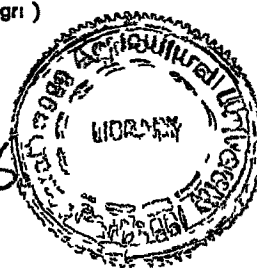


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**PROCESSING OF MOTH BEAN (*Phaseolus aconitifolius* Jacq)
FOR IMPROVED NUTRITIONAL FUNCTIONALITY AND
ELIMINATION OF CERTAIN ANTINUTRITIONAL FACTORS**

BY
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M Sc (Agri)

T 1516



**DISSERTATION
SUBMITTED TO THE MARATHWADA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN FOOD SCIENCE AND TECHNOLOGY**

**COLLEGE OF AGRICULTURAL TECHNOLOGY
MARATHWADA AGRICULTURAL UNIVERSITY
PARBHANI**

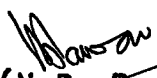
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Candidate's declaration

I hereby declare that the
dissertation or part thereof has
not been previously submitted
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University/Institute.

Parbhani.

Dated: 29th September, 1986


(V.D. Pawar)

Affectionately dedicated
to my late father
Deoraoji Pawar
and
my beloved mother

Dr. U.M.Ingle
M.Sc.(Agri), Ph.D.
Associate Dean and Principal
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MAU, Parbhani.

CERTIFICATE I

Shri Vitthal Deora Pawar has satisfactorily prosecuted his course of research and that the dissertation entitled "Processing of moth bean (Phaseolus aconitifolius Jacq) for improved nutritional functionality and elimination of certain antinutritional factors" submitted by him is the result of the original research work and is of sufficiently high standard to warrant its presentation to the Ph. D. examination. I also certify that the dissertation or part thereof has not been previously submitted for the degree of any University or Institute.

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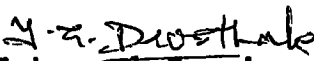
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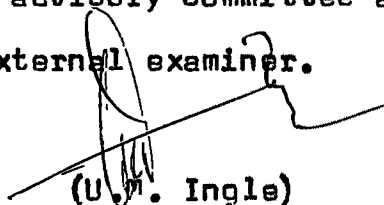


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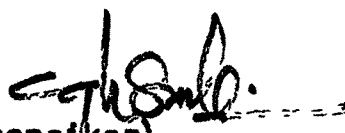
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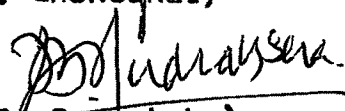
This is to certify that the dissertation entitled "Processing of moth bean (Phaseolus aconitifolius Jacq) for improved nutritional functionality and elimination of certain antinutritional factors" submitted by Shri Vitthal Deorao Pawar to the Marathwada Agricultural University in partial fulfilment of the requirement for the degree of Doctor of Philosophy in Food Science and Technology has been approved by the candidate's advisory committee after viva voce examination with the external examiner.


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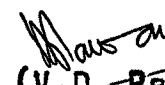
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PARBHANI.

Dated: 29th September, 1986.


(V.D. Pawar)

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INTRODUCTION

1. INTRODUCTION

Legumes are widely grown throughout the world, and their dietary and economic importance is globally appreciated and recognised. They are important sources of proteins, carbohydrates including fibre, certain minerals (calcium, magnesium, zinc, iron, potassium and phosphorus), and B-complex vitamins. Legumes are consumed in many forms. 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 and/or toxic factors, and possible interaction of nutrients with other food components i.e. polyphenolic compounds, phytic acid, certain minerals etc. (Reddy et al., 1982).

Compared to animal proteins, legume foods have secondary nutritional value and low utilization. This has been partially attributed to: (i) inherent presence of beany flavors, (ii) prolonged preparation and cooking required prior to consumption, (iii) deficiency of sulphur containing amino acids (particularly methionine), (iv) presence of several heat-stable and heat-labile antinutritional and/or toxic factors including enzyme inhibitors (trypsin, chymotrypsin, and α -amylase inhibitors), phytohemagglutinins, cyanogenic glycosides, lathrogens, saponins, estrogens, allergens, antivitamin

and favism factors, and polyphenolic compounds, (v) presence of phytic acid (phytate), and flatulence causing oligosaccharides (Salunkhe, 1982).

Moth bean has been identified as potential food source for the tropics by the National Academy of Sciences of the United States of America (NAS, 1979). It is commonly grown in arid areas of India. While a number of legumes viz. greengram, cowpea, chickpea, lentils, beans, peas, etc. are widely used in diets; human consumption of moth bean is limited to some states in India (Proteins Foods Association of India, 1973). Though extensive information is available on the nutritive value of many common legumes, information on the nutritive value of moth bean is limited (Subbulakshmi et al., 1976; Meshram et al., 1980, and Shastri et al., 1982).

A fair portion of moth bean is soaked, sprouted, fried and cooked before it is used in human diet.

Soaking usually forms an integral part of bean processing methods such as cooking, germination, fermentation and toasting. Soaking of beans facilitates quicker cooking. This is particularly true when dry beans are used for canning in commercial production (Nordstrom and Sistrunk, 1977). Soaking media include water, salt (or a combination of salts), and alkali and the soak water may or may not be discarded prior to cooking depending on regional

preferences. Such practices might influence the nutritional quality of beans. Certain water-soluble, nutritionally important minerals and vitamins may also be lost to soak water, if discarded, alongwith undesirable components such as flatulence causing oligosaccharides, phytates, and tannins.

Iyer et al. (1980 a) used soak solutions which contained 2.5 per cent sodium chloride, 1.5 per cent sodium bicarbonate, 1.0 per cent sodium tripolyphosphate and 0.5 per cent sodium carbonate in 100 ml distilled water (all percentages w/v), and a combination of the above salt solutions to prepare quick-cooking dry beans. They observed 80 to 85 per cent reduction in cooking time on soaking dry beans in mixed salt solution for 24 h over the control. Similarly, Iyer et al. (1980b), by using mixed salt solution as above, observed a greater reduction in antinutritional factors of dry beans on soaking. A similar mixed salt solution, in composition, was used by Deshpande and Cheryan (1983) and observed a greater reduction in antinutritional factors of dry beans on soaking in mixed salt solution or in sodium bicarbonate and in distilled water.

Kadam et al. (1981) reported that soaking of horse gram in a salt solution of 1.5 per cent NaHCO_3 , 0.5 per cent Na_2CO_3 and 0.75 per cent citric acid for 12 h caused

reduction in cooking time from 145 to 27 min. They also observed 69 to 78 per cent improvement in protein digestibility and 35 per cent less polyphenols in cooked horse gram. The effects of pre-soaking of pulses in the salt solution of several chemicals in reducing the cooking time of pigeonpea splits (Narsimha and Desikachar, 1978b), peas (Bongirwar and Sreenivasan, 1977), beans (Rockland and Mertzler, 1967 and Iyer et al., 1980a), winged bean (Narayana, 1981) and pigeonpea, chickpea, black bean, mung bean and lentil dhals (Chavan et al., 1983b) have been reported.

Several workers have reported that the nutritive value of legumes increased after germination. Subbulakshmi et al. (1976) reported an increase in non-protein nitrogen and in vitro protein digestibility and decrease in trypsin inhibitory activity of moth bean after germinating for 24 and 72 h. Reddy et al. (1978) observed reduction in phytate content in black gram after germinating for 10 days. A reduction in the polyphenols content in sorghum grains was also reported by Chavan et al. (1979).

Cooking improves the palatability and nutritional quality of food legumes; however, prolonged cooking results in a decrease in the protein quality and the loss of nutrients such as vitamins and minerals (Kadam et al., 1981). Bressani et al. (1963) reported that cooking of

black beans for 10-30 min at 121°C improved their protein utilization as compared to raw beans. Longer cooking, however, caused a drop in the nutritive value of beans as judged by the amount of available lysine in the sample studied, and the weight gain of the rat and PER values obtained. Other reports also indicated that excess heat treatment caused a reduction in nutritive value of legume seeds (Patwardhan, 1962).

Dehulling of beans into cotyledons has been shown to reduce the cooking time considerably. In certain legumes like moth bean this practice is not effective. Pressure cooking, addition of chemicals to cooking water or soaking of common legumes in solution of chemicals have been recommended for reducing the cooking time (Kadam et al., 1981) and producing instant cooking beans (Rockland and Metzler, 1967).

A major portion of phosphorus in many legumes is present as phytate phosphorus. It accounts for about 80 per cent of total phosphorus in beans (Reddy et al., 1982) and primarily present as a complex salt of minerals complexed with proteins (Rackis and Anderson, 1977), phytate has a strong binding capacity to form complex with multivalent cations and proteins (Cheryan, 1980; Maddaiah et al., 1964 and Reddy and Salunkhe, 1981). Most of the

phytate-metal complexes are insoluble at physiological pH and hence render several complexed minerals (Ca^{++} , Zn^{++} , Mg^{++} and $\text{Fe}^{++}/\text{Fe}^{+++}$) biologically unavailable to humans and animals (Erdman, 1981 and O'Dell and de Boland, 1976). Published reports indicate that phytic acid interacts with proteins to form complexes at acidic pH and low cation concentration by ionic interactions, while at alkaline pH, divalent cations mediate such formation of complexes between phytic acid and proteins (Cheryan, 1980). These protein-phytate complexes are more resistant to proteolytic digestion as compared to protein alone at low pH of the stomach and are thought to be responsible for the reduced bioavailability of minerals (de Rham and Jost, 1979 and Erdman, 1979). Phytase dephosphorylates phytic acid into free inositol and phosphates (Reddy et al., 1978). Deshpande and Cheryan (1983) reported decrease in phytic acid in dry beans on soaking in water, sodium bicarbonate and mixed salt solution. Reduction in phytic acid is also reported on germinating black gram by Reddy et al. (1978). Chang et al. (1977) studied the effect of preheating at 60°C on phytate hydrolysis and observed 60 per cent hydrolysis of phytate in beans. They further reported that soaking in water and preheating at 60°C could cause 43.0-80.5 per cent reduction in phytate.

Recently, polyphenolics in food legumes have been recognised as antiphenological substances (Bressani and

Elias, 1979 and Salunkhe et al., 1982). These appear to be present in beans, primarily in the seed coat of the grains with low or negligible amounts in cotyledons. Relationship between polyphenolics and protein digestibility has been reported (Bressani and Elias, 1979). The adverse effect of polyphenols on protein digestibility could be due to: (i) binding of proteins to polyphenols thus conferring resistance towards enzyme susceptibility, and (ii) inhibition of proteolytic and other hydrolytic enzymes such as α -amylase, cellulase etc. Many animal studies indicated that these compounds affect nutritional quality. A significant reduction (72 per cent) in tannin content of mung beans after 4 days of germination was reported by Noor et al. (1980). Reduction in tannins and trypsin inhibitor activity was also reported by Deshpande and Cheryan (1983) in dry beans on soaking in water and mixed salt solution. Similarly, Subbulakshmi et al. (1976) found decrease in trypsin inhibitor activity and Satwadhkar et al. (1981) found decrease in polyphenols in moth bean on germination. Cooking does not destroy polyphenolic compounds; however, about 30-40 per cent reduction in polyphenols on cooking of dry beans has been reported by Bressani and Elias (1979) which could be due to discarding of cooking water. Iyer et al. (1980b) also observed a reduction in phytic acid and trypsin inhibitor activity

in dry beans on cooking and on soaking and cooking.

A large number of trypsin inhibitors have been isolated from the plant sources, especially from the leguminous seeds. Though, these trypsin inhibitors have been found to be distributed in different parts of the plant like seeds, leaves, roots and stems; depending upon the plant species, they are mostly present in seeds. As the legumes form an important part of the diet in the developed and the developing countries like India, a number of attempts have been made to improve their nutritional value by using heat treatments to destroy the trypsin inhibitor (Liener, 1962; Soni et al., 1978). Soaking (Kakade and Evans, 1966) and germination (Subbulakshmi et al., 1976) of the legumes have also been used to enhance the nutritive value of the legumes.

Keeping in view the above facts, the present investigation was undertaken with the following objectives:

1. Effect of soaking moth bean in water and mixed salt solution on cooking characteristics and functional properties of flours.
2. Effect of soaking moth bean on phytate, minerals, polyphenols, trypsin inhibitor activity, electrophoresis of soluble proteins and in vitro protein digestibility.
3. Effect of germination on functional properties of moth bean flours.

4. Effect of germination on phytate, minerals, polyphenols, trypsin inhibitor activity, electrophoresis of soluble proteins and in vitro protein digestibility.
5. Effect of cooking on functional properties of moth bean flours.
6. Effect of cooking on phytate, minerals, polyphenols, trypsin inhibitor activity, electrophoresis of soluble proteins and in vitro protein digestibility.
7. Phytate-protein-minerals complexes in moth bean flour.

2. REVIEW OF LITERATURE

The research pertaining to the present investigation carried out so far on moth bean and other legumes is reviewed herewith under the following different headings.

2.1 Structure and proximate composition of moth bean:

The seeds of moth bean are brown, whitish green, or yellow brown, rarely mottled, cylindrical to rectangular and distinctly elongate subtruncate at both ends (National Academy of Sciences, 1978).

The proximate composition of moth bean is very much comparable with commonly cultivated legumes. Legume seeds have three major components. These are seed coat, cotyledon and embryo. The embryo is rich in proteins and lipids (Singh et al., 1968) whereas the seed coat contains significant amounts of crude fiber and calcium in moth bean.

The per cent proximate composition of moth bean as reported by several researchers are as follows;

	Moisture	Protein (N x 6.25)	Fat	Crude fiber	Ash	Carbo- hydrates
Gopalan <u>et al.</u> (1981)	10.8	23.6	1.1	4.5	3.5	56.5
Borhade <u>et al.</u> (1984)	10.0	21.9	3.48	-	3.4	59.1
Meshram <u>et al.</u> (1980)	9.5	28.4	1.0	4.6	3.2	53.3

2.2 Antinutritional factors of moth bean:

2.2.1 Phytates:

In legumes, a major portion of the total phosphorus is present in the form of phytic acid. The availability of phosphorus, when present in the form of phytate, depends on the species, the age of the experimental animal and the level of phytase activity in the intestinal tracts of specific species. Phytic acid complexes with essential minerals such as calcium, zinc, iron and magnesium and makes them biologically unavailable for absorption (Oberleas, 1973). Borhade et al. (1984) reported 0.65 per cent phytic acid content in moth bean.

2.2.2 Polyphenols:

Polyphenols have been recognised as one of the antinutritional factors in legumes. These compounds are known to interact with proteins leading to either the inactivation of enzymes or making the proteins insoluble (Salunkhe et al., 1982). The inhibitory effect of these compounds on the activities of digestive enzymes like trypsin, lipase, and amylase is well known. They are well known to decrease the digestibilities of protein and carbohydrates and the availability of vitamins and minerals (Salunkhe et al., 1982). Satwadhari et al. (1981) reported 1.30 per cent polyphenols content in moth bean.

2.2.3 Trypsin inhibitor:

These are the substances which have the ability to inhibit trypsin activity. The presence of trypsin inhibitor has been reported in moth bean (Subbulakshmi et al., 1976 Ghorpade et al., 1986 and Mehta and Simlot, 1982).

Mehta and Simlot (1982) purified the trypsin inhibitor from moth bean by employing DEAE cellulose chromatography and gel filtration. The inhibitor was a glycoprotein, highly hygroscopic, and contained phosphorus. The molecular weight of the inhibitor was found to be 9000 by its elution pattern on a G-50 Sephadex column. The inhibitor was found to bind rapidly (in 40 sec) with trypsin. Alkaline conditions at 97°C and autoclaving destroyed its activity within 5 to 10 min. The inhibitor was also stable at higher temperatures, losing only 40 per cent of its activity in 2 h at 80°C and almost all in 2 h at 97°C. The stability of trypsin inhibitor was attributed to its smaller molecular size and simple structure like a polypeptide.

2.3 Processing of moth bean:

Grain legumes are important sources of food in the vegetarian diet in developing countries. Legumes contain about 2-3 times more protein as compared to cereals, but the protein digestibility of legumes is very poor (Liener, 1962). Factors are many. Most of the legumes, therefore are subjected to heat treatment before consumption, but

moth bean have poor cooking quality. They are therefore soaked either in distilled water or soak solution of several chemicals for overnight or germinated and either fried or cooked before consumption. Most commonly followed methods are; soaking and producing the quick cooking beans, autoclaving, and germination.

2.4 Effect of soaking on:

2.4.1 Cooking characteristics:

The effect of presoaking of legume seeds in the soak solution of several chemicals in reducing the cooking time of peas and beans has been reported.

Rockland and Metzler (1967) soaked lima and other dry beans in mixed salt solution of 1.5 per cent NaHCO_3 , 0.5 per cent Na_2CO_3 , 2.5 per cent NaCl , and 1 per cent sodium polyphosphate and observed significant effect of this soak solution in reducing the cooking time.

Bongirwar and Sreenivasan (1977) developed a process for the production of quick cooking dehydrated peas from dry commercial peas. It involved hydrating the peas by soaking in 0.25 per cent sodium bicarbonate solution for 5 h, pricking the soaked peas for 10 min followed by precooking for 4 min at 1.05 kg/cm² gauge pressure and dehydrating the precooked peas in a standard tray drier at 55 to 60°C for 2 to 2.5 h to reduce the moisture content to about 8 per cent.

The presoaked peas reconstitute in boiling water in 5 to 6 min, thus reducing the cooking time by about 80 per cent. The product keeps well for over one year at ambient temperature.

Narsimha and Desikachar (1978a) determined the (I) cooking time, (II) water uptake, (III) dispersed solids into cooking water, (IV) contents of minerals, (V) protein and (VI) PCMP (a) Pectin ($\text{Ca} + \frac{1}{2} \text{mg}$)/phytin or (b) pectin + ($\text{Ca} + \frac{1}{2} \text{mg}$) - phytin numbers for 10 varieties of pure bred pearled tur (Cajanus cajan). The hydration and the dispersed solids during progressive cooking (0-80 min) of 4 varieties of polished (15 per cent) and unpolished tur were also determined. They observed much variation in the values (range) for (I) (32-68 min) (II) (0.52-1.50 g/g) (III) (47.8-82.4 per cent) (IV) (18.15-22.00 per cent) and (V) (a) (0.81-3.60) and (IV) (b) (3.42-7.10). Further, they observed high correlation between (I) and the Ca, Mg, pectin, phytin and (IV) (a) and (b). Dispersed solids per cent and hydration were always higher for polished grains than for whole grains.

Narsimha and Desikachar (1978b) used chemicals for reducing the cooking time of split red gram (Cajanus cajan). (I) Sodium carbonate, (II) sodium bicarbonate, (III) trisodium phosphate and (IV) ammonium carbonate either alone or in combination were either (a) added to cooking

water (0.5-1 per cent level) or (b) coated on to the dhal and dried or (c) the dhal was soaked in chemical solutions (0.75-1.5 g/100 g dhal) for 2 h, drained and washed off the soaked dhal prior to cooking. Combination of (III) and (IV) or (I) and (II) reduced cooking time by 50 per cent (control 60 min). Treatment (b) was more effective than (a), (III) was not preferred as it left bad taste. Cooked dhal was acceptable when (I) or (II) was mixed with (IV) at 0.5 per cent concentration, at higher concentration (1 and 0.75 per cent) the cooked dhal had alkaline taste. Dispersed solids per cent and the water uptake (g/g) were higher (79.8-90.4, 0.98-1.33) with (b) than the control (47.8, 0.82) or (a) (59.3-69.2, 0.95-1.28). In (c) chemicals (III) and (IV) gave acceptable product without alkaline taste.

Muller (1967) found that the main factor affecting the cooking quality of seeds of several pea and bean varieties was found to be the composition of the cell wall as expressed by a "PCMP number" relating to the contents of phytin, Ca, Mg and free pectin. Other factors probably involved were the thickness of the seed coat, palisade layer and the contents of lignin and alpha-cellulose in the seed coats. Cell contents had no detectable effect.

Ankrah and Dorlo (1978) used trona (hydrated, basic sodium carbonate containing Na_2CO_3 , $\text{NaHCO}_3 \cdot 2\text{H}_2\text{O}$ with some impurities) for cooking cowpeas and observed reduction in

cooking time from 75 to 50 min.

Rizley and Sistrunk (1979) observed that peas when soaked in pyrophosphate solution gave a lighter color, whereas soaking in bicarbonate solution gave a less desirable color, but a softer texture and better flavor. They further observed that longer soaking time results in greater discoloration.

Varriano-Marston and de Omana (1979) observed that absorption of water by black beans was not significantly affected by the amounts of sodium salts present in soaking water. Water absorption was linearly related to initial pH of soaking solution; the pH after soaking period, however, was more highly correlated with water imbibed by the beans.

Kon (1979) soaked California small white beans at different temperatures, in 10°C increments, from 20 to 90°C, until maximum imbibition was achieved and measured losses of total solids, nitrogen compounds, total sugars, oligosaccharides, Ca, Mg and three water soluble vitamins (thiamin, riboflavin and niacin). Losses were found to be very small at soaking temperatures upto 50°C. An increase in these losses from three to four fold was found when the soaking temperature was raised to 60°C or above. Cooking rates followed a bell-shaped pattern with the peak, longest cooking time, being for beans soaked at 70°C and shortest

cooking time for beans soaked at 90°C. Cooking rates corresponded closely to the amounts of organic phosphate left in the beans after soaking at the different temperatures.

Bueno et al. (1980) used sodium bicarbonate, trisodium phosphate and ammonium carbonate as cooking aids for kidney bean dhal. These were found to be more effective when pre-coated on the dhal than when added directly to the cooking water. Pre-soaking of the dhal in the salt solution also reduced the cooking time by about 50 per cent without affecting the organoleptic qualities.

Shinde and Shiralkar (1980) used (I) ammonium chloride (II) sodium acetate (III) ammonium phosphate (IV) sodium dihydrogen phosphate (V) EDTA (VI) sodium bicarbonate (VII) sodium chloride (VIII) EDTA + Sodium bicarbonate at 0.5 per cent w/v to reduce the cooking time of dry beans of Rajmah (Phaseolus vulgaris). Of these chemicals only (VI) and (V) alone or in combination reduced the cooking time from 10 to 3 min.

Iyer et al. (1980a) developed quick-cooking beans by soaking three bean cultivars viz. Great Northern, kidney and pinto in mixed salt solution containing 2.5 per cent sodium chloride, 1.5 per cent sodium bicarbonate, 1.5 per cent sodium tripolyphosphate and 0.5 per cent sodium carbonate. They observed 80-85 per cent reduction in cooking time over corresponding controls and high

temperature (37°C and 45°C) and pH (9.0) caused greater water imbibition and total solids loss than at room temperature (22°C) during soaking.

Narayana (1981) determined cooking characteristics such as hydration, dispersion of solids and cooking time of winged bean dhal. Ammonium carbonate (0.5 per cent) with either sodium carbonate or bicarbonate (0.5 per cent) reduced the cooking time nearly 50 per cent. However, direct addition of these chemicals to the cooking water imparted an alkaline taste and undesirable color. Coating of the dhal with the chemicals or presoaking in solution of these salts eliminated these disadvantages.

Kadam et al. (1981) reported that soaking of horse gram in a solution of 1.5 per cent NaHCO_3 , 0.5 per cent Na_2CO_3 and 0.75 per cent citric acid for 12 h was found to be effective in reducing cooking time from 147 to 27 min. They also observed 67 per cent reduction in cooking time of moth bean on soaking in salt solution for 12 h.

Silva et al. (1981) found that a salt combination of soaking solution was most effective in promoting bean softening during cooking compared to no soaking or a distilled water soak to black beans (Phaseolus vulgaris).

Sathe and Salunkhe (1981c) reported the leaching losses of total solids and proteins in soaking medium.

They soaked the winged beans in distilled water and 2 per cent solutions of NaOH and KOH, 1:10 w/v, for 24 h at room temperature (21°C). They reported per cent loss of total solids and proteins as 2.30-0.15, 2.52-0.37 in distilled water, 17.76-23.80, 13.93-21.27 in 2 per cent NaOH and 20.60-27.10 and 21.92-26.22 in 2 per cent KOH, respectively.

Onwuka (1983) used kaun (an admixture of 98 per cent trona (Na_2CO_3 , $\text{NaHCO}_3 \cdot 2\text{H}_2\text{O}$) and traces of silica, sodium and magnesium chloride, aluminium oxide and calcium carbonate) at different concentrations (0.1 to 0.5 g) to cook cowpeas for 5-20 min at 115°C. He observed decrease in the concentration of kaun. He also observed increased water uptake with an increase in the concentration of kaun.

Chavan et al. (1983a) observed that soaking of legume seeds in soak solution (1.5 per cent NaHCO_3 , 0.5 per cent Na_2CO_3 and 0.75 per cent citric acid) for 12 h at 25°C improved the water uptake by the resultant dhal during cooking and decreased their cooking time by 50 to 75 per cent. However, soaking of dry dhal in water for 1 h prior to cooking was essential. The soak treatment to seeds also resulted in an increased dispersion of solids during cooking of dhal of chickpea and pigeonpea while the same was found decreased in mung bean and black gram. Losses in protein and ash contents due to cooking were prominent when the cooking fluids were discarded.

Chavan et al. (1983b) reported that pre-soak treatment of different legumes seed dhals in water or soak solution (1.5 per cent NaHCO_3 , 0.5 per cent Na_2CO_3 and 0.75 per cent citric acid) at 25°C decreased the cooking time substantially. Soaking in soak solution was found to be more effective method in reducing cooking time than that in water. The optimum soaking time in soak solution for maximum reduction in cooking time was 6 h for pigeonpea, 5 h for chickpea, 3 h for mung bean and 2 h for black bean and lentil dhals.

2.4.2 Functional properties:

Beuchat et al. (1975) studied physicochemical properties of peanut flour as affected by proteolysis. They hydrolysed defatted peanut flour with pepsin, bromelin and trypsin. Nitrogen solubility was increased substantially in water at pH 4.0-5.0, and in 0.03 M Ca^{++} at pH 4.0-11.0, Water absorption by the flour when exposed to various relative humidities was increased as a result of hydrolysis. Emulsion capacities in water and in 0.5 M NaCl were completely destroyed during digestion and water and oil retaining properties were reduced when compared to control samples.

McWatters and Holmes (1979a) measured nitrogen solubility in 2 per cent dispersions and emulsifying properties in 8 per cent dispersions of unheated peanut

flour (50.9 per cent protein) in water, 0.1 M NaCl (low salt) and 1.0 M NaCl (high salt over a pH range of 2.0 to 10.0. Flour concentration effects were measured over the same range in water only at 2,4,6 and 8 per cent flour levels (1, 2, 3 and 4 per cent protein). Both nitrogen solubility and emulsion forming and thickening properties of the water and low-salt suspensions were lower at pH 4.0 than at levels below and above 4.0, increases in solubility being quite substantial below pH 3.0 and above pH 6.0. In the high salt suspensions, the nitrogen solubility was low at pH 3.0 and below, high at pH 6.0 and above, with linear increase between 3.0 and 6.0; no emulsions formed at pH 2.0 to 4.0, with generally low viscosity emulsions at pH 5.0 to 10.0. A flour concentration of 2 per cent (1 per cent protein) in water could support emulsion formation only at pH levels of 2,3,8,9 and 10 and the quality of these emulsions was extremely poor (low viscosity). At higher flour concentrations (4,6 and 8 per cent) emulsion capacities and viscosities were improved substantially by adjusting the pH to levels below or above 4.0 than above.

McWatters and Holmes (1979b) measured nitrogen solubility and emulsifying properties of soy flour over a pH range of 2.0 to 10.0 and in three dispersion media including water, 0.1 M NaCl (low salt) and 1.0 M NaCl (high salt). Nitrogen solubility and emulsion capacity

of water and low salt suspensions were similar and were quite low at pH levels below or above 4.0. In high salt suspensions nitrogen solubility and emulsion capacity generally increased from pH 2.0 to pH 10.0. Whereas, no emulsions were formed by water and low salt suspensions at pH 4.0, thick-mayonnaise like emulsions were formed by the high salt treatment at this pH. Except for the water and low salt suspensions at pH 4.0, and the low salt suspensions at pH 5.0 all emulsions formed were mayonnaise like in consistency and compared favourably to a commercial mayonnaise. Multiple regression analyses showed that pH was the primary determinant of nitrogen solubility and emulsifying properties of soy flour.

del Rosario and Flores (1981) studied the functional properties of mung bean flour after soaking the beans for 3 h in water and boiling for 15 min. They observed no significant differences in nitrogen solubility in raw and blanched mung bean flours. However, they observed a better water absorption capacity in blanched mung bean flours than raw bean flours.

Sathe and Salunkhe (1981b) studied the solubilization and electrophoretic characterization of the Great Northern bean proteins. They reported that isoelectric pH of the NaCl extracted proteins was about 4.4. Several salts,

NaOH and HCl were employed to solubilize the Great Northern bean proteins. Amongst all the protein solubilizing agents, Na_2CO_3 , K_2SO_4 , sodium-dodecyl sulfate (SDS) and NaOH at respective concentrations of 0.5, 5, 5 per cent (all w/v) and 0.02 N were found to be better protein solubilizers than the rest; solubilizing 93.6 g of Lowry protein per 100g Kjeldahl protein.

Borhade et al. (1984) reported that minimum solubility of defatted flour proteins of horse gram and moth bean was at pH 4.0 and 4.5, respectively. The water and oil absorption and foaming capacities in case of horse gram and moth bean flours were 2.0 g/g, 2.2 g/g, 23.0 per cent and 2.0 g/g, 1.6 g/g, 27.6 per cent, respectively. They further reported that NaCl at 10 per cent (w/v) and NaCO_3 at 0.5 per cent (w/v) were found to be effective in extracting 89 and 80 per cent of horse gram and moth bean proteins respectively from defatted flour.

2.4.3 Phytates:

There are many reports on the presence of a phytase in cereals and legumes. McCance and Widdowson (1944) have demonstrated that a pH of 4.50 and a temperature of 43-58°C are the best for phytase action.

The effects of time, temperature, pH, soaking and heating on the autolysis of phytate of California small white beans were evaluated by Chang et al. (1977). At

50°C, the hydrolysis of phytate from the beans was 31.0 per cent and reached a maximum of 49 per cent at 60°C. However, little effect was found at temperatures below 50°C or above 70°C. These results suggest an initiation of enzyme activity at about 60°C and inactivation of enzyme at 70°C. They also reported that after 10 h of incubation of beans at 60°C, only a negligible amount of phytic acid was found in the beans, approximately 75 per cent of the total phytic acid being hydrolysed and 25 per cent being diffused into the water in which beans were incubated. They further found an apparent decrease in phytate throughout the pH range of 4.0-5.8 with greater decrease between range of 5.0-5.5 (about 56-61 per cent of phytate hydrolysis during 36 h of incubation. A considerable amount of phytate from the beans was also diffused into the medium under these time and temperature conditions. Hydrolysis of phytate was higher in beans when they were incubated in distilled water at 60°C for 4 h than when they were incubated at 50°C either in distilled water alone or in acetate buffer for 3 h. The low hydrolysis of the phytate in rehydrated beans exposed to water-saturated air at 60°C for 6 h was also reported by these investigators. The effect of 60°C preheating treatment has been studied in several beans (California small white, mung, soy and lima beans) and wheat by Chang et al. (1977). A 36.9 per cent decrease in phytate content

was observed in soybean when incubation was carried out at room temperature instead of at 60°C by them. Greater hydrolysis of phytate was noted in lima beans, California small white beans and wheat slurries when incubated at 60°C. However, Ferrel (1978) found that the autolysis of phytate in beans was slow at both 35 and 55°C.

Reports of Rao and Prabhavati (1982) showed, however, that soaking green gram dhal and bengal gram in water for 24 h did not cause a marked decrease in phytate phosphorus over their corresponding controls.

T Tabekia and Luh (1980) demonstrated a decrease in phytate content of 7.7, 8.1, 13.2 and 19.1 per cent, respectively for black-eyed, red kidney, mung and pink beans on soaking these beans for 12 h at 24°C in tap water.

Iyer et al. (1980b) found that when pinto, Great Northern and red kidney beans were soaked in distilled water for 18 h at room temperature, the phytate content of the beans was appreciably reduced (52.7, 69.6 and 51.7 per cent, respectively). However, they noticed a somewhat lessened phytate hydrolysis when the beans were soaked in a mixed salt solution (2.5 per cent sodium chloride + 1.5 per cent sodium bicarbonate + 0.5 per cent sodium carbonate + 1.0 per cent sodium tripolyphosphate) at pH 7.0 and room temperature of 21°C.

Deshpande and Cheryan (1983) observed that losses in

phytic acid in four dry bean (Phaseolus vulgaris) cultivars on soaking in sodium bicarbonate and a mixed salt solution for 12 h each were much greater as compared to those for beans soaked in distilled water for 6-18 h. The average loss in phytic acid content was 1.3 per cent for beans soaked in distilled water for 18 h, and 3.9 and 6.9 per cent, respectively, for those soaked for 12 h each in sodium bicarbonate and mixed salt solutions. As compared to the different soaking media, phytic acid lost to soaking solution indicated similar trends among the cultivars, with a maximum reduction in phytic acid content for beans soaked in the mixed salt solution.

Ologhobo and Fetunga (1984a) reported 24.4 per cent decrease in phytic acid content on soaking of cowpeas in distilled water for 3 days. Similarly, Ologhobo and Fetunga (1984b) observed that soaking and germination of cowpeas, lima beans and soybeans were most effective in decreasing phytate contents.

2.4.4 Minerals:

Rao and Deosthale (1983) observed minerals losses in pigeonpea, green gram and black gram during overnight soaking in water. They observed that the loss in ash content was 48 per cent in black gram and 34 per cent in green gram, the corresponding losses in copper content in these two legumes were 44 per cent and 27 per cent and

that of iron about 20 per cent in both. The loss of nutrients, in pigeonpea were, however, relatively less. Loss of total phosphorus content of the three pulses occurred during overnight soaking. On the other hand, phytin phosphorus content of the three legumes remained constant during soaking and germination upto 24 h.

2.4.5 Polyphenols:

Iyer et al. (1980a) studied the effects of soaking dry beans of Great Northern, kidney and pinto in water and mixed salt solution for 18 h at 22°C on polyphenols content. They reported 46.6, 45.2 and 39.6 per cent decrease in polyphenols in Great Northern, kidney and pinto beans, respectively on soaking in water for 18 h at 22°C. The corresponding decrease on soaking in mixed salt solution was 65.9, 67.9 and 88.4 per cent respectively.

de Lumen and Salamat (1980) reported reduction in tannins of Chimbu cultivar of winged bean on soaking (6 h, room temperature) to be 34 per cent (water soaking), 65 per cent (1 N NaOH soaking), 49 per cent (NaHCO₃ and CaCO₃ soaking), 52 per cent (acetic acid soaking) and 63 per cent (ash solution soaking).

