decreased iron content in all legumes except green gram. No large effects of processing treatments on the levels of calcium and magnesium were observed in

these legumes. Meiners *et al.* (1976) reported considerable mineral losses during cooking of different legumes. Such losses were attributed to leaching of minerals in cooking water which was discarded. The present results suggest that cooking would not result in mineral losses if cooking water is not discarded.

Effect of Processing on Availability of Iron from Legumes .

Germination resulted in an increase of available iron in all the legumes. This confirms earlier reports that showed enhancement of iron availability due to germination (Latunde-Dada, 1991). Prabhavati and Narasinga Rao (1979) also observed increases in ionisable and soluble iron when green gram was germinated. Bau and Debry (1979), apart from showing losses of phytate during the germination of soybeans, also demonstrated increased levels of ascorbic acid in these beans (ascorbic acid being an enhancer of iron availability). In the present study, germination beyond 48 hrs. was accompanied by a considerable reduction in the phytic acid content. The increase in ionisable iron observed as a result of germination can be attributed to the decrease in phytic acid and probably an increase in ascorbic acid content. Hallberg *et al.* (1989) reported that the inhibitory effect of phytate on iron absorption was markedly counteracted by ascorbic acid. This neutralising effect of ascorbic acid was related to the amount of ascorbic acid given the amount of phytates present. Increase in ionisable iron as a result of germination was higher for genotypes with low levels of phytic acid.

Fermentation improved the *in vitro* availability of iron significantly in all the legumes studied (Tables 40-44). Moeljopawiro *et al.* (1987) reported that fermentation by either lactic acid producing bacteria or *R. oligosporus* increased the relative biological value of iron in soybeans. It was hypothesized that the increase in the relative biological value of iron by lactic acid fermentation may be due to : 1) Release of iron from complexes by enzymes, such as proteases and phytases, produced by lactic acid microorganisms, 2) lactic acid produced by microorganisms acts as a chelator for iron, and 3) other chelating agents, such as lysine are produced by lactic acid microorganisms. The present results indicate that fermentation of legumes could strongly increase the availability of iron as estimated *in vitro*. The increase could mainly be attributed to a corresponding degradation of phytate of these legumes by endogenous phytase or by adding exogenous phytase during fermentation.

Autoclaving positively influenced the *in vitro* availability of iron, increasing its levels in all the legumes studied (Tables 40-44). Roasting improved the *in vitro* availability of iron significantly in chickpea, green gram, black gram and soybean (Tables 41-44). In pigeonpea roasting resulted in significant improvement in ionisable iron only in ICPL 88046 (Table 40). Rodriguez *et al.* (1985) reported that the beneficial effects of heat treatment upon iron bioavailability from soy protein was probably due to inactivation of trypsin inhibitors and unfolding of the protein molecular structures to increase the susceptibility of the proteins and phytate to digestion by proteolytic enzymes and phytase respectively. These latter processes would then facilitate release of iron

from the protein-Fe-phytate complex with concomitant improvement in bioavailability.

5.8 EFFECT OF STORAGE ON PHYTIC ACID, PROTEIN DIGESTIBILITY, MINERALS AND COOKING QUALITY

5.8.1 Phytic Acid in Stored Pulses

Phytic acid content of all the legume species decreased with storage time. The decrease in phytic acid after 12 months of storage was the highest in chickpea (56-67%) stored at 25 and 37°C (Table 46) and the lowest in soybean (29%) stored at 25 and 37°C (Table 49). *Dhal* samples of all the legume species stored at higher temperatures (i.e 25 and 37°C) exhibited a greater loss in phytic acid than samples stored at 5°C (Figs 10-14). This observation agrees with previous studies in which phytic acid of *Phaseolus vulgaris* varieties decreased during storage in high temperature, high humidity (Kon and Sanshuck, 1981; Hernandez-Unzon and Ortega-Delgado, 1989). Kon and Sanshuck (1981) reported a 65% reduction in phytic acid content of California small white beans stored for 10 months at 32°C. Hernandez-Unzon and Ortega-Delgado observed that beans stored for 8 years had from 94% to 98% less phytic acid than recently harvested common beans. Long cooking times for legume seeds have been related to low phytate contents (Kon and Sanshuck, 1981). In a warm, moist environment, there would be increased metabolic activity, phytase activation, and membrane degradation. Hentges *et al.* (1991) reported that phytase activity

decreased during storage of cowpeas and other bean varieties. The decrease in phytase activity strongly correlated with increased storage time, temperature, and humidity. Hydrolysis by phytase would result in conversion of phytate to inorganic phosphorus. Phytase activity decreased during storage but retained sufficient activity to continue phytate hydrolysis throughout the storage period. Enzymatic hydrolysis was shown to increase the conversion of white bean phytate (Chang *et al.*, 1977) to inorganic phosphorus during storage at high temperatures and high humidities. The reduction in phytic acid during storage at high temperatures in the present study probably resulted from hydrolysis by phytase.