Sathe and Salunkhe (1981c) soaked two cultivars of winged bean (Chimbu and HF-10) in soak solution, 1:10 ratio, for 24 h at room temperature (21°C). The soak solution comprised of distilled water, 2 per cent (w/v)

KOH, or 2 per cent (w/v) NaOH. They reported per cent reduction in tannin content, in Chimbu and HF-10 varieties in 2 per cent NaOH and 2 per cent KOH, 86.83, 87.24 and 66.00, 82.27 respectively. The reduction in tannin content was higher when soaked in either NaOH or KOH than in distilled water.

Rao and Deosthale (1982) studied the tannin content of pigeonpea, green gram, black gram and chickpea and observed a 50 per cent reduction in tannin content in pigeonpea and chickpea and 25 per cent in black gram and green gram by soaking the pulses overnight in water.

Deshpande and Cheryan (1983) studied changes in tannins in dry beans (Phaseolus vulgaris) and observed that leaching of tannins during soaking treatment apparently were a function of tannin content of raw beans. The leaching of bean tannins on soaking in distilled water increased with the time of soaking. Tannin losses averaged 17, 41 and 49 per cent when beans were soaked in water for 6, 12 and 18 h respectively. Those beans after soaking in sodium bicarbonate and mixed salt solutions were much greater and averaged 78.7 and 83.5 per cent, respectively.

Ologhobo and Fetunga (1984b) soaked the seeds of cowpea in distilled water for 3 days and observed decrease in tannic acid by 13.4 per cent.

Recently, Barroga et al. (1985) reported 24 to 50 per cent reduction in assayable polyphenols content in

mung bean (Vigna radiata) on soaking in water for 18 h at 5, 10 and 15°C. In contrast to this, Narsinga Rao and Prabhavati (1982) did not find a marked decrease in tannin content in green gram and bengal gram on soaking in water for 24 h at 37°C.

2.4.6 Trypsin inhibitor:

Kakade and Evans (1966) studied the trypsin inhibitor activity of navy beans after soaking the beans in distilled water for 1-4 days. They reported decrease in trypsin inhibitor activity from 23.25 to 16.75 TIU/g bean and 98.5 to 83.7 TIU/g protein.

Soni et al. (1978) studied antitryptic activity of 11 common Indian pulses. They observed a reduction in antitryptic activity in many of the pulses on soaking in water or 1 per cent solutions of different salts for 6 h. All the solutions were, however, not equally effective in lowering the antitryptic activity. The solutions of sodium bicarbonate, zinc chloride and manganese chloride had significantly more effect than the other salts used.

Iyer et al. (1980b) observed 96.7, 89.5 and 86.7 per cent decrease in residual trypsin inhibitory activities in Great Northern, kidney and pinto beans respectively on soaking these beans in mixed salt solution for 18 h at 22°C.

de Lumen and Salamat (1980) reported atleast 95 per cent reduction in trypsin inhibitor activity in winged

bean on soaking in water for 6 h and then boiling for 1 h.

Sathe and Salunkhe (1981c) soaked winged bean in 2 per cent solutions of KOH or NaOH for 24 h and observed reduction in trypsin inhibitor activity by 77 and 92 per cent or 84 and 92 per cent, respectively.

AL-Bakir et al. (1982) studied occurrence and stability of trypsin inhibitors in Iraqi local beans, lentils and mung bean and observed decrease in trypsin inhibitor activity after soaking the legumes in water for 24 h.

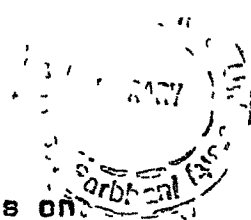
Deshpande and Cheryan (1983) observed greater reduction in trypsin inhibitor activity in dry beans (Phaseolus vulgaris) after soaking in sodium bicarbonate and mixed salt solutions than after soaking in water.

Ologhobo and Fetunga (1984b) soaked the seeds of cowpea in distilled water for 3 days and observed decrease in trypsin inhibitor activity by 31.2 per cent.

Ghorpade et al. (1986) observed that soaking of seeds in distilled water at 4°C for 8 h decreased trypsin inhibitor activity of horse gram by about 50 per cent.

2.4.7 Electrophoresis of soluble proteins:

Iyer et al. (1980a) studied the electrophoretic patterns of soluble proteins of dry beans (Phaseolus vulgaris) soaked in water and mixed salt solution for 18 h



at 22°C. They evaluated electrophoretic patterns on the basis of the presence or absence of protein bands as compared with the respective controls pattern.

Disappearance of an existing band or appearance of a new band in a processed bean protein sample compared with the corresponding band of the respective control was considered as evidence of the hydrolysis of that protein subunit.

In all samples, quick-cooking cooked beans showed greater susceptibility toward enzymatic hydrolysis than the conventionally cooked beans. This suggested that quick cooking cooked beans (salt-soaked and cooked) were superior to conventionally cooked beans in in vitro digestibility, which may result in improved protein utilization.

2.4.8 In vitro protein digestibility:

Iyer et al. (1980a) studied susceptibility of raw and processed bean proteins towards sequential enzymatic hydrolysis by: a) trinitrobenzene sulfonic acid (TNBS) assay, which determines release of alpha-amino groups; and b) electrophoresis on the PAMU-PAGE (phenol-acetic acid-mercaptoethanol-urea poly acrylamide gel electrophoresis) system. They observed that Great Northern bean proteins undergo a higher per cent peptide hydrolysis than kidney and pinto bean proteins. Cooked quick-cooking Great Northern, kidney and pinto bean proteins had 97.5, 51.7 and 41.3 per cent increase in peptide hydrolysis,

respectively; compared with conventionally cooked beans.

Sathe and Salunkhe (1981c) studied the in vitro protein digestibility of winged bean after soaking the beans in distilled water and 2 per cent NaOH and KOH solutions 1:10 ratio, w/v, for 24 h at room temperature (21°C). They observed 3-4 per cent improvement in in vitro protein digestibility by soaking the seeds in alkali.

2.5 Effect of germination on proximate composition:

Germination is the sequential differentiation of oxidative and synthetic pathways (Jann and Amen, 1977). The metabolic changes that occur during germination are complex and vary among the plant species. During germination seed rapidly changes from a quiescent to a dynamic stage. Proteins, carbohydrates and lipids are hydrolysed and the products thus formed are used for synthesis of protoplasm, structural components and subsequent growth. The storage proteins are hydrolysed either partially or completely by certain proteases to free amino acids, The fats are hydrolysed by certain lipases. Starch hydrolysing enzymes like amylase, amylopectin 1-6 glucosidases break down reserve starch. The changes in reducing sugars directly reflect on the amylolytic activity of germinating seeds.

Various morphological and chemical factors influence the process of germination. The nature of seed coat has a significant influence on the seed germination. The seed

coats of the normally impermeable species contain a rather high level of phenolic compounds and also of catechol oxidase (Mayer, 1977). It has been further suggested that the impermeability of seed coat is a result of a tanning reaction of proteins.

Subbulakshmi et al. (1976) studied changes in the nitrogenous constituents of horse gram and moth bean before and after germination for 24 to 72 h. They reported decrease in total nitrogen and protein nitrogen and increase in non-protein nitrogen in both the legumes during germination. However, Satwadhar et al. (1981) reported very little effect of germination on crude protein content of horse gram and moth bean.

Reddy et al. (1978) reported decrease in dry weight and increase in total ash content during a 10 day germination period of black gram seeds.

Noor et al. (1980) studied the effect of germination on the nutritive value of mung bean in raw and cooked samples after 0, 2 and 4 days of germination. They reported increase in water content and decrease in crude protein, lipids, crude fibre, ash and carbohydrates with germination.

Jaya and Venkataraman (1980) also observed a regular decrease with increasing germination period from 24 to 72 h for protein, fat, crude fibre, carbohydrates and ash in

green gram and chickpea. Similarly, they reported decrease in calcium, phosphorus and iron content in both the legumes as the germination period progressed.

Prudente and Mabesa (1981) studied the nutritional quality of flours from mungo (Vigna radiata) sprouts and observed increase in moisture and crude protein and decrease in ash, crude fibre, crude fat and carbohydrate contents of sprouted mungo bean as germination time progressed.

2.5.1 Functional properties:

During germination, seed proteins are slightly decreased due to increased proteolytic activity. Either hydrolysis or chemical modification of proteins cause a marked changes in the functional properties.

Beuchat et al. (1975) hydrolysed defatted peanut flour with pepsin, bromelin and trypsin and reported that nitrogen solubility was increased substantially in water at pH 4.0 to 5.0 and in 0.03 M Ca^{++} at pH 4.0 to 11.0. Water absorption by the flour when exposed to various relative humidities was increased as a result of hydrolysis. Emulsion capacities in water and in 0.5 M NaCl were completely destroyed during digestion and water and oil retention properties were reduced when compared to control samples.

Beuchat (1977) modified peanut flour protein with

various amounts of succinic anhydride at pH 7.4 to 8.0 and reported decrease in nitrogen solubility at pH < 4 and increase at pH 6 to 7. He further reported increase in water retaining capacity, water absorption capacity and emulsion capacity in modified peanut flour protein.

del Rosario and Flores (1981) studied the functional properties of mung bean flours after sprouting for 36 h. The nitrogen solubility profile showed no significant differences in raw and sprouted mung bean flours. The sprouted mung bean flour, however, was exceptionally high in water and fat absorption capacity compared to raw mung bean flour. Moreover, they observed more or less same gelation and foam capacity in raw and sprouted mung bean flour. However, they observed less foam stability in sprouted mung bean flour than raw mung bean flour.

2.5.2 Phytates:

Germination reduces and/or eliminates considerable amounts of phytate from the seeds or grains. Hydrolysis of phytate, however, in legumes varied greatly during 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 into inositol and phosphate. Several seeds or grains are known to contain phytase and its activity varies widely.

Banerjee and Nandi (1949) studied the phytin content and phytase activity of 4 varieties of pulses viz. black gram, green gram, chickpea and field bean before and during germination upto 5 days. They reported in all instances a gradual decrease in phytin content, the maximum decrease occurred on the 3rd or 4th day, on the other hand, the phytase activity increased, the maximum activity occurred when the phytin content was a minimum.

Belawady and Banerjee (1953) estimated total, phytin and water soluble phosphorus contents and phytase and phosphatase activities in ungerminated and 5 days germinated seeds of 7 pulses. During germination phytin phosphorus diminished and water soluble phosphorus increased. Phytase activity was absent before but appeared after germination. Phosphatase activity was present in ungerminated and increased in germinated seeds.

In legumes, complete hydrolysis of phytate did not take place during 5 days of germination. Disappearance of phytate during germination depends on the phytase activity. A rapid rise in phytase activity was observed after 48 h germination of bush beans (Walker, 1974). Chen and Pan (1977) found that phytase activity increased 227,

807 and 3756 per cent in soybeans, dwarf grey peas and early Alaska peas, respectively after 5 day germination.

There exists a good correlation between phytate breakdown and phytase activity of the seeds during germination. Such correlations have been demonstrated for mung bean (Mandal et al., 1972), navy bean (Lolas and Markakis, 1977) and soybean (Chen and Pan, 1977) during germination. Reddy et al. (1978) found a negative significant correlation between total phosphorus and phytate phosphorus during germination of black gram.

Reddy et al. (1978) observed in black gram that 50 per cent of the phytate phosphorus had disappeared on 10th day of germination with simultaneous increase in the quantities of available inorganic phosphorus.

Kumar et al. (1978) observed decrease in phytin content on cooking of germinated green gram, chickpea and cowpea.

Tabekhia and Luh (1980) studied the effects of germination for 96 h or longer on the changes in total phosphorus, inorganic phosphorus and phytate retention in black-eyed, red kidney, mung and pink beans. They observed a significant breakdown in phytate and an increase in inorganic phosphorus during germination.

Rao and Deosthale (1983) reported decrease in phytin

phosphorus in green gram and black gram on germinating for 24 h.

Sathe et al. (1983) reported on Great Northern beans that during 5 day germination the phytic acid was reduced to 57.8 per cent.

Borhade et al. (1984) reported 57 and 55 per cent of the total phosphorus as phytate phosphorus in horse gram and moth bean which were reduced to 20 and 26 per cent, respectively at 48 h germination.

Thompson and Serraino (1985) reported 13 per cent reduction in phytic acid in rapeseed on germination for 2 days and 65 per cent for 7 days.

Similarly, the decrease in the phytin content on germination of green gram, horse gram and red gram (Giri et al., 1981), fabe bean (Michael and Wiebe, 1983), cowpeas (Ologhobo and Fetunga, 1984b) and peas (Chen and Pan, 1977) had been reported.

2.5.3 Minerals:

Prabhavathi and Rao (1979) investigated, using in vitro method, effects of simple processing on ionisable iron from cereals and legumes. They observed ten fold increase when legumes and cereals were germinated for different periods. In legumes, increase was observed only with the whole grain, whereas no such changes were observed

with decorticated legume. Such an increase in total available, protein-free and protein-bound iron is also reported by Singh and Banerjee (1955) in different pulses germinated for 2 to 3 days.

Giri et al. (1981) reported decrease in ash content during germination of green gram, horse gram and red gram. Further, they showed that the mineral content was constant and the availability of iron and calcium was increased.

EL-Mahdy and EL-Sebaiy (1982) studied the changes in phytate and minerals during germination of fenugreek seeds. They reported that raw and germinated seeds for 96 h, contained total phosphorus 207, 269, phytic acid 414, 132, phytate phosphorus 88, 28 and inorganic phosphorus 21, 187 mg/100g dry matter respectively. The contents of the individual minerals generally increased after germination because of loss of dry matter by 25 per cent.

Rao and Deosthale (1983) studied the effect of germination on mineral composition of three pulses. Dehusked (i) green gram and (ii) black gram and converted into dhal split bran; and (iii) unhusked pigeonpea were germinated for 24 and 48 h and the mineral content (ash, P, phytin P, Mg, Fe, Zn and Cu) of germinated seeds were studied. Germination per se affected only the phytin P content during 48 h of germination in (i) and (ii).

Jaya and Venkataraman (1980) observed decrease in

calcium, phosphorus and iron content in chickpea and green gram on germination for 24 to 72 h.

2.5.4 Polyphenols:

Noor et al. (1980) observed reduction in tannin content in mung bean from 2.5 to 0.7 mg catechin/g beans after germinating mung bean for 4 days. They further concluded that the higher values obtained in raw beans is primarily due to presence of seed coat.

Satwadhar et al. (1981) studied changes in polyphenols and IVPD due to germination and cooking in horse gram and moth bean. Polyphenols content in horse gram decreased from 1.6 to 1.1 per cent at 48 h germination but in moth bean there was a reduction in polyphenols content from 1.3 to 1.0 per cent at 24 h germination with a significant increase from 1.0 to 1.7 per cent at 36 h germination. Seeds germinated for 48 h also exhibited higher amounts of polyphenols than in raw moth bean.

Rao and Prabhavathi (1982) germinated green gram dhal and black gram for 24, 48 and 72 h at 37°C and observed 20 to 30 per cent reduction in tannin content during germination.

Rao and Deosthale (1982) estimated the tannin content of different varieties of pigeonpea, chickpea, black gram and green gram. They observed 60 per cent loss of tannin

in pigeonpea and chickpea and 50 per cent in black gram and green gram varieties during germination for 48 h. They claimed that loss of tannin during germination of these pulses may be due to enzymatic degradation.

Similarly, Barroga et al. (1985) studied the determination and removal of polyphenols in mung bean (Vigna radiata) and observed 23 to 36 per cent reduction in polyphenols content during germination, with maximum polyphenols removal occurring after 48 h. However, they observed that continued germination upto 120 h resulted in a decrease in polyphenols removal or an increase in polyphenols content.

2.5.5 Trypsin inhibitor:

Kakade and Evans (1966) studied the trypsin inhibitor activity of navy bean after germinating the beans for 1-4 days. They reported decrease in trypsin inhibitor activity from 23.25 to 24.50 TIU/g bean and 98.5 to 93.1 TIU/g protein.

Subbulakshmi et al. (1976) found 4×10^3 TIU/g seed of moth bean. They observed reduction in the trypsin inhibitor activity to the extent of about 40 per cent on germinating moth bean for 72 h.

Gupta and Wagle (1978) reported an increase in trypsin inhibitor activity of Phaseolus mungoreus on germination for 84 h or more. But, Gupta and Wagle (1980)

reported decrease in trypsin inhibitor activity in Phaseolus mungoreus over a period of 9 h of germination.

Noor et al. (1980) observed increase in trypsin inhibitor activity in mung bean (Vigna radiata) with germination when expressed in TIU/ml extract and TIU/mg protein. They further reported that germinated and cooked mung bean have a even higher values as compared to raw beans; the difference, however, was not statistically significant.

Jaya and Venkataraman (1980) reported decrease in trypsin inhibitor activity in chickpea upto 24 h germination whereas in green gram such a decrease was observed upto 48 h germination. However, trypsin inhibitor activity was found increased after 48 h germination in both the legumes.

Kamalakannan et al. (1981) also reported decrease in trypsin inhibitor activity in black gram during last 48 h of germination and an increase was observed later.

Sathe et al. (1983) reported on Great Northern bean that during 5 day germination the trypsin inhibitory activity was reduced by 62.9 per cent.

Ghorpade et al. (1986) reported no significant effect on the activity of trypsin inhibitor in horse gram seeds on germination for 24 and 48 h.

2.5.6 Electrophoresis of the soluble proteins:

Kumar and Venkataraman (1975) studied by ultracentrifugation and polyacrylamide gel electrophoretic changes in the reserve proteins of cowpea, chickpea and green gram during germination. The seeds were germinated for 24 and 72 h. Three fractions with S_{20W} values of 2.5, 8.5 and 11.6 S were observed in ungerminated cowpea, two of 6.3 and 10.2 S in chickpea and only one of 5.8 S in green gram; the values decreased during germination, suggesting degradation of the proteins. Breakdown of globulins was seen at 72 h of germination in all the legumes. In gel electrophoresis ungerminated chickpea proteins gave about 20 bands, cowpea about 18 and green gram 14 bands. With germination the number of fast moving components increased. Both sedimentation analysis and gel electrophoresis indicated breakdown of the proteins during germination upto 72 h though the changes were not drastic.

Subbulakshmi et al. (1976) studied the effect of germination of moth bean for 24 and 72 h on the polyacrylamide gel electrophoretic pattern of proteins. The gel electrophoretic pattern indicated breakdown of proteins with an increase in the fast moving components during germination. They further observed that moth bean globulins amounted to approximately 70 per cent of the total proteins.

Krishnamurthy and Venkataraman (1983) monitored, by the standard physicochemical techniques, the changes in the reserve proteins of green gram during germination. On gel filtration, the total proteins were resolved to give distinct fractions. The S_{20}^W values were 5.9 and 8.8 of which 5.9 S fraction was a major component. There was degradation of higher molecular weight storage proteins to lower molecular weight fraction as a result of germination. The degradation was more pronounced at 72 h of germination.

2.5.7 In vitro protein digestibility:

It is well known that in vitro protein digestibility of seeds increases on germination. Subbulakshmi et al. (1976) germinated horse gram and moth bean for 24 and 72 h and observed increase in in vitro protein digestibility. The per cent in vitro protein digestibility of ungerminated horse gram and moth bean were 80.0 and 69.0, respectively, which were increased in 24 and 72 h germinated samples of horse gram and moth bean to 84.0, 87.0 and 72.0, 94.0, respectively. Similarly, Satwadhkar et al. (1981) observed increase in in vitro protein digestibility of horse gram from 76.0 to 81.0 per cent upto 36 h of germination, while in moth bean from 71.0 to 77.0 per cent upto 48 h of germination.

Jaya and Venkataraman (1980) observed increase in in vitro digestibility of proteins of germinated chickpea

and green gram for 24 to 72 h.

Sathe et al. (1983) reported that during 5 day germination the major reserve proteins of Great Northern bean were substantially hydrolysed. In vitro trypsin digestion of the extracted proteins upto 4 h at 37°C indicated that the major storage proteins in germinated and the ungerminated seeds, although substantially hydrolysed, could not be completely degraded.

2.6 Effect of cooking:

2.6.1 Functional properties:

Yasumatsu et al. (1972) have reported that heat processing diminished the nitrogen solubility of soy proteins by denaturation and also reduced the foam capacity.

Wu and Inglett (1974) have reported that water absorption capacity of soy flour increased due to heat processing. High molecular weight proteins, namely 7S and 11S, constitute nearly 70 per cent of soy proteins (Naismith, 1955). These proteins consists of subunit structure and the proteins are dissociated on heating (Catsimpooulas et al., 1970).

Fleming et al. (1975) investigated the gel forming and viscosity characteristics of flours, concentrates and isolates from oilseed and legume seeds by heating 10 per cent protein slurries in sealed containers at 90°C for 45 min. Heating caused marked but variable increases in

slurry viscosity. Only one soybean isolate developed a firm gel with high viscosity. Although sunflowerprotein did not form a gel, faba bean and field bean concentrates developed gels with minimum viscosity characteristics, and were somewhat less elastic than that produced by soy isolate. A soft gel was developed by soybean flour after treatment by pH activation. Albumin and globulin protein fractions were isolated from soybean and sunflowerflour and from faba bean and field pea concentrate. While albumin fractions showed no thickening ability, globulins from soybean, faba bean and field pea produced gel structures of variable consistency to support the theory that gelation occurs in the globulin fraction.

McWatters and Holmes (1979c) determined nitrogen solubility and emulsifying properties of unheated and heated (live steam at 100°C for 10, 20 and 30 min) soy and peanut flours. Results showed that each of these functional properties was reduced as heating time increased. Thick salad dressing-like emulsions were produced by soy flour heated for 30 min and peanut flour heated for 10 or 20 min. Poor emulsifying properties were exhibited by peanut flour steamed for 30 min where a thin pourable emulsion was formed. High levels of nitrogen solubility were not necessarily associated with maximum emulsifying capacity. Multiple regression analyses confirmed that heating time

was the primary determinant in reduction of functionality of both flours and that flours derived from peanuts and soybean responded differently to moist heat treatment.

Narayana and Rao (1982) studied nitrogen solubility, emulsification capacity, foam capacity, fat and water absorption capacity of raw and heat processed winged bean flour and raw soy flour as a function of pH. Nitrogen solubility versus pH profile showed only one minimum at pH 4.5. Heat processing of winged bean flour lowered nitrogen solubility and increased water and fat absorption capacity by 38 and 57 per cent, respectively. Heat processing diminished emulsification and foam capacity of winged bean flour by about 35 and 18 per cent, respectively.

Shastri et al. (1982) studied the nitrogen profile of raw and parched moth bean in the pH range of 2-10. They observed minimum nitrogen solubility at pH 5.0 (9 per cent) and maximum at pH 11 (84 per cent) in both raw and parched samples.

Chen and Morr (1985) developed a pilot scale ion exchange process to produce a 75-77 per cent phytate reduced soy protein isolate. The solubility and foaming properties of this isolate were compared to those of control and commercial soy protein isolates as a function of protein concentration (5 and 10 per cent w/v), pH

(3, 6 and 9) and preheat temperature (25, 60 and 80^o C). Phytate reduced soy protein extract exhibited minimum solubility at pH 4.8-5.0 compared to 4.2-4.5 for control soy extract. Phytate reduced soy protein isolate was most soluble and functional at pH values below its isoelectric point (pH 3.0) whereas control and commercial soy protein isolates were generally most soluble and functional at pH values above their isoelectric point (pH 6 and 9).

2.6.2 Phytates:

The correlation of phytate with the cooking quality of peas was first suggested by Mattson (1946). He showed that the cooking quality of peas deteriorated when the level of phytate phosphorus in peas was low. He also suggested that phytic acid acted as a precipitant for calcium and magnesium, which were then unable to cross-link the uronic acid groups of pectin.

Crean and Haisman (1963) studied the interaction between phytic acid the divalent cations-calcium and magnesium-during the cooking of dried peas. They found that phytic acid in dried peas exists wholly as a water soluble salt (probably potassium phytate) but on cooking some of it combines with the calcium and magnesium in the pea to form insoluble calcium and magnesium phytate. They further studied the rate of conversion of soluble

phytate to its insoluble form in peas during cooking in distilled water with the addition of calcium chloride. Equilibrium was approached within 5-10 min, but even at high concentrations of calcium chloride (2×10^{-2} Ca eq/litre) only 60 per cent of the available phytate in the peas was converted to the insoluble form. They obtained similar results with magnesium chloride, a maximum of 43 per cent of the phytate being complexed when the peas were cooked in 2×10^{-2} Mg eq/litre. Finally they concluded that the influence of phytate on the texture of cooked peas was small.

Rosenbaum and Baker (1969) studied the distribution of phytic acid in pea cotyledons and the movement of calcium ions during cooking. Their results indicated that the higher cookability of the interior of the pea is not associated with a higher phytic acid content.

Kumar et al. (1978) investigated the effects of cooking on characteristics of legumes—green gram, cowpea and chickpea. They observed that the cooking process decreased both water and acid extractable phytate phosphorus in all three legumes. They also observed a little change in the ratio of phytate phosphorus/total phosphorus in the uncooked and cooked legumes (only in HCl extracted legumes). The poor extractability of phytate phosphorus with water and HCl noticed by them in all the three cooked legumes could be due 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 find any breakdown of phytate phosphorus during cooking of black gram seeds and cotyledons. They observed during short time cooking that the losses in total phosphorus and phytate phosphorus were due to leaching of these components into the cooking water. Cooking for 45 min at 115°C caused small losses of phosphorus and phytate into cooking water which may have been due to reabsorption of phytate by beans from cooking water.

Tabekia and Luh (1980) studied the effects of cooking beans, red kidney beans, mung bean and pink beans. They observed that the cooking of beans in water containing 3 per cent NaCl (beans:water 1:4 w/v) at 100°C for 3 h resulted in a slight decrease in phytic acid in black-eyed and red kidney beans, but more reduction in mung and pink beans.

Iyer et al. (1980b) observed a reduction in the phytate content of pinto, Great Northern and red kidney beans during a combined process of soaking and cooking. They soaked the beans for 18 h in distilled water or a combined salt solution and found higher hydrolysis of

phytate in beans soaked and cooked in distilled water than those soaked and cooked in salt solution.

Ologhobo and Fetunga (1984b) studied the effect of cooking on phytic acid content in cowpeas and observed slight reduction in phytic acid content on cooking.

Borhade et al. (1984) observed a significant decrease in phytate phosphorus content in horse gram and moth bean after cooking in water for 10 min. However, they observed no net change in the total phosphorus content in both the legumes.

The main factor affecting the cooking quality of seeds of several pea and bean varieties was found to be the composition of the cell wall as expressed by a "PCMP number" relating to the contents of phytin, Ca, Mg and free pectin (Muller, 1967). The cookability of peas (Rosenbaum et al., 1966) and pigeonpea (Sharma et al., 1977) was found associated with the phytic acid.

2.6.3 Minerals:

Kumar et al. (1978) observed in three legumes viz. green gram, cowpea and chickpea that calcium content was reduced on germination and cooking, while magnesium content was relatively unaltered. Further they indicated that a combined interaction of phytins, calcium, magnesium and pectins, expressed as "PCMP number" was found to correlate with the cooking behaviour of the legume studied.

Meiners et al. (1976) have observed significant losses of mineral nutrients on cooking of several dry legumes which they attributed to the leaching of the nutrients into the cooking broth.

Reddy et al. (1978) studied changes of minerals in black gram seeds during cooking. They cooked black gram seeds at 116°C for 5 to 45 min at 10 lb pressure. Beans to water ratio was 1:4 and they observed that cooking of beans for 5 min resulted in a major loss of minerals viz. Ca, Mg, Zn and Fe in cooking water. At 40 min or at prolonged durations of cooking, losses were negligible after initial leaching.

Chang et al. (1979) studied the retention of minerals in California small white beans after heating the beans at 60°C and they indicated that the retention of iron was increased from control 13 to 20 per cent and of manganese from 43 to 58 per cent, the calcium and magnesium were not affected due to cooking.

EL-Mahdy and EL-Sebaïy (1982) studied the changes in phytate and minerals during boiling and roasting of fenugreek seeds. They reported that raw, boiled and roasted seeds contained total phosphorus 207, 255 and 252 phytic acid 414, 304 and 249 phytate phosphorus 88, 65 and 53 and inorganic phosphorus 21, 30 and 74 mg/100 g dry matter, respectively. The contents of the individual

minerals generally increased after cooking because of loss in dry matter by 19 per cent.

Rao and Deosthale (1983) cooked the seeds of dehusked (i) green gram and (ii) black gram and converted into dhal split bran; and (iii) pigeonpea in distilled water for 15 min at 1.1 kg/cm^2 pressure in an autoclave. Cooked seeds were drained of water and dried and they found that the losses of minerals (ash, P, phytin P, Mo, Fe, Zn and Cu) during cooking of raw and germinated (i) and (ii) were significant, while in (iii) the losses were relatively low because of the intact seed coat during soaking, germination and cooking.

Recently, Borhade et al. (1984) observed a significant decrease in calcium content in ungerminated and 12 h germinated moth bean seeds. They observed a similar effect only in ungerminated seeds (soaked in water for 6 h at 4°C) of horse gram. However, they noticed no significant changes in magnesium, iron, manganese and zinc contents in both the legumes due to cooking in water for 10 min.

2.6.4 Polyphenols:

Bressani and Elias (1979) reported 30-49 per cent decrease in polyphenols expressed as tannic acid on cooking of black, red and white beans of Phaseolus vulgaris. The cooked material was analysed without removing the cooking water.

The decrease in polyphenols was reported by Iyer et al. (1980a) in dry beans of Great Northern, kidney and pinto after conventional cooking (90 min) and quick cooking (15 min) (soaking in mixed salt solution for 18 h at 22°C and cooking). The per cent decrease reported was from 78.7 to 95.8 and 72.4 to 91.8 in conventionally cooked and quick cooking cooked beans, respectively.

Satwadhar et al. (1981) reported 20 to 25 per cent reduction in polyphenols on cooking horse gram and moth bean in boiling distilled water.

Rao and Deosthale (1982) cooked raw whole grains (2-3 g) of pigeonpea, chickpea, black gram and green gram in 10 ml water for 15 min at 15 lb pressure in an autoclave and removed cooking broth from the seeds and analysed for tannin content. They reported significant loss of tannin content in cooked grains than that of the raw grains. In pigeonpea, loss of tannin on cooking was about 59 per cent and in the other three legumes it was about 70 per cent.

Ologhobo and Fetunga (1984b) reported 31.0-47.3 per cent reduction in tannic acid contents on cooking and autoclaving of cowpea seeds. Similarly, boiling for 30 min and roasting for 10 min resulted in 73 to 17 per cent reduction in assayable polyphenols, respectively, in mung bean (Vigna radiata) (Barroga et al., 1985).

The amount of assayable polyphenols reported by Ekpenyong (1985) was higher in Nigerian legumes than in cereal grains. Cooking reduces significantly the tannin levels in legumes. The loss of tannin is attributed to the destruction of polyphenolic compounds by moist heat. It is also due to the formation of some insoluble complexes between the tannin and protein.

2.6.5 Trypsin inhibitor:

Sohonie and Bhandarkar (1954) reported inactivation of the trypsin inhibitory activity of winged bean by moist heat treatment (autoclaving for 10 min at 130°C or by boiling for 30 min after soaking in water for 10 h).

Jaffe and Flores (1975) reported that cooking of bean (Phaseolus vulgaris) at 85°C for 2 h, previously soaked for 2 h, destroyed trypsin and chymotrypsin inhibitor activities. They further reported that beans autoclaved for 30 min at 15 lb pressure had low or nil inhibitors.

Soni et al. (1978) autoclaved the seed extract at 15 psi for 15 min of 11 common Indian pulses and observed reduction in antitryptic activity of the pulses to almost negligible amounts except in the case of red gram.

Ekpenyong and Borchers (1981b) reported that roasting, autoclaving and cooking for 30 min or 5 h modify the chemical composition of winged bean. Heat

inactivated the trypsin inhibitors thereby improving the digestibility in vitro.

AL-Bakir et al. (1982) reported that heating soaked seeds of chickpea, broad bean, lentil and mung bean at 121°C for 30 min completely destroyed trypsin inhibitor activity.

Tan and Wong (1982) observed that autoclaving at 120°C for 10 min decreased trypsin inhibitor activity in six varieties of winged bean meals by 53 to 75 per cent and whole bean meals were similarly affected.

Mehta and Simlot (1982) purified ninety fold trypsin inhibitor from moth bean. They observed that autoclaving at 97°C destroyed its activity within 5-10 min. The inhibitor was also stable to higher temperatures losing only 40 per cent of its activity in 2 h at 80°C and almost all in 2 h at 97°C.

Ologhobo and Fetunga (1984b) reported on cowpea seeds that trypsin inhibitor activity was completely eliminated by cooking and autoclaving.

Ghorpade et al. (1986) studied the thermal stability of trypsin inhibitor from horse gram and found that application of dry heat at 80°C did not inactivate the inhibitor upto 60 min. The inhibitor lost only 20 per cent activity when subjected to 100°C for 60 min. The

autoclaving (moist heat) of meal at 120°C and 15 psi destroyed inhibitor activity almost completely within 10 min.

2.6.6 Electrophoresis of soluble proteins:

Cherry et al. (1975) used gel electrophoresis to evaluate changes in solubility and structural components of proteins in peanuts subjected to moist heat (temperature 50-120°C, time intervals 15-20 min). Temperature of 100 and 120°C were used to decrease markedly the amount of soluble protein. However, extended heating at 120°C increased the proportion of soluble proteins. Heated peanuts stored for different times (i.e. holding time which included frozen immediately, stored overnight, or stored overnight and ground prior to lypophilization) showed protein solubilities which varied among treatments. Polyacrylamide gel electrophoresis showed only minor qualitative changes in proteins heated at 50 or 75°C. However, it became increasingly difficult to detect nonarachin proteins in gel patterns of peanut heated for 15-74 min at 100°C. Simultaneously, a new group of protein bands was formed in these gel patterns. When heating was > 105 min at 100°C, arachin became less defined in gel patterns and was not present in soluble fraction after heating for 120 min. Prolonged heating produced new components detectable by gel electrophoresis. At 120°C, similar changes were observed between 15 and 90 min. A broad

diffuse band of intermediate migration was prevalent in gels of peanuts heated at 120°C for 105 to 210 min. Although moist heat at high temperature denatured the proteins, many new structural components remained soluble even after peanuts were heated at 120°C for 210 min.

Sathe and Salunkhe (1981c) studied the solubilization of California small white bean proteins. They reported 25.9 per cent crude protein on dry weight basis. Albumins accounted for 11.3 per cent of the total crude proteins. Alkali (1 N NaOH) extractable proteins had an isoelectric pH of about 4.0. About 88 per cent of the total crude proteins were recovered employing acid/alkali extraction. Polyacrylamide gel electrophoretic analysis indicated that the acid soluble proteins were comprised of at least five components whereas the alkali soluble proteins consisted of atleast two components.

Sathe et al. (1982a) while studying the functional properties of winged bean proteins, used sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the bean flour proteins and protein concentrates. They observed 9 sub units each with apparent molecular weight ranges from 27,000-3,80,000 and 14,200-1,43,000 daltons, respectively.