There was an increase in the calcium content of pigeonpea during storage (Table 50). The calcium content of the other legumes studied did not change noticeably during storage. The magnesium content of all the legumes studied increased significantly during storage. Jones and Boulter (1983b) reported that during storage phytate hydrolyses phytin to release bound calcium and magnesium.

5.8.2 *Effect of Storage on Protein Digestibility and Mineral Content of Legumes*

Chickpea, green gram, and soybean stored at 25 and 37°C showed marked decreases in digestibility of proteins (*in vitro*) (Tables 51, 52 and 54). Conditions of storage had a significant effect on the loss of digestibility from stored pulses. *In vitro* protein digestibility of *dhal* samples stored at 25 and 37°C decreased at a faster rate than samples stored at 5°C. These results are in agreement with the

findings of Bressani (1983) who reported that after a 6 month storage at 5°C, beans suffered a 6% loss of protein digestibility. At 30 and 40°C storage, there was a 15% loss. Sievwright and Shipe (1986) demonstrated a decrease in protein digestibility (*in vitro*) accompanied by changes in tannins and phytates, in black beans stored at 30 or 40°C and 80% relative humidity. According to these workers, protein digestibility was reduced by interactions between protein and tannins, especially low molecular weight tannins. Firmness increased and protein digestibility decreased as the phytic acid content decreased. It was suggested that as beans become firm due to phytate losses, proteins may become less susceptible or exposed to proteolytic action.

Tuan and Phillips (1991) reported that storage of cowpeas under conditions that produced the hard-to-cook defect reduced protein digestibility as determined by both an *in vitro* technique and analysis of rat ileal contents. The observed exacerbation of the negative effect of hard-to-cook development on protein digestibility was attributed to interaction between proteins and phenolic acids.

The present results indicate that the post harvest physiological changes that contribute to poor *in vitro* protein digestibility can be reduced by storage at 5°C. The reduction in IVPD of legumes observed in the present study may be attributed to interactions between protein and tannins due to which the proteins may have become less exposed to proteolytic action. Secondly as the legumes became firm due to phytate losses proteins may become less susceptible to proteolytic action.

5.8.3 Effect of Storage on Cooking Quality

The term cooking quality or cookability refers to the condition in which dry legume seeds achieve a degree of tenderness acceptable to the consumer during cooking. A characteristic property of nearly all dried legumes is the long cooking time required to attain the desirable degree of softness and palatability. The loss of cookability during storage of legume seed has been reported by several workers (Kon and Sanshuck, 1981; Moscoso *et al.*, 1984). This was mainly attributed to the decrease in hydration rate (Sefa-Dedeh *et al.*, 1979), changes in microstructure of the seeds (Sefa-Dedeh *et al.*, 1979) and chemical and/or enzymatic changes that occur in the seed coat and the cotyledon during storage (Hentges *et al.*, 1991).

In the present study the cookability of stored pulses as a function of storage time was studied. The pulses stored at 25 and 37°C required prolonged cooking times; however, pulses stored at 5°C showed only a slight increase in cooking time (Tables 55-59). These results confirm previous reports that high temperature during storage increased the time required to soften legume seeds during cooking (Moscoso *et al.*, 1984). Long cooking times for legume seeds have been related to low phytate contents (Kon and Sanshuck, 1981). Hentges *et al.* (1991) observed that in a warm, moist environment, there would be increased metabolic activity, phytase activation and membrane degradation. Since membranes were degraded, calcium and magnesium hydrolyzed from phytin by phytase could diffuse to the pectic substances located in the middle lamella to form insoluble pectate salts. A decrease in pectin solubility would increase

cooking time. The slower cooking rates of the pulses stored at 25 and 37°C in the present study may be attributed to the more gradual leaching of divalent cations within the middle lamella by the phytic acid and other naturally occurring chelating agents contained within the intracellular cytoplasm.

The water absorption of chickpea samples remained unaffected (Table 56), however in case of the other legumes there was a slight increase in water absorption during storage. These findings are similar to the observations of Hernandez-Unzon and Ortega-Delgado (1989) who reported that water absorption in older seeds of *Phaseolus vulgaris* was more than in the younger seeds. It was suggested that recently harvested seeds were more resistant to water absorption because their membranes were still intact. Other workers (Jones and Boulter, 1983a; Moscoso *et al.*, 1984) reported decreased water uptake in stored beans. Sefa-Dedeh *et al.* (1979) stated that the seed coat is the main factor affecting the initial water uptake in dry legumes.