2.6.7 In vitro protein digestibility:

Iyer et al. (1980a) studied susceptibility of raw

and processed bean proteins towards sequential enzymatic hydrolysis by pepsin followed by trypsin + chymotrypsin. They reported 413.4, 166.0 and 208.6 per cent increase in hydrolysis in conventionally cooked Great Northern, kidney and pinto beans, respectively. The corresponding figures in these beans after soaking in mixed salt solution for 18 h at 22°C and cooking were 862.7, 305.5 and 336.2 per cent, respectively.

Autoclaving winged bean for 30 min caused a marked improvement in the in vitro digestibility (Ekpenyong and Borchers, 1981b). In vitro digestibility of the raw black gram proteins was found to be lower than autoclaved black gram and autoclaved black gram supplemented with native black gram trypsin inhibitor (Kamalakannan et al., 1981).

Satwadhkar et al. (1981) studied the in vitro protein digestibility of raw, cooked and germinated for 12-36 h and cooked horse gram and moth bean. They reported that cooking decreased the protein digestibility of horse gram either in ungerminated or germinated form, but such a decrease was noticed in moth bean at only 36 h germination. Moth bean either in raw form or germinated for 24 h did not exhibit a decrease in in vitro digestibility after cooking.

Sathe et al. (1982b) studied functional properties of Great Northern bean proteins and in vitro digestibility. They reported that heating improve in vitro susceptibility

of bean proteins to enzymatic hydrolysis. Moist heat was more effective than dry heat in improving the in vitro digestibility of the bean proteins.

Shastri et al. (1982) studied the in vitro protein digestibility of moth bean and reported that in vitro digestibility of protein by trypsin in raw moth bean was 19.4 per cent when compared to that of casein. This was improved to about 44 to 45 per cent by soaking in water for 4-8 h and roasting at 250°C. The dry roasting also increased the in vitro digestibility but to a lesser extent as compared with that by parching.

Sathe et al. (1983) observed that the major reserve proteins were substantially hydrolysed during 5 day germination of Great Northern beans. In vitro trypsin digestion of the extracted proteins upto 4 h at 37°C indicated that major storage proteins in germinated and ungerminated seeds, although substantially hydrolysed, could not be completely degraded. In vitro susceptibility of the extracted proteins was improved on moist heat treatment (97°C, 30 min).

2.7 Phytate-protein-minerals interactions:

The ability of phytic acid to complex with proteins and particularly with minerals has been a subject of investigation for several reasons but predominantly from chemical and nutritional view points.

Crystalline preparation of phytate-protein complex at pH 4.0 has been found by Bourdilon (1951) in the Great Northern bean (Phaseolus vulgaris) and several physicochemical properties were reported by him. The interaction between phytic acid and proteins is thought to be of ionic type (de Rham and Jost, 1979). The interaction between phytate and proteins leads to decreased solubility of proteins (Bourdilon, 1951; Smith and Rackis, 1957; Cheryan, 1980). It has also been shown that calcium ions interact with proteins and phytate to decrease further the solubility of proteins (Wolf and Briggs, 1959; Saio et al., 1967). Barre' (1956) reported that phytate protein complexes are less subject to proteolytic attack than the same protein alone.

The titration curve for phytic acid has been reported to have two inflections (Maddaiah et al., 1964; Crean and Haisman, 1963; Vohra et al., 1965). Crean and Haisman (1963) reported that the two inflections were at pH 4.9 and 8.1 respectively. They also stated that of the 12 replaceable hydrogen atoms of phytic acid, six are strongly dissociated with a pK value of about 1.8, two are weakly dissociated (pK value of about 6.3), and the remaining four are so feebly acidic (pK value of about 9.7) that they can not be determined. Blank et al. (1971) reported the structural characterization of phytate employing X-ray crystallography. They reported that phytate has the hexaorthophosphate ester

structure and that the phosphates at positions C-1, 3,4,5 and 6, are axially disposed with that of the C-2 equatorial. Phytic acid in free form is quite unstable (Vohra et al., 1965) and decomposes to yield orthophosphoric acid.

Phytic acid is known to form complexes with proteins at both acidic and alkaline pH. Omosaiya and Cheryan (1979) and Cheryan (1980) offered the following explanation:

Low pH: This is attributed to strong charge effects. Six of 12 dissociable protons of phytic acid are strongly dissociated with a pK of about 1.8 (Crean and Haisman, 1963). Thus phytate is strongly negatively charged below the acidic pH range (pH of about 2.0), while proteins are strongly positively charged at pH 2.0. As a result, phytate protein complex could be formed under such circumstances. This complexation, it is suggested (Smith and Rackis, 1957) is rapid and followed by nonionic irreversible reactions. It infers, therefore, that this nonionic irreversible reaction confers the stability of the phytate-protein complex.

High pH: Strong protein-phytate interaction is suggested at alkaline pH (O'Dell and de Boland, 1976) which is apparently different than at low pH, in that the electrostatic effects are minor at alkaline pH. Multivalent cations such as Ca^{++} (at certain necessary concentrations) are thought to mediate such phytate-protein complexes (Saio et al., 1967; Okubo et al., 1976). It is therefore suggested that such ternary complexes of phytic acid be formed by the

following mechanisms;

Omosaiye and Cheryan (1979):

Cation + phytic acid $\longrightarrow$ (cation-phytic acid)

Protein + cation + phytic acid $\longrightarrow$ (protein-cation-phytic acid).

Saio et al. (1967) suggested that these ternary complexes are fairly labile in alkaline solution.

Conceivably, if proteins are to form complexes with phytic acid, they must have some electrical charge on them, which is possible at below or above the isoelectric pH of the protein. This is evident from several studies indicating involvement of lysyl, histidyl, arginyl and amino terminal groups in the formation of phytate-protein complexation (Okubo et al., 1975; Omosaiye and Cheryan, 1979; de Rham and Jost, 1979; Saio et al., 1967). Reddy and Salunkhe (1981) suggested that phytate-protein complexes did not form in black gram albumins at pH 6.40 (which is near to the isoelectric pH of black gram albumins), supporting the view that protein must be in a charged state to form the phytate-protein complex.

Nutritional implications of phytate-protein complexes are still under scrutiny. The reduced solubility of proteins as a result of phytate-protein complexes can adversely affect certain functional properties of proteins which are dependent upon their hydration and solubility, such as hydrodynamic properties (viscosity, gelation, etc),

emulsifying capacity, foaming and foam performance and dispersibility in aqueous media. In addition reduced bioavailability of phosphorus is a distinct possibility.

O'Dell and de Boland (1976) observed complexation of phytate with proteins and cations in corn germ and oilseed meals and reported that by gel electrophoresis at pH 9.0 or more, several proteins in an aqueous extract of soybean flakes complex with phytate whereas those of corn germ do not. Gel filtration on Sephadex G-50 at pH 4.0 also clearly separated phytates from corn germ proteins.

Rackis and Anderson (1977) reported that reduced bioavailability of essential minerals by either phytate or phytate protein complexes in legumes and other protein foods depends on several factors viz. (I) the ability of endogenous carriers in the intestinal mucosa to absorb essential minerals bound to phytate and other dietary substances, (II) the concentration of phytic acid in food stuff, (III) the concentration of minerals in food stuff, (IV) the digestion or hydrolysis of phytate by phytase in the intestine, (V) phytase inhibition and (VI) processing of products or methods of processing.

Phytate-mineral complexes are well documented (Erdman, 1979). Phytate, being a strong acid, forms a variety of salts with heavy metals. The solubility of such metal complexes of phytate is pH dependant. The

nutritionally important minerals such as calcium, magnesium, copper, iron, zinc and others form complexes with phytic acid resulting in reduced solubility of the metals.

Reddy and Salunkhe (1981) while studying the interactions between phytate, protein and minerals in whey fractions of black gram, reported that 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 per cent, respectively, after 48 h 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.

Cheryan et al. (1983) studied the solubility characteristics of magnesium phytate complexes as a function of pH and molar ratio of magnesium (Mg) to phytic acid (PA) in pure (in vitro) systems. Complexes were soluble below pH 5 at molar ratios studied (0.5-12). Above pH 5, solubility decreased rapidly. The higher the Mg:PA molar ratio, the lower the pH at which the solubility drop was observed. At Mg:PA ratios of 4 or less, phytate phosphorus was relatively more soluble than magnesium at pH 5. However, at Mg:PA ratios greater than 6, the magnesium was more soluble than phytate.

Murthy and Rao (1984) studied binding of phytic acid with the mustard 12S protein in the pH range 5.0-1.0 by the techniques of precipitation, turbidimetry, electrophoresis and electrometric titration. They found increased binding with increased pH. At pH 3.0 maximum binding of 170 mol of P/mol of protein was observed, which aged with the basic amino acid content of the protein. Only one class of binding sites was indicated. Electrophoretic studies indicated that even at pH 3.0 some amount of protein was still left in solution. Electrometric titration studies indicated the possibility of the presence of soluble protein-phytate complexes in the system. At pH 3.0, calcium inhibited the formation of protein-phytate complex.

Serraino and Thompson (1984) studied the effect of pH on the disruption of protein-phytate complexes and hence on the removal of phytic acid from rapeseed flour and protein concentrate in a dialysis system. Phytic acid removal from these dispersions was also tried by using various amounts of EDTA (0.25-0.75 M) at pH 9.0, CaCl_2 (0.25-0.75 M) at pH 3.5 or phytase (3 per cent) at pH 5.15. Disruption of rapeseed flour and protein concentrate were dialysed against distilled water for 7 days. While the greatest removal of phytic acid (100 per cent) with the least loss of nitrogen was observed with phytase treatment, near adjustment of protein concentrate to pH 3.5 and

flour to pH 5.15 resulted in 73 and 88 per cent losses of phytic acid, respectively. Dialysis of flour dispersions without pH pre-adjustment i.e. at pH 5.8, removed upto 67 per cent of the phytic acid.

Honig et al. (1984) observed that phytate-protein complexes formed during the manufacture of soy protein isolates reportedly affect mineral bioavailability in foods. They estimated the amounts of such complexes by determining the phytic acid (PA) content by anion-exchange high performance liquid chromatography (HPLC) before and after dialysis. Protein fractions analysed include 1) commercial soybean protein isolates, 2) laboratory prepared isolates, 3) the two major components of the isolates, the 7S and 11S globulins, 4) the pH 8 insoluble material formed on neutralization of the isoelectric (pH 4.5) form of the isolate and 5) the pH 8 soluble fraction of protein isolates. After dialysis, the PA content of various soybean protein isolates was 0.95-1.75 per cent. The 11S globulins were essentially devoid of PA, whereas 7S globulins contained 0.34 per cent. PA values for dialysed pH 8 insoluble and soluble protein fractions were 0.22-1.41 per cent and 0.36-1.29 per cent, respectively. Yields of pH insoluble fractions were 10.2-28.6 per cent of the total protein isolate. This data indicated that certain protein components of the soybean protein isolate were strongly

complexed with PA, especially at pH 8. However, variability in the nature and yield of insoluble proteins and in the amount of phytic acid bound indicated that factors other than the pH and protein type were involved in the formation of phytate-protein complexes. Processing conditions appeared to be important because extensive dialysis of the commercial samples at pH 8 failed to remove appreciable amounts of PA.

3. MATERIALS AND METHODS

3.1 Materials:

3.1.1 Moth bean:

In the present investigation, pure seeds of moth bean (Phaseolus aconitifolius Jacq) were purchased from the local market of Parbhani. They were cleaned for extraneous matter, if any and stored in clean glass bottles at 4°C.

3.1.2 Chemicals:

Most of the chemicals used in this investigation were of analytical grade. These were obtained from Glaxo Laboratories (India) Ltd. Bombay. The fine chemicals were obtained from V.P. Chest Institute, New Delhi.

3.2 Methods:

3.2.1 Soaking and production of quick-cooking beans:

Quick-cooking beans were prepared as per the procedures described by Rockland and Metzler (1967), Rockland et al. (1977), Bongirwar and Sreenivasan (1977) and Kadam et al. (1981). Beans were blanched in boiling distilled water for 5 min to facilitate hydration and to loosen the seed coats. The blanched beans were soaked for 3, 6, 9 and 12 h in salt solution containing 1.5 per cent NaHCO_3 , 0.5 per cent Na_2CO_3 and 0.75 per cent citric acid, pH 7.0 ± 0.05 at room temperature ($25^\circ\text{C} \pm 2$). Throughout, the bean salt solution ratio was 1:3 (wt/vol). One lot was soaked in distilled water for 12 h and taken

as the control. The soaked beans were subsequently removed from the soaking medium and rinsed with distilled water. The amount of water imbibed during soaking of beans in salt solution was determined at intervals of 3, 6, 9 and 12 h by weight gain. Total solids leached during soaking were determined by drying aliquots of salt solution to constant weight. The soaked beans were cooked traditionally in distilled water at 100°C (beans: water ratio 1:4 wt/vol) until they were softened to a uniform mass when pressed between thumb and forefinger, as described by Sharma et al. (1977). The time required to obtain such a consistently cooked product was recorded as the cooking time. Soaking was carried out in duplicate. One set was used to note the cooking time and another was dried in cabinet tray dryer to a final moisture content of 10-12 per cent. An air velocity of 14 m/min and a temperature not more than 50°C were maintained during drying. The dried beans were ground in Waring Blender (Sumset) to pass through 60 mesh sieve and stored in air tight glass bottles at 4°C for further analysis.

3.2.2 Germination:

100 g seeds of uniform size were sterilized with 0.1 per cent mercuric chloride and were soaked in water for 6 h at 4°C. The seeds were placed on a double layer of filter paper in petriplates and incubated at 30°C.

The filter papers were moistened at regular intervals of 12 h till 72 h. The germination was carried out at room temperature ($25^{\circ}\text{C} \pm 2$) for 24, 48 and 72 h. The germinated beans were dried, ground as described above and stored in air tight glass bottles at 4°C for further analysis.

3.2.3 Cooking:

Whole moth bean seeds were cooked from the dry state by using an autoclave at set conditions of temperature and pressure. 100 g seeds were placed in 1 litre beakers and 400 ml of water were added. The beaker tops were closed by aluminium foil. The samples were autoclaved for varying lengths of time (10, 15, 20, 25 and 30 min). Beans to water ratio was 1:4 (wt/vol beans were used as such). The temperature (120°C) and steam pressure (10 psi) were kept constant in autoclave during cooking. The cooked beans were dried, ground as described above and stored in air tight glass bottles at 4°C for further analysis.

Moth bean seeds soaked either in distilled water or salt solution, germinated and cooked in autoclave were used for studying the improvement in nutritional functionality and removal of certain antinutritional factors like phytates, polyphenols and trypsin inhibitor.

3.2.4 Proximate composition:

Moisture, total minerals, crude fat (ether extract)

and total nitrogen (Kjeldahl N) of all the samples were determined according to the standard procedures of AOAC (1975). The protein contents were calculated by using conversion factor of 6.25. Total carbohydrates were determined by difference.

3.2.5 Functional properties:

3.2.5.1 Water and oil absorption:

Oil (Postman vegetable refined cooking oil, Ahmed Mills, Bombay) and water absorption capacities were determined by the method of Beuchat (1977). 1 g sample was mixed with 10 ml of oil or distilled water for 30 sec in a Waring Blendor (Sumeet) at high speed. The samples were then allowed to stand at room temperature ($25^{\circ}\text{C} \pm 2$) for 30 min, centrifuged at $5,000 \times g$ for 30 min, and the volume of the supernatant was measured in a 10 ml graduated cylinder. Density of water was assumed to be 1 g/ml and that of oil was determined to be 0.88 g/ml. Water and oil absorption capacities are expressed as g/g of the flour or protein.

3.2.5.2 Gelation:

Least gelation concentrations of moth bean flours were determined by the method of Coffmann and Garcia (1977) with slight modifications as described by Deshoande et al. (1982). Appropriate sample suspensions of 2,4,6,

8, 10, 12, 14 and 16 per cent (wt/vol) were prepared in 5 ml of distilled water. The test tubes containing these suspensions were then heated for 1 h in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were then further cooled for 2 h at 4°C. The least-gelation concentration percentage was determined as that of the sample that did not fall down or slip from the inverted test tube. The gelation is expressed in percent of the flour.

3.2.5.3 Nitrogen solubility:

Nitrogen solubility of moth bean flours was determined according to the method of Narayana and Rao (1982) in the pH range of 2-12, using 1 g flour with a meal:water ratio of 1:60 and shaking for 2 h at room temperature. The pH of the suspension was adjusted by the addition of 0.1 N HCl or 0.1 N NaOH. After extraction the suspension was centrifuged at 3,000 x g for 20 min at room temperature (25°C $\pm$ 2) and nitrogen in the supernatant was estimated by the micro-kjeldahl method. Nitrogen extracted is expressed as per cent of the flour nitrogen content.

3.2.5.4 Foaming properties:

Foaming capacity and stability of moth bean flours were studied according to the method of Coffmann and Garcia (1977). 1 g sample was whipped with 100 ml of distilled water for 5 min in a Waring Blendor (Sumeet) and

then poured in 250 ml graduated cylinder. The foam volumes were recorded at intervals of 30,60,90 and 120 min at room temperature ($25^{\circ}\text{C} \pm 2$). Volume increase (per cent) was calculated according to the following formula;

$$\text{Volume increase (per cent)} = \frac{\text{Volume after whipping (ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

Specific volumes of foams were determined as an index of air uptake during whipping. Weights were taken before and after whipping and specific volume calculated according to the method of Balduin and Sinthavalai (1974) as follows;

$$\text{Specific volume} = \frac{\text{Volume after whipping (ml)}}{\text{Weight after whipping (g)}}$$

To study the effects of concentration on foamability, 1,2,3,4,5,7 and 10 per cent (wt/vol) aqueous dispersions of flour samples were prepared. Effects of concentration were evaluated for raw and processed moth bean flours.

A 2 per cent (wt/vol) aqueous dispersion was employed to study the effects of pH on foaming capacity of raw and processed moth bean flours because it gave a noticeable increase in volume. The pH was adjusted to a desired value by using 0.1 N HCl or 0.1 N NaOH or both before the dispersions were whipped.

3.2.5.5 Emulsion properties:

Emulsifying activity and emulsion stability of the samples were evaluated by the method of Yasumatsu et al. (1972) with slight modifications as described by Deshpande et al. (1982). A 7 per cent (wt/vol) aqueous dispersion of the flours were prepared in distilled water. Twenty ml of dispersion was mixed with 20 ml of oil (Postman vegetable refined cooking oil, Ahmed Mills, Bombay) and blended in Waring Blendor (Sumeet) for 5 min. An aliquot was centrifuged at 3,000 x g for 5 min. Emulsifying activity was expressed as the percentage of the total mixture that remained emulsified after centrifugation at room temperature ($25^{\circ}\text{C} \pm 2$). The emulsion stability was evaluated by recentrifugation following heating at 80°C for 30 min in a water bath.

The emulsification capacity was determined in 25 ml of 7 per cent (wt/vol) aqueous dispersion of the sample at pH 2 - 12 by titrating with oil to the breakpoint of emulsion as per the procedure of Beauchat et al. (1975). The pH was adjusted to a desired value by 0.1 N HCl or 0.1 N NaOH. The emulsification capacity determinations were carried out at room temperature ($25^{\circ}\text{C} \pm 2$) and the values are expressed as g of oil emulsified by 1 g of protein in the sample.

3.2.6.1 Determination of phytate phosphorus:

For estimation of phytate phosphorus, a combination

of three methods was employed. Extraction and precipitation of phytate phosphorus were performed according to the method of Wheeler and Ferrel (1981). The latter steps in phytate phosphorus estimation were carried out according to the method of Makower (1970). Finally, the iron content was measured by the AOAC (1975) method using O-phenanthroline reagent. Contents of iron and phosphorus were assumed to be in 4:6 ratio in order to calculate phytate phosphorus content.

On the basis of phytate phosphorus contents, the phytate (phytic acid) was calculated assuming 28.20 per cent phosphorus in the molecule.

3.2.7.1 Determination of minerals:

Total phosphorus was measured by the colorimetric method described by Fiske and Subba Row (1925). Calcium, magnesium, iron and zinc were determined as per the procedures described in a manual of laboratory techniques of National Institute of Nutrition (1983) by using the atomic absorption spectrophotometer (Model Varian Techtron 1100).

3.2.8.1 Determination of polyphenols:

Polyphenols content was estimated according to the method of AOAC (1975) as modified by Cristensen (1974).

Reagents:

1. **Folin-Denis reagent:** One hundred g of sodium tungstate, 20 g of phosphomolybdic acid and 50 ml conc. phosphoric acid were added to 750 ml distilled water. The mixture was allowed to reflux for 2 h on hot water bath and made upto 1000 ml.
2. **Alkaline reagent:** Sodium carbonate (350 g) was dissolved in 1000 ml distilled water at 80°C, allowed to stand for overnight at room temperature and then filtered through glass wool.
3. **Standard tannic acid solution (0.1 per cent):** One hundred mg of tannic acid was dissolved in 100 ml distilled water.

One g of 60 mesh ground sample of moth bean flour was weighed into 500 ml conical flasks and 150 ml water were added to each flask and replicated four times. Internal standards were prepared by adding 10, 15 and 20 ml of standard tannic acid solution in the first three flasks and the fourth flask was left as control. The contents in the conical flasks were gently heated and boiled for 30 min and centrifuged at 5000 x g for 20 min. The supernatant was collected in a 250 ml volumetric flasks and volume was adjusted with distilled water.

Color development: Ten ml of supernatant were taken in 100 ml flask to which 75 ml water, 2.5 ml Folin-Denis reagent and 5 ml of standard sodium carbonate solution

were added and volume was made to 100 ml and allowed to stand for 30 min. Optical density was read at 740 nm on spectrophotometer. A standard curve was prepared by plotting optical density versus tannic acid concentration. Optical density of the control wherever it cuts 'Y' axis was taken as new origin. Difference in optical density in first and second origin was taken as a measure of tannin content in the sample.

3.2.9.1 Determination of trypsin inhibitor activity:

The method developed by Kakade et al. (1969) was used for determining the trypsin inhibitor activity in the crude preparations (phosphate buffer and water extracts). A 2 per cent casein solution in phosphate buffer (0.1 M, pH 7.6) was used as substrate, while the enzyme used was trypsin (E. Merk) (5 mg/ml). The incubation mixture consisted of 0.5 ml trypsin solution, 2 ml of 2 per cent casein, phosphate buffer (pH 7.6, 0.1 M) of volumes 0.6 ml to 1.0 ml, HCl solution (0.001 M) of volume 0.1 ml to 0.5 ml, flour extracts of phosphate buffer and water extracts of volumes 0.2 ml and 0.4 ml. In all cases the total volume of incubation mixture was kept at 4 ml. Incubations were carried out at 37°C for 30 min after which 6.0 ml of 5 per cent TCA solution was added to stop the reaction and corresponding blanks were run concurrently. In this method, one trypsin unit (TU) was arbitrarily

defined as an increase of 0.01 absorbance unit at 280 nm in 30 min for 10 ml of reaction mixture under the conditions described and the trypsin inhibitory activity as the number of trypsin units inhibited (Kakade et al., 1969).

3.2.10.1 Electrophoresis of soluble proteins:

Polyacrylamide gel disc electrophoresis was used to separate soluble proteins because of its high resolving power, transparency and chemical inertness.

The soluble proteins of soaked, germinated and cooked moth bean flours were separated. For this purpose, anionic system (Ornstein, 1964 and Davis, 1964) was employed in 6 mm (i.d.) x 7.5 cm (height) gel columns. The composition of 7.5 per cent running gel (per 20 ml) was as follows;

1 N HCl	1.2 ml
Tris	0.91 g
TEMED	6.0 μ l
Acrylamide	1.4 g
Bisacrylamide	0.036 g
Ammonium persulphate	0.014 g
Sucrose	1.0 g
pH	8.9

Running gel was polymerised in a column using fluorescent light for 30 min. At the top of running gel,

water was layered to prevent meniscus formation.

Composition of 2.5 per cent spacer gel (per 20 ml)
was as follows;

1 N HCl	1.2 ml
Tris	0.15 g
TEMED	11.5 μ l
Acrylamide	0.5 g
Bisacrylamide	0.125 g
Riboflavin	0.100 g
Sucrose	4.0 g
pH	6.7

The spacer gel (0.3 cm height) was polymerised at the top of the running gel by exposure to fluorescent light for 15 min. At the top of the spacer gel water was layered to prevent meniscus formation.

Extraction of soluble proteins:

One g of moth bean seeds were immersed in distilled water for 6 h at 4°C and washed thoroughly with distilled water and homogenised in 7 ml Tris-HCl buffer (pH 7.2, 0.1 M). The contents were centrifuged at 12,000 x g for 60 min and the supernatants were collected and stored at 4°C.

Sample containing about 150 to 200 μ g proteins was layered on the spacer gel and electrophoresis was conducted

in Tris-glycine buffer (pH 8.3). The composition of electrode buffer was as follows;

Glycine	2.88 g
Tris	0.60 g
Volume	1000 ml
pH	8.3

Electrophoresis was conducted at 4°C by applying 2 mA current per gel tube until tracking dye (bromothynol blue) entered in running gel and subsequently 4 mA per gel tube. After completion of electrophoresis as indicated by movement of tracking dye to the bottom of gels, the gels were stained for 30 min in 0.1 per cent amido black (in 4 per cent acetic acid) and destained by diffusing out excess stain in 7 per cent (vol/vol) acetic acid. The Rf value of each band was calculated as follows;

$$Rf = \frac{\text{Distance travelled by band from the top of the running gel}}{\text{Distance travelled by tracking dye}}$$

3.2.11.1 In vitro protein digestibility:

The samples of soaked, germinated and cooked moth bean flours were defatted by petroleum ether. The in vitro protein digestibility of these samples was studied by two different methods.

A. The rate of digestion was expressed in terms of Δ

moles of amino acid formed per g fat-free powdered material in 4 h after digesting the material with digestive enzymes namely pepsin and trypsin separately, under the experimental conditions and was correlated to zero time control.

1. Pepsin: The in vitro protein digestibility of samples was estimated by the method of Fasold and Gundlach(1964).

200 mg of finely powdered fat-free sample was suspended in 10 ml of 0.075 N HCl and incubated with 100 mg of pepsin at 37°C. An aliquot of 2 ml was taken out at 0 and 4 h of incubation and mixed with equal volume of 10 per cent TCA. After 1 h, the suspension was filtered and 0.1 ml of filtrate was subjected to amino acid determination by ninhydrin method (Rosen, 1957).

2. Trypsin: The method of Fasold and Gundlach (1964) was followed. The procedure remained same as described for digestion with pepsin except a change in buffer (0.01 M phosphate buffer, pH 7.0) and the digestive enzyme (trypsin) used in the reaction mixture.

B. The in vitro protein digestibility was determined by calculating the difference between the amount of nitrogen in the sample before and after hydrolysis with pepsin (AOAC, 1975).

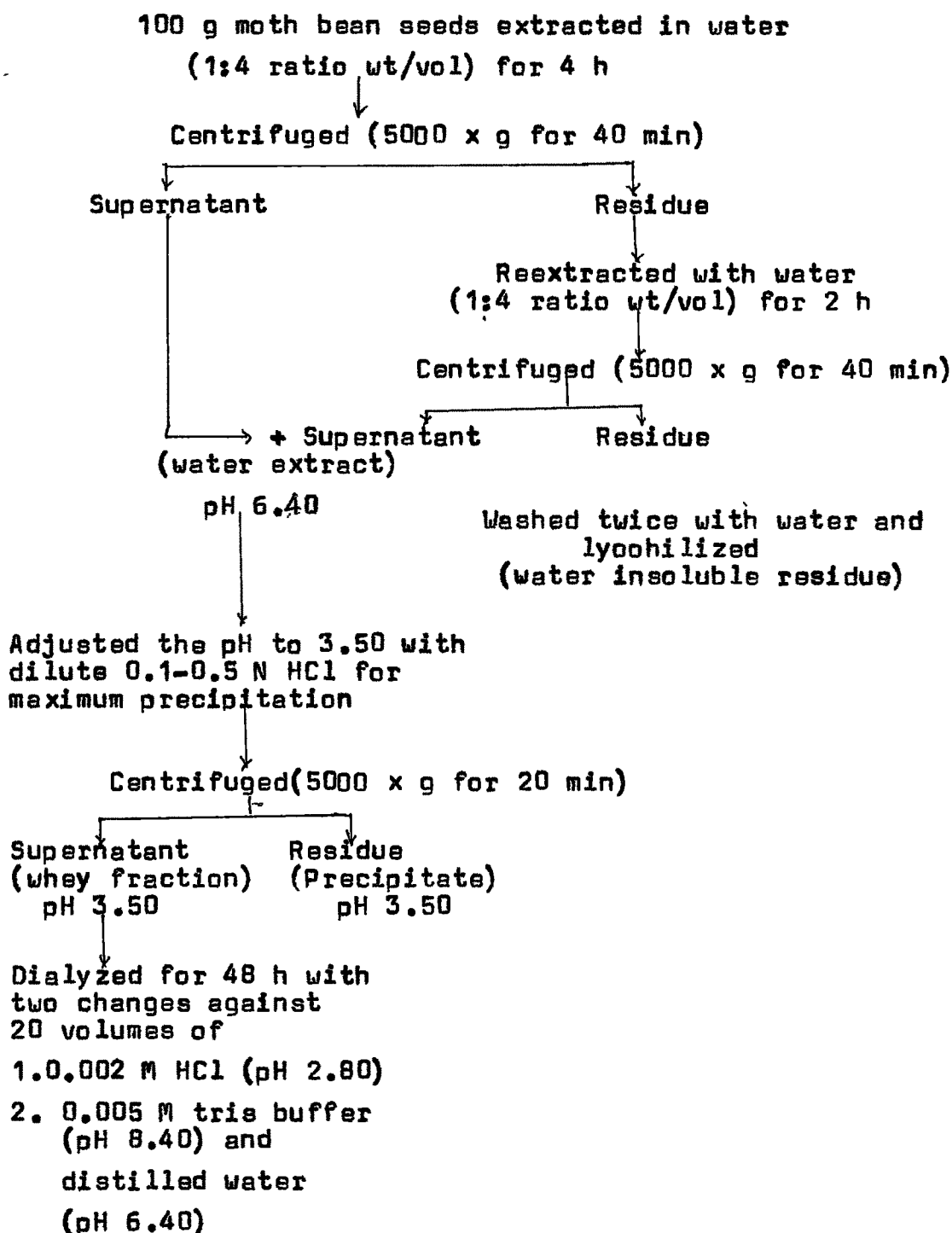
200 mg of finely powdered flour was incubated with

50 ml of 0.2 per cent pepsin in 0.075 N HCl for 24 h at 37°C. Digestion was performed in duplicate. The digests were filtered through Whatman No.2 filter paper. Nitrogen in the residue was washed with warm water on the filter. Nitrogen was estimated by micro-kjeldahl method. The in vitro protein digestibility was obtained by calculating the difference between the amount of total nitrogen in the sample before and after in vitro digestion with pepsin.

3.2.12.1 Identification of phytate-protein-minerals complexes:

Phytate-protein-minerals complexes in the moth bean flours were identified as per the method of Reddy and Salunkhe (1981) with slight modifications.

100 g of meal of raw moth bean were extracted for 4 h at room temperature with distilled water (1:10 meal to water wt/vol). The slurry was centrifuged at 5,000 x g for 40 min and the supernatant was collected. Residue was reextracted with distilled water for 2 h at room temperature and centrifuged at 5,000 x g for 40 min. The supernatants were combined and referred to as water extract. Residue (water insoluble residue) was washed twice with distilled water and lyophilized. The pH (6.20) of the water extract was adjusted to the pH of maximum precipitation (pH 3.50) with dilute HCl (0.1-0.5 N) and centrifuged at 5,000 x g for 20 min. Insoluble precipitate at pH 3.50 was collected

Flow diagram for phytate-protein-minerals complexes

They were freeze dehydrated and are hereafter
referred to as fraction I, fraction II and fraction III.

and freeze dried. The supernatant remaining at pH 3.50 referred to as whey fraction and was used for interaction studies between phytate and protein by dialyzing against three different pH solutions.

Four whey fraction sets were prepared similarly as mentioned above by employing 100 g meal for each set. One set of whey fraction was freeze dried without dialysis. The other three sets of whey fraction were dialyzed for 48 h with two changes against 20 volumes of 0.002 M HCl (pH 2.80), 0.005 M Tris buffer (pH 8.40) and distilled water (pH 6.40), respectively. The dialyzed samples were centrifuged at 5000 x g for 20 min and the supernatants were freeze dried and are here after referred to as fraction I, fraction II and fraction III in the subsequent test.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

The results obtained in the present investigation on processing of moth bean for improved nutritional functionality and elimination of certain antinutritional factors are discussed. Unless otherwise mentioned, each value given in a Table is a result of triplicate analysis and is expressed on dry weight basis within a given experiment. The results of these studies for purposes of convenience are presented and discussed one by one under the following different headings.

4.1 Proximate composition of moth bean flour:

The representative moth bean flour samples were studied to find out the proximate composition. The results are presented in Table 1. Moth bean flour contained 23.5 per cent protein ($N \times 6.25$) and 3.32 per cent ash which were fairly comparable to the reports of Borhade et al. (1984) and Meshram et al. (1980). Like many other legumes, moth bean was found good source of protein and total minerals.

Table 1. Proximate composition of moth bean flour.

Per cent composition	On as is basis	On dry weight basis
Moisture	10.5	10.5
Crude protein ($N \times 6.25$)	21.0	23.5
Crude fat	1.44	1.61
Ash	2.97	3.32
Total carbohydrates	64.0	71.5

4.2 Effect of soaking moth bean on proximate composition:

The data on changes in proximate composition on soaking moth bean seeds in either distilled water or mixed salt solution are presented in Table 2. A net loss of dry weight occurred as a result of soaking and oxidation of stored compounds with concomittant increase of moisture.

Table 2. Effect of soaking moth bean on proximate composition.

Treatment	Per cent proximate composition				
	Moisture	Protein (N x 6.25)	Crude fat	Ash	Carbo- hydrates
Control (dry bean)	10.5	23.5	1.61	3.32	71.5
Soaking ^a	11.4	21.3	1.60	3.28	73.8
Soaking ^b for					
3 h	10.7	22.9	1.55	3.30	72.2
6 h	11.2	22.2	1.50	3.30	73.0
9 h	11.8	21.6	1.50	3.26	73.6
12 h	12.0	21.1	1.60	3.24	74.0

^aSoaking in distilled water for 12 h at 25°C ± 2.

^bSoaking in mixed salt solution for 3,6,9 and 12 h at 25°C ± 2.

At the end of 12 h soaking period, the dry weight was reduced to 98.9 and 98.3 per cent in case of distilled

water soaked and salt soaked moth bean seeds, respectively. The total ash content also gradually decreased throughout the soaking period (Fig 1). Rao and Deosthale (1983) also reported loss in ash content of green gram and black gram during overnight soaking in distilled water. This may be probably because of leaching of total minerals in the soaking medium. The protein content decreased from 23.5 to 21.3 per cent in 12 h distilled water soaked and 21.1 per cent in 12 h salt soaked moth bean flours. This is supported by the results of Kakade and Evans (1966) on navy beans. Crude fat and total dry matter were also found decreased during soaking in water and salt solution.

4.3 Effect of soaking moth bean on cooking characteristics:

4.3.1 Water uptake and leaching losses:

The rate of water imbibition and leaching losses of solids are presented in Fig 2. It is seen from the results that soaking the beans in distilled water and salt solution both for 12 h. had nearly comparable water uptake and losses of total solids in salt solution. Initial rates of water uptake and leaching losses of total solids were high, when beans were soaked in distilled water for 12 h but decreased when soaked in salt solution for 3 h. However, the rates of water uptake and leaching losses of total solids increased progressively, when beans were soaked in salt solution for 6,9 and 12 h. Loss of solids during

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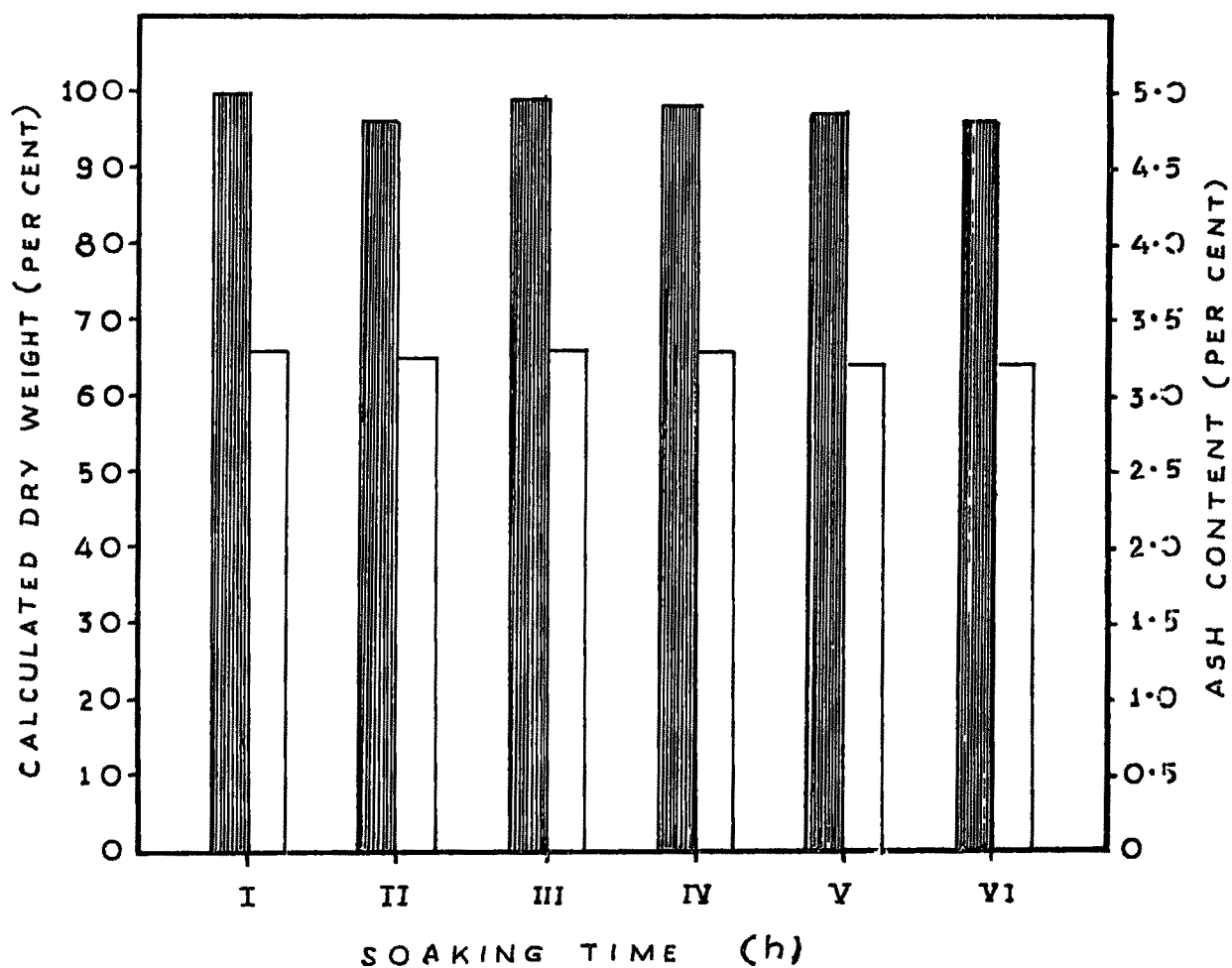


Fig.1 EFFECT OF SOAKING ON DRY WEIGHT AND ASH CONTENT
IN MOTH BEAN FLOURS

soaking may be influenced by a concentration gradient (difference in chemical potential), which governs the rate of diffusion and the physical barriers in cotyledon cell walls and seed coat, which limit diffusion of water. The increased rate of water imbibition may be due to increased swelling of proteins and carbohydrates. Iyer et al. (1980a) studied rate of hydration and leaching losses of total solids during soaking of Great Northern, kidney and Pinto beans in mixed salt solution containing sodium chloride (2.5 per cent) + sodium carbonate (1.5 per cent) + sodium bicarbonate (1.5 per cent) + sodium tripolyphosphate (1.0 per cent) and observed increased rate of both hydration and leaching losses with increased soaking time from 6 to 24 h. Similar observations were made by Nordstrom and Sistrunk (1977) on dry beans.

4.3.2 Cooking time:

It is observed from Table 3 that time required for cooking dry moth bean was 75 min when the beans to cooking water ratio was 1:4 (wt/vol). When the beans were soaked in distilled water for 12 h or in salt solution for 3, 6, 9 and 12 h, the time required for cooking drastically decreased from 75 min to 40, 15, 12, 10 and 8 min respectively. Soaking the beans in distilled water and salt solution both for 12 h caused reduction in cooking time 46.6 and 89.3 per cent, respectively. Narsimha and Desikachar (1978b) observed nearly 50 per cent reduction in cooking time in

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WATER UPTAKE
LEACHING LOSSES

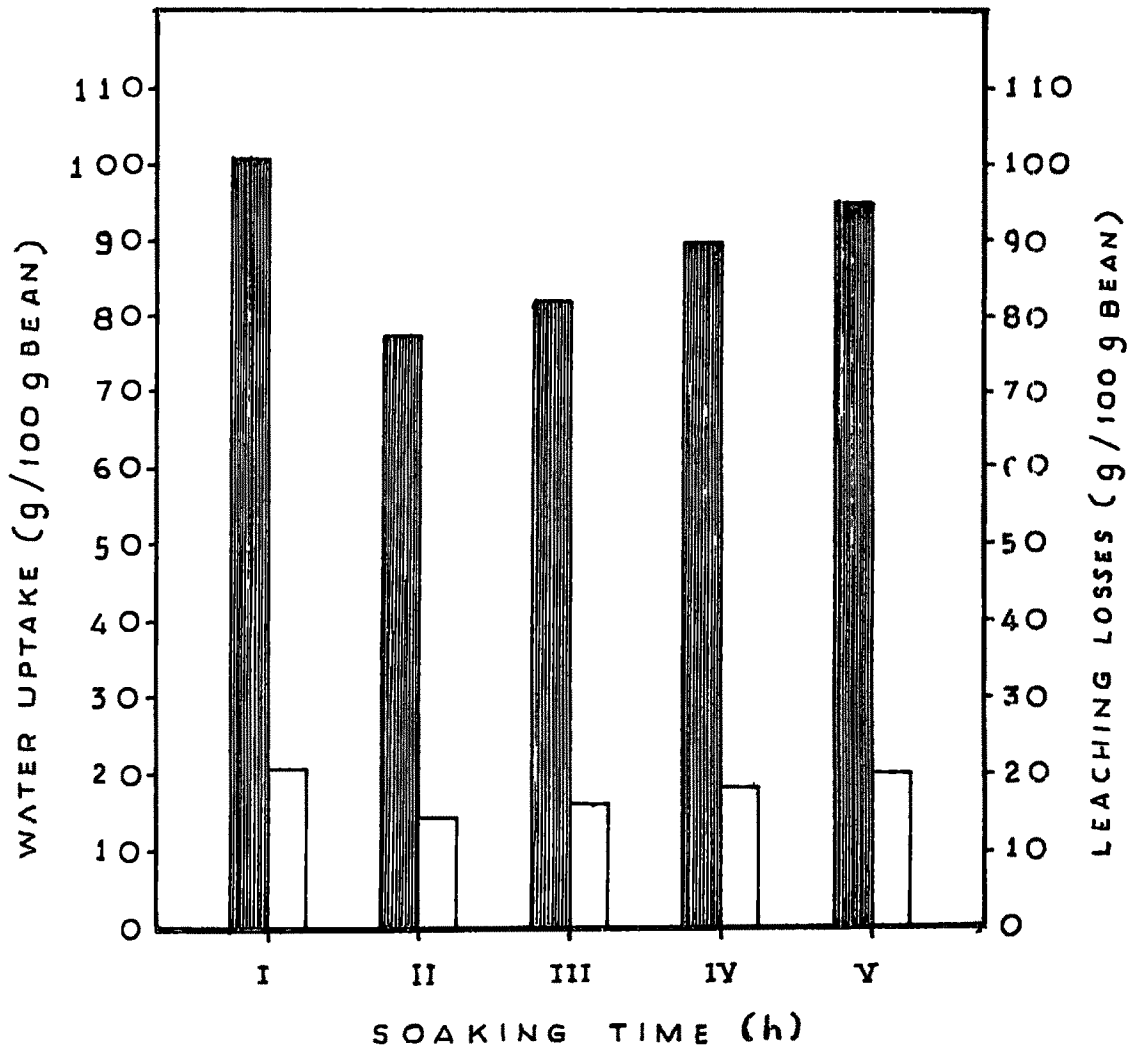


Fig 2 EFFECT OF SOAKING ON WATER UPTAKE AND
LEACHING LOSSES OF SOLIDS IN MOTH BEAN

Table 3. Effect of soaking moth bean seeds on cooking time.

Treatment	Cooking time (min)	Per cent reduction in cooking time
Control (dry bean)	75	-
Soaking ^a	40	46.6
Soaking ^b for		
3 h	15	80.0
6 h	12	84.0
9 h	10	86.6
12 h	8	89.3
SE $\pm$	0.57	
LSD (P=0.05)	1.77	

^aSoaking in distilled water for 12 h at 25°C $\pm$ 2.

^bSoaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

red gram splits when presoaked for 2 h in salt solution. Narayana (1981) studied the cooking time of winged bean (Psophocarpus tetragonolobus) dhal and reported that soaking the dhal in salt solution for 2 h reduced the cooking time by nearly 50 per cent. Kadam et al. (1981) reported 67 per cent reduction in cooking time over 12 h distilled water soaked moth bean when soaked in a mixed salt solution for 12 h. Iyer et al. (1980a) on dry beans (Phaseolus vulgaris)

reported 80 to 85 per cent reduction in cooking time on soaking the beans in mixed salt solution for 24 h. The results of the present investigation are in conformity with the results reported above. Bongirwar and Sreenivasan (1977) have reported that soak treatment softens the pellicle or bran by its tenderizing effect in addition to its effect on solubilization of protein and starch components. These observations suggest that divalent cations, such as calcium and magnesium, are dissociated from polymeric protein and carbohydrate complexes by interaction of ion exchange mechanisms with pectic substances that plasticize the middle lamella and enable softening of the cotyledons (Marston and De Omana, 1979; Rockland, 1979 and Rockland and Jones, 1974). Sodium bicarbonate and sodium carbonate of the salt solution have solubilizing effect on the pectic substances. This facilitates easy penetration and faster hydration on interior starch and protein molecules resulting into quick-cooking beans.

4.4 Effect of soaking moth bean on functional properties:

4.4.1 Water and oil absorption:

Water and oil absorption capacity of flour and protein increased on soaking either in distilled water or in salt solution (Table 4). Soaking in salt solution for 12 h had little better water absorption capacity (2.5 g/g flour and 11.8 g/g protein) than soaking in distilled water

Table 4. Effect of soaking moth bean seeds on water and oil absorption capacity.

Treatment	Per cent crude protein ^a (N x 6.25)	Water absorption capacity (g/g)		Oil absorption capacity (g/g)	
		Flour	Protein ^b	Flour	Protein ^b
Control (dry bean)	23.5	2.4	10.2	1.7	7.2
Soaking ^c	21.3	2.5	11.7	1.7	7.9
Soaking ^d for					
3 h	22.9	2.4	10.4	1.7	7.4
6 h	22.2	2.5	11.2	1.7	7.6
9 h	21.6	2.5	11.5	1.8	8.3
12 h	21.1	2.5	11.8	1.8	8.5
SE $\pm$		0.007		0.008	
LSD(P=0.05)		0.021		NS	

^a Expressed on dry weight basis.

^b Expressed on crude protein basis.

^c Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^d Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

for 12 h (2.5 g/g flour and 11.7 g/g protein). The increase in water and oil absorption capacity in g/g protein was observed to be 15.6 and 18.1 per cent respectively in beans

soaked in salt solution as against 14.7 and 9.7 per cent respectively in beans soaked in water both for 12 h.

Though, there was apparent decrease in protein content in salt soaked flours the water absorption capacity increased significantly. This increase may be due to dissociation of proteins during blanching of moth bean before soaking in salt solution. The subunits have more water binding sites than the oligomeric proteins. Increase may also be due to more hydrophilic groups on soaking in salt solution for 12 h. Wu and Inglett (1974) have reported increased water absorption capacity of soy flour due to heat processing. del Rosario and Flores (1981) also reported increase in water absorption capacity of mung bean flour on blanching in boiling water for 15 min.

The increase in oil absorption capacity was observed to be 1.8 g/g flour and 8.5 g/g protein on soaking beans in salt solution as against 1.7 g/g flour and 7.9 g/g protein on soaking beans in distilled water both for 12 h. However, increase in all the treatments was found nonsignificant. This increase could be attributed to the increased capacity of the flour to hold the fat globules as the amount of lipophilic proteins increases on soaking. Moreover, dissociation of proteins may occur on blanching and denaturation of proteins is expected to unmask the nonpolar residues from the interior of the protein molecule which results into increased oil absorption capacity.

Among the water and oil absorption capacity, the oil absorption capacity was lower compared to water absorption capacity. This may be due to hydrophilic nature of legume proteins.

4.4.2 Gelation:

The data on per cent least gelation concentration of soaked moth bean flours (Table 5) showed that gelation was

Table 5. Effect of soaking on least gelation concentrations of moth bean flours^a.

Treatment	Per cent crude protein (N x 6.25)	Least gelation concentration (per cent wt/vol)
Control (dry bean)	23.5	9.06 $\pm$ 0.01 ^b
Soaking ^c	21.3	8.62 $\pm$ 0.01
Soaking ^d for		
3 h	22.9	9.01 $\pm$ 0.02
6 h	22.2	9.04 $\pm$ 0.01
9 h	21.6	9.05 $\pm$ 0.03
12 h	21.1	9.03 $\pm$ 0.02

^a Each value is the average of nine determinations.

^b Mean plus or minus standard deviation.

^c Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^d Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

more or less constant (9.0 per cent) on soaking in salt solution for 3,6,9 and 12 h. Coffman and Garcia (1977) reported that gelation of mung bean protein isolates depends on protein concentration and that gel formed by a 10 per cent concentration of protein heated at 80°C for 10 min exhibited a more exceptional gelling ability than soybean proteinate even on higher protein concentration. Results of the present study showed that even the flours of soaked moth bean which had a much lower protein content due to blanching prior to soaking formed stable gels at these conditions using this simple gelation test. It is possible that besides protein concentration, other constituents, most probably starch, contribute to the gelling capacity of bean flours.

4.4.3 Nitrogen solubility:

Raw moth bean flour had minimum nitrogen solubility of 18 per cent around pH 4.5 (Fig 3). On either side of this pH it increased. At pH 2.0 about 80 per cent of nitrogen was soluble and at pH 12.0 it was about 96 per cent. The results of present investigation partly agrees with the reports of Borhade et al. (1984) who observed minimum nitrogen solubility at pH 4.5 and maximum at pH 2.0 and 9.0 in case of moth bean. The decrease in nitrogen solubility beyond pH 9.0 observed by Borhade et al. (1984) may be due to salting in effects of extractants used by them in extracting soluble nitrogen from moth bean.

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- RAW
- +—+ SOAKED IN WATER FOR 12 h
- SOAKED IN SALT SOLUTION FOR 3 h
- SOAKED IN SALT SOLUTION FOR 6 h
- SOAKED IN SALT SOLUTION FOR 9 h
- SOAKED IN SALT SOLUTION FOR 12 h

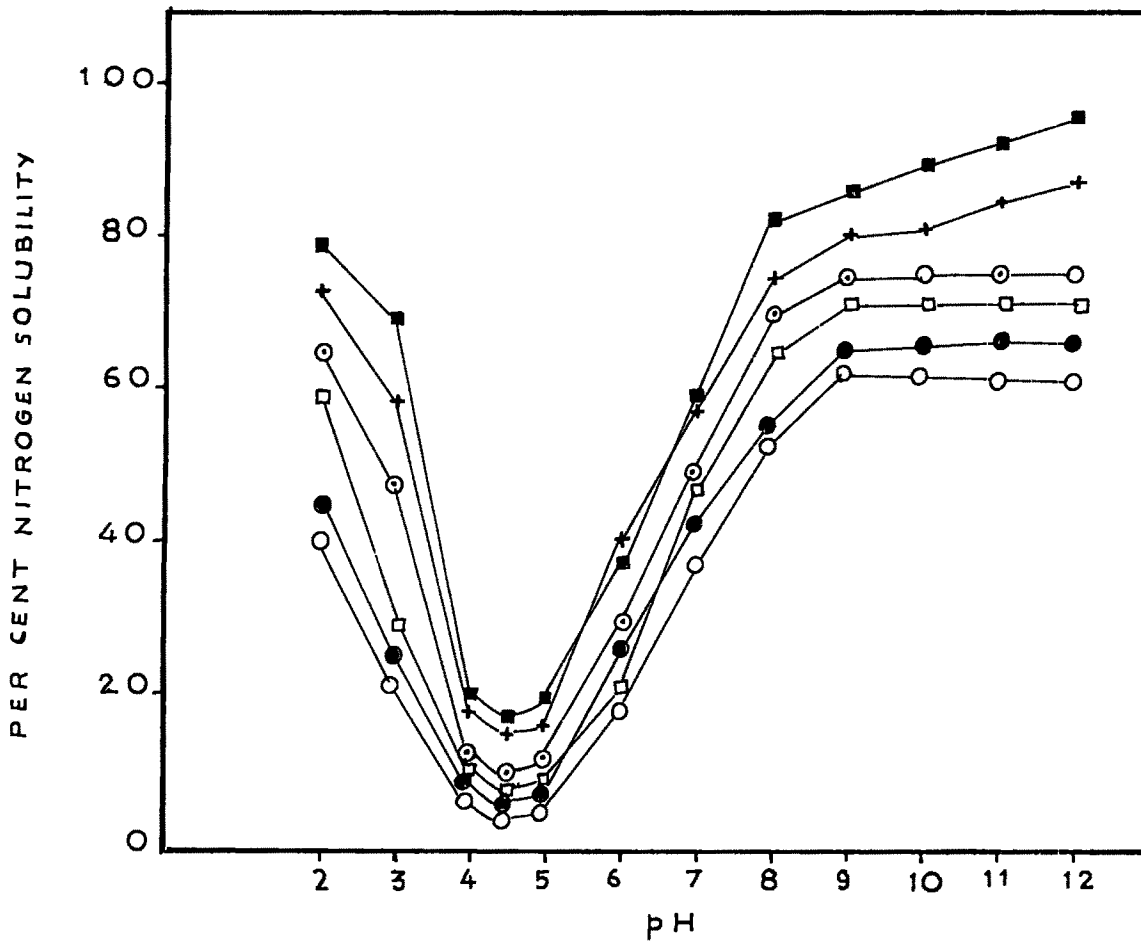


Fig 3 NITROGEN SOLUBILITY Vs pH PROFILE OF RAW AND SOAKED MOTH BEAN FLOURS

In the present investigation, nitrogen solubility of unsoaked and 12 h water soaked moth bean flours was found increased even beyond pH 9.0 but there was no significant improvement in nitrogen solubility in rest of the cases (salt soaked samples). However, it remained more or less constant upto pH 12.0. Narayana and Rao (1982) observed a minimum nitrogen solubility of 23 per cent around pH 4.5 and maximum of 95 per cent at pH 10.5 in case of heat processed winged bean. Smith and Circle (1972) also observed similar nitrogen solubility behaviour of soy flour with a minimum of 10 per cent around pH 4.0 and a maximum of 95 per cent at pH 10.0.

The nitrogen solubility decreased in water- and salt soaked moth bean flours at all pH's studied (Fig 3). As the time of soaking in salt solution increased the solubility of nitrogen decreased at all pH's studied. The main difference in nitrogen solubility was at pH 2.0, 3.0, 8.0, 9.0, 10.0, 11.0 and 12.0 in all soaked flours. Decrease in the nitrogen solubility of 12 h water soaked flour may be due to decrease in protein content and of salt soaked flours at 3,6,9 and 12 h may be partly due to decrease in protein content and partly due to protein denaturation during blanching prior to soaking. The results of the present investigation fairly agrees with the results of del Rosario and Flores (1981) on mung bean flours.

They reported lower nitrogen solubility of mung bean protein at the isoelectric point (pH 4.0-5.0) and at pH 2.0 and 6.0 on blanching mung bean in boiling water for 15 min. However, they did not find improvement in nitrogen solubility beyond pH 6.0.

4.4.4 Foaming properties:

Soaking moth bean in either distilled water or salt solution caused significant decrease in foam volume of the flour (Table 6). The per cent volume increase on whipping 1 per cent aqueous dispersions was less in both distilled water and salt soaked moth bean flours than unsoaked moth bean flour. The probable reason for decrease in per cent foam volume in soaked moth bean flours was decrease in the protein content since foaming capacity of flours depend on the nature and amount of proteins.

Soaking also significantly decreased the specific volume of foams (Table 6) indicating poor foaming and air uptake during whipping.

The foam stability results are presented in Table 7. The decrease in total volumes after soaking moth bean in distilled water and salt solution for 12 h was 8.9 and 9.0 per cent, respectively, compared to 7.0 per cent for unsoaked moth bean flour. The corresponding decrease in foam volume was 35.4, 37.9 and 31.4 per cent, respectively. The foam stability was comparatively more in unsoaked

Table 6. Effect of soaking on foaming properties of moth bean flours.

Treatment	Volume after whipping (ml)		Per cent volume increase	Specific volume (ml/g)
	Total volume	Foam volume		
Control (dry bean)	127	35	27	1.30
Soaking ^a	123	31	23	1.23
Soaking ^b for				
3 h	126	34	26	1.28
6 h	125	32	25	1.25
9 h	124	31	24	1.22
12 h	121	29	21	1.20
SE $\pm$	0.86	0.81		0.010
LSD(P=0.05)	2.64	2.50		0.030

^a Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^b Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

moth bean flours than water- or salt soaked moth bean flours indicating that foam stability was more in flours containing native proteins. The same observations were also reported by Yasumatsu et al. (1972) and Lin et al. (1974) wherein they correlated foam stability with the amount of native proteins present.

Table 7. Foam stability of soaked moth bean flours.

Treatment	Volume (ml) at room temperature (25°C ± 2) after min.										per cent decrease over 120 min	
	0		30		60		90		120			
	Total	Foam	Total	Foam	Total	Foam	Total	Foam	Total	Foam		
Control (dry bean)	127	35	126	33	123	31	120	28	118	24	7.0	31.4
Soaking ^a	123	31	120	29	118	27	115	23	112	20	8.9	35.4
Soaking ^b for												
3 h	126	34	124	32	122	28	120	26	116	23	7.9	32.3
6 h	125	32	131	30	120	28	116	24	114	21	8.8	34.3
9 h	124	31	122	29	118	27	116	23	113	20	8.8	35.4
12 h	121	29	118	27	115	25	113	23	110	19	9.0	37.9
SE ±	0.86		0.81				0.86		1.07			
LSD(P=0.05)	2.64		2.50				NS		NS			

^a Soaking in distilled water for 12 h at 25°C ± 2.

^b Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C ± 2.

The increased concentration of the flours in aqueous dispersions improved the foaming capacity (Table 8).

Table 8. Effect of concentration on foaming capacity of soaked moth bean flours.

Treatment	Per cent increase in volume after whipping						
	Concentration (per cent wt/vol)						
	1	2	3	4	5	7	10
Control (dry bean)	27	55	61	65	70	78	98
Soaking ^a	23	45	52	58	63	75	91
Soaking ^b for							
3 h	26	50	55	62	69	78	96
6 h	25	48	54	60	67	77	95
9 h	24	46	53	61	65	75	90
12 h	21	45	51	58	63	74	88
SE $\pm$	0.49						
LSD(P=0.05)	1.50						

^aSoaking in distilled water for 12 h at $25^{\circ}\text{C} \pm 2$.

^bSoaking in mixed salt solution for 3,6,9 and 12 h at $25^{\circ}\text{C} \pm 2$.

However, the increase was nearly double in 2 per cent aqueous dispersions in all the treatments. The increase in foaming capacity was lowest in 12 h salt soaked moth bean flour than 9,6,3 h salt soaked and 12 h water soaked

moth bean flours. The decrease in foaming capacity with increasing solids concentrations in salt soaked moth bean flours may be attributed to the decreased protein contents. Beyond 2 per cent concentration, the increase in foam volumes in all the cases was not so conspicuous. Deshpande et al. (1982) reported nearly double increase in foaming capacity in dry beans at 2 per cent solids concentration. The results of the present investigation are in conformity with the results of Deshpande et al. (1982).

Foaming capacities of soaked and unsoaked moth bean flours were found to be pH dependent (Fig 4). Soaking considerably lowered the foaming capacity of moth bean flours at all pH's studied. At isoelectric point of moth bean proteins (pH 4.5), a volume increase of 26.0 per cent was observed for 12 h salt soaked flour compared to 31.0 per cent for raw flour. On either side of the pH 4.5, the foaming capacity was increased in all the samples. The increase was observed continuously upto pH 12.0 in all the samples. The decrease in foaming capacity in salt soaked moth bean flours than raw and water soaked moth bean flours at all pH's studied may be due to decrease in the protein content.

4.4.5 Emulsion properties:

Emulsion properties of raw and soaked moth bean flours are presented in Table 9. Emulsifying activity in per cent of raw control dry bean flour and protein was 20.2 and

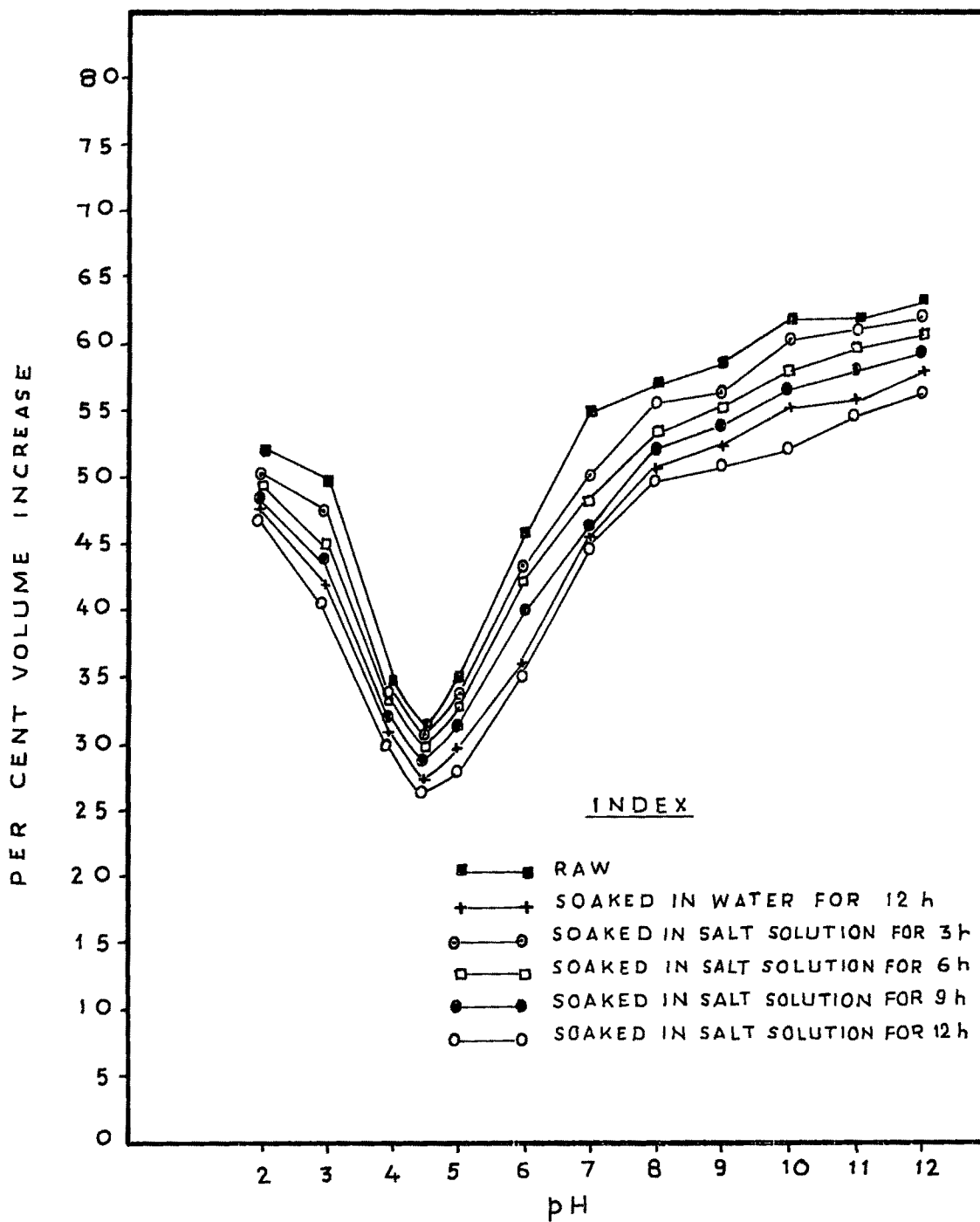


Fig 4 FOAMING CAPACITY VS pH PROFILE OF 2 PER CENT (W/V) AQUEOUS DISPERSIONS OF RAW AND SOAKED MOTH BEAN FLOURS

Table 9. Effect of soaking on emulsion properties of moth bean flours.

Treatment	Emulsifying activity (per cent)		Emulsion stability ^a	
	Flour	Protein ^b	Flour	Protein ^b
Control (dry bean)	20.2	85.9	12.0	51.0
Soaking ^c	16.5	77.4	10.5	49.2
Soaking ^d for				
3 h	18.1	79.0	11.2	48.9
6 h	17.4	78.3	11.0	49.5
9 h	16.8	77.7	10.6	49.5
12 h	16.0	75.8	10.2	48.3
SE $\pm$	0.078		0.088	
LSD (P=0.05)	0.240		0.272	

^a Per cent of the original emulsifying activity after heating at 80°C for 30 min.

^b Expressed on crude protein basis (the oil emulsified for protein).

^c Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^d Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

85.9, respectively, which was found decreased significantly on soaking moth bean either in distilled water or salt solution. However, both emulsifying activity and stability

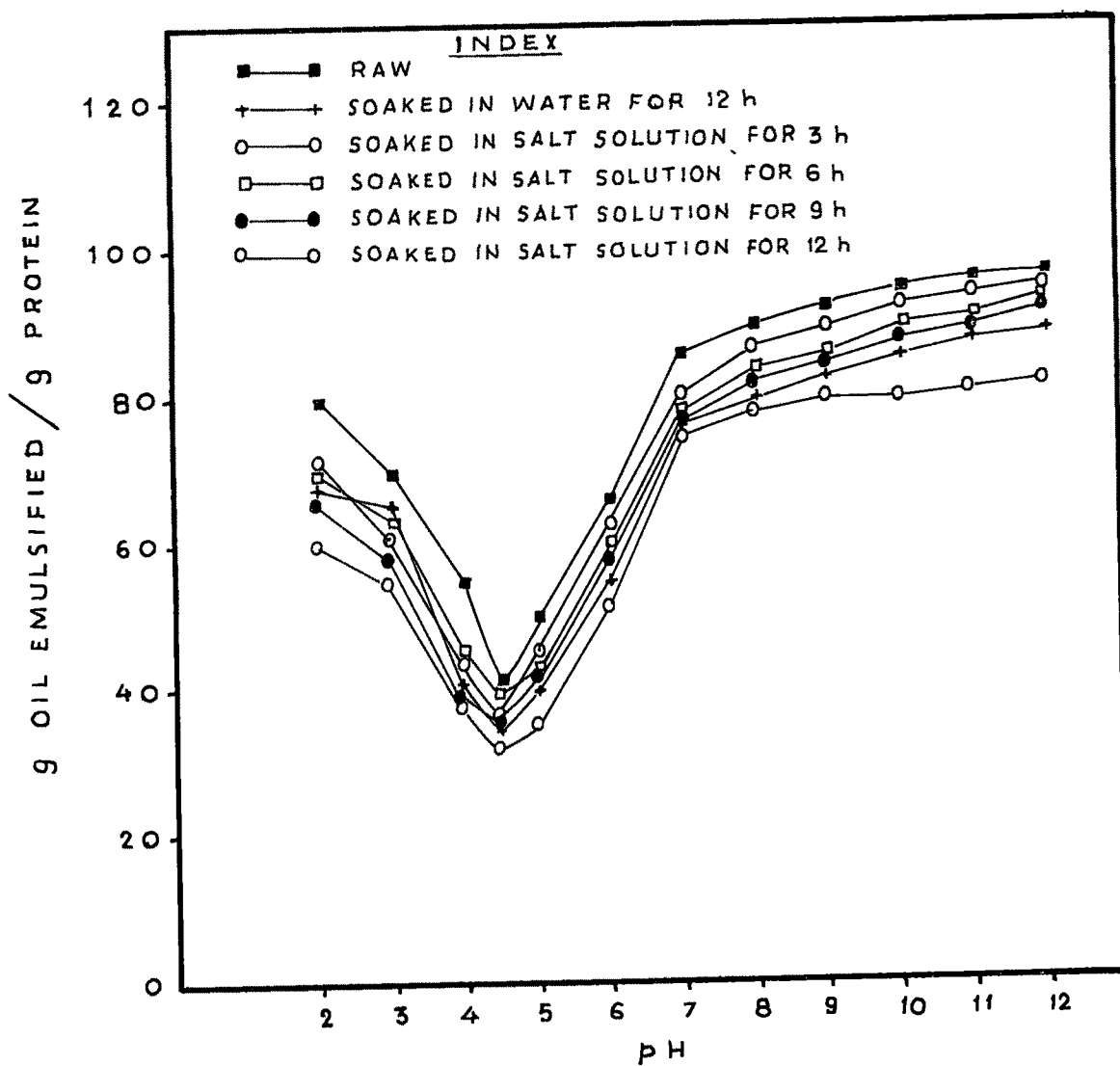


Fig.5 EMULSIFICATION CAPACITY VS pH PROFILE OF RAW AND SOAKED MOTH BEAN FLOURS

of 12 h water soaked flour were comparatively better than 12 h salt soaked flours. As the time of soaking in salt solution increased from 3 to 12 h, the emulsion properties decreased significantly. The decrease in emulsion properties of soaked moth bean flours may be due to decrease in protein content.