There was an increase in solid dispersion during the first six months of storage in all the legume species (Tables 55-59). Jones and Boulter (1983b) reported an increase in leakage of solids in stored legumes due to membrane deterioration.

There was a statistically significant increase in pectic substances in all the legume species at the end of the storage period (Tables 55-59). These findings are in agreement with the findings of Hernandez-Unzon and Ortega-Delgado (1989) who observed an increase in pectic substances during the first three years of storage. Pectic substances content then levelled off in seeds stored from 4 to 5

years. This finding suggests that the metabolism of phytic acid is in the pathway of pectic substances biosynthesis. The results of the present study support the theory that changes in the pectic substances are responsible for the changes in the cooking properties of stored legumes.

PCMP number relating the contents of pectin, calcium, magnesium and phytin increased during storage. This was noticed in all the legume species. Muller (1967) observed that the hardness of the seed can be directly correlated to higher PCMP number. The present results were consistent with the proposed theory that the hard-to-cook defect involves interactions between phytate, minerals and pectin. The mechanism responsible for the development of the hard-to-cook defect theory involves associations among phytic acid, minerals, and pectin. Phytic acid, located in the protein bodies of the cotyledons, chelates divalent cations. In high temperature, high relative humidity conditions, there would be increased metabolic activity, phytase activity and membrane degradation. Phytase hydrolyses phytin to release bound calcium and magnesium. The present results indicate that the magnesium content of all the legumes studied increased significantly during storage. The increase in PCMP number based on phytic acid, calcium, magnesium and pectin content was lower for samples stored at 5°C.

During prolonged storage of the legumes studied, the most notable change observed was the loss of phytic acid. When phytic acid disappears during prolonged storage, chelation diminishes and Ca and Mg are freed as cations. Probably the free Ca and Mg associate with pectic substances or proteinaceous

materials, causing the hard-to-cook phenomena. However under storage conditions of low temperature (5°C), these changes were minimized. The phytic acid level can thus be indicative of the cookability of legumes.

SUMMARY

CHAPTER VI

SUMMARY

Phytic acid is widely distributed in legumes and can function as an antinutrient when legumes are consumed in large amounts. The present study was undertaken to determine the variability in phytic acid content of legumes, and to investigate the effect of processing on phytic acid, *in vitro* protein digestibility, and iron availability (*in vitro*) of legumes and to study the effect of storage on phytic acid, protein digestibility and cooking quality.

A large variability was observed in the phytic acid contents and *in vitro* protein digestibility (IVPD) values among and within the legume species. Phytic acid (mg/g) was higher in soybean (36.4) followed by black gram (13.7), pigeonpea (12.7), green gram (12.0) and chickpea (9.5). There was a significant negative correlation between phytic acid and IVPD, of all the legumes studied, implying that phytic acid would adversely influence the protein quality of grain legumes. The negative association observed between phytic acid and IVPD in the present study could be attributed to the inhibition of enzyme activity by phytate. Phytic acid probably reduced the protein digestibility by interfering with enzyme activity. The formation of a complex with protein did not appear to be a strong factor in the present study as there was no noticeable correlation between nitrogen solubility (as an index of protein solubility) and phytic acid.

Genotypic differences were observed in the ionisable iron content of the legumes studied. Phytic acid was negatively and significantly correlated with the

percent ionisable iron. There was a significant negative correlation between phytic acid and ionisable iron in both pigeonpea (r = 0.81) and chickpea (r = 0.73) genotypes tested separately. The present findings strongly suggest that phytic acid is a major factor inhibiting iron absorption in legumes. The negative association of phytic acid with ionisable iron observed in the present study was probably due to the insoluble complex formation of phytic acid with iron at gastro intestinal pH.

The decrease in phytic acid as a result of germination was the highest in chickpea (87%) followed by pigeonpea (69%), black gram (48%), green gram and soybean (46%). This indicated that the treatment will be more beneficial in chickpea and pigeonpea as compared with other legumes. The breakdown of phytate during germination was attributed to an increase in the activity of endogenous phytase. Fermentation also resulted in the reduction of phytic acid of these legumes, though it was less effective as compared to germination. In India, black gram and chickpea are the choice ingredients in the fermented steamed food products 'idli' and 'dhokla' respectively. As a result of fermentation, phytic acid was reduced by 30% in black gram and 67% in chickpea. The loss of phytate during fermentation might have been due to the activity of the enzyme phytase present in the fermentative microorganisms or endogenous phytase naturally present in these legumes. Both wet-heating (autoclaving) and dry-heating (roasting) also reduced the phytic acid levels in these legumes. The heat-processing might have reduced the extractability of phytic acid in the present study.