Emulsion stability in per cent of the original emulsifying activity was also decreased significantly on soaking either in distilled water or salt solution.

The effect of pH in the range of 2-12 on the emulsification capacity of raw and soaked moth bean flour (Fig 5) showed that emulsification capacity curve resembled the nitrogen solubility curve (Fig 3). The emulsification capacity of salt soaked flour decreased at all pH's studied, probably because of decrease in protein content.

4.5 Effect of soaking on phytate phosphorus and phytate of moth bean flours:

Data on phytate phosphorus, total phosphorus and phytic acid of dry raw and soaked moth bean flours are presented in Table 10. The total phosphorus remained more or less constant during soaking. The phytate phosphorus, however, decreased significantly from 61.7 per cent of dry bean flour to 51.5 per cent of 12 h salt soaked bean flours. Reduction in phytic acid on soaking moth bean in salt solution for 12 h was much greater (18.9 per cent) as compared to those for beans soaked in distilled water for

Table 10. Effect of soaking on phytate phosphorus and phytate of moth bean flours.

Treatment	Total P (mg/g)	Phytate P (mg/g)	Phytate P (per cent of total)	Calculated phytic acid ^a (mg/g)	Per cent reduc- tion in phytic acid
Control (dry bean)	3.4	2.1	61.7	7.4	-
Soaking ^b	3.4	2.0	58.8	7.0	5.4
Soaking ^c for 3 h	3.4	1.9	55.8	6.7	9.4
6 h	3.3	1.8	54.5	6.3	14.8
9 h	3.4	1.8	52.9	6.3	14.8
12 h	3.3	1.7	51.5	6.0	18.9
SE $\pm$	0.010	0.042			
LSD(P=0.05) NS		0.130			

^a Calculated phytic acid (phytate) assuming 28.20 per cent phosphorus in the molecule.

^b Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^c Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

12 h (5.4 per cent). It was further interesting to note that phytic acid reduction in beans soaked in salt solution for 3 h was also greater (9.4 per cent) as compared to those for beans soaked in distilled water for 12 h

(5.4 per cent). Deshpande and Cheryan (1983) observed on dry beans (Phaseolus vulgaris) that the losses of phytic acid on soaking in 2 per cent sodium bicarbonate and a mixed salt solution (2.5 per cent NaCl + 1.0 per cent sodium tripolyphosphate + 1.5 per cent NaHCO_3 + 0.5 per cent NaCO_3) for 12 h each, were much greater as compared to those for beans soaked in distilled water for 6-18 h. The average loss of phytic acid content observed by them was 1.3 per cent for beans soaked in distilled water for 18h, and 3.9 and 6.9 per cent, respectively, for those soaked for 12 h each in 2 per cent sodium bicarbonate and mixed salt solutions. The results of the present investigation are in agreement with those of Deshpande and Cheryan (1983) but contradictory to the results of Iyer et al. (1980b) who observed greater reduction in phytate in dry beans (Phaseolus vulgaris) on soaking in distilled water for 18 h than on soaking in mixed salt solution, similar in composition, for 18 h. However, they reported 20 to 30 per cent reduction in phytate content in beans soaked in mixed salt solution over control dry beans. They claimed that less reduction in phytate in salt soaked beans than in water soaked beans may be due to lower diffusibility of phytate during rehydration when beans were soaked in salt solution and it was possible that divalent cations, i.e. calcium and magnesium, released from protein-carbohydrate complexes during rehydration in the

salt solution, react with soluble phosphate-phytate to form insoluble or nondiffusible calcium and/or magnesium phytate compounds.

The data presented here indicated that mixed salt solution, when used as soaking medium, effectively remove phytate of moth bean to a greater degree than when water was used. Soaking in sodium bicarbonate and mixed salt solutions is known to reduce the cooking times of beans (Iyer et al. 1980a; Beuno et al. 1980; Rockland and Metzler, 1967 and Kadam et al. 1981). Quick-cooking bean processes usually involve soaking of beans in mixed salt solutions as a preliminary step (Iyer et al. 1980b; Rockland and Metzler, 1967 and Kadam, et al. 1981). According to Mattson (1946), loss of solids during soaking may be influenced by the concentration gradient (difference in chemical potential), which governs the rate of diffusion, and by the physical barriers in cotyledon cell walls and seed coat, which limit the diffusion of water or the soaking medium. The greater loss of phytic acid observed in beans soaked in salt solution, in the present investigation, may be due to the effects of these chemicals in creating an ionic environment. Under such conditions, changes in the seed coat permeability may be much greater and rapid, thus allowing higher losses.

4.6 Effect of soaking on calcium, magnesium iron and zinc content of moth bean flours:

The data on divalent cations viz. calcium, magnesium, iron and zinc presented in Table 11 revealed that there were little changes in their concentrations due to soaking

Table 11. Effect of soaking on calcium, magnesium, iron and zinc content of moth bean flours.

Treatment	Calcium (mg/g)	Magnesium (mg/g)	Iron (mg/g)	Zinc (mg/g)
Control (dry bean)	2.00	2.32	0.105	0.035
Soaking ^a	1.82	2.30	0.100	0.025
Soaking ^b for				
3 h	1.87	2.25	0.095	0.030
6 h	1.87	2.30	0.098	0.032
9 h	1.80	2.21	0.095	0.035
12 h	1.78	2.20	0.090	0.035
SE $\pm$	0.044	0.008	0.001	0.0003
LSD(P=0.05)	0.135	0.025	0.004	0.0009

^a Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^b Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

the beans either in distilled water or salt solution. The little loss of each mineral may be due to leaching of minerals from beans into external medium. The little more loss of calcium and magnesium in beans soaked in salt solution than in beans soaked in distilled water may

be attributed to the insoluble complexes between calcium-phytate, calcium-protein and magnesium-phytate and magnesium-protein because of cationic environment change on soaking in salt solution.

4.7 Effect of soaking on polyphenols of moth bean flours:

The polyphenols expressed as tannic acid decreased from 1.45 mg/g to 0.88 mg/g in beans soaked in distilled water for 12 h and to 0.51 mg/g in beans soaked in salt solution for 12 h (Table 12). The per cent reduction in polyphenols was 39.3 in water soaked beans and 64.8 in salt soaked beans, both for 12 h. The reduction of bean polyphenols on soaking in salt solution increased significantly with the time of soaking. Iyer et al. (1980a) observed on dry beans (Phaseolus vulgaris) 45 to 59 and 65 to 88 per cent loss of phenolics content on soaking in distilled water and mixed salt solution, respectively, both for 18 h. Deshpande and Cheryan (1983) reported on dry beans (Phaseolus vulgaris) the average loss of tannins as 17, 41 and 49 per cent when beans were soaked in water for 6, 12 and 18 h, respectively, and as 78.7 and 83.5 per cent when beans were soaked in sodium bicarbonate and mixed salt solution, respectively. Barroga et al. (1985) observed 25 to 50 per cent reduction in polyphenols content in mung bean on soaking in distilled water for 18 h at 30°C. The reports of the present investigation coincide with the reports of Deshpande and Cheryan (1983) and

Table 12. Effect of soaking on polyphenols content of moth bean flours.

Treatment	Polyphenols (mg of tannic acid/g)	Per cent reduction in polyphenols
Control (dry bean)	1.45	-
Soaking ^a	0.88	39.3
Soaking ^b for		
3 h	1.00	31.0
6 h	0.80	44.8
9 h	0.63	56.5
12 h	0.51	64.8
SE $\pm$	0.12	
LSD (P=0.05)	0.39	

^a Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^b Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

Iyer et al. (1980a). The loss of polyphenols observed on soaking moth bean may be due to leaching of polyphenols in soaking medium. Polyphenols are characteristically located in the seed coat of certain pigmented cultivars of millets and legumes. Since they are present in the outermost layers of the seed, they may be more prone to leaching losses. Reduction in polyphenols content of legumes after soaking in different media has been reported

by several workers (Sathe and Salunkhe, 1981; de Lumen and Salamat, 1980 and Bressani and Elias, 1979). However, a caution must be exerted in interpreting the results. It is possible that at least some polyphenols may diffuse into the seed and bind with proteins. When bound to proteins, polyphenols are usually not detected by the procedures routinely employed for polyphenols analysis and results into lower estimates (Butler et al., 1979). Price and Butler (1979) observed poor weight gains for rats fed a cooked high-tannin sorghum than did rats fed the uncooked grains, although the former had a lower assayable polyphenols content. These authors suggested that the apparent loss of tannin was caused by the formation of insoluble tannin-protein complexes and which were not assayed by the method employed to determine the tannin content.

The greater removal of polyphenols from beans soaked in salt solution than from beans soaked in water could be because of difference in chemical potential and creating an ionic environment. The changes in seed coat permeability may be much greater and rapid, thus allowed higher losses of polyphenols.

4.8 Effect of soaking on trypsin inhibitor activity of moth bean flour:

Data on trypsin inhibitor activity of raw and soaked beans are summarised in Table 13. Similar trends i.e.

Table 13. Effect of soaking on trypsin inhibitor activity of moth bean flours.

Treatment	Trypsin inhibitor activity			
	TIU ^a /g flour $\times 10^3$	Per cent decrease	TIU ^a /g protein $\times 10^3$	per cent decrease
Control (dry bean)	4.2	-	17.8	-
Soaking ^b	4.1	2.3	16.2	8.9
Soaking ^c for				
3 h	3.8	9.5	16.5	7.3
6 h	3.7	11.9	16.6	6.7
9 h	3.6	14.2	16.6	6.7
12 h	3.4	19.0	16.1	9.5
SE $\pm$	0.011			
LSD (P=0.05)	0.036			

^a Trypsin inhibitor unit expressed in terms of tryptic units inhibited, the tryptic unit being defined as the increase of 0.01 absorbance unit at 280 nm in 30 min of digestion under the experimental conditions.

^b Soaking in distilled water for 12 h at 25°C $\pm$ 2.

^c Soaking in mixed salt solution for 3,6,9 and 12 h at 25°C $\pm$ 2.

greater losses after soaking beans in mixed salt solution than after soaking in water, were also observed for the trypsin inhibitor and its activity was decreased from

4.2×10^3 to 3.4×10^3 TIU/g flour during 12 h soaking in salt solution. Similar results were also reported by Deshpande and Cheryan (1983) and Iyer et al. (1980b) on dry beans. The greater losses in trypsin inhibitor in beans soaked in salt solution than in beans soaked in water may be due to much greater and rapid seed coat permeability, which might have allowed to trypsin inhibitor loss.

4.9 Effect of soaking on electrophoretic properties of soluble proteins of moth bean flours:

The phytate and polyphenols are associated with all the protein fractions viz. albumin, globulin, prolamin and glutelin. In moth bean, globulins amounted to approximately 70 per cent of the total protein (Subbulakshmi et al., 1976). It has been shown that phytate and polyphenols reduce the solubility of all the protein fractions. The reduction in solubility of proteins is due to binding of phytate with proteins (Reddy and Salunkhe, 1981 and de Rham and Jost, 1979) and polyphenols with proteins (Bressani and Elias, 1979). By using polyacrylamide gel electrophoretic technique, attempts have been made to study the properties of phytate-protein and polyphenols-protein complexes formed when phytate and polyphenols of moth bean get mixed with its protein.

The soluble proteins from raw and processed moth bean flour were extracted in Tris-HCl buffer (0.05 M, pH 7.2)

and electrophoresed in 7.5 per cent polyacrylamide gel. The results are presented in Table 14 and Fig 6. The bands having Rf value (electrophoretic mobility) less than 0.3 were grouped as slow moving and more than 0.7 as fast moving while bands with Rf value between 0.3 to 0.7 were grouped as intermediate mobility bands.

Table 14. Rf values of soluble proteins of raw and soaked moth bean flours.

	Soaking time (h)				
0	12 ^a	3	6	9	12
0.04	-	-	-	-	-
0.08	-	-	-	-	-
0.12	-	-	-	-	0.13
0.16	-	0.15	0.16	0.17	0.18
0.36	0.36	0.36	0.35	0.36	0.35
0.40	0.40	0.41	0.40	0.42	0.42
0.70	0.68	0.69	0.70	0.70	0.71
0.77	-	-	0.78	0.79	0.78
0.84	-	-	-	-	-
0.97	-	-	-	-	-

^a Soaking in distilled water for 12 h at 25°C $\pm$ 2. Remaining soaking times in mixed salt solution are 3,6,9 and 12 h at 25°C $\pm$ 2.

The soluble proteins from raw and processed moth bean flour resolved into 10 bands with 4 slow moving and 4 fast

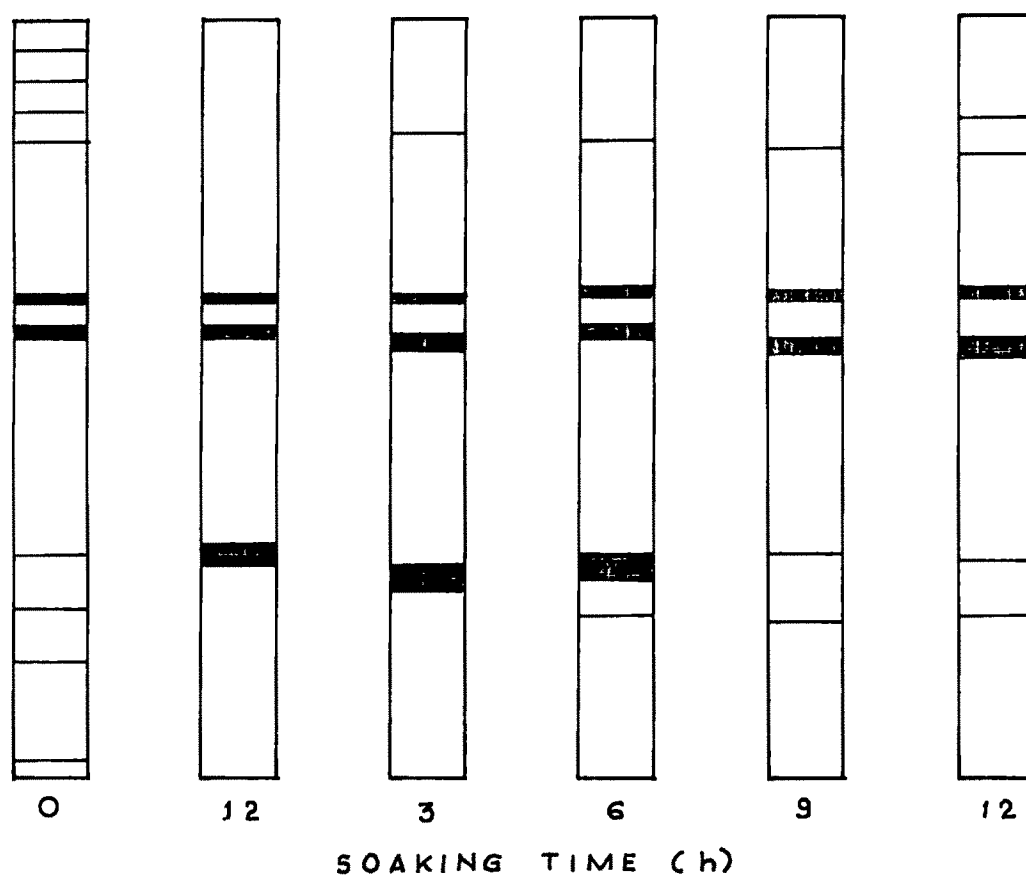


Fig.6 GEL ELECTROPHORETIC PATTERNS OF SOLUBLE PROTEINS OF SOAKED MOTH BEAN FLOURS

moving bands. The remaining 2 bands were the major bands while 8 bands were faint. The band with Rf 0.40 exhibited highest intensity.

Electrophoretic analysis of moth bean proteins extracted in phosphate buffer (0.03 M, pH 7.5) containing 1 M NaCl had revealed 8 bands of which 2 were the major bands (Subbulakshmi et al., 1976). The separation pattern of soluble proteins obtained in the present investigation fairly agrees with the pattern reported by Subbulakshmi et al. (1976). However, 1 more slow and fast moving faint band each were detected in moth bean flours. This may be due to varietal differences or the extraction medium.

The separation pattern of soluble proteins of soaked moth bean flours at different time (Table 14 and Fig 6) showed that proteins separate into less than 10 bands as observed in raw unsoaked bean flours but more than 3 bands as observed in 12 h water soaked flour. The total bands observed in 3,6,9 and 12 h mixed salt soaked flours were 4,5,5 and 6, respectively.

4.10 Effect of soaking on in vitro protein digestibility of moth bean flours:

Results of earlier experiments have shown that the phytate and polyphenols present in the moth bean can be effectively removed by soaking. Therefore, it was of interest to study the effect of soaking on the in vitro protein digestibility of moth bean flours.

The IVPD of moth bean flours after soaking is shown in Table 15. The water and salt soaked flours showed higher IVPD values than control dry bean flour.

Table 15. Effect of soaking on IVPD of moth bean flours.

Treatment	In vitro protein digestibility		Per cent IVPD
	μ moles of leucine formed per g per 4 h		
	Pepsin	Trypsin	
Control (dry bean)	124	132	69.0
Soaking ^a	131	138	77.0
Soaking ^b for			
3 h	125	133	72.0
6 h	130	136	73.4
9 h	133	140	73.5
12 h	138	147	78.2
SE $\pm$	0.65	0.78	
LSD (P=0.05)	2.00	2.40	

^a Soaking in distilled water for 12 h at $25^{\circ}\text{C} \pm 2$.

^b Soaking in mixed salt solution for 3,6,9 and 12 h at $25^{\circ}\text{C} \pm 2$.

The 12 h salt soaked flour also showed better IVPD than 12 h water soaked flour. The IVPD was increased progressively with soaking period. The results of IVPD

obtained for pepsin and trypsin were almost similar. In all samples salt soaked flours showed greater susceptibility towards enzymatic hydrolysis than dry beans.

The per cent IVPD values were also more in salt soaked flours than control. Shastri et al. (1982) reported increase in IVPD of moth bean during combined process of soaking for 4 and 8 h and roasting. Similarly, Iyer et al. (1980a) reported better IVPD of salt soaked cooked beans than conventionally cooked beans (Phaseolus vulgaris). Romero and Ryan (1978) have shown that tannins affect IVPD of bean proteins. In the present study salt soaked bean flours registered higher susceptibility to enzymatic hydrolysis than did unsoaked bean flour. This may be due to the interactions between polyphenols and proteins and phytate and proteins, rendering the latter less susceptible to enzymatic hydrolysis. The improvement in IVPD may be partially due to reduction in phytate, tannins and trypsin inhibitor during soaking.

4.11 Effect of germination on proximate composition of moth bean flour:

It is well known that during the initial stages of germination, seeds utilize many stored substances to provide substrate and energy for various processes of growth. A net loss of dry weight occurs as a result of oxidation and breakdown of stored components (Mayer and Poljakoff-Mayber, 1963). The data in Table 16 indicates

the changes in proximate composition of moth bean during 72 h germination. There was net increase in moisture and ash content. Increase in moisture content may be due to gradual absorption of moisture by the beans with corresponding decrease in the dry weight. During 72 h germination period, the changes in dry weights and ash contents of moth bean seeds are shown in Fig 7. At the end of 72 h germination period, the dry weight was reduced to 97.98 per cent. This concurs with the earlier observations of Hall and Hodges (1966), Mayer (1956) and Reddy et al. (1978). The total ash content increased gradually throughout

Table 16. Effect of germination on proximate composition of moth bean flour.

Per cent composition	Period of germination (h)			
	0	24	48	72
Moisture	10.5	12.0	12.8	13.6
Crude protein (N x 6.25)	23.5	24.1	24.4	24.5
Crude fat	1.61	1.44	1.43	1.42
Ash	3.32	4.18	4.32	4.44
Total carbohydrates	71.5	70.2	69.8	69.6

the germination period. Reddy et al. (1978) have also observed increase in ash content in black gram during 10 day germination period. Kylan and McCready (1975) studied the nutrients in seeds and sprouts of alfalfa,

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DRY WEIGHT
ASH

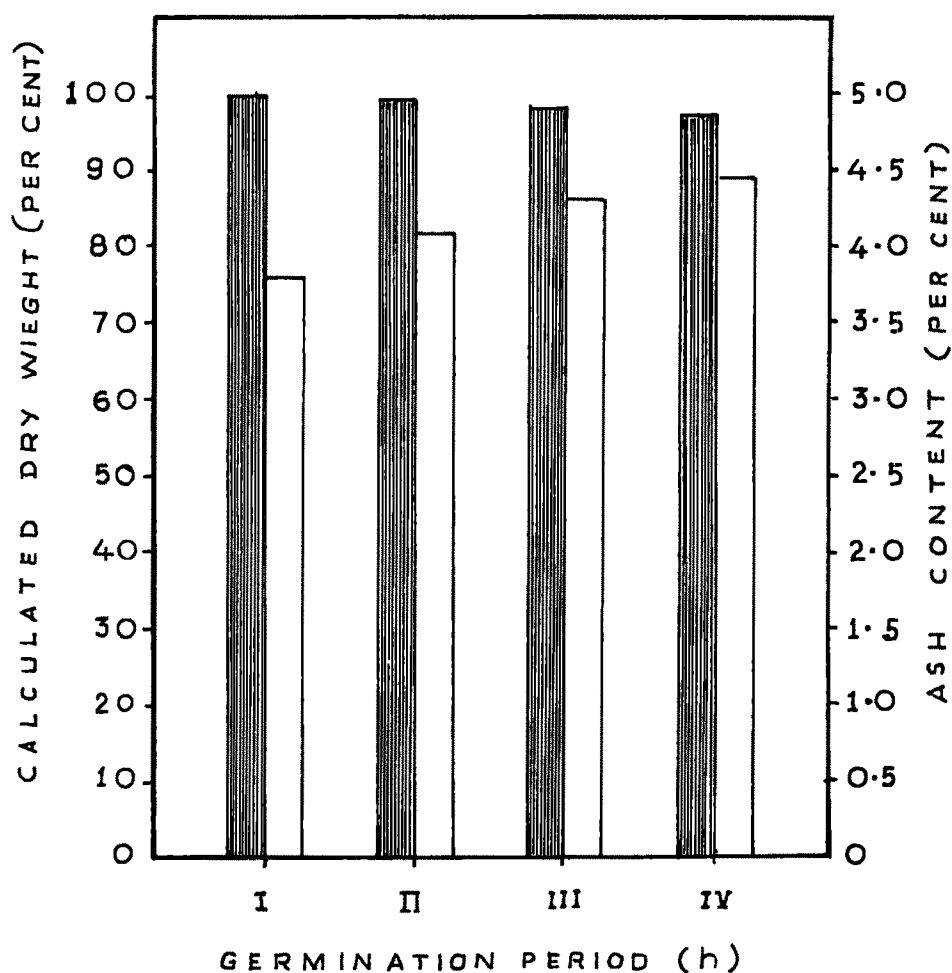


Fig. 7 EFFECT OF GERMINATION ON DRY WEIGHT
AND ASH CONTENT IN MOTH BEAN FLOURS

lentils, murg bean and soybean and attributed the increase in ash content of sprouts to the minerals imbibed from water.

Germination had very little effect on crude protein (Table 16) content except little increase. Similar findings are reported by Kylen and McCready (1975) who attributed the increase to protein synthesis at the time of sprouting. It is also possible though that the increase in protein was due to changes resulting from the uptake of water during sprouting. Kakade and Evans (1966) have also reported increase in crude protein content in navy beans (Phaseolus vulgaris) on germination for 4 days.

The decrease in carbohydrates content in moth bean during germination may be due to increased activity of carbohydrases. Subbulakshmi et al. (1976) reported decrease in starch and non-reducing sugars and increase in reducing sugars in moth bean and horse gram during 72 h germination period. Decrease in carbohydrate content during germination was also observed by Jaya and Venkataraman (1980) on chickpea and green gram and Noor et al. (1980) on mungo bean.

The decrease in fat content in moth bean during germination may be due to apparent increase in the lipase activity. These results are in confirmity with the results of Jaya and Venkataraman (1980) and Noor et al. (1980).

4.12 Effect of germination on functional properties of moth bean flours:

4.12.1 Water and oil absorption:

It is seen from the Table 17 that dry ungerminated moth bean flour had a water absorption capacity of 2.4 g/g flour and oil absorption capacity of 1.7 g/g flour. The corresponding values in g/g protein were 10.2 and 7.2, respectively. The values of water and oil absorption

Table 17. Effect of germination on water and oil absorption capacity of moth bean flours.

Germination time (h)	Per cent crude protein ^a (N x 6.25)	Water absorption capacity (g/g)		Oil absorption capacity(g/g)	
		Flour	Protein ^b	Flour	Protein ^b
0	23.5	2.4	10.2	1.7	7.2
24	24.1	2.8	11.6	2.1	8.7
48	24.4	3.0	12.2	2.4	9.8
72	24.5	3.6	14.4	2.6	10.4
SE $\pm$		0.008		0.003	
LSD(P=0.05)		0.026		0.010	

^a Expressed on dry weight basis.

^b Expressed on crude protein basis.

capacity of dry ungerminated flour coincides with values reported by Borhade et al. (1984). They reported 2.2 g/g flour and 1.6 g/g flour water and oil absorption capacity,

respectively. The germinated moth bean flours had significantly higher water and oil absorption capacity than that of ungerminated moth bean flours.

During 72 h germination period, the water absorption capacity was exceptionally increased to 3.6 g/g flour and 14.4 g/g protein. This could be attributed to the increased protein content and changes in the quality of proteins upon germination. Increase in water absorption capacity may also be due to more hydrophilic groups of proteins during germination. del Rosario and Flores (1981) also reported increase in water absorption capacity in 36 h sprouted mung bean flour.

The ability of proteins to bind fat is important since fats act as flavor retainer and increase the mouth feel of foods (Kinsella, 1976). The oil absorption capacity was also increased to 2.6 g/g flour and 10.4 g/g protein content. This could be attributed to the increased capacity of the flour to hold the fat globules as the amount of lipophilic proteins increases. del Rosario and Flores (1981) also reported increase in fat absorption capacity in 36 h sprouted mung bean flour.

4.12.2 Gelation:

The germination improved the gelation property of moth bean flours (Table 18). The least gelation concentration for the control dry bean and 72 h germinated bean flours were 9.06 and 9.08 per cent, respectively. The

increased least gelation concentration of the germinated moth bean flours may be due to increased protein content. However, del Rosario and Flores (1981) did not find any increase in gelation capacity of 36 h sprouted mung bean flour over the control.

Gelation involves the formation of a continuous network that exhibits order. Higher protein may enhance the rate at which such a network is formed. The seed coat fractions containing complex carbohydrates might interfere

Table 18. Effect of germination on least gelation concentrations of moth bean flours^a.

Germination time (h)	Per cent crude protein (N x 6.25)	Least gelation concentration (per cent wt/vol)
0	23.5	9.06 $\pm$ 0.01 ^b
24	24.1	9.07 $\pm$ 0.02
48	24.4	9.07 $\pm$ 0.01
72	24.5	9.08 $\pm$ 0.01

^a Each value is the average of nine determinations.

^b Mean plus or minus standard deviation.

in the formation of such continuous network of molecules to form gels. A higher protein concentration thus may be required to overcome their interference with gel formation. During germination, seed coats are loosened, complex

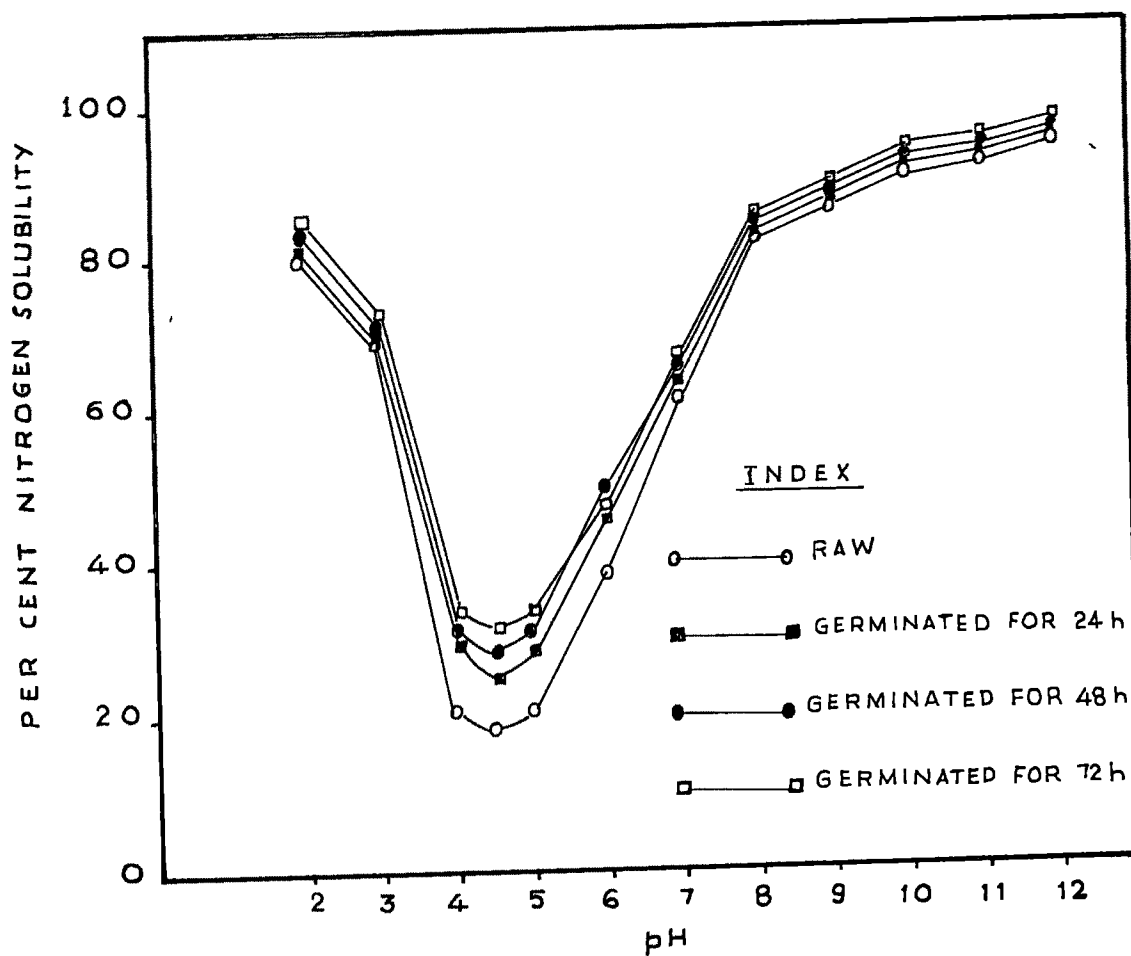


Fig. 8 NITROGEN SOLUBILITY Vs pH PROFILE OF RAW AND GERMINATED MOTH BEAN FLOURS

carbohydrates are minimised, and gelling capacity increased.

The gelation capacity of legume proteins is mainly because of globulin fraction which is the major protein fraction in moth bean flour. The finding and the hypothesis is supported by Fleming et al. (1975) who observed that gelation occurs in the globulin fraction in soybean, fababean and field pea. It was suggested that this property would make the proteins useful in food systems such as weiners and sausage emulsions, custard type puddings and sauces, which require thickening and gelling.

4.12.3' Nitrogen solubility:

Nitrogen solubility versus pH profile of ungerminated and germinated moth bean flours is depicted in Fig 8. It was observed that nitrogen solubility was pH dependent. Ungerminated moth bean flour had minimum nitrogen solubility of 18 per cent around pH 4.5. It increased on either side of pH 4.5. At pH 2.0 about 80 per cent of nitrogen was soluble and at pH 12.0 it was about 96 per cent. Nitrogen solubility behaviour of moth bean flours in the present investigation fairly agrees with the reports of Narayana and Rao (1982) on winged bean and Smith and Circle (1972) on soybean.

The nitrogen solubility increased in germinated moth bean flours at all pH's studied. The increase was more conspicuous in 72 h germinated moth bean flour than 24 h

and 48 h germinated moth bean flours. The main difference in nitrogen solubility was between pH 4 to 6. Legume proteins like those in soybeans whose major components are globulins, are known to be soluble in dilute salt solutions except at their isoelectric points, as reported by Wolf (1970). Nitrogen solubility, however, is affected by germination which results in protein synthesis. The results agreed with those reported by del Rosario and Flores (1981) who showed a narrow pH range of 4 to 5 at the isoelectric point for raw and 36 h sprouted mung bean flours. Similar results were also reported by Fan and Sosulski (1974) for mung bean and field pea. The applicability of these flours in food preparations where maximum solubility of proteins is desired, as in aqueous emulsions, and gel food preparations, looks very promising. Seed protein concentrates, for example, which are soluble at about pH 4-7 could be used in such beverage as vegetable milk.

4.12.4 Foaming properties:

Germination significantly improved both the total and foam volumes of moth bean flours (Table 19). Volume increase on whipping of 1 per cent aqueous dispersions of the ungerminated moth bean flour was 27 per cent which was found increased progressively during germination. The increase in foam volume during germination for 24, 48 and 72 h was respectively 27, 30 and 40 per cent. This suggests that foaming property was dependent on the

solubilized protein. During germination the solubilized proteins increase. del Rosario and Flores (1981), however, did not find any change in foaming capacity of raw and 36 h sprouted mung bean flour. But they reported the increase in the volume of air-classified high protein flour suspension forming a very stable white foam after whipping for 6 min. The same observations were also reported by Yasumatsu et al. (1972) and Lin et al. (1974) wherein they correlated foaming capacity with the amount of native proteins present.

Table 19. Effect of germination on foaming properties of moth bean flours.

Germination time (h)	Volume after whipping (ml)		Per cent volume increase	Specific volume (ml/g)
	Total	Foam		
0	127	35	27	1.30
24	130	38	30	1.33
48	136	41	36	1.38
72	140	45	40	1.43
SE <u>±</u>	0.68	0.28		0.005
LSD (P=0.05)	2.23	0.93		0.017

Germination improved significantly the specific volume of foams, indicating better foaming and air uptake during whipping. The lower air uptake during whipping of

ungerminated moth bean flour was accompanied by a decrease in its foam volume. Specific volume of a foam for a given set of conditions is an indication of air uptake only and is not necessarily a measure of foam quality and stability.

The foam stability results are presented in Table 20. The decrease in total volumes over 120 min was 7.0 per cent for ungerminated and 10.7 per cent for 72 h germinated moth bean flours. The corresponding decrease in foam volumes was 31.5 and 37.7 per cent, respectively. The lower foam stability of 36 h sprouted mung bean flour than the raw mung bean was also reported by del Rosario and Flores (1981). Dashpande et al. (1982) observed 7.8 to 15.5 per cent and 61.8 to 80.0 per cent decrease in total and foam volume, respectively, over 120 min for whole dry bean flours. Similarly, Sosulski et al. (1976) observed a decrease of 18.2 to 38.6 per cent in foam volumes of 10 legume flours over 120 min.

The foam stability is dependent on the amount of native proteins present. del Rosario and Flores (1981) observed a 530 per cent decrease in volume of the flour suspension forming a very stable white foam after whipping of air classified high protein mung bean flour for 6 min over the raw mung bean flour. In the present investigation, though the protein content during germination was apparently increased, the foam stability of germinated moth bean flours was comparatively poor than the ungerminated

Table 20. Foam stability of germinated moth bean flours.

Germination time (h)	Volume (ml) at room temperature 25°C $\pm$ 2 after min.				per cent decrease over 120 min.			
	0	30	60	90	120	Total	Foam	Total
	Total	Foam	Total	Foam	Total	Foam	Total	Foam
0	127	35	126	33	123	31	120	28
					118	24	7.0	31.4
24	130	38	127	33	125	31	122	29
					120	25	7.6	34.2
48	136	41	132	38	130	33	124	30
					122	26	10.2	36.5
72	140	45	135	40	132	35	128	32
					125	28	10.7	37.7
SE $\pm$	0.68 0.28				0.28 0.28			
LSD(P=0.05)	2.23 0.93				0.66 0.93			

moth bean flour indicating thereby that the foam stability is dependent on native proteins present. The same observations were also reported by Yasumatsu et al. (1972) and Lin et al. (1974) wherein they correlated foam stability with the amount of native proteins present.

Foaming capacities of raw and germinated moth bean flours were greatly improved by increasing the concentration of the flour in aqueous dispersions (Table 21). At 10 per cent (wt/vol) solids concentration, the foaming capacity

Table 21. Effect of concentration on foaming capacity of germinated moth bean flours.

Germination time (h)	Per cent volume after whipping						
	Concentration (per cent wt/vol)						
	1	2	3	4	5	7	10
0	27	55	61	65	70	78	98
24	30	60	66	72	76	84	96
48	36	70	74	78	82	95	105
72	40	77	82	86	96	112	120
SE $\pm$	0.40						
LSD($p=0.05$)	1.32						

increased from 27 to 98 per cent in raw and from 40 to 120 per cent in 72 h germinated moth bean flours. The increase in foaming capacity with increasing solids

concentrations in germinated moth bean flours may be attributed to the increased protein contents. However, the increase in foaming capacity was highest at 2 per cent (wt/vol) aqueous dispersion than the rest of the concentrations in all the test samples. It was nearly doubled at 2 per cent concentration. Beyond 2 per cent concentration, the increase in foam volumes in all the cases was not so conspicuous. Deshoande et al. (1982) reported increase in foaming capacity in dry beans when they increased flour concentration from 1 to 10 per cent (wt/vol) aqueous dispersions. Similarly, an increase in foaming capacity and stability of glandless cotton seed flour was reported by Cherry and McVatters (1981) when they increased the flour concentration from 2 to 6 per cent in suspensions at natural pH (about 6.5). These authors observed a decrease in both foaming capacity and stability, however, beyond 6 per cent concentration of the flour. The improvement in foaming properties with increased flour concentration was largely attributed to the increased protein concentration.

Foaming capacity of raw and germinated moth bean flours was pH dependent (Fig 9). The foam capacity versus pH profile of 2 per cent (wt/vol) aqueous dispersions of raw and germinated moth bean flours closely resembled in shape its nitrogen solubility versus pH profile (Fig 8), suggesting that foaming property was also dependent on

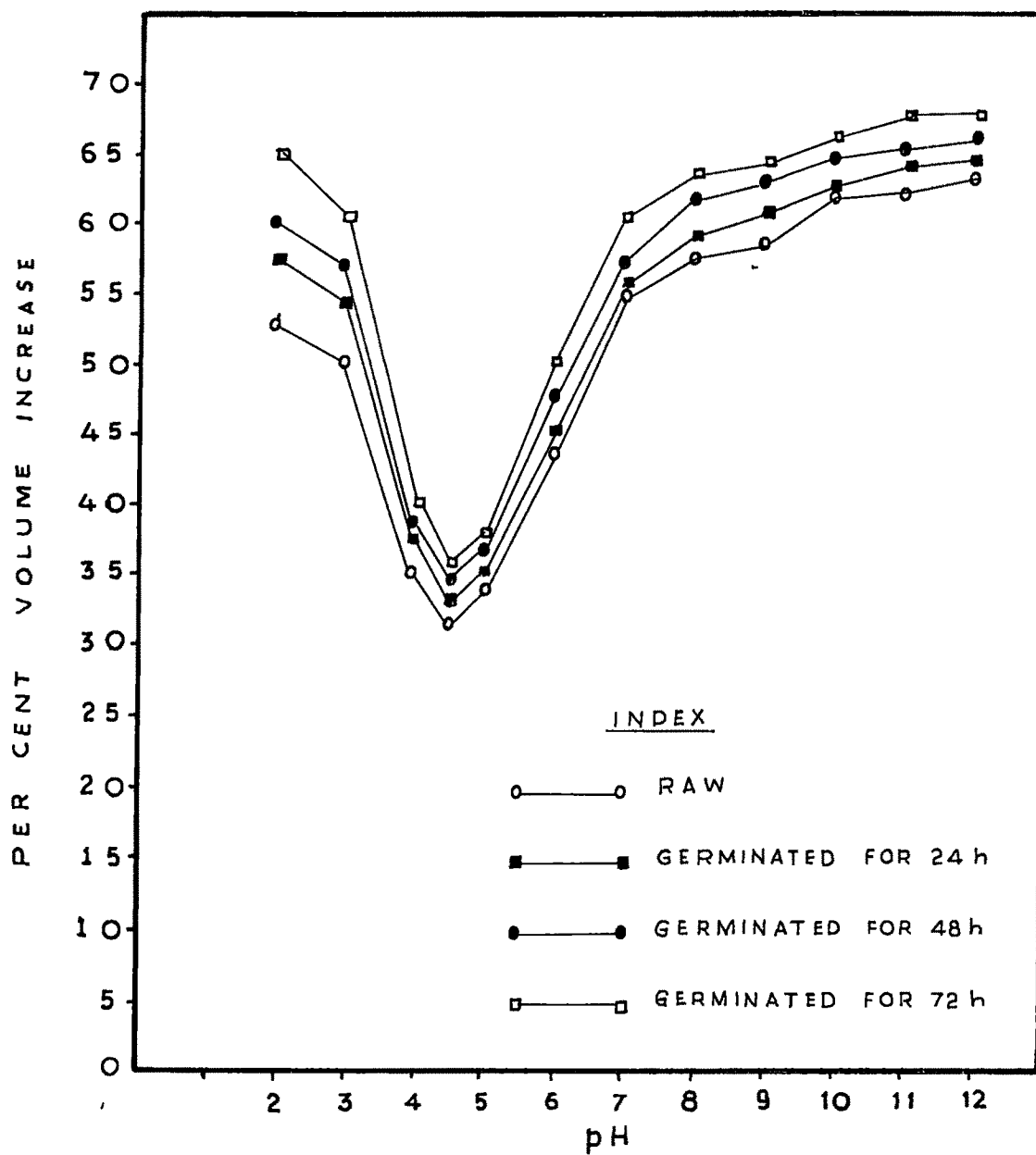


Fig. 9 FOAMING CAPACITY VS pH PROFILE OF 2 PER CENT (W/V) AQUEOUS DISPERSION OF RAW AND GERMINATED MOTH BEAN FLOURS

the amount and solubilized protein. The trend of nitrogen solubility at different pH levels varied with the period of germination. At all the pH values investigated, 72 h germinated moth bean flour had a greater increase in volume than did the corresponding raw moth bean flour. At pH 4.5, the apparent isoelectric pH of moth bean proteins, minimal foaming was observed for all samples. Invariably, the greatest increase in volume on whipping of 2 per cent aqueous dispersions of both raw and germinated moth bean flours was observed at pH 2, 8, 9 and 11. Cherry and McWatters (1981) reported a similar trend (higher foaming at about pH 2.0 and above 8.0) for glandless cotton seed flour. They attributed the optimum foaming properties of glandless cotton seed flour at pH 1.5 and 11.5 to the dissociated proteins and increased solubility of major storage globulins at these pH values. Such pH dependence of foaming characteristics was also reported for soy and sunflower proteins (Lin et al., 1974), succinylated and acetylated sunflower proteins (Cancella et al., 1979), peanut proteins (Sekul et al., 1978) and winged bean proteins (Narayana and Rao, 1982).

4.12.5 Emulsion properties:

Emulsion properties of the raw and germinated moth bean flours are summarised in Table 22. Emulsifying activity (g/g) of raw moth bean flours and protein was

20.2 and 85.9, respectively, which was found increased significantly (27.5 and 110.4 respectively) during germination at all periods. The increase in the emulsifying activity during germination of moth bean may be ascribed partly to more protein being in the suspension for a given amount of oil and partly to the lipolytic

Table 22. Effect of germination on emulsion properties of moth bean flours.

Germination time (h)	Emulsifying activity (per cent)		Emulsion stability ^a	
	Flour	Protein ^b	Flour	Protein ^b
0	20.2	85.9	12.0	51.0
24	23.0	95.4	11.8	48.9
48	25.0	102.4	11.3	46.3
72	27.5	110.4	11.0	44.1
SE $\pm$	0.10		0.064	
LSD(P=0.05)	0.32		0.200	

^a Per cent of the original emulsifying activity after heating at 80°C for 30 min.

^b Expressed on crude protein basis (the oil emulsified, for protein)

products (mono-di-and triglycerides) formed during germination as lipase activity increase considerably. Moreover, increased amount of solubilized proteins

during germination has positive correlation with emulsifying activity. In the case of soybean (Crenwelge et al., 1974), groundnut (Ramanathan et al., 1978) guar (Nath and Rao, 1981) and winged bean proteins (Narayana and Rao, 1982) similar relationship between emulsification capacity and solubilized proteins has been reported.

The emulsion stability of germinated moth bean flours, however, was dropped considerably from 12.0 to 11.0 and 51.0 to 44.1 per cent for flour and protein, respectively, upto 72 h germination time (Table 22). The decrease in emulsion stability during germination may be due to increased proteolytic activity which is responsible for protein hydrolysis. The emulsion properties of flours can not be solely attributed to the proteins and other food components such as carbohydrates and lipids may also contribute aporeciably, possibly through protein-carbohydrate and protein-lipids interactions. These interactions are reduced during germination with concomittant decrease in the emulsion stability. This explanation is also supported by the observations of McWatters and Cherry (1977) who reported that emulsion properties of cowpeas were influenced by high carbohydrate content (about 60 per cent). These authors stated that the starch components may interact with protein and other food components in the system.

The effect of pH on the emulsification capacity of the raw and germinated moth bean flour (Fig 10) showed

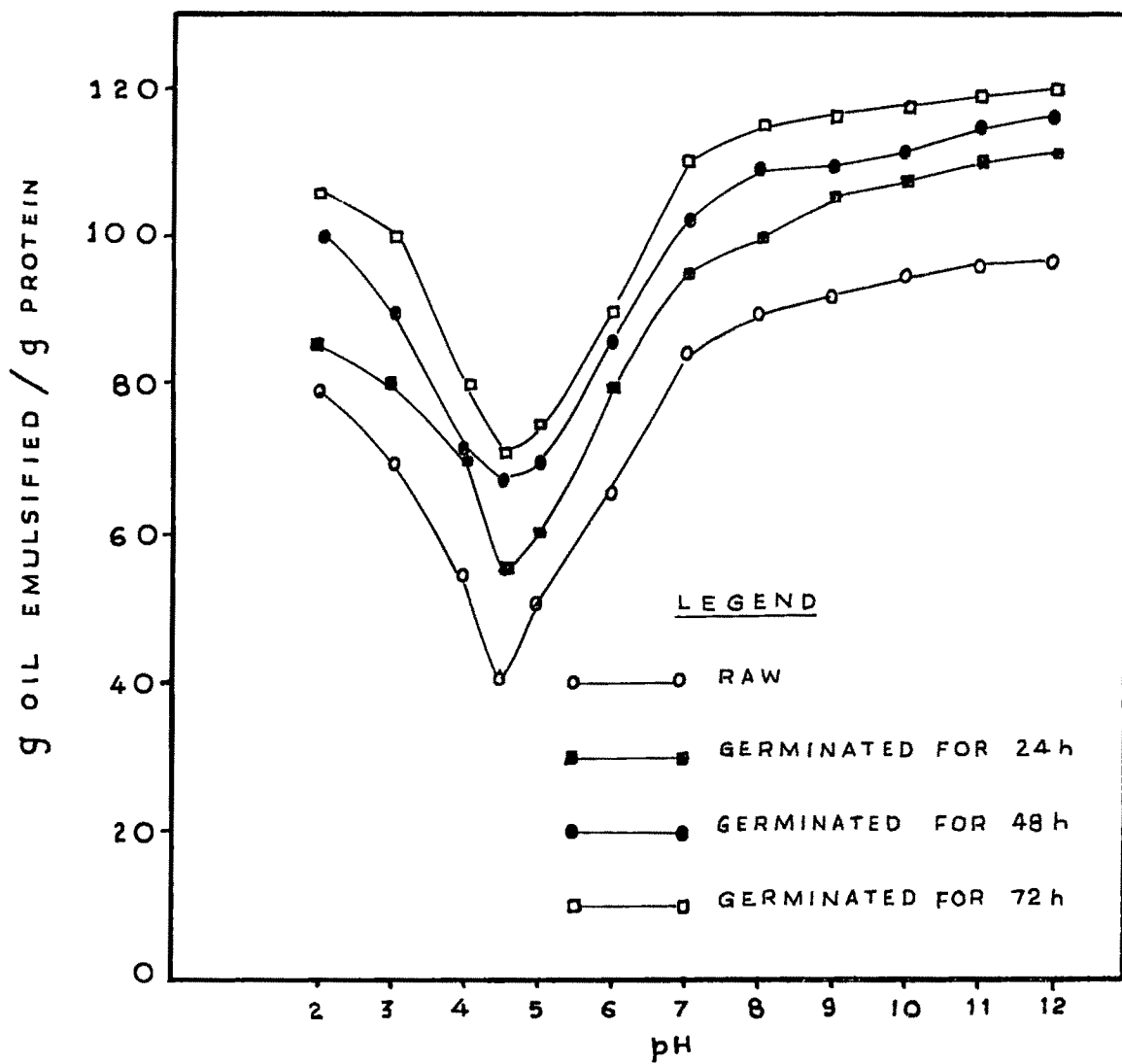


Fig. 10 EMULSIFICATION CAPACITY VS pH PROFILE OF RAW AND GERMINATED MOTH BEAN FLOURS

that emulsification capacity of germinated flours was higher than raw flour. The emulsification capacity of 72 h germinated flour was 106 g/g protein at pH 2.0, 71 g/g protein at pH 12. The emulsification capacity was increased progressively with increase in germination time. At all pH's studied, the emulsification capacity of germinated flours was higher compared to raw flour. The graph of emulsification capacity versus pH profile also resembled the nitrogen solubility versus pH profile. This suggested that emulsification capacity was due to solubilized proteins.

4.13 Effect of germination on phytate phosphorus and phytate of moth bean flours;

It is observed from Table 23 that moth bean, among the legumes, is a good source of phosphorus, as it contains 0.34 per cent phosphorus which is primarily present in seeds. Phytate phosphorus is the primary storage form of phosphates in moth bean seeds (61.7 per cent of total phosphorus). It was reported that phytate phosphorus represents 53.6 to 81.0 per cent of total phosphorus in beans (Phaseolus vulgaris) (Lolas and Markakis, 1975), 78.6 per cent in black gram (Reddy et al., 1978), 68 to 70 per cent in winged bean (Kadam et al., 1982) and 55 and 57 per cent in moth bean and horse gram, respectively (Borhade et al., 1984).

In seeds, the phytate phosphorus is considered to as

Table 23. Effect of germination on phytate phosphorus and phytate content of moth bean flours.

Germination time (h)	Total P (mg/g)	Phytate P (mg/g)	Phytate P (per cent of total)	Calculated phytic acid ^a (mg/g)	Per cent reduction in phytic acid
0	3.4	2.1	61.7	7.4	-
24	3.3	1.6	48.8	5.6	23.7
48	3.2	1.2	37.5	4.2	42.8
72	3.3	1.0	30.3	3.5	52.4
SE $\pm$	0.013	0.009			
LSD(P=0.05)	0.042	0.030			

^a Calculated phytic acid (phytate) assuming 28.20 per cent phosphorus in the molecule.

a store of inorganic phosphorus. Sobolev (1963) has reported that the enzymatic hydrolysis of phytate phosphorus during germination of seeds is accomplished by two phosphatases. One of these phosphatases, namely phytase, is responsible for the initial breakdown of phytate phosphorus upto the stage of formation of inositol monophosphate and the other enzyme accomplishes a complete dephosphorylation of inositol phosphate into inositol and phosphate.

In the present study the phytate phosphorus decreased gradually during initial stages of germination and reached

its nearly 50 per cent on 3rd day of germination. As the germination progressed, there was a continuous decrease in the phytate phosphorus. The phytic acid was also reduced to its nearly 50 per cent during 72 h germination. However, the total phosphorus content remained almost constant during germination. Rao and Deosthale (1983) reported a slight reduction in phytin phosphorus in 48 h germinated green gram and black gram. Borhade et al. (1984) observed a decrease in phytate phosphorus from 55 to 26 per cent in moth bean and from 57 to 20 per cent in horse gram, during 48 h germination. However, Belavady and Banerjee (1953) have observed significant reduction in phytin phosphorus content of pulses only after 72 h germination. This decrease in phytate phosphorus can be attributed to the development of phytase activity during germination. These observations thus indicate that phytase which hydrolyses phytate was probably induced in appreciable amounts in pulses after 48 to 72 h of germination. The development of phytase activity stimulates hydrolysis of phytic acid into inositol and inorganic phosphate (Reddy et al., 1978). The phytate ions form complexes with di-or trivalent metallic ions and in many cases, it forms insoluble compounds. This results in decreases in the availability of minerals. Therefore, breakdown of phytate by the phytase during germination is desirable (Vandersteop, 1981).

4.14 Effect of germination on calcium, magnesium, iron and zinc of moth bean flours:

Data on changes in mineral content of raw and germinated moth bean flours are presented in Table 24.

Table 24. Effect of germination on calcium, magnesium, iron and zinc content of moth bean flours.

Germination time (h)	Calcium (mg/g)	Magnesium (mg/g)	Iron (mg/g)	Zinc (mg/g)
0	2.00	2.32	0.105	0.035
24	1.88	2.35	0.112	0.032
48	1.76	2.40	0.100	0.036
72	1.79	2.54	0.100	0.034
SE $\pm$	0.044	0.012	0.001	0.0007
LSD(P=0.05)	0.145	0.041	0.005	0.0022

Calcium and iron contents in the raw moth bean flour observed to be were 200 mg/100 g and 10.5 mg/100g, respectively which were fairly comparable to the reports of Gopalan et al. (1981). However, the value reported by Borhade et al. (1984) for calcium content in moth bean was extremely less being only 120 mg/100g. The variation in mineral content may be in part due to cultivar differences.

It was observed that during germination, there was

no drastic change in minerals content they were decreased significantly. The decrease was more in calcium and magnesium content. Reddy et al. (1978) also did not find drastic changes in mineral content except slight increase in magnesium content in black gram during 10 day germination period. Jaya and Venkataraman (1980) observed no significant change in calcium and iron content in chickpea and green gram. Rao and Deosthale (1983) observed no significant change in magnesium, iron, zinc and copper content of pigeonpea, green gram and black gram during 48 h germination. However, Borhade et al. (1984) reported significant decrease in calcium content and increase in magnesium content in 48 h germinated moth beans and horse gram. The results of present investigation are in agreement with those of Borhade et al. (1984). The slight decrease in calcium observed in germinated moth bean might be due to leaching in the external medium. However, a possibility of interaction of calcium with other compounds such as phytate or polyphenols to form insoluble complexes can not be ruled out. The slight increase in magnesium content in germinated moth bean seeds may be due to reabsorption of magnesium by beans from external medium.

4.15 Effect of germination on polyphenols of moth bean flours:

Data on polyphenols as tannic acid content of raw and germinated moth bean flours are presented in Table 25. During germination, there was a continuous decrease in polyphenols content of moth bean flours from 1.45 to

Table 25. Effect of germination on polyphenols content in moth bean flours.

Germination time (h)	Polyphenols (mg of tannic acid/g)	Per cent reduction in polyphenols
0	1.45	-
24	1.20	17.2
48	1.16	20.0
72	1.42	2.0
SE $\pm$	0.016	
LSD (P=0.05)	0.054	

1.16 mg/g upto 48 h of germination. Beyond 48 h of germination, however, there was a significant increase in polyphenols from 1.16 to 1.42 mg/g at 72 h of germination. The data showed that maximum reduction (20 per cent) in polyphenols content in moth bean flour occurred at 48 h of germination. Satwadhari *et al.* (1981) observed maximum decrease in polyphenols content in moth bean flour upto 24 h of germination. They, however, observed increase in polyphenols content when moth beans were germinated for more than 24 h. Rao and Deosthale (1982) reported 3 to 20 per cent loss of tannin in the pigeonpea, chickpea, black gram and green gram on 24 h germination over that of 0 h germination. They further reported that continuation

of germination upto 48 h further decreased the tannin content to 40 per cent in chickpea and 50 per cent in green gram and black gram. Rao and Prabhavathi (1982) noted a 20 to 30 per cent loss of tannin content on germination of green gram dhal and bengal gram for 48 h. Recently, Barroga et al. (1985) also reported 23 to 36 per cent reduction in polyphenols content by germination in mung beans. However, they reported maximum reduction (36 per cent) in polyphenols content upto 48 h of germination. Beyond 48 h of germination, they further observed decrease but at a decreasing rate. Continued germination upto 120 h resulted in a decrease in polyphenols removal or an increase in polyphenols content. Kruger (1976) has demonstrated increasing activity of the enzyme polyphenol oxidase with increasing period of germination of wheat. Loss of polyphenols on germination observed here in the moth beans may be attributed to the presence of similar enzyme activity.

In the present experiment the decrease in polyphenols content in moth bean flours upto 48 h of germination may be partly due to increase in the polyphenol oxidase activity and partly due to leaching of polyphenols in the external medium. However, a significant increase in polyphenols content of moth bean flours germinated beyond 48 h may be due to a fresh synthesis of polyphenols or polymerization of existing phenolic compounds or degradation of high molecular insoluble polymers into

small molecular weight soluble polymers that give a color reaction with the Folin-Denis reagent (Satwadhari et al., 1981).

4.16 Effect of germination on trypsin inhibitor activity of moth bean flours:

Changes in trypsin inhibitor activity of raw and germinated moth bean flours are shown in Table 26.

Table 26. Effect of germination on trypsin inhibitor activity of moth bean flours.

Germination time (h)	Trypsin inhibitor activity			
	TIU ^a /g flour x 10 ³	Per cent decrease	TIU ^a /g protein x 10 ³	Per cent decrease
0	4.2	-	17.8	-
24	4.0	4.7	16.5	7.3
48	3.3	21.4	13.5	24.1
72	3.2	23.8	12.8	28.0
SE $\pm$	0.016			
LSD(P=0.05)	0.053			

^a Trypsin inhibitor unit expressed in terms of tryptic units inhibited, the tryptic unit being defined as the increase of 0.01 absorbance unit at 280 nm in 30 min of digestion under the experimental conditions.

The raw moth bean flour contained 4.2 TIU/g flour x 10³ which was found to be lower compared to some other legumes

like soybean (Liener, 1973), horse gram (Subbulakshmi et al., 1976), chickpea and green gram (Jaya and Venkataraman, 1980). Trypsin inhibitor units per g flour $\times 10^3$ and per g protein $\times 10^3$ were found decreased significantly from 4.2 to 3.2 and 17.8 to 12.8, respectively. The per cent decrease in TIU per g flour and protein was increased with increase in germination time. Subbulakshmi et al. (1976) also reported similar decrease (about 40 per cent) in trypsin inhibitor units in 72 h germinated moth bean flours. However, Jaya and Venkataraman (1980) reported decrease in trypsin inhibitor activity of chickpea and green gram only upto 48 h germination period. Beyond 48 h, it was found increased. Noor et al. (1980) also did not find any significant change in trypsin inhibitor activity in mung bean during germination. They found decrease in trypsin inhibitor activity upto 48 h germination and increase beyond 48 h. Similar trend in trypsin inhibitor activity was observed by Kakade and Evans (1966) on navy bean.

The biological implications of germination as reflected in the decrease and/or increase in the trypsin inhibitor activity of many beans are very difficult to rationalize at present. Chattopadhyay and Banerjee (1953) found that although germination did not influence the amount of trypsin inhibitor in the five legumes tested by them, it did bring about an improvement in the biological

value of three of the legumes to the extent of 10 to 20 per cent, but had no effect on the other two.

4.17 Effect of germination on electrophoretic properties of soluble proteins of moth bean flours;

As reported earlier (Table 14 and Fig 6) the soluble proteins of unprocessed moth bean flour resolved into 10 bands of which 2 were the major bands. It has been shown earlier that germination of moth bean removes phytate (Table 23) and polyphenols (Table 25) from beans. The soluble proteins were extracted from germinated moth bean flours and electrophoresis was carried out under identical conditions to know the changes in the electrophoretic mobility of soluble proteins of germinated moth bean flours from which phytate and polyphenols are removed.

The separation pattern of soluble proteins of germinated moth bean flour at different times (Table 27 and Fig 11) showed that the proteins separate into less than 10 bands viz. 4,3, and 3 bands in 24,48 and 72 h germinated moth bean flours, respectively. The band having Rf 0.48, 0.49 and 0.50 had highest intensity in 24, 48 and 72 h germinated flours, respectively, as observed 0.40 in raw flour and were the fast moving components in all. Thus, separation of soluble proteins of germinated moth bean flours into less than 10 bands confirmed that phytate-proteins and polyphenols-proteins complexes are broken down during germination and protein subunits are hydrolysed.

Table 27. Rf values of soluble proteins of moth bean flours during germination.

Germination time (h)			
0	24	48	72
0.04	-	-	-
0.08	0.09	-	0.10
0.12	-	0.13	-
0.16	-	-	-
0.36	0.45	0.45	0.45
0.40	0.48	0.49	0.50
0.70	-	-	-
0.77	-	-	-
0.84	-	-	-
0.97	0.98	-	-

Due to these complexes, distinct bands in unprocessed flour were seen whereas on germination gel electrophoretic pattern of soluble proteins showed a breakdown with increasing period of germination. The major bands in germinated flours mainly due to globulins showed a degradation at 24,48 and 72 h germination with increase in low molecular proteins. Subbulakshmi *et al.* (1976) observed similar gel electrophoretic pattern of moth bean

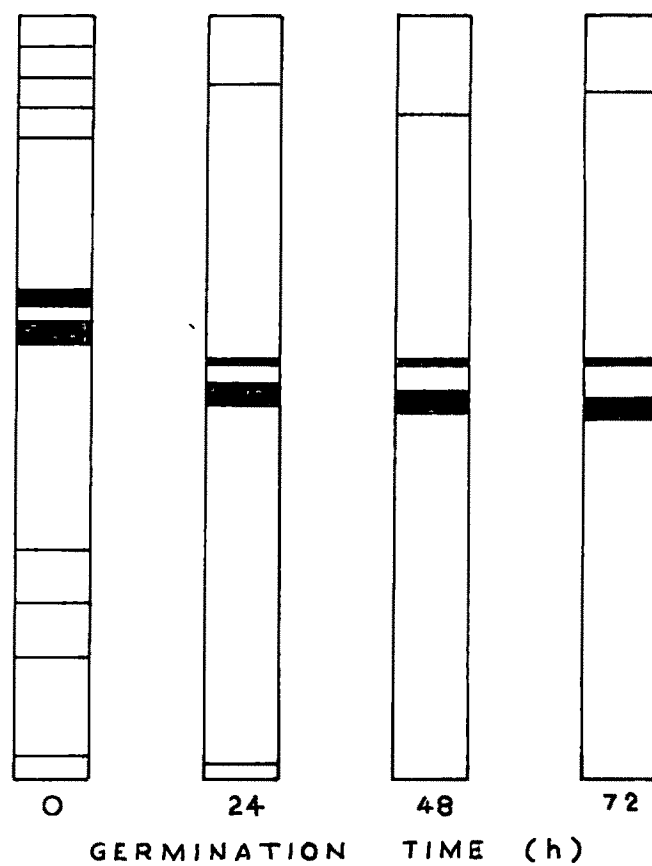


FIG 11 GEL ELECTROPHORETIC PATTERNS OF SOLUBLE PROTEINS OF GERMINATED MOTH BEAN FLOURS

globulins on germination for 24 and 72 h. The solubility of proteins was increased during germination mainly due to breakdown of complexes and proteins and as such increase in low molecular weight components which did not show distinct bands.

4.18 Effect of germination on in vitro protein digestibility of moth bean flours:

The IVPD values when expressed as μ moles of leucine formed per g in 4 h were significantly improved (Table 28)

Table 28. Effect of germination on IVPD of moth bean flours.

Germination time (h)	In vitro protein digestibility		Per cent IVPD
	μ moles of leucine formed per g per 4 h		
	Pepsin	Trypsin	
0	124	132	69.0
24	133	148	81.0
48	150	172	84.3
72	134	140	78.1
SE $\pm$	1.04	0.86	
LSD (P=0.05)	3.38	2.81	

during germination for 48 h. However, a slight decrease in IVPD was observed beyond 48 h of germination. The values for pepsin and trypsin were also almost similar.

The per cent IVPD was improved from 69.0 to 84.3 upto 48 h germination. The increase in IVPD was 78.1 per cent in 72 h germinated flours which was lower than that of 48 h germinated flour. A progressive increase in IVPD (22.1 per cent) was observed with germination period upto 48 h. However, beyond 48 h germination period it was 13.0 per cent.

Subbulakshmi et al. (1976) reported 25 per cent increase in IVPD of moth bean flour on germination for 72 h whereas Satwadhar et al. (1981) reported 15.49 per cent increase in IVPD of moth bean flour on germination only upto 36 h. However, they reported only 8.4 per cent increase in 48 h germinated flour. The results of the present investigation little agrees with the results of Satwadhar et al. (1981) but are contradictory to those of Subbulakshmi et al. (1976). In the present study decrease in IVPD over 48 h germinated sample was observed. The hydrolysis of the protein during germination probably accounts for the better digestibility of proteins. Decrease in per cent IVPD of 72 h germinated flour might be due to polyphenols-proteins interactions since polyphenols are increased in 72 h germinated flour. The increase in polyphenols, as previously postulated, may be due to fresh synthesis of polyphenols or polymerization of existing phenolic compounds or degradation of high molecular insoluble polymers into small molecular weight soluble

polymers that give a color reaction with the reagent (Satwadhkar et al., 1981).

4.19 Effect of cooking on proximate composition of moth bean flours:

The proximate composition of raw and cooked (autoclaved at 120°C at 10 psi for 10,15,20,25 and 30 min) moth bean flours are presented in Table 29. The moisture and total carbohydrates were found increased whereas protein, fat and

Table 29. Effect of cooking on proximate composition of moth bean flours.

Per cent composition	Cooking time (min)					
	0	10	15	20	25	30
Moisture	10.5	11.6	12.2	13.6	13.7	13.9
Crude protein (N x 6.25)	23.5	23.4	22.1	21.0	20.6	19.4
Crude fat	1.61	1.40	1.42	1.20	1.05	0.90
Ash	3.32	3.25	3.20	3.00	2.85	2.70
Total carbohydrates	71.5	72.0	73.2	74.8	75.5	77.0

ash were found decreased during cooking. The moisture content was increased from 11.6 to 13.9 per cent with decrease in dry matter from 88.4 to 85.9 per cent during 30 min of cooking period. The changes in dry matter and ash contents of moth bean during cooking of moth bean

are shown in Fig 12. Ekpenyong and Borchers (1981b) also observed increase in water and total carbohydrate content and decrease in protein, fat and ash content in winged bean on autoclaving for 30 min at 110°C at 15 psi. del Rosario and Flores (1981) also reported increase in moisture, total carbohydrates and fat content and decrease in protein and ash content in mung beans on blanching in boiling water for 15 min. Decrease in ash content during cooking (autoclaving for 15 min at 1.1 kg/cm²) of pigeonpea, green gram and black gram was also reported by Rao and Deosthale (1983).

4.20 Effect of cooking on functional properties of moth bean flours:

4.20.1 Water and oil absorption capacity:

Heat processed moth bean flours had higher water absorption capacity than that of the raw flour (Table 30).

Wu and Inglett (1974) have reported that water absorption capacity of soy flour increased due to heat processing. High molecular weight proteins, namely 7S and 11S constitute nearly 70 per cent of soy proteins (Naismith, 1955). These proteins consist of subunit structure and the proteins are dissociated on heating (Catsimpoles et al., 1970). It is possible that these proteins are also dissociated on heating perhaps the subunits have more water binding sites than the oligomeric proteins.