The *in vitro* protein digestibility (IVPD) values were greatly influenced by these processing practices. Both germination and fermentation remarkably increased the IVPD in all the legume species. Effects were more pronounced in pigeonpea as compared to the other legumes. Both germination and fermentation appeared to be equally effective in increasing IVPD of these legumes. The hydrolysis of seed proteins, protease inhibitors, phytic acid and polyphenols during germination may have accounted for increased IVPD in these legumes. Production of certain proteolytic enzymes by microflora during fermentation may be responsible for improved protein digestibility of legumes.

Roasting and autoclaving did not noticeably change the levels of protein in these legumes, but resulted in considerable increases in IVPD values in chickpea, pigeonpea, green gram and black gram. In the present study, an increase in IVPD was associated with a decrease in phytic acid due to various processing practices indicating that phytic acid interferes with protein digestibility. Among the processing practices, germination and fermentation appear to be more effective in lowering phytic acid content of legumes and achieving a corresponding increase in protein digestibility. When compared with soybean, these processes appear more beneficial in chickpea and pigeonpea, the most important legume crops in India.

Germination and fermentation processes significantly reduced the total dietary fiber (TDF) contents of all the legumes. In pigeonpea, chickpea and green gram autoclaving and roasting resulted in slight increases in TDF values. Germination and fermentation did not bring about any apparent changes in the

calcium, magnesium, and iron contents of pigeonpea, chickpea, green gram and black gram. Calcium and iron content were slightly decreased as a result of germination in all the legume species. The heating processes noticeably deceased iron content in all the legumes except green gram.

Germination resulted in an increase of available iron in all the legumes. The increase in ionisable iron observed as a result of germination can be attributed to the decrease in phytic acid and probably an increase in ascorbic acid content. Fermentation improved the *in vitro* availability of iron significantly in all the legumes studied. The increase could mainly be attributed to a corresponding degradation of phytate of these legumes by endogenous phytase or by exogenous phytase produced by microorganisms during fermentation. Autoclaving positively influenced the *in vitro* availability of iron, increasing its levels in all the legumes studied. Roasting improved the *in vitro* availability of iron significantly in chickpea, green gram, black gram and soybean.

Phytic acid content of all the legume species decreased with storage time. The decrease in phytic acid after 12 months of storage was the highest in chickpea (56-67%), stored at 25 and 37°C and the lowest in soybean (29%) stored at 25 and 37°C. *Dhal* samples of all the legume species stored at higher temperatures (i.e. 25 and 37°C) exhibited a greater loss in phytic acid than samples stored at 5°C. The reduction in phytic acid during storage at high temperatures, in the present study, probably resulted from hydrolysis by phytase. Chickpea, green gram and soybean stored at 25 and 37°C showed marked decreases in digestibility of proteins (*in vitro*). Conditions of storage had a significant effect on the loss of

digestibility from stored legumes. *In vitro* protein digestibility of *dhal* samples stored at 25 and 37°C decreased at a faster rate than samples stored at 5°C. The present results indicate that the post harvest physicochemical changes that contribute to poor *in vitro* protein digestibility can be reduced by storage at 5°C. The reduction in IVPD of legumes observed in the present study may be attributed to interaction between protein and tannins due to which the proteins may have become less exposed to proteolytic action. Secondly as the legumes became firm due to phytate losses proteins may have become less susceptible to proteolytic action.

In the present study, the cookability of stored pulses as a function of storage time was studied. A good correlation was observed between cooking time and phytic acid in pigeonpea, chickpea, green gram and black gram. During prolonged storage of the legumes studied, the most notable change observed was the loss of phytic acid. When phytic acid disappears during prolonged storage, chelation diminishes and Ca and Mg are freed as cations. Probably the free Ca and Mg associate with pectic substances or proteinaceous materials causing the hard-to-cook phenomena. However under storage conditions of low temperature (5°C), these changes were minimised. The phytic acid level can thus be indicative of the cookability of legumes.

To conclude, it may be mentioned that large variability exists in phytic acid content of different legume genotypes and this must be exploited in breeding programs to develop genotypes with reduced level of phytic acid. Results indicate that genotypes with higher protein content may have higher phytic acid, but low

magnitude of correlation between these two traits in chickpea and pigeonpea would suggest that it may be possible to select low phytic acid line without reducing its protein content. Additional efforts to study the influence of growing environments on phytic acid contents in these legumes will be very helpful for breeding programs.

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