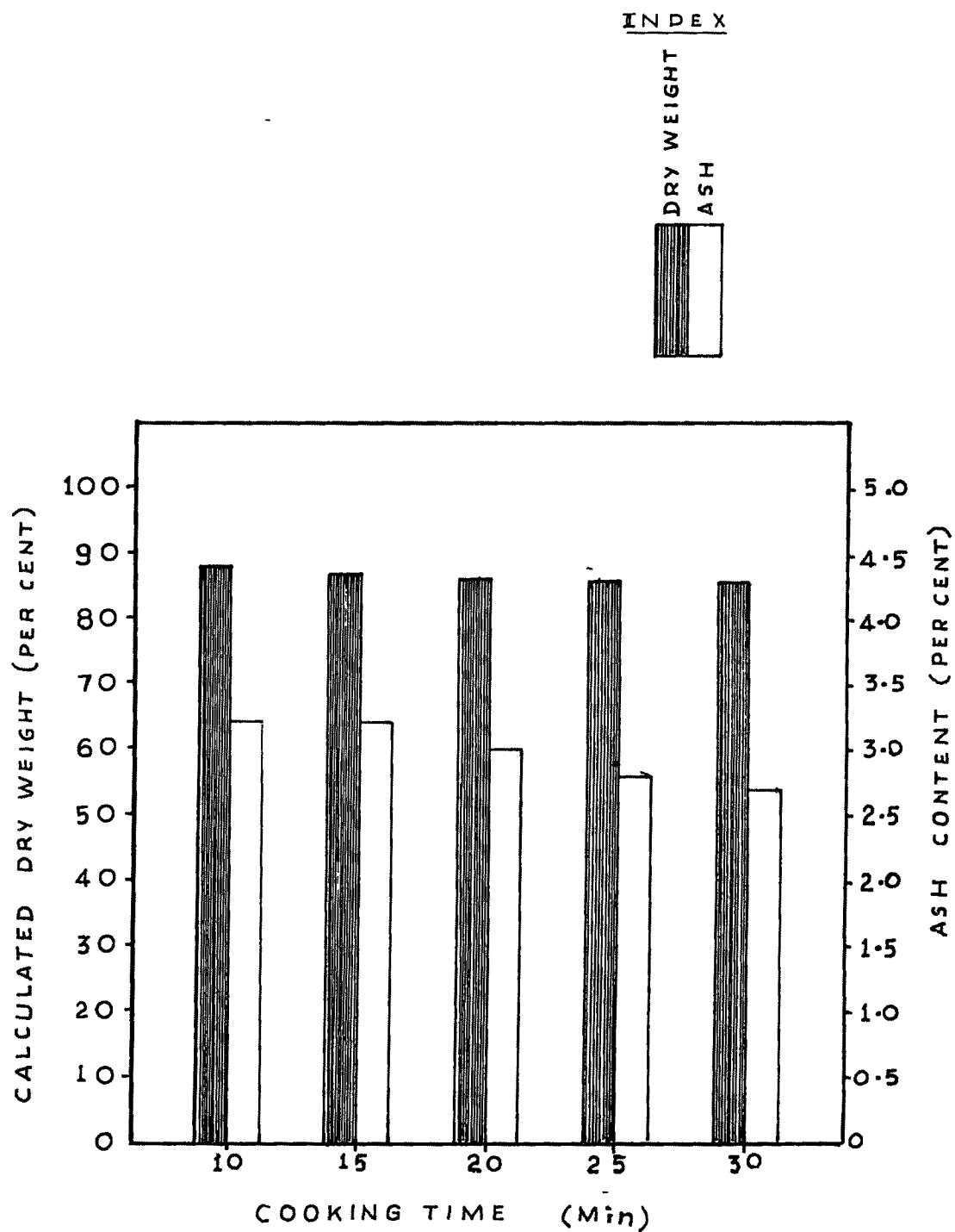


Fig. 12 EFFECT OF COOKING TIME ON DRY WEIGHT
AND ASH CONTENT IN MOTH BEAN FLOURS

Table 30. Effect of cooking on water and oil absorption capacity of moth bean flours.

Cooking time(min)	Per cent crude protein ^a (N x 6.25)	Water absorption capacity(g/g)		Oil absorption capacity(g/g)	
		Flour	Protein ^b	Flour	Protein ^b
0	23.5	2.4	10.2	1.7	7.2
10	23.4	2.4	10.2	1.9	8.1
15	22.1	2.6	11.7	2.0	9.0
20	21.0	2.5	11.9	2.3	10.9
25	20.6	2.7	13.1	2.5	12.1
30	19.4	2.7	13.8	2.6	13.4
SE $\pm$		0.007		0.026	
LSD(P=0.05)		0.023		0.081	

^a Expressed on dry weight basis.

^b Expressed on crude protein basis.

This could be the reason for the observation that heat-processed moth bean had higher water absorption capacity. Carbohydrates may also play a role in water absorption. During autoclaving, gelatinization of the carbohydrates and swelling of the crude fiber may occur which could also lead to increased water absorption. The results of the present investigation are in agreement with those of Narayana and Rao (1982) who observed increase

in water absorption capacity in heat processed winged bean and soybean flours. del Rosario and Flores (1981) also reported increase in water absorption capacity of mung bean flour on blanching in boiling water for 15 min. These results suggest that heat treatment affected the water absorption capacity of the native proteins. This is supported by Lin et al. (1974) who determined that heat denaturation improved the water imbibino capacity of sunflower proteins. The increase in water absorption capacity in heat processed soy flour (Wu and Inglett, 1974) has also been reported.

The same could be said of oil absorption capacity. The data presented (Table 30) showed that oil absorption of both flour and protein increased significantly with cooking time. Heat processing for 30 min increased the oil absorption capacity of moth bean flour from 1.7 g/g flour to 2.6 g/g flour and from 7.2 g/g protein to 13.4 g/g protein. Narayana and Rao (1982) also observed increase due to heat processing in the fat absorption capacity of winged bean flour from 1.4 g/g flour to 2.2 g/g flour and from 3.3 g/g protein to 5.1 g/g protein. Similar increase in fat absorption capacity from 0.84 g/g flour to 1.12 g/g flour was also reported in mung bean flours blanched in boiling water for 15 min (del Rosario and Flores, 1981). This increase in fat absorption capacity could be due both to the dissociation of the proteins that may occur

on heating and also to denaturation which is expected to unmask the non polar residues from the interior of the protein molecule.

4.20.2 Gelation:

The least gelation concentration of raw and cooked moth bean flours in this study revealed more or less constant gelation at 9.06 per cent except an apparent decrease on cooking (Table 31). del Rosario and Flores (1981) reported 10 per cent gelation of raw and blanched mung bean flours. They did not find any difference in gelation capacity in mung bean flour after heat treatment. The slight decrease in gelation capacity observed in the present investigation may be due to lower protein content of the cooked moth bean flours. When compared on crude protein content, the gelation per cent observed in raw and cooked moth bean flours was more or less constant. Factors that affect gelation properties include protein concentration, other protein components in the complex food system, nonprotein components, pH, ionic and reducing agents and heat treatment. Though the protein content during cooking in moth bean flour was slightly decreased, it formed stable gels on heating. The results of the present investigation fairly agrees with the results of Coffman and Garcia (1977) who observed more stable gels in mung bean flours after heating even with the lower protein content. Results from this study showed, as previously reported, that even the flours which had a

Table 31. Effect of cooking on least gelation concentration of moth bean flours^a.

Cooking time (min)	Per cent crude protein (N x 6.25)	Least gelation concentration (per cent wt/vol)
0	23.5	9.06 $\pm$ 0.01 ^b
10	23.4	9.04 $\pm$ 0.01
15	22.1	8.96 $\pm$ 0.03
20	21.0	8.94 $\pm$ 0.02
25	20.6	8.91 $\pm$ 0.03
30	19.4	8.90 $\pm$ 0.01

^a Each value is the average of nine determinations.

^b Mean plus or minus standard deviation.

lower protein content formed stable gels at these conditions using this simple gelation test. Fleming et al. (1975) reported that gelation capacity of legume proteins is attributed to the globulin fraction. During cooking, the change in the protein fraction (increase in globulin) occurs which may be responsible for maintaining, at least, gelation more or less constant.

4.20.3 Nitrogen solubility:

Nitrogen solubility decreased in heat-processed moth bean flour at all pH's studied (Fig 13). As the time of cooking increased, the solubility of nitrogen decreased

INDEX

- RAW
- +—+ COOKED FOR 10 MIN
- COOKED FOR 15 MIN.
- COOKED FOR 20 MIN
- COOKED FOR 25 MIN
- COOKED FOR 30 MIN.

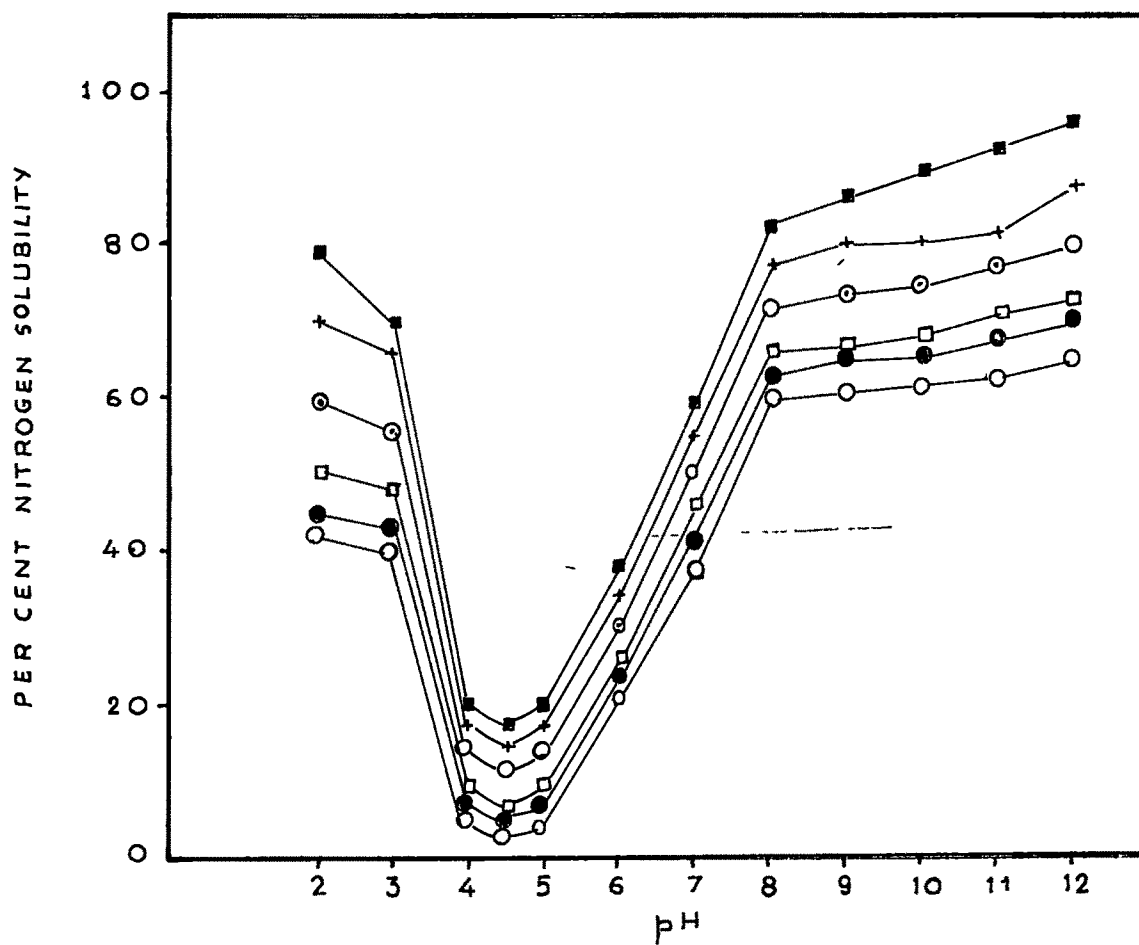


Fig. 13 NITROGEN SOLUBILITY Vs pH PROFILE OF RAW AND COOKED MOTH BEAN FLOURS

at all pH's studied. Reduction in nitrogen solubility was more conspicuous at pH 2.0, 9.0, 10.0 and 11.0 in all cooked samples. Reduction in nitrogen solubility due to heat processing has been reported in the case of soy and pea nut flours (McWatters and Holmes, 1979a) and winged bean flours (Narayana and Rao, 1982). del Rosario and Flores (1981) also reported lower nitrogen solubility at the isoelectric point in case of blanched mung bean flours in boiling water for 15 min. In the present study, probably autoclaving denatured the proteins of moth bean flour and reduced their solubility in water at different pH's studied.

Shastri et al. (1982) reported that on parching moth bean at 250°C, less nitrogen was soluble from the parched sample as compared to the raw sample below pH 3.0. However, they did not find significant difference at pH 7.0. At pH 8.0 and above, there was continuous increase in the nitrogen solubility of parched sample over that of raw sample. They claimed that this might be due to the partial denaturation and other changes in proteins occurring as a result of heat treatment and decrease in water soluble proteins and corresponding increase in alkali soluble proteins during parching.

4.20.4 Foaming properties:

The whippability and foaming capacity of cooked moth bean flours was found significantly decreased (Table 32).

Table 32. Effect of cooking on foaming properties of moth bean flours.

Cooking time (min)	Volume after whipping (ml)		Per cent volume increase	Specific volume (ml/g)
	Total	Foam		
0	127	35	27	1.30
10	125	31	25	1.20
15	121	28	21	1.10
20	118	26	18	0.95
25	117	24	17	0.88
30	114	20	14	0.76
S.E. $\pm$	0.59	0.60		0.009
LSD($P=0.05$)	1.82	1.86		0.028

The per cent volume increase on whipping of 1 per cent aqueous dispersions of the raw moth bean flour was 27 which was found decreased progressively to 14 (nearly 50 per cent) as the cooking time increased to 30 min. del Rosario and Flores (1981) reported less foaming capacity of blanched mung bean flour than raw mung bean flour. Similarly, Narayana and Rao (1982) reported decrease in foaming capacity of winged bean flour on autoclaving. Decrease in whippability and foaming property is correlated to the amount of native proteins present. During

autoclaving, proteins are denatured and thus foam capacity decreased. This hypothesis is supported by the observations of Yasumatsu et al. (1972) who reported that heat processing diminished the foam capacity of soy proteins by denaturation.

Cooking also decreased significantly the specific volume of foams (Table 32) indicating poor foaming and air uptake during whipping.

The foam stability results are presented in Table 33. The decrease in total volumes over 120 min was 16.6 per cent for 30 min cooked moth bean flour compared to 7.0 per cent for raw flour. The corresponding decrease in foam volumes was 55.0 and 31.4 per cent, respectively. The lower foam stability of blanched mung bean flour than raw mung bean flour was also reported by del Rosario and Flores (1981). Narayana and Rao (1982) also reported less foam stability of heat processed winged bean flour. It has been suggested that foam stability is also related to protein denaturation. Native proteins give higher foam stability than the denatured proteins (Yasumatsu et al., 1972).

The increased concentration of the flours from 1 to 10 per cent (wt/vol) in aqueous dispersions increased the foam volume (Table 34). As observed earlier the increase was almost double with 2 per cent solids concentration. The differences in per cent volume increase during cooking 1 per cent solids concentration were significant.

Table 33. Foam stability of cooked moth bean flours.

Cooking time (min)	Volume (ml) at room temperature 25°C ± 2 after min								per cent decrease over 120 min			
	0		30		60		90			120		
	Total	Foam	Total	Foam	Total	Foam	Total	Foam		Total	Foam	
0	127	35	126	33	123	31	120	28	118	24	7.0	31.4
10	125	31	121	28	116	24	114	21	108	18	13.6	41.9
15	121	28	117	25	115	21	110	18	108	15	14.2	46.4
20	118	26	114	23	112	19	107	14	100	12	15.2	53.8
25	117	24	111	20	108	15	105	12	98	11	16.2	54.1
30	114	20	110	17	106	14	102	11	95	9	16.6	55.0
SE ±	0.59		0.60				0.78		0.83			
LSD(P=0.05)	1.82		1.86				2.40		2.57			

Table 34. Effect of concentration of foaming capacity of cooked moth bean flours.

Cooking time (min)	Per cent volume after whipping						
	Concentration (per cent wt/vol)						
	1	2	3	4	5	7	10
0	27	55	61	65	70	78	98
10	25	51	57	61	64	71	91
15	21	40	50	56	61	70	85
20	18	35	42	48	55	66	78
25	17	35	43	46	55	67	75
30	14	30	34	41	42	52	71
SE $\pm$	0.38						
LSD(P=0.05)	1.18						

The foam capacity versus pH profile of 2 per cent (wt/vol) aqueous dispersions of raw and cooked moth bean flours closely resembled in shape its nitrogen solubility versus pH profile (Fig 14), suggesting that foaming property of cooked moth bean flour was also dependent on the solubilized protein. Minimum foam capacity of raw moth bean flour was observed to be 31.0 per cent at pH 4.5 and maximum of 63.0 per cent at pH 12.0. Similar observations have been reported in the case of soy protein isolate, caseinate and whey protein concentrate (Harmansson, 1975) and winged bean (Narayana and Rao, 1982).

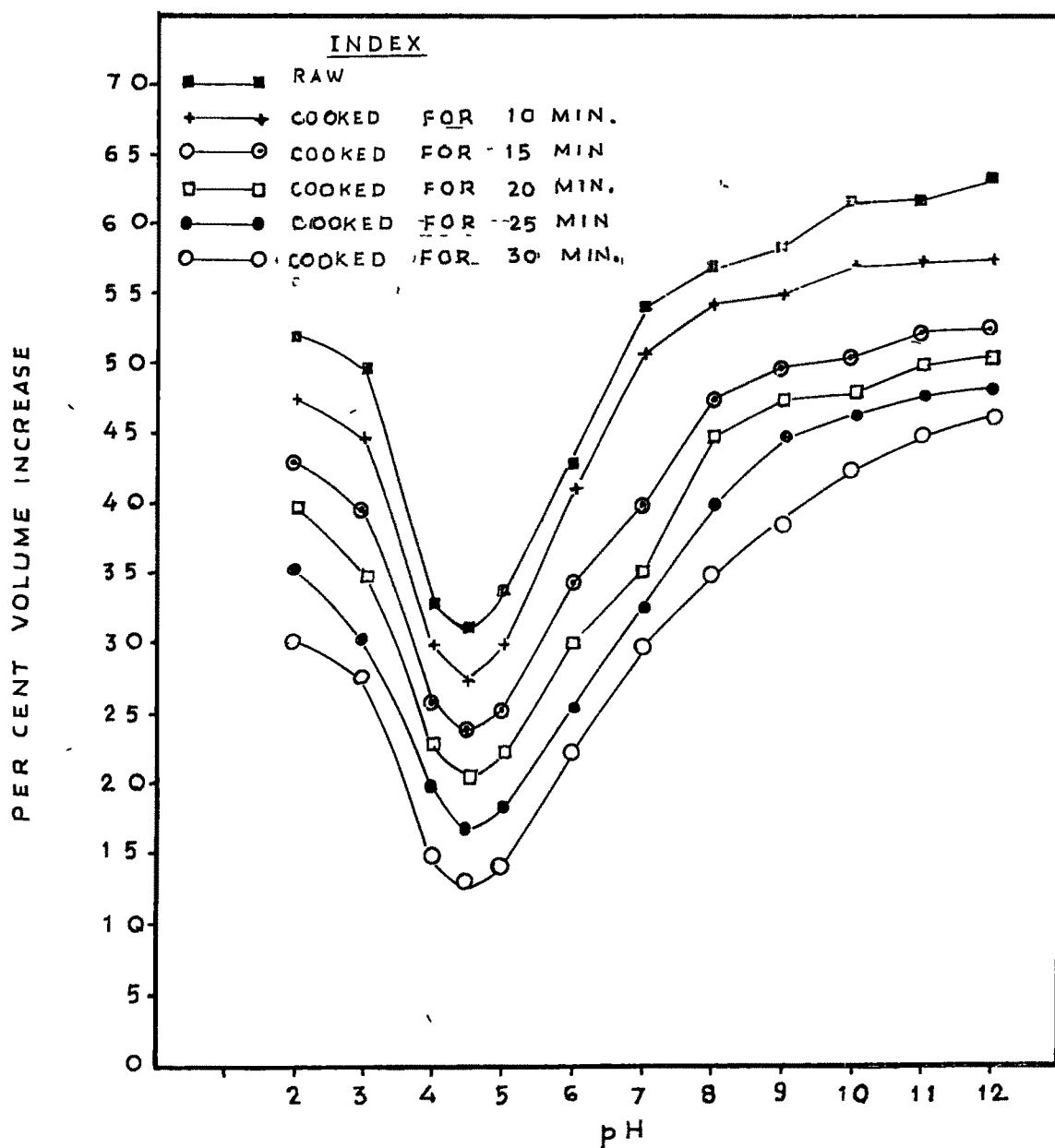


Fig. 14 FOAMING CAPACITY VS pH PROFILE OF 2 PERCENT (W/V) AQUEOUS DISPERSIONS OF RAW AND COOKED MOTH BEAN FLOURS

Heat processing considerably lowered the foam capacity of moth bean flours at all pH's studied. At pH 4.5, a volume increase of 13.0 per cent was observed for heat processed flour compared to 31.0 per cent for raw flour. Above pH 4.5, heat processed moth bean flours had continuous increase in foam capacity upto pH 12.0. Yasumatsu et al. (1972) have reported that heat processing diminished the nitrogen solubility of soy proteins by denaturation and also reduced the foam capacity.

4.20.5 Emulsion properties:

Emulsion properties of raw and heat processed moth bean flours are summarised in Table 35. The emulsifying(per cent) activity of raw moth bean flour was observed to be 20.2 for flour and 85.9 for protein which was found decreased significantly on cooking at all periods. The decrease was in increasing order as the time of cooking was increased from 10 min to 30 min. The emulsifying activity of 30 min cooked moth bean flour was 14.0 for flour and 71.9 for protein. The emulsion stability was also decreased significantly from 12.0 per cent for flour and 51.0 per cent for protein of raw flour to 9.0 per cent for flour and 46.2 per cent for protein of 30 min cooked moth bean flour. Narayana and Rao (1982) reported a considerable reduction in emulsification capacity of heat processed winged bean flour. McWatters and Holmes (1979a) have also reported that soy and peanut flours are sensitive to moist heating

Table 35. Effect of cooking on emulsion properties of moth bean flours.

Cooking time (min)	Emulsifying activity (per cent)		Emulsion stability ^a	
	Flour	Protein ^b	Flour	Protein ^b
0	20.2	85.9	12.0	51.0
10	18.4	78.6	11.5	49.1
15	17.1	77.3	11.0	49.7
20	16.0	76.1	10.5	50.0
25	15.3	74.2	9.5	46.1
30	14.0	71.9	9.0	46.2
SE $\pm$	0.033		0.034	
LSD(P=0.05)	0.101		0.106	

^a Per cent of the original emulsifying activity after heating at 80°C for 30 min.

^b Expressed on crude protein basis (the oil emulsified, for protein)

and the heating time was the primary determinant in the reduction of nitrogen solubility and emulsification capacity.

The effect of pH on the emulsification capacity of the cooked moth bean flours was determined over the pH range of 2 to 12 (Fig 15). At pH 2.0 emulsification capacity of 30 min cooked moth bean flour was 48 g/g

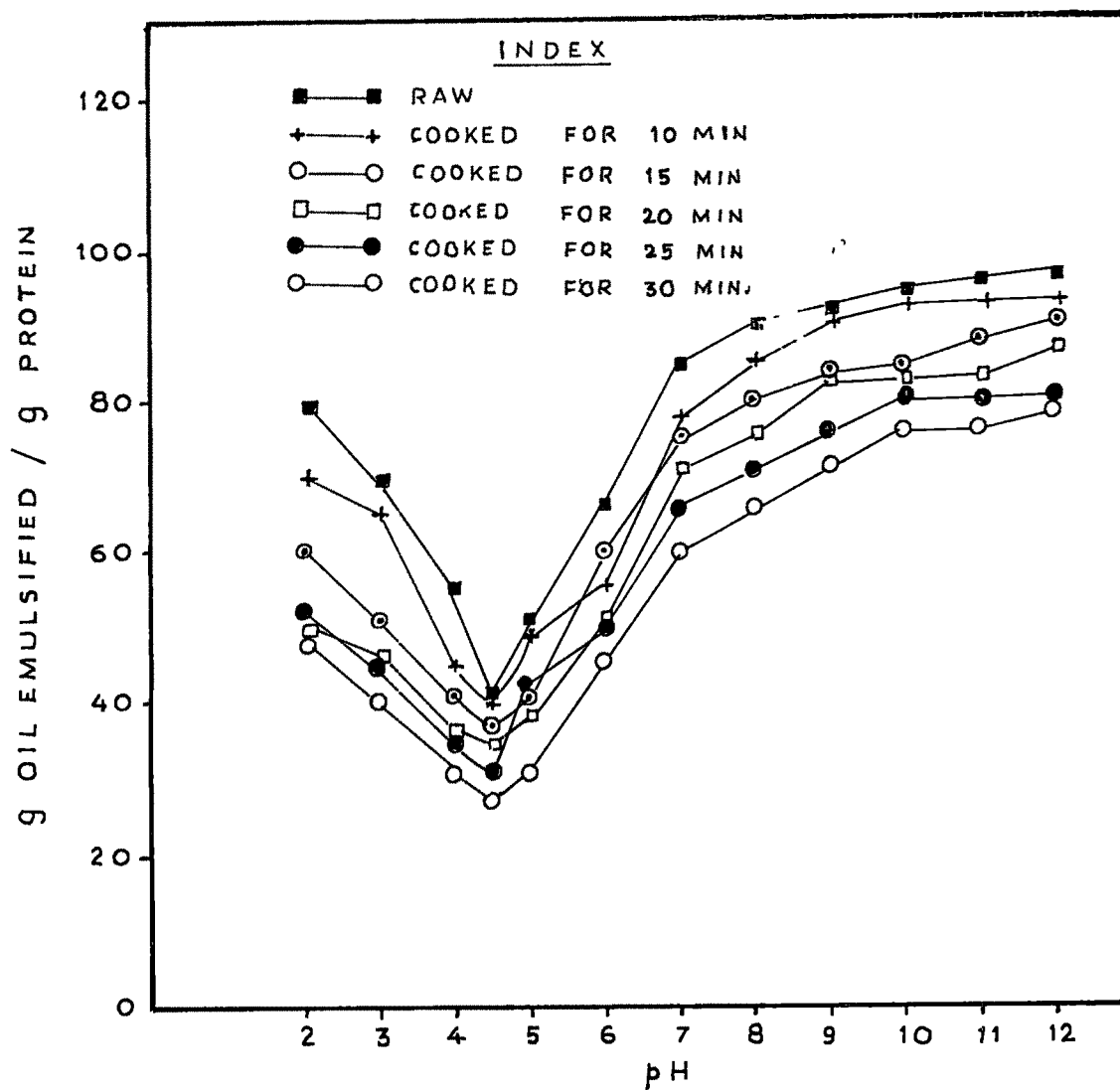


Fig 15 EMULSIFICATION CAPACITY VS pH PROFILE OF RAW AND COOKED MOTH BEAN FLOURS

protein as compared to 80 g/g protein for the raw flour. At the pH of minimum solubility (pH 4.5) emulsification capacity of 30 min cooked flour was only 26 g/g protein which was lower than raw flour. With the progressive increase of pH on the alkaline side, the emulsification capacity increased to a maximum of 96 g/g protein of raw flour at pH 11 and 80 g/g protein of 30 min cooked at pH 10. In general, emulsification capacity of heat processed moth bean flour was found lower at all pH's studied and was correlated with the time of cooking.

The emulsification capacity versus pH profile also closely resembled the nitrogen solubility versus pH profile of all samples, suggesting that emulsification was due to the solubilized proteins. In the case of soybean (Crenwelge et al., 1974), groundnut (Ramanathan et al., 1978) guar (Nath and Rao, 1981) and winged bean proteins (Narayana and Rao, 1982), similar relationship between emulsification capacity and pH has been reported.

4.21 Effect of cooking on phytate phosphorus and phytate of moth bean flours:

The data on effect of cooking on total phosphorus and phytate phosphorus of moth bean flours are presented in Fig 16. Cooking of moth bean with water in 1:4 ratio for 10 min resulted in a major loss of total phosphorus and phytate phosphorus in cooking water. This may be due to leaching of phosphorus from beans into cooking water.

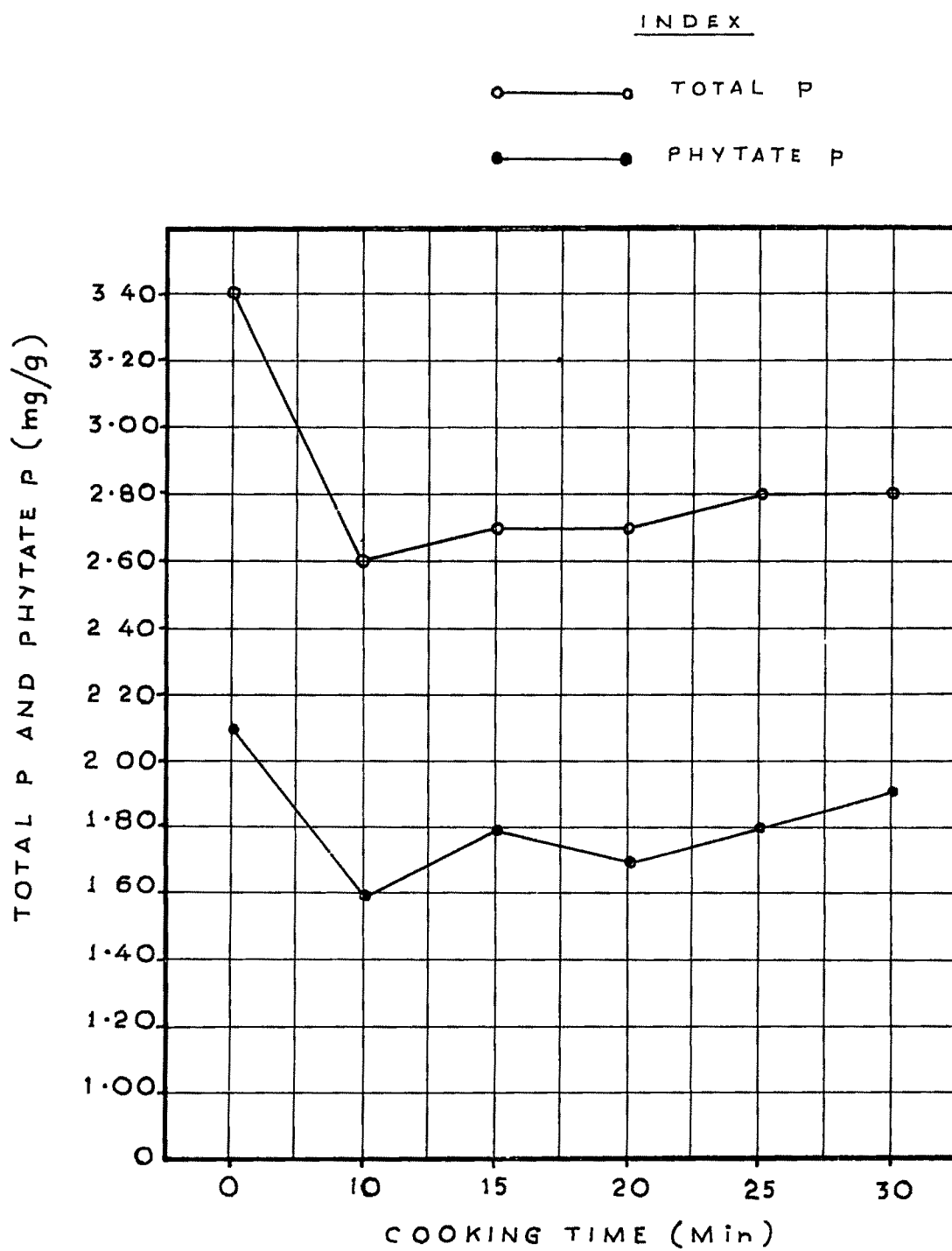


Fig 16 EFFECT OF COOKING ON TOTAL PHOSPHORUS AND
PHYTATE PHOSPHORUS CONTENT OF MOTH BEAN
FLOURS

After 10 min of cooking, losses of phosphorus were not found after initial leaching. On the contrary, the increase in both total and phytate phosphorus was observed as the time of cooking was increased. The increase in phosphorus content in beans when cooked for prolonged time may be due to reabsorption of phosphorus by beans from the cooking water. Cooking of beans with water in 1:4 ratio did not show significant effects on phytate phosphorus breakdown but during short time cooking the losses of phytic acid phosphorus and total phosphorus were greater. The losses of phytate phosphorus at short time cooking may be attributed to water soluble nature of bean phytic acid. Reddy et al. (1978) reported that after cooking for 45 min, whole black gram seeds did not show any breakdown in the total and phytate phosphorus after initial leaching upto 5 to 10 min of cooking when the beans to water ratio was 1:4. Borhade et al. (1984) also reported decrease in phytate phosphorus during cooking of both ungerminated and germinated seeds of horse gram and moth bean. They, however, did not find significant loss of total phosphorus content in both the legumes during cooking. Kumar et al. (1978) noted that the cooking process decreased both water and acid extractable 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 phytate and other components during cooking. Iyer et al. (1980b) also observed a

reduction in phytate content of phaseolus beans during the combined process of soaking and cooking. The rate of phytate destruction during cooking is influenced by its protein and/or cation environment (de Boland et al., 1975). The observed decrease in moth bean due to cooking can be attributed to the formation of insoluble complexes between phytate and other components of seeds as observed in the case of cowpea, chickpea and green gram.

4.22 Effect of cooking on calcium, magnesium, iron and zinc content of moth bean flours:

The data on changes in mineral content of moth bean flour during cooking are depicted in Fig 17 and 18. Same could be said for losses of minerals during cooking. The maximum loss of calcium, magnesium (Fig 17) and iron (Fig 18) occurred at 10 min cooking time. Whereas of zinc (Fig 18) occurred at 25 min cooking. This may be due to leaching of minerals from beans into external medium. After 10 min cooking time the increase in calcium, magnesium and iron may be due to reabsorption of minerals from the cooking broth. Meiners et al. (1976) have also observed significant losses of mineral nutrients on cooking of several dry legumes which they attributed to the leaching of mineral nutrients into the cooking broth. Similarly, Rao and Deosthale (1983) observed minerals losses of pigeonpea, green gram and black gram in cooking broth.

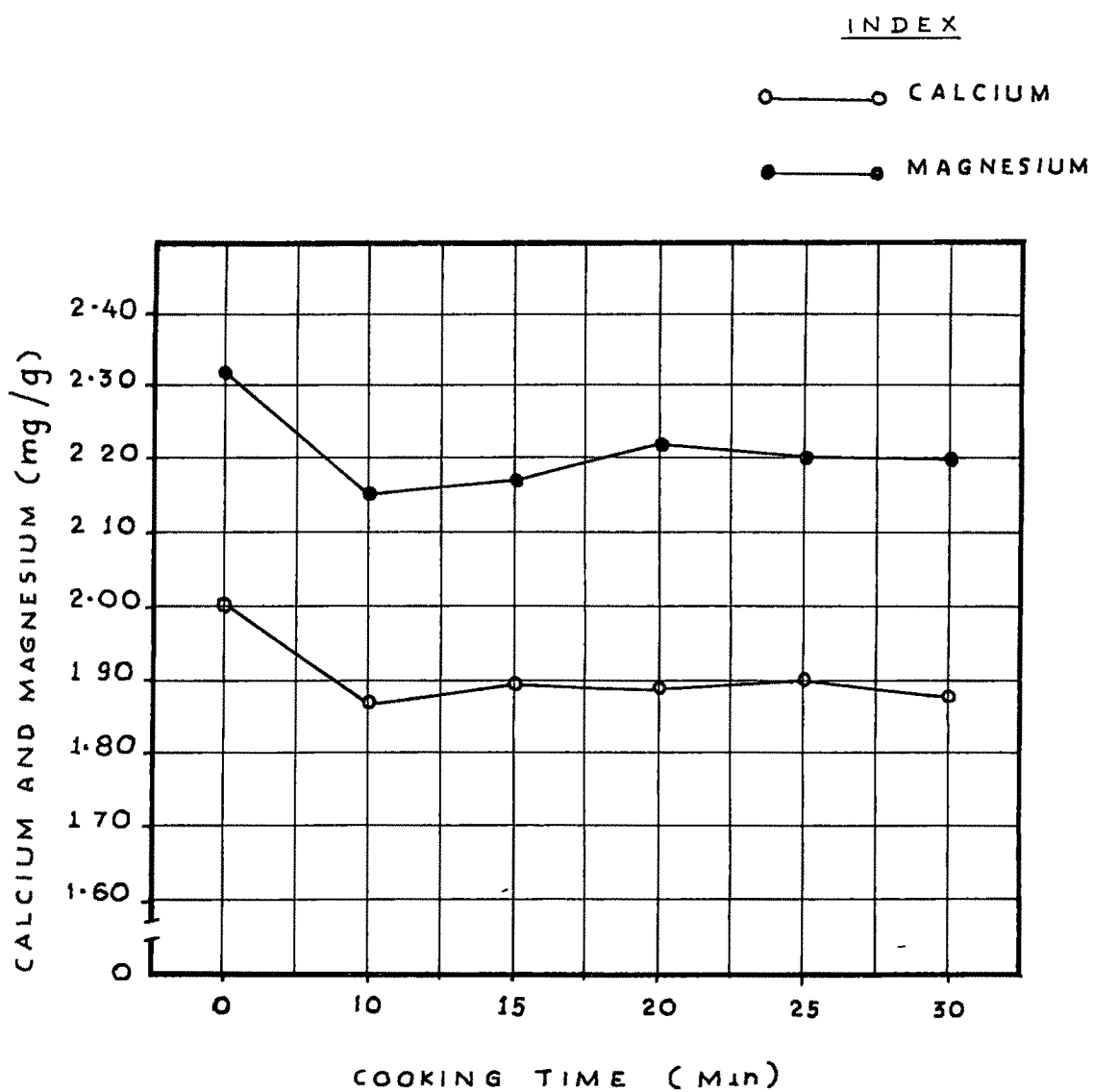


Fig 17 EFFECT OF COOKING ON CALCIUM AND MAGNESIUM
CONTENT OF MOTH BEAN FLOURS

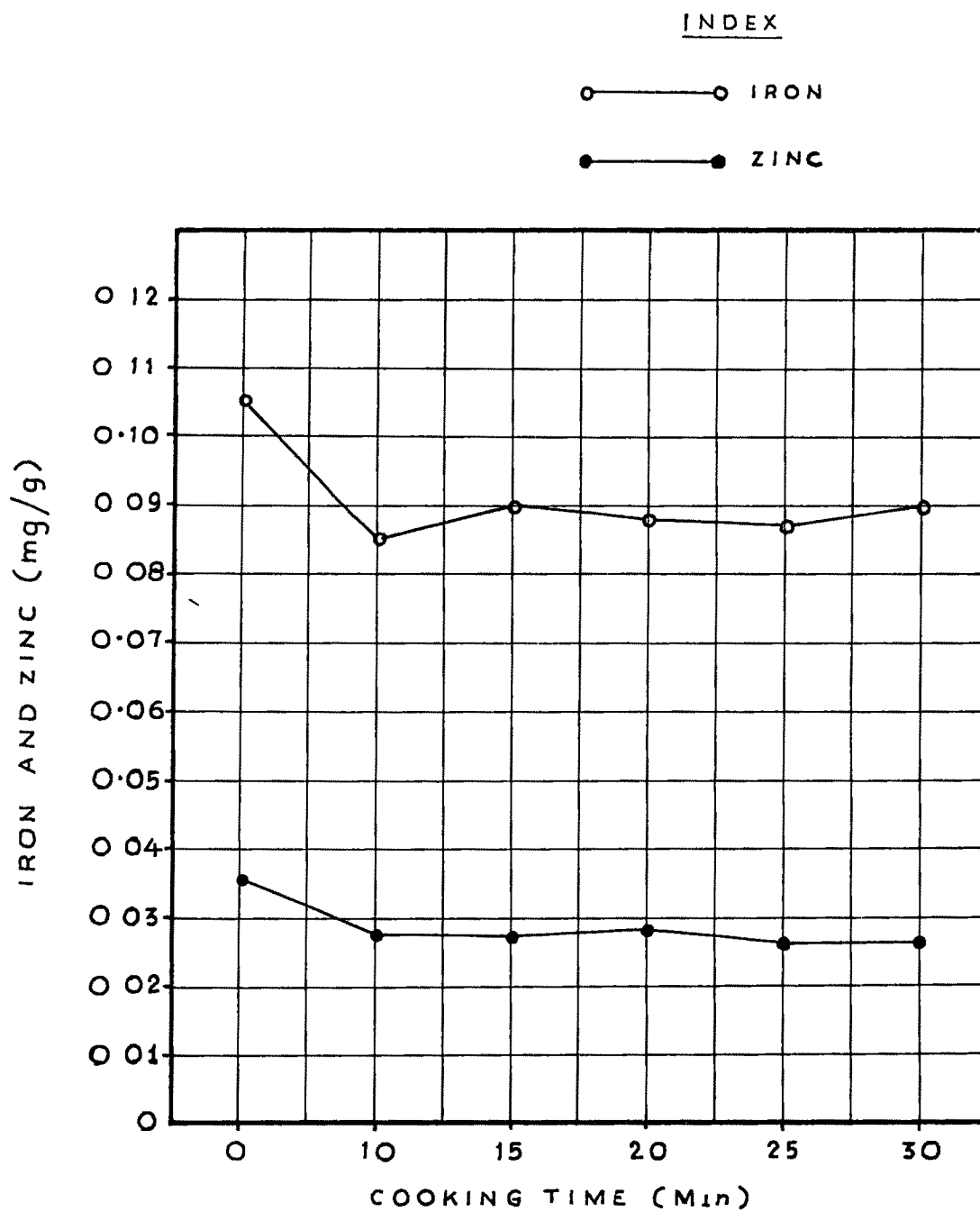


Fig 18 EFFECT OF COOKING ON IRON AND ZINC CONTENT
OF MOTH BEAN FLOURS

4.23 Effect of cooking on polyphenols of moth bean flours:

Data on polyphenols as tannic acid content of cooked moth bean flour are presented in Table 36. Autoclaving for 30 min reduced polyphenols upto 77.5 per cent. Rao and Deosthale (1982) reported 58, 77, 69 and 72 per cent reduction of polyphenols in pigeonoes, chickpea, black gram and green gram dhals, respectively, on cooking in an autoclave for 15 min. Barroca et al. (1985) reported 91 per cent reduction in polyphenols in mung bean on boiling in water for 90 min. Elias et al. (1979) reported that cooking decreased tannin content in whole seeds while a high concentration was found in the cooking broth. In moth bean and horse gram the per cent reduction in polyphenols on cooking in boiling water for 80 min was 50 and 37.5, respectively (Satwadhkar et al., 1981).

The greater reduction in polyphenols in moth bean in the present investigation was due to short time cooking in water with the bean to water ratio of 1:4. Butler et al. (1979) observed a large decrease in assayable polyphenols in sorghum grains on drying only if processed by boiling. This was attributed to a disruption of cellular compartments in the process. On disruption, polyphenols could become accessible to protein or to some other components and form insoluble complexes that on drying would be strongly bound. It was suggested that the apparent decrease in polyphenols was most likely not due to actual

Table 36. Effect of cooking on polyphenols of moth bean flours.

Cooking time (min)	Polyphenols (mg of tannic acid/g)	Per cent reduction in polyphenols
0	1.45	-
10	0.58	59.4
15	0.53	62.8
20	0.49	66.0
25	0.41	71.3
30	0.32	77.5
SE $\pm$	0.013	
LSD(P=0.05)	0.041	

decrease in polyphenols, but to a change in their solubility or chemical reactivity. Thus, the observed decrease in polyphenols content due to cooking in moth bean may have resulted from the binding of polyphenols with other organic substances or from alterations in the chemical structure of polyphenols that rendered them incapable of giving the chemical color reaction. The leaching loss of polyphenols in cooking broth can not be ruled out. A high amounts of tannin were found in the cooking broth of winged bean (de Lumen and Salamat, 1980) and field bean (Elias *et al.*, 1979).

4.24 Effect of cooking on trypsin inhibitor activity of moth bean flours:

It was observed that autoclaving moth bean at 120°C and 10 psi steam pressure for 10 min resulted into more than 50 per cent reduction in trypsin inhibitor activity (Table 37). When autoclaved for 15 min antitryptic activity

Table 37. Effect of cooking on trypsin inhibitor activity of moth bean flours.

Cooking time (min)	Trypsin inhibitor activity			
	TIU ^a /g flour x 10 ³	Per cent decrease	TIU ^a /g protein x 10 ³	Per cent decrease
0	4.2	-	17.8	-
10	2.7	35.7	11.5	35.3
15	ND ^b		ND	
20	ND		ND	
25	ND		ND	
30	ND		ND	
SE ±	0.043			
LSD(P=0.05)	0.133			

^a Trypsin inhibitor unit expressed in terms of tryptic units inhibited, the tryptic unit being defined as the increase of 0.01 absorbance unit at 280 nm in 30 min of digestion under the experimental conditions.

^b Not detectable.

was found negligible and not detected. Soni et al. (1978) observed 2.42 trypsin inhibitor units/g flour of moth bean when autoclaved at 15 psi for 15 min as against 1442.8 trypsin inhibitor units/g flour of raw, uncooked moth bean. Mehta and Simlot (1982) also reported complete disappearance of trypsin inhibitor in moth bean when autoclaved at 15 psi for 10 min.

4.25 Effect of cooking on electrophoretic properties of soluble proteins of moth bean flours:

As reported earlier (Table 14 and Fig 6) the soluble proteins of unprocessed moth bean flour resolved into 10 bands of which 2 were the major bands. Cooking is known to decrease phytate and polyphenols. The Fig 16 and Table 36 showed removal of phytate and polyphenols respectively from flours during cooking. The soluble proteins from cooked moth bean flours and electrophoresis was carried out under identical conditions to know the changes in the electrophoretic mobility of soluble proteins of cooked moth bean flours from which phytate and polyphenols are removed to some extent.

The separation pattern of soluble proteins of cooked moth bean flours at different times (Table 38 and Fig 19) showed that the proteins separate into less than 10 bands viz. 6,5,4,4 and 3 bands in 10,15, 20,25 and 30 min cooked moth bean flours, respectively. The band having Rf 0.40 in 10,15 and 30 min cooked flour and band having

Table 38. Rf values of soluble proteins of moth bean flours during cooking.

Cooking time (min)					
0	10	15	20	25	30
0.04	-	-	-	-	-
0.08	0.07	-	-	-	-
0.12	0.11	0.12	-	-	-
0.16	-	-	-	0.15	0.16
0.36	0.35	0.36	0.34	0.35	0.35
0.40	0.40	0.40	0.42	0.41	0.40
0.70	-	0.72	0.73	-	-
0.77	0.75	-	-	-	-
0.84	-	-	-	-	-
0.97	0.94	0.96	0.97	0.94	-

Rf 0.42 and 0.41 in 20 and 25 min cooked flours, respectively, had the highest intensity. Disappearance of an existing band or appearance of a new band in a cooked moth bean flours compared to the bands of uncooked flour was considered as evidence of the hydrolysis of the protein subunit. Protein subunits became accessible for breakdown after cooking and as such bands decrease in number. In uncooked samples, phytate-protein and protein-polyphenols complexes might have given distinct bands. During cooking complexes are broken and globulins (major protein

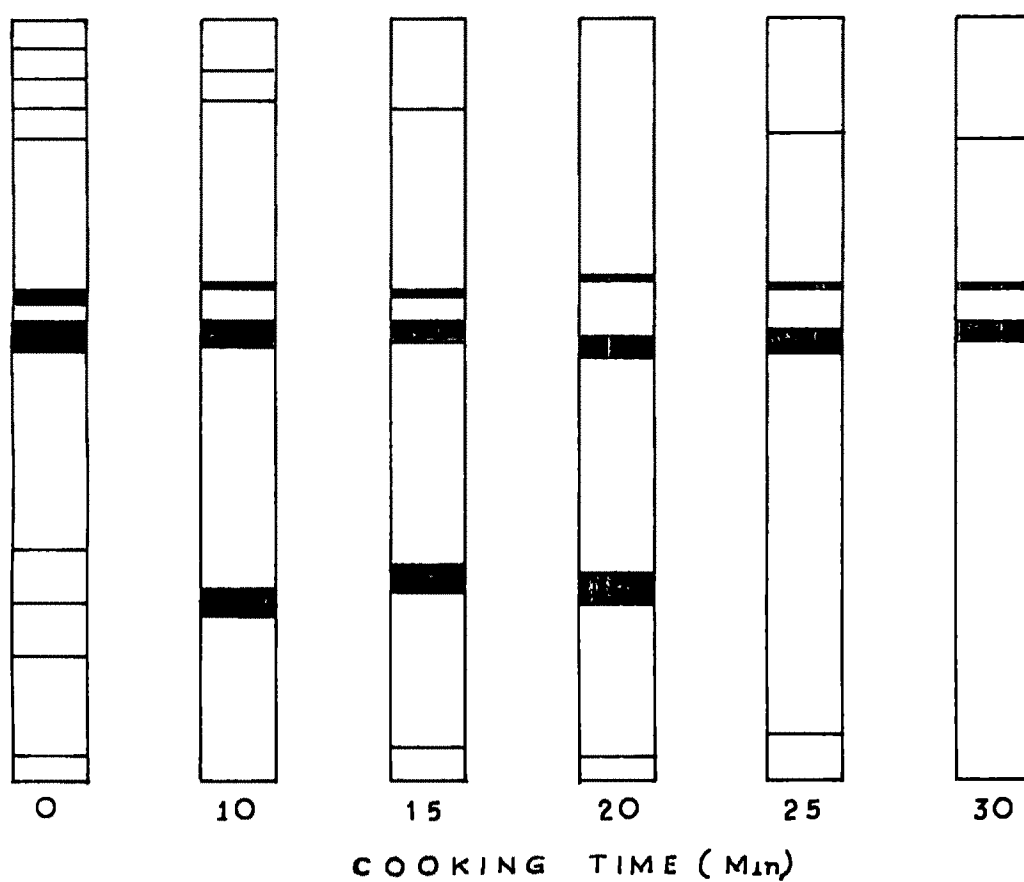


Fig.19 GEL ELECTROPHORETIC PATTERNS OF SOLUBLE PROTEINS OF COOKED MOTH BEAN FLOURS

fraction in moth bean) are degraded and failed to show the distinct bands. The results of the present investigation fairly agrees with the results of Iyer et al. (1980a) who reported on dry beans (Phaseolus vulgaris) the disappearance of bands during cooking due to hydrolysis of protein subunits.

4.26 Effect of cooking on IVPD of moth bean flours:

The progressive increase in IVPD of moth bean flours during cooking was observed (Table 39). The increase in

Table 39. Effect of cooking on IVPD of moth bean flours.

Cooking time (min)	In vitro protein digestibility		Per cent IVPD
	μ moles of leucine formed per g per 4 h		
	Pepsin	Trypsin	
0	124	132	69.0
10	124	133	71.6
15	130	135	74.7
20	131	135	76.3
25	140	148	80.0
30	155	158	83.5
SE $\pm$	0.33	0.81	
LSD(P=0.05)	1.02	2.51	

IVPD was 9.0 to 25.0 per cent when expressed in terms of μ moles of leucine formed per g in 4 h by pepsin and trypsin,

respectively. The increase was almost similar in both the enzymes. As reported earlier, the action of proteolytic enzymes on removal of phytate and polyphenols during cooking was found increased.

The IVPD was increased from 69.0 to 83.5 per cent during cooking upto 30 min. A progressive increase upto 21.0 per cent in IVPD was observed in 30 min cooked flour. Moth bean in cooked form contain less phytate and polyphenols which probably are responsible for improved IVPD. This is supported by the observations of Liener (1976) who reported that cooking improve the protein digestibility of several legume proteins by inactivating protease inhibitors and lowering the levels of antinutritional factors.

4.27 Phytate-protein-minerals complexes in moth bean flour:

4.27.1 Extraction of phytate from moth bean flour:

The efficiency of different extractants on extractability of phytate from moth bean flour is summarised in Table 40. Extraction with 3 per cent trichloroacetic acid (TCA) was more complete and yielded higher phytate compared to other extractants. Moth bean flour had a phytate phosphorus content of 2.10 ± 0.07 mg/g on a dry weight basis (equivalent to 0.74 per cent phytic acid). Phytate phosphorus accounts for 61.7 per cent of the total phosphorus in moth bean flour. The extractants,

Table 40. Effects of different extractions on extractability of phytate from moth bean flour^a.

Extractant	pH of extract	Phytate phosphorus (mg/g)	Calculated ^b phytic acid (mg/g)
Distilled water	6.20	1.95 $\pm$ 0.06 ^c	6.91
3 per cent TCA	1.40	2.10 $\pm$ 0.07	7.44
2 per cent HClO ₄	0.80	1.72 $\pm$ 0.04	6.09
2 per cent HCl	0.80	1.55 $\pm$ 0.05	5.49
Tris buffer (0.05 M, pH 8.40)	8.30	1.60 $\pm$ 0.03	5.67

^a Each value is the average of six determinations and expressed on dry weight basis. Moth bean flour contained total phosphorus content of 3.40 mg/g dry weight basis.

^b Calculated phytic acid (phytate) assuming 28.20 per cent phosphorus in the molecule.

^c Mean plus or minus standard deviation.

2 per cent HCl, Tris buffer (0.05 M, pH 8.40) and 2 per cent HClO₄ solubilized 73.8, 76.1 and 81.9 per cent of phytate, respectively, compared to complete solubilization with 3 per cent TCA. Phytate solubility in distilled water was 92.8 per cent. Low extractability of phytate with 2 per cent HCl and tris buffer (0.05 M, pH 8.40) may be due to formation of insoluble complexes between phytate, protein and minerals. Chang et al. (1977) also

observed low solubility of phytate with HCl because as a result of formation of insoluble complexes between phytate and proteins in California Small White beans. Similar results were also observed by Wheeler and Ferrel (1971) in wheat protein concentrate and by Reddy and Salunkhe (1981) in black gram.

Extractability of phytate from any food and/or seeds depends on: 1) phytate association with other components i.e. the nature of proteins and mono- and divalent cations with which the phytate is associated; 2) pH ; 3) the type of solvent and ionic strength to be used for extraction; and 4) presence of endogenous iron. The presence of relatively high amounts of endogenous iron in moth bean flour might have precipitated the phytate during acid extraction. Anderson (1963) observed a recovery of only 80 per cent phytate in barley when the soluble iron to phytate phosphorus ratio was 0.33:1.0. In the present study, the ratio of iron to phytate phosphorus was about 0.05:1.0. Therefore, the interference of soluble iron on the phytate extractability by different acid solvents would be negligible.

Results of distribution of recovered solids, phytate and proteins in water extract and residue of moth bean flours are presented in Table 41. Moth bean flour had a total crude protein content of 23.5 per cent and phytic acid content of 0.74 per cent on dry weight basis. The

Table 41. Distribution of recovered solids, phytate and proteins in water extract and residue of moth bean flour^a.

Sample	Recovered solids(g)	Phytate ^b (mg/g)	Per cent protein (N x 6.25)
Moth bean flour	100.00	7.44	23.50
Water extract	17.04	38.33 (87.78)	22.13 (16.04)
Water insoluble residue	76.30	0.85 (8.71)	33.41 (71.85)
Precipitate (pH 3.50)	5.70	41.50 (31.79)	44.46 (10.78)
Whey, undialyzed (pH 3.50)	11.34	36.73 (55.98)	10.90 (5.26)
Whey, dialyzed (pH 3.50)	3.00	2.64 (1.06)	33.81 (4.31)

^a Each value is the average of three determinations.

^b Calculated phytic acid (phytate) assuming 28.20 per cent phosphorus in the molecule.

Values in parenthesis are the percentages of the original component recovered.

water extract contained 87.7 and 16 per cent of the original phytate and proteins (albumins) respectively. Lolas and Markakis (1975) observed 99.6 per cent phytate in water-soluble form compared to 87.7 per cent in moth bean flour in the present study. The water-insoluble residue contained the majority of water-insoluble

proteins (mainly globulins, prolamins and glutelins) and 8.7 per cent phytates. The water extract was rich in phytates and albumins. When the water extract was adjusted to pH 3.50 (point of maximum insolubility) 67.2 per cent of the albumins precipitated alongwith 36.2 per cent phytates. The moth bean flour whey (pH 3.50) still contained 32.8 and 63.8 per cent water extractible proteins (albumins) and phytates, respectively, and was employed for interaction studies between phytate, protein and minerals at different pH conditions.

4.27.2 Influence of pH on phytate-protein-minerals binding in moth bean flour:

The data on binding of phytate-protein in different pH and media as measured by dialysis are shown in Table 42. Fractions, I, II and III contained 0.62, 1.15 and 0.28 g protein compared to 1.24 g in undialyzed whey. On dialysis for 2 days, retention of proteins was found more in fraction II than in fractions I and III. Same could be said of phytate retention, being more in fraction II than in fraction I and III. The amount of phytate retained during dialysis was a function of pH. Similar observations were reported by Okubo et al. (1975) on soybean and by Reddy and Salunkhe (1981) on black gram.

At acidic pH 2.80 (below the isoelectric point of moth bean albumins) fraction I retained 22.11 per cent phytate during 2 days dialysis (Table 42). Crean and

Table 42. Binding of phytate with proteins in whey fractions I, II and III as measured by dialysis at different pH and media^a.

Sample	pH	Medium	Phytate ^b (mg)	Protein (g)	Per cent phytate retained after 2 days dialysis	Ratio mg phytate /g protein at 2 days
Whey undialyzed (pH 3.50)	-	-	416.51	1.24	-	335.8
Whey dialyzed Fraction I	2.80	HCl (0.002M)	92.13	0.62	22.11 (12.38)	148.6
Fraction II	8.40	Tris buffer (0.005M)	131.10	1.15	31.47 (17.62)	114.0
Fraction III	6.40	Distilled water	6.38	0.28	1.53 (0.85)	22.8

^a Each value is the average of three determinations.

^b Calculated phytic acid (phytate) assuming 28.20 per cent phosphorus in the molecule.

Values in parenthesis are the percentages of the original component recovered.

Haisman (1963) reported that of the 12 replaceable protons in phytic acid molecule, 6 are strongly dissociated with pK value of about 1.80 and 2 are weakly dissociated and had

a pK value of about 6.30. Hence, phytate would exist as a strongly negatively charged molecule at pH values of 2.80, 6.40 and 8.40. Moth bean albumins on the other hand exist as positively charged molecules at pH 2.80 and a protein-phytate binding was possible as a result of strong electrostatic interaction. Several investigators showed that phytate binding below isoelectric point of proteins occurs as a result of strong electrostatic interactions between positively charged parts of protein (lysyl, histidyl, arginyl and amino terminal groups) and negatively charged phosphate groups of phytate (Okubo et al. 1975; Omosaiye and Cheryan, 1979; de Rham and Jost, 1979 and Reddy and Salunkhe, 1981). Below the isoelectric point of proteins, ionic interaction between phytate and protein would also depend upon the number of positively charged groups available in the proteins that are free to react with anionic phosphate groups of phytate. Hence, as a result of phytate protein complexes formation at acidic pH, 22.11 per cent of the phytates remained during dialysis and the ratio between phytate and proteins was relatively high i.e. 148.6 (Table 42). Loss of phytate during dialysis at acidic pH (2.80) may be partly due to removal of calcium phytate or other phytate salts (which exists in acidic pH in a soluble and deionised form) through the pores of dialysis tubing as it was further evidenced by presence of low concentration

of calcium in acidic pH dialysed fraction I (Table 43).

At alkaline pH, 8.40 (above the isoelectric point of moth bean albumins) fraction II retained 31.47 per cent phytate after 2 days dialysis against Tris-buffer (0.005M, pH 8.40) (Table 42) which may be due to association of phytate with proteins and/or minerals. At alkaline pH, 8.40 both the phytate and proteins exist in ionized form (negatively charged). O'Dell and de Boland (1976) reported that a strong phytate protein interaction occurs at high pH. Numerous studies have showed that the divalent cations such as calcium and other ions mediated the complexation between proteins (negatively charged at alkaline pH) and phytate (also negatively charged at alkaline pH) (de Rham and Jost, 1979; Okubo et al., 1975 and Reddy and Salunkhe, 1981) and a certain minimum concentration of such cations was necessary for maintaining this complex (Saio et al., 1967). They have also reported that the interactions between phytate and proteins occur only above isoelectric points of proteins through the mediation of calcium ions. In fraction II, the ratio of phytate to protein was 114.0. As expected, fraction II had high concentrations of divalent cations i.e. 43.6, 45.3 and 60.0 per cent calcium, magnesium and iron, respectively, compared to the other two fractions I and III (Table 43). These divalent cations might have been involved in binding between phytate and proteins and

Table 43. Mineral content of moth bean flour, whey undialyzed and whey dialyzed fraction I, II and III (mg/100 g)^a.

Minerals	Moth bean flour	Whey undialyzed	Whey dialyzed		
			Fraction I	Fraction II	Fraction III
Calcium	200	58.2	2.0 (3.4)	25.4 (43.6)	4.1 (7.0)
Magnesium	232	67.3	13.2 (19.6)	30.5 (45.3)	14.8 (21.9)
Iron	10.5	1.5	0.3 (20.0)	0.9 (60.0)	0.2 (13.3)
Zinc	3.5	0.2	ND ^b	ND	ND

^a Each value is the average of three determinations.

^b Not detectable.

Values in parenthesis are the percentages recovered from the original water extracted components.

therefore fraction II retained high concentrations of divalent cations calcium, magnesium and iron, zinc, however, was not detectable in whey fractions being its very less concentration in whey.

At pH 6.40 (which is near to the isoelectric point of moth bean proteins), the complexes between phytate and proteins did not form; as a result of 1.53 per cent phytate retention and 98.47 per cent phytate removal in

fraction III during 2 days dialysis against distilled water. At this pH, the phytate molecule also exists in anionic form, since it had a pK value of about 1.80. While the proteins exist in neutral form, in fraction III, the phytate to protein ratio was 22.8. This fraction also had appreciable amounts of divalent cations such as calcium, magnesium and iron.(Table 43). Similar observations were reported by Reddy and Salunkhe (1981) on black gram.

A hand-drawn L-shaped line consisting of a vertical line on the left and a horizontal line at the bottom, intersecting at the origin.

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSIONS

Seeds of moth bean were processed by three most commonly used methods viz. soaking in distilled water and mixed salt solution, germination and cooking and the effects of these processes were independently studied on proximate composition, functional properties, phytate, minerals, polyphenols, trypsin inhibitor activity, electrophoresis of soluble proteins and in vitro protein digestibility. Effects of soaking moth bean in distilled water and mixed salt solution on cooking time and water uptake and leaching of total solids in soaking medium were also studied.

The interactions between phytate, proteins and minerals in the undialyzed and dialyzed whey fractions of moth bean were also studied.

1. It was observed that on soaking moth bean in either distilled water or mixed salt solution, there was decrease in protein, fat, ash and dry ~~matter~~ and increase in moisture content. These observations have indicated that there was increased hydrolysis of proteins, fats and carbohydrates due to the increased enzyme activities.

2. The water uptake and leaching losses of total solids in soaking medium were found higher in distilled water soaked beans than in mixed salt solution soaked beans, when soaked for 12 h. Both were found increased with increase in soaking time in mixed salt solution from

3 to 12 h. It may be concluded that salts influence the concentration gradient which limit the diffusion of water in seeds.

3. The cooking time was found drastically decreased from 75 to 8 min (89.3 per cent reduction) when the beans were soaked in mixed salt solution for 12 h. However, the per cent reduction in cooking time on soaking in distilled water for 12 h was 46.6. It may be concluded that salts soften the bran and solubilize protein, starch and pectic substances and thus reduce the cooking time.

4. The functional properties viz. water and oil absorption capacity of flour and protein were found increased on soaking the beans in mixed salt solution. However, the gelation, nitrogen solubility, foamability and emulsion properties were found decreased. Since the protein content in salt soaked moth bean flours decreased, the functional properties also decreased.

5. The phytate phosphorus and phytate (phytic acid) were found reduced from 2.1 to 1.7 mg/g and from 7.4 to 6.0 mg/g, respectively, during 12 h soaking in mixed salt solution. The per cent decrease in phytic acid in beans soaked in mixed salt solution was 18.9 as against 5.4 for those soaked in distilled water. It may be concluded that phytic acid loss on soaking in salt solution was comparatively more than on soaking in distilled water which might be because of more seed coat permeability.

6. No drastic changes were observed in calcium, magnesium, iron and zinc contents in bean flours due to soaking, either in distilled water or mixed salt solution. The slight decrease in minerals might be due to their leaching into the soaking medium.

7. The per cent reduction in polyphenols on soaking the beans in distilled water and mixed salt solution, both for 12 h, was 39.3 and 64.8, respectively. The reduction in bean polyphenols on soaking in mixed salt solution was found increased with the time of soaking. The greater reduction in polyphenols in salt soaked beans might be due to difference in chemical potential and creating an ionic environment. Moreover, it may be concluded that more reduction was due to increased seed coat permeability for diffusion of polyphenols in the soaking medium.

8. The trypsin inhibitor was found significantly decreased on soaking beans in mixed salt solution. Greater loss of trypsin inhibitor in beans soaked in mixed salt solution than in beans soaked in distilled water, both for 12 h, was due to increased seed coat permeability, which might have allowed to trypsin inhibitor loss.

9. The separation pattern of soluble proteins of soaked moth bean flours was found different than of soluble proteins of unsoaked moth bean flour. The soluble proteins of distilled water and mixed salt solution soaked bean flours were resolved into less than 10 bands.

However, number of bands observed in mixed salt solution soaked bean flour proteins were more than in distilled water soaked bean flour protein. The decrease in number of bands on soaking the beans was due to degradation/hydrolysis of protein. The smaller protein subunits might have failed to give the clear bands.

10. The IVPD was observed more (77.0 per cent) in distilled water soaked bean flour and still more in mixed salt solution soaked bean flour (78.2 per cent) both for 12 h than unsoaked bean flour. It may be concluded that during soaking, the antinutritional factors such as phytate, polyphenols and trypsin inhibitor get lost due to leaching and this might have improved the IVPD.

11. On germinating moth bean for 24, 48 and 72 h, the proximate composition was found changed. Crude fat and carbohydrates were found slightly decreased whereas moisture, crude protein and ash were found slightly increased.

12. The functional properties viz. water and oil absorption capacity of flour and protein, gelation, nitrogen solubility, foamability and emulsion properties were found increased on germinating the beans at all times. It may be concluded that since the protein content in germinated bean flours increased, the functional properties were also increased.

13. The phytate phosphorus and phytate (phytic acid) were found reduced from 2.1 to 1.0 mg/g and from 7.4 to 3.5 mg/g, respectively, during 72 h germination. The per cent decrease in phytic acid during 72 h germination period was observed 52.4. These observations indicated that during germination phytate hydrolysis occurred due to increased activity of phytase.

14. No drastic changes were observed in calcium, magnesium, iron and zinc contents in germinated bean flours except slight decrease in calcium due to leaching and increase in magnesium content due to absorption from external medium.

15. The polyphenols were found decreased significantly, from 1.45 to 1.16 mg/g (20.0 per cent) during 48 h, germination. However, they were found increased to 1.42 mg/g during 72 h germination. It may be concluded that during germination upto 48 h, the polyphenols decreased due to increased activity of polyphenol oxidase and leaching of polyphenols in the external medium. However, beyond 48 h germination, the polyphenols were synthesised or polymerised and gave color reaction and resulted into increased amount.

16. The trypsin inhibitor was found decreased significantly during germination upto 72 h. This observation indicated that during germination trypsin inhibitor was lost or leached out in water.

17. The separation pattern of soluble proteins of germinated moth bean flours resulted into less than 10 bands as observed in ungerminated bean flour protein. It may be concluded that protein solubility was increased during germination due to protein breakdown and increased in low molecular weight components which might have not shown distinct bands.

18. The IVPD was found improved during germination. It was improved from 69.0 to 84.3 per cent during 48 h germination. However, IVPD was found decreased to 78.1 per cent during 72 h germination. These observations indicated that decrease in IVPD beyond 48 h germination might be due to increased polyphenols which bind the proteins through polyphenols-protein interactions.

19. On cooking moth bean for 10,15,20,25 and 30 min, the proximate composition was found changed. There was decrease in crude protein, crude fat and ash whereas there was increase in moisture and carbohydrates content during cooking.

20. The functional properties viz. water and oil absorption capacity of flour and protein were found increased whereas nitrogen solubility, gelation, foamability and emulsion properties were found decreased during cooking at all times. This observation concluded that increase in water absorption capacity of flour and protein was due to heat denaturation of protein which might have resulted

into improved water imbibing capacity. The same could be said of oil absorption capacity. Heat denaturation might have unmasked the non polar residues from the interior of the protein molecule. The decrease in rest of the functional properties might have been due to decrease in crude protein content during cooking.

21. The phytate phosphorus was found decreased during cooking upto 10 min. Beyond 10 min of cooking, however, it increased. This result showed that cooking upto 10 min was desirable. The increase in phytate phosphorus beyond 10 min cooking might be due to its reabsorption in cooking water.

22. Similar results were obtained in case of minerals. The calcium, magnesium, iron and zinc were found decreased during 10 min cooking and increased beyond 10 min cooking. Initially they might have been leached in cooking water and later reabsorbed.

23. The polyphenols were found decreased from 1.45 to 0.32 mg/g (77.5 per cent) during 30 min cooking. There was continuous decrease in polyphenols content during cooking. This observation indicated that decrease in polyphenols during cooking might have been due partially to their leaching in cooking water and partially a change in their solubility or chemical reactivity because of disruption in polyphenols during cooking.

24. The trypsin inhibitor was also found decreased on cooking which might have been partially due to its leaching in cooking water and partially its disruotion during cooking.

25. The separation pattern of soluble proteins of cooked moth bean proteins also resulted into less than 10 bands as observed in control bean flour proteins. It may be concluded that during cooking proteins were degraded/ denatured into subunits which become accessible further for breakdown after cooking and as such bands decrease in number.

26. The IVPD was found increased from 69.0 to 83.5 per cent on cooking moth bean for 30 min. The IVPD was found increased with increasing time of cooking. This observation concluded that during cooking protease inhibitors and other antinutritional factors might have been partially eliminated and resulted into improved IVPD.

27. Among the extractants used for extracting the phytate from moth bean flour, 3 per cent TCA was found most effective, extracting mostly complete phytate as against 73.8, 76.1, 81.9 and 92.8 per cent extraction, respectively, by 2 per cent HCl, Tris buffer (0.05 M, pH 8.40), 2 per cent HClO_4 and distilled water. It may be concluded that low extractability of phytate with 2 per cent HCl and Tris buffer (0.05 M pH 8.40) might have been due to formation

of insoluble complexes between phytate, protein and divalent cations.

28. It was observed that 87.7 per cent of phytate was present in water soluble form in moth bean flour. On treatment with HCl, 67.2 and 36.2 per cent of protein and phytates, respectively, were found precipitated. Recovery of phytate in fraction I (pH 2.80), fraction II (pH 8.40) and fraction III (pH 6.40) was 22.1, 31.4 and 1.5 per cent respectively, after 2 days 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 iron) than the other two fractions I and III.

